# DEVELOPMENT AND EVALUATION OF NOVEL EXTENDED RELEASE FORMULATION OF ANAGESIC DRUGS

A thesis submitted to
**THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA**
for the Degree of

**DOCTOR OF PHILOSOPHY**
**IN**
**PHARMACY**

By
**Bhavik Shah**

Supervised by
**Prof. Ambikanandan Misra**
Professor of Pharmaceutics
Dean, Faculty of Technology and Engineering


**Pharmacy Department**
Faculty of Technology and Engineering
The Maharaja Sayajirao University of Baroda
Baroda

**August 2013**

# DECLARATION

I hereby declare that the thesis on the topic entitled "Development and evaluation of novel extended release formulation of analgesic drugs" which is submitted herewith to 'The Maharaja Sayajirao University of Baroda, Vadodara' for the award of Ph.D. in Pharmacy is the result of work done by me in the Pharmacy Department, Faculty of Technology & Engineering, The Maharaja Sayajirao University of Baroda, under the able guidance of Prof. Ambikanandan Misra, Professor in Pharmaceutics, Pharmacy Department and Dean Faculty of Technology & Engineering, The Maharaja Sayajirao University of Baroda. I further declare that the result of this work has not been previously submitted for any degree or fellowship.

(Bhavik Shah)

Certified by and forwarded through the research guide,

Ambikanandan Misra,
Professor in Pharmaceutics,
Dean, Faculty of Technology & Engineering,
The Maharaja Sayajirao University of Baroda,
Vadodara-390001

# Pharmacy Department

**Faculty of Technology & Engineering**
**The Maharaja Sayajirao University of Baroda**
Post Box No.: 51, Kalabhavan,
Vadodara – 390 001, GUJARAT, INDIA.
Phone: (+91-0265) 2434187
Fax: (0265) 2423898/2418927
E-mail: head-pharm@msubaroda.ac.in


August 30, 2013

## CERTIFICATE

This is to certify that the thesis entitled "Development and evaluation of novel extended release formulation of analgesic drugs", submitted for Ph.D. degree in Pharmacy by Bhavikkumar I shah comprises the original research work carried out by him under my guidance and supervision.

Guide
Prof. Ambikanandan Misra
Pharmacy Department

Head
Pharmacy Department

Dean
Faculty of Technology and Engineering

# ***Acknowledgements***

*For getting a dream come true, one need to convert it to an ambition and give enough effort towards proper direction to accomplish the goal. The research requires desire, guidance, inspiration, patience, perseverance, financial & mental support and good wishes. The task is not simple, but the journey can be made easy by guidance, support constant stimulation of dear one. Words are limited to express my gratitude to people who have helped and supported me. This is done as an accustomed gesture of acknowledgement. I am heartily gratified to:*

*Honorable Sir Sayajirao Gaekwad, the founder of The Maharaja Sayajirao University of Baroda, for establishing such a wonderful and multidisciplinary education foundation.*

*Pharmacy Department, The Maharaja Sayajirao University of Baroda, which nurtured me from high school pass out to graduate, graduate to post graduate and post graduate to PhD research fellow.*

*My mentor and esteemed guide, Dr. Ambikanandan Misra, Professor in Pharmaceutics, Pharmacy Department, The Maharaja Sayajirao University of Baroda for his privileged guidance, freedom to explore, encouragement, judicious moves, requisite facilities, careful consideration, unstated industrial, mental, social, and emotional support needed throughout course of this work. No words can compare his concern for students, his kind support for getting the financial assistance during course. It is a privileged to work under his guidance and I feel of getting improved intellectual and innovative abilities.*

*Dr. M.R.Yadav, Head and Professor, Pharmacy Department, The Maharaja Sayajirao University of Baroda for providing me the necessary facilities for the research work and for selecting me for Temporary Lectureship for some time which indirectly help me managing my finance.*

*Dr. (Mrs.) Rajani Giridhar, Professor in Pharmaceutical Chemistry, Dr. (Mrs.) K.K. Sawant, Professor in Pharmaceutics, Dr. (Mrs.) S.J. Rajput, Professor in Pharmaceutical Quality Assurance and Dr. RC Mashru, Reader of Pharmaceutical Quality Assurance, Pharmacy Department, The Maharaja Sayajirao University of Baroda for their valuable support during the research work.*

*The non-teaching staff of Pharmacy Department, for their kind cooperation during the work*

*I am thankful to TheraQuest Bioscience Inc. for gift sample of Butorphanol Tartrate, Cadila Healthcare Ltd for gift sample of Lornoxicam, Colorcon Asia Pvt. Ltd, Goa, India forproviding gift sample of Methocel and Polyox.AsahiKASEI for providing free gift sample of MCC, Signet Chemical Corporation Pvt.Ltd for providing initial free gift sample of cellulose Acetate 398-10 and Kollidon.*

*Dr.Vandana Rao, Metallurgical engineering department of M.S University, Baroda for helping analyze samples for SEM.*

*Dr. Yogesh Raichandani to whom I shared my day to day experiences for providing motivation, help and encouragement for the work and thesis compilation.*

*Dr. Jigar Lalani, my friend and colleague, from support to manuscript preparation.*

*Dr. S Ganesh, DGM, Sun Pharma Advanced Research Company Ltd. for his motivating gesture especially thesis compilation and helping to cross psychological barriers for thinking of solution.*

*I am thankful to Dr. Divyen, Dr. Hamsaraj, Mr Kushal and Mr Ronak for inspiration.*

*I express my thanks for the support of my family especially may father for his blessings and for dreaming me achieving this. My affectionate gratitude to my respectable mother, didi, jijaji, wife, for their unconditional love, constant inspiration and tacit patience for being the backbone for achievement of this hercules task. I thank them for many a times bearing with me. Special thanks to my pretty daughter and nieces for their childish gestures which made me feel free of lots of worries. I am also thankful to my in-laws for believing me for my abilities.*

*Last but not the least, I thank almighty God for empowering me with courage, perseverance and strength to take up and accomplish the task.*

*Finally, my sincere apologies to all whose name could not be included in this section. No names have been intentionally missed out. My heartfelt thanks to one and all*

*Bhavik Shah*

## *INDEX*

*LIST OF TABLES*

*LIST OF FIGURES*

# 1.0 INTRODUCTION

The pharmaceutical industry is facing problems in development of new drug molecule for prevention and treatment of existing and newer diseases. Additionally, the development cost for NCEs is very high and escalating, and today the cost is more than 1 billion US $ for developing one NCE and bring it to market. Drug delivery system helps in providing competent drug product of existing drugs. The earliest studies in the field of controlled drug delivery date back to the 1950s. Since then, a large number of drug products with controlled release characteristics, have been introduced. The incredible growth can be attributed to several advantages that these products offer, including improved patient compliance, better therapeutic efficiency, potential for cost saving, patentability and opportunity for extending product life-cycle. Various technologies have been investigated in order to achieve different kinds of modified release, e.g. sustained, delayed, pulsatile, targeted and programmed release. Regardless of the delivery type, the main mechanisms associated with drug transport in these systems include diffusion, swelling, erosion, ion exchange, and osmotic effect. Figure 1.1 shows schematic of plasma profiles attained through different types of delivery systems

**BENEFITS OF EXTENDED RELEASE DRUG DELIVERY SYSTEMS**

By improving the way in which drugs are delivered, an extended release drug delivery system is capable of achieving the following benefits.

1. Maintenance of optimum therapeutic drug concentration in the blood with minimum fluctuations for extended period of time.
2. Predictable and reproducible release rates for extended duration.
3. Enhancement of activity duration for short half-life drugs.
4. Elimination of frequent dosing, inconvenience of night time administration of drug.
5. Optimized therapy and better patient compliance.
6. Improve efficacy/safety ratio
7. Reduction total dose
8. Uniform drug effect
9. Reduction of the incidences and degree of toxic and side effects such as irritation of gastro-intestinal tract caused by some orally administrated drugs.

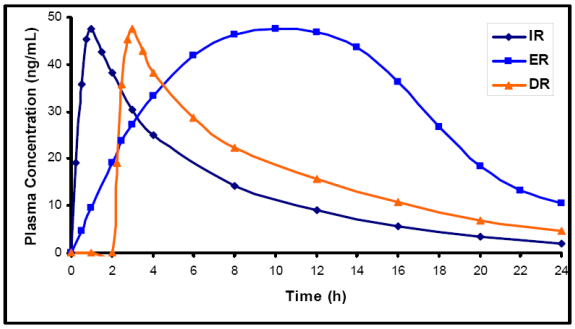



**Figure 1.1 Schematic IR – Immediate Release; ER – Extended Release; DR – Delayed Release**

Acute pain is usually a consequence of an identifiable situation, such as surgery or other trauma, or a consequence of a disease, e.g. mechanical low back pain, sprain, strain, stone etc. The treatment of acute pain, including post-surgical pain has not significantly improved despite the growing recognition that adequate pain relief is a foremost important for improving patient's life. Better pain management is supreme need. Chronic non-cancer pain is a major health problem that afflicts a significant number of patients, resulting in personal suffering, reduced productivity and substantial health care costs. Musculoskeletal conditions such as low back pain, osteoarthritis, and peripheral neuropathic pain and idiopathic chronic pain joint pain are the leading causes of disability in individuals of working age. Patient who undergoes surgery has more fears about post-surgical pain rather any other concern, including whether or not the surgery would be effective. Survey data indicate that chronic pain is poorly managed and that many patients experience moderate to severe pain and reduced activities of daily living despite seeking the assistance of clinicians. In the absence of established medical or surgical procedures to significantly influence many conditions that result in acute and chronic pain, current management is symptomatic and directed primarily towards relief of pain, optimisation of function and minimization of disability.

Non-pharmacologic management of pain is expected to reduce inflammation (with ice and/or heat), rest, exercise, improving range of motion, increasing muscle strength, restoring favourable mechanics and improving coping skills. There is, therefore, a need for optimised pharmacologic and non-pharmacologic treatment strategies for the management of chronic non-cancer pain.

Drug treatment includes nonsteroidal anti-inflammatory drugs (NSAIDs), COX-2 selective inhibitors and opioid analgesics.

Although NSAIDs like COX-2 selective inhibitors and paracetamol are effective in ameliorating the symptoms of acute musculoskeletal pain and mild chronic pain. The inhibition of COX-1 during long-term NSAID therapy is believed to be responsible for a number of common and severe adverse effects, including coagulopathies, gastrointestinal injury and renal impairment.

Lornoxicam, is a short-acting nonsteroidal anti-inflammatory drug (NSAID) from the oxicam group with analgesic, anti-inflammatory and antipyretic properties. It works like other NSAIDs, the inhibitory action on prostaglandin synthesis, via inhibition of cyclo-oxygenase(COX) activity. It is used in the form of injectable, suppository, tablet formulations for relieving postoperative pain following gynaecological or orthopedic surgery, and as effective as other NSAIDs after oral surgery. Lornoxicam was also as effective as other NSAIDs in relieving symptoms of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, acute sciatica and low back pain. Patients suffering from diseases such as acute pain, mild to moderate pain and/or inflammatory conditions and/or related conditions very often require a dosage and a formulation which enable a fast onset of the therapeutic effect of the non-steroidal anti inflammatory drugs (NSAID)
The twice daily dosing regimen for immediate-release Lornoxicam tablets is well tolerated with few incidences of adverse events which are proportionate to the drug plasma level and therefore for improving the therapeutic efficacy, reducing incidences of adverse events and enhancing patient compliance an extended release once-daily regimen is needed. An important goal of analgesic therapy is to achieve continuous relief of chronic pain. Regular administration of an analgesic is generally required to ensure that the next dose to be given before the effects of the previous dose dilapidates.

The objective here is to provide an extended release pharmaceutical composition of lornoxicam, which upon ingestion results in blood plasma levels having pharmacological effect for an extended period of time. It is needed to produce a pharmaceutical composition which releases Lornoxicam in predetermined manner. It is also needed to provide extended release pharmaceutical composition of Lornoxicam for once daily dosage regimen.

Extended therapeutically effective plasma levels over a twenty four hour period with lesser incidences of adverse events by eliminating the troughs and peaks of drug concentration in a patient blood plasma, which comprises administering orally to a patient in need thereof, an

extended release tablet that provides a peak blood plasma level of Lornoxicam for extended duration.

The use of high viscosity grade hydrophilic and the hydrophobic polymers to produce extended or controlled release pharmaceutical composition is very well known. For extending the release, the tablet comprising the drug also comprises of high viscosity grade hydrophilic polymer. On contact with gastric fluid, it enters the tablet core and results into the hydration of the polymer which also controls the release of the drug. Control of the rate of release benefits therapy by producing constant blood plasma levels of the active ingredient and by decreasing the frequency of administration, thereby improving the patient compliance to the dosage regimen. It is imminent to develop pharmaceutical composition of extended release tablets of Lornoxicam suitable for once daily administration with immediate burst effect.

Like most of the NSAIDs, lornoxicam is very sparingly soluble in water. Since lornoxicam is a weak acid (pKa of 4.7), the aqueous solubility of acidic lornoxicam is pH dependent. Fast absorption of drugs into the circulating blood is generally required in managing pain relief, like in the case of lornoxicam. Therefore, for oral dosage forms it is of utmost importance to have the drug dissolved, completely or partially, already when present in the gastric fluid. Thus, in the event where the drug is not absorbed from the gastric mucosa, it may be ready for being absorbed already when entering the upper intestinal tract, such as duodenum. Duodenum itself has a limited amount of liquid, thus resulting in slow dissolution of the drug in duodenum, although the weak acid may be more soluble in the intestinal fluid. Concordantly, the major problem associated with the formulation and effectiveness of the lornoxicam is its variable oral absorption due to insufficient aqueous solubility at gastrointestinal pH, thus making solubility the rate-determining step in the gastric absorption of it followed by controlled rate of release to produce constant blood plasma levels leading to decreasing the frequency of administration, thereby improving the patient compliance to the dosage regimen.

Presently Lornoxicam is administered to adults as conventional immediate release tablets. The current dosing regimen includes twice daily administration. Lornoxicam is available as an immediate release and is approved for sale in various countries.

Opioid analgesics are highly effective in treating moderate to severe pain. Although the role of opioid analgesics in cancer pain is now well established, there is continued resistance to their use in acute, and particularly, chronic non-cancer pain, due to a perceived lack of

efficacy, concerns about analgesic tolerance, side effects, addiction and adverse regulatory sanctions. Many clinicians utilize opioid analgesics half-heartedly, often at suboptimal doses in patients with significant pain that is only partially responsive to non-opioid analgesics.

A major snag in the evaluation of opioid analgesic therapy for non-cancer pain is the possible risk of addiction. The perception that patients with chronic pain receiving opioid analgesics are at high risk of addiction has significantly influenced the approach to treatment for many painful chronic non-cancer conditions. The terms physical dependence, psychological dependence (addiction) and tolerance are often used inconsistently, resulting in considerable misunderstanding among clinicians, patients and regulators. Physical dependence is characterised by an abstinence syndrome following the abrupt cessation of an opioid or following administration of opioid antagonists. Physical dependence to opioids is to be expected with long-term opioid therapy at therapeutic doses. In such patients, symptoms of opioid withdrawal can be avoided or minimised by gradually tapering the dose of an opioid. Psychological dependence (addiction), on the other hand, is a behavioural syndrome that is characterised by an intense desire for the opioid, evidence of compulsive use, and acquisition of opioids by manipulation of the medical system or from a non-medical source. According to the American Academy of Pain Medicine, the American Pain Society and the American Society of Addiction Medicine, addiction is a neurobiological disease that is characterised by behaviours with at least one of the following: impaired control over drug use, compulsive use, use despite harm and craving for the drug. Tolerance is a phenomenon resulting from continued exposure to the drug, resulting in a decreased pharmacological effect over time.

The use of opioids for non-medical purposes has existed throughout recorded human history. Pharmaceutical dosage forms containing opioids have been used for non-medical purposes like by patients with pain who had a pre-existing addiction disorder; by patients with an addiction disorder seeking opioids for their non-analgesic properties; and by recreational drug users looking for periodic mood-altering effects of opioids.

Experience with the use of opioid analgesics in cancer pain and, more recently, in patients with chronic non-cancer pain indicates that in patients with no prior history of drug abuse, the risk of addiction to opioids is low. In depth understanding of the clinical pharmacology of opioids and well-controlled clinical trials data resulted in their more widespread use in patients with non-cancer pain in regulated market. This, in turn, has led to concerns about the

increased non-medical use of opioids through both licit and illicit channels. For instance, unsuspecting clinicians may prescribe opioids for pain to individuals with an addiction disorder or individuals with pain who divert a portion of their prescribed dose to other persons. There have also been documented cases of inappropriate prescribing or dispensing of opioids by physicians and pharmacists, with its eventual diversion into the non-medical marketplace. In addition, the non-medical supply of pharmaceutical-grade opioids is often achieved through prescription forgeries and theft from pharmacies. Non-medical users of opioids analgesics are either recreational drug users who may use such agents episodically, or individuals with an addiction disorder who may require frequent maintenance doses. Opioid analgesics may be ingested whole, crushed and ingested, crushed or vaporised and inhaled, or injected intravenously after attempted extraction of the active pharmaceutical ingredient.

A number of strategies have been introduced to minimise the abuse of opioid analgesics. Primary among these schemes is a legal infra-structure that controls the manufacture, distribution and sale of such drugs. Excessive controls on the manufacture, distribution and particularly the sale of opioids has the unintentional effect of causing physicians, fearful of being accused of permitting opioid overuse, to prescribe suboptimal doses of opioids to patients. This phenomenon is described in the literature as opiophobia or narcophobia. It is also evident that controls on the manufacture, distribution and sale of opioids alone are not adequate to deter the abuse of opioid analgesics.

There is greater resistance on the part of physicians and patients to the use of the more potent opioid analgesics for non-cancer pain. There is also a greater risk of drug abuse and drug diversion with the full opioid agonists, such as morphine, fentanyl, meperidine, methadone, oxycodone and hydromorphone.

Butorphanol is a unique synthetically derived opioid agonist-antagonist analgesic of the phenanthrene series. Unique characteristic of butorphanol is that it is an agonist at the κ opioid receptor and an antagonist at the μ opioid receptor which is not the case for commercially available opioid analgesics morphine, hydromorphone, oxycodone, hydrocodone, fentanyl and pethidine. Butorphanol was pioneered in the U.S. as an injectable analgesic and as an intranasal formulation later. Both injection and nasal spray formulations of butorphanol tartrate are approved in the U.S. for the management of pain when the use of an opioid analgesic is appropriate. The injection formulation is also indicated as a

preoperative or pre-anesthetic medication, as a supplement to balanced anesthesia, and for the relief of pain during labor.

Butorphanol is not a controlled substance in most countries, including India and it is not a listed drug in Schedules I and II of the 1961 Single Convention on Narcotic Drugs, as amended by the 1972 Protocol Amending the Single Convention. The International Narcotics Control Board (INCB), an independent and quasi-judicial control organ monitoring the implementation of the United Nations drug control conventions does not monitor its import or export.

At the time of its commercial introduction in the U.S., butorphanol was an unscheduled narcotic. In 1997, as a result of increased rates of abuse, it was reclassified in the U.S. as a Schedule IV controlled substance. According to the Drug Enforcement Administration (DEA), which enforces the controlled substances laws and regulations of the United States, a Schedule IV drug has a low potential for abuse relative to other substances in schedule III, II or I. For comparison purposes, morphine, fentanyl, meperidine, methadone, oxycodone and hydromorphone, the most commonly used opioids are all Schedule II opioids.

The agonist properties of butorphanol at the κ opioid receptor and antagonist properties at the μ opioid receptor give rise to its unique pharmacological profile when compared with μ opioid receptor agonists such as morphine, fentanyl, meperidine, methadone, oxycodone and hydromorphone, including:

1. A "ceiling" to the respiratory depressant effects of butorphanol;
2. A reduced propensity to produce physical dependence;
3. Significantly reduced reinforcing properties in subjects;
4. Reduced drug liking by drug addicts and recreational drug users;
5. Different opioid receptor binding profile

It will not be wrong to conclude that butorphanol provides a safer alternative to the μ-opioid receptor agonists, both in terms of opioid effects and in terms of the risk of physical dependence, addiction and drug diversion.

The oral route of administration (i.e., oral ingestion) is the most widely used and most widely preferred method of drug administration. It is simple, reliable and readily accessible. Under

most conditions of use, particularly outside the hospital setting, it is the recommended method of drug administration. Even in settings of skilled nursing care, where there are technical and human resources to initiate and manage parenteral therapy, the goal is to rapidly transition patients from parenteral medications to oral medications. Some generally cited exceptions to the use of the oral route include: (i) drugs with poor oral bioavailability; (ii) drugs requiring a rapid onset of effect; (iii) where venous access already exists (e.g., in the peri-operative or intensive care setting); and (iv) where the oral route provides unreliable or inconsistent clinical effects.

Administration of butorphanol by the oral route provides significantly greater flexibility in dosage form design, clinical utility and patient acceptability. When compared with intranasal administration, oral butorphanol may be associated with reduced peak to trough fluctuation in concentrations and clinical effects, such as drug craving. Furthermore, in many cases, such dosage forms may have a reduced potential for abuse and diversion than intranasal solutions of butorphanol, thereby reducing subsequent abuse by the intravenous route.

Well controlled clinical trials also demonstrate the efficacy of oral immediate release butorphanol.

Oral extended release opioids such as morphine are widely utilized for the management of chronic severe pain (Babul et al., J Clinical Pharmacol, 1998) but due to adverse effects they required discontinuation (Bruera et al., Journal of Clinical Oncology, 1998). Recent clinical experience suggests that patients who have failed to obtain adequate analgesia due to intolerable and unmanageable side effects while taking one opioid may benefit from switching to an alternative opioid. Clinicians can exploit this variability in drug response by empirically offering sequential trials of different opioids in order to optimize analgesia and minimize side effects (opioid rotation). Butorphanol is uniquely suited for use in opioid rotation regimens due to its receptor binding properties, which differentiate it from other morphine like molecules.

The apparent low bioavailability of butorphanol (about 17%) has also been referred as a barrier to effective oral therapy and has short half life. However, by developing an extended

release formulation activity duration for short half-life drugs can be enhanced. Several clinical trials have established efficacy of oral immediate release butorphanol for different pain i.e. moderate to severe musculoskeletal pain, postsurgical pain dose from 2 mg to 16 mg. Additionally, low oral bioavailability with the parent drug is usually associated with higher concentration of its principal metabolite. For butorphanol, both hydroxybutorphanol and norbutorphanol appear to be pharmacologically active and may contribute disproportionately to the analgesic efficacy of oral butorphanol. Some parallels can be drawn between the low oral bioavailability of butorphanol and the low oral bioavailability of oral oxymorphone, a μ opioid receptor agonist, which has an oral bioavailability of less than 10 % and has demonstrated robust oral efficacy in acute and chronic pain in immediate release and extended release forms.

The duration of analgesic action of intranasal and injectable butorphanol is approximately 2 to 4 hours. Previous well controlled clinical trials in chronic cancer and non-cancer pain with other opioids have demonstrated that scheduled administration of extended release analgesic results in significant reductions in pain intensity, breakthrough pain and pain related disability, when compared with "unlimited" analgesic administration of immediate release formulation.

An important goal of analgesic therapy is to achieve continuous relief of chronic pain. Regular administration of an analgesic is generally required to ensure that the next dose to be given before the effects of the previous dose dilapidates. Uninterrupted pain suppression through the day use of opioid analgesics is recommended in chronic pain treatment guidelines (American Pain Society, 2002; American Pain Society, 2005). Conventional release so called "immediate-release", "short acting" or "normal release" opioid analgesics have been demonstrated to provide in consistent plasma levels leads to dosing interval of 4-6 hours for treatment of chronic pain. In the case of butorphanol, the duration of analgesic effect when administered parenterally or intranasally is approximately 2 to 4 hours. In contrast, twice-a-day Morphine Sulphate & Oxycodone respectively MS Contin™ controlled release, OxyContin™ timed release or once-a-day Morphine Sulphate preparations of opioid analgesics vinza™ and Extended release Hydromorphone capsule, Jurnista™ prolonged-release tablets are designed to maintain effective plasma levels throughout a 12 or 24-hour dosing interval using various modified release drug delivery systems.

Extended release formulations are the standard in chronic pain. An extended release formulation of butorphanol has the potential to provide better sleep, reduced dependence on caregivers, improved compliance, enhanced quality of life outcomes, and increased control over the management of their pain. In addition, such a formulation may provide more constant plasma concentrations and clinical effects, less frequent peak to trough fluctuations and fewer side effects, compared with short acting opioids. Furthermore, butorphanol, a schedule IV opioid associated with less abuse potential than the Schedule II opioid agonists like morphine, fentanyl, meperidine, methadone, oxycodone and hydromorphone molecules.

## 2.0 LITERATURE REVIEW

The influence of feeding and temporal patterns on GI transit is of great relevance in attempting to optimize drug absorption because the physiology of the digestive process is not suitable for the competent absorption of many of the modern therapeutic entities to administer. There is a short lag phase before the mixing movements in the lower part of the stomach and the pyloric antrum which increase in upper part. There is a sharp contrast between the activity in the top and bottom parts of the stomach. In the small intestine, contact time with the epithelium where absorption is possible is limited, and a small-intestinal transit time of 3.5–4.5 h in healthy volunteers. The Holy Grail of drug delivery would be to discover a mechanism that extended the period of contact with this area of the gastrointestinal tract. Various approaches are there, although a universal solution is not evident, and extend GI residence not proven fruitful. Transit through the lower part of gut is approximated at about 24 hours still in reality the ascending colonic environment has sufficient fluid which facilitate dissolution. The anatomy of the distal colon, with its thick muscular walls, supports a predominant activity. Studies with single administrations of pellets or Pulsincap devices recommended that this area is difficult to reach because the second half of the transverse colon and the descending colon function only as a channel but absorptive.

The time of dosing is an important factor in maximizing colonic contact, particularly in the ascending colon. Morning dosing without fasting is a common practice in clinical trials. It is well accepted that early-morning dosing, a non disintegrating unit clears the stomach in 1–2 h and has a small-intestinal transit time of 3–4 h. Thus, after noon the unit will be expected to reach the ileocecal junction or may have just entered the colon. Colonic transit through the proximal colon of intact objects such as capsules is usually 5–7 h. For a non-disintegrating oral formulation, dosed in the morning, the unit will have arrived at the hepatic flexure by 7–8 pm. The drug gets absorbed in the colon, the maximum time window for absorption is 6–8 h following morning dosing with a monolith. Transit of a dispersed particulate phase through the proximal colon is longer, about 12 h. The maximum time window for absorption in this case is approximately 12–15 hours.

If a delayed release formulation is taken in the afternoon, it will have progressed through to the ascending colon by the time the patient goes to bed. Propulsive movements in the large bowel during the night are relative stagnant and units remain in the ascending colon. Potentially, this can increase the time of contact to 11–13 h even for a slowly dissolving

matrix. On rising, the change in posture stimulates mass movements, experienced by the subject as an urge to defecate, and contents move from the right to the left side of the colon. For poorly soluble substances, the reserve time is an important determinant of bioavailability. Moving away from the current practice of dosing sustained- release formulations in the mornings might allow a reduction in the dosing frequency and increased efficacy of colon-targeted drugs, and would be especially suitable for formulations used to prevent acute disease episodes at night and in the early morning.

The specificity of most drug actions suggests a bond formation between the drug and some cellular constituent, generally referred to as a receptor. Drug receptor interactions may initiate responses by altering the permeability of membranes, by interfering with carrier mechanisms, by modifying templates, or by acting on enzymes. Current trends in fundamental research on drug effects focus on transport of ions and the binding and release of endogenous mediators, with much

Work being done in isolated systems. Agonistic or antagonistic effects demonstrated in the test tube can, however, be completely different when we start to evaluate the action of the drug in the human body. Various physiological factors must be considered to achieve beneficial and therapeutic effects from a drug. The goal is not only to have an effect on ‘‘a man’s heart’’ but on the entire organism.

Two major functions of the small intestine are, a) efficient absorption of nutrients, fluids, electrolytes, and drugs, and b) the simultaneous exclusion of potentially antigenic or toxic inflammatory substances. The overall ability of the intestinal epithelium to provide a barrier to the absorption of these potentially harmful compounds is often referred to as selective permeability.

The oral administration of many drugs and drug candidates is prevented by their poor absorption through the intestine. Several highly potent polypeptides and protein drugs belong to this group of compounds, and it is of considerable interest to improve the understanding of this barrier to enable and optimize oral administration of such substances.

The oral route of administration is preferred for many drugs categories; ease of administration and patient compliance are the main reasons. Estimating oral bioavailability in

humans for the selection of the best development molecule is a considerable challenge to the pharmaceutical industry. Several promising in vitro models became available for the study of the absorption potential of new compounds. Systemic bioavailability is influenced by a variety of factors (Table 1), with poor solubility, poor permeation, intestinal and liver metabolism, and P-glycoprotein (P-gp)-mediated efflux being among the most common detrimental influences on drug absorption. Physicochemical properties of a drug and their influence on the overall bioavailability are major area of interest. These properties can have a particularly important effect on the absorption. The main uptake mechanism through mucosal membranes is by passive diffusion, using the transcellular pathway. However, several alternative processes needs to be considered. These processes may not be independent and take place in parallel. Membrane diffusion by the transcellular and paracellular pathways is a physicochemical process; therefore, physicochemical properties are believed to have an important influence on these membrane transport. Physicochemical properties can be a helpful guide in the selection of compounds that will have sufficient oral absorption in humans and therapeutic effects.

In vitro–in vivo correlations (IVIVC) are important because bioequivalence of changes in drug products can be approved by in vitro. The generic drug products approval decision will be solely on the basis of in vitro data if an IVIVC established. To establish IVIVC it is necessary that the chosen in vitro method can reflect the in vivo plasma profile. A precise knowledge of the in vivo situation is a key to an in-vivo in-vitro correlation (IVIVC and drug solubility, dissolution, and gastrointestinal permeability as the fundamental parameters for correlating the in vitro with the in vivo data. On the in vivo side, we have to consider that, in addition to the permeability; the motility can also impinge on the availability of the drug. The average passage time of this dosage form was about 1 day to 30 h, shows that the transit time in the major absorption site, the small intestine is less than one-sixth of the total residence time in the GI tract. In the colon, the absorption as well as enzymatic and bacterial decomposition of drugs varies compared with the small intestine. Presently, for the low-solubility, high-permeability drugs that commonly come from drug discovery, we expect an IVIVC if the in vitro dissolution rate is similar to the in vivo dissolution rate. Here in vitro dissolution and in vivo dissolution are important parameters unless the dose is very high. In contrast with poorly soluble drugs and controlled-release dosage forms, the gastric-emptying and small-intestine–transit time will not influence the fraction dose absorbed of highly soluble, highly permeable drugs because those drugs will normally be absorbed within the

gut passage. For these compounds, the gastric emptying will alter $T_{max}$ and probably also $C_{max}$, and, if this occurs, it is unlikely that a satisfactory IVIVC can be achieved.

IVIVC can be expected if the dissolution rate of a drug is slower than gastric emptying particularly for class II and IV drugs. Due to variable gastric emptying regulates the absorption rate of a class I drug and the permeability limits the uptake of class III drug.
The choice of dissolution test for an IR dosage form can be facilitated by BCS classification considerations. Compounds with good solubility (Class 1 & 3) the medium and method should be kept simple. For low solubility drug with and good permeability (class 2) the choice of test will depend on the objective to be met. For the development of IVIVCs, the new biorelevant media appear to offer significant advantages over the traditional compendial media in terms of being able to forecast the in vivo dissolution behaviour. In the majority of cases, *USP* paddle are the method of choice for the dissolution test, with medium volume chosen according to the intended administration conditions (fasted or fed) and the site where the drug will most likely dissolve in the GI tract. No data predicts supports correlating rotational speed and in vivo hydrodynamics conditions. Normally, rotational speeds of 50–100 rpm yields data that can be successfully used to develop IVIVCs. Slow release of the drug from the dosage form, unstable drug in the GI tract, lack of permeability of the GI mucosa to the drug, and metabolism due to first pass effect of drug in the gut wall or liver. In principle, dissolution tests can be used to predict the in vivo performance of the dosage form when release of the drug is the limiting factor in the absorption process. There are two classic cases in which release is limiting to absorption: controlled-release (CR) dosage forms and immediate-release (IR) dosage forms containing drugs that are poorly soluble. The biopharmaceutics Classification Scheme is useful as a guide to determine whether an IVIVC can be expected for an immediate release product. Figure 1- 2 depicts Oral absorption process in Gastro-intestinal track.

Extended-release formulations are created to ensure a limited, controlled release of the drug dissolution rate. Only the amount of released drug is available for solubilisation and permeation through the gut wall.

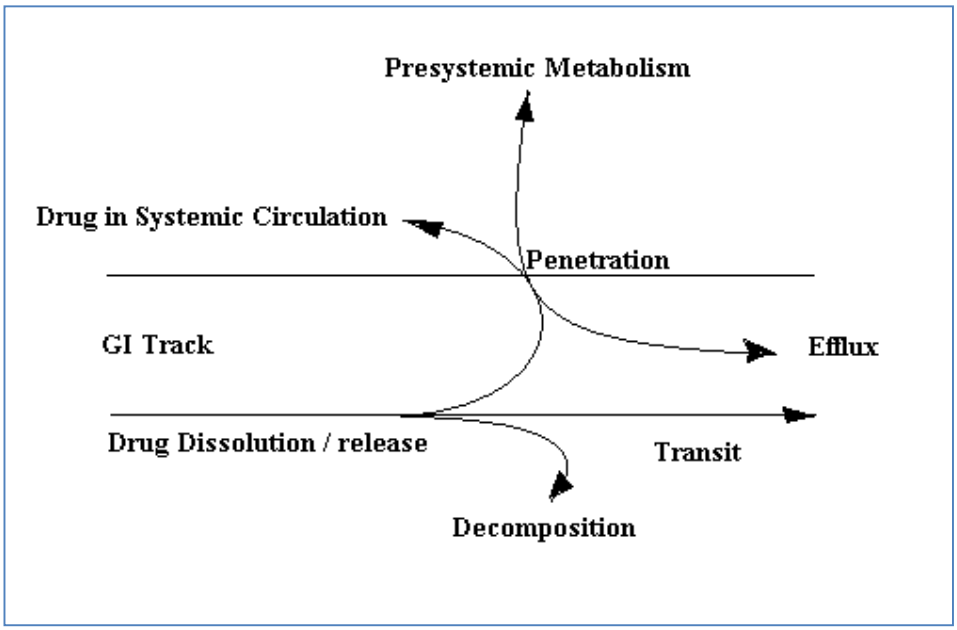



**Figure 1 - 2 Oral absorption process in Gastro-intestinal tract**

In vitro dissolution is a tool in development of solid dosage forms provided that in vivo predictive results are obtained. To fulfil this requirement, physicochemical and pharmacokinetic properties of the substances, as well as the function of the dosage form and physiological factors, must be taken into consideration in design of dissolution tests and in predictions of in vivo performance based on in vitro data.

**Extended release Drug Delivery System**

Extended release (ER) dosage forms have been extensively used because of their significantly improved efficacy clinically and better patient acceptability. Among the various controlled release (CR) drug delivery systems available in market, oral controlled release systems hold the major market share because of their obvious advantages of ease of administration and better patient compliance. A number of design options are available to control or modulate the drug release from an oral dosage form. Majority of oral CR dosage forms falls in the following categories,

- Matrix systems
- Reservoir systems and

- Osmotic systems.

**2.1 Matrix Systems**

In matrix systems, the drug is embedded in a polymer matrix and the release takes place by partitioning of drug into the polymer matrix and the release medium. In contrast, reservoir systems have a drug core surrounded/ coated by a rate controlling membrane. However, factors like pH, presence of food, and other physiological factors may affect drug release from conventional CR systems (matrix and reservoir). Osmotic systems utilize the principles of osmotic pressure for the delivery of drugs. Drug release from these systems is independent of pH and other physiological parameters to a large extent and it is possible to modulate the release characteristics by optimizing the properties of drug and system. Osmotic pumps are well known for delivering drug at a zero order rate.

Hydrophilic matrix tablets are the frequently developed ER dosage forms for oral use. Hydrophilic matrices do not disintegrate and are formulated for drug to release over a pre-defined period of time following exposure to water on oral administration. The goal for n oral extended release matrix is reduction in dosing frequency compared to a conventional dosage form. Preparation of matrix tablets that may involve direct compression of a blend of a drug with release retarding polymers and other excipients is most direct approach for extended release delivery of drugs for oral administration. Various polymeric materials have been explored as release retarding agents in hydrophilic matrix systems. There are different oral matrix formulations such as inert matrices and wax or hydrophobic matrices are available, formulation and design of hydrophilic matrices is important to understand due to better shelf life. Wax or lipid matrices are prepared by adding the drug and excipients to the molten fat or wax, congealing, granulating, and compressing into matrix cores. Substances that produce these matrices include carnauba wax, fatty alcohol, GPS, stearyl alcohol, beeswax, aluminium mono-stearate, and GMS. The mechanism of drug release from wax matrix may be diffusion of drug through liquid-filled pores. On the other hand, there are erodible through digestion in totality lipid-based (Wax) matrix systems that control the release of drug through combination of diffusion and erosion. Inert matrices using polymers such as ethylcellulose, methylacrylate, methylmethacrylate, polyvinyl chloride, and polyvinyl acetate are prepared through wet granulation and compression into matrices. Drug release from these matrices is by simple diffusion through water-filled pores.

The ingredients of a hydrophilic matrix HM can be either directly compressed or granulated to aid flow and compression or improve content uniformity. On exposure to water, in the hydrophilic matrix polymer on or near surface of the matrix hydrates & form a gel like layer. The gel layer controls water ingestion into the matrix and controls the mechanism of drug release. Therefore, the mechanism of drug release from HM systems is a combination of hydration and swelling of the formulation, drug dissolution, drug diffusion, and erosion of surface polymers. Polymers used in the manufacture of hydrophilic matrices alone or in combinations include Cellulose Derivatives and Non-cellulose Derivatives. Cellulose derivatives are methylcellulose (MC), hydroxypropylmethylcellulose (hypromellose, HPMC), sodium carboxymethylcellulose (Na CMC), and hydroxypropylcellulose (HPC) and Non-cellulose Derivatives are carbomers, sodium alginate, xanthan gum, guar gum and carrageenan. The choice of the polymer used in the matrix formulation depends on the chemistry of the drug, desired release profile. Hypromellose is available commercially from Dow Chemical Company under the trade name METHOCEL. It is available in different chemistries depending on the degree of hydroxypropoxyl and methoxyl group substitutions. Hypromellose 2910, USP (METHOCEL E) and (METHOCEL K ) hypromellose 2208, USP are most widely used in extended release formulations and are distributed worldwide by Colorcon Inc. The USP classification code is based on the substitution type with first two digits representing mean percent methoxyl substitution and the last two digits representing the mean percent hydroxypropyl substitution. Hypromellose is the most commonly used polymer for the preparation of hydrophilic matrix systems. Hypromellose quickly forms uniform gel that protects the matrix from disintegration, formation of a strong, viscous gel layer which controls release. In addition, HPMC has a long history of application in marketed products with wide global regulatory acceptance. As described above, HPMC polymers may differ in their degree of methoxyl or hydroxypropoxyl substitution and/or degree of polymerization. Varying the ratios of methoxyl and hydroxypropoxyl substitution and molecular weights influences properties of formulation like organic solubility, thermal gelation temperature of their aqueous solutions, swelling, flow properties, compressibility and compactability, diffusion behaviour, and drug release properties. Figure 2 – 2 depicts schematic representation of hydrating swellable hydrophilic matrix formulation.

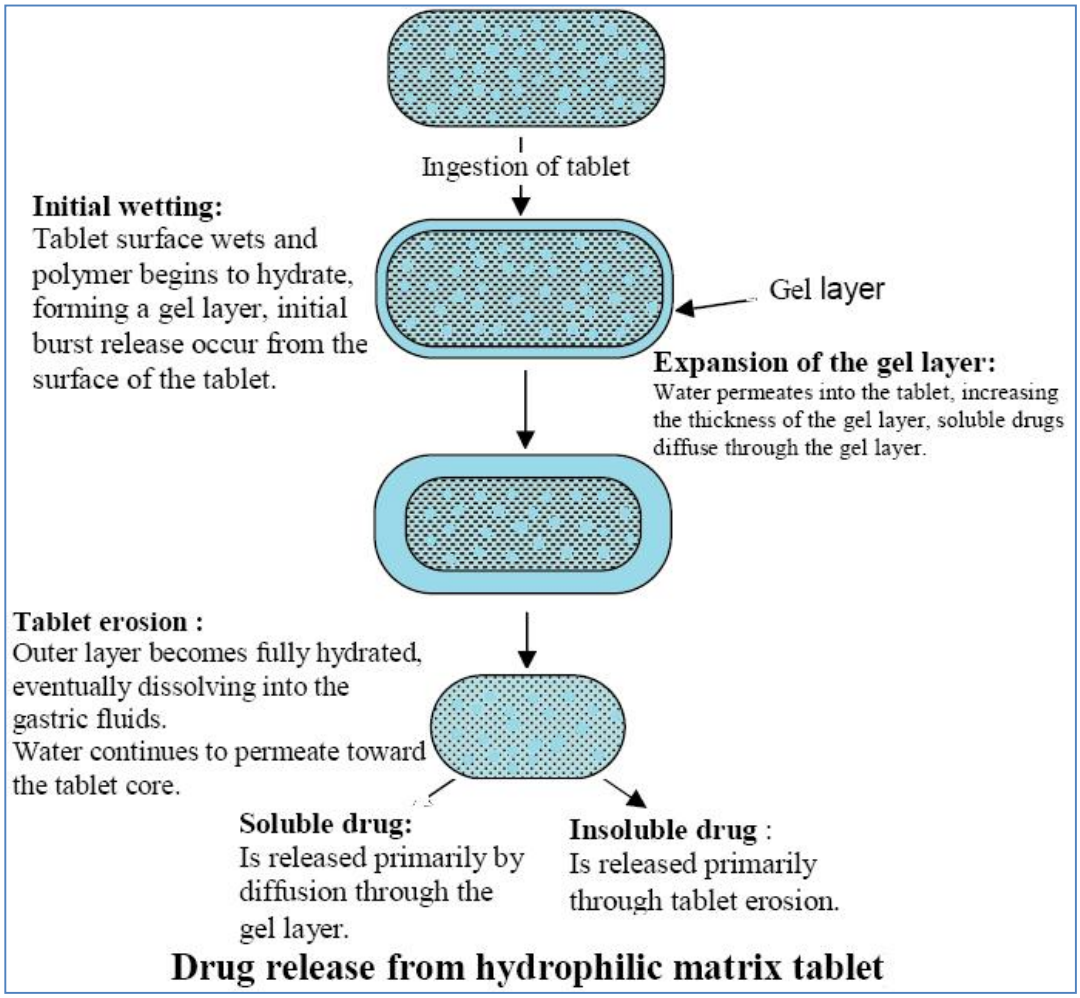



**Figure 2 - 2 Schematic representation of hydrating swellable hydrophilic matrix tablet**

Extended drug release from HM systems is achieved through rapid hydration of the polymer on the outer tablet surface to form a gelatinous layer. Very fast formation of a gelatinous layer is essential to retain structural integrity, prevent water ingress to the interior of the matrix, and inhibit immediate disintegration of formulation. Once gel layer is formed which is protective in nature, that controls the water movement in the gel layer and further imbibitions of water into the formulation. Logically, hydrophilic polymers are of small particle size range and so ensuring rapid and consistent hydration of the polymer. Immediately once the outer gel layer hydrates fully, the polymer starts disentangles from the surface, which is continuously replaced with the hydrated polymer from within the core to control drug release. Hydroxypropylmethylcellulose (HPMC) used in many successful controlled release systems, mainly due to generally recognized as safe (GRAS) status and biodegradable behaviour. Compatibility is proven with several drugs, high drug loading is possible using it and can be easily incorporated to form matrix tablets by through established

manufacturing techniques like direct blending or granulation. Wide range of viscosity grades of HPMC are available allows the developer to modify the release of drugs from HPMC matrix tablets according to therapeutic requirement. Increasing the HPMC concentration in the tablet or using higher viscosity grades increases the strength of the gel layer and retards the penetration of water into the dry glassy core Outcome, decrease in release of both water-soluble and water-insoluble drugs. Modification of drug release from HPMC matrix tablets has been attained by modifying the polymer concentration and by using different viscosity grades of HPMC. HPMC and polyethylene oxide are well known for their rapid hydration and gel formation. The release of drug from a hydrophilic matrix system relies on swelling of the matrix, dissolution of the drug, and diffusion and erosion properties of the gel layer. The solubility and dose of the drug, type and quantity of fillers, and the polymer influence the mechanism of drug release. Mechanism and rate of drug release from hydrophilic matrices depend not only on the type and level of the polymer, choice of filler, and size of the matrix, but also on the physic-chemical properties of the drug substance. Researchers have used quantitative structure–property relationship (QSPR) methods for prediction of mechanism and rate of drug release from HPMC matrices.

The microenvironment pH control may enhance solubility or stability of the formulated drug in the matrix and within the gel layer of a hydrated system. Compression coating (tablet in tablet) technology containing hydrophilic polymers has been applied to generate lag time followed by either fast release or slow release. Mini-matrix is examples of application of hydrophilic matrices in multiparticulate formulations. Various technologies have been investigated to modulate drug release from hydrophilic matrices. Use of polymer blends may provide an alternative approach to modulate drug release compared to conventional single polymer matrices. Matrices containing Polyethylene Oxide (MW from 600,000 to 7,000,000), polymer concentrations, and carbopol combinations also made, polymer concentrations and molecular weight, had a significant impact on drug release rate and profile. Matrices containing polymer blends of HPMC, Carbopol, are also available. It is postulated that there may be strong hydrogen bonding between the carboxyl groups of Carbopol and hydroxyl groups of HPMC leading to stronger interactions between the two polymers and therefore slower drug release than a matrix with a single polymer. Blends of HPMC and polyvinyl pyrrolidone (PVP) in a hydrophilic matrix shows a biphasic release of caffeine as a water-soluble model drug. The initial release is controlled by HPMC, but because of faster release of drug from the matrix, the PVP becomes rich progressively. The

breakup of the HPMC gel by enriched PVP resulted in a bimodal release profile with faster release at the terminal phase. The breakup happens at different time points depending on the PVP level in the matrix.

Blending of polyvinyl acetate phthalate (PVAP), an enteric polymer, HPMC, and Carbopol also studied as release retardant. Changing the ratio of the polymers in the blend, it was possible to modulate the drug release profile. Use of polymer blends at lower concentrations, providing the desired release profiles and maintaining formulation robustness, would be a suitable approach for drugs with higher dose. The retardation of drug release has been attributed to the synergistic interactions between PVA, Carbopol, and HPMC leading to formation of a stronger gel layer and slower diffusion and erosion rates.

Formulation of drugs with pH-dependent solubility in hydrophilic matrices may be helpful to release profiles that alter with variation in the pH of the media. In most situations, a desirable release profile should be pH independent to withstand the physiological pH changes in the gastrointestinal tract. Buffers and polymers are added in the formulation that maintains pH within the gel structure of hydrophilic matrix. The majority of the frequently used low molecular weight pH modifiers tend to diffuse out of the hydrated matrix faster than the drug and maintaining the desired pH over the entire duration of release can be a challenge. The effectiveness of different types of acids, fumaric acid, citric acid, succinic acid, and ascorbic acid, to maintain a microenvironment pH for the duration of release is studied. Even sodium hydroxide and melamine are studied. pH modifier leaches out of the matrix system immediately, ionic polymers that contribute to microenvironment pH are used and due to their high molecular weight they stay within the gel structure until eroded from the surface of the matrix.

Minitablets having a diameter around 2 to 3 mm can be encapsulated or compressed into larger tablets. Mini-tabs are advantageous because of reduced inter- and intra-subject variability, formulation flexibility, and appropriate dosage forms for drug delivery for lower age group. Miniaturises (2.5mm in diameter and 12 mg in weight) have been evaluated for biphasic drug release (fast/slow) HPMCK100M and EC can be used as release retardant matrix formers. Miniaturises (3mm in diameter and 2mm in height) using polymer blends of Polyethylene Oxide and polyethylene glycol (PEG) and ethylcellulose containing metoprolol

tartrate have been are also used. The minimatrices can be made through hot melt extrusion to get 24 h drug release profiles.

Hydrophilic matrix systems are widely considered, simple, robust, and versatile extended release technology. Different chemistries and viscosity grades of hydrophilic polymers and more specifically different grades of HPMC, allow this technology for ER formulations of drugs with wide range of solubility and dose strengths. Various approaches have been used to modulate drug release for having predicted pharmacological profiles. HPMC matrix can be used as a platform for blending other polymers to provide flexibility in formulation for achieving desired formulation characteristics. Ionic, nonionic, and insoluble polymers have been used in HPMC matrices as blends or as film or compression coating to successfully modulate the release profile of various drugs. The addition of ionic polymers may not only modify the drug release profile but also allow micro-environmental pH control of the gel layer, which may enhance solubility or stability of drugs.

The use of polymer blends for controlled drug delivery systems can offer major advantages, including: (i) Fabrication of desired drug release, mechanical properties and drug release mechanisms, (ii) improved matrix formation and on storage stability, and (iii) the possibility to develop novel strategies for site specific drug delivery within the gastro intestinal tract (e.g., colon targeting).

Multiple factors affect drug release from hydrophilic polymeric matrix

1. Drug
- Molecular weight
- Solubility
- Drug particle size
- Drug Dose

2. Polymer
- Molecular weight
- Polymer Particle Size
- Type of polymer
- Polymer blend
- Substitute of the polymer side chain

- Radius of gyration
- Ionic strength of medium
- Percentage of the polymer
- Intrinsic viscosity
- Dissolution medium
- Dose-dumping effect
- Temperature

3. Formulation

- Amount of water penetrating the matrix
- Characteristics of the tablet : Geometry, other drug, excipients
- Micro environmental pH
- Ait trapped in the matrix
- Resistance of matrix to breakage
- Manufacturing process

HPMC concentrations as low as 10% (w/w) can be used to modulate drug release from matrix tablets, polymer percolation threshold for robust HPMC matrix tablets is a good idea. The percolation threshold for any component (A) in a binary system (e.g. A–B) is defined as the concentration at which "individual isolated clusters" of this component (A) change to "an infinite cluster". In HPMC matrix tablet formulation, below and above this percolation threshold sudden changes in matrix integrity and/or dug release mechanism can be observed. It has been reported that the HPMC concentration has an effect on the robustness of formulation, and should be more than 30% (w/w) in matrix tablets to eliminate the effect of small variations in manufacturing method or raw material. Even viscosity deviation within the same viscosity grade and polymer chemistry in raw materials can drastically contributes drug release from erosion based HPMC (100 cP) matrix tablets. HPMC percolation threshold for controlled release application was found to be between 30 and 35% (w/w) for HPMC (100 cP) in HPMC–mannitol matrix tablets. Robust tablet performance above percolation threshold is observed, whereas disintegration was observed below this polymer concentration under stressed conditions. The low viscosity HPMC grades (50 cP and 100 cP) in combination with water-soluble generates consistent erosion controlled systems for water-insoluble drugs. Recommendations on minimum HPMC concentration for robust matrix tablets are supported by in-vitro observations. However, mostly the results obtained from in-

vitro studies cannot be directly correlated to the in-vivo environment due to in-consistency in the physiological conditions of the GI tract experienced by controlled release formulations. Evaluation of erosion and disintegration properties of tablet of this type in in-vivo, with HPMC content at 20 and 40% (w/w), representing values above and below the proposed percolation threshold is key parameter.

### 2.1.1 Mechanism of Drug Release from Hydrophilic Matrices

Controlled drug release from hydrophilic matrix systems is achieved through rapid hydration of the polymer (e.g., HPMC) on the outer tablet surface to form a gelatinous layer. This rapid formation of a gelatinous layer is important for retaining structural & functional behaviour, prevent water to the interior of the matrix, and inhibit disintegration of the tablet. Once the protective gel layer is created, it controls the water progress in the gel layer and further entrance into the tablet. Therefore, hydrophilic polymers are usually supplied in small particle size (CR Grade) range to ensure rapid and consistent hydration of the polymer. As the outer gel layer fully hydrates, the polymer disentangles from the surface, which is continuously replaced with the hydrated polymer from within the core to control drug release. HPMC and polyethylene oxide are well known for their rapid hydration and gel formation. The release of drug from a hydrophilic matrix system relies on swelling of the matrix, dissolution of the drug, and diffusion and erosion properties of the gel layer. The solubility and dose of the drug, type and quantity of fillers, and the polymer influence the mechanism of drug release. The release behaviour from an insoluble inert matrix can be mathematically expressed by the following equation:

$$\frac{dM}{dh} = C_0 dh - \left(\frac{C_s}{2}\right) \text{.....(1)}$$

Where, dM is the change in the amount of drug release per unit area and dh denotes the change in the thickness of the zone of matrix of the drug that has depleted. C0 and Cs are the total amount of drug in a unit volume of matrix and saturated concentration of the drug within the matrix, respectively. According to diffusion theory, dM is proportional to the diffusion coefficient (Dm) and Cs; therefore,

$$dM = \left(\frac{C_m C_s}{h}\right) dt \text{.....(2)}$$

Combination and integration of Equations 1 and 2 lead to

$$M = [CsDm(2Co - Cs)t]^{0.5} \text{....(3)}$$

When the amount of drug is in excess of the saturated concentration, Equation 3 can be refined to Eq (4)

$$M = (2C_s D_m t)^{0.5} \quad \text{.....(4)}$$

This equation (4) is known as the Higuchi equation and initially was valid only for planar matrix systems, and later it was modified to consider different geometrical shapes and matrix characteristics including porous structures. It is important to keep in mind that the classical Higuchi equation was derived under pseudo-steady-state assumptions and cannot be applied to real controlled release systems. The final equation shows that if a system is predominantly diffusion controlled, then it is expected that a plot of the drug release against square root of time will result in a straight line. For the purpose of data treatment, the Higuchi equation can be simply written as Eq (5)

$$M = kt^{0.5} \quad \text{.....(5)}$$

where M is the portion of drug released at time t and k is a constant. If the predominant mechanism of drug release from this type of matrix is diffusion controlled, then the drug release can be controlled by varying the factors such as porosity, tortuosity, initial concentration of drug in the matrix, solubility of the drug, and polymer system forming the matrix. Since a hydrophilic matrix structure in an aqueous medium undergoes dynamic alterations due to the polymer hydration and swelling, drug dissolution, diffusion, and erosion, mathematical models describing drug release from these systems are complex. But for simplicity a series of transport phenomena involved in drug release have been reported. Figure 3 -2 summarizes a schematic representation of three situations described in the literature: the swelling, the erosion, and the diffusion.

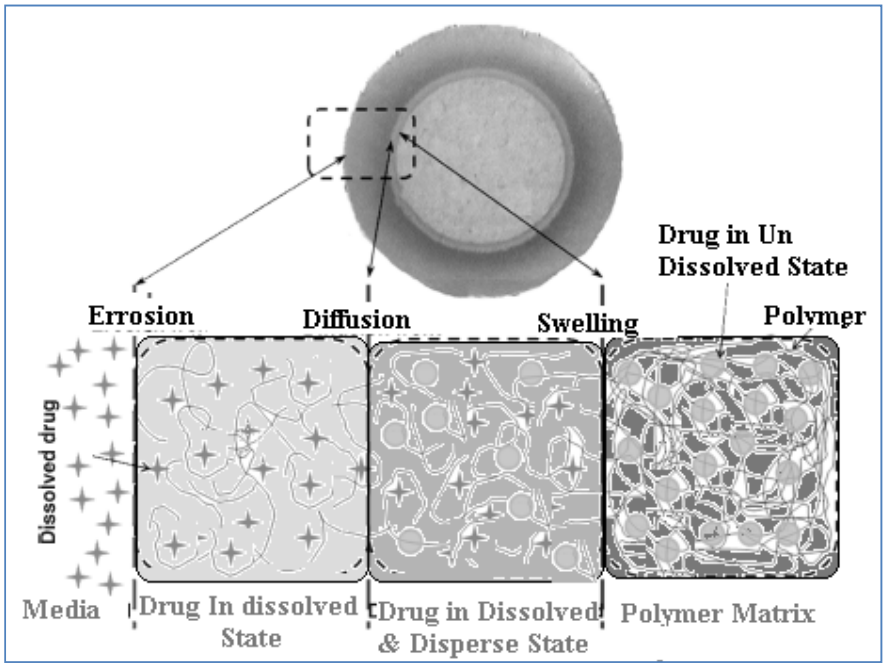



**Figure 3- 2 Schematic representation of three situations swelling ,erosion and diffusion**

Each situation indicates a change in physical condition from the adjoining front. The swelling separates the rubbery region (gel layer) from the glassy region (dry core) and has absorbed enough water to allow macromolecular mobility and swelling of polymer. Diffusion that separates the areas of undissolved drug from the area of dissolved drug is located between the swelling and erosion. Finally, the erosion is the border between the unstirred gel layer and the well-stirred medium separating the matrix from the bulk solution. A large number of mathematical models have been developed to describe drug release profiles from matrices. The simple and more widely used model is the one derived Eq (6)

$M_t/M_a = k\mathrm{t}^{\,n}$.....(6)

Where Mt/Ma is the fraction of drug released, k is the diffusion rate constant, t is the release time, and n is the release exponent indicative of the mechanism of drug release. The equation was modified by Ford et al. to account for any lag time l or initial burst release of the drug:

$M_t t/M_a = k(\mathrm{t}-1)^{\,n}$.....(7)

It is clear from Equations 6 and 7 that when the exponent n takes a value of 1.0, the drug release rate is independent of time. This case corresponds to zero-order release kinetics (also known as case II transport). Here, the polymer relaxation and erosion are the rate-controlling steps. When n¼0.5, Fickian diffusion is the rate-controlling step (case I transport). Values of

n between 0.5 and 1 indicate the contribution of both the diffusion process and polymer relaxation in controlling the release kinetics (non-Fickian, anomalous, or first-order release). It should be noted that the two extreme values of n ¼, 0.5 or 1 are valid only for slab geometry. For cylindrical tablets, these values range from 0.45<n<0.89 for Fickian, anomalous, or case II transport. In order to describe relaxation transport, Peppas and Sahlin modified Equation 7 to account for relaxation transport: Eq (8)

$Q = k_1 t^n + k_2 t^{2n}$ .....(8)

where $k_1$ and $k_2$ were Fickian diffusion constant and relaxation mechanism constant, respectively. If the surface area of the system is fixed, which is unlikely, the value of n should be 0.5 and the above equation is transformed to Eq (9):

$Q = k_1 t^{0.5} + k_2 t$ .....(9)

The first term of the equation represents the diffusion phenomenon while the second term accounts for polymer erosion.

### 2.2 Osmotic Systems

Since pharmaceutical agents can be delivered in a controlled pattern over a long period by osmotic pressure, past two decades have witnessed increasing interest in the development of osmotic systems.

#### 2.2.1 Principle of osmosis

Osmosis is defined as a process in which the solvent molecules move through a semipermeable membrane from a pure solvent to a solution or from a dilute solution to a concentrated solution. Abbe Nollet first reported osmotic effect in 1748, but Pfeffer pioneered the field by quantification of osmotic effect. He measured the pressure in 1877 by utilizing a membrane, which was selectively permeable to water but impermeable to sugar (Fig. 4 -2). A semi-permeable membrane separated sugar solution from pure water allowing entry of water. He observed a movement of water in to the sugar solution that stopped when a pressure ($\pi$) applied to the sugar solution, and hence postulated that this pressure, the osmotic pressure ($\pi$) of the sugar solution directly proportional to the concentration of solution and absolute temperature.

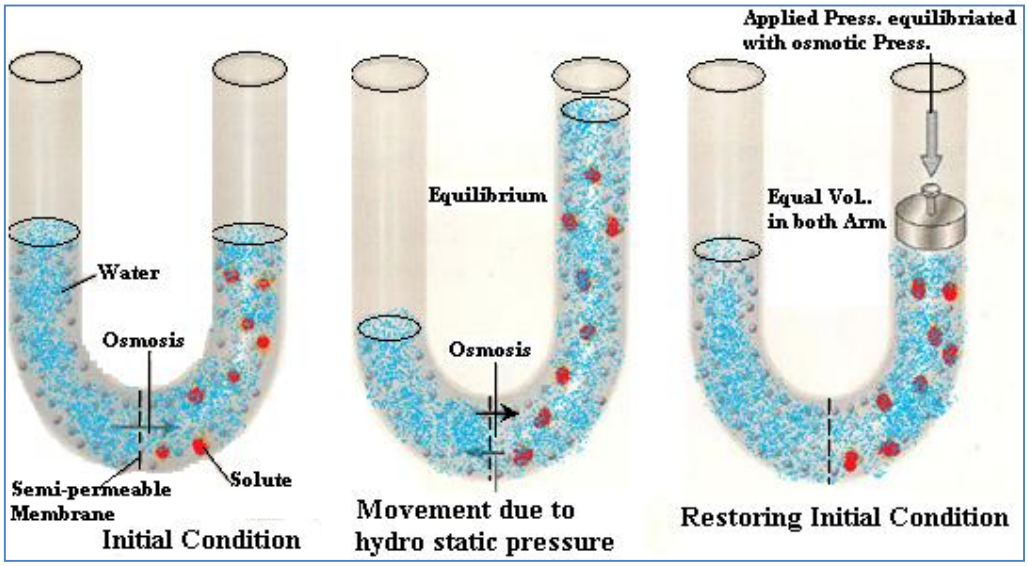



**Figure 4 - 2 Osmotic movement and the osmotic equilibrium and osmotic pressure**

Van't Hoff established the analogy between the Pfeffer results and the ideal gas laws by the expression, Eq (10)

$\pi V = nRT$ .....(10)

Where,

$\pi$ - osmotic pressure in atmosphere

V - volume of solutions in liters

n - number of moles of solutes

R - gas constant equal to 0.082 liter atm/mole

T - absolute temperature.

Osmotic pressure can be obtained to a good approximation vapour pressure measurements by using the expression.

$\pi = RT/V \ In \ (P_0/P)$ .....(11)

Where,

$P_0$ is the vapour pressure of pure solvent

P is the vapour pressure of the solution

V is the molar volume of the solvent

The osmotic water flow across the membrane is given by,

$d_v/d_t = A\theta\Delta\pi/l$.....(12)

Where,

$d_v/d_t$ - water flow across the membrane area A and thickness l, whose permeability is $\theta$.

$\Delta\pi$ - osmotic pressure difference between the two solutions on either side of the membrane. Osmosis is the phenomenon that makes controlled drug delivery a truth. Osmotic pressure created due to imbibition of fluid from external environment regulates the delivery of drug from the osmotic device. There are various factors that guide of drug delivery like nature of semi-permeable membrane, diameter of delivery orifice, surface area of semipermeable membrane, nature and concentration of osmogen etc.

Pharmaceutical solutes used in osmotic pumps and the osmotic pressures of these saturated solutions are presented in Table 1 - 2 .

| **Compound** | **Osmotic pressure (atm)** | **Compound Mixture** 50: 50 | **Osmotic pressure ( atm)** |
|---|---|---|---|
| Sodium chloride | 356 | Lactose- fructose | 500 |
| Fructose | 355 | Dextrose- fructose | 450 |
| Potassium chloride | 245 | Sucrose- fructose | 430 |
| Sucrose | 150 | Mannitol- fructose | 415 |
| Dextrose | 82 | Lactose- sucrose | 250 |
| Potassium sulphate | 39 | Lactose- dextrose | 225 |
| Mannitol | l38 | Mannitol- dextrose | 225 |
| Sodium phosphate tribasic | 36 | Dextrose- sucrose | 190 |
| Sodium phosphate dibasic | 31 | Mannitol- sucrose | 170 |
| Sodium phosphate monobasic | 28 | Mannitol- lactose | 130 |

**Table 1 - 2 Osmotic Pressures of Saturated Solutions of Commonly Used Pharmaceutical Solutes**

**Advantages of osmotic drug delivery systems**

Osmotic drug delivery systems for oral and parenteral use offer distinct and practical advantages over other means of delivery.

- Constant (Zero Order) delivery rate is achieved with osmotic systems as shown by in vitro and in vivo experiments.
- Delivery may be delayed or pulsed, as per requirements.
- Drug release is independent of gastric pH and hydrodynamic conditions for orals.
- Fast release is possible with osmotic systems compared with conventional diffusion-controlled drug delivery systems.
- The release rate of osmotic systems is predictable and can be preprogrammed by modulating the release control parameters.
- A high degree of in vivo-in vitro correlation (IVIVC) is obtained in osmotic system.
- The release from osmotic systems is minimally affected by the presence of food in the gastrointestinal tract (GIT).

### 2.2.2 Types of Osmotic Pump System

#### 2.2.2.1 Asymmetric Membranes

Use of asymmetric membranes in osmotic drug delivery that consist of very thin, dense skin structure supported by a thicker, porous structural layer is also described in the literature. These membranes have high flux characteristics and thus, higher release rates for poorly water-soluble drugs can be obtained. Moreover, the permeability of the membranes to water can be easily adjusted by controlling the membrane structure and porosity. The asymmetric membranes can be applied to tablets, capsules, or multi-particulate formulations.

Herbig and co workers in 1995 developed a new type of membrane coating for osmotic drug delivery which offers significant advantage over the membrane coatings used in conventional osmotic tablets. These new coatings have an asymmetric structure, similar to asymmetric membranes made for reverse osmosis or ultra filtration, in that the coating consists of a porous substrate with a thin outer skin. These asymmetric membrane coatings can be used to make osmotic drug delivery formulations with several unique characteristics. High water fluxes can be achieved, facilitating osmotic delivery of drugs with low solubility and making higher release rates possible. The permeability of the coating to water can be adjusted by controlling the membrane structure, allowing altering release kinetics without any change in coating material or coating thickness. In addition

the porosity of the film can be controlled, minimizing the time lag before drug delivery begins and allowing the drug to be released from a large number of delivery ports. This type of coating has also been applied to capsule and multi particulate formulations.

Lin and Ho formulated asymmetric membrane coated capsules with in-situ formation of delivery orifice. The capsule wall membrane was produced by phase inversion process in which an asymmetric membrane was formed on stainless steel mold pins by dipping the mold pins into a coating solution containing a polymeric material followed by dipping into a quenching solution. Permeability across the asymmetric membrane of the capsule was determined for drugs with water solubility in a moderate to high range. Poorly soluble drug could not generate enough osmotic pressure to activate drug release. Solubilization either by the addition of solubility enhancer, SLS, or by a solid dispersion with HPMC could increase the solubility of nifedipine to a sufficient extent to activate drug release. Synergistic action of both HPMC and SLS increased the solubility of nifedipine resulting in release from the system.

**Osmotic pumps and solubility of drugs**

Osmotic pumps are well known for delivering drugs at a constant rate. Formulation of both highly water soluble and highly water insoluble drugs are not suitable candidates for osmotic drug delivery. The kinetics of osmotic drug release is directly related to the solubility of the drug within the core. Assuming a tablet core of pure drug, the fraction of core released with zero-order kinetics is given by the following equation (Eq.13),

$$F(z) = 1 - S\rho \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (13)$$

Where $F(z)$ is the fraction released by zero-order kinetics, $S$ is the drug's solubility (g/ $cm^3$ ), and $\rho$ is the density (g / $cm^3$) of the core tablet. Drugs with a solubility of $\leq 0.05$ g/ $cm^3$ would be released with $\geq 95\%$ zero-order kinetics. Hence a solubiliser for the drug can be included in the core formulation in case of water insoluble drugs. It is also possible that the drug is very highly soluble and the water flux is too great to provide sustained release. In this case, the core can include a component that suppresses the solubility of the active agent.

Some of the approaches used to deliver drugs having extremes of solubility in the literature:

**Co-compression of drug with excipients:**

Incorporation of excipients that modulate the solubility of drug within the core can be one approach to control the release of drugs from the osmotic systems. McClelland et al. and Zentner et al. reported CPOP of a highly water-soluble drug, diltiazem hydrochloride (solubility more than 590 mg/ml at 37.7ºC). Because of very high water-solubility, the majority of the drug fraction was released predominantly at a first-order rather than the desired zero-order rate. The solubility of diltiazem hydrochloride was reduced to 155 mg/ml by incorporation of sodium chloride (at 1 M concentration) into the core tablet formulation. The modification resulted in more than 75% of the drug to be released by zero-order kinetics over a 14–16-h period.

Controlled porosity solubility modulated osmotic pumps for delivery of drugs having low water solubility are described in US Patents. The composition described consists of controlled release solubility modulating agents, which are either surfactants (e.g. sodium dodecyl sulfate) or complexing agents (e.g. sodium salicylate). In order to prolong the availability of these excipients within the device, they were either surrounded by a rate controlling membrane or dispersed in a matrix. In the examples, tablet cores of two different drugs, namely, simvastatin and lovastatin, along with the solubility modulating agents were prepared and coated with a microporous membrane. The release of drug from the systems was controlled for an extended period of 4–24 h.

Prabakaran with his co worker formulated elementary osmotic pump for diltiazem hydrochloride. The drug candidate selected shows higher aqueous solubility, and hence is an unfit candidate for the formulation of elementary osmotic pumps. To control the solubility of the drug in the core various hydrophilic polymers (HPMC & NaCMC) were incorporated and the otherwise fast dissolving core was altered to release the drug for the prolonged period. Ingredients of the system were optimized for parameters like drug polymer ratio and amount of osmogent, for the desired release pattern. The coated tablets were drilled mechanically in the centre of each pump. The aperture diameter and coating thickness were measured microscopically using empty shells obtained after complete dissolution of the contents. Different dissolution models were applied to drug release data in order to establish release mechanism and kinetics. Criteria for selecting the most appropriate model were based on best goodness of fit and smallest sum of squared residuals.

**Use of encapsulated excipients**

Thombre and co-workers described a capsule device coated with asymmetric membranes to deliver drugs having poor water-solubility. In the examples, solubility of a poorly water-soluble drug, glipizide, was improved by incorporation of encapsulated excipients (pH-controlling excipients) within the capsule device. The solubility modifier (meglumine), in the form of mini-tablets, was coated with a rate controlling membrane to prolong its availability within the core. Thus, the solubility of glipizide was improved leading to its prolonged release from the device.

**Use of swellable polymers**

Swellable polymers can be utilized for osmotic delivery of drugs having poor aqueous solubility. Examples using this approach are reported in US Patent by Khanna for carbamazepine, theophylline, acetylsalicylic acid, and nifedipine. The formulation mainly consists of a compartment, containing the drug, swelling agents, and osmogents, coated with a rate controlling membrane. Vinylpyrrolidone / vinyl acetate copolymer (Kollidon VA 64, BASF) and polyethylene oxide (MW: 53  10 , Polyox -coagulant, Union Carbide) were used as swelling agents. Uniform rate of swelling of these polymers ensures that the drug is released at a relatively constant rate. Also, the pressure produced during swelling does not lead to rupture of the system.

Sastry and his co worker prepared and evaluated an optimized, osmotically controlled formulation of atenolol. Preparation involved the fabrication of biconvex, bilayered tablets containing drug, an osmotic agent and other additives. Studies on the screening of several variables have revealed that orifice size, coating level and the amount of carbopol have pronounced effects on the in vitro release kinetics of atenolol. For formulation optimization a three factor, three level Box-Behnken design was employed with independent variables of orifice size, coating level and the amount of carbopol. The response variables was cumulative per cent of atenolol released with constrains of time for certain percentage release. Preparation of optimized formulations showed a good correlation between predicted and observed values

**Use of effervescent mixtures**

Use of effervescent mixture, can be another approach to deliver poorly water-soluble drugs from osmotic dosage forms. After administration, the effervescent mixture containing the drug is delivered under pressure through the delivery orifice in the membrane. This method

of enhancing release of poorly water-soluble drug is reported in US Patent assigned to Theeuwes. In one of the examples, citric acid and sodium bicarbonate were used as the effervescent couple for the delivery of acetyl salicylic acid. The formulation imbibes aqueous fluids across the membrane causing the couple to generate an effervescent solution that dispenses the drug in a suspension form.

**Use of Cyclodextrin derivatives**

Incorporation of the cyclodextrin–drug complex has also been used as an approach for delivery of poorly water-soluble drugs from the osmotic systems. A CPOP has been described for testosterone (having a solubility of 0.039 mg/ml at 37 °C), solubility of which was improved to 76.5 mg/ml through complexation with sulfobutyl ether-β-cyclo dextrin sodium salt. In a comparative study with hydroxypropyl-β-cyclodextrin (HP-β-CD) and a sugar mixture, it was found that testosterone release from the device in the presence of sulfobutyl ether-β-cyclo dextrin sodium salt was mainly due to osmotic pumping while for HP-β-CD, the major contribution was due to diffusion. In case of the sugar mixture, the drug was poorly released due to the absence of solubilizer. Similar results were obtained with prednisolone and chlorpromazine. It was reported that sulfobutyl ether-β-cyclo dextrin sodium salt could serve both as a solubilizer and osmotic agent.

Okimoto defined membrane controlling factors responsible for drug release from a controlled porosity osmotic pump tablet that utilizes a sulfobutyl ether-β-cyclodextrin, as both a solubilizing and osmotic agent. Chlorpromazine was used as a model drug. The core tablets were coated with cellulose acetate solutions varying the amount and size of micronized lactose, the amount of triethyl citrate and composition ratio of dichloromethane to ethanol. The membrane surface area of the coated tablet was measured with multi point analysis by the gas absorption method. The release rate of drug from osmotic pumps increased with increasing amounts of micronized lactose and decreasing amount of TEC and lactose particle size in the membrane. Also, release rates from the formulations using mixtures of varying ratios of dichloromethane to ethanol were almost identical.

**Modulation of resin**

Release of a highly water-soluble drug, diltiazem hydrochloride from a CPOP was modulated effectively using positively charged anion-exchange resin, poly (4-vinyl pyridine) Zentner and co-workers. Pentaerythritol was used as osmotic agent and citric and adipic acids were added to maintain a low core pH to assure that both the drug and resin

carry a positive charge. The solubility of diltiazem hydrochloride was reduced for an extended period and pH-independent zero-order release was obtained.

**Using alternative salt form**

For an ionic drug, an alternative salt form can also be used as reported for metoprolol and oxprenolol. Hydrochloride salt used in commercial formulations of oxprenolol was found to have high water solubility (70% w/v) making it difficult to achieve extended zero-order delivery from osmotic systems. The authors replaced it by the less soluble succinate salt. In case of metoprolol, they used fumarate salt form as drug and osmotic driving agent, instead of tartrate salt. These salt forms were found to have optimum solubility and provided extended release up to 24 h.

**Using crystal habit modifiers**

If the drug exists in more than one crystal form, each having different aqueous solubility, it is beneficial to include a crystal modifying agents. One such example is reported in US Patent inventor Koparkar and Shah, 1994, wherein a slightly soluble drug, carbamazepine, along with crystal modifying agents (combination of hydroxymethyl cellulose and hydroxyethyl cellulose) and other excipients was formulated in the form of osmotic pumps that were able to provide approximately zero-order release for the desired period of time.

**Use of lyotropic crystals**

Use of lyotropic liquid crystals, to assist osmotic delivery of poorly water soluble drugs, is also reported in the literature (Curatolo, 1989 and 1992). The lyotropic liquid crystals are non-polymeric compounds, generally in the molecular weight range of 200–1500. Also known as amphipathic compounds, these form mesophases and swell in presence of water. Compounds that can be used as lyotropic liquid crystals include natural phosphatides such as phosphatidyl- choline (lecithin), phosphatidyl ethanolamine, phosphatidylserine, phosphatidylglycerol, and the like. Few examples using this approach are mentioned in US Patent no. 5,108,756 and 5,030,452. In these examples, Alcolec lecithin (American Lecithin Co., Atlanta, GA) and mixture of soybean phospholipids was utilized for osmotic delivery of two insoluble drugs, namely, glipizide and prazosin. The inventors claimed that the extended drug release up to 24 h was achieved.

**Use of wicking agents**

Inclusion of wicking agents in the osmotic formulations has also been reported as an approach for poorly water-soluble drugs. A wicking agent is dispersed throughout the composition that enhances the contact surface area of drug with the incoming aqueous

fluids. Thus, the drug is released predominantly in a soluble form through the delivery orifice in the membrane. The authors delivered nifedipine using this approach and some of the reported wicking agents are colloidal silicon dioxide, PVP, sodium lauryl sulfate, etc.

### 2.2.2.2 Elementary osmotic pump (EOP)

The concept of osmotic delivery through elementary osmotic pump (EOP) was first introduced by Theeuwes . The EOP consists of an osmotic core with the drug, surrounded by a semipermeable membrane with a delivery orifice. Figure 5 -2 shows schematic diagram of elementary osmotic pump (EOP), which in its simplest design, consists of an osmotic core (containing drug with or without an osmogent) coated with a semipermeable membrane.

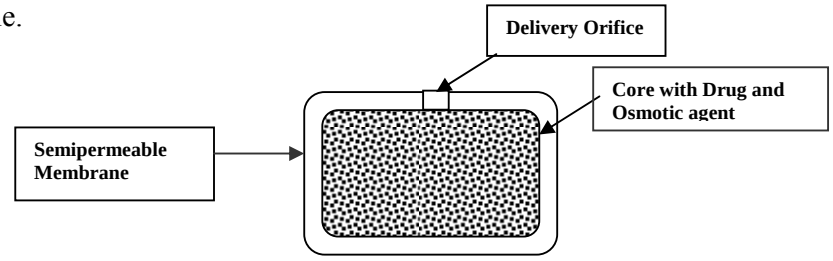



**Figure 5 - 2 : Elementary Osmotic Pump**

The device in fact represents a coated tablet with a hole and may be ultimate simplification of the original Rose-Nelson pump, when this coated tablet is exposed to an aqueous environment the osmotic pressure of the soluble drug inside the tablet draws water through the semipermeable coating resulting in the formation of a saturated aqueous solution inside the device. The membrane is non-extensible and the increase in volume due to imbibition of water raises the hydrostatic pressure inside the tablet, eventually leading to flow of saturated solution of active agent out of the device through small orifice.

The dosage form, after coming in contact with the aqueous fluids, imbibes water at a rate determined by the fluid permeability of the membrane and osmotic pressure of core formulation. This osmotic imbibition of water results in formation of a saturated solution of drug within the core, which is dispensed at a controlled rate from the delivery orifice in the membrane. Though 60–80% of drug is released at a constant rate from EOP, a lag time of 30–60 min is observed in most of the cases as the system hydrates before zero-order delivery from the system begins. These systems are suitable for delivery of drugs having moderate water solubility.

Solubility of drug in water plays a critical role in functioning of osmotic pump. Typically the solubility of drug delivered by these pumps are at least 10 to 15% w/w, example of drugs with this property are sodium indomethacin, potassium chloride, metoprolol and acetazolamide

Elementary osmotic pump for Indomethacin is reported in detail in a literature (Theeuwes 1982) that explains the determination of theoretical release rate of Indomethacin elementary osmotic pumps. Using that concept, delivery of any agent in solution form from the elementary osmotic pump can be achieved at a rate proportional to the solubility of the agent inside the system (Sd) and the osmotic pressure of the formulation inside the system ($\pi_t$). Given a tablet size with surface area (A), and given the membrane permeability and thickness, the desired rate can be obtained by incorporating into the core formulation substances that affect either Sd or $\pi_t$. Such a formulation can be called as the composite core.

Delivery of potent agents may require the incorporation of formulating agents to permit fabrication of a system of acceptable size (these agents are also added during the formulation of conventional tablets). If these agents are water soluble, system performance can be predicted from the knowledge of certain parameters and the theoretical considerations. The zero order release rate of drug, $(dm_d/dt)_z$, from such a system, assuming a negligible osmotic pressure of the environmental fluid, is then given by,

$$Z = \left(\frac{dm_d}{dt}\right)_z = K \frac{A}{h} \pi_t \quad \text{.....(13)}$$

Where,

K - osmotic permeability coefficient of the membrane,

A - membrane area and h is the membrane thickness. The zero order rate will persist from time t=0 to $t=t_z$, at which time the solids, drug and osmotic agent have gone into solution. The non-zero order rate will decline parabolically as a function of time.

The above equation provides a convenient way of calculating the membrane permeability (k) for a set of systems with the same release rate. Alternatively for systems with different membrane thickness and release rates, the slope of the line of the release rate versus the inverse of the membrane thickness provides a means of calculating the membrane

permeability. Consequently, the release rate can be expressed as a function of membrane weight (w), since this weight is related to membrane thickness.

$w = \rho_m Ah$. ……(14)

Where $\rho_m$ is the membrane density, and by substituting above equation to the first equation,

$$Zd = K \frac{A^2}{w} \rho_m \pi_t S_d \text{……..……..…(15)}$$

The membrane permeability, therefore, can be obtained from the slope of the line $Z_d$ versus 1/w.

The final equation indicates the parameters to which the average zero order release rate will be sensitive. These parameters are, membrane permeability (k), tablet core surface are (A), membrane weight (W), density ($\rho_m$), total osmotic pressure ($\pi_t$) and drug solubility ($S_d$). When a composite composition is chosen, $\pi_t$ and $S_d$ become fixed for the zero-order release period. The fixed composition also determines the total surface area (A) of the tablet core. When the membrane is chosen and applied reproducibility, values for K and $\pi_m$ are fixed. Therefore, when testing is conducted at a constant temperature, the average zero order release rate should be a function of the weight of the membrane applied.

Alza is the leading pharmaceutical concern which developed the elementary osmotic pump under the trade name OROS®, for oral controlled release. The first elementary osmotic pump that hit international market was Osmosin® (controlled release Indomethacin).

**PRODUCTS INCORPORATING ALZA'S OROS® TECHNOLOGY INCLUDE**

- Alpress™ LP (prazosin) once-daily extended-release tablet sold in France for the treatment of hypertension.
- Cardura® XL (doxazosin mesylate) sold in Germany for the treatment of hypertension.
- Concerta® (methylphenidate hydrochloride) CII once-daily extended-release tablet for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in patients age six and older.
- Covera-HS® (verapamil) a Controlled Onset Extended Release (COER-24™) system for the management of hypertension and angina pectoris.

- Ditropan XL® (oxybutynin chloride) extended-release tablet for the once-a-day treatment of overactive bladder characterized by symptoms of urge urinary incontinence, urgency and frequency.
- DynaCirc CR® (isradipine) once-daily, extended-release tablet for the treatment of hypertension.
- Efidac 24® (chlorpheniramine) over-the-counter, extended-release tablet providing 24-hour relief from allergy symptoms and nasal congestion.
- Glucotrol XL® (glipizide) extended-release tablet used as an adjunct to diet for the control of hyperglycemia in patients with non-insulin-dependent diabetes.
- Sudafed® 24 Hour (pseudoephedrine) over-the-counter nasal decongestant for 24-hour relief of colds, sinusitis, hay fever and other respiratory allergies.
- Procardia XL® (nifedipine) extended-release tablet for the treatment of angina and hypertension.
- Volmax® (albuterol) extended-release tablet for relief of bronchospasm in patients with reversible obstructive airway disease.

In the OROS tablets, semipermeable membrane coating of the device must be 200-300 microns thick to withstand the pressure generated within the device. These thick coverings however, lower the water permeation rate, particularly for moderately water-soluble drugs. In general it could be predicted that these thick coating devices are suitable for highly water-soluble drugs. The delivery rate attained with moderately soluble drugs is generally low, even with the most water permeable membrane also. The above problem can be resolved by utilizing a coating material having very high water permeability, such as addition of plasticizers and a water-soluble additive to the cellulose acetate membranes which increased the permeability of latter up to tenfold. (Theeuwes and Ayer, 1978).

The second approach of Theeuwes involves the multi layer composite coating around the tablet (Figure 6 - 2). The first layer is made up of thick microporous film that provides the strength required to withstand the internal pressure, while second layer is composed of thin semi permeable membrane that produces the osmotic flux. The support layer is formed by including the coating of the tablets with a layer of cellulose acetate containing 40 to 60% of pore forming agent such as sorbitol.

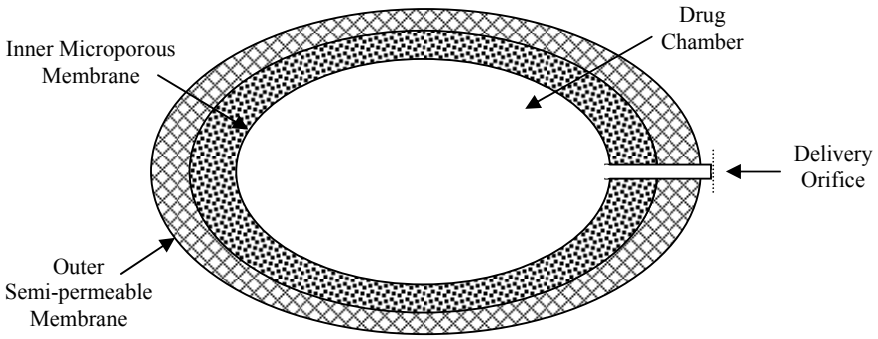



**Figure 6 - 2 Composite membrane coating to deliver moderately soluble drugs**

Another modification includes the addition of a carbonate or bicarbonate salt to the drug chamber, which eventually leads to effervescence when exposed to water due to formation of carbon dioxide at stomach pH.

The simple elementary osmotic pump suffers from the disadvantage that it can only deliver relatively soluble drugs, which are capable of developing an osmotic pressure greater than physiological fluids. Incorporation of water-soluble compound into the tablet formulation such as, sodium chloride, sucrose, fructose or other common tableting aids can be used, which serves as osmotic attractants and overcomes this limitation.

Several coated tablet have been reported by Zentner et al*,* 1985 in which the drug escapes, following leaching of water soluble components, such as lactose or polyethylene glycol from the coating material. Once the tablet has been swallowed, water-soluble component dissolves in external fluid, resulting in initiation of pumping system.

Shokri et al., 2008 designed a new type of elementary osmotic pump (EOP) tablet for efficient delivery of poorly water-soluble/practically insoluble drugs. The drug release

profile from osmotic devices showed that the type of polymer in the core formulation could markedly affect the drug release. The results also demonstrated that aperture size is a critical parameter and should be optimized for each swellable EOP system. This study also revealed that optimization of semipermeable membrane thickness is very important for approaching zero order kinetics.

### 2.2.2.3 Push–pull osmotic pump (PPOP)

Push–pull osmotic pump (PPOP) can be used for delivery of drugs having extremes of water solubility. As shown in Figure 7 - 2, it is a bilayer tablet coated with a semipermeable membrane. Drug along with osmogen is present in the upper compartment whereas lower compartment consists of polymeric osmotic agents Swanson et al., 1987, Wong et al., 1986. The drug compartment is connected to the outside environment via a delivery orifice. After coming in contact with the aqueous environment, polymeric osmotic layer swells and pushes the drug layer, thereby delivering the drug in the form of a fine dispersion via the orifice by Grundy in 1996.

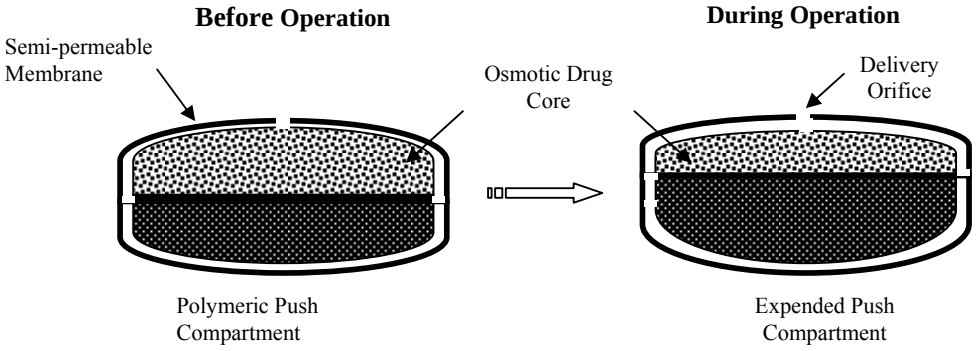



**Figure 7 - 2 Drug delivery process from two-chamber osmotic tablet**

Pumps with two chambers separated by an elastic or movable barrier are particularly interesting and valuable because they allow delivery of drugs with limited solubility. This class of osmotic pump can further be classified into two groups, one with internal film that moves from a rest to an expanded state leading to change in volume of chamber. The second group has fixed volume chamber communicating through opening provided in between.

Swanson et al. (1987) formulated a dosage form based on the gastrointestinal therapeutic system (GITS) push pull osmotic pump configuration in three strengths with different drug delivery rates(mg/hour) per dose (mg), as 1.7/30, 3.4/60, and 5.1/90. The delivery rates of drug from these systems are controlled by drug loading, composition of osmotic components, membrane properties, and dimensions. The release rates were independent of pH in the range from gastric pH 1.2 to intestinal pH 7.5. *In vitro* release studies were carried out at different stirring rates (50, 100, 150 rpm). The release rates are independent of stirring rate and therefore unlikely to be influenced by motility in the gastrointestinal tract. The in-vivo release tests in dogs were found to be equal to the release rate in-vitro. Nifedipine GITS dosage forms were administered to human subjects, absorption rates, calculated from resulting plasma concentrations, indicate that the cumulative amount of drug absorbed in humans over 24 hours is proportional to the amounts of drug delivered in-vitro. Plasma concentrations are therefore predictable and remain relatively constant for 24 hour dosing interval. Weight of drug layer, weight of push layer, membrane thickness, and membrane permeability along with the delivery rates was reported. Comparison of in-vitro and in-vivo cumulative amount released was shown.

Among the successful approaches incorporation of finely dispersed drug in hydrogel present a most valuable alternative. Many of the useful hydrogel polymers are ionic materials such as sodium carboxy methylcellulose, which contains ionizable groups, which provide most of the osmotic pressure required to draw water through the semipermeable membrane. These polymers possess dual property of being compressed in dry conditions and become fluid gels, which are easily extrudable through the small delivery hole in hydrated conditions. A number of modifications are available for this type of system such as delayed push–pull system (as used in Covera HS, extended release formulation for verapamil), multi-layer push–pull system (for pulsatile or delayed drug delivery), and push–stick system (for delivery of insoluble drugs requiring high loading, with an optional delayed, patterned, or pulsatile release profile)

**2.2.2.4 Controlled porosity osmotic pumps (CPOP)**

CPOP contain water-soluble additives in the coating membrane, which after coming in contact with water, dissolve resulting in an in situ formation of a microporous membrane (Fig. 8). The resulting membrane is substantially permeable to both water and dissolved

solutes and the mechanism of drug release from these systems was found to be primarily

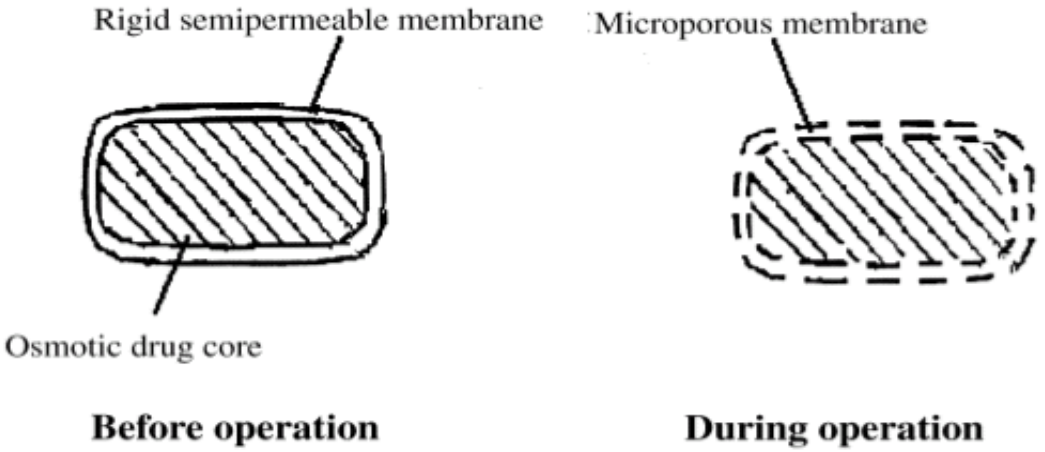



**Figure 8 - 2 Cross- sectional diagram of L–OROS delivery system before and during operation**

osmotic, with simple diffusion playing a minor role by Zentner et al., 1985.

Zentner et al., (1985a) developed a controlled porosity osmotic pump of cyclobenzaprine hydrochloride. The coating solution applied to the core was Cellulose Acetate-398-30: sorbitol : polyethylene glycol 400 (10 : 7.5 : 1 by parts) dissolved in dichloromethane:methanol:water.

Verma et al., (2003) developed extended release formulations of isosorbide mononitrate based on osmotic technology. Formulation variables like type (PVP, PEG 4000 & HPMC) and level of pore former, per cent weight gain were found to affect the drug release from the developed formulations. Drug release was inversely proportional to the membrane weight but directly related to the initial level of pore former in the membrane. Burst strength of the exhausted shells was inversely proportional to the level of pore former, but directly affected by the membrane weight. The release from the developed formulations was independent of pH and agitation intensity, but depended on the osmotic pressure of the release media. Results of SEM studies showed the formation of pores in the membrane from where the drug release occurred.

Gondaliya and Pundarikakshudu in 2003 developed a controlled porosity osmotic pump of diltiazem hydrochloride. *In vitro* dissolution studies of tablets were conducted in osmotically active media. The external osmotic pressure was maintained at higher levels than the osmotic pressure generated inside the tablet. The drug release rate was tested in a

2.4% (by weight) magnesium sulfate solution (6 atm pressure) and water (0 atm pressure). An *in vitro* release rate was found to be 2.2 mg/h in a magnesium sulfate solution, although in water it was found 10.6 mg/h. In the magnesium sulfate solution, the drug release rate was mainly attributed only to diffusion through the membrane, although in water, the drug release rate was mainly attributed to diffusion and osmosis. High *in vitro* drug release was mainly attributed to osmotic pressure generated inside the osmotic tablets. In an osmotically active medium, the osmosis phenomenon is stopped. These results were further confirmed by performing in *vitro* drug release study by changing the medium instead of the method. An *in vitro* drug release study was conducted for 0- 4 h in water (0 atm pressure) followed by 4- 8 h in a 2.4% (by weight) magnesium sulfate solution (~6 atm pressure) followed by 8- 12 h in water. The results showed a significant difference in the release rate in different media. From these results, one may conclude that the drug release from the tablets was mainly caused by diffusion in an osmotically active medium. *In vitro* release was carried out in a USP type II dissolution test apparatus at 100 rpm. Operating condition was 900 ml of distilled water at 37$^{o}$C. Coating membrane was composed of cellulose acetate as film forming polymer, glycerol as pore forming agent and dibutylpthalate as a plasticizer. An equal portion of isopropyl alcohol and acetone was used as a coating solvent.

Verma and Garg developed controlled porosity osmotic pump of glipizide. To assure a reliable performance of this formulation independent of pH, release studies were conducted according to pH change method. The release media was simulated gastric fluid (SGF, pH 1.2) for first 2 h, acetate buffer (pH 4.5) for next 2 h, followed by SIF (pH 6.8) for the remaining period of 24 h. In order to study the effect of agitational intensity of the release media, release studies of the formulation were carried out in dissolution apparatus at various rotational speeds using USP-I dissolution apparatus (rotating basket) at 50, 100, and 150 rpm. In another experiment, stirred and stagnant conditions were induced in a single run using USP-I apparatus. The rotational speed was kept at 100 rpm (stirred conditions), which was stopped intermittently to induce the stagnant conditions. The protocol used was stirred conditions for first 3 h (0–3 h), stagnant conditions for next 2 h (3–5 h), stirred condition for next 3 h (5–8 h), and stagnant condition for next 2 h (8–10 h). In order to confirm the mechanism of drug release, release studies of the formulation were conducted in media of different osmotic pressure. To increase the osmotic pressure of the release media, sodium chloride (osmotically effective solute) was added in SIF and the pH

was adjusted to 6.8 ± 0.05. Release studies were carried out in 1000 ml of media using USP-I dissolution apparatus (100 rpm). Release from the formulation was inversely proportional to the osmotic pressure of the release media, conforming osmotic pumping to be the major mechanism of drug release.

**Reported in-vitro evaluation of osmotic system in literature**

Rani et al., in 2003 developed an elementary osmotic pump for diclofenac sodium. To study the effect of pH, dissolution was carried out in USP II apparatus in different release media (pH 7.4, pH 6.8, and distilled water) maintained at 37 + 0.2 °C and 100 rpm which resulted in a non-significant difference in release. To study the effect of agitation intensity, in vitro studies were performed at 50 rpm, 100 rpm, and under static conditions. Under static conditions, samples were taken at different times after uniform mixing of the media. Studies under stirred and static conditions exhibited no significant difference in the rate and extent of release. In vitro studies were done using a USP 24 dissolution apparatus II at 100 rpm.

Zentner developed a controlled porosity osmotic pump of potassium chloride. To study the effect of pH of release media on drug release, release study was conducted in deionized water and various other aqueous receptor media at 37 $^{o}$C. Experiments at pH 5, 7.4, and 8 were conducted in 0.07 M Sorensen's phosphate buffer. Studies at pH 1 were in 0.1 N hydrochloric acid. Wherever required, pH adjusted media were made iso-osmotic to normal saline by adding sodium chloride. The following patterns of stirring were employed in the release studies, 100 rpm continuously and 100 rpm interrupted with a 2 hour period of no stirring at the midpoint of the steady state release profiles. The effects of receptor media osmotic pressure on potassium chloride release were studied in 1.64, 3.42, 7.06, and 11.63 molar aqueous solutions of urea at 25 $^{o}$C. Coating solution composed of cellulose acetate, sorbitol and PEG 400 as film forming polymer, pore forming agent, and plasticizer, respectively. Solvent was a quaternary mixture of dichloromethane, methanol, water, and polyethylene glycol 400 mixed 150: 100: 10: 1 by weight respectively, as dictated by the solubility of the solid components that were incorporated.

Makhija and Vavia in 2003 developed a controlled porosity osmotic pump of pseudoephedrine. As a proof of an osmotically controlled release system, (delivers its contents independent of external variables) the *in vitro* release studies were conducted in buffers of different pH, i.e., pH 1.2 buffer, pH 4.5 phosphate buffer and pH 7.2 phosphate buffer as well as in distilled water. The system exhibits a media independent release. Thus, the fluid in different parts of the GI tract will scarcely affect drug release from the osmotic system. The *in vitro* release from the coated tablets was studied using USP dissolution apparatus type I at 100 rpm. The dissolution medium used was 500 ml of phosphate buffer of pH 7.2. The tablets were coated with cellulose acetate as semipermeable film forming polymer containing different channeling agent viz. diethylphthalate, dibutylpthalate, dibutylsebacate, and polyethylene glycol 400. Talc and titanium dioxide were used as antiadherant and opacifier respectively. Acetone: isopropyl alcohol (80:20) was used as a coating solvent.

Verma and Garg 2004 developed controlled porosity osmotic pump of glipizide. The *in vitro* release was carried out in a USP type 1 dissolution apparatus at 100 rpm. Dissolution medium was simulated intestinal fluid (SIF, pH 6.8, 1000 ml) maintained at 37 $\pm$ 0.5$^{o}$C. Coating membranes was consisting of cellulose acetate as film forming polymer. PVP was used as a water-soluble component. PEG 400 and triacetin were used as a water-soluble and water insoluble plasticizer respectively. Dichloromethane:methanol (3:1) were used as a coating solvent.

Garg et. al., in 2007 studied the effect of formulation parameters on the release characteristics of propranolol from asymmetric membrane coated tablets. A zero order release of propranolol was obtained from the coated tablets of propranolol. The release was independent of the pH and the rate of agitation of the dissolution medium ($p > 0.05$). Asymmetric membranes could be successfully utilized in the controlled delivery of highly water soluble drugs like propranolol and by modifying preparation parameters like polymer concentration, pore former concentration and temperature of the precipitation bath, desired release rates can be obtained.

**RESEARCH ENVISAGED** The aim of present research was to design and optimize the Extended release drug delivery systems for water soluble drug Butorphanol Tartrate and low water soluble drug Lornoxicam.

- Controlled Porosity Osmotic System (CPOP) – Butorphanol tartrate
- Extended release matrix Tablet ( ER) - Lornoxicam

**CPOP**

The aim of the current study was to design a controlled porosity osmotic system (CPOP) see figure 9 – 2 based drug delivery system for controlled release of highly water soluble drug, Butorphanol Tartrate.

The current study is also focused on the effect of concentration of pore formers, Sorbitol, and PEG-400. Controlled porosity osmotic pumps (CPOP), contain water-soluble additives in the coating membrane, which after coming in contact with water, dissolve resulting in an in situ formation of a microporous membrane. The resulting membrane is substantially permeable to both water and dissolved solutes and the mechanism of drug release from these systems was found to be primarily osmotic, with simple diffusion playing a minor role .

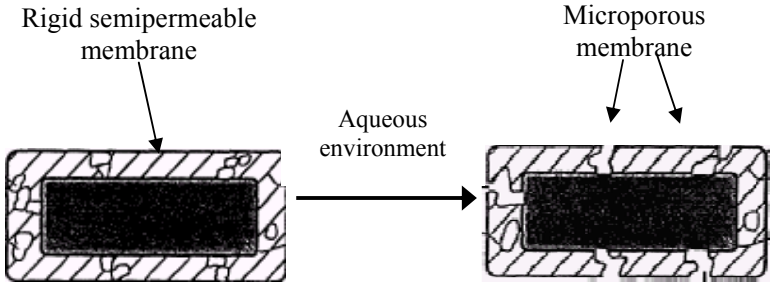



**Figure 9– 2 Release mechanism from a CPOP**

The mechanisms by which drug release is controlled in CPOP are dependent on many variables. One of the principles of drug release would be osmotic pressure. It is possible that one can modulate the release profile of the water soluble, sparingly soluble and poorly soluble active agents.

Matrix

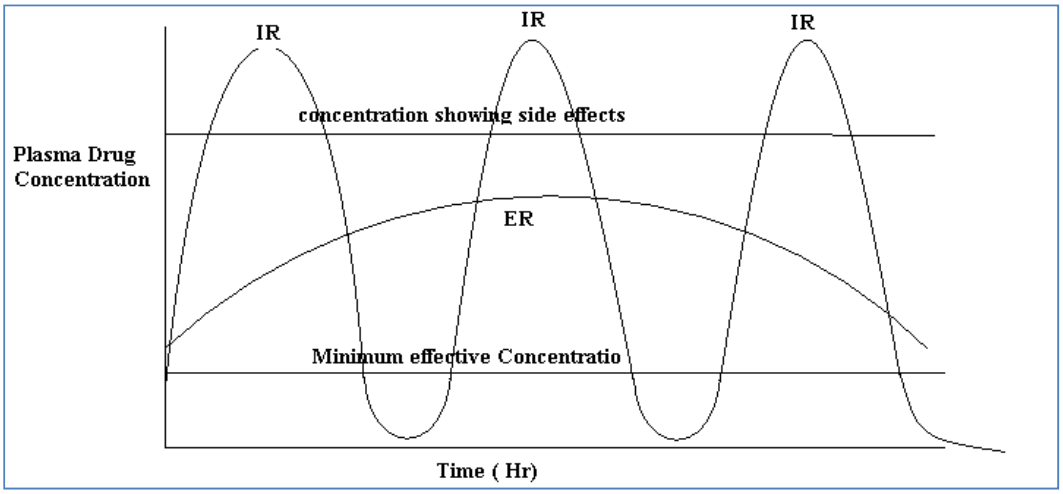



**Figure 10 - 2 Simulation of ER plasma concentration versus time profile based on IR formulation PK**

The release of drug in vivo can be analyzed by de-convolution of the plasma concentrations from the CR formulations with release profiles. A common approach for de-convolution is to use an orally administered immediate release formulation as a reference, which then allows one to calculate a unit impulse function from IR Formulation to obtain the input rate for the CR dosage form. Once the in vivo release profiles of the CR dosage forms are obtained, correlation of in vivo release profiles & corresponding in vitro release profiles can be established as point-to-point Level A correlation.

PK simulation employing PK principles to predict CR dosage form is an important element of CR feasibility assessment. Simulation of CR oral dosage forms requires altering the dose and release rate in an attempt to maintain selected plasma concentrations within a desired dosing interval. Initial CR formulation development, PK simulation is performed to assess if a specific drug is a candidate for CR formulation and whether it is possible to alter the release rate and dose to achieve that goal. So, plasma concentrations following administration of various CR dosage forms can be simulated if the PK data from an IR dosage form are available Figure 10 - 2.

However, simulation is conducted by employing PK principle & it encompasses biopharmaceutic properties of the drug, for example, solubility and permeability, and allows modelling of drug input rates, small and large intestinal transit time variations, and position-dependent absorption rates. The drug colonic absorption potential can be revealed

from the simulation. By inputting the in vitro dissolution profile and drug PK parameters, projected performance of a CR formulation represented by a simulated plasma concentration profile can be illustrated. If information on colonic permeability or absorption is not available, simulations can be carried out assuming low, medium, and high colonic absorption to predict the performance.

In totality, the projected performance of a CR formulation (single-dose or steady state PK) can be simulated. Simulation is conducted based on the physicochemical, biopharmaceutic, and/or PK properties of the drug. The results of simulations reveal the projected performance of a CR formulation and indicate the dose range and release rates that are reasonable to meet the clinical target.

**PLAN OF WORK**

1. Preformulation studies of

i. Butorphanol tartrate

ii. Lornoxicam

2. Controlled Porosity Osmotic System (CPOP)

i. Prediction of extended release profile from immediate release profile.

ii. Target drug release profile

iii. Selection of formulation

iv. Optimization of core and coating components

v. *In-vitro* drug release

vi. *Characterization studies*

3. Hydrophilic Matrix System

vii. Target drug release profile

viii. Selection of formulation

ix. Optimization of core components

x. *In-vitro* drug release

xi. *Characterization studies*

4. Performance evaluation of optimized formulations

i. Effect of pH

ii. Effect of agitational intensity

iii. Effect of osmotic pressure

iv. Scanning electron microscopy

6. *In vivo* studies

7. Stability studies

8. Compilation, analysis and interpretation of results

References


- D.A. Alderman, A review of cellulose ethers in hydrophilic matrices for oral controlled-release dosage forms, Int. J. Pharm. Technol. Prod. Manuf 5 (1984) 1–9.
- J.L. Ford, M.H. Rubinstein, J.E. Hogan, Formulation of sustained release promethazine hydrochloride tablets using hydroxypropyl–methylcellulose matrices, Int. J.Pharm. 24 (1985) 327–338.
- J.L. Ford, M.H. Rubinstein, J.E. Hogan, Propranolol hydrochloride and aminophylline release from matrix tablets containing hydroxypropylmethylcellulose, Int. J. Pharm. 24 (1985) 339–350.
- S.K. Baveja, K.V. Ranga Rao, K. Padmalata Devi, Zero-order release hydrophilic matrix tablets of β-adrenergic blockers, Int. J. Pharm. 39 (1987) 39–45.
- J.L. Ford, M.H. Rubinstein, F. McCaul, J.E. Hogan, P.J. Edgar, Importance of drug type, tablet shape and added diluents on drug release kinetics from hydroxypropylmethylcellulose matrix tablets, Int. J. Pharm. 40 (1987) 223–234.
- N. Shah, G. Zhang, V. Apelian, F. Zeng, M.H. Infeld, A.W. Malick, Prediction of drug release from hydroxypropyl methylcellulose (HPMC) matrices: effect of polymer concentration, Pharm. Res. 10 (1993) 1693–1695.
- P. Gao, J.W. Skoug, P.R. Nixon, T.R. Ju, N.L. Stemm, K.C. Sung, Swelling of hydroxypropyl methylcellulose matrix tablets. 2. Mechanistic study of the influence of formulation variables on matrix performance and drug release, J.Pharm. Sci. 85 (1996) 732–740.
- K. Tahara, K. Yamamoto, T. Nishihata, Overall mechanism behind matrix sustained release (SR) tablets prepared with hydroxypropyl methylcellulose 2910, J.Controlled Release 35 (1995) 59–66.
- A. Miranda, M. Millan, I. Caraballo, Investigation of the influence of particle size on the excipient percolation thresholds of HPMC hydrophilic matrix tablets (HPMCparticle size), J. Pharm. Sci. 96 (2007) 2746–2756.
- P.R. Ravi, S. Ganga, R.N. Saha, Design and in vitro evaluation of zidovudine oral controlled release tablets prepared using hydroxypropyl methylcellulose, Chem.Pharm. Bull. (Tokyo) 56 (2008) 518–524.
- T. Goncalves-Araujo, A.R. Rajabi-Siahboomi, I. Caraballo, Application of percolation theory in the study of an extended release Verapamil hydrochloride formulation, Int. J. Pharm. 361 (2008) 112–117.

- A. Miranda, M. Millan, I. Caraballo, Study of the critical points of HPMC hydrophilic matrices for controlled drug delivery, Int. J. Pharm. 311 (2006) 75–81.
- F. Tajarobi, S. Abrahmsen-Alami, M. Hansen, A. Larsson, The impact of dose and solubility of additives on the release from HPMC matrix tablets—identifying critical conditions, Pharm. Res. 26 (2009) 1496–1503.
- H. Leuenberger, The application of percolation theory in powder technology, Adv. Power Tech. 10 (1999) 323–352.
- S.A. Mitchell, K.M. Balwinski, Investigation of hypromellose particle size effects on drug release from sustained release hydrophilic matrix tablets, Drug Dev. Ind. Pharm. 33 (2007) 952–958.
- Fuertes, I. Caraballo, A. Miranda, M. Millan, Study of critical points of drugs with different solubilities in hydrophilic matrices, Int. J. Pharm. 383 (2010) 138–146.
- S.B. Tiwari, A.R. Rajabi-Siahboomi, Extended-release oral drug delivery technologies: monolithic matrix systems, in: K.K. Jain (Ed.), Drug Delivery Systems, Human Press, Totowa, 2007, pp. 217–243.
- M.Z.I. Khan, Dissolution testing for sustained or controlled release oral dosage forms and correlation with in vivo data: challenges and opportunities, Int. J.Pharm. 140 (1996) 131–143.
- B. Abrahamsson, M. Alpsten, M. Hugosson, U.E. Jonsson, M. Sundgren, A. Svenheden, J. Tolli, Absorption, gastrointestinal transit, and tablet erosion of felodipine extended-release (ER) tablets, Pharm. Res. 10 (1993) 709–714.
- B. Abrahamsson, M. Alpsten, B. Bake, A. Larsson, J. Sjogren, In vitro and in vivo erosion of two different hydrophilic gel matrix tablets, Eur. J. Pharm. Biopharm. 46 (1998) 69–75.
- J. Davis, J. Burton, A.L. Connor, R. Macrae, I.R. Wilding, Scintigraphic study to investigate the effect of food on a HPMC modified release formulation of UK-294,315, J. Pharm. Sci. 98 (2009) 1568–1576.
- M. Ghimire, et al., In-vitro and in-vivo erosion profiles of hydroxypropylmethylcellulose (HPMC) matrix tablets, J. Control. Release (2010)
- T. Ishikawa et al. Effect of hydroxypropylmethylcellulose (HPMC) on the release profiles and bioavailability of a poorly water-soluble drug from tablets prepared using macrogol and HPMC International Journal of Pharmaceutics 202 (2000) 173–178

- Oral Drug Absorption Prediction and Assessment edited by Jennifer B. Dressman Marcel Dekker,
- Hong Wen, Kinam Park. Oral controlled release Formulation design and drug Delivery theory to practice John Wiley & Sons,
- http://www.dow.com/dowexcipients/products/methocel.htm
- http://www.uspnf.com/uspnf/login
- Zhou M, Notari RE. A nomogram to predict the best biological half-life values for candidates for oral prolonged-release formulations. J. Pharm. Sci. 1996;85(8):791–795.
- Irvin JR, Notari RE. Computer-aided dosage form design. III. Feasibility assessment for an oral prolonged-release phenytoin product. Pharm. Res. 1991;8(2):232–237.
- ChenY, McCallTW, Baichwal AR, MeyerMC. The application of an artificial neural network and pharmacokinetic simulations in the design of controlled-release dosage forms. J. Control. Release 1999;59(1):33–41.
- Cheung WK, Yacobi A, Silber BM. Pharmacokinetic approach to the rational design of controlled or sustained release formulations. J. Control. Release 1988;5(3):263–270.
- Oral Controlled Release Formulation Design and Drug Delivery: Theory to Practice, Edited by Hong Wen and Kinam Park Hua zhang and jean m. Surian "biopharmaceutic consideration

  And assessment for oral controlled release formulations" Publishers John Wiley & Sons (2010)
- Gilbert MM, Sanzari NP, Losada M, Caruso FS. J Int Med Res. 1978;6(1):14-23. "Double-blind oral analgesic study of butorphanol in musculoskeletal pain: a comparison with codeine and placebo"
- Smith B.E. and Walsh S. "A study of analgesic characteristics of oral butorphanol administered for post episiotomy pain" Pharmacologist (1977) 19:2 No. 503.
- Levin HM. J Int Med Res. 1978;6(1):24-33. "Double-blind oral analgesic study of butorphanol in episiotomy pain: a comparison with codeine and placebo"
- Eduardo Bruera, David Hui, Shalini Dalal, Isabel Torres-Vigil, Joseph Trumble, Joseph Roosth, Susan Krauter, Carol Strickland, Kenneth Unger, J. Lynn Palmer, Julio Allo, Susan Frisbee-Hume and Kenneth Tarleton JCO Jan 1, 2013:111-

118 "Parenteral Hydration in Patients With Advanced Cancer: A Multicenter, Double-Blind, Placebo-Controlled Randomized Trial" Journal of Clinical Oncology, 1998

- Cebere I, Dorrell L, McShane H, Simmons A, McsCormack S, Schmidt C, Smith C, Brooks M, Roberts JE, Darwin SC, Fast PE, Conlon C, Rowland-Jones S,McMichael AJ, Hanke T. Vaccine. 2006 Jan 23;24(4):417-25 "Phase I clinical trial safety of DNA- and modified virus Ankara-vectored human immunodeficiency virus type 1 (HIV-1) vaccines administered alone and in a prime-boost regime to healthy HIV-1-uninfected volunteers"
- Theeuwes F. J Pharm Sci. (1975) "Elementary osmotic pump" Dec ; 64 (12) 1987-91.
- Theeuwes F. & S.I Yum Annals of Biomedical engineering Vol.4 No. 4 (1976) "Principal of design and operation of generics osmotic pumps for the delivery of semi solids or liquid drug formulations"
- Korsmeyer RW, Gurny R, Doelker E M, Buri P and Peppas NA. Int J Pharm., 15: 25-35 (1983). Mechanism of solute release from porous hydrophilic polymers.
- Khurahashi, H., Kami, H., Sunada, H., 1996. Influence of physicochemical properties on drug release rate from Hydroxypropyl methylcellulose matrices. Chem. Pharm. Bull. 44, 829–832.
- Colombo, P., Bettini, R., Peppas, N.A., 1999. Observation of swelling process and diffusion front position during swelling in hydroxypropylmethylcellulose (HPMC) matrices containing a soluble drug. J. Control. Release 61, 83–91
- Bettini, R., Catellani, P.L., Santi, P., Massimo, G., Peppas, N.A., Colombo, P., 2001. Translocation of drug particles in HPMC matrix gel layer: effect of drug solubility and influence on release rate. J. Control. Release 70, 383–391.
- Narasimhan, B., 2001. Mathematical models describing polymer dissolution : consequences for drug delivery. Adv. Drug Deliv. Rev. 48, 195–210.
- Durig, T., Fassihi, R., 1999. "An investigation into the erosion behavior of a high drug-load (85%) particulate system designed for an extended-release matrix tablet. Analysis of erosion kinetics in conjunction with variations in lubrication, porosity, and compaction rate". J. Pharm. Pharmacol. 51, 1085–1092.
- Durig, T., Fassihi, R., 2000. "Evaluation of floating and sticking extended release delivery systems : an unconventional dissolution test". J. Control.Release 67, 37–44.

- Sako, K., Sawada, T., Nakashima, H., Yokohama, S., Sonobe, T., 2002. "Influence of water soluble fillers in hydroxypropylmethylcellulose matrices on in vitro and in vivo drug release". J. Control. Release 81, 165–172.
- Turner, S., Federici, C., Hite, M., Fassihi, R., 2004. Formulation development and human in vitro-in vivo correlation for a novel, monolithic controlledrelease matrix system of high load and highly water-soluble drug niacin. Drug Dev. Ind. Pharm. 30, 797–807.

  Drug Dev. Ind. Pharm. 30, 797–807 Higuchi T., (1963). Mechanism of sustained-action medication: theoretical analysis of rate of release of solid drug dispersed in solid matrices, J Pharm. Sci.,52: 1145 -1149.
- Higuchi T., (1961). Rate of release of medicaments from ointment bases containing drugs in suspension, J Pharm. Sci., 50: 874-875
- Joseph R. Robinson, Vincent H. L. Lee Controlled Drug Delivery: Fundamentals and Applications Marcel Dekker Incorporated, 1987
- Shahla Jamzad, Reza Fassihi. International Journal of Pharmaceutics (2006) 312(1-2) : 24-32. Development of a controlled release low dose class II drug – Glipizide
- Arkinstall, W.A., Sandler, A., & Goughnor, B. (1995). Efficacy of controlled-release codeine in chronic non-malignant pain: A randomized, placebo-controlled clinical trial. Pain, 62, 169–178.
- American Academy of Pain Medicine, American Pain Society, & American Society of Addiction Medicine. (2001). Definitions related to the use of opioids for the treatment of pain. Glenview, IL: American Academy of Pain Medicine, American Pain Society, & American Society of Addiction Medicine.
- USPTO patent No. US 7858122 B2
- European patent application EP 2394641 A1
  (Santus and Baker, 1995)
- Rajan K Verma, Divi Murali Krishna, Sanjay Garg "Formulation aspects in the development of osmotically controlled oral drug delivery systems "Journal of Controlled Release Volume 79, Issues 1–3, 19 February 2002, Pages 7–27.
- McClelland GA, Sulton SC, Engle K, Zentner GM. The solubility–modulated osmotic pump: invitro /invivo release of diltiazem hydrochloride. Pharma Res 1991; 8:88-92

- Zentner GM, McClelland GA, Sutton SC. Controlled porosity solubility- and resin-modulated osmotic drug delivery systems for release of diltiazem hydrochloride. J Control Release, 1991; 16:1-2:237–243.
- Korsemeyer RW, Lo J, Smith KL, Thombre AG. Asymmetric membranes in delivery devices. US Patent No. 56, 1997;:98, 220
- Thombre AG, Cardinal JR, DeNoto AR, Herbig SM, Smith KL. Asymmetric membrane capsules for osmotic drug delivery I. Development of a manufacturing process. J Control Release 1999; 57: 55-64
- Khanna, S.C. Therapeutic system for sparingly soluble active ingredients. U. S. Patent No. 4992278, 1991.
- Siepmann J, Kranz H, Bodmeier R, Peppas NA. HPMC-matrices for controlled drug delivery: a new model combining diffusion, swelling, and dissolution mechanisms and predicting the release kinetics. Pharm. Res. 1999;16:1748–1756.
- Colombo P, Bettini R, Santi P, Peppas NA. Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance. Pharm. Sci. Technol. Today 2000;3: 198–204.
- Siepmann J, Siepmann F. Mathematical modelling of drug delivery. Int. J. Pharm. 2008;364:328–343.
- Bajwa GS, Hoebler K, Sammon C, Timmins P, Melia CD. Microstructural imaging of early gel layer formation in HPMC matrices. J. Pharm. Sci. 2006;95:2145–2157.
- Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv. Drug Deliv. Rev. 2001;48:139–157.
- Peppas NA, Sahlin JJ. A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. Int. J. Pharm. 1989;57:169–172.
- Ford JL, Rubinstein MH, Hogan JE. Propranolol hydrochloride and aminophylline release from matrix tablets containing hydroxypropylmethylcellulose. Int. J. Pharm. 1985;24:339–350.
- Ford JL, Rubinstein MH, Hogan JE. Dissolution of a poorly water soluble drug, indomethacin, from hydroxypropylmethylcellulose
  controlled release tablets. J. Pharm. Pharmacol. 1985. 37:33P.
- Tahara K, Yamamoto K, Nishihata T. Application of modelindependent and model analysis for the investigation of effect of drug solubility on its release rate from

hydroxypropyl methylcellulose sustained release tablets. Int. J. Pharm.1996;133:17–27.

- Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int. J. Pharm. 1983;15:25–35.
- Baveja SK. Ranga Rao KV, Singh A, Gombar VK. Release characteristics of some bronchodilators from compressed hydrophilic polymeric matrices and their correlation with molecular geometry. Int. J. Pharm. 1988;41:55–62.
- Shah N, Zhang G, Apelian V, Zeng F, Infeld MH, Malick AW. Prediction of drug release from hydroxypropylmethylcellulose
  (HPMC) matrices: effect of polymer concentration. Pharm. Res. 1993;10:1693–1695.
- Gao P, Nixon PR, Skoug JW. Diffusion in HPMC gels. Prediction of drug release rates from hydrophilic matrix extended release dosage forms. Pharm. Res. 1995;12:965–971.
- Ford JL, Mitchell K, Rowe P, Armstrong DJ, Elliott PNC, Rostron C, Hogan JE. Mathematical modelling of drug release from hydroxypropylmethylcellulose matrices: effect of temperature. Int. J. Pharm. 1991;71:95–104.
- Arun D. Koparkar, Shailesh B. Shah Oral osmotic system for slightly soluble active agents
  us patent US 5284662 A
- Dorica, J., Wong, A., and Garner, B.C., "Complete Effluent Recycling in the Bleach Plant with Ultrafiltration and Reverse Osmosis", Tappi Journal, 69, 122 (1986).
- R.K. Verma et al. Formulation aspects in the development of osmotically controlled oral drug delivery systems Journal of Controlled Release 79 (2002) 7 –27
- Santus C, Baker (1995) Osmotic drug delivery: a review of the patent literature. J Control Release 134:74–80.
- Herbig SM, Cardinal JR, Korsmeyer KL, Smith KL. Asymmetric-membrane tablet coatings for osmotic
  drug delivery. J Control Release, 1995; 35:127-136.
- Lin YK, Ho HO. Investigations on the drug releasing mechanism from an asymmetric membrane-coated capsule with an in situ formed delivery orifice. J Control Release. 2003;89:57–69.
- Sastry SV, DeGennaro MD, Reddy IK, Khan MA. Atenolol gastrointestinal therapeutic system. I. Screening of formulation
  variables. Drug Dev. Ind. Pharm. 1997;23(2):157–165.

- U.S. Pat. No. 3,845,770, 5,108,756 and 5,030,452
- Okimoto, K., Rajewski, R. A., and Stella, V. J. 1999.Release of testosterone from an osmotic pump tablet utilizing (SBE)7m-β-cyclodextrin as both a solubilizing and an osmotic pump agent. J. Controlled Rel. 58: 29–38.
- K. Okimoto, M. Miyake, N. Ohnishi, R.A. Rajewski, V. J. Stella, T. Irie and K. Uekama. Design and evaluation of an osmotic pump tablet for prednisolone usng (SBE)-$_{7m}$-ß-CD, Pharm. Res. 15, 1562-1568; 1998.
- K. Okimoto, Y. Tokunaga, R. Ibuki, T. Irie, K. Uekama, R. A. Rajewski and V. J. Stella, Applicability of (SBE)(7m)-β-CD in controlled-porosityosmotic pump tablets (OPTs), Int. J. Pharm., Vol. 286 ... 189, pp.227-234 1999
- F Theeuwes, DR Swanson, G Guittard, A Ayer, S Khanna Osmotic delivery systems for the beta-adrenoceptor antagonists metoprolol and oxprenolol: design and evaluation of systems for once- daily administration. British Journal of Clinical Pharmacology Volume 19, Issue S2, pages 69S–76S, April 1985
- F. Theeuwes. Elementary Osmotic Pump, J. Pharm. Sci. 4(12), 1987-1991; 1975.
- F. Theeuwes. Osmotic dispenser with gas generating means. US Patent 4036228; 1977.
- Prabakaran D, Singh P, Kanaujia P, Vyas SP. Effect of hydrophilic polymers on the release of diltiazem hydrochloride from elementary osmotic pumps. Int J Pharm. 2003;259:173–9
- Rudnic, E.M., et al., Osmotic drug delivery system, US Patent 6110498, 2000
- R. Cortese and F. Theeuwes. Osmotic device with hydrogel driving member. US Patent 4327725; 1982
- F. Theeuwes, R.J. Saunders and W.S. Mefford. Process for forming outlet passageways in pills using a laser. US Patent 4088864; 1978.
- G.M. Zentner, G.S. Rork and K.J. Himmelstein. The controlled porosity osmotic pump. J. Control. Release, 1, 269-282; 1985.
- G.M. Zentner, G.S. Rork and K.J. Himmelstein. Osmotic flow through controlled porosity films: An approach to delivery of water soluble compounds. J. Control. Release, 2, 217-229; 1985.

- J. Shokri et al. Swellable elementary osmotic pump (SEOP): An effective device for delivery of poorly water-soluble drugs European Journal of Pharmaceutics and Biopharmaceutics 68 (2008) 289–297
- Swanson DR., Barclay BL., Wong PSL., Theuwes F. Asymmetric membrane capsule for osmotic delivery of flurbiprofen. American. Med 1987; 83: 3-9.
- Wong PSL, Barclay B, Deters JC, Theeuwes F. Osmotic device with dual thermodynamic activity. US patent 4,612,008. Sep 16, 1986.
- G.M. Zentner, G.S. Rork, K.J. Himmelstein, Osmotic flow through controlled porosity films: an approach to delivery of water soluble compounds, J. Control. Release 2 (1985) 217–229.
- Grundy, J.S., Foster, R.T., 1996. The nifedipine gastrointestinal therapeutic system (GITS). Evaluation of pharmaceutical, pharm
- R.K. Verma, S. Garg Development and evaluation of osmotically controlled oral drug delivery system of glipizide European Journal of Pharmaceutics and Biopharmaceutics 57 (2004) 513–525.
- R.K. Verma, A.M. Kaushal, S. Garg, Development and evaluation of extended release formulations of isosorbide mononitrate based on osmotic technology, Int. J. Pharm. 263 (2003) 9–24.
- Gondaliya D, Pundarikakshudu K (2003): The Fabrication and evaluation of the formulation variables of a controlled-porosity osmotic drug delivery System with Diltiazem Hydrochloride, Pharmaceutical technology, 58-68.
- Garg A, Gupta M, Bhargava HN. Effect of formulation parameters on the release characteristics of propranolol from asymmetric membrane coated tablets. Eur J Pharm Biopharm. 2007 Nov; 67(3):725-31. Epub 2007 Apr 21.
- M. Ranibet al.: Development and biopharmaceutical evaluation of osmotic pump tablets for controlled delivery of diclofenac sodium, Acta Pharm. 53 (2003) 263–273.
- Makhija SN, Vavia PR. Controlled porosity osmotic pump-based controlled release systems of pseudoephedrine. I. Cellulose acetate as a semipermeable membrane. J Control Release. 2003 Apr 14;89(1):5-18.

# 3.0 Analytical Methods (Butorphanol Tartrate)

The analytical methods developed for testing of Butorphanol Tartrate (BT) in bulk powder and extended release formulation of are employed in this section. Table 1 - 3 and 2 - 3 show list of material and equipment used respectively. Method for Assay, content uniformity, dissolution studies and determination of drug during in-vivo studies are discussed in this section.

| Sr. No. | Material | Source |
|---|---|---|
| 1. | Butorphanol Tartrate | Theraquest Bioscience Corporations |
| 2. | Dichloromethane | Merck Limited, Mumbai, India |
| 3. | Methanol | Merck Limited, Mumbai, India |
| 4. | Acetone | Merck Limited, Mumbai, India |
| 5. | Isopropyl alcohol | Merck Limited, Mumbai, India |
| 6. | Glacial acetic acid | S. D. Finechem Limited, Mumbai, India |
| 7. | Hydrochloric acid | S. D. Finechem Limited, Mumbai, India |
| 8. | Potassium dihydrogen phosphate | S. D. Finechem Limited, Mumbai, India |
| 9. | Sodium hydroxide | S. D. Finechem Limited, Mumbai, India |
| 10. | Sodium lauryl sulphate | S. D. Finechem Limited, Mumbai, India |
| 11. | HPLC grade Methanol, Acetonitrile, Acetic acid | S. D. Finechem Limited, Mumbai, India |
| 12. | Ethanol (99.5%V/V) | Baroda Chem. Ind. Ltd., Baroda, India |
| 13. | Water (distilled) | Prepared in laboratory by distillation |

**Table 1 - 3 List of materials**

| | Equipments | Source/Make |
|---|---|---|
| 1. | Digital weighing balance | AG-64, Mettler Toledo, Switzerland |
| 2. | pH meter | Mettler Toledo, Switzerland |
| 3. | Bath sonicator | DTC 503, Ultra Sonics |
| 4. | HPLC system | LC 20-AT prominence, Shimadzu Corp., Japan |

| | | |
|---|---|---|
| 5. | UV-Visible Spectrophotometer | Shimadzu UV-1601, Japan |
| 6. | Calibrated pipettes of 1.0 ml, 5.0 ml and 10.0 ml. | Schott & Corning (India) Ltd., Mumbai |
| 7. | volumetric flasks of 10 ml, 25 ml, 50 ml and 100 ml capacity. | Schott & Corning (India) Ltd., Mumbai |
| 8. | Funnels (i.d. 5.0 cm) | Schott & Corning (India) Ltd., Mumbai |
| 9. | Beakers (250 ml) and other requisite glass wares | Schott & Corning (India) Ltd., Mumbai |
| 10. | Nuclepore Polycarbonate membrane 2 µm 25mm | Whatman, USA |

**Table 2 - 3 List of Equipments**

## 3.1. Preparation of reagents and buffers

### 3.1.1 Preparation of Simulated Gastric Fluid (SGF - pH 1.2)

2 gm of sodium chloride and 200 ml of distilled water was placed in a 1000 ml volumetric flask. 8.5 ml of concentrated hydrochloric acid was added and the volume was adjusted with distilled water upto 1000 ml. pH was adjusted to 1.2. (USP 30, 2007).

### 3.1.2 Preparation of Acetate Buffer (pH 4.5)

2.99 gm of sodium acetate trihydrate and 200 ml of distilled water was placed in a 1000 ml volumetric flask. 14 ml of acetic acid solution was added and the volume was adjusted with distilled water upto 1000 ml. (USP 30, 2007).

### 3.1.3 Preparation of Simulated Intestinal Fluid (SIF - pH 6.8)

250 ml of 0.2 M potassium phosphate monobasic was placed in a 1000 ml volumetric flask, 112 ml of 0.2 M sodium hydroxide was added and volume was adjusted with distilled water upto 1000 ml. (USP 30, 2007).

## 3.2. Estimation of Butorphanol tartrate

### 3.2.1. Estimation of Butorphanol tartrate in SGF pH 1.2, SIF pH 6.8 & PBS 4.5

Butorphanol tartrate shows strong absorbance in UV-Visible region. Hence, the estimation of Butorphanol tartrate was performed by UV-visible spectrophotometry. A UV-visible spectroscopic method for estimation of Butorphanol Tartrate for assay, uniformity of drug

content and *in-vitro* drug release in formulation was developed. Estimation method for the assay, uniformity of drug content was developed in water and method for *in-vitro* drug release was developed in SGF pH 6.8, SIF pH 1.2 & PBS pH 4.5.

### 3.2.1.1 Preparation of standard stock solutions of Butorphanol tartrate in SGF pH 1.2

50 mg of Butorphanol tartrate was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of SGF pH1.2 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with SGF pH1.2 to produce 1000 µg per ml of Butorphanol tartrate.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with SGF pH1.2 to prepare stock solution of 250 µg per ml of Butorphanol tartrate

.

### 3.2.1.2 Calibration curve of Butorphanol tartrate in SGF pH 1.2

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with release media to give final concentrations of 1, 2.5, 6.25, 12.5, 25, 50, 75, 100, 150, 200 µg/ml and analyzed by UV spectrophotometry at 280nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. Figure 1-3 show the linearity, of Butorphanol Tartrate in SGF pH 1.2, results are tabulated in table 3-3.

| Concentration (µg/ ml) | **Average*** | **SD** | **RSD** |
|---|---|---|---|
| 1 | 0.0160 | 0.0002 | 1.2471 |
| 2.5 | 0.0320 | 0.0001 | 0.3622 |
| 6.25 | 0.0438 | 0.0006 | 1.3772 |
| 12.5 | 0.0581 | 0.0004 | 0.6891 |
| 25 | 0.1114 | 0.0010 | 0.9341 |
| 50 | 0.2232 | 0.0003 | 0.1544 |

| | | | |
|---|---|---|---|
| 75 | 0.3215 | 0.0004 | 0.1178 |
| 100 | 0.4238 | 0.0008 | 0.1885 |
| 150 | 0.6422 | 0.0008 | 0.1195 |
| 200 | 0.8437 | 0.0014 | 0.1655 |

**Table 3 - 3 Calibration for Butorphanol tartrate in SGF pH 1.2**

Regression equationY= 0.0042X + 0.0122; Correlation coefficient = 0.9999

*Mean of 3 values

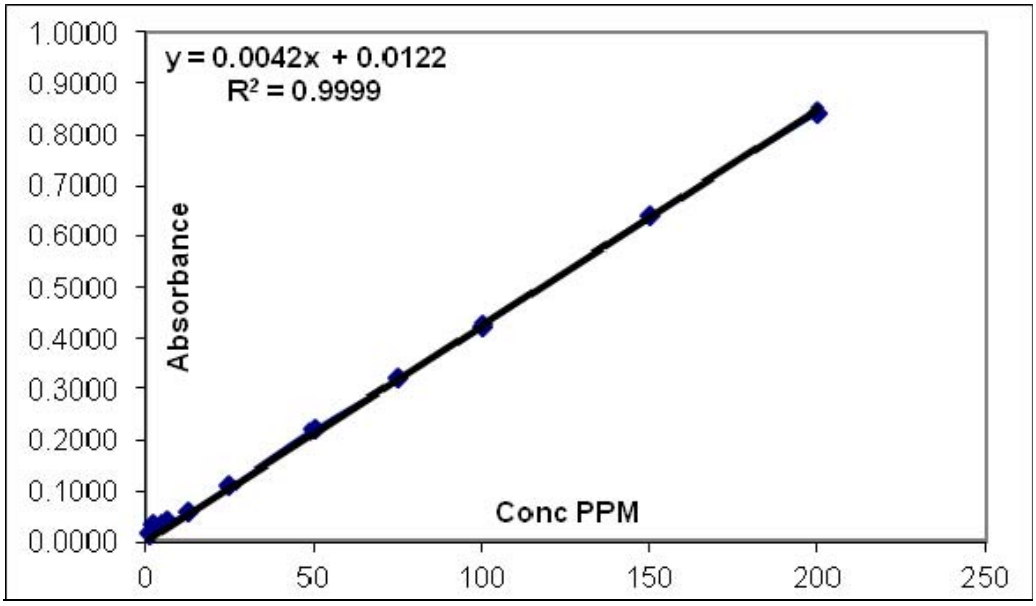



**Figure 1 - 3 Calibration curve for estimation of Butorphanol tartrate in SGF pH 1.2**

***Accuracy and repeatability***

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of butorphanol tartrate in SGF pH 1.2 is shown in Table 4 - 3.

In order to determine the accuracy of the developed method, known amounts of Butorphanol Tartrate (20µg/mL, 100µg/mL and 200µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (20, 100, 200 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 4 -3.

| Conc. of BT (µg/ml) Std. | AVG Recovery (µg/ml) | % Recovery | SD | RME | Confidence | RSD Intra Day | RSD Inter Day |
|---|---|---|---|---|---|---|---|
| 1.00 | 1.0 | 103.4 | 0.0230 | 0.0103 | 1.0 $\pm$ 0.0642 | 2.23 | 2.81 |
| 100 | 100.3 | 100.3 | 0.1914 | 0.0856 | 100.3 $\pm$ 0.5336 | 0.19 | 0.80 |
| 200 | 201.9 | 101.0 | 1.2571 | 0.5622 | 201.9 $\pm$ 3.5039 | 0.62 | 0.41 |

**Table 4 - 3 Evaluation of accuracy and repeatability of the estimation method of BT in SGF pH 1.2**

** At 95% Confidence level; $t_{tab}$ = 3.18 for 4 degrees of freedom (n=5)*

### 3.2.1.3 Preparation of standard stock solutions of Butorphanol tartrate in SIF pH 6.8

50 mg of Butorphanol tartrate was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of SIF pH6.8 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with SIF pH 6.8 to produce 1000 µg per ml of Butorphanol tartrate. 25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with SIF pH 6.8 to prepare stock solution of 250 µg per ml of Butorphanol tartrate.

### 3.2.1.4 Calibration curve of Butorphanol tartrate in SIF pH 6.8

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 100 ml with release media to give final concentrations of 3.125, 6.25, 12.5, 25, 50, 75, 100, 150, 200, 250 µg/ml and analyzed by UV spectrophotometry at 280nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed

analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Figure 2-3 show the linearity, of Butorphanol Tartrate in SIF pH 6.8, results are tabulated in table 5 - 3.

| Concentration (µg/ ml) | **Average*** | **SD** | **RSD** |
|---|---|---|---|
| 3.125 | 0.0221 | 0.0010 | 4.5256 |
| 6.25 | 0.0460 | 0.0007 | 1.5584 |
| 12.5 | 0.0591 | 0.0010 | 1.6091 |
| 25 | 0.1200 | 0.0011 | 0.9177 |
| 50 | 0.2010 | 0.0017 | 0.8343 |
| 100 | 0.4050 | 0.0033 | 0.8026 |
| 150 | 0.6010 | 0.0040 | 0.6572 |
| 200 | 0.7990 | 0.0036 | 0.4549 |
| 250 | 0.9850 | 0.0035 | 0.3517 |

**Table 5 - 3 Calibration for Butorphanol tartrate in SIF pH 6.8**

Regression equation** Y= 0.0039X + 0.0141; Correlation coefficient = 0.9998

*Mean of 3 values

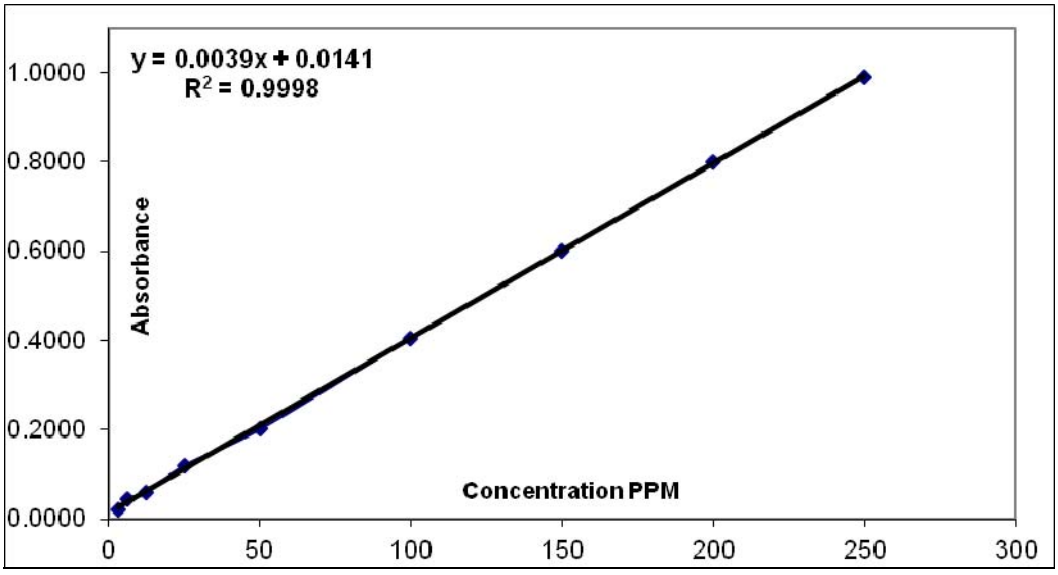



**Figure 2- 3 Regressed calibration curve for estimation of Butorphanol tartrate in SIF pH 6.8**

***Accuracy and Precision***

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of Butorphanol Tartrate in SIF pH 6.8 is shown in Table 6 - 3.

In order to determine the accuracy of the developed method, known amounts of Butorphanol Tartrate (10µg/mL, 100µg/mL and 200µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (10, 100, 200 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 6 - 3.

| Conc. of BT (µg/ml) Std. | AVG Recovery (µg/ml) | % Recovery | SD | RME | Confidence | RSD Intra Day | RSD Inter Day |
|---|---|---|---|---|---|---|---|
| 10 | 10.1 | 101.3 | 0.0283 | 0.0126 | 10.1 + 0.0788 | 0.28 | 0.74 |
| 100 | 100.3 | 100.3 | 0.1914 | 0.0856 | 100.3 + 0.5336 | 0.19 | 0.21 |
| 200 | 200.2 | 100.1 | 0.1002 | 0.0448 | 200.2 + 0.2794 | 0.05 | 0.09 |

**Table 6 - 3 Evaluation of accuracy and precision of the estimation method of BT in PBS 6.8**

** At Alphaa 0.05 Confidence level; $t_{tab}$ = 3.18 for 4 degrees of freedom*

### 3.2.1.5 Preparation of standard stock solutions of Butorphanol tartrate in acetate Buffer pH 4.5

50 mg of Butorphanol tartrate was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of acetate Buffer pH 4.5 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and

then the final volume of the flask was made up to 50 ml with acetate Buffer pH 4.5 to produce 1000 µg per ml of Butorphanol tartrate.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with PBS pH 4.5 to prepare stock solution of 250 µg per ml of Butorphanol tartrate.

### 3.2.1.6 Calibration curve of Butorphanol tartrate in acetate buffer pH 4.5

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with release media to give final concentrations of 20, 25, 50, 75, 100, 125, 150, 175, 200 µg/ml and analyzed by UV spectrophotometry at 280nm. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. Figures 3 – 3 show the calibration curve of Butorphanol Tartrate in acetate buffer pH 4.5, the values are tabulated in table 7 – 3.

| Concentration (µg/ ml) | **Average*** | **SD** | **RSD** |
|---|---|---|---|
| 20 | 0.1292 | 0.0010 | 0.7753 |
| 25 | 0.1434 | 0.0007 | 0.5000 |
| 50 | 0.2291 | 0.0010 | 0.4149 |
| 75 | 0.3448 | 0.0011 | 0.3195 |
| 100 | 0.4373 | 0.0017 | 0.3836 |
| 125 | 0.5550 | 0.0033 | 0.5856 |
| 150 | 0.6574 | 0.0017 | 0.2551 |
| 175 | 0.7599 | 0.0036 | 0.4784 |
| 200 | 0.8424 | 0.0010 | 0.1128 |

**Table 7 - 3 Calibration for Butorphanol tartrate in acetate buffer pH 4.5**

Regression equation** Y= 0.0041X + 0.0392; Correlation coefficient = 0.999

*Mean of 3 values

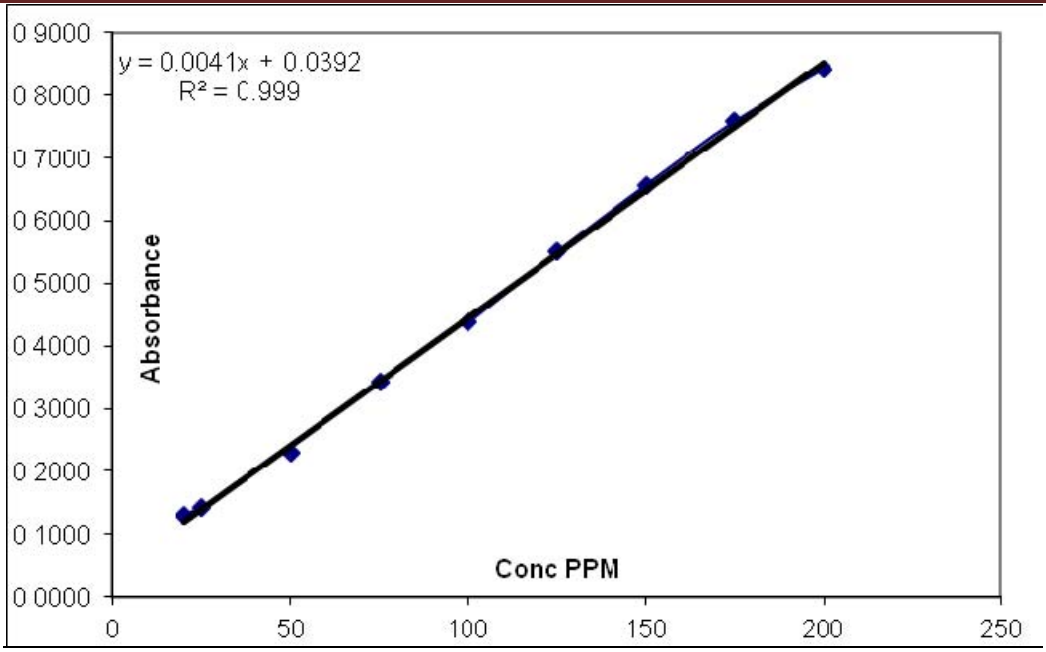



**Figure 3 - 3 Regressed calibration curve for estimation of Butorphanol tartrate in acetate buffer pH 4.5**

***Accuracy and Precision***

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of Butorphanol Tartrate in acetate buffer pH 4.5 is shown in Table 8 - 3.

In order to determine the accuracy and precision of the developed method, known amounts of Butorphanol Tartrate (10µg/mL, 100µg/mL and 200µg/mL) were subjected to recovery studies as per the procedure described above. The results obtained are tabulated in table 3.3.

| Theoretical Conc of BT (µg/mL) | AVG Recovery (µg/mL) | % Recovery | SD | RME | Confidence | RSD Intra Day | RSD Inter Day |
|---|---|---|---|---|---|---|---|
| 20 | 20.1 | 100.7 | 0.1741 | 0.0779 | 20.1 ± 0.3254 | 0.86 | 0.91 |
| 100 | 100.7 | 100.7 | 0.3401 | 0.1521 | 100.7 ± 0.6355 | 0.34 | 0.80 |
| 200 | 201.4 | 100.7 | 0.8833 | 0.3950 | 201.4 ± 1.6506 | 0.44 | 0.41 |

**Table 8 - 3 Evaluation of accuracy and precision of the estimation method of BT in acetate Buffer 4.5**

** At Alphaa 0.05 Confidence level; $t_{tab}$ = 3.18 for 4 degrees of freedom*

### 3.2.1.7 Preparation of standard stock solutions of Butorphanol tartrate in water

50 mg of Butorphanol tartrate was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of water was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with water to produce 1000 µg per ml of Butorphanol tartrate. 25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with water to prepare stock solution of 250 µg per ml of Butorphanol tartrate.

### 3.2.1.8 Calibration curve of Butorphanol tartrate in water

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with release media to give final concentrations of 10, 50, 100, 150, 200, 250 µg/ml and analyzed by UV spectrophotometry at 280nm. The above procedure was repeated three times. The data was recorded in Table 3.2 along with standard deviation. Figures 4 - 3 calibration curve of Butorphanol tartrate in water, the values are tabulated in table 9 -3.

| Concentration (µg/ ml) | Average* | SD | RSD |
|---|---|---|---|
| 10 | 0.0590 | 0.0004 | 0.6421 |
| 50 | 0.2289 | 0.0008 | 0.3491 |

| | | | |
|---|---|---|---|
| 100 | 0.4404 | 0.0008 | 0.1743 |
| 150 | 0.6516 | 0.0014 | 0.2143 |
| 200 | 0.8608 | 0.0003 | 0.0300 |

**Table 9 - 3 Calibration curve of butorphanol Tartrate in water**

Regression equation** Y= 0.0042X + 0.0190; Correlation coefficient = 1.0000

*Mean of 3 values

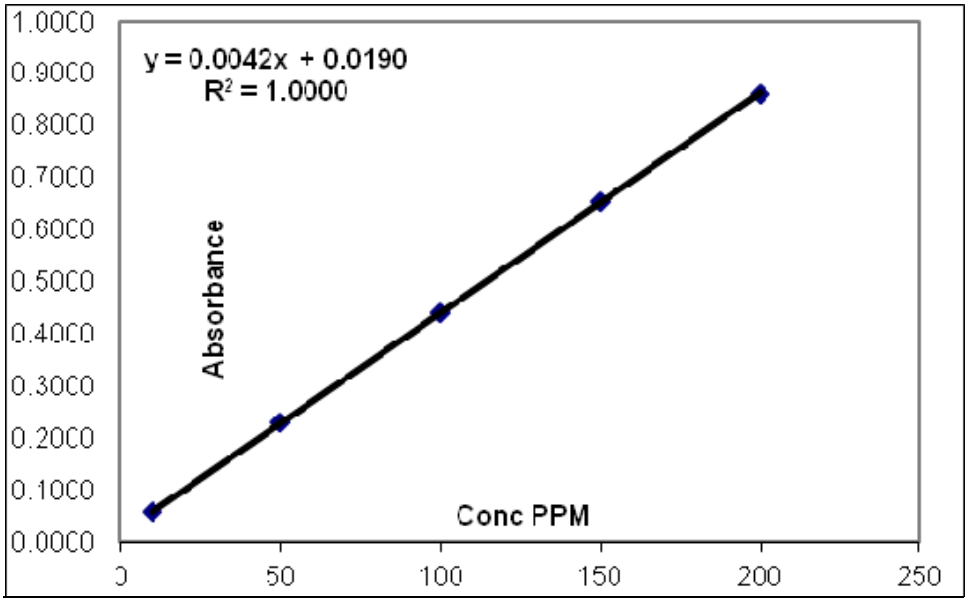



**Figure 4 - 3 Regressed calibration curve for estimation of Butorphanol tartrate in water**

***Accuracy and Precision***

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of Butorphanol tartrate in water is shown in Table 10 - 3.

In order to determine the accuracy and precision of the developed method, known amounts of Butorphanol Tartrate (10µg/mL, 100µg/mL and 250µg/mL) were subjected to recovery studies as per the procedure described above. The results obtained are tabulated in table 10.3.

| Theoretical Conc of BT (µg/mL) | AVG Recovery (µg/mL) | % Recovery | SD | RME | Confidence | RSD Intra Day | RSD Inter Day |
|---|---|---|---|---|---|---|---|
| 10 | 10.1 | 100.7 | 0.0891 | 0.0398 | 10.1 $\pm$ 0.1664 | 0.88 | 0.85 |
| 100 | 100.6 | 100.6 | 0.3750 | 0.1677 | 100.6 $\pm$ 0.7008 | 0.37 | 0.80 |
| 200 | 201.4 | 100.7 | 0.8833 | 0.3950 | 201.4 $\pm$ 1.6506 | 0.44 | 0.41 |

**Table 10 - 3 Evaluation of accuracy and precision of the estimation method of BT in water**

** At Alphaa 0.05 Confidence level; $t_{tab}$ = 3.18 for 4 degrees of freedom*

## 3.3 Estimation of BT in Formulation for Assay

To determine the amount of BT in the Coated Tablet, 20 tablets were crushed and added to 100 ml of water and subjected to shaking at room temperature for 5 mins for complete disintegration of excipients for extraction of the drug from the formulation. The filtered supernant was further diluted with water and estimated at 280 nm.

## 3.4 Estimation of BT in formulation for content uniformity

To determine the amount of BT in the Coated Tablet, 10 tablets were crushed individually and added to 100 ml of water and subjected to shaking at room temperature for 5 mins for complete disintegration of excipients for extraction of the drug from the formulation. The filtered supernant was further diluted with water and estimated at 280 nm.

## 3.5 Estimation of BT for in-vitro release

The release studies for BT coated formulation in different release media. One tablet containing 10mg drug was placed in dissolution vessel containing 250 ml of release medium maintained using paddle at 50 RPM at 37 $\pm$ 2 °C. 5 ml aliquots were taken out at different time and replace with same quantity of release media. The dissolved drug in release medium analyzed as per the method above. The amount of the drug released and cumulative percentage release was calculated.

The spectrophotometeric determination of placebo formulation consisting of all ingredients except drug showed no any absorbance under discussed methods. The capacity of the method to separate Butorphanol tartrate the non-interference with Butorphanol tartrate indicates the specificity of the methods. Stability of the Butorphanol Tartrate in the solution was demonstrated to be stable in solvent during the period of 24 h since the change in the $\lambda_{max}$ was not significant with the RSD value.

### 3.5.1 Test for alcohol dose dumping

In order to study the effect of alcohol on drug release or to verify weather alcohol leads to dose dumping 4 % V/V, 20 % V/V and 40 %V/V alcohol was added in the release media and drug release was verified against control ( without alcohol).

### 3.5.2 Mechanism of drug release

To ensure the major mechanism of drug release, release studies of the optimized formulation were conducted in media of varying concentration of osmotically active solute. To increase the osmotic pressure of the release media, sodium chloride was added in dissolution medium because it's saturated solution produces osmotic pressure in the range of 356 atm. The pH of the medium was adjusted to 6.8 ± 0.05. Release studies were carried out in 250 ml of media using USP II dissolution test apparatus (50 rpm). Release profiles of the optimized formulations at different concentration of osmotically active agent was plotted and compared.

### 3.5.3 Multimedia Testing

The optimized formulation was subjected to in vitro release studies in buffers with different pH like SGF pH 1.2, SIF pH 6.8 and acetate buffer pH 4.5 for multimedia testing.

### 3.5.4 Effect of agitation intensity

To study the effect of agitation intensity of the release media, release studies of the optimized formulation of CPOP were carried out in USP dissolution apparatus type II at varying rotational speed (50, 100 and 150 rpm).

### 3.5.5 HPLC method for estimation of Butorphanol Tartrate

The plasma concentration of the butorphanol was determined by extraction and HPLC method. For determination of linearity 100 µg of Butorphanol Tartrate was accurately

weighed using single pan electronic balance and transferred to Mathanol to produce primary standard of Butorphanol, which was used to make working standard solutions of Butorphanol Tartarte by diluting primary standard solution with Mathanol. Rat plasma calibration standards (1.00, 5.00, 15.0, 20 µg/ml) of Butorphanol were prepared by spiking the working standard in to the drug free rat plasma. The samples were aliquoted (300 µL) into polypropylene tubes and stored at -20° C until analysis. 0.3 ml rat plasma was mixed with 1.0 ml 1N NaOH, and 7 ml chloroform. The samples were vortexed for 20 seconds, followed by centrifugation. The upper aqueous layer was aspirated to waste. The organic layer was transferred to a conical centrifuge tube and evaporated to dryness. The samples were reconstituted in 0.2 mL mobile phase to get 1, 5 15, 20 µg per ml concentration. 10 µl of the said stock was injected in HP in following condition. Butorphanol plasma standards were analyzed simultaneously with the samples.

Method Type : Isocratic System Method

Mobile Phase :Ammonium Acetate 0.05 M: Acetonitrile (3:1) adjusted pH to 4.1 with glacial acetic acid

Guard Column : Required. ( For Plasma Analysing)

Column : Phenomenex - Luna 5µ C 18 (2) 100 A 250 x 4.60 mm x 5 µ ( Size)

Flow Rate : 1.5 ml/min

$\lambda_{max}$ : 280 nm

Thermostat : Not required (Room temperature)

Retention time:7.5 min

The mobile phase was prepared freshly, filtered through a 0.45 µm membrane filter.

| **Concentration ( µg/ml)** | **Area µV .S** | **RSD** |
|---|---|---|
| **1** | 1134.0 | 1.0494 |
| **5** | 1092.1 | 0.8516 |
| **15** | 37703.0 | 0.6419 |
| **20** | 50722.0 | 0.5560 |

**Table 11- 3 Calibration curve of Butorphanol tartrate using HPLC**

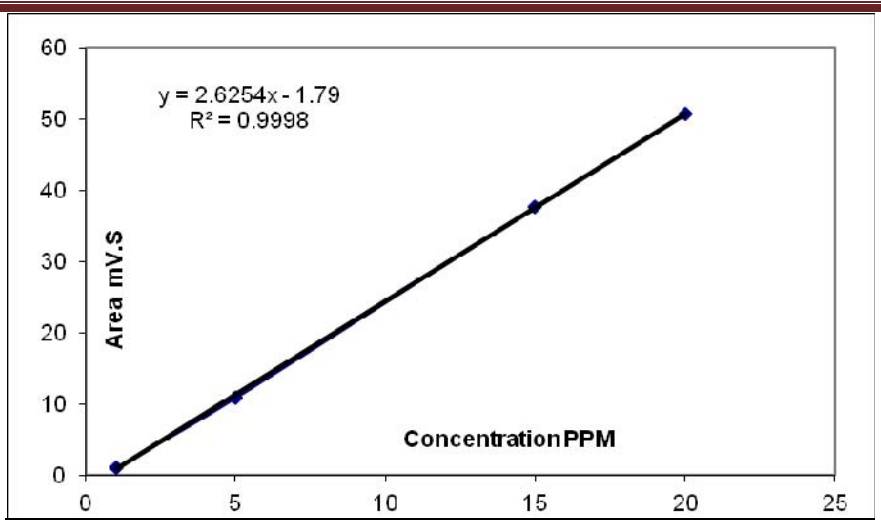



**Figure 5 - 3 Regressed calibration curve for estimation of Butorphanol tartrate using HPLC**

Figures 5 - 3 calibration curve of Butorphanol tartrate in using HPLC, the values are tabulated in table 11 -3.

***Accuracy and Precision***

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated. Accuracy of method for analysis of Butorphanol Tartrate is shown in table 12 - 3.

In order to determine the accuracy and precision of the developed method, known amounts of Butorphanol Tartrate (1µg/mL, 5µg/mL and 20µg/mL) were subjected to recovery studies as per the procedure described above. The results obtained are tabulated in following table.

| Theoretical Conc of BT (µg/mL) | AVG Recovery (µg/mL) | % Recovery | SD | RME | Confidence | RSD Intra Day | RSD Inter Day |
|---|---|---|---|---|---|---|---|
| 1 | 1.0 | 99.5 | 0.0098 | 0.0044 | 0.0184 | 0.99 | 0.84 |
| 5 | 5.0 | 101.0 | 0.0476 | 0.0213 | 0.0889 | 0.94 | 0.81 |
| 20 | 20.0 | 100.2 | 0.1477 | 0.0661 | 0.2760 | 0.74 | 0.76 |

**Table 12 - 3 Evaluation of accuracy and precision of the estimation method of BT using HPLC**

** At Alphaa 0.05 Confidence level; $t_{tab}$ = 3.18 for 4 degrees of freedom*

The developed isocratic high performance liquid chromatographic method was rapid and suitable for the estimation of Butorphanol Tartrate in plasma. Specificity, linearity, precision, accuracy and robustness were verified. The stability of analytical solutions was sufficient for the whole analytical process. Using the established method, the amount of Butorphanol Tartrate in plasma was determined. The chromatographic determination of placebo formulation consisting of all ingredients except drug showed no any absorbance under discussed methods. The capacity of the method to separate Butorphanol the non-interference with Butorphanol Tartrate indicates the specificity of the methods. Stability of the Butorphanol Tartrate in solution Butorphanol Tartrate was verified and found to be stable in solvents during the period of 24 h since the change in the $\lambda_{max}$ was not significant with the maximum RSD value of 0.84%. It was also confirmed that retention time of Butorphanol Tartrate not shifted with the adjustment of the proportion of acetonitrile and the flow rate. But the final result did not show significant change. Considering the stability in the system suitability parameters, the method conditions would be concluded to be robust.

## 3.6 SEM Analysis

In order to elucidate the mechanism of drug release from the developed formulations, surface of coated tablets before and after dissolution studies, was studied using scanning electron microscope (SEM). The samples were placed on a spherical brass stub (12 mm diameter) with a double backed adhesive tape. Small sample of the coating membrane was carefully cut from the exhausted shells (after dissolution studies) and dried at 50°C for 2 h. Membranes were dried at 45-C for 12 hours and stored between sheets of wax waper in a dessicator until examination. The morphology of the exhausted osmotic pump shell was analyzed using SEM (Surface Electron Microscopy). The mounted samples were examined under SEM (JSM-6360, Jeol, Japan).

## 3.7 Discussion

The DSC of samples was carried out by scanning the samples using differential scanning colorimeter (Mettler) for pre-formulation studies to find out any incompatibility in advance.

The UV spectroscopic method was developed for the BT estimation in SGF pH 1.2, SGF pH 6.8, Acetate Buffer pH 4.5 and water. The measurement was done at $\lambda_{max}$ 280 nm for all four solvents. There was no interference observed with any excipient used. The methods were validated for linearity, accuracy and repeatability. The validation parameters were found to meet the "readily pass criteria" specified in the USP and % RSD were found less than 1%.

The absorbance for BT in SGF pH 1.2 was found to be linear in the range of 1 - 200 µg /ml with $r^2$ value of 0.9999. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown in Table.

The absorbance for BT in SIF pH 6.8 was found to be linear in the range of 3.125 - 250 µg /ml with $r^2$ value of 0.9990. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown in Table.

The absorbance for BT in acetate buffer pH 4.5 was found to be linear in the range of 20 - 200 µg /ml with $r^2$ value of 0.9998. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown in Table.

The absorbance for BT in water was found to be linear in the range of 10 - 200 µg /ml with $r^2$ value of 1.0000. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown in Table.

The invitro release study was performed using type II dissolution apparatus using 250 ml release medium. At different time intervals, the samples were removed, replaced with same medium and analysed for the drug. The cumulative percentage drug released was calculated.

For Butorphanol Tartrate, the calibration curve was established using HPLC for estimation of drug in plasma with Ammonium Acetate 0.05 M: Acetonitrile (3:1) adjusted pH to 4.1 with glacial acetic acid as mobile phase and detection at 280nm. The linearity of Temozolomide was found to be 1-20 µg/ml ($R^2$=0.9998). The recovery studies for accuracy and precision were carried out at 1, 5 and 20 µg/ml and The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. 1 µg/ml of drug was spiked in plasma as concentration of the drug in plasma was below the quantification limit.

**3.8 References**


- Mohamed, M. E. (1986) 'First-Derivative Spectrophotometric Determination of a Mixture of Pirbuterol Hydrochloride and Butorphanol Tartrate, Analytical Letters, 19:11, 1323 — 1339.
- 2008 USPC, Inc. Official 8/1/05 - 12/31/05 USP Monographs: Butorphanol Tartrate
- Mohamed, M. E. (1983) 'Spectrophotometric and Fluorimetric Determination of Butorphanol Tartrate', Spectroscopy Letters, 16:10, 731 — 739
- Tracy A. Willey, Glenn F. Duncan, Lee K. Tay, Kenneth A. Pittman, Raymond H. Farmen High-performance liquid chromatographic method for the quantitative determination of butorphanol, hydroxybutorphanol, and norbutorphanol in human urine using fluorescence detection. Journal of Chromatography B, 652 (1994) 171-178
- Ed, Mohamed E. Mo Ham, Gad-Kariem, El Rasheed A. and Aboul-enein, Hassan Y. (1983) 'Polarographic etermination of Butorphanol Tartrate', International Journal of Environmental Analytical Chemistry, 16:3, 197 — 203

- Maristela H. Andrausa, Maria Elisa P.B. Siqueira Journal of Chromatography B, 704 (1997) 143–150 “Determination of butorphanol in horse race urine by immunoassay and gas chromatography–mass spectrometry”

- Robert J. Meyer and Ajaz S. Hussain, Office of New Drugs and Office of Pharmaceutical Science Center for Drug Evaluation and Research, FDA FDA’s ACPS Meeting, October 2005 Awareness Topic: Mitigating the Risks of Ethanol Induced Dose Dumping from Oral Sustained/Controlled Release Dosage Forms

# 4.0 Formulation Development (Butorphanol Tartrate)

The main objective of the present work is to develop extended release solid oral formulation of Butorphanol Tartrate ( BT) for maintaining therapeutic blood levels of the drug for extended period of time and improve patient compliance by minimizing local and systemic adverse effect. Table 1-4 lists the equipment used for development.

| Sr. No. | Instruments | Make |
|---|---|---|
| 1. | Compression | 8-station compression machine, KMP-8, Cadmach Engg, Ahmedabad, India. |
| 2. | Coating | Perforated pan, Solace Autocoater, India. |
| 3. | Digital weighing balance | AG-64, Mettler Toledo, Switzerland |
| 4. | Tap density tester | ETD-1020, Electrolab, Mumbai, India. |
| 5. | Hardness tester | 6-D, Dr Schleuniger Pharmatron, Manchester, NH, USA |
| 6. | pH meter | Mettler Toledo, Switzerland |
| 7. | Tray dryer | Bombay Eng. Works, Mumbai, India |
| 8. | Friability tester | EF-2, Electrolab, Mumbai, India |
| 9. | Thickness gauge | Digimatic Caliper, Mitutoyo, Japan |
| 10. | Bath sonicator | DTC 503, Ultra Sonics, Vetra, Italy |
| 11. | Stability chamber | Thermolab, Mumbai, India |
| 12. | Differential Scanning Calorimeter (DSC) | Mettler DSC 20, Mettler Toledo, Switzerland |
| 13. | Scanning electron microscope (SEM) | JSM-6360, Jeol, Japan |
| 14. | Stability oven | Shree Kailash Industries, Vadodara |
| 15. | HPLC system | LC 20-AT prominence, Shimadzu Corp., Japan |
| 16. | UV-Visible Spectrophotometer | Shimadzu UV-1601, Japan |

| | | |
|---|---|---|
| 17. | Nuclepore Polycarbonate membrane 2 µm 25mm | Whatman, USA |

**Table 1 - 4 Equipments Used**

### 4.1 Drug Substance

**Butorphanol Tartrate**

The drug substance attribute, affects the drug product development, manufacture, performance and stability. The following aspects were considered during the product development:

**Physiochemical properties of Drug**

As physicochemical drug properties plays an important role in the manufacturing of a dosage form and its therapeutic activity, the characterization of the powder properties of Butorphanol Tartrate among all the physicochemical properties was found to be important.

Particle size distribution of drug substance: Butorphanol Tartrate water soluble drug, particle size would not have significant impact on the rate of dissolution of drug and hence on the bioavailability

Bulk density and tapped density: Concentration of the drug in the formulation is less 5% w/w so density of the formulation is not going to be affected majorly by density of drug.

The stability of the Butorphanol tartrate is not affected by aqueous media and hence wet granulation method was adopted for the product development. Stability of the Butorphanol tartrate is not affected by aqueous media and hence wet granulation method was adopted for the product development. Butorphanol tartrate is not light sensitive and hence processing was done under normal light. Commonly used excipients were employed for development of tablet dosage form of Butorphanol tartrate.

Partition Coefficient: The n-octanol/aqueous buffer partition coefficient of butorphanol is 180:1 at pH 7.5. (34th ECDD 2006/4.1)

Physical Description: A white or almost white crystalline powder

Melting Point: It melts at 217°C to 219°C. (34th ECDD 2006/4.1)

Stability: Butorphanol Tartrate is very stable compound no individual degradation products were identified during forced degradation studies.

Biopharmaceutical Classification System (BCS) category: BCS has categorized Butorphanol Tartrate in Class I, i.e. High solubility High permeability.

**4.2 Pharmacokinetics**

Butorphanol is rapidly absorbed, widely distributed, undergoes extensive hepatic first-pass metabolism, and is excreted primarily via the kidneys. (34th ECDD 2006/4.1)

Peak plasma concentrations of 2.2 ng/mL butorphanol occur between 30-60 min after a single 2-mg i.m. administration. Peak plasma concentrations of 1.5 ng/mL butorphanol occur almost immediately after a single 1-mg i.v. administration. Apparent plasma half-life of butorphanol is between 6 and 10 h. (Boulton et al., 2002)

Butorphanol exhibits partial agonist and antagonist activity at the μ opioid receptor and agonist activity at the κ opioid receptor. Stimulation of these receptors on central nervous system neurons causes an intracellular inhibition of adenylate cyclase, closing of influx membrane calcium channels, and opening of membrane potassium channels. This leads to hyperpolarization of the cell membrane potential and suppression of action potential transmission of ascending pain pathways. Because of its κ-agonist activity, at analgesic doses butorphanol increases pulmonary arterial pressure and cardiac work. Additionally, κ-agonism can cause dysphoria at therapeutic or supertherapeutic doses; this gives butorphanol a lower potential for abuse than other opioid drugs. (Wikipedia)

Oral absorption of Butorphanol (Tartrate) is found to be 70% ±20. Volume of distribution is found to be 12l/kg and plasma protien binding is 80% and metabolism is reported Hepatic. Renal Excretion accounts for 1.05% and plasma half life is 2.5 - 4 hr. (http://druginfosys.com 17th March 2011) Peak plasma concentration of 0.7 ng/ml was achieved 1 to 1.5 hr following the 8 mg of oral dose.

Butorphanol is completely absorbed form gastro-intestinal track and following intra- muscular injection.

After intramuscular or intravenous administration, butorphanol is widely distributed to tissues with an estimated volume of distribution ranging from 300-900 mL. The extent of plasma protein binding is approximately 80%. (Gaver et al.,1980).

Butorphanol rapidly crosses the placenta and neonatal serum concentrations are 0.4-1.4 times maternal concentrations. Butorphanol is distributed into breast milk although breastfed infants would receive a negligible amount. Doses of 8 mg intramuscular to 12 healthy nursing mothers resulted in neonatal exposure of only 4 mcg. (Pittman et al., 1980a; Pittman et al., 1980b)

Following oral administration mean oral bioavailability of unchanged butorphanol is 17% due to hepatic first-pass metabolism. Due to the extensive hepatic metabolism of butorphanol, oral bioavailability is approximately 5 to 17%. Sublingual tablet and buccal disk formulations only increased mean absolute bioavailability to 19 and 29%. Butorphanol is metabolized by hydroxylation and N-dealkylation to form the major metabolite hydroxybutorphanol (45-50% of parenterally administered dose) and norbutorphanol (5-10% of parenterally administered dose). (Vachharajani et al., 1997a; Vachharajani et al., 1997b). Neither metabolite appears to have any pharmacological effects. (Gaver et al.,1980).

The major route of elimination for Butorphanol and its metabolite is renal. Less than 5% of butorphnol is excreted unchanged in urine, and principal urinary metabolite being free hydroxybutorphanol.

**4.3 Pharmacodynamic Properties**

Butorphanol (Tartrate) is opioid analgesic. Butorphanol (Tartrate) is used to relieve the pain. It has some narcotic activity. Butorphanol (Tartrate) is used to relieve pain of surgical procedures and labor and to enhance the effects of anesthesia. Long term use of butorphanol tartrate can produce dependence. (http://druginfosys.com 17th March 2011)

Studies of oral administration have demonstrated analgesic activity with doses of 4 to 16mg of butorphanol. 8 or 16mg oral doses produced a peak analgesic effect similar to that seen with 60mg of codeine when given for a few days to patients with acute musculoskeletal or episiotomy pain, but butorphanol appeared to be longer acting and was thus often statistically superior to codeine at 4 to 6 hours after administration of a dose. Similarly, a single 8mg dose of oral butorphanol was more effective than 50mg of pentazocine (usual oral dose 50 to 100mg) at several evaluation times in postoperative patients, and appeared to be longer acting. (Butorphanol 1978)

Only a small group of patients with chronic pain have received repeated intramuscular or oral doses of butorphanol over an extended period. Treatment was discontinued due to side effects (sedation, nausea, confusion, dizziness, rash) in 18 of 63 patients receiving repeated injections (usually 2 or 4mg) for up to 34 weeks. In a small number of patients who received oral treatment over a 6 to 8 month period, no drug related changes in laboratory or physical examination findings occurred. However, further studies in which larger numbers of patients are treated for

extended periods are needed to more clearly determine the drug's tolerability and safety when used in the treatment of chronic pain. (Butorphanol 1978)

Oral butorphhanol is comparable to oral midazolam in children but analgesia alongwith sedation is an additional advantage which makes it better than midazolam without a significant increase in side effects. (Singh et al., 2005)

**Mechanism of Action**

Butorphanol, a synthetic morphinan derivative, was developed so as to minimize side effects associated with normal narcotic analgesics. Butorphanol is a mixed agonist-antagonist with low intrinsic activity at receptors of the μ-opioid type (morphine-like). It is also an agonist at k-opioid receptors. The analgesic potency of butorphanol is approx 5 times to that of morphine, 35 times that of meperidine, 17 times that of pentazocine, and very less than that of naloxone. Butorphanol also has a strong antitussive effect that is approx 100 times that of codeine.

## 4.4 Excipients:

### *4.4.1 Excipient used in drug product:*

Following table describes the list of excipients. Table 2-4 includes all raw materials used in the manufacture of the drug product, whether they appear in the finished product or not. All excipients used in fabrication of drug product matches with specifications commonly used in design of oral products.

| Ingredients | Functional Category | Source |
|---|---|---|
| Butorphanol Tartrate | Active | Theraquest (Supplied By Teva) |
| Lactose Monohydrate | Osmogen | Granulac 200, Meggle. |
| Microcrystalline cellulose | Diluent | Celphere CP-102, AsahiKASEI, Japan |
| Polyvinyl Pyrrolidone | Binder | Kollidon, BASF, Germany |
| Mannitol | Osmogen | Pearlitol 200 SD, Roquette, France |
| Sodium chloride | Osmogen | S.D. Fine Chemicals Ltd, Mumbai, India |
| Purified Talc | Antiadherant | Luzenac |
| Tartaric acid | pH Modifier | S.D. Fine Chemicals Ltd, Mumbai, India |
| Magnesium stearate | Lubricant | Mallinckrodt, USA |

| | | |
|---|---|---|
| Colloidal silicon dioxide | Glidant | Aerosil 200, Degussa, Frankfurt, Germany |
| Polyethylene Glycol 400 (Liquid form) | Plasticizer | S.D. Fine Chemicals Ltd, Mumbai, India |
| Sorbitol | Pore Former | Qualigens Fine Chemicals, Mumbai, India |
| Cellulose acetate (CA-398-10 NF) | Film Former | Eastman Chemical Inc, Kingsport, TN, USA |
| HPMC ( Methocel Series) | Matrix Forming Agent | Colorcon Asia Pvt. Ltd, Goa, India |
| Polyethylene oxide (Polyox WSR Series) | Cross Linker | The Dow Chemical Company, MI, USA |
| Purified Water * | Processing solvent | Prepared in laboratory by distillation |
| Acetone * | Processing solvent | S.D. Fine Chemicals Ltd, Mumbai, India |
| Isopropyl Alcohol * | Processing solvent | S.D. Fine Chemicals Ltd, Mumbai, India |

**Table 2 - 4 List of Excipients**

*Used as processing agent, does not remain in the final product.

### *4.4.2 Suppliers Specifications of the excipients*

**Microcrystalline cellulose**

| **Specifications** | |
|---|---|
| Loss on drying | % 3.0 - 5.0 |
| Bulk density | 0.26 - 0.31 g/cc |
| Identification | A, B Passes |
| Degree of polymerization, units | NMT 350 |
| pH | 5.5 - 7.0 |
| Conductivity, | NMT 75 µS/cm |
| Residue on ignition, | % NMT 0.05 |
| Water soluble substances, | mg/5g NMT 12.5 |
| Water soluble substances, | % NMT 0.25 |
| Ether soluble substances, | NMT 5.0 mg/10g |
| Heavy metals, | NMT 0.001 % |
| Solubility in Copper Tetrammine Hydroxide | Soluble |
| **Microbial limits:** | |
| Total aerobic microbial count | NMT 100 cfu/g |
| Total yeast and mold count, cfu/g * | NMT 20 cfu/g |
| Pseudomonas aeruginosa in a 10g sample | Absent |

| | |
|---|---|
| Escherichia coli in a 10g sample | Absent |
| Staphylococcus aureus in a 10g sample | Absent |
| Salmonella species in a 10g sample | Absent |
| Coliform species in a 10g sample | Absent |
| **Additional FMC Specifications** | |
| Particle size (Air Jet): | |
| wt. % + 60 mesh (250 microns) | NMT 1.0 |
| wt. % + 200 mesh (75 microns) | NMT 30 |

**Cellulose Acetate CA 398-10 NF-EP**

| **Specifications** | |
|---|---|
| ASTM A Viscosity (mPa s) | 8.0 to 13.0 |
| NF Loss on drying | % 3.0 - 5.0 |
| NF NF Acetyl (%) | 39.2 to 40.3 |
| NF Loss on drying | 5 % W Max |
| NF Free Acid | 0.1% Max |
| NF Infrared Identity | Pass |
| NF Residue on ignition, | 0.1% Max |
| NF Residual Solvents | Pass |
| EP Heavy metals, | 0.001 % Max |
| **Microbial limits:** | |
| EP Total aerobic microbial count | $10^3$ cfu/g Max |
| EP Total yeast and mold count, cfu/g * | $10^2$ cfu/g Max |
| EP Escherichia coli in a 10g sample | Absent |
| EP Salmonella species in a 10g sample | Absent |

**Lactose Monohydrate**

| **Specifications USP NF 23** | |
|---|---|
| Identification | Pass |
| Appearance/color of solution | Pass |
| Optical rotation | +54.4 to +55.9° |
| Acidity or alkalinity | Pass |
| Heavy metals | ≤5 µg/g |
| Absorbance 210–220 nm | ≤0.25 |
| Absorbance 270–300 nm | ≤0.07 |
| Loss on drying | ≤0.5% |
| Water | ≤1.0% |
| Residue on ignition | ≤0.1% |
| Heavy metals, | NMT 0.001 % |

| | |
|---|---|
| Solubility in Copper Tetrammine Hydroxide | Soluble |
| **Microbial limits:** | |
| Total aerobic microbial count | NMT 100 cfu/g |
| Total yeast and mold count, cfu/g * | NMT 50 cfu/g |
| Escherichia coli in a 10g sample | Absent |
| Isomer ratio | Pass |
| Salmonella species in a 10g sample | Absent |

| **PERLITOL Mannitol** | |
|---|---|
| **Specifications USP NF 23** | |
| Melting range | 164–169°C |
| Specific rotation | +137° to +145° |
| Acidity | + |
| Loss on drying | ≤0.3% |
| Chloride | ≤0.007% |
| Sulfate | ≤0.01% |
| Arsenic | ≤1 ppm |
| Reducing sugars | + |
| Assay (dried basis) | 96.0–101.5% |
| mean diameter 180 µm | |

| **Sodium Chloride** | |
|---|---|
| **Specifications USP 28** | |
| Identification | Pass |
| Appearance of solution | Pass |
| Acidity or alkalinity | Pass |
| Loss on drying | 0.50% |
| Arsenic | 1 µg/g |
| Bromides | ≤0.01% |
| Chloride | Pass |
| Barium | Pass |
| Nitrites | Pass |
| Aluminum | ≤0.2 µg/ga |
| Magnesium and alkaline earth metals | ≤0.01% |
| Iodide | Pass |
| Iron | ≤2 µg/g |
| Sulfate | ≤0.020% |
| Ferrocyanides | Pass |
| Heavy metals | ≤5 ppm |
| Phosphate | ≤0.0025% |

| | |
|---|---|
| Potassium | ≤0.05%[ab] |
| Sterility | Pass |
| Assay (dried basis) | 99.5–100.5% |

| **Polyox** | |
|---|---|
| **Specifications USP NF 23** | |
| Identification | Pass |
| Loss on drying | ≤1.0% |
| Silicon dioxide and nonsilicon dioxide residue on ignition | ≤2.0% |
| Silicon dioxide | ≤3.0% |
| Heavy metals | ≤0.001% |
| Free ethylene oxide | ≤0.001% |
| Organic volatile impurities | Pass |
| Viscosity | Pass |

| **Methocel** | |
|---|---|
| **Specifications USP 28** | |
| Identification | Pass |
| Apparent viscosity | Pass |
| Loss on drying | ≤5.0% |
| For viscosity grade >50 mPa s | ≤1.5% |
| For viscosity grade ≤50 mPa s | ≤3.0% |
| For type 1828 of all viscosities | ≤5.0% |
| Heavy metals | ≤0.001% |
| Organic volatile impurities | Pass |
| **Methoxy content** | |
| Type 1828 | 16.5–20.0% |
| Type 2208 | 19.0–24.0% |
| Type 2906 | 27.0–30.0% |
| Type 2910 | 28.0–30.0% |
| **Hydroxypropoxy content** | |
| Type 1828 | 23.0–32.0% |
| Type 2208 | 4.0–12.0% |
| Type 2906 | 4.0–7.5% |
| Type 2910 | 7.0–12.0% |

| **Tartric Acid** | |
|---|---|
| **Specifications USP 23** | |
| Identification | Pass |
| Specific rotation | +12.0° to +13.0° |

| | |
|---|---|
| Loss on drying | ≤0.5% |
| Residue on ignition | ≤0.1% |
| Organic volatile impurities | Pass |
| Oxalate | Pass |
| Sulfate | Pass |
| Heavy metals | ≤0.001% |
| Assay (dried basis) | 99.7–100.5% |

| **PEG 400** | |
|---|---|
| **Specifications USP 23** | |
| CAS # | 25322-68-3 |
| Description | Clear colourless liquid |
| Sp gravity @ 27/27 deg cel | 1.110 - 1.120 |
| Cloud Point in deg cel | Report |
| BP in deg cel | > 200 |
| Flash Point in deg cel | 235 |
| pH of 1% solution | 4.5 -7.0 |
| Solubility | Soluble in water and ethanol |

| **Polyvinyl Pyrrolidone** | |
|---|---|
| **Specifications USP NF** | |
| Appearance @ 25°C | White to creamy white powder |
| Identification Tests | Meets all ID tests |
| Appearance @ 25°C ( 5% as is aqueous solution) | Free of haze |
| European Colour Test – B Colour | Pass |
| European Colour Test – BY6 Colour | BY6 Min |
| European Colour Test – R Colour | R6 Min |
| % Moisture ( Karl Fischer) | 5.0 Max |
| pH ( 5% as is aqueous solution) | 3.0-5.0 |
| % Ash ( Residue on Ignition or Sulphated) | 0.02 Max |
| ppm Vinyl Pyrrolidone ( HPLC) | 5.0 Max |
| % 2-Pyrrolidone | 3.0 Max |
| ppm Heavy Metals (as Lead) | 5 Max |
| ppm Aldehydes ( Calculated as acetaldehyde) | 500 Max |
| % Nitrogen ( anhydrous basis) | 12.0-12.8 |
| K-Value ( 1% solids w/v aqueous solution) | 29-32 |
| ppm Peroxide Content ( Titanyl Sulfate Method) | 400 Max |
| ppm Hydrazine | 1.0 Max |
| Salmonella species in a 10g sample | Absent |
| Coliform species in a 10g sample | Absent |
| **Micorbial Limit Test Specifications** | |
| Total Aerobic Plate Count, CFU/g | 100 Max |

| | |
|---|---|
| Mould/Yeast, CFU/g | 100 Max |
| Salmonella, CFU/g | Negative |
| Staphylococcus Aureus CFU/g | Negative |
| Pseudomonas aeruginosa, CFU/g | Negative |
| E.Coli, CFU/g | Negative |

| **Purified Talc** | |
|---|---|
| **Specifications USP NF** | |
| Identification | Complies with EP/BP tests |
| Acidity or alkalinity | |
| Change colour to pink | NMT 0.3 ml of 0.01 M NaOH |
| Water-soluble substances | Max 0.2% |
| Aluminium | Max 2.0% |
| Calcium | Max 0.90% |
| Iron | Max 0.25% |
| Lead | Max 10.0 ppm |
| Magnesium | 17.0% to 19.5% |
| Loss on ignition | Max 7.0% |
| **Microbial contamination** | |
| Total viable aerobic count | NMT total of $10^2$ bacteria and fungi per gram. |

| **Aerosil 200** | |
|---|---|
| **Specifications USP NF 23** | |
| Identification | Pass |
| pH (4% w/v dispersion) | 3.5–5.5 |
| Arsenic | 8 µg/g |
| Loss on drying | 2.50% |
| Loss on ignition | 2.00% |
| Organic volatile impurities | Pass |
| Assay (on ignited sample) | 99.0–100.5% |
| Specific Surface Area | 200 ± 25 $m^2/g$ |
| Tapped Density | 0.05 $g/cm^3$ |

| **Sorbitol** | |
|---|---|
| **Specifications USP NF 23** | |
| Identification | Pass |
| pH | 3.5 – 7.0 |
| Appearance of solution | Pass |
| Chloride | 0.005% |
| Sulfate | 0.01% |
| Bacterial | $10^3/g$ |

| | |
|---|---|
| Fung | $E10^2/g$ |
| Bacterial endotoxins | Pass |
| Nickel | 1 µg/g |
| Organic volatile impurities | Pass |
| Reducing sugars | 0.30% |
| Residue on ignition | 0.10% |
| Water | 1.50% |
| Assay (anhydrous basis) | 91.0–100.5% |

| **Magnesium Stearate** | |
|---|---|
| **Specifications USP NF 23** | |
| Identification | Pass |
| **Microbial limits** | |
| Aerobic microbeal Count | $10^3/g$ |
| Fungi and yeasts | 500/g |
| Acidity or alkalinity | Pass |
| Specific surface area | Pass |
| Loss on drying | 6.00% |
| Chloride | 0.10% |
| Sulfate | 1.00% |
| Lead | 0.001% |
| Relative stearic/palmitic content | Pass |
| Organic volatile impurities | Pass |
| Assay (dried, as Mg) | 4.0–5.0% |

| Ingredients | Approval Status ( USFDA) | Qty ( mg) |
|---|---|---|
| Sorbitol | Oral; tablet | 337.28 |
| Cellulose acetate CA-398-10 | Oral; tablet, extended release ( pending) | 47.49 |
| Polyethylene glycol 400 | Oral; tablet | 105.065 |
| Cellulose, microcrystalline | Oral; table | 1385.3 |
| Lactose monohydrate | Oral; tablet, film coated | 587.44 |
| Sodium chloride | Oral; tablet, extended release | 335.1 |
| Talc | Oral; tablet, extended release | 80 |
| Silicon dioxide, colloidal | Oral; tablet | 99 |
| Mannitol | Oral; tablet | 991.77 |
| Magnesium Stearate | Oral; tablet | 150 mg |
| Sodium Chloride | Oral; tablet | 148 mg |

**Table 3 - 4 Approval status of the inactive ingredients used for Oral Route**

***4.4.3 Drug excipients compatibility study:***

At an early stage of proposed drug product development, drug-excipient compatibility study performed, to identify the potential incompatibilities of drug with the excipients intended to be used for product development. The pre-formulation study was based on the excipients to be used in the finished product. All the inactive ingredient guide (USFDA) approved ingredients were selected for the study as mentioned in table 3-4.

Butorphanol tartrate was mixed with Lactose and mannitol in Drug to Excipient ratio of 1:5 W/W and Other excipients in Drug to Excipient ratio of 5:1 proportion. Binary mixture and the blend were exposed to 40°C /75%RH temperatures for 4 weeks to accelerate drug degradation and interaction with excipients in USP type I amber glass vials with LDPE (low density polyethylene) stopper for evaluation of their compatibility at stress condition. The blends exposed to stress conditions were compared with their respective initial blend stored at controlled condition by physical observation. The samples are then characterized for the drug content, which were determined quantitatively using developed analytical method after dissolving the drug in water and percentage drug content verified initially and after 4 weeks and compared.

Results of drug-excipient compatibility study are described below in table

| **Drug : Excipients** | **Initial** | | **4 Week 40°C /75%RH** | |
|---|---|---|---|---|
| | **Observation** | **% Drug Content** | **Observation** | **% Drug Content** |
| Butorphanol Tartrate | White to off-white Crystalline powder. | 100.35 | White to off- white, free flowing powder. No change in physical appearance observed | 99.49 |
| Butorphanol Tartrate & CA 398-10 | White to off-white Crystalline powder. | 100.28 | White to off- white, free flowing powder. No change in physical appearance observed | 101.34 |
| Butorphanol Tartrate & Magnesium Stearate | White to off-white, free flowing powder. | 101.34 | White to off- white, free flowing powder. No change in physical appearance observed | 100.85 |
| Butorphanol | White free | 100.32 | White to off- white, free | 99.62 |

| Drug : | Initial | | 4 Week 40°C /75%RH | |
|---|---|---|---|---|
| Tartrate & Kollidon K 30 | flowing powder. | | flowing powder. No change in physical appearance observed | |
| Butorphanol Tartrate & Sodium Chloride | White to off-white, free flowing powder. | 100.74 | White to off- white, free flowing powder. No change in physical appearance observed | 100.64 |
| Butorphanol Tartrate & Lactose | White to off-white, free flowing powder. | 102.75 | White to off- white, free flowing powder. No change in physical appearance observed | 101.73 |
| Butorphanol Tartrate & Purified Talc | White to off-white, free flowing powder. | 99.25 | White to off- white, free flowing powder. No change in physical appearance observed | 97.35 |
| Butorphanol Tartrate & D-Sorbitol | White to off-white, free flowing powder. | 100.34 | White to off- white, free flowing powder. No change in physical appearance observed | 101.24 |
| Butorphanol Tartrate & Colloidal Silica | White to off-white, free flowing powder. | 100.48 | White to off- white, free flowing powder. No change in physical appearance observed | 102.42 |
| Butorphanol Tartrate & Mannitol | White to off-white, free flowing powder. | 97.51 | White to off- white, free flowing powder. No change in physical appearance observed | 95.20 |
| Butorphanol Tartrate & PEG 400 | White to off white colour lump. | 100.31 | White to off white colour lump. No change in physical appearance observed | 101.52 |
| Butorphanol | White to off- | 101.60 | White to off- white, free | 100.30 |

| Drug : | Initial | | 4 Week 40°C /75%RH | |
|---|---|---|---|---|
| Tartrate & MCC 101 | white, free flowing powder. | | flowing powder. No change in physical appearance observed | |
| Butorphanol Tartrate & Acetone | White to off white colour lump. | 99.66 | White to off white colour lump. No change in physical appearance observed | 97.63 |
| Butorphanol Tartrate & Isopropyl alcohol | White to off white colour lump. | 101.88 | White to off white colour lump. No change in physical appearance observed | 100.61 |
| Butorphanol Tartrate & Purified water | White to off white colour lump. | 101.36 | White to off white colour lump. No change in physical appearance observed | 103.52 |

**Table 4 - 4 Drug excipient compatibility Results**

**Conclusion :** Under mentioned accelerated conditions (40°C /75%RH for 4 weeks), butorphanol tartrate did not reveal show sharp fall in content so it can be concluded that it do not show any incompatibilities with the proposed excipients as summarized in table 4 -4. Preformulation studies show that the selected excipients are compatible with Butorphanol tartrate

## Differential scanning Calorimetry

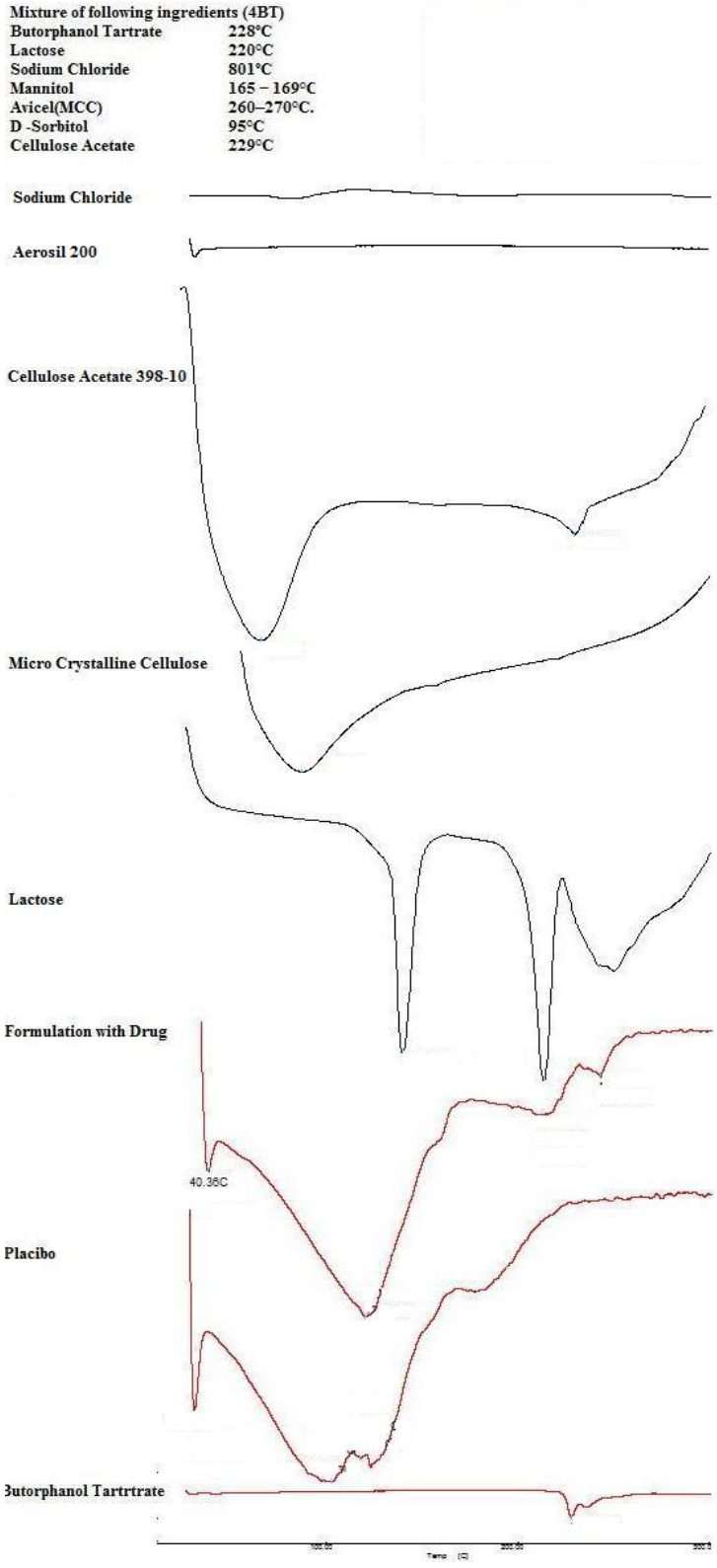



**Figure 1 - 4 DSC Study**

The DSC of samples was carried out by scanning the samples using differential scanning colorimeter (Mettler). Thermograms were analyzed using Mettler Toledo star SW 7.01. An empty aluminium pan was used as the reference for all measurements. During each scan, 2 to 3 mg of sample was heated, in a hermetically sealed aluminium pan. DSC studies were performed under nitrogen flush at heating rate of 10 ºC from 35 ºC to 300 ºC to investigate the any incompatibility between drug and excipients.

DSC curve of lornoxicam exhibited sharp endothermic peak at 228° C, which is due to melting of Butorphanol Tartrate.

For that DSC of plain drug was done followed by DSC study of individual excipients to be used in dry form. i.e. aerosil 200, Cellulose acetate 398-10, Sodium Chloride, Lactose, MCC and drug mixed with placebo formulation was done.

**Conclusion:**

Formulation composition DSC shows exhibited a sharp melting endotherm & plain drug melting endotherm can be observed in figure 1 – 4 that shows no changes observed in the formulation & the original plain drug melting endotherm which exhibited a sharp melting endotherm at (228°C). Hence it was clear that there is no specific interaction between the drug and excipients used in the formulation. Melting endotherm of Cellulose acetate and drug are nearer but it is not showing any signs interaction, no change in intensity of peak observed. Minor shift in melting point can be observed which is not indicative of interaction. Melting peak of PVP k 30, magnesium Stearate, Mannitol, Sorbitol, Micro Crystalline Cellulose observed at 150, 89, 167,95, 280 ºC respectively in the placebo.

**4.5 Drug Product Formulation Development**:

The proposed target for formulation development must be easy to manufacture, chemically and physically stable throughout the manufacturing process, product shelf life and bio-available in predicted manner. During design of the formulation, critical formulation and manufacturing variables were identified and adjusted to yield quality product. Design of experiments was used to improve and establish the robustness of the formulation around target formulation.

Butorphanol tartrate osmotic Pump 10 mg, the proposed drug product is intended to have following primary attributes:

- Product may to be formulated as coated tablet dosage form or matrix tablet.
- Product is developed as an coated dosage form where coating shall semi permeable in nature and must comply with the following release specifications:

### *4.5.1 Predicted Plasma concentration of Butorphanol Tartrate*

From data of oral IR plasma concentration of drug available in literature total amount of drug required to achieve steady state level in particular time frame is found out. The target release profile was decided from the AUC of the oral immediate release blood concentration data by Wagner nelson de-convolution process and is shown in figure 2-4. The target drug release profile is depicted in table 5 -4 & 6 – 4.

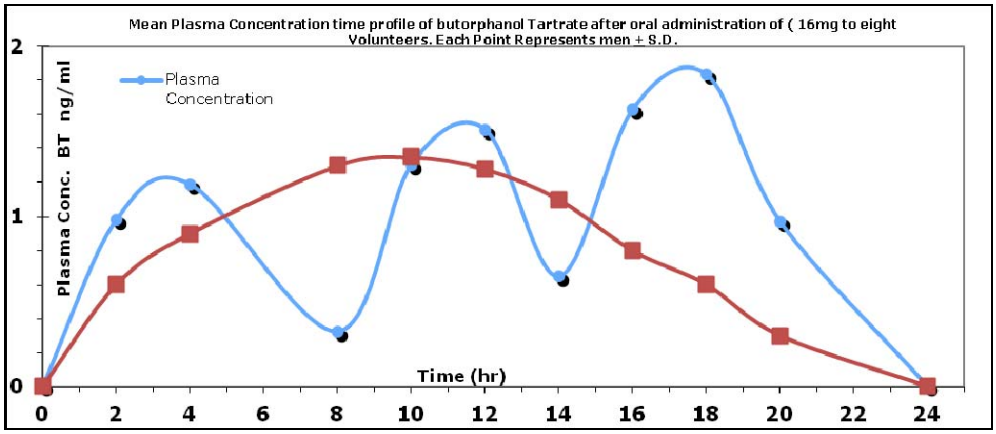



**Figure 2 - 4 Predicted Plasma concentration of Butorphanol Tartrate**

Above mentioned graph shows actual plasma concentration time profile after administration of three IR formulation administration at the interval of 7 hours and from the plasma concentration of the IR formulation plasma concentration time profile of ER formulation predicted considering pharmacokinetic data. From the data of ER plasma drug concentration profile In-vitro target release profile was calculated through deconvolution.

| Time Hr | Target *In-vitro* Drug release |
|---|---|
| 0 | 0.00 |
| 2 | 3.00 |
| 4 | 15.00 |
| 8 | 28.00 |
| 10 | 57.00 |
| 12 | 70.00 |
| 14 | 82.00 |
| 16 | 90.00 |
| 18 | 95.00 |
| 20 | 99.00 |
| 22 | 99.00 |

| | |
|---|---|
| 24 | 100.00 |

**Table 5 - 4 Target release profile (Detail)**

| **Time (hr)** | **Target Range Cum. % release profile** |
|---|---|
| 2 | NMT 15 |
| 4 | 10 to 30 |
| 8 | 30 to 55 |
| 14 | 50 to 80 |
| 21 | NLT 90 |

**Table 6 - 4 Target release profile (Brief)**

| **Drug Name :** Butorphanol Tartrate | | | **Dosage Form** : Extended Release | | |
|---|---|---|---|---|---|
| **Stage** | **USP Apparatus** | **Speed** | **Medium** | **Volume (ml)** | **Recommended Sampling Times** |
| Stomach | II (Paddle) | 50 | 0.1 N HCl Followed by | 250 ml | 120 minutes Followed by |
| Intestine | II (Paddle) | 50 | 6.8 pH Phosphate Buffer | 250 ml | up to 24 Hrs in buffer stage |

**Table 7 - 4 Dissolution conditions**

### *4.5.2 Quality Target Product Profile (QTTP) :*

As a target for the development of a manufacturing process, the following attributes were identified that will ensure the desired product quality to match all aspects of Quality target product profile:

1. Correct amount (assay) of drug substance in the drug product.
2. Content Uniformity
3. Weight variation of the drug product.
4. Dissolution (Conditions mentioned in table 7 – 4) of drug substance from the drug

product. (Target release profile table 5 -4 & 6 – 4).

5. Type and concentration of excipients that directly influences the quality and performance of the drug product.
6. Container closure system to provide intended protection to drug product.
7. Overages if required
8. Hardness : 2 – 5 kg/$cm^2$
9. Friability NMT 1.0 %
10. Bulk density and tapped density ( Granules)

**4.5.3 Selection of Manufacturing Process:**

Trials were initiated with dry granulation method but sufficient hardness could not be achieved dry granulation method so further trails were done with wet granulation method.

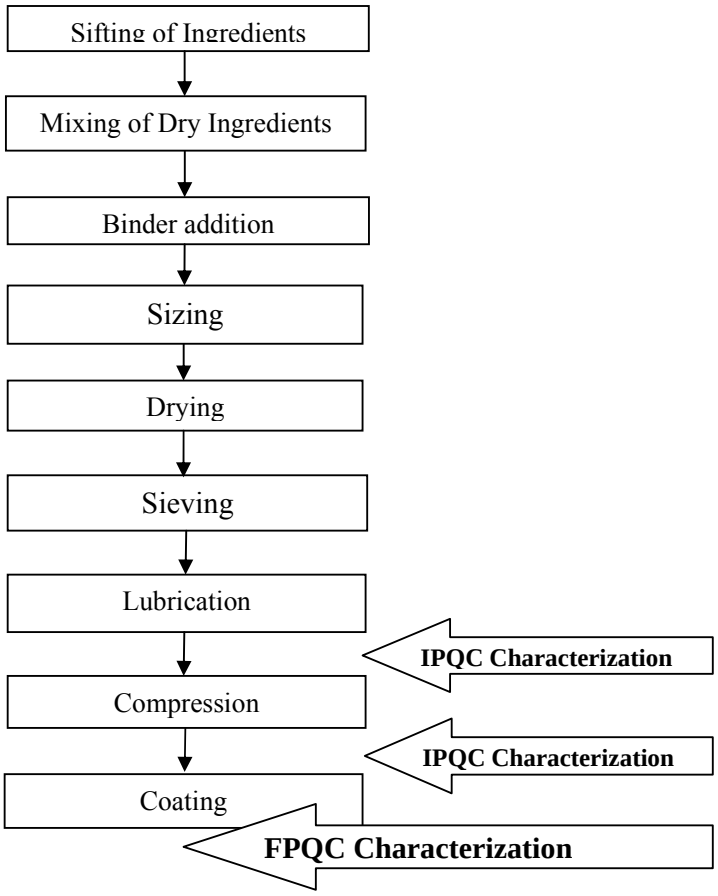
The wet granulation method was selected for the manufacturing of core tablets.

Drug substance sourced as USP grade and evaluated for physio-chemical and analytical parameters as per IH method of analysis.

**Preparation method of core tablets**

Core tablets were prepared by wet granulation and the composition is given in table 1. For preparation of core tablets, the batch size was kept as 1000 tablets. The mixture was moistened with PVP solution in isopropyl alcohol and granulated by passing through 18 sieve. The granules were dried at 50°C (approximately 1 h) after which they were passed through 25 sieve. These sized granules were then blended with magnesium stearate, talc and aerosol 200 (all 100 sieve passed) and compressed into tablets using a rotary tablet compression machine (General Machinary Company, India) fitted with 9 mm std concave punches.

### *4.5.4 Process Flow Chart*




### *4.5.5 Unit Operations of the Preparation process*

**Granulation**

Sieving : All the excipients were sieved before use to break the agglomerates sieve sizes used for sieving are mentioned in table 8 - 4.

Following sieve was used for the same.

| Excipients | Sieve Size |
|---|---|
| Butorphanol Tartarate | 40 # |
| Mannitol | 60 # |
| Lactose | 40 # |
| NaCl | 40 # |
| MCC 101 | 40 # |
| PVP K 30 | 40 # |
| Mg. Stearate | 100 # |

| | |
|---|---|
| Talc | 100 # |
| Aerosil 200 | 100 # |

**Table 8 - 4 Sieving Details**

Dry Mixing : Drug was mixed with other excipients using geometric dilution method followed by sieving through 40 # twice to have uniform mixing.

Binding : For binding Polyviny Pyrrolide was dissolved in IPA 10% w/v and 120 ml of this solution was used for binding. Binding was performed till wet mass with granular consistency obtained.

Sizing : Sizing was done in 18# sieve. ( Wet Milling)

Drying : Drying was done at 50˚till LOD is achieved NMT 3%.

Sieving : Dry granules were again passed through 25.

Lubrication : Talk,aerosil and Magnesium stearate were added in sequence respectively and blended for 5 min in poly bag to get uniform coating on the granules.

**Characterization :** Assay, Blend uniformity, LOD, Bulk Density, Tapped Density.

**Compression :** Compression was done on 8 Station rotary tablet compression machine from general machinery company.

Compression Parameters : 9 mm standard concave punch plain on both sides

Turret Speed : 9 RPM

Compaction Force: 4 – 5 kg

Thickness Adjustment Lever: Optimised to 3.1 to 3.3 mm thickness.

### *4.5.6 Optimisation of osmogen composition in the core*

| **Core Formula mg/Tab** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
| Butorphanol Tartrate | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Mannitol | | | 100.00 | 100.00 | | 100.00 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Lactose | | 100.00 | | 100.00 | 100.00 | | |
| NaCl | 100.00 | | | | 100.00 | 100.00 | |
| MCC 101 | 100.00 | 100.00 | 100.00 | | | | 200.00 |
| PVP K 30 | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 |
| Mg. Stearate | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Purified Talc | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Aerosil 200 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| **Total** | **230.00** | **230.00** | **230.00** | **230.00** | **230.00** | **230.00** | **230.00** |

**Table 9 - 4 Composition of core for optimisation of osmogen**

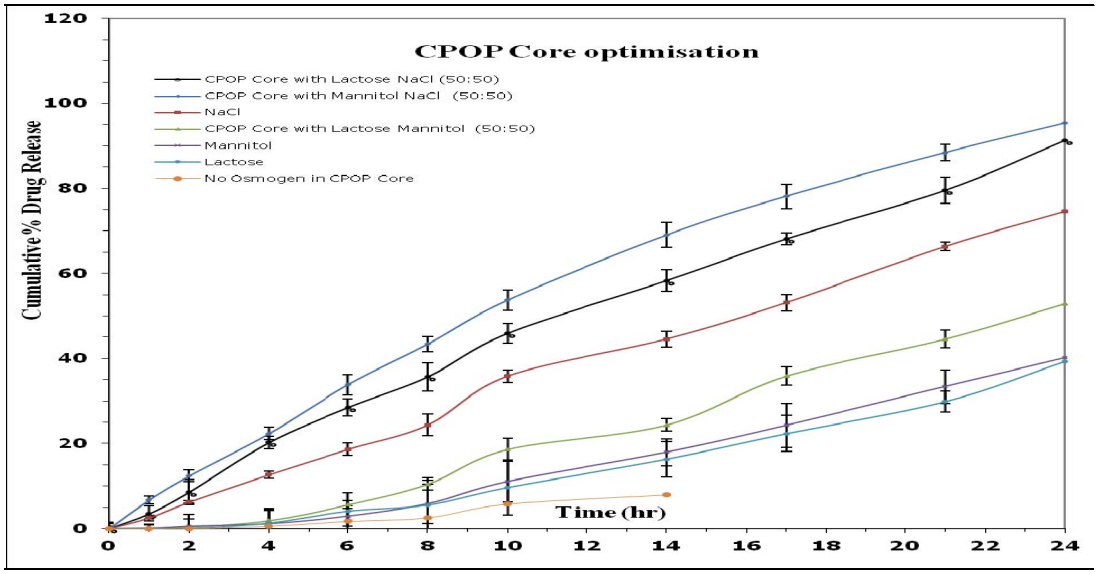



**Figure 3 - 4 Drug release from CPOP with different osmogen in core**

**Effect of osmogen and combination thereof on drug release:**

During initial development it was necessary to fix the osmogen within the core and core composition with its manufacturing procedure. Osmotic pressure of some osmogen and combination thereof were evaluated for degree of osmosis keeping manufacturing process and composition (Table 9-4) except osmogen trials were taken and subjected to dissolution as mentioned in table 7 -4, the graph Figure 3 -4 plotted to optimised the prototype batch.

| Sr. No. | Compound/ mixture | Osmotic pressure (Atm) |
|---|---|---|
| 1 | Sodium Chloride: Mannitol (1:1) | 365++ |

| | | |
|---|---|---|
| 2 | Sodium chloride :Lactose (1:1) | 365+ |
| 3 | Sodium chloride | 356 |
| 4 | Mannitol:Lactose (1:1) | 130 |
| 5 | Mannitol | 38 |
| 6 | Lactose | 23 |

**Table 10 - 4 Osmotic pressure exerted by saturated solution of compounds**

Table 10 – 4 shows Osmotic pressure exerted by saturated solution of compounds used for developmental studies. Lactose, mannitol and sodium chloride separately and in combination with each other in equal ratio were used as osmogen in the core tablet and its effect on drug release was determined keeping other parameters constant i.e thickness of coating (weight gain) and pore forming agent level constant. Theoretically osmotic pressure exerted by the osmogen is in following order. NaCl & Lactose ( 1: 1 Combination ) > NaCl & Mannitol ( 1: 1 Combination ) > NaCl > Lactose & Mannitol ( 1: 1 Combination ) > Lactose > Mannitol. It is apparent from Figure 3 -4 that as osmotic pressure of osmogen enhances the drug release when used in constant amount and thus had a direct effect which might be due to the increased water uptake and hence increased driving force for drug release. Combination of Lactose and NaCl in equal proportion was showing fastest drug release and was considered further for optimization.

### *4.5.7 Optimisation of coating*

**Coating process**

Coating process varies with type of coating system, manual pan coating with gun spray is Require skills and may induce variability specifically for functional coating. Therefore, Perforated pan make Solace Autocoater was used for coating for better reproducibility in functional coating. It requires coating load of at-least 1 kg. To increase the coating load dummy tablets with smaller size were used with active tablets. Increasing coating load leads to decreased coating variation and increased uniformity of coating. Due to previous experience and coating process optimised on dummy tablet

**Coating Ingredients**

Following substance were opted for preparation of Coating Solution

Cellulose acetate is widely used in pharmaceutical formulations both in sustained-release applications and for taste masking. Cellulose acetate is used as a semipermeable coating on tablets, especially on osmotic pump type tablets and implants. This allows ~~for~~ controlled, extended release of actives from the osmotic pump. Cellulose acetate films, in conjunction with other materials, also offer sustained release without the necessity of drilling a hole in the coating as is typical with osmotic pump systems.

Cellulose acetate occurs as a white to off-white powder, free-flowing pellets, or flakes. It is tasteless and odourless in nature. The solubility of cellulose acetate is greatly influenced by the level of acetyl groups present. In general, cellulose acetates are soluble in acetone–water blends of varying ratios, dichloromethane–ethanol blends, dimethyl formamide, and dioxane. The cellulose acetates of higher acetyl level are generally more limited in solvent choice than are the lower-acetyl materials. Cellulose acetate is compatible with the following plasticizers: diethyl phthalate, polyethylene glycol, triacetin, and triethyl citrate.

In this formulation Cellulose acetate 398-10 was used with acetyl content of 39.8%.

Sorbitol occurs as an odorless, white or almost colorless, crystalline, hygroscopic powder with density 0.448 g/cm$^3$. It is used as a diluent in tablet formulations. Sorbitol is a very hygroscopic powder and relative humidities greater than 60% at 25°C. Sorbitol also has been used as a plasticizer in film formulations. Sorbitol is soluble in water and insoluble in ether and slightly soluble in ethanol.

Plasticizers are used in polymeric coating dispersions to optimize properties of the film such as permeability, hydrophobicity, adhesiveness, flexibility and brittleness. Three commonly used plasticizer types are polyethylene glycols (PEGs), fixed oils (e.g.: Castor oil, Oleic acid) and organic esters (e.g.: Triacetin, Tributyl citrate). Plasticizers are known to affect the Tg of the polymer. Some plasticizers such as glycerin or PEG 400 are water soluble while others are not. PEG 400 (Plastisizer) Polyethylene glycols are stable, hydrophilic substances The presence of polyethylene glycols in film coats, especially of liquid grades, tends to increase their water permeability. Polyethylene glycols are useful as plasticizers pharmaceutical products to avoid rupture of the coating film.

Dibutyl Sebacate which is is a clear, colorless, oily liquid used in oral pharmaceutical formulations as a plasticizer soluble in ethanol (95%), isopropanol, and mineral oil; practically insoluble in water.

**Solvent Selection**

Combination of Acetone and water in different proportions were tried to have coating solution with cellulose Acetate CA 398-10, D-Sorbitol and Dibutyl Secabate. So, acetone and water were selected as final solvents.

**Solid content**

This parameter has immense impact on uniformity of coating. 4% solid content was confirmed through literature and trial of coating was conducted on dummy tablet to confirm the same.

**4.5.7.1 Optimisation of level of pore former in the coating membranes.**

To study the effect of increasing level of pore former sorbitol, core tablets were coated with varying coating composition 17, 27, 37 and 47% (w/w) of D - sorbitol. For this study lactose and NaCl in equal proportion were taken as optimised before. And coating thickness (% weight gain) was kept constant at 5 %.

| Coating Components | | Formulation Code | | | |
|---|---|---|---|---|---|
| | | Ratio of Polymer, pore former and plasticizer in solid | | | |
| | | BT1 | BT2 | BT3 | BT4 |
| Total Solids (4 %W/W) | CA | 40 % | 50 % | 60 % | 70 % |
| | D –Sorbitol | 47 % | 37 % | 27 % | 17 % |
| | PEG 400 | 13 % | 13 % | 13 % | 13 % |
| Acetone | | 86 % w/w | | | |
| Water | | 10 % w/w | | | |
| Solid | | 4 % w/w | | | |

**Table 11 - 4 Composition of coating for optimisation of coating solution composition**

Sorbitol being soluble in nature, on contact with dissolution medium dissolves immediately and forms channels in the coating through which drug can come out though simple diffusion. It was found that pore-former ratio has direct impact on drug release and level of pore former increases simultaneously (Figure 4-4). The drug release increases with the increase in the concentration of the pore former. At levels up to 27% and 37% (w/w) of pore former, numbers of pores were not sufficient to contribute to significant drug release. On the other hand, membrane that contained 47% (w/w) of pore former; the release profile was faster since it became highly porous after coming in contact with aqueous media. On further increasing the pore-forming agent the coating membrane lost its release retardant properties. Considering that 37% of pore former ( D – sorbitol ) was considered for further optimisation of formulation.

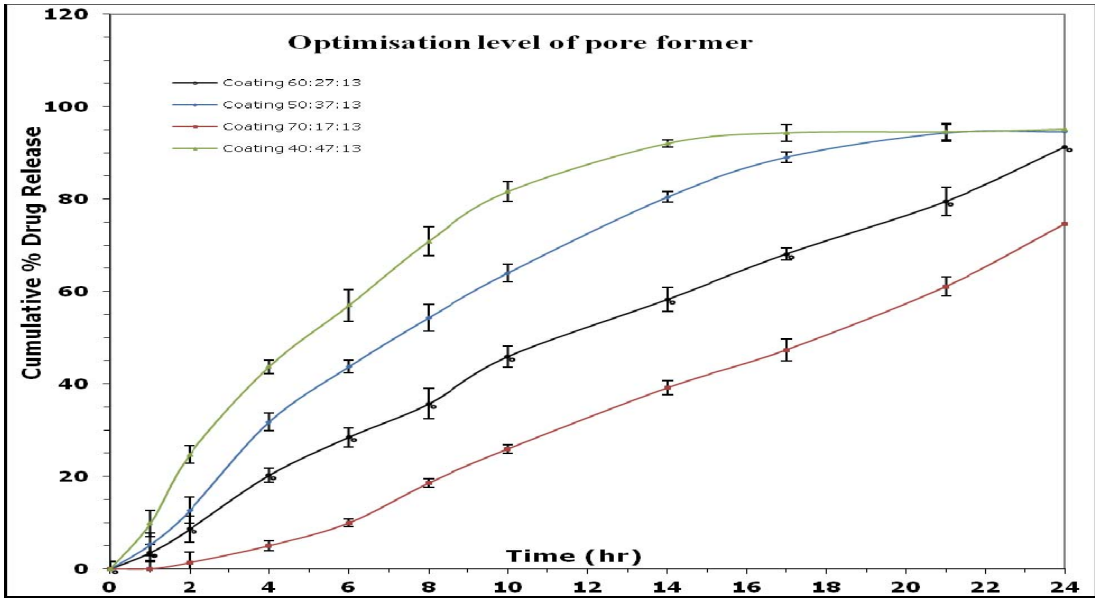



**Figure 4 - 4 Drug release from various coating compositions**

**4.5.7.2 Optimisation of coating thickness (Weight gain)**

In many cases coating has barrier properties and coating in-process is verified by weight gained by the system in stipulated time period. Because of barrier properties coating thickness will have direct impact on drug release. To study the effect of weight gain during coating on drug release, core tablets containing equal proportions of Lactose and Sodium chloride and were coated with coating solution containing 37 % w/w of pore forming agent to achieve a weight gain of 3, 6, 9, 12, 15 % w/w of the total solid contents of coating. The *in vitro* release was shown in figure 5-4. It was observed that drug release decreases with an increase in weight gain (Coating Thickness) of the membrane. 3 and 6 % of coating shows higher variations (Higher SD) where as 9, 12 and 15 % don't show such variations.

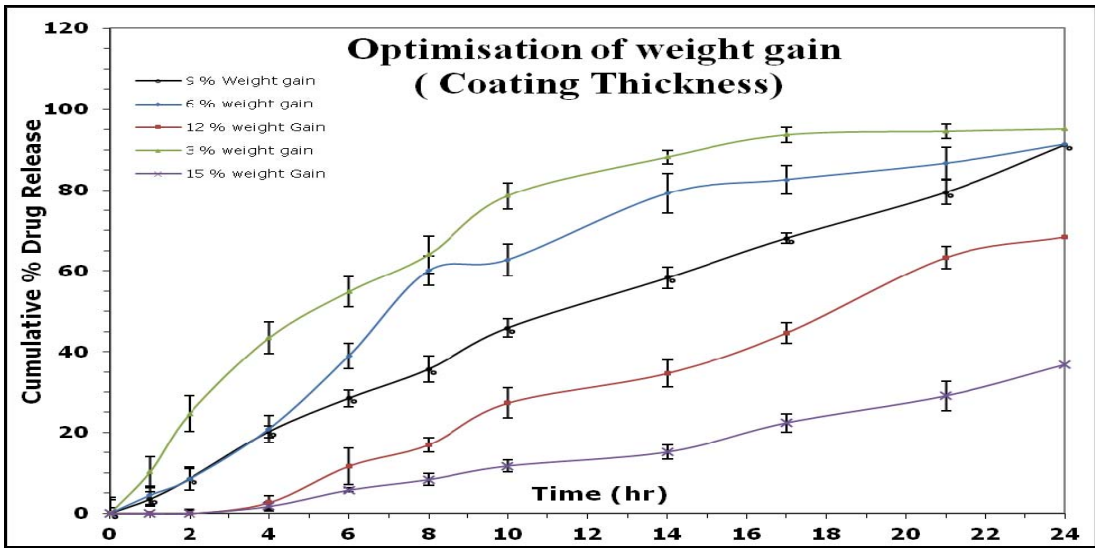



**Figure 5 - 4 Drug release from variable coating thickness**

Thickness of cross section of exhaused shell after dissolution was measured in SEM analysis which shows that optimised formulation has coating thickness of 100 $\pm$ 10 µm.

4.5.7.3 Optimised Coating Formula & Preparation method

Table 12 – 4 shows optimised coating composition based on previous experimentation.

| **Coating Formula** | |
|---|---|
| Cellulose Acetate | 50 % w/w |
| D -Sorbitol | 37 % w/w |
| PEG - 400 | 13 % w/w |
| **Ratio of Acetone : Water : Solid** | |
| Acetone | 86.00 % w/w |
| Water | 10.00 % w/w |
| Solid | 4.00 % w/w |

**Table 12 - 4 Optimised coating composition**

**Coating solution preparations:**

Step 1: Cellulose acetate CA 398-10 was dissolved in Acetone.

Step 2: PEG 400 dissolved in 25% quantity of water.

Step 3: D-solrbitol dissolved in 25% quantity of water.

Step 4: PEG 400 solution was added in D-Sorbitol Solution slowly. Make clear solution by addition of more 25% of water.

Step 3: Very slowly add solution received in Step-4 to solution received in Step 1 drop by drop. Add remaining quantity of water. Do not add further water if precipitates remains for fore then 5 Sec.

| Parameter | Value |
|---|---|
| Solvent | Acetone : Water ( 86 : 10) |
| Solids content (%w/w) | 4 % |
| Weight gain (%) | 6 % |

**Table 13 - 4 Optimised Coating solution parameters**

**Coating Machine Parameters**

| Parameter | Value | Justification |
|---|---|---|
| Fluid nozzle (mm) | 1.5 | Fixed Machine Parameter |
| Spray pan size (Inch) | 12 " | Fixed Machine Parameter |
| Baffles (Nos.) | 6 | Fixed Machine Parameter |
| Inlet air CFM | 28-32 | Shall be lower than Outlet |
| Outlet air CFM | 42-46 | Shall be higher than Inlet to carry solvent outside. |
| Inlet air temperature (°C) | 50 - 55 °C | Selected considering solvent to be evaporated |
| Out let air temperature (°C) | 45-50 °C | Output |
| Pre-warm tablet bed (°C) | 40-43 °C | Curing |
| Tablet surface bed temp (°C) | 39-42 °C | Curing effect Result |
| Atomizing air pressure ( $kg/cm^2$) | 3 -4 $kg/cm^2$ | Studied on dummy considering Broadness of the spray from the spray gun |

**Table 14 - 4 Coating machine parameters**

**Gun-to-bed distance (Inches)**: Furthest possible Gun-to-bed distance was used on reducing this distance the spray pattern was observed getting narrow.

**Spray Rate / Paristaltic Pump RPM:** Least achievable paristaltic pump speed was 6 RPM at which the spray was pulsative and Very high speed i.e. 16 RPM was resulting in very high spray rate. On 10 to 12 RPM paristaltic pump speed was optimum to have uniform coating.

**Perforated Coating pan Speed:** Through coating trials on dummy tablet Perforated Coating pan speed was optimised to 3.8 to 4.2 RPM.

| Coating Parameters | |
|---|---|
| Gun-to-bed distance (inches) | 3.5 ± 0.5 |
| Spray rate (g/min) | 4.0 ± 0.5 g\min |
| Pan speed (rpm) | 4.0 ± 0.2 RPM |
| Peristaltic Pump RPM | 11 ± 1 RPM |

**Table 15 - 4 Coating Variables**

**Tablet Surface bed Temperature:**

An acceptable product temperature range was identified. In the studied range, drug-layered beads with consistent quality were produced. Spray drying and agglomeration were minimized. Product temperature is a scale-independent parameter and can be applied to other scales. The risk of product temperature to impact the assay of the drug-layered beads is low.

**Air volume**

Air volume range was identified and an optimal fluidization pattern was achieved. In the studied range, drug-layered beads with consistent quality were produced. Air volume is a scale-dependent parameter.

**Spray rate**

Spray rate is critical process parameter. Spray rate range 3.5 to 4.5 g/min was studied for it's impact on drug release, coating with consistent quality was produced at the 600gm scale. Spray rate is a scale dependent parameter. The spray rate per nozzle shall be kept the same if processing equipment change from an 12″ perforated coating pan. The total spray rate can be increased to any fold by multiplying spray guns.

**Atomization air pressure**

Atomization air pressure was identified as critical process parameter affecting coating quality and so drug release critical quality attribute. The range of Atomization air pressure studied for consistent coating quality at the 600 gm scale. Attrition was minimized. Atomization air pressure

is an equipment-dependent parameter. As this is a scale-out process and each of the three nozzles used in the 12″ solace coater shall be kept constant.

Table 13-4, 14-4 and 15-4 shows coating parameters and machine parameters as discussed are critical process parameters for coating to achieve quality target product profile (QTTP).

**4.6 Results:**

The results of characterization at pre-compression ( granules) stage for Assay, Bulk Uniformity (BU), Water content(WC), Loss On Drying ( LOD), Bulk Density (BD) & Tap Density(TD) are summarized in table 16-4.

The results of characterization after compression ( Core Tablet) for Description, Average Weight (mg), Assay (%), Water Content, Hardness (kg/cm2), Friability (% ), Thickness (mm), Diameter (mm), Uniformity of content are mentioned in table 17-4.

The results of characterization after coating (coated Tablet)all above parameters and dissolution are mentioned in table 18-4.

**4.6.1 Pre-compression characterization:**

| Parameter | Limits | Result |
|---|---|---|
| Assay | 95.00 to 105.00 % | 101.23 |
| Bulk Uniformity | Minimum 90 % | Minimum 97 % |
| | Maximum 110 % | Maximum 107 % |
| | Mean 95 % to 105 % | Mean 102.97 % |
| | RSD 5 % | RSD 2.71 % |
| Water Content | NMT 5% | 3.0 |
| LOD | NMT 3% | 1.6 % |
| Bulk Density | 0.40 - 0.50 gm/ml | 0.44 gm/ml |
| Tapped Density | 0.55 - 0.65 gm/ml | 0.61 gm/ml |

**Table 16 - 4 Results of analysis at precompression stage**

### *4.6.2 Characterization of Core Tablets*

| Parameters | Limit | Result |
|---|---|---|
| **Observed in stability** | | |
| Description | White colored, circular, biconvex, uncoated tablet. plain on both side | Complies |
| Average Weight (mg) | Target – 230 mg<br>230 ± 3% | 233.60 mg |
| Assay (%) | 95 to 105 | 100.26 |
| Water Content | NMT 5% | 2.9 |
| **Not observed in stability** | | |
| Hardness ($kg/cm^2$) | 4 - 6 | 5 |
| Friability (% ) | NMT 0.10 | 00.01 |
| Thickness (mm) | 3.10 – 3.30 | 3.18 mm |
| Diameter (mm) | 9.0 | 9.00 mm |
| Uniformity of content | Minimum : 90.00 % | Minimum : 100.24 |
| | Maximum : 110.00 % | Maximum : 102.03 |
| | Average : 95 to 105 % | Average : 101.55 |
| | % RSD NMT 5% | % RSD : 3.02 |

**Table 17 - 4 Results of analysis of croe tablet**

### *4.6.3 Characterization of Coated CPOP:*

| Parameters | Limit | Result |
|---|---|---|
| **Observed in stability** | | |
| Description | White colored, circular, biconvex, coated tablet. plain on both side | Complies |
| Average Weight (mg) | Target – 245 mg<br>245 ± 3% | 242.60 mg |
| Assay (%) | 95 to 105 | 101.62 |
| Water Content | NMT 5% | 2.9 % |

| | | | |
|---|---|---|---|
| % Drug Dissolved | 2 Hr | NMT 15% | 9.1 % |
| | 4 hr | 10-30% | 20.3% |
| | 8 hr | 30 to 55 % | 36.2 % |
| | 14 hr | 50 to 80 % | 58.8% |
| | 24 hr | NLT 80 | 91.3 % |
| **Not observed in stability** | | | |
| Hardness (kg/cm$^2$) | | 5-8 | 7 |
| Friability (% ) | | NMT 0.10 | 00.00 |
| Thickness (mm) | | 3.40 – 3.60 | 3.52 mm |
| Diameter (mm) | | 9.2 | 9.20 mm |
| Uniformity of content | | Minimum : 90.00 % | Minimum : 99.72 |
| | | Maximum : 110.00 % | Maximum : 101.98 |
| | | Average : 95 to 105 % | Average : 100.97 |
| | | % RSD NMT 5% | 2.86 |

**Table 18 - 4 Finished product analysis results**

Developed formulation meets the predefined quality target product profile at can be formulated repetitively through effective control of critical process parameters however; large scale production may require some changes in the process depending on scale.

#### 4.6.3.1 Thickness of exhausted Coating membrane through SEM analysis:

Exhausted shell after dissolution was broken and cross section of that was observed under SEM. Thickness of cross section of exhausted shell after dissolution was measured in SEM analysis (figure 6-4)which shows that optimised formulation has coating thickness of 100 $\pm$ 10 µm.

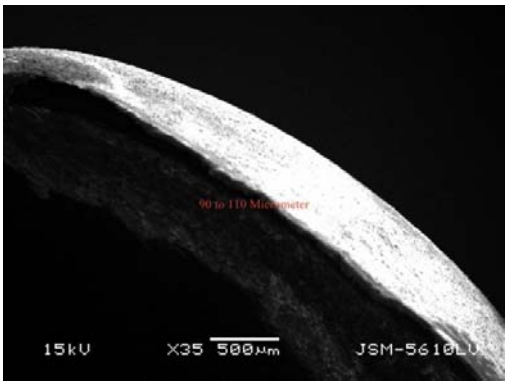



**Figure 6 - 4 SEM Image of cross sectionof exhausted shell after dissolution**

#### 4.6.3.2 Effect of pH on drug release

Optimised formulation was subjected to drug release at different pH i.e. PBS 6.8 (SIF), 0.1 N HCL (SGF), acetate buffer pH 4.5, water and combination of SGC for 2 hr followed by SIF. The resulted were plotted against time the figure 7-4 shows graph plotted and table 19-4 shows summarized results.

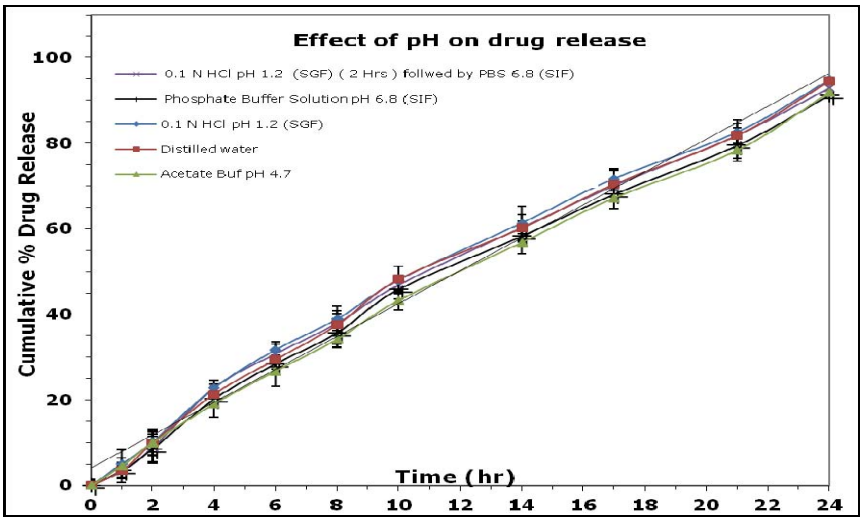



**Figure 7 - 4 Drug release - effect of pH**

| Effect of pH on drug release | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time (hr) | PBS pH 6.8 (SIF) | SD | 0.1 N HCl pH 1.2 (SGF) | SD | Acetate Buf pH 4.7 | SD | SGF 2 Hrs + SIF | SD | DW | SD |
| 1 | 3.51 | 1.46 | 5.04 | 1.38 | 4.57 | 1.19 | 3.33 | 1.33 | 3.47 | 1.46 |
| 2 | 8.57 | 1.77 | 10.21 | 1.47 | 10.01 | 3.79 | 8.83 | 1.30 | 9.96 | 1.89 |
| 4 | 20.23 | 2.89 | 22.84 | 3.07 | 19.09 | 2.07 | 22.84 | 3.63 | 21.42 | 2.89 |
| 6 | 28.48 | 1.47 | 31.73 | 1.79 | 26.78 | 3.06 | 30.79 | 1.85 | 29.49 | 2.42 |
| 8 | 35.69 | 2.06 | 38.90 | 1.89 | 34.18 | 3.60 | 37.89 | 2.33 | 37.54 | 2.34 |
| 10 | 45.91 | 3.29 | 48.04 | 1.96 | 43.29 | 2.00 | 46.89 | 2.34 | 48.23 | 4.40 |
| 14 | 58.30 | 2.28 | 61.35 | 3.17 | 56.81 | 2.24 | 60.26 | 2.00 | 60.17 | 2.99 |
| 17 | 68.11 | 2.57 | 71.69 | 3.97 | 67.21 | 2.57 | 70.03 | 1.47 | 70.46 | 3.16 |
| 21 | 79.50 | 1.34 | 82.55 | 2.09 | 78.33 | 2.58 | 81.80 | 1.10 | 81.82 | 3.65 |
| 24 | 91.24 | 3.09 | 94.76 | 2.83 | 91.87 | 2.58 | 92.85 | 2.72 | 94.51 | 1.66 |

**Table 19 - 4 Drug release data effect of pH**

**Discussion**

Osmotic drug delivery is considered to be a delivery system which gives constant drug release irrespective of pH of the release media. The drug release from optimised formulation was confirmed in various release media i.e. PBS pH 6.8 (SIF), 0.1 N HCl pH 1.2 (SGF), Acetate Buf pH 4.7, SGF 2 Hrs + SIF and distilled water.

It is clearly evident from (table) that the release of BT from CPOP is independent of the pH of the medium.

The *f*1 and *f*2 values were found to be *f*1 5.09 and *f*2 76.02 (between PBS pH 6.8 (SIF) and 0.1 N HCl pH 1.2), *f*1 8.09 and *f*2 69.86 (between 0.1 N HCl pH 1.2 (SGF) and Acetate Buf pH 4.7), *f*1 6.19 and *f*2 74.72 (between Acetate Buf pH 4.7 and SGF 2 Hr followed by SIF), *f*1 1.72 and *f*2 92.55 (between SGF 2 Hr followed by SIF and distilled water) and *f*1 4.01and *f*2 82.91 (between distilled water and PBS pH 6.8)

Hence, it can be expected that the release from the developed formulation will be independent of the pH of the absorption site.

#### 4.6.3.3 Effect of Agitation intensity

Optimised formulation was subjected to drug release at different rotational speed of 50, 100 and 150 RPM under PBS 6.8 (SIF) combination of SGC for 2 hr followed by SIF. The resulted were plotted against time the figure 8-4 shows graph plotted and table 20-4 shows summarized results.

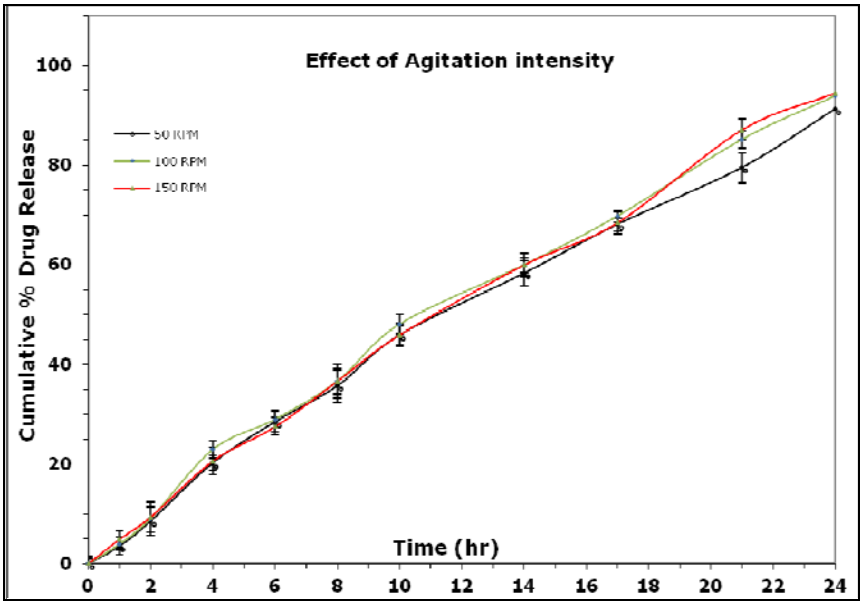



**Figure 8 - 4 Drug release - effect of agitation intensity**

| Effect of Agitation intensity | | | | | | |
|---|---|---|---|---|---|---|
| Time (hr) | 50 RPM | SD | 100 RPM | SD | 150 RPM | SD |
| 1 | 3.51 | 1.46 | 3.96 | 0.87 | 4.89 | 1.01 |
| 2 | 8.57 | 1.77 | 9.07 | 1.28 | 9.35 | 1.71 |
| 4 | 20.23 | 2.89 | 22.91 | 3.37 | 20.60 | 2.95 |
| 6 | 28.48 | 1.47 | 28.95 | 1.67 | 27.48 | 2.64 |
| 8 | 35.69 | 2.06 | 36.60 | 1.71 | 36.60 | 1.71 |
| 10 | 45.91 | 3.29 | 48.06 | 2.65 | 45.91 | 3.29 |
| 14 | 58.30 | 2.28 | 59.95 | 1.99 | 59.95 | 1.99 |
| 17 | 68.11 | 2.57 | 69.73 | 1.38 | 68.44 | 2.38 |
| 21 | 79.50 | 1.34 | 85.08 | 1.31 | 87.08 | 2.16 |
| 24 | 91.24 | 3.09 | 93.81 | 1.77 | 94.48 | 2.19 |

**Table 20 - 4 Drug release data - effect of agitation intensity**

**Discussion**

Osmotic drug delivery is considered to be a delivery system which give constant drug release irrespective of agitation intensity. The drug release from optimised formulation was confirmed at various agitation speed i.e. 50, 100, and 150 RPM.

It is clearly evident from (table) that the release of BT from CPOP is independent of the agitation intensity. The $f1$ and $f2$ values were found to be $f1$ 4.05 and 79.47 (between 50 RPM and 100 RPM), $f1$ 2.44 and 88.38 (between 100 RPM and 150 RPM) for $f1$ 3.92 and $f2$ 76.75 (between 150 RPM and 50 RPM).

Hence, it can be expected that the release from the developed formulation will be independent of the agitation conditions of the absorption site.

#### 4.6.3.4 Effect of dissolution volume

Optimised formulation was subjected to drug release at 50 RPM in combination of SGC for 2 hr followed by SIF in different dissolution volume. The resulted were plotted against time the figure 9-4 shows graph plotted and table 21-4 shows summarized results.

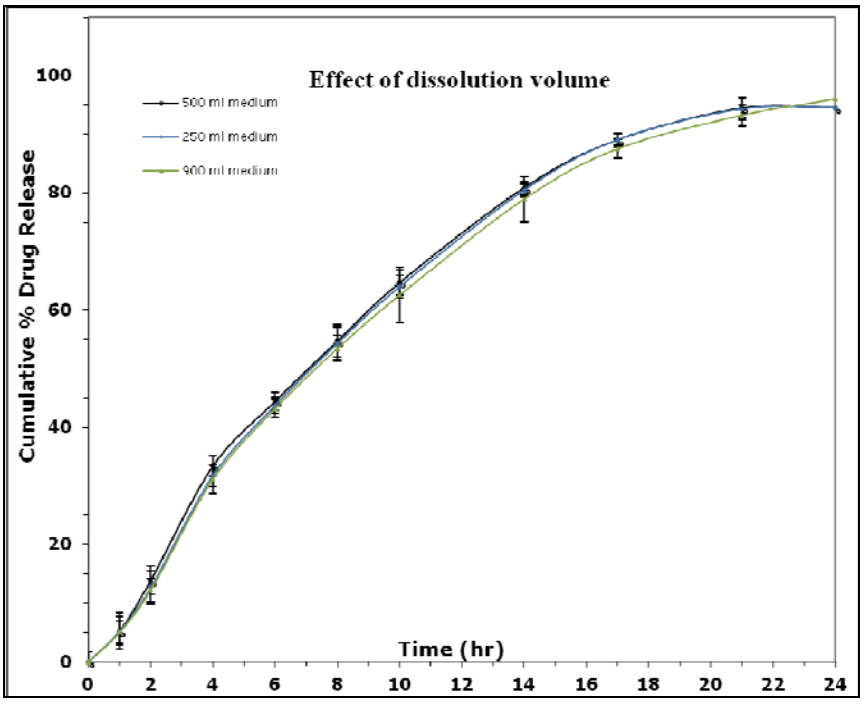



**Figure 9 - 4 Drug release effect of dissolution volume.**

| Time (hr) | 500 ml | SD | 250 ml | SD | 900 ml | SD |
|---|---|---|---|---|---|---|
| 1 | 5.23 | 1.65 | 5.23 | 1.65 | 5.03 | 1.58 |
| 2 | 13.89 | 3.12 | 12.64 | 2.42 | 12.22 | 2.00 |
| 4 | 33.40 | 2.45 | 31.76 | 2.81 | 31.14 | 2.07 |
| 6 | 44.44 | 1.66 | 43.72 | 1.88 | 43.20 | 2.43 |
| 8 | 54.71 | 1.52 | 54.29 | 1.37 | 53.43 | 1.55 |
| 10 | 64.68 | 2.83 | 63.97 | 2.85 | 62.56 | 2.20 |
| 14 | 80.88 | 2.21 | 80.42 | 1.95 | 78.94 | 4.74 |
| 17 | 89.05 | 1.13 | 89.03 | 1.13 | 87.51 | 3.85 |
| 21 | 94.49 | 0.96 | 94.29 | 1.17 | 93.23 | 1.68 |
| 24 | 94.55 | 1.82 | 94.55 | 1.82 | 96.00 | 1.82 |

**Table 21 - 4 Drug release data effect of dissolution volume**

**Discussion :** Osmotic drug delivery is considered to be a delivery system which give constant drug release irrespective of volume if sink condition is maintained. The drug release from optimised formulation was confirmed at different release volume i.e. 250, 500, and 900 ml.

It is clearly evident from (table) that the release of BT from CPOP is independent of the dissolution volume. The *f*1 and *f*2 values were found to be *f*1 0.94 and 95.10 (between 250 ml and 500 ml), *f*1 2.60 and 86.27 (between 900 ml and 500 ml) and *f*1 1.67 and *f*2 91.78 (between 900 ml and 250 ml).

Hence, it can be expected that the release from the developed formulation will be independent of the volume at the absorption site.

#### 4.6.3.4 Dose Dumping Study

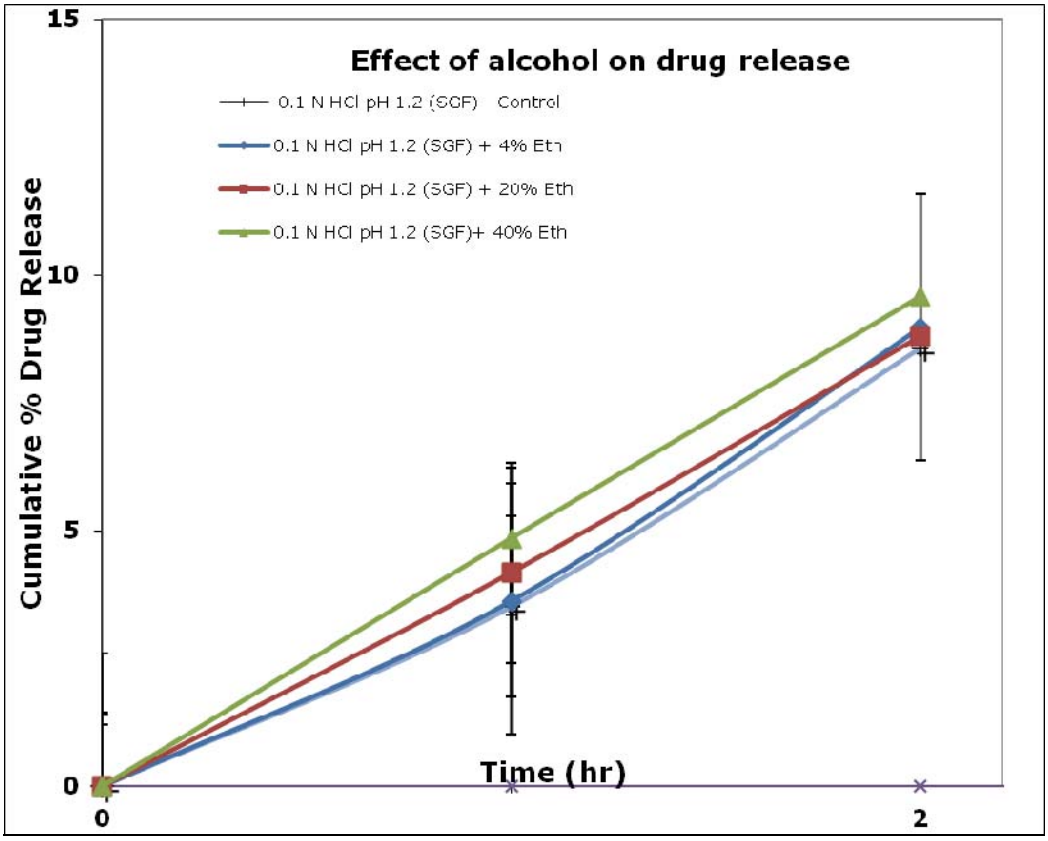



**Figure 10 - 4 Dose dumping study**

**Discussion**

In order to study the effect of alcohol on drug release or to verify weather alcohol leads to dose dumping 4 % V/V, 20 % V/V and 40 %V/V alcohol was added in the release media and drug release was verified against control ( without alcohol).

Dose dumping is most commonly seen in drugs taken by mouth and digested in the gastrointestinal tract. Around the same time patients take their medication, they can also ingest other substances like fatty meals or alcohol that increase drug delivery. The substances may act on the drug product to speed up drug release, or they may stimulate the body's absorptive surfaces to increase the rate of drug uptake. Developed formulation was subjected to ethanol induced dose dumping study to emulate a “worst case” scenario. It was observed that no major

impact of alcohol on drug release. Hence, it is expected that accidental co-administration of alcohol will not lead to any dose dumping.

***Curve fitting analysis***

In order to describe the kinetics of drug release from controlled release preparations various mathematical equations have been proposed in the literature. Release data obtained was applied to different release models in order to establish the drug release mechanism and kinetics. Best goodness of fit test (R2) was taken as criteria for selecting the most appropriate model. The values are tabulated below.

| **Model** | Zero Order | First Order | Higuchi Model | Peppas |
|---|---|---|---|---|
| **Calculated $R^2$** | 0.9876 | 0.7400 | 0.9623 | 0.9834 |

**Table 22 - 4 Calculated R2 Values for Developed formulation for differnt models**

Discussion

Calculated $R^2$ for Zero order of drug release is nearest to 1, and osmotic pumps shows zero order of drug release. It can be concluded that the drug release from developed controlled porosity osmotic pump gives constant drug release.

**4.6.3.5 Surface analysis of coating before dissolution and exhausted shell after dissolution use SEM**

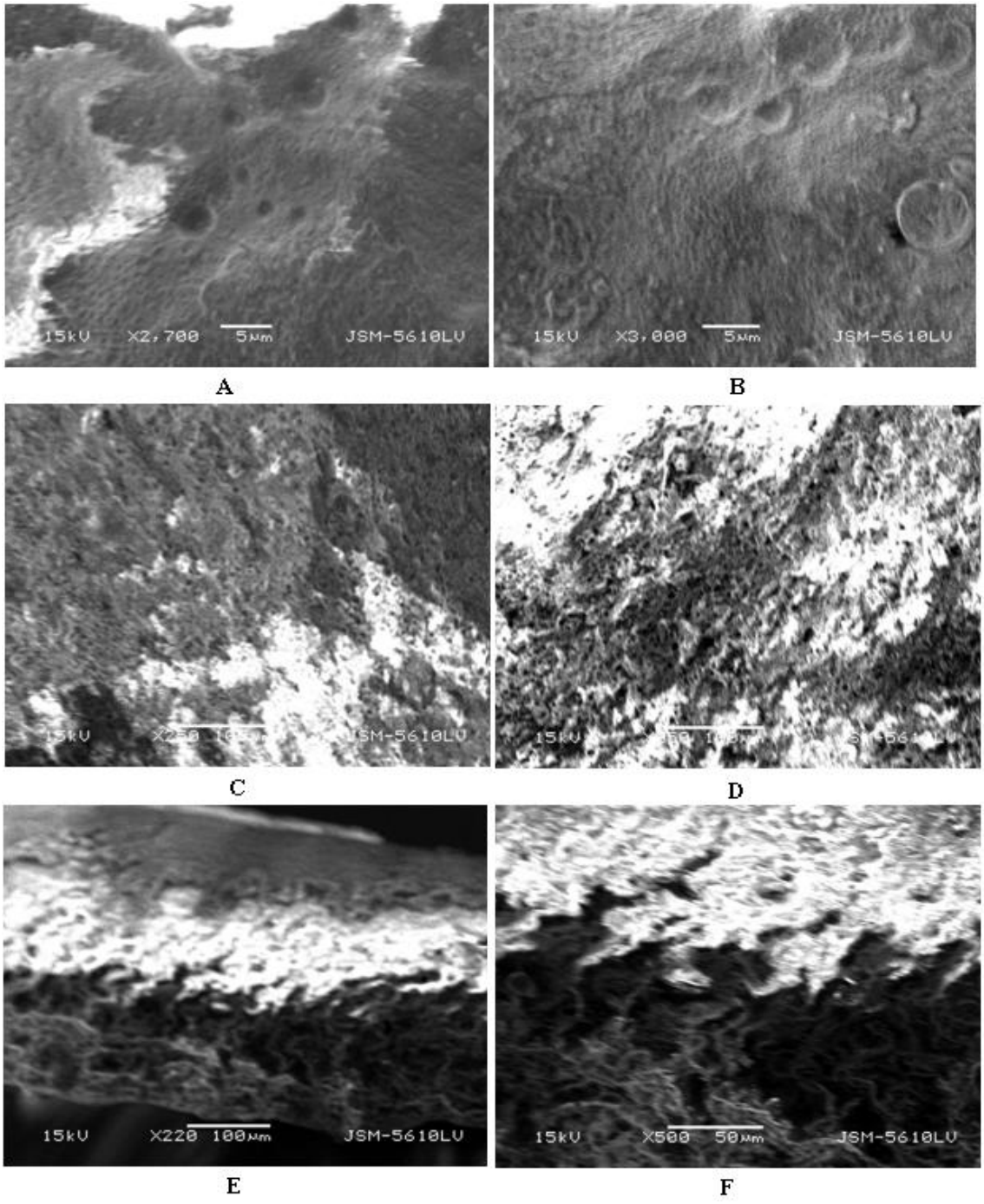



**Figure 11 - 4 SEM images of Surface analysis of coating membrene before dissolution and exhausted shell after dissolution:**

Surface of coated tablets before and after dissolution studies, was observed using scanning electron microscope (SEM). The morphology of the exhausted osmotic pump shell was analyzed using SEM (Surface Electron Microscopy).

**Discussion :** The drug release from the CPOP is anticipated due to formation of channels which forms on solubility of the pore former i.e Sorbitol in this membrane rendering the coating semi permeable allowing ingress of dissolution media followed by release of drug due to osmotic pressure. Formation of channels was confirmed by observing the coating membrane before and after the dissolution under surface electron microscopy. First two pictures Figure 11- 4 A (captured at 15kv X 2700) & Figure 11- 4 C (captured at 15kv X 250) shows no channel formation before dissolution. Second two pictures Figure 11- 4 B (captured at 15kv X 3000) & Figure 11- 4 D (captured at 15kv X 250) shows formation of pores in the coating membrane top view after dissolution. Third two pictures Figure 11- 4 E (captured at 15kv X 220) & Figure 11- 4 F (captured at 15kv X 500) shows pores are actually channels in the coating membrane side view of the coating membrane after dissolution.

Hence, it is confirmed that drug release in the developed formulation is through pores and channels formed due to pore former in the coating composition.

#### 4.6.3.6 Observation of before and after dissolution


**Figure 12 - 4 Physical observation of CPOP before and after dissoltion**

Discussion : Physical change in the dimension of the tablet was observed. It was observed that it bulge after dissolution. Diameter of the tablet after & before dissolution was 12 mm and 9 mm respectively. The shape of enlarged table after dissolution changed to oval which was hollow filled with liquid inside and intact coating membrane see figure 12-4.

Hence, it was confirming the drug release through dissolution and movement through coating membrane in solution using osmotic pressure as driving force.

**Drug release mechanism**

To ensure the major mechanism of drug release, release studies of the optimized formulation were conducted in media containing saturated solution of osmotically active solute. Sodium chloride was added in dissolution medium. Saturated solution of sodium chloride produces osmotic pressure in the range of 356 atm. The pH of the medium was adjusted to 6.8 ± 0.05. Release studies were carried out in 250 ml of media using USP II dissolution test apparatus (50 rpm).

## 4.7 Result of Development studies

**Optimised Dosages Form:**

Butorphanol Tartrate Controlled porosity Osmotic pump is developed as white coloured, circular, biconvex, coated tablet dosage form for oral administration.

**Formulation Details:**

Controlled porosity Osmotic Pump is white colored, circular, biconvex coated tablet plain on both sides containing 10mg of Butorphanol Tartrate and following are inactive ingredients Sodium Chloride, Lactose Monohydrate, Microcrystalline Cellulose, Polyvinyl Pyrrolidone, Magnesium Stearate, Silicon Dioxide, PEG, D – Sorbitol etc and Isopropyl Alcohol, water and Acetone as solvent which shall not be part of final product.

**Packing Profile:**

Dosage form to be packed in HDPE Bottle pack of 100s.

### 4.7.1 Components of the Drug Product:

The quantitative composition ( per tablet and % W/W), compandial status and function of each component used in the developed drug product is provided below in table 23-4.

| Formula ingredients | Specification | Function(s) | Quantity (mg/tablet) | Quantity (% w/w) |
|---|---|---|---|---|
| **Dry Mixing Stage :** | | | | |
| Butorphanol tartrate | USP | API | 10.00 | 4.10 |
| Sodium Chloride | USP NF | Osmogen | 100.00 | 40.99 |
| Lactose Monohydrate* | USP NF | Osmogen | 100.00 | 40.99 |
| PVP K 30 | NF | Binder | 12.00 | 4.92 |

| Formula ingredients | Specification | Function(s) | Quantity (mg/tablet) | Quantity (% w/w) |
|---|---|---|---|---|
| Isopropyl Alcohol** | USP NF | Processing Solvent | Q.s. | Processing Solvent |
| **Total weight of core tablet** | | | **222.00** | **91.00** |
| **Lubrication Stage :** | | | | |
| Mg. Stearate | USP NF | Glidant | 3.00 | 1.23 |
| Talc/Purified Talc | USP NF | Antiadherent | 3.00 | 1.23 |
| Colloidal silicon dioxide | USP NF | Lubricant | 2.00 | 0.82 |
| **Total weight of core tablet** | | | **230.00** | **94.00** |
| **Coating Solution : For 6 % Weight Gain ( Calculated values)** | | | | |
| Cellulose CA398-10 | USP NF | Film Forming Agent | 7.34 | 2.86 |
| PEG 400 | Ph.Eur | Plasticizer | 5.43 | 2.11 |
| D – Sorbirol | IP | Pore Forming Agent | 1.91 | 0.74 |
| Purifies Water ** | IH | Processing Solvent | Q.s. | Processing Solvent |
| Acetone** | IP | Processing Solvent | Q.s. | Processing Solvent |
| **Total weight of coated tablet after coating** | | | **245** | **100.00 %** |

**Table 23 - 4 Optimised compostion after development studies**

* Quantity is compensated depending on the potency of Butorphanol tartarte to maintain tablet weight constant.** Used as processing solvent, does not remain in the final product.

### 4.7.2 Optimised Coating Solution composition

Ratio of solids used in coating solution is Critical Process parameter and it has direct impact on drug release optimsed coating formulation is summarized in table 24-4.

| Ratio of solids used in coating solution | |
|---|---|
| Cellulose Acetate | 50 % w/w |
| D -Sorbitol | 37 % w/w |
| PEG - 400 | 13 % w/w |
| Ratio (Acetone : Water : Solid) | |
| Acetone | 86.00 % w/w |
| Water | 10.00 % w/w |
| Solid | 4.00 % w/w |

**Table 24 - 4 Optimised coating compostion - development studies**

### *4.7.3 Critical Quality Attributes: (CQAs)*

1. Blend Uniformity
2. Uniformity of weight
3. Assay
4. Dissolution
5. Uniformity of Dosage unit
6. Stability

### *4.7.4 Critical Process Parameters (CPPs)*

1. Blending process (Time, Speed, Agitation intensity, Room Temperature ( Humidity).
2. Dry Mixing time (Geometric Mixing and Sieve size)
3. Compression ( Speed, Compression force)
4. Coating (Pan RPM, Broader spray pattern, Inlet and outlet air CFM, Inlet and outlet air temperature)

**4.8 Conclusion:**

Stable 10 mg of butorphanol tartrate extended release COPO formulation is developed which gives drug release independent of agitation intensity, pH and dissolution volume, rendering the drug release through osmotic pressure at constant and is expected to provide desired in-vivo performance.

## References


- Critical review of BUTORPHANOL 34th ECDD 2006/4.1
- Boulton D W, Duncan G F and Vachharajani N N (2002) Validation and application of a sensitive assay for butorphanol in human plasma by high-performance liquid chromatography with tandem mass spectrometry detection. J Chromatogr B Analyt Technol Biomed Life Sci 775:57-62.
- Vachharajani N N, Shyu W C and Barbhaiya R H (1997a) Pharmacokinetic interaction between butorphanol nasal spray and oral metoclopramide in healthy women. *J Clin Pharmacol* **37**:979-985.
- Vachharajani N N, Shyu W C, Greene D S and Barbhaiya R H (1997b) The pharmacokinetics of butorphanol and its metabolites at steady state following nasal administration in humans. *Biopharm Drug Dispos* **18**:191-202.
- Gaver R C, Vasiljev M, Wong H, Monkovic I, Swigor J E, Van Harken D R and Smyth R D (1980) Disposition of parenteral butorphanol in man. *Drug Metabolism Disposition* **8**:230-235.
- Pittman K A, Smyth R D, Losada M, Zighelboim I, Maduska A L and Sunshine A (1980a) Human perinatal distribution of butorphanol. *Am J Obstet Gynecol* **138**:797-800.
- Pittman K A, Smyth R D and Mayol R F (1980b) Serum levels of butorphanol by radioimmunoassay. *J Pharm Sci* **69**:160-163.
- Adis Online: Drugs - Evaluations on New Drugs: PDF Only Volume 16 Issue 6 (1978) "Butorphanol: A Review of its Pharmacological Properties and Therapeutic Efficacy"
- Ricardo Rangel – Guerra, Journal of International medical research( 1981) 9, 120 " An open evaluation of oral butorphanol as long tern therapy in out-patients suffering from moderate to severe chronic pain."
- F.S. Caruso, A.W. Pircio, H. Medissoo, R. D. Smyth, R .J . Pachter. " BUTORPHANOL" Pharmaceutical and Biological properties of drug substance Volume 2 Edited by :Morton E Goldberg. Americal Pharmaceutical Association, Academy of Pharmaceutical Science.

- Michael M Gilbert, Neil P Sanzari, manual Losada, Frank S Caruso. Journal of International medical research( 1978) 6, 14 "Double blind oral analgesic study of Butorphanol in Musculoskeletal pain : A comparison with Codeine and Placebo.
- A.W. Pircio, J.P. Buyniski and L. E. Roebel. "Pharmacological effects of a combination of Butorphanol and Acetaminophen" Archives Int. Pharmacodynamics 235 PP 116 – 123 (1978)
- F.M. GALLOWAY, j. HaDLmKA, M. LOSADA, R.J. NOVECK, AND F.S. CARUSO Canad. Anaesth. Soe. J., vol. 24, no. 1, January 1977 "Comparison Of Analgesia By Intravenous Butorphanol And Meperidine In Patients With Post-Operative Pain"
- Vinita Singh, Manu Pathak and G.P. Singh Indian Journal of Pediatrics, Volume 72—September, 2005 Oral Midazolam and Oral Butorphanol Premedication
- Ricardo Rangel – Guerra, Journal of International medical research( 1981) 9, 120 – 123 " An open evaluation of oral butorphanol as long term therapy in Out- patients suffering from moderate to aevere pain.
- H. C. Chang and L.C.Li Drug Development and Industrial Pharmacy, 26(8), 829–835 (2000) "Sustained-Release Butorphanol Microparticles "
- www.drugs.com( assessed on 17/03/3013)
- Francisco V R, Juan Piscina Casas, Francisco F M. Current Therapeutic Research Vol.12 No.1 July Section 2, 1977 " Human Safety and tolerance study of multi dose oral administration of Butorphanol"
- Chang et. Al. Butorphanol sustained release formulations – Unites States Patent 6197344 B1 March 06, 2011
- Hendeles L, Wubbena P, Weinberger M. Food-induced dose dumping of once-aday theophylline. Lancet. 22: 1471 (1984).
- Guidance for Industry: Food effect guidance Food-Effect Bioavailability and Fed Bioequivalence Studies (Issued 12/2002, Posted 1/30/2003). http://www.fda.gov/cder/guidance/5194fnl.pdf

## 5. Analytical Methods (Lornoxicam)

The analytical methods developed for testing of Lornoxicam in bulk powder and extended release formulation of are employed in this section. Table 1 - 5 and 2 - 5 show list of material and equipment used respectively. Method for Assay, content uniformity, dissolution studies and determination of drug during in-vivo studies are discussed in this section.

| Sr. No. | Material | Source |
|---|---|---|
| 1. | Lornoxicam | Cadila Healthcare Ltd., Moraiya, Ahmedabad, Gujarat, India |
| 2. | Dichloromethane | Merck Limited, Mumbai, India |
| 3. | Methanol | Merck Limited, Mumbai, India |
| 4. | Acetone | Merck Limited, Mumbai, India |
| 5. | Isopropyl alcohol | Merck Limited, Mumbai, India |
| 6. | Glacial acetic acid | S. D. Finechem Limited, Mumbai, India |
| 7. | Hydrochloric acid | S. D. Finechem Limited, Mumbai, India |
| 8. | Potassium dihydrogen phosphate | S. D. Finechem Limited, Mumbai, India |
| 9. | Sodium hydroxide | S. D. Finechem Limited, Mumbai, India |
| 10. | Sodium lauryl sulphate | S. D. Finechem Limited, Mumbai, India |
| 11. | HPLC grade Methanol, Acetonitrile, Acetic acid | S. D. Finechem Limited, Mumbai, India |
| 12. | Ethanol (99.5%V/V) | Baroda Chem. Ind. Ltd., Baroda, India |
| 13. | Water (distilled) | Prepared in laboratory by distillation |

**Table 1 - 5 List of materials**

| Sr. No. | Equipments | Source/Make |
|---|---|---|
| 1. | Digital weighing balance | AG-64, Mettler Toledo, Switzerland |
| 2. | pH meter | Mettler Toledo, Switzerland |
| 3. | Friability tester | EF-2, Electrolab, Mumbai, India |
| 4. | Bath sonicator | DTC 503, Ultra Sonics |
| 5. | Dissolution apparatus | Electrolab, Mumbai, India |
| 6. | HPLC system | LC 20-AT prominence, Shimadzu Corp., Japan |
| 7. | UV-Visible Spectrophotometer | Shimadzu UV-1601, Japan |
| 8. | Calibrated pipettes of 1.0 ml, 5.0 ml and 10.0 ml. | Schott & Corning (India) Ltd., Mumbai |
| 9. | volumetric flasks of 10 ml, 25 ml, 50 ml and 100 ml capacity. | Schott & Corning (India) Ltd., Mumbai |
| 10. | Funnels (i.d. 5.0 cm) | Schott & Corning (India) Ltd., Mumbai |
| 11. | Beakers (250 ml) and other requisite glass wares | Schott & Corning (India) Ltd., Mumbai |
| 12. | Nuclepore Polycarbonate membrane 2 µm 25mm | Whatman, USA |

**Table 2 - 5 List of Equipments**

## 5.1 Preparation of reagents and buffers

### 5.1.1 Preparation of Acetate Buffer pH 4.5

2.99 gm of sodium acetate trihydrate and 200 ml of distilled water was placed in a 1000 ml volumetric flask. 14 ml of acetic acid solution was added and the volume was adjusted with distilled water upto 1000 ml. (USP 30, 2007).

**5.1.2 Preparation of Phosphate Buffer pH 7.5**

Dissolve 6.8 g of monobasic potassium phosphate and 1.6 g of Sodium Hydroxide in 1000 ml of water. Adjust the pH to 7.5 with 2 N Sodium Hydroxide. (USP 30, 2007)

**5.2 Estimation of Lornoxicam**

**5.2.1 Estimation of Lornoxicam in acetate buffer 4.5, Phosphate Buffer 7.5 and PBS 7.5 : Methanol ( 50: 50)**

Lornoxicam shows strong absorbance in UV-Visible region. Hence, the estimation of Lornoxicam was performed by UV-visible spectrophotometry.. For determination of Lornoxicam for assay and content uniformity 50:50 mixture of PBS and Methanol was used and UV spectrophotometric method for estimation of *in-vitro* drug release was developed in acetate buffer 4.5 and Phosphate Buffer 7.5. An analytical method for determination of lornoxicam in plasma during *in-vivo* studies was developed using HPLC.

**5.2.1.1 Preparation of standard stock solutions of Lornoxicam in PBS pH 7.5**

50 mg of Lornoxicam was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of PBS 7.5 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with PBS pH 7.5 to produce 1000 µg per ml of Lornoxicam.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with PBS 7.5 to prepare stock solution of 250 µg per ml of Lornoxicam.

**5.2.1.2 Calibration curve of Lornoxicam in PBS pH 7.5**

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with PBS 7.5 to give final concentrations of 5,10, 15, 20, 25, 30 µg/ml and analyzed by UV spectrophotometry at 278 nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation.

| Concentration (µg/ ml) | Average* | SD | RSD |
|---|---|---|---|
| 5 | 0.0910 | 0.0013 | 0.9428 |
| 10 | 0.2500 | 0.0021 | 0.8556 |
| 15 | 0.4190 | 0.0040 | 0.9633 |
| 20 | 0.5870 | 0.0036 | 0.6193 |
| 25 | 0.7710 | 0.0034 | 0.4381 |
| 30 | 0.9340 | 0.0029 | 0.3954 |

**Table 3 - 5 Calibration for Lornoxicam in PBS 7.5**

Regression equation** Y= 0.034X - 0.0859; Correlation coefficient = 0.9996

*Mean of 3 values

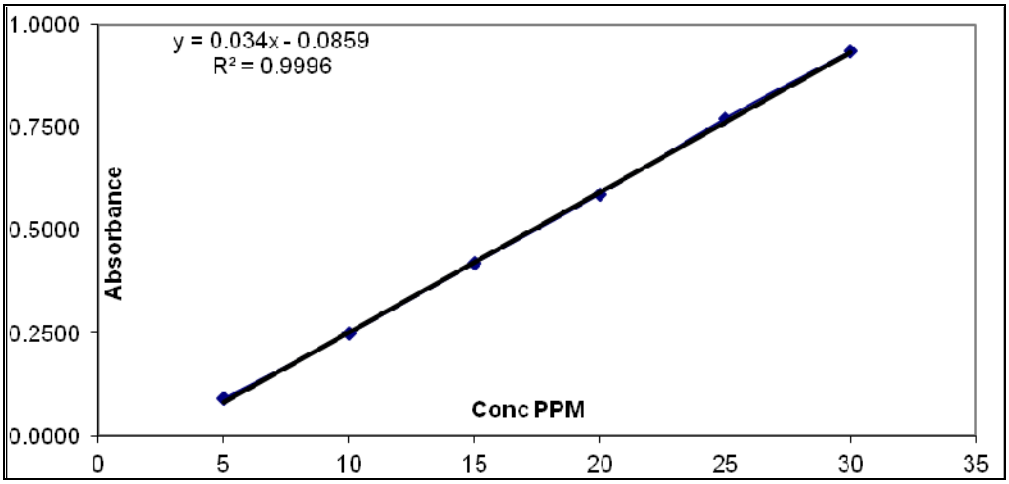



**Figure 1 - 5 Regressed calibration curve for estimation of Lornoxicam in PBS 7.5**

Figure 1-5 shows Regressed calibration curve for estimation of Lornoxicam in PBS 7.5, values as mentioned in table 4 - 5.

**Accuracy and repeatability**

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples by the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable

concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated.

Accuracy of method for analysis of Lornoxiam in PBS 7.5 was show in 5 -5.

In order to determine the accuracy of the developed method, known amounts of Lornoxicam (5 µg/mL, 10 µg/mL and 30 µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (5, 10, 30 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 5 -5.

| Conc. of LOR (µg/ml) Std. | AVG Recovery (µg/ml) | % Recovery | SD | RME | Confidence | RSD Intra Day | RSD Inter Day |
|---|---|---|---|---|---|---|---|
| 5 | 5.1428 | 102.9 | 0.0607 | 0.0272 | 5.1428 + 0.1693 | 1.18 | 1.57 |
| 10 | 9.8828 | 98.8 | 0.2898 | 0.1296 | 9.8828 + 0.8079 | 2.93 | 1.76 |
| 30 | 29.9802 | 99.9 | 0.1155 | 0.0516 | 19.9802 + 0.3219 | 0.39 | 0.34 |

**Table 4- 5 Evaluation of accuracy and repeatability of the estimation method of LOR in PBS pH 7.5**

** At 95% Confidence level; $t_{tab}$ = 3.18 for 4 degrees of freedom (n=5)*

### 5.2.1.3 Preparation of standard stock solutions of Lornoxicam in Acetate Buffer pH 4.5

50 mg of Lornoxicam was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of Acetate Buffer pH 4.5 was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with Acetate Buffer pH 4.5 to produce 1000 µg per ml of Lornoxicam.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with Acetate Buffer pH 4.5 to prepare stock solution of 250 µg per ml of Lornoxicam.

### 5.2.1.4 Calibration curve of Lornoxicam in Acetate Buffer pH 4.5

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with Acetate Buffer pH 4.5 to

give final concentrations of 10, 20, 40, 50,60 µg/ml and analyzed by UV spectrophotometry at 282 nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. Figure 2-5 shows Regressed calibration curve for estimation of Lornoxicam in acetate buffer pH 4.5, values as mentioned in table 6 - 5.

| Concentration (µg/ ml) | Average* | SD | RSD |
|---|---|---|---|
| 10 | 0.224 | 0.0030 | 1.3367 |
| 20 | 0.370 | 0.0026 | 0.7027 |
| 40 | 0.680 | 0.0022 | 0.3235 |
| 50 | 0.825 | 0.0034 | 0.3312 |
| 60 | 0.965 | 0.0031 | 0.3253 |

**Table 5 - 5 Calibration for Lornoxicam in Acetate Buffer pH 4.5**

Regression equation** Y= 0.0149X + 0.0752; Correlation coefficient = 0.9997

*Mean of 3 values

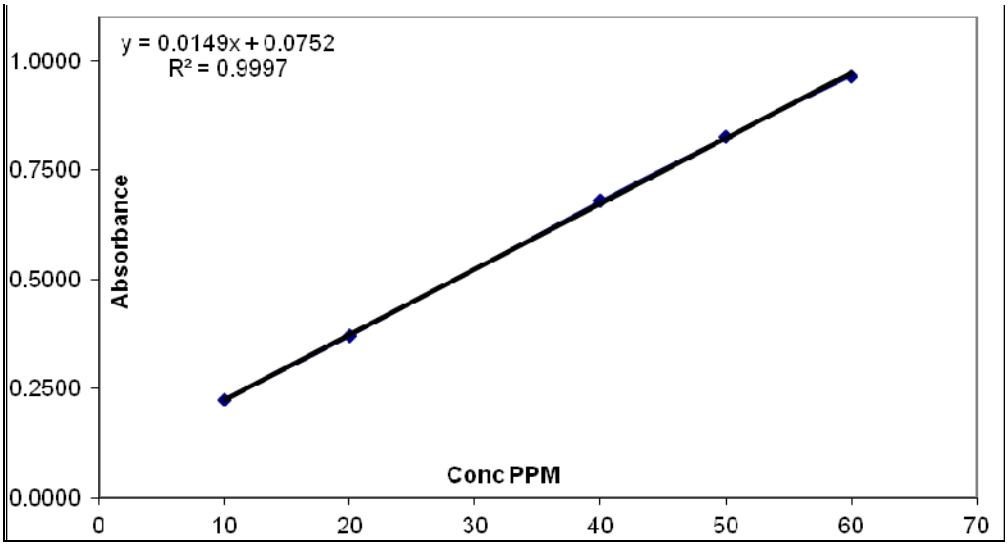



**Figure 2- 5 Regressed calibration curve for estimation of Lornoxicam in Acetate Buffer pH 4.5**

**Accuracy and Repeatability**

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three

replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. Accuracy of method for analysis of Lornoxiam in acetate buffer 4.5 is shown in Table 6 - 5.

1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated.

In order to determine the accuracy of the developed method, known amounts of Lornoxicam (10 µg/mL, 20 µg/mL and 60 µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (10, 20, 60 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 6 - 5.

| Conc. of LOR (µg/ml) Std. | AVG Recovery (µg/ml) | % Recovery | SD | RME | Confidence | RSD Intra Day | RSD Inter Day |
|---|---|---|---|---|---|---|---|
| 10 | 10.1325 | 101.3 | 0.1784 | 0.0798 | 10.1325 ± 0.4972 | 1.76 | 2.93 |
| 20 | 19.9802 | 99.9 | 0.1155 | 0.0516 | 19.9802 ± 0.3219 | 0.58 | 0.52 |
| 60 | 59.9379 | 99.9 | 0.0842 | 0.0376 | 59.9379 ± 0.2346 | 0.14 | 0.17 |

**Table 6 - 5 Evaluation of accuracy and precision of the estimation method of lor in Acetate Buffer 4.5**

** At 95% Confidence level; $t_{tab}$ = 3.18 for 4 degrees of freedom (n=5)*

**5.2.1.5 Preparation of standard stock solutions of Lornoxicam in PBS 7.5 : Methanol ( 50 : 50)**

50 mg of Lornoxicam was accurately weighed using single pan electronic balance and transferred to 50 ml volumetric flask. 25 ml of PBS 7.5 : Methanol ( 50 : 50) was accurately measured and transferred to the above volumetric flask, the drug was dissolved properly and then the final volume of the flask was made up to 50 ml with PBS 7.5 : Methanol ( 50 : 50) to produce 1000 µg per ml of Lornoxicam.

25 ml of the above solution was accurately measured by calibrated graduated pipette and transferred to the 100 ml volumetric flask. The final volume was made up to 100 ml with PBS: Methanol (50 : 50) to prepare stock solution of 250 µg per ml of Lornoxicam.

### 5.2.1.6 Calibration curve of Lornoxicam in PBS 7.5 : Methanol

Suitable aliquots of standard stock solution were accurately measured and transferred to the 10 ml of volumetric flasks. The final volume was made up to 10 ml with PBS 7.5 : Methanol ( 50 : 50) to give final concentrations of 10, 12, 14, 18, 20, 25 µg/ml and analyzed by UV spectrophotometry at 278 nm. No interference due to excipients used in the formulation was observed. The above procedure was repeated three times. The data was recorded in Table along with standard deviation. Figure 3-5 shows Regressed calibration curve for estimation of Lornoxicam in PBS 7.5 : Methanol, values as mentioned in table 7 - 5.

| Concentration (µg/ ml) | Average* | SD | RSD |
|---|---|---|---|
| 10 | 0.3007 | 0.0026 | 0.8714 |
| 12 | 0.3540 | 0.0029 | 0.8166 |
| 14 | 0.4007 | 0.0037 | 0.9244 |
| 18 | 0.5040 | 0.0039 | 0.7741 |
| 20 | 0.5490 | 0.0038 | 0.6878 |
| 25 | 0.6667 | 0.0048 | 0.7140 |

**Table 7 - 5 Calibration for Lornoxicam in PBS : Methanol**

Regression equation** Y= 0.0244X - 0.0598; Correlation coefficient = 0.9995

*Mean of 3 values

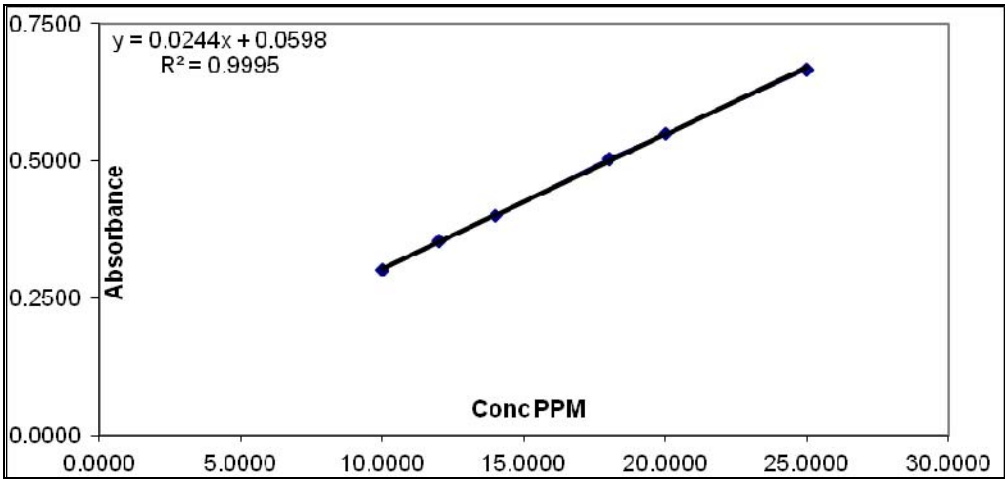



**Figure 3 - 5 Regressed calibration curve for estimation of Lornoxicam in PBS 7.5 : Methanol**

**Accuracy and repeatability**

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. Accuracy of method for analysis of Lornoxiam in Medium was show in Table 8 - 5.

1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated.

In order to determine the accuracy of the developed method, known amounts of Lornoxicam (10µg/mL, 14µg/mL and 25µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (10, 14, 25 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 8 -5.

| Theoretical Conc of BT (µg/ml) | AVG Recovery (µg/ml) | % Recovery | SD | RME | Confidence | RSD Intra Day | RSD Inter Day |
|---|---|---|---|---|---|---|---|
| 10 | 10.0428 | 100.4 | 0.1493 | 0.0668 | 10.0428 ± 0.4162 | 1.49 | 0.87 |
| 14 | 14.0084 | 100.1 | 0.0685 | 0.0306 | 14.0084 ± 0.1910 | 0.49 | 0.74 |
| 25 | 25.0270 | 100.1 | 0.0601 | 0.0269 | 25.0270 ± 0.1676 | 0.24 | 1.43 |

**Table 8 - 5 Evaluation of accuracy and repeatability of the estimation method of LOR in PBS 7.5 : Methanol**

** At 95% Confidence level; $t_{tab}$ = 3.18 for 4 degrees of freedom (n=5)*

**5.3 Estimation of Lornoxicam in Formulation for Assay**

To determine the amount of Lornoxicam in the tablet, 20 tablets were crushed and added to 10 ml mixture of PBS 7.5 : Methanol (50:50) and subjected to shaking at room temperature for 5 mins for complete disintegration of excipients & extraction of the drug from the formulation. The filtered solution was further diluted with PBS 7.5 : Methanol (50:50) to get detectable concentration and estimated at 278 nm.

**5.4 Estimation of Lornoxicam in formulation for content uniformity**

To determine the amount of Lornoxicam in the Tablet , 10 tablets individually were crushed and added to 100 ml of PBS 7.5 : Methanol ( 50 : 50) and subjected to shaking at room temperature for 5 mins for complete disintegration of excipients and extraction of the drug from the formulation. The filtered supernant was further diluted with PBS 7.5 : Methanol ( 50 : 50) to get detectable concentration and estimated at 278 nm.

**5.5 Estimation of LOR for in-vitro release**

The release studies for Lornoxicam formulation in different release media. One tablet containing 16 mg drug was placed in dissolution vessel containing 900 ml of release medium maintained using paddle at 50 RPM at 37 ± 2 °C. 5 ml aliquots were taken out at different time and replace with same quantity of release media. The dissolved drug in release medium analyzed as per the method above. The amount of the drug released and cumulative percentage release was calculated.

The spectrophotometeric determination of placebo formulation consisting of all ingredients except drug showed no any absorbance under discussed methods. The capacity of the method to separate Lornoxicam the non-interference with Lornoxicam indicates the specificity of the methods. Stability of the Lornoxicam in the solution was demonstrated to be stable in solvent during the period of 24 h since the change in the $\lambda_{max}$ was not significant with the RSD value.

**5.6 HPLC method for estimation of Lornoxicam**

For determination of linearity 100 µg of Lornoxicam was accurately weighed using single pan electronic balance and transferred to aconitrile to produce primary standard of Lornoxicam, which was used to make working standard solutions of lornoxicam by diluting primary standard solution with acetonitrile. Rat plasma calibration standards (0.50, 1.00, 2.00, 3.00, 4.00, 5.00 µg/ml) of lornoxicam were prepared by spiking the working standard in to the drug free rat plasma. The aliquot samples were (200 µL) stored at -20° C into polypropylene tubes until analysis.

200 µL of blank plasma, calibration standards and QC samples were mixed with 200 µL of 0.5 M HCl. The samples were extracted with 900 µL of ethyl acetate in 2.0 ml polypropylene tubes by vortex-mixing for 5 min at high speed and centrifuged at 5000 g for 5 min at room temperature. 850 µL of the organic layer was transferred and evaporated to dryness using reduced pressure. The residues were dissolved in 40 µL of 0.1 N methanolic NaOH by vortex-mixing for 2 min, centrifuged at 5000 g for 5 min, transferred to injection vials. To 40 µl of above solution 10 µl of 50 µg/ml solution of drug in 0.1 N methanolic sodium hydroxide was spiked to achieve minimum quantifiable concentration and 10 µL were injected into the HPLC column using the discussed analytical method. 1 ml of 50 µg/ml solution of drug was spiked to 4 ml of sample to achieve minimum quantifiable concentration using the discussed analytical method. After measuring concentration through discussed method spiked 50 µg amount was deducted and amount available in 4 ml of sample was calculated.

Method Type : Isocratic System Method

Buffer Solution pH 7.3: Dissolve 2.88 mg Ammonium Dihydrogen Phosphate in 1000ml of water, and adjust pH to 7.3 $\pm$ 0.05 with triethylamine.

Mobile Phase : sodium acetate (pH 7.3) and methanol (45:55) mix and sonicate for 5 min and filter through a 0.45 µm Poly Tetra Fluoro Ethylene (PTFE) membrane filter.

Column : Phenomenex - Luna C 18 100 A 250 x 4.60 mm x 5 µ

Sample Injection Volume: 10 µl

Flow Rate : 1.5 ml/min

$\lambda_{max}$ : 275 nm

Thermostat : 30ºC

Retention time: 8.5 min

Run Time : 15 min

Column was equilibrated with mobile phase until stable baseline is obtained.

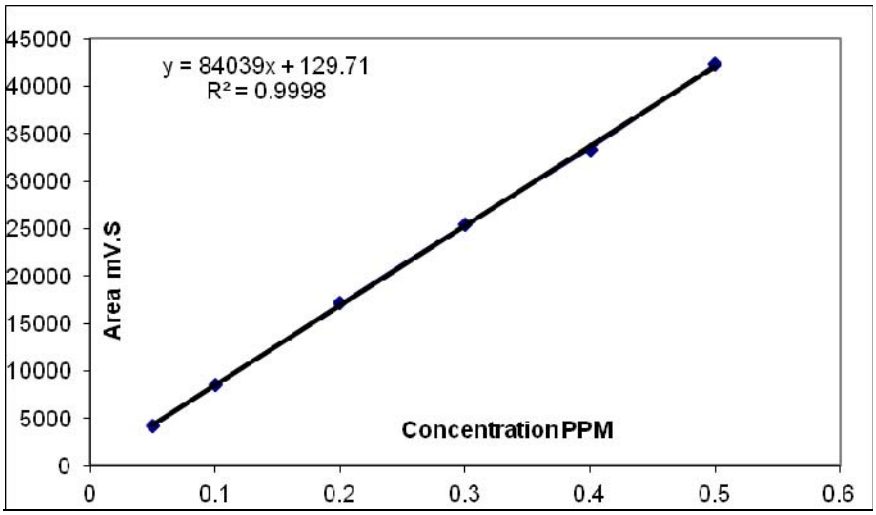



**Figure 4 - 5 regressed calibration curve of Lornoxicam (HPLC)**

Calibration curve of Lornoxicam

| **Concentration ( µg/ml)** | **Area mV .S** | **SD** | RSD |
|---|---|---|---|
| 0.050 | 4250 | 39 | 0.9176 |
| 0.100 | 8500 | 72 | 0.8471 |
| 0.200 | 17142 | 139 | 0.8109 |
| 0.300 | 25468 | 234 | 0.9188 |
| 0.400 | 33381 | 281 | 0.8418 |
| 0.500 | 42298 | 319 | 0.7542 |

**Table 9 - 5 Calibration curve of lornoxim Using HPLC**

Figures 4 - 5 calibration curve of Lornoxicam tartrate in using HPLC, the values are tabulated in table 9 -5.

**Accuracy and repeatability**

Accuracy of an analytical method is the closeness of test results obtained by that method to true value (USP30-NF25, 2007). Accuracy is calculated from the test results as the

percentage of analyte recovered by assay. Accuracy was calculated by analysis of three replicate samples for the above described methods. The observed concentrations of the drug were then back calculated using the equation of standard calibration curve and compared with actual concentrations. Accuracy of method for analysis of Lornoxiam in Medium was show in Table 10 - 5.

In order to determine the accuracy of the developed method, known amounts of Lornoxicam (0.05 µg/mL, 0.30 µg/mL and 0.50 µg/mL) were subjected to recovery studies as per the procedure described above. To determine the repeatability of the analytical method Intraday result for three different concentrations (0.05, 0.30, 0.50 µg/ml) determined 5 times at two different days and RSD for results were compared. The results obtained are tabulated in table 10-5.

Stability of the Lornoxicam in solution was verified and found to be stable in solvents during the period of 24 h since the change in the $\lambda_{max}$ was not significant with the maximum RSD value of 2.90% as mentioned in table 10-5.

It was also confirmed that retention time of Lornoxicam not shifted with the adjustment of the proportion of methanol and the flow rate. But the final result did not show significant change. Considering the stability in the system suitability parameters, the method conditions would be concluded to be robust.

| Theoretical Conc of LOR (µg/ml) | AVG Recovery (µg/ml) | % Recovery | SD | RME | Confidence | RSD Intra Day | RSD Inter Day |
|---|---|---|---|---|---|---|---|
| 0.0500 | 0.0497 | 99.5 | 0.0008 | 0.0004 | 0.0497 $\pm$ 0.0022 | 1.62 | 2.90 |
| 0.3000 | 0.2984 | 99.5 | 0.0056 | 0.0025 | 0.0497 $\pm$ 0.0156 | 1.87 | 2.75 |
| 0.5000 | 0.5032 | 100.6 | 0.0107 | 0.0048 | 0.5032 $\pm$ 0.0299 | 2.13 | 2.68 |

**Table 10 - 5 Evaluation of accuracy and precision of the estimation method of LOR using HPLC**

** At 95% Confidence level; $t_{tab}$ = 3.18 for 4 degrees of freedom (n=5)*

**System Suitability verified by following parameters**

- % RSD for three replicate was not more then 3.0.
- The tailing factor is not more than 2.0.

The developed isocratic high performance liquid chromatographic method was rapid and suitable for the estimation of Lornoxicam in rat plasma. Linearity, repeatability, accuracy and

robustness were verified. The stability of analytical solutions was sufficient for the whole analytical process. Using the established method, the amount of Lornoxicam in plasma was determined.

The chromatographic determination of placebo formulation consisting of all ingredients except drug showed no any absorbance under discussed methods. The capacity of the method to separate Lornoxicam is verified. The non-interference with Lornoxicam indicates the specificity of the methods.

**5.7 Discussion**

The UV spectroscopic method was developed for the LOR estimation in PBS pH 7.5, Acetate Buffer pH 4.5 and PBS : Methanol ( 50 : 50), measurement was done at $\lambda_{max}$ 278 nm, 282 nm and 278 nm respectively for solvents. There was no interference observed with any excipient used. The method was validated for linearity, accuracy and repeatability. The validation parameters were found to meet the "readily pass criteria" and % RSD were found less than 2%.

The absorbance for BT in PBS pH 7.5 was found to be linear in the range of 5 - 30 µg /ml with $r^2$ value of 0.9996. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate LOR in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels do not show significant difference.

The absorbance for LOR in acetate buffer pH 4.5 was found to be linear in the range of 10 - 60 µg /ml with $r^2$ value of 0.9997. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate LOR in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels were shown do not show significant difference.

The absorbance for LOR in PBS : Methanol ( 50 :50) was found to be linear in the range of 10 - 25 µg /ml with $r^2$ value of 0.9995. The recovery was found to be more than 90%, indicating the reliability accuracy to estimate BT in the mentioned range. The repeatability of the measurement was expressed in terms of % RSD and the % RSD for intra-day and inter-day of BT at 3 different concentration levels do not show significant difference.

The invitro release study was performed using type II dissolution apparatus using 900 ml release medium. At different time intervals, the samples were removed, replaced with same medium and analysed for the drug. The cumulative percentage drug released was calculated.

For Lornoxicam, the calibration curve was established using HPLC for estimation of drug in plasma with sodium acetate (pH 7.3) and methanol (45:55) mix as mobile phase and detection at 275nm. The linearity of LOR was found to be 0.050 – 0.500 µg/ml ($R^2$=0.9998). The recovery studies for accuracy and precision were carried out at 0.050, 0.300 and 0.500 µg/ml and the recovery was found to be more than 90%, indicating the reliability accuracy to estimate LOR in the mentioned range.

## 5.8 References


- Jianjun Zhang, Li Li, Yuan Gao and Weiming Fan "Development and validation of a stability indicating HPLC method for the analysis of lornoxicam in powder for injection" Pak. J. Pharm. Sci., Vol.25, No.2, April 2012, pp.371-375

- Bhupendra Singh*, Geetanjali Saini, Devendra N Naath Sharma, Saumendu Deb Roy and Nishant Gautam "Estimation Of Lornoxicam In Tablet Dosage Form By Uv Spectrophotometric Method" IJPSR, 2011; Vol. 2(1): 116-120

- Emirhan Nemutlu, Şeyda Demircan, Sedef Kır Determination Of Lornoxicam In Pharmaceutical Preparations By Using Spectrophotometric And Chromatographic Methods Adnan Menderes University, 4th AACD Congress, 29 Sept-3 Oct.2004, Kuşadası-AYDIN/ TURKEY Proceedings Book 069.DOC 134

- Prasad Byrav D S, B Medhi, A Prakash, S Patyar, S Wadhwa "Lornoxicam: a Newer NSAID" *IJPMR 2009; 20 (1):27-31*

- S. Radhofer-Welte, P. Dittrich "Determination of the novel non-steroidal anti-inflammatory drug lornoxicam and its main metabolite in plasma and synovial fluid" Journal of Chromatography B, 707 (1998) 151–159

- E.venumadhav, t.neeha, p.bhargavi, amreen nishat, a.swetha and g.devala rao International journal of applied biology and pharmaceutical technology "New spectrophotometric methods for the determination of Lornoxicam in pharmaceutical dosage forms" volume: 2: issue-1: Jan-mar -2011

- Y. Gao, J. J. Zhang, W. M. Fan, B. J. Ren, Q. N. Ping Solubilization study of lornoxicam for parenteral formulation

- Robert J. Meyer and Ajaz S. Hussain, Office of New Drugs and Office of Pharmaceutical Science Center for Drug Evaluation and Research, FDA FDA's ACPS Meeting, October 2005 Awareness Topic: Mitigating the Risks of Ethanol Induced Dose Dumping from Oral Sustained/Controlled Release Dosage Forms

# 6.0 Formulation Development Lornoxicam (LXM):

The main objective of the present work is to develop extended release solid oral formulation of Lornoxicam with sufficient burst release for maintaining therapeutic blood levels of the drug for initial and extended period of time which can reduce dosing frequency and improve patient compliance by minimizing local and systemic adverse effect. Table 1-6 lists the equipment used for development.

**Equipments**

| Sr. No. | Instruments | Make |
|---|---|---|
| 1. | Compression | 8-station compression machine, KMP-8, Cadmach Engg, Ahmedabad, India. |
| 2. | Digital weighing balance | AG-64, Mettler Toledo, Switzerland |
| 3. | Tap density tester | ETD-1020, Electrolab, Mumbai, India. |
| 4. | Hardness tester | 6-D, Dr Schleuniger Pharmatron, Manchester, NH, USA |
| 5. | pH meter | Mettler Toledo, Switzerland |
| 6. | Tray dryer | Bombay Eng. Works, Mumbai, India |
| 7. | Friability tester | EF-2, Electrolab, Mumbai, India |
| 8. | Thickness gauge | Digimatic Caliper, Mitutoyo, Japan |
| 9. | Bath sonicator | DTC 503, Ultra Sonics, Vetra, Italy |
| 10. | Stability chamber | Thermolab, Mumbai, India |
| 11. | Differential Scanning Calorimeter (DSC) | Mettler DSC 20, Mettler Toledo, Switzerland |
| 12. | Dissolution apparatus | Electrolab, Mumbai, India |
| 13. | Stability oven | Shree Kailash Industries, Vadodara |
| 14. | HPLC system | LC 20-AT prominence, Shimadzu Corp., Japan |
| 15. | UV-Visible Spectrophotometer | Shimadzu UV-1601, Japan |
| 16. | Nuclepore Polycarbonate membrane 2 µm 25mm | Whatman, USA |

**Table 1 - 6 Equipment Used**

## 6.1 Drug Substances:

**Lornoxicam**

The drug substance attribute, affects the drug product development, manufacturing, performance and stability. The following aspects were considered during the product development:

**Physiochemical properties of Drug**

As physicochemical drug properties plays an important role in the manufacturing of a dosage form and its therapeutic activity, the characterization of the powder properties of Lornoxicam among all the physicochemical properties was found to be important.

Particle size & size distribution of drug substance : Lornoxicam is poorly soluble in water drug (Bramhane D. M., 2011) , particle size can affect the formulation properties and subsequently bioavailability but because the drug is used in soluble form in the formulation it is not critical parameter for this development. Bulk density and tapped density:

Bulk Density: 0.36 gm/ml; Tap Density : 0.48 gm/ml

Partition coefficient: Partition Coefficient of lornoxicam is 1.8 in n-octanol and phosphate buffer (pH 7.4) (Ahmed M. O et al., 2011)

Physical Description

Orange to yellow crystalline powder

Melting Point: 225°C to 230°C (Ahmed M. O et al., 2011).

Solubility

Since lornoxicam is a weak acid (pKa of 4.7), the aqueous solubility of lornoxicam is pH dependent. Increasing pH leads to decrease in the ratio of non-ionized to ionized drug, and in solubility-pH profiles, the solubility of lornoxicam decreases exponentially with the increase of pH from alkaline pH 9.0 to acidic pH 3.0.

Stability

Lornoxicam is very stable compound no individual degradation products were identified during forced degradation studies.

Biopharmaceutical Classification System (BCS) category: BCS has categorized Lornoxicam in Class II, i.e. Low solubility High permeability.

## 6.2 Pharmacokinetics

Lornoxicam is dissolves slowly, absorbed rapidly and completely from the GIT. $C_{max}$ is within 2 to 2.5 hrs considered as BCS Class II drug. On repeated administration, peak

plasma concentration is increased in dose related manner. No drug accumulation occurs if repeated drug administration. Food related reduction in absorption of the drug is observed. Almost 99% is protein bound exclusively to albumin. No first-pass effect has been observed. Lornoxicam is found in the plasma in unchanged form and as its hydroxylated metabolite. The hydroxylated metabolite exhibits no pharmacological activity. CYP2C9 (CYP450) has been shown to be the primary enzyme responsible for the biotransformation of the lornoxicam to its major metabolite, 5'-hydroxy lornoxicam unlike other oxicams, the plasma half-life of lornoxicam is about 3 to 5 hours.. Approximately 2/3 of drug is eliminated via the liver and 1/3 of drug via the kidneys as inactive substance. It does not undergo enterohepatic recirculation. Glucuroconjugated metabolites are excreted in urine and faeces with a half-life of about 11 hours. It readily penetrates into synovial fluid, the proposed site of action in chronic inflammatory arthropathies. Lornoxicam synovial fluid: plasma AUC ratio is 0.5, after administration of 4 mg twice daily. (Byrav D S Prasad, 2009)

Absolute oral bioavailability of LOR is more than 90%. Lornoxicam is found in the plasma in hydroxylated and unchanged form. It readily penetrates into synovial fluid, the proposed site of action in chronic inflammatory arthropathies. In elderly patients the clearance of lornoxicam is reduced by about 30% to 40%; thus the half-life is somewhat higher. Even in the presence of impaired kidney and liver function, no major differences in pharmacokinetics have been observed. On account of its short half-life, no accumulation happens on repeated administration. The maximum plasma concentration of lornoxicam that produce therapeutic analgesic activity is 1µg/ml. After administration of lornoxicam 4 mg tablets to healthy volunteers, mean peak serum concentrations of 300 to 360 ng/mL were reported at 1.6 to 3 hours

Food protracts the average time to maximum concentration and can reduce the area under the curve (AUC) by up to 20% (Ahmed M. O et al., 2011)

The absolute bioavailability of Lornoxicam is 90–100%. No first-pass effect was observed (Pruss T.P.at al, 1999)

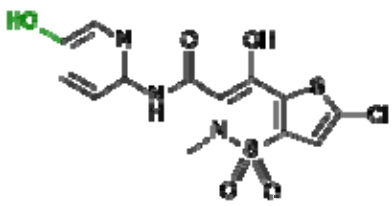


**Figure 1 - 6 Lornoxicam Structure**

### 6.3 Pharmacodynamic Properties

Lornoxicam is an active substance from the group of acidic anti-pyretic analgesics. The accumulation of acidic analgesics in the inflamed tissue is considered to be a significant aspect of their anti-inflammatory effect. In cases of painful inflammatory reactions, the capillaries in the inflamed tissue are damaged and plasma proteins along with bound pharmaceutical substances are discharged into the extravascular space. On account of the reduced pH value in inflamed tissue, analgesic acids are able to move from the extracellular space and enter the cells more easily. This also explains why the duration of action of acidic substances is generally longer than one would expect in consideration of their plasma half-life. The inflamed tissue probably behaves like a deep compartment whose filling and depletion adjust to the plasma concentrations with substantial delay.

**Mechanism of Action**

Lornoxicam is a non steroidal anti-inflammatory drug, analgesic in nature and it fit in to the class of oxicams. Same as that of other NSAIDs, lornoxicam acts by biosynthesis inhibition of prostaglandin through blocking the cyclooxygenase enzyme. Lornoxicam inhibits both COX-1 and COX-2 enzymes. It exerts analgesic action by inhibiting cyclooxygenase, which suppresses the production of thromboxanes & prostaglandins leading to reduction of pain and inflammation. The analgesic activity shows almost equal inhibition of COX- 1 and COX-2 and release endogenous dynorphin and β endorphin with reported central analgesic activity. Unlike some NSAIDs, the inhibition of cyclooxygenase by lornoxicam does not result in an increase in leukotriene formation and not allow unexpected shunting of arachidonic acid to the 5-lipoxygenase cascade, which reduces some potential adverse events, e.g. allergic reactions.

## 6.4 Excipients:

### *6.4.1 Excipient used in drug product:*

Following table describes the list of excipients. Table 2-6 includes all raw materials used in the manufacture of the drug product, whether they appear in the finished product or not. All excipients used in fabrication of drug product matches with specifications commonly used in design of oral products.

| Ingredients | Functional Category | Source |
|---|---|---|
| Lornoxicam HCl | Active | Cadila Healthcare Ltd., Moraiya, Gujarat, India |
| Lactose Monohydrate | Filler | Granulac 200, Meggle. |
| Microcrystalline cellulose | Filler | Celphere CP-102, AsahiKASEI, Japan |
| Purified Talc | Antiadherant | Luzenac |
| Magnesium stearate | Lubricant | Mallinckrodt, USA |
| Colloidal silicon dioxide | Glidant | Aerosil 200, Degussa, Frankfurt, Germany |
| HPMC K4M | Matrix forming agent | Methocel K4M, Colorcon Asia Pvt. Ltd, Goa, India |
| HPMC K15M | Matrix forming agent | Methocel K15M, Colorcon Asia Pvt. Ltd, Goa, India |
| HPMC K100M | Matrix forming agent | Methocel K100M, Colorcon Asia Pvt. Ltd, Goa, India |
| HPMC K100LV | Matrix forming agent | Methocel K100LV, Colorcon Asia Pvt. Ltd, Goa, India |
| Polyvinyl Pyrrolidone | Binder | Kollidon, BASF, Germany |
| Polyethylene oxide (Polyox WSR Series) | Matrix forming Agent | Polyox WSR N750 and WSR N10, The Dow Chemical Company, MI, USA |
| Sodium Hydroxide | Buffering agent | S.D. Fine Chemicals Ltd, Mumbai, India |
| Meglumine | Buffering agent/Stabilizer | S.D. Fine Chemicals Ltd, Mumbai, India |
| Distilled Water * | Processing solvent | Prepared in laboratory by distillation |

**Table 2 - 6 List of excipients**

*Used as processing agent, does not remain in the final product.

### *6.4.2 Supplier specifications of the excipients*

**Microcrystalline cellulose**

| **Specifications** | |
|---|---|
| Loss on drying | % 3.0 - 5.0 |
| Bulk density | 0.26 - 0.31 g/cc |
| Identification | A, B Passes |
| Degree of polymerization, units | NMT 350 |
| pH | 5.5 - 7.0 |
| Conductivity, | NMT 75 µS/cm |
| Residue on ignition, | % NMT 0.05 |
| Water soluble substances, | mg/5g NMT 12.5 |
| Water soluble substances, | % NMT 0.25 |
| Ether soluble substances, | NMT 5.0 mg/10g |
| Heavy metals, | NMT 0.001 % |
| Solubility in Copper Tetrammine Hydroxide | Soluble |
| **Microbial limits:** | |
| Total aerobic microbial count | NMT 100 cfu/g |
| Total yeast and mold count, cfu/g * | NMT 20 cfu/g |
| Pseudomonas aeruginosa in a 10g sample | Absent |
| Escherichia coli in a 10g sample | Absent |
| Staphylococcus aureus in a 10g sample | Absent |
| Salmonella species in a 10g sample | Absent |
| Coliform species in a 10g sample | Absent |
| **Additional FMC Specifications** | |
| Particle size (Air Jet): | |
| wt. % + 60 mesh (250 microns) | NMT 1.0 |
| wt. % + 200 mesh (75 microns) | NMT 30 |

**Lactose Monohydrate**

| **Specifications USP NF 23** | |
|---|---|
| Identification | Pass |
| Appearance/color of solution | Pass |
| Optical rotation | +54.4 to +55.9° |
| Acidity or alkalinity | Pass |
| Heavy metals | ≤5 µg/g |
| Absorbance 210–220 nm | ≤0.25 |
| Absorbance 270–300 nm | ≤0.07 |
| Loss on drying | ≤0.5% |
| Water | ≤1.0% |
| Residue on ignition | ≤0.1% |
| Heavy metals, | NMT 0.001 % |
| Solubility in Copper Tetrammine Hydroxide | Soluble |

| **Microbial limits:** | |
|---|---|
| Total aerobic microbial count | NMT 100 cfu/g |
| Total yeast and mold count, cfu/g * | NMT 50 cfu/g |
| Escherichia coli in a 10g sample | Absent |
| Isomer ratio | Pass |
| Salmonella species in a 10g sample | Absent |

| **Polyox WSR 303** | |
|---|---|
| **Specifications USP NF 23** | |
| Identification | Pass |
| Loss on drying | ≤1.0% |
| Silicon dioxide and nonsilicon dioxide residue on ignition | ≤2.0% |
| Silicon dioxide | ≤3.0% |
| Heavy metals | ≤0.001% |
| Free ethylene oxide | ≤0.001% |
| Organic volatile impurities | Pass |
| Viscosity | Pass |

| **Methocel** | |
|---|---|
| **Specifications USP 28** | |
| Identification | Pass |
| Apparent viscosity | Pass |
| Loss on drying | ≤5.0% |
| For viscosity grade >50 mPa s | ≤1.5% |
| For viscosity grade ≤50 mPa s | ≤3.0% |
| For type 1828 of all viscosities | ≤5.0% |
| Heavy metals | ≤0.001% |
| Organic volatile impurities | Pass |
| **Methoxy content** | |
| Type 1828 | 16.5–20.0% |
| Type 2208 | 19.0–24.0% |
| Type 2906 | 27.0–30.0% |
| Type 2910 | 28.0–30.0% |
| **Hydroxypropoxy content** | |
| Type 1828 | 23.0–32.0% |
| Type 2208 | 4.0–12.0% |
| Type 2906 | 4.0–7.5% |
| Type 2910 | 7.0–12.0% |

| **Purified Talc** | |
|---|---|
| **Specifications USP NF** | |
| Identification | Complies with EP/BP tests |
| Acidity or alkalinity | |
| Change colour to pink | NMT 0.3 ml of 0.01 M NaOH |
| Water-soluble substances | Max 0.2% |
| Aluminium | Max 2.0% |
| Calcium | Max 0.90% |
| Iron | Max 0.25% |
| Lead | Max 10.0 ppm |
| Magnesium | 17.0% to 19.5% |
| Loss on ignition | Max 7.0% |
| **Microbial contamination** | |
| Total viable aerobic count | NMT total of $10^2$ bacteria and fungi per gram. |

| **Aerosil 200** | |
|---|---|
| **Specifications USP NF 23** | |
| Identification | Pass |
| pH (4% w/v dispersion) | 3.5–5.5 |
| Arsenic | 8 µg/g |
| Loss on drying | 2.50% |
| Loss on ignition | 2.00% |
| Organic volatile impurities | Pass |
| Assay (on ignited sample) | 99.0–100.5% |
| Specific Surface Area | 200 ± 25 $m^2/g$ |
| Tapped Density | 0.05 $g/cm^3$ |

| **Magnesium Stearate** | |
|---|---|
| **Specifications USP NF 23** | |
| Identification | Pass |
| **Microbial limits** | |
| Aerobic microbeal Count | $10^3/g$ |
| Fungi and yeasts | 500/g |
| Acidity or alkalinity | Pass |
| Specific surface area | Pass |
| Loss on drying | 6.00% |
| Chloride | 0.10% |
| Sulfate | 1.00% |
| Lead | 0.001% |
| Relative stearic/palmitic content | Pass |
| Organic volatile impurities | Pass |
| Assay (dried, as Mg) | 4.0–5.0% |

| Meglumin | |
|---|---|
| **Specifications USP NF 28** | |
| Identification | Pass |
| Loss on drying | 1.00% |
| Melting Range | 128–132°C |
| Specific Optical rotation | 5.7 to 7.3° |
| Residue on ignition | 0.100% |
| Absence of reducing substances | Pass |
| Heavy metals | 0.002% |
| Assay | 99.0–100.5% |

| Sodium Hydroxide | |
|---|---|
| **Specifications USP NF** | |
| Identification | Pass |
| Insoluble substances and organic matter | Pass |
| Potassium | Pass |
| Heavy metals | 0.003% |
| Assay (total alkali calculated as NaOH) | 95.0–100.5% |

| Ingredients | Approval Status ( USFDA) | Qty ( mg) |
|---|---|---|
| Cellulose, microcrystalline | Oral; table | 1385.30 |
| Lactose monohydrate | Oral; tablet, film coated | 587.44 |
| Talc | Oral; tablet, extended release | 80.00 |
| Silicon dioxide, colloidal | Oral; tablet | 99.00 |
| Meglumine | Oral; tablet | 24.00 |
| Sodium hydroxide | Oral; tablet | 6.72 |
| Hydroxypropyl Methyl cellulose | Oral; tablet | 100.4 |
| Sodium Hydroxide | Oral; tablet | 6.72 |
| Polye ethylene oxide | Oral; tablet | Not Approved |
| Meglumine | Oral; tablet | 24.0 mg |

**Table 3 - 6 Approval Status of ingredients used**

### *6.4.3 Drug excipients compatibility study:*

At an early stage of proposed drug product development, drug-excipient compatibility study was performed, to identify the potential incompatibilities of drug with the excipients intended to be used for product development. The pre-formulation study was based on the

excipients to be used in the finished product. All the inactive ingredient guide (USFDA) approved ingredients were selected for the study as mentioned in table 3-6.

Lornoxicam was mixed with Lactose and Microcrystalline cellulose in ratio of 1:5 w/w, with water in 1:5 w/v proportion with distilled water and other excipients in drug to Excipient ratio of 5:1 proportion of binary mixture and the blend were exposed to 40°C /75%RH temperatures for 4 weeks to accelerate drug degradation and interaction with excipients in USP type I amber glass vials with LDPE (low density polyethylene) stopper for evaluation of their compatibility at stress condition. The blend exposed to stress conditions and then compared with their respective initial blend stored at controlled condition by physical observation. The samples are then characterized for the drug content, which were determined quantitatively using developed analytical method after dissolving the drug in equal proportion mixture of PBS 7.5 : Methanol and percentage drug content verified initially and after 4 weeks and compared.

Results of drug-excipient compatibility study are described below in table 4-6.

| **Drug : Excipients** | **Initial** | | **4 Week 40°C /75%RH** | |
|---|---|---|---|---|
| | **Observation** | **% Drug Content** | **Observation** | **% Drug Content** |
| Lornoxicam | Orange to yellow crystalline powder. | 101.72 | Orange to yellow crystalline powder. No change in physical appearance observed | 102.67 |
| Lornoxicam & PVP k 30 | Orange to yellow crystalline powder. | 102.14 | Orange to yellow crystalline powder. No change in physical appearance observed | 101.24 |
| Lornoxicam & Magnesium Stearate | Orange to yellow crystalline powder. | 101.78 | Orange to yellow crystalline powder. No change in physical appearance observed | 101.41 |
| Lornoxicam & Lactose Monohydrate | Orange to yellow crystalline powder. | 102.52 | Orange to yellow crystalline powder. No change in physical appearance observed | 100.61 |
| Lornoxicam & Purified Talc | Orange to yellow crystalline powder. | 102.59 | Orange to yellow crystalline powder. No change in physical appearance observed | 99.40 |
| Lornoxicam & Colloidal Silica | Orange to yellow crystalline | 100.38 | Orange to yellow crystalline powder. No change in physical appearance observed | 99.56 |

| Drug : | Initial | | 4 Week 40°C /75%RH | |
|---|---|---|---|---|
| | powder. | | | |
| Lornoxicam & MCC 101 | Orange to yellow crystalline powder. | 101.33 | Orange to yellow crystalline powder. No change in physical appearance observed | 99.49 |
| Lornoxicam & HPMC | Orange to yellow crystalline powder. | 100.21 | Orange to yellow crystalline powder. No change in physical appearance observed | 100.94 |
| Lornoxicam & Polyethylene Oxide | Orange to yellow crystalline powder. | 96.53 | Orange to yellow crystalline powder. No change in physical appearance observed | 94.52 |
| Lornoxicam & Meglumin | Orange to yellow crystalline suspension. | 101.39 | Orange to yellow crystalline powder. No change in physical appearance observed | 100.41 |
| Lornoxicam & Purified water | Orange to yellow crystalline suspension. | 99.67 | Orange to yellow crystalline suspension. Probable degradation of Lornoxicam may be there. | 77.36 |
| Lornoxicam & Sodium Hydroxide 1M Solution | Yellowish solution. | 98.65 | Yellowish solution. No change in physical appearance observed | 93.51 |

**Table 4 - 6 Drug excipients compatibility study results**

**Conclusion :** Under 40°C /75%RH for 4 weeks, lornoxicam did not reveal show sharp fall in content so it can be concluded that it do not show any incompatibilities with the proposed excipients as summarized in table 4 -6. It seems not stable in aqueous vehicle for after one week of time. Preformulation studies show that the selected excipients are compatible with Lornoxicam.

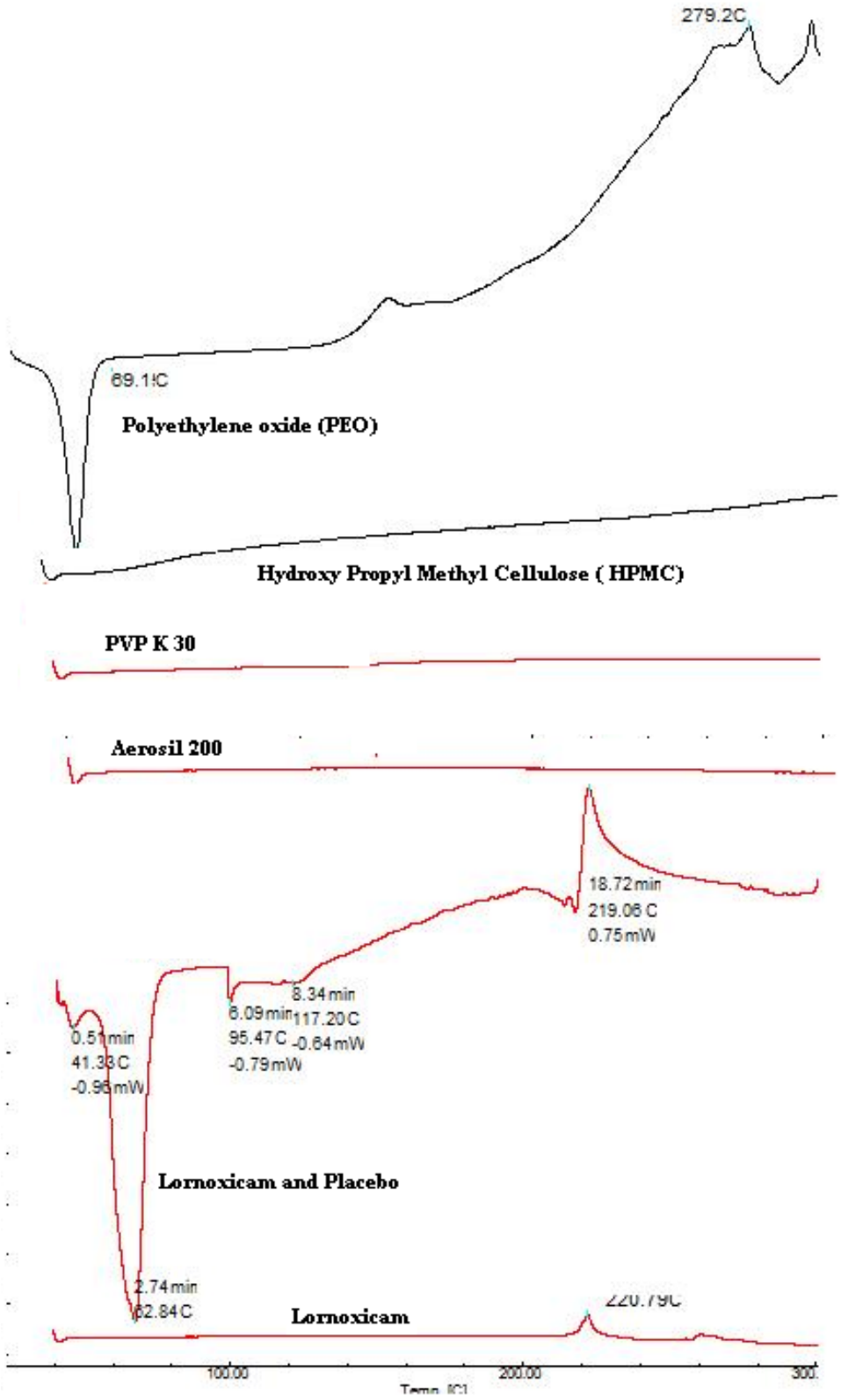



**Figure 2 - 6 DSC Study**

**Differential scanning Calorimetry**

The DSC of samples was carried out by scanning the samples using differential scanning colorimeter (Mettler). Thermograms were analyzed using Mettler Toledo star SW 7.01. An empty aluminium pan was used as the reference for all measurements. During each scan, 2 to 3 mg of sample was heated, in a hermetically sealed aluminium pan. DSC studies were performed under nitrogen flush at heating rate of 10 ºC from 35 ºC to 300 ºC to investigate the any incompatibility between drug and excipients.

DSC curve of lornoxicam exhibited sharp endothermic peak at 220.79° C, which is due to melting of lornoxicam with decomposition.

For that DSC of plain drug was done followed by DSC study of individual excipients to be used in dry form. i.e. aerosil 200, PVP K 30, HPMC and PEO and then DSC of drug mixed with placebo was done. NaOH and Meglumine are to be used in solution form so DSC study was not performed.

**Conclusion:**

It is evident from figure 2 - 6 (DSC study graph) that no changes observed in the DSC of drug mixed with placebo and the original melting endotherm of the crystalline form of drug which exhibited melting endotherm at (220°C). Hence it is clear that there is no specific interaction between the drug and excipients found used in the formulation.

**6.5 Drug Product Formulation Development:**

The proposed target for formulation development must be easy to manufacture, chemically and physically stable throughout the manufacturing process, product shelf life and bio-available in predicted manner. During design of the formulation, critical formulation and manufacturing variables were identified and adjusted to yield quality product. Design of experiments was used to improve and establish the robustness of the formulation around target formulation.

The proposed drug product (Lornoxicam Extended release formulation16 mg) is intended to have following primary attributes:

- Product to be formulated as coated tablet or matrix tablet.
- Product to be developed as an coated dosage form where coating shall be nonfunctional and must comply with predicted release specifications.

### *6.5.1 Predicted Plasma concentration of Lornoxicam*

From data of oral immediate release formulation's plasma drug concentration of available in literature total amount of drug required to achieve steady state level in particular time frame is found out. The target release profile was decided from the AUC of the oral immediate release blood concentration data by Wagner nelson de-convolution process and is shown in figure 3-6. The target drug release profile is depicted in table 5 -6 & 6 - 6

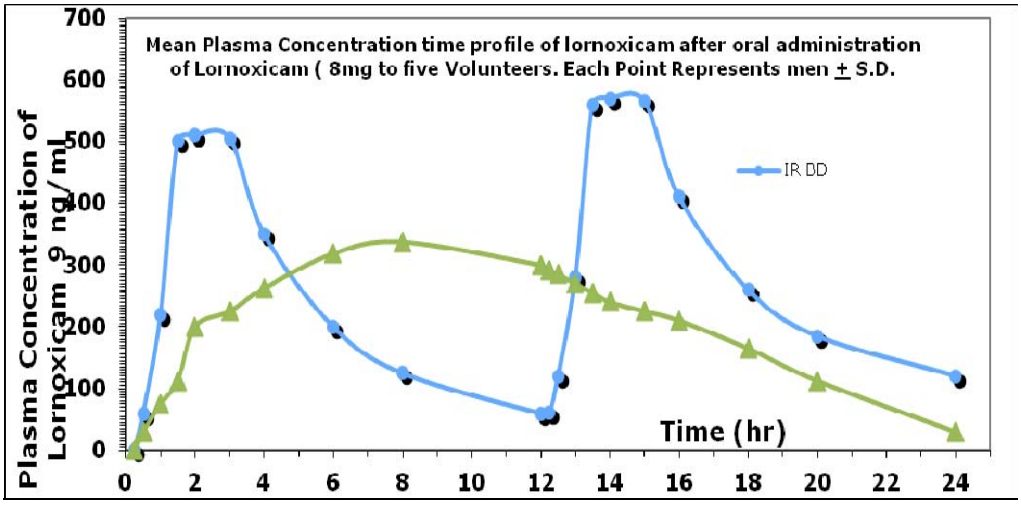



**Figure 3 - 6 Predicted plasma concentration of Lornoxicam**

Above mentioned graph (Figure 3-6) shows actual plasma concentration time profile after administration of two IR formulation administration at the interval of 12 hours (Young Hoon Kim et al., 2007) and from the plasma concentration of the IR formulation plasma concentration time profile of ER formulation predicted considering pharmacokinetic data. From the data of ER plasma drug concentration profile In-vitro target release profile was calculated through deconvolution using Microsoft excel. The method is discussed in detail in literature (F. Langenbucher., 1985).

| Time ( Hr) | Target Profile (Dose 16mg) | Actual IR Plasma Profile ng/ml | Predicted XR Plasma Profile ng/ml |
|---|---|---|---|
| 0.50 | 4.42 | 60 | 60 |
| 1.00 | 11.64 | 220 | 150 |
| 2.00 | 27.86 | 510 | 330 |
| 3.00 | 37.31 | 505 | 390 |

| | | | |
|---|---|---|---|
| 4.00 | 47.63 | 350 | 450 |
| 6.00 | 63.08 | 200 | 480 |
| 8.00 | 76.80 | 125 | 480 |
| 12.00 | 96.21 | 60 | 400 |
| 13.00 | 97.12 | | 340 |
| 14.00 | 98.82 | | 300 |
| 15.00 | 101.52 | | 280 |
| 16.00 | 100.79 | | 220 |
| 18.00 | 101.91 | | 160 |
| 20.00 | 101.31 | | 100 |
| 24.00 | 100.00 | | 30 |

**Table 5 - 6 Final Target release profile (Detail)**

| **Time (hr)** | **Target Range Cumulative % release profile** |
|---|---|
| 0 | 0 |
| 2 | NMT 40 |
| 4 | 40 to 60 |
| 8 | 70 to 80 |
| 12 | NLT 85 |

**Table 6 - 6 Final Target release profile (Brief)**

The release studies for Lornoxicam formulation in different release media. One tablet containing 16 mg drug was placed in dissolution vessel containing 900 ml of release medium using paddle (USP Type II) at 50 RPM maintained at 37 $\pm$ 2°C. Aliquots were taken out at different time interval and replaced with equal quantity of release media. The dissolved drug in release medium analyzed as per the method discussed analytical method section. The amount of the drug released and cumulative percentage release was determined.

| **Drug Name : Lornoxicam** | | | **Dosage Form** : Extended Release | | |
|---|---|---|---|---|---|
| **Stage** | **USP Apparatus** | **Speed (RPM)** | **Medium** | **Volume (ml)** | **Recommended Sampling Time points** |
| Stomach | II (Paddle) | 50 | PBS pH 7.5 | 900 | up to 12 Hrs in buffer stage |

**Table 7 - 6 Dissolution Conditions**

### *6.5.2 Quality of target product profile (QTTP) :*

As a target for the development of a manufacturing process, the following attributes were identified that will ensure the desired product quality to match all aspects of Quality target product profile:

1. Correct amount (Assay) of drug substance in the drug product.
2. Content Uniformity ( represents compression uniformity)
3. Dissolution (Conditions mentioned in table 7 – 6) of drug substance in the drug product (Target release profile table 5 - 6 & 6 – 6).
4. Weight variation of the drug product.
5. Type and concentration of excipients that directly influences the quality and performance of the drug product.
6. Container closure system to provide intended protection to drug product.
7. Overages requirement if product loss during process.
8. Hardness : 2 – 5 $kg/cm^2$
9. Friability NMT 1 %
10. Bulk density and tapped density

### *6.5.3 Selection of Manufacturing Process:*

Lornoxicam is poorly soluble in water and slightly soluble in simulated gastric fluid. Its poor aqueous solubility can lead to absorption rate limiting step and thus delay in onset of action. Solubility being an important parameter for absorption of water insoluble drugs it is a key rate-limiting step. An enhancement in the solubility and the dissolution rate may result in the higher bioavailability and it lead to improved therapeutic efficacy. Various efforts have been made to enhance the solubility of poorly water soluble drugs including the use of surfactants, amorphous form of drug, micronisation and incorporation of alkalizing agents in formulation ( US patent : 6599529 and US patent application US 2006/0024365 A1) are some of the options available micronisation do not provide sufficient fast release, excess of surfactants may be damaging to body and amorphous form may not be stable enough to give stability to formulation so further trials were initiated incorporating an alkylating agent Meglumine in the formulation, in addition of dissolution another objective is to have drug release over extended period of time so matrix forming polymers i.e. HPMC K15 M CR were used in the initial formulation.

Drug substance sourced as USP grade and evaluated for physio-chemical and analytical parameters as per IH method of analysis.

**Preparation method of tablets**

Core tablets were prepared by wet granulation method and the composition is given in table 8-6. For preparation of core tablets, the batch size was kept as 750 tablets. The drug (Sieved through 40 #) was added to the mixture of lactose (Sieved through 40 #), MCC (Sieved through 40 #) and meglumine (Sieved through 40 #) through geometric dilution method and sifting three times. The blend was mixed with 10 % W/V PVP K 30 solution in isopropyl alcohol for binding and granulated by passing through 22 # sieve. The granules were dried at 50°C (approximately 1 h) after which they were passed through 40 # sieve. These sized granules were then blended with extra granular matrix forming agent (HPMC K15 M CR) (40 # passed) for 5 minutes followed by talc (Sieved through 100 #), aerosol 200 (Sieved through 60 #) and magnesium stearate (Sieved through 100 #) and blending for 3 min after addition of each. The blend was compressed to tablets using a rotary tablet compression machine (General Machinary Company, India) fitted with 7 mm standard concave punches. Initial development composition made with formula mentioned in table 8-6 and subjected to dissolution studies as mentioned in table 7 -6, the results are summarized in table 9-6 and plotted as graph in figure 4-6.

**6.5.3.1 Initial Development composition**

| | A | B | C |
|---|---|---|---|
| **Material** | **mg** | **mg** | **mg** |
| Lornoxicam | 16.00 | 16.00 | 16.00 |
| Lactose Monohydrate | 55.75 | 60.00 | 51.50 |
| Micro crystalline Cellulose | 55.75 | 60.00 | 51.50 |
| Meglumin | 25.50 | 17.00 | 34.00 |
| Binding | | | |
| PVP k 30 | 5.00 | 5.00 | 5.00 |
| Extra-granular | | | |
| HPMC K15M CR | 17.00 | 17.00 | 17.00 |
| Mg. Stearate | 2.00 | 2.00 | 2.00 |
| Purified Talc | 2.00 | 2.00 | 2.00 |
| Aerosil 200 | 1.00 | 1.00 | 1.00 |
| **Total** | 180.00 | 180.00 | 180.00 |

**Table 8 - 6 Initial Development composition**

Bulk uniformity for above mentioned composition was within 90 to 110 % but the RSD of 10 results was 10.23 %, 9.59 5 and 11.46 % with mean 102%, 98% and 103.71 % for composition a, B and C respectively. RSD for blend uniformity was targeted less than 5%. Drug uniformity in the powder blend using above mentioned procedure was not achieved and desired initial release was not observed additionally tablets were showing mottling because drug being insoluble. Many other formulation developers have tried to solve the issue by micronisation of drug but that could not completely solve the issue. Further trials were taken using drug in binder solution made by addition of sodium hydroxide and meglumine which was expected to solve the mottling, uniformity issue and initial fast release profile requirement.

Dissolution Studies

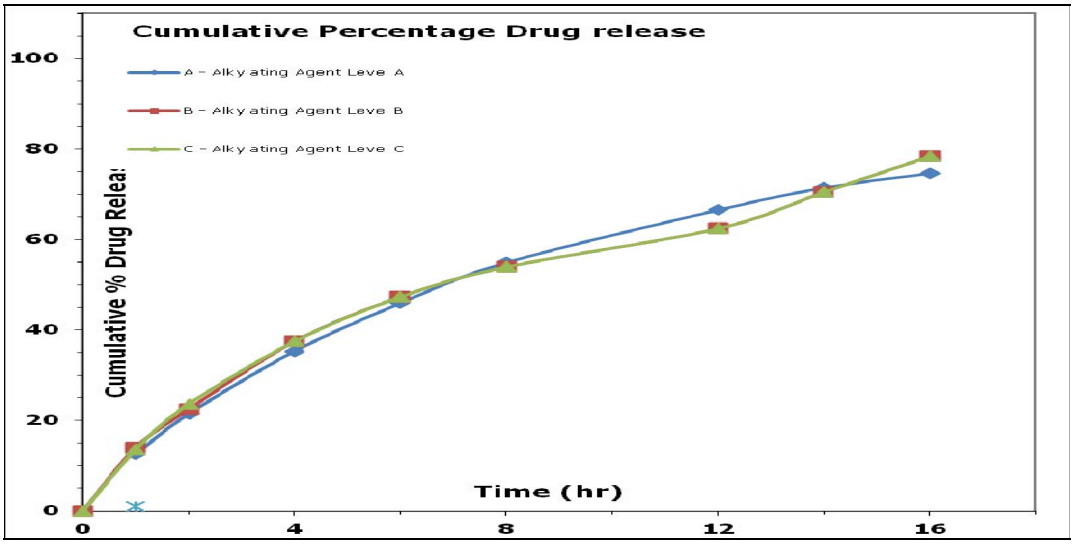



**Figure 4 - 6 Dissolution Studies graph of initial development composition**

| Time (hr) | Cumulative % Drug release | | |
|---|---|---|---|
| | A | B | C |
| 0 | 0 | 0 | 0 |
| 1 | 12.52 | 14 | 13.6 |
| 2 | 21.45 | 22.5 | 23.6 |
| 4 | 35.28 | 37.5 | 37.5 |
| 6 | 45.92 | 47.3 | 47.3 |

| | | | |
|---|---|---|---|
| 8 | 54.87 | 53.9 | 53.9 |
| 12 | 66.49 | 62.4 | 62.4 |
| 14 | 71.41 | 70.5 | 70.5 |
| 16 | 74.59 | 78.4 | 78.4 |

**Table 9 - 6 Dissolution Studies results initial development composition**

**6.5.3.2 Optimisation of core for achieving initial release**

Best possible answer to this issue of uniformity and initial release requirement was to use the drug in solution form, drug being soluble at basic pH further trail was conducted by using the dissolved form of drug containing Sodium Hydroxide and meglumine for binding of granules. For that 12 g of drug was dissolved in 80 ml aqueous solution of 2.25 g of sodium hydroxide & 25.5 g of meglumin and using it for binding of 81 gm of dry mix of Lactose Monihydrate & MCC using wruster coater. The above granules further mixed with extra granular matrix forming agent different grades of HPMC (40 # passed) for 5 minutes followed by talc (Sieved through 100 #), aerosol 200 (Sieved through 60 #) and magnesium stearate (Sieved through 100 #) and blending for 3 min after addition of each. The blend was compressed to tablets using a rotary tablet compression machine (General Machinary Company, India) fitted with 7 mm standard concave punches.

| | N | D | E | F | G |
|---|---|---|---|---|---|
| Lornoxicam | 16.00 | 16.00 | 16.00 | 16.00 | 16.00 |
| HPMC K15M CR | 17.00 | | | | |
| HPMC K100M CR | | 17.00 | | | |
| HPMC K100LV | | | 17.00 | | |
| HPMC E50LV | | | | 17.00 | |
| Polyox WSR 303 | | | | | 17.00 |
| PVP k 30 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Lactose Monohydrate | 54.25 | 54.25 | 54.25 | 54.25 | 54.25 |
| Micro crystalline Cellulose | 54.25 | 54.25 | 54.25 | 54.25 | 54.25 |
| Meglumin | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 |
| NaOH | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Mg. Stearate | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Purified Talc | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Aerosil 200 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 180.0 | 180.0 | 180.0 | 180.0 | 180.0 |

**Table 10 - 6 Initial release optimisation trial dissolution studies Composition**

Further trials were made to optimize the viscosity of matrix forming agent once initial burst effect was achieved mentioned in table 10-6 and subjected to dissolution studies as mentioned in table 7 -6, the results are summarized in table 11-6 and plotted as graph in figure 5-6.

Bulk uniformity for above mentioned composition was within 90 to 110 % and the RSD of 10 results was 3.45 %, 2.19, 2.74, 3.12 and 2.41 % with mean 102%, 101%, 100%, 99% and 102 % for composition N, D, E, F and G respectively. RSD for blend uniformity was targeted less than 5% was achieved. Drug uniformity in the powder blend using above mentioned procedure was achieved and desired initial release was observed and tablets were not showing mottling

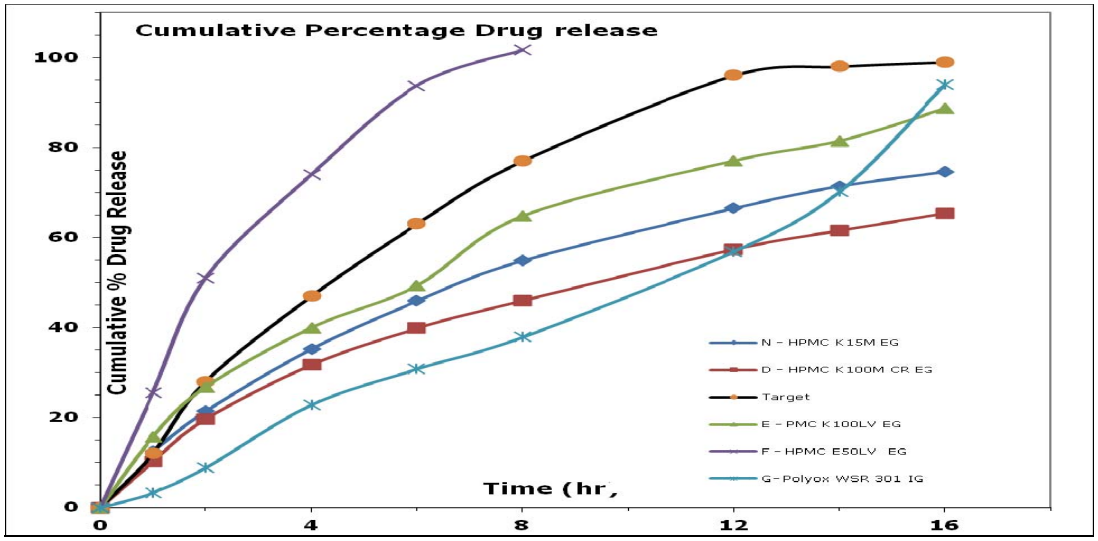



**Figure 5 - 6 Fast release part release optimisation trial dissolution studies graph**

| Time (hr) | Cumulative % Drug release | | | | |
|---|---|---|---|---|---|
| | N | D | E | F | G |
| **0** | 0 | 0 | 0 | 0 | 0 |
| 1 | 12.52 | 10.2 | 15.7 | 25.45 | 3.33 |
| 2 | 21.45 | 19.7 | 26.8 | 51 | 8.83 |
| 4 | 35.28 | 31.7 | 40 | 74 | 22.8 |
| 6 | 45.92 | 39.9 | 49.3 | 93.72 | 30.8 |
| 8 | 54.87 | 46 | 64.7 | 101.6 | 37.9 |
| 12 | 66.49 | 57.4 | 77 | | 56.9 |
| 14 | 71.41 | 61.6 | 81.4 | | 70.3 |
| 16 | 74.59 | 65.4 | 88.6 | | 94 |

**Table 11 - 6 Dissolution studies results fast release part optimisation trials**

**6.5.3.3 Optimisation of extended release part**

Trials were conducted to optimize the ratio of viscosity of matrix forming agent to optimize extended release effect once initial release profile was achieved composition is mentioned in table 12-6.

Formulation were subjected to dissolution studies as mentioned in table 7 -6, the results are summarized in table 13-6 and plotted as graph in figure 6-6.

| | H | I | J | K | L | M |
|---|---|---|---|---|---|---|
| Lornoxicam | 16.00 | 16.00 | 16.00 | 16.00 | 16.00 | 16.00 |
| HPMC K15M CR | 8.50 | | | 8.50 | 8.50 | |
| HPMC K100M CR | 8.50 | 8.50 | | | | 8.50 |
| HPMC K100LV | | 8.50 | 8.50 | | 8.50 | |
| HPMC E50LV | | | 8.50 | 8.50 | | 8.50 |
| PVP k 30 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Lactose Monohydrate | 54.25 | 54.25 | 54.25 | 54.25 | 54.25 | 54.25 |
| Micro crystalline Cellulose | 54.25 | 54.25 | 54.25 | 54.25 | 54.25 | 54.25 |
| Meglumin | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 |
| NaOH | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Mg. Stearate | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Purified Talc | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Aerosil 200 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 180.0 | 180.0 | 180.0 | 180.0 | 180.0 | 180.0 |

**Table 12 - 6 Extended release optimisation trial composition**

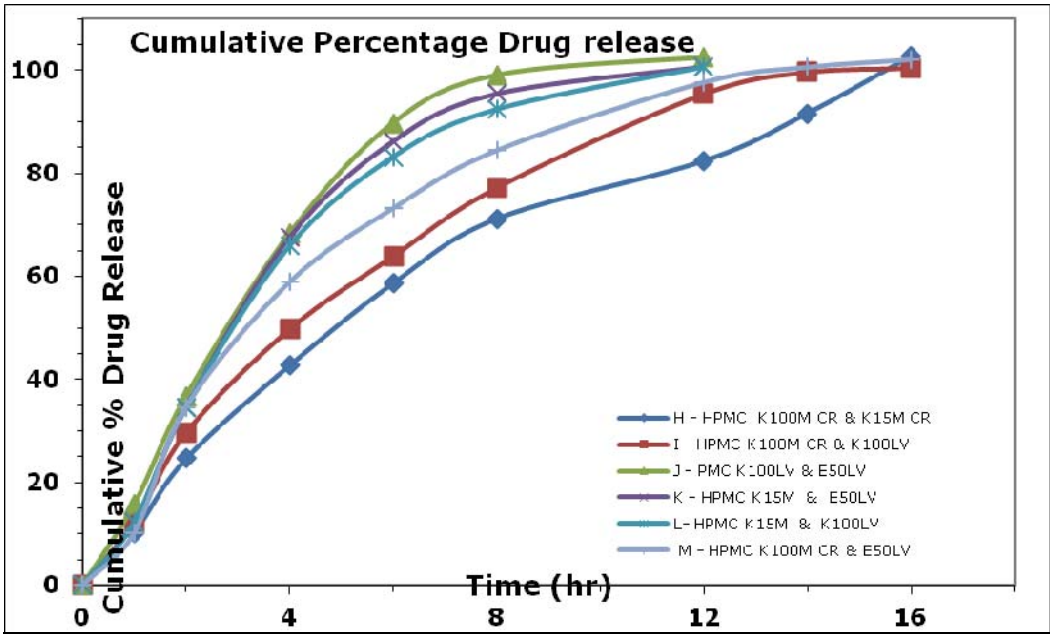



**Figure 6 - 6 Extended release optimisation trial dissolution studies graph**

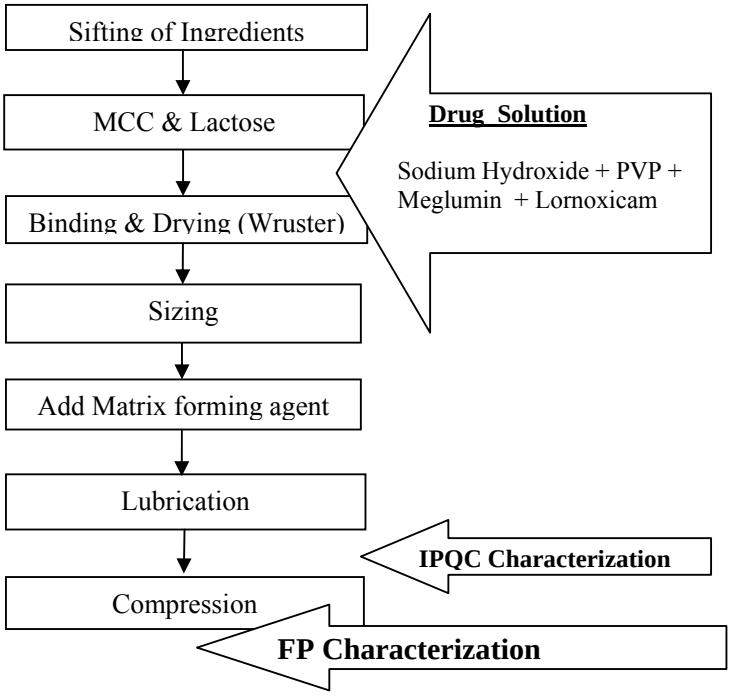
Bulk uniformity for above mentioned composition was within 90 to 110 % and the RSD of 10 results was 2.35 %, 3.16, 3.54, 2.42 and 3.13 %, 2.12 with mean 100%, 102%, 101%, 99%,102% and 103 % for composition H, I, J, K, L and M respectively. RSD for blend uniformity was targeted less than 5% was achieved. Drug uniformity in the powder blend using above mentioned procedure was achieved and desired initial release was observed and tablets were not showing mottling.

| Time (hr) | Cumulative % Drug release | | | | | |
|---|---|---|---|---|---|---|
| | H | I | J | K | L | M |
| **0** | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 10.214 | 12.2 | 15.7 | 12.21 | 12.2 | 10.21 |
| 2 | 24.65 | 29.7 | 36.8 | 34.65 | 34.7 | 34.65 |
| 4 | 42.732 | 49.7 | 68.3 | 67.52 | 65.9 | 58.87 |
| 6 | 58.675 | 63.9 | 89.7 | 86.16 | 83.2 | 73.16 |
| 8 | 71.16 | 77.2 | 99 | 95.38 | 92.4 | 84.38 |
| 12 | 82.38 | 95.4 | 102 | 100.6 | 101 | 97.6 |
| 14 | 91.6 | 99.6 | | | | 100.5 |
| 16 | 102.69 | 100 | | | | 102 |

**Table 13 - 6 Extended release optimisation trial dissolution studies results**

**Discussion & Conclusion:** Lornoxicam is poor solubility in aqueous solution drug optimisation trails were aimed at initial drug release profile and then extended release profile. The target release profile was achieved in optimised formulation 'I". During initial trial only meglumine was added to the formulation but that was not proven suffice for providing sufficient basic microenvironment so combination sodium hydroxide and meglumine were added with dissolved drug in binder solution. Quantity of extra-granular hydrophilic matrix forming agent was optimised to get release up to extended period of time. Formulation "I" containing equal amount of HPMC K100M CR and HPMC K100LV was showing optimum drug release consistently hence, that was considered for further characterisation studies. With good blend uniformity, initial release profile and extended release profile was achieved and tablets were not showing any motteling.

### *6.5.4 Process Flow Chart of the optimised process*




### *6.5.5 Unit Operations of the Preparation process*

**Sieving :** All the excipients were sieved before use to break the agglomerates sieve sizes used for sieving are mentioned in table 14 - 4.

Following sieve was used for the same.

| Excipients | Sieve Size |
|---|---|
| HPMC | 40 # |
| Lactose Monohydrate | 40 # |
| MCC 101 | 40 # |
| Polyethylene Oxide | 40 # |
| Mg. Stearate | 100 # |
| Talc | 100 # |
| Aerosil 200 | 60 # |

**Table 14 - 6 Sieving details of ingredients**

**Sieving :** Dry granules were again passed through 40# to break the agglomerates.

**Matrix forming agent**: HPMC was added to the granules and blended for 5 min poly bag for uniform distribution of HPMC.

**Lubrication :** Glident, lubricant and anti adherent were added in sequence respectively and blended for 3 min in poly bag to get uniform coating on the granules.

**Top spray granulation process ( Fluid bed processor)**

Binding, granulation and drying process varies with type of system, binding solution was added through top spray granulation process. Henceforth, palmglatt autocoater was used for granulation. For top spray granulation palmglatt requires minimum load of approx 100 gm.

**Coating process:** Coating process optimised on equal proportion powder mixture of Lactose Monohydrate and Microcrystalline cellulose.

**Solvent Selection**

Considering solubility of drug 0.62 M Sodium Hydroxide solution was used PVP k 30, meglumine and lornoxicam was added in it.

**Solid content**

| Binding Composition 389.50 gm (Total) | |
|---|---|
| Ratio within Solid content | |
| Sodium Hidroxide | 32.32 % w/w |
| PVP K 30 | 10.10 % w/w |
| Meglumin | 51.51 % w/w |

| | |
|---|---|
| Lornoxicam | 6.06 % w/w |
| **Ratio of Water : Solid** | |
| Water | 82.90 % w/w |
| Solid | 17.10 % w/w |

**Table 15 - 6 Composition of the binder solution**

**Binder solution preparations:**

Step 1: Dissolved sodium hydroxide in water.

Step 2: PVP k 30 dissolved in step – 1 Solution.

Step 3: Meglumin dissolved in step – 2 Solution.

Step 4: Drug dissolved in step – 3 Solution

Table 15-6 summaries composition of binder solution used in palm glatt wurster process the critical process parameters for the process are given in table 16-6, figure 7-6 shows schematic diagram of wurster coating process.

**Characterization of Granules :** Assay, Blend uniformity, Loss On drying, Bulk Density, Tapped Density, compressibility index.

**Compression :** Compression was done on 8 Station rotary tablet compression machine from general machinery company.

Compression Parameters :

Turret Speed : 9 RPM

Compaction Force: 4 – 5 kg

Thickness Adjustment Lever: Optimised to 3.1 to 3.3 mm thickness.

| Parameter | Value | Justification |
|---|---|---|
| Fluid nozzle (mm) | 0.7 | Fixed Machine Parameter |
| Inlet air temperature (°C) | 65 - 70 °C | Selected considering solvent to be evaporated |
| Out let air temperature (°C) | 50-55 °C | Output |

| | | |
|---|---|---|
| Product Temp (°C) | 55 - 60 °C | Curing |
| Atomizing air pressure ( $kg/cm^2$) | 0.6 - 0.8 $kg/cm^2$ | Studied on dummy |
| Inlet Opening | 50 - 70 | Studied on dummy |
| Spray rate (g/min) | 2.0 $\pm$ 0.5 | Observed optimum rate |
| Peristaltic Pump RPM | 9 $\pm$ 1 RPM | Observed optimum speed |
| Purging Time | 2 Sec | Observed optimum |

**Table 16 - 6 Fluid Bed processor critical process parameters (CPP)**

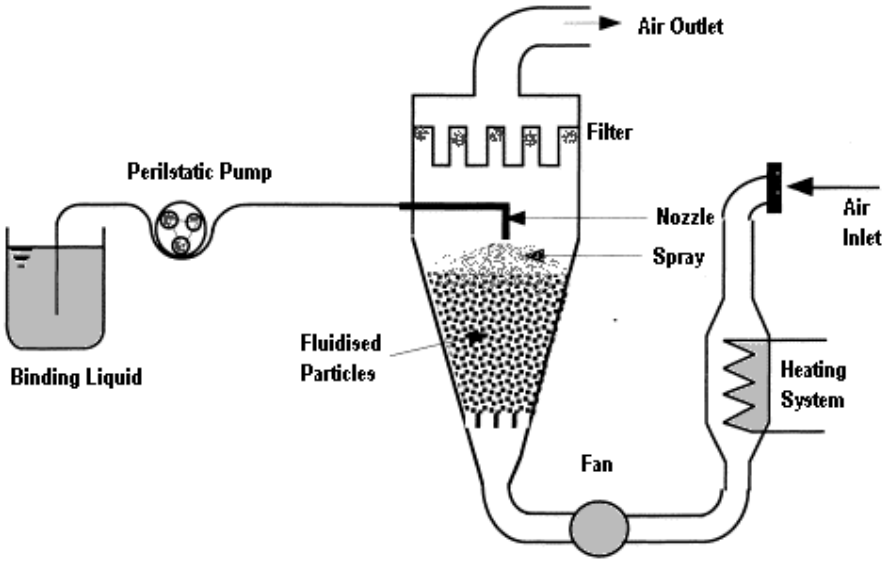



**Figure 7 - 6 Schematic top spray fluid bed processor**

**Spray Rate / Paristaltic Pump RPM:** Least achievable peristaltic pump speed was 6 RPM at which the spray was pulsative and Very high speed i.e. 16 RPM was resulting in very high spray rate which may result in non uniform distribution. On 8 to 10 RPM peristaltic pump speed was optimum to have continuous flow with minimum speed.

**Spray rate**

Spray rate is critical process parameter. Spray rate range 1.5 to 2.5 g/min was studied for it's impact on drug release, coaitng with consistent quality was produced at the 100 gm scale. Spray rate is a scale dependent parameter. The spray rate per nozzle shall be kept the same. The total spray rate can be increased to any fold by multiplying spray guns. However, a processing equipment can be change from a 6″ palm glatt.

**Product Temperature:**

An acceptable product temperature range was identified 55 - 60 °C. In the studied range, drug-layered granules with consistent quality were produced at the 100 gm scale. Spray drying and agglomeration were minimized. Product temperature is a scale-independent parameter and can be applied to other scales. The risk of product temperature to impact the assay of the drug-layered beads is low.

**Air volume**

Air volume range was identified and an optimal fluidization pattern was achieved. In the studied range, drug-layered beads with consistent quality were produced at the 100 gm scale. Air volume is a scale-dependent parameter.

**Atomization air pressure**

Atomization air pressure 0.6 - 0.8 kg/cm$^2$ was identified as critical process parameter affecting coating quality and so drug release critical quality attribute. The range of Atomization air pressure studied for consistent coating quality at the 100 gm scale. Atomization air pressure is an equipment-dependent parameter.

## 6.6 Results:

The results of characterization at pre-compression ( granules) stage for Assay, Bulk Uniformity (BU), Water content(WC), Loss On Drying ( LOD), Bulk Density (BD) & Tap Density(TD) are summarized in table 17-6.

The results of characterization after compression ( Core Tablet) for Description, Average Weight (mg), Assay (%), Water Content, Hardness (kg/cm2), Friability (% ), Thickness (mm), Diameter (mm), Uniformity of content are mentioned in table 18-6.

### *6.6.1 Pre-compression characterisation*

Bulk powder of the optimsed formulation before compression was subjected to characterization studies and results are mentioned in the table 17-6

| Parameter | Limits | Result |
|---|---|---|
| Assay | 90.00 to 110.00 % | 99.51 |
| Bulk Uniformity | Minimum 90 % | Minimum 97 % |
| | Maximum 110 % | Maximum 107 % |
| | Mean 95 % to 105 % | Mean 102.97 % |
| | RSD 5 % | RSD 3.16 % |
| LOD | NMT 3% | 2.4 % |
| Initial Bulk Density of powder before binding | 0.30 - 0.40 gm/ml | 0.34 gm/ml |
| Final Bulk Density of granules after wuruster coating | 0.40 - 0.50 gm/ml | 0.46 gm/ml |
| Tapped Density | 0.50 - 0.60 gm/ml | 0.54 gm/ml |
| Water Content | NMT 5 % | 2.8 |
| Hausner ratio | NMT 1.25 | 0.85 |
| Carr's Index | NMT 20 | 17.39 |

**Table 17 - 6 Characterization of optimised batch "I" bulk powder:**

Optimised formulation was subjected to characterization studies and results are tabulated in the table 18-6.

***6.6.2 Compressed Formulation***

| Parameters | Limit | Result |
|---|---|---|
| Description | Yellow colored, circular, biconvex, uncoated tablet. plain on both side | Complies |

| | | |
|---|---|---|
| Uniformity of Weight (mg) | Target - 180 mg<br>(180 ± 3%) | 181.83 mg/ tablet |
| Hardness (kg/cm$^2$) | 5-8 | 7 kg/cm$^2$ |
| Friability (% wt loss) | NMT 0.10 | 0.01 % |
| Thickness (mm) | 3.1 – 3.3 | 3.03 mm |
| Diameter (mm) | 7 | 7 mm |
| Dissolution (%) | 2 Hr – NMT 40 % | 29.7 |
| | 4 Hr – 40 to 60 % | 49.7 |
| | 8 Hr – 70 to 80 % | 77.2 |
| | 12 Hr – NLT 85 | 95.4 |
| Assay (%) | 95 to 105 % | 100.20 % |
| Water Content | NMT 5% | 2.4 |
| Content Uniformity (%) | Minimum : 90.00 % | Minimum : 100.51 |
| | Maximum : 110.00 % | Maximum : 102.18 |
| | Average : 95 to 105 % | Average : 101.37 |
| | % RSD :<br>(RSD NMT 5%) | % RSD : 3.86 |

**Table 18 - 6 Characterization of optimised batch "I" Tablet:**

### *6.6.3 Effect of agitation intensity (RPM)*

Optimised formulation was verified for impact of agitation intensity (RPM) on drug release in USP type II (Paddle) dissolution test apparatus 900 ml PBS pH 7.5 as release media, impact of dissolution media volume on drug release was verified using USP type II (Paddle ) dissolution test apparatus PBS pH 7.5 as media at 50 RPM, figure 8 - 6 and figure 9 – 6 represents graphical representation of the results obtained and results are tabulated in table 19 - 6 and table 20 - 6.

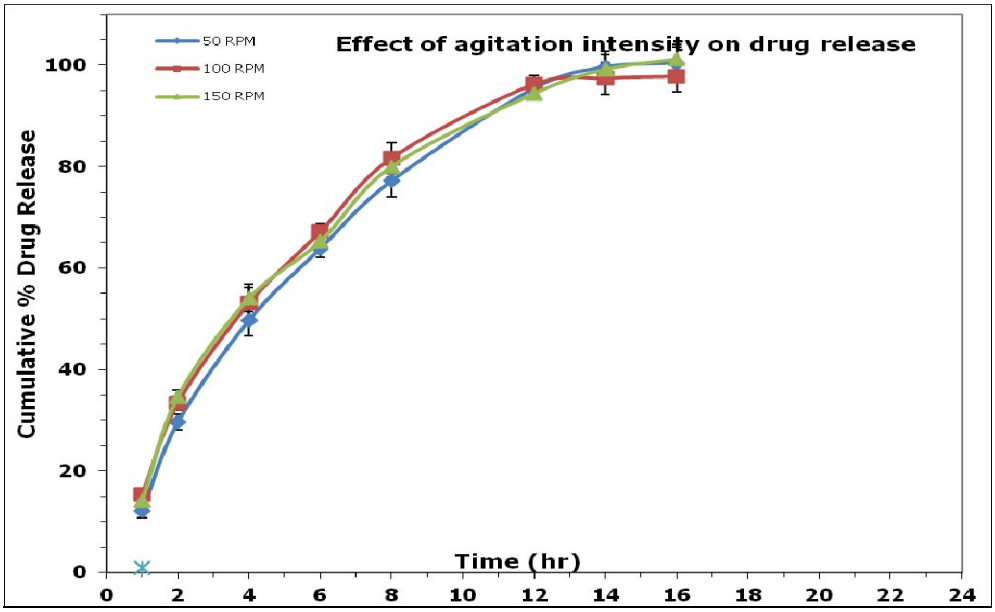



**Figure 8 - 6 Effect of Agitation intensity graph**

| Time (hr) | 50 RPM | SD | 100 RPM | SD | 150 RPM | SD |
|---|---|---|---|---|---|---|
| 1 | 12.21 | 1.38 | 15.37 | 1.27 | 14.21 | 1.67 |
| 2 | 29.65 | 1.47 | 33.41 | 1.34 | 34.65 | 1.27 |
| 4 | 49.73 | 3.07 | 53.05 | 3.03 | 54.05 | 2.70 |
| 6 | 63.87 | 1.79 | 67.14 | 1.55 | 65.25 | 1.19 |
| 8 | 77.16 | 3.16 | 81.63 | 3.11 | 79.99 | 2.03 |
| 12 | 95.38 | 1.96 | 96.18 | 1.74 | 94.38 | 1.19 |
| 14 | 99.60 | 3.17 | 97.39 | 3.10 | 99.13 | 2.93 |
| 16 | 100.39 | 3.17 | 97.74 | 3.00 | 101.14 | 2.93 |

**Table 19 - 6 Effect of agitation intensity results**

**Discussion :** It needs to be verified that if developed hydrophilic matrix formulation gives constant drug release irrespective of agitation intensity. The drug release from optimised formulation was confirmed at various agitation speed i.e. 50, 100, and 150 RPM.

It is clearly evident from (table 19-6) that the release from hydrophilic matrix formulation is independent of the agitation intensity. The *f*1 and *f*2 values were found to be *f*1 4.36 and *f*2 74.17 (between 50 RPM and 100 RPM), *f*1 2.55 and *f*2 83.69 (between 100 RPM and 150 RPM) for *f*1 3.27 and *f*2 76.87 (between 50 RPM and 150 RPM).

Hence, it can be expected that the release from the developed hydrophilic matrix formulation will not have much impact of agitation conditions at the absorption site.

### *6.6.4 Effect of dissolution volume*

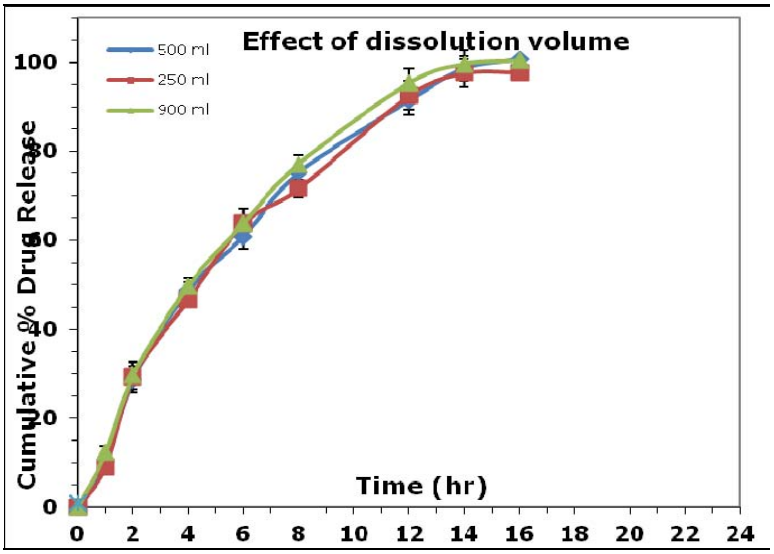



**Figure 9 - 6 Effect of Dissolution Volume Graph**

| Time (hr) | 500 ml | SD | 250 ml | SD | 900 ml | SD |
|---|---|---|---|---|---|---|
| **0** | 0.00 | | 0.00 | | 0.00 | |
| 1 | 10.21 | 1.30 | 9.14 | 1.38 | 12.21 | 1.38 |
| 2 | 28.65 | 1.43 | 29.18 | 1.60 | 29.65 | 1.47 |
| 4 | 48.73 | 2.97 | 46.73 | 3.35 | 49.73 | 3.07 |
| 6 | 60.87 | 1.77 | 63.87 | 1.79 | 63.87 | 1.79 |
| 8 | 75.16 | 2.80 | 71.63 | 3.16 | 77.16 | 3.16 |
| 12 | 91.38 | 1.50 | 92.62 | 1.96 | 95.38 | 1.96 |
| 14 | 98.60 | 3.08 | 97.60 | 3.17 | 99.60 | 3.17 |
| 16 | 100.63 | 2.85 | 97.82 | 3.17 | 100.39 | 3.17 |

**Table 20 - 6 Effect of dissolution volume results**

**Discussion:** It needs to be verified that if developed hydrophilic matrix formulation gives constant drug release irrespective of volume if sink conditions are maintained. The drug release from optimised formulation was confirmed at different release volume i.e. 250, 500, and 900 ml.

It is clearly evident from (table 20-6) that the release of LOR from hydrophilic matrix formulation is independent of the dissolution volume. The *f*1 and *f*2 values were found to be *f*1 2.99 and 81.16 (between 500 ml and 250 ml), *f*1 2.70 and 81.48 (between 900 ml and 500 ml) and *f*1 3.82 and *f*2 75.63 (between 900 ml and 250 ml).

Hence, it can be expected that the release from the developed formulation will not have drastic impact of the volume available if sink conditions are maintained at the absorption site.

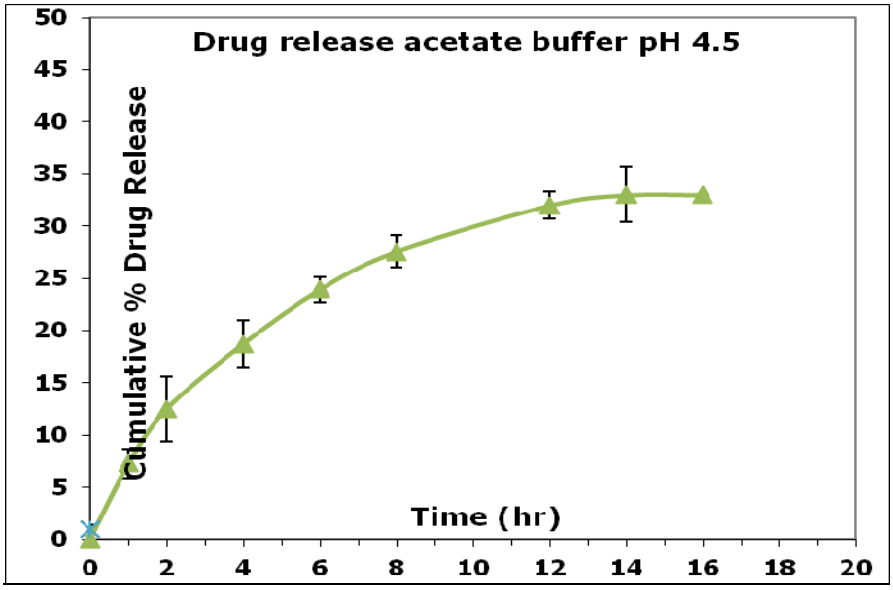



**Figure 10 - 6 Drug release acetate buffer**

Effect of pH on drug release : Drug shows pH dependent solubility having no solubility in water and acidic pH, good solubility at basic pH which was known fact since beginning of the development and affected the manufacturing process so the dissolution studies are done in PBS pH 7.5 an attempt was made to perform dissolution studies at different pH i.e acetate buffer pH 4.5, figure 10-6. Limited drug release up to 30% due to non sink condition observed.

Lornoxicam (pKa 4.7) shows pH dependent solubility having poor solubility in the acidic pH henceforth, multimedia testing at pH 1.2 was not verified, limited drug release up to 30% due to non sink condition observed at pH 4.5 and to maintain the sink condition dissolution was carried out in PBS 7.5 pH.

### 6.6.5 Dose Dumping Study

**Discussion :** Dose dumping is most commonly seen in drugs taken orally and digested in the gastrointestinal tract. Around the same time patients take their medication, they may also ingest other substances like fatty meals or alcohol that increase drug delivery. The substances may act on the drug product to speed up drug release, or they may stimulate

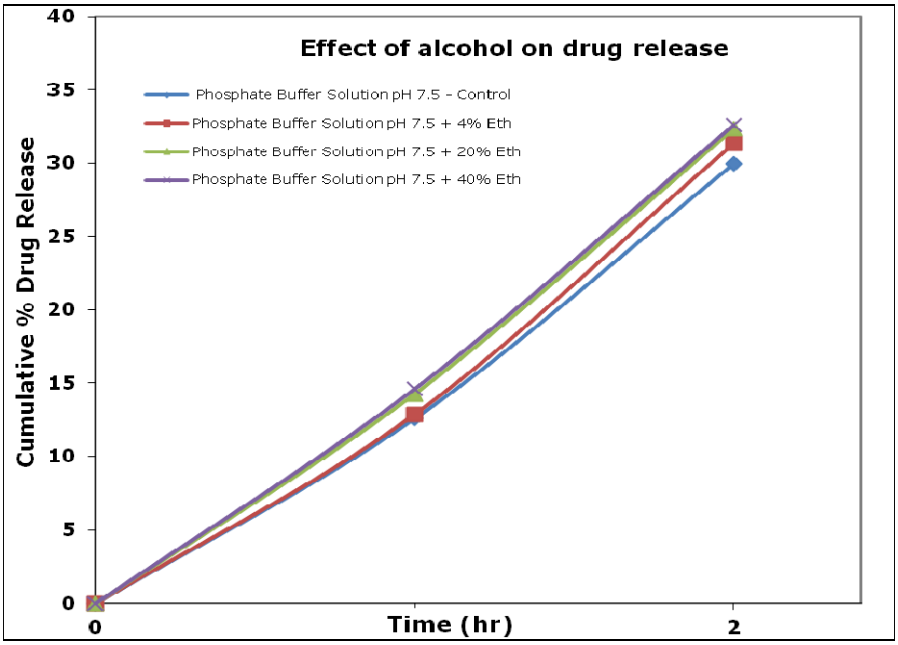



**Table 21 - 6 Drug release in presence of various concentration alcohol**

the body's absorptive surfaces to increase the rate of drug uptake. Developed formulation was subjected to ethanol induced dose dumping study to emulate a "worst case" scenario. Figure 21-6 the graph showing drug release under various concentration of ethanol. It was observed that no major impact of alcohol on drug release. Hence, it is expected that accidental co-administration of alcohol will not lead to any dose dumping.

***Curve fitting analysis***

In order to describe the kinetics of drug release from controlled release preparations various mathematical equations have been proposed in past. Release data obtained was applied to different release models in order to establish the drug release mechanism and kinetics. Best goodness of fit test (R2) was taken as criteria for selecting the most appropriate model. For calculation of best model Microsoft excel was used. The values are tabulated in table 22-6.

| **Model** | Zero Order | First Order | Higuchi Model | Peppas. |
|---|---|---|---|---|
| **Calculated R2** | 0.9838 | 0.7863 | 0.9405 | 0.9676 |

**Table 22 - 6 Calculated R2 Values for Developed formulation for different models**

Discussion: Calculated R2 for Zero order of drug release is nearest to 1, It can be concluded that the drug release from developed formulation gives constant drug release.

**6.7 Result of Development studies**

**Optimised Dosages Form:**

Hydrophilic matrix formulation is developed as Yellow colored matrix, circular, biconvex uncoated tablet plain on both sides containing 16mg of Lornoxicam for oral administration.

**Formulation Details:**

Yellow colored matrix , circular, biconvex uncoated tablet plain on both sides containing 16mg of Lornoxicam and following are inactive ingredients Lactose Monohydrate, Microcrystalline Polyvinyl Pyrrolidone, Magnesium Stearate, Silicon Dioxide, HPMC, Polyethylene Oxide, Sodium Hydroxide and Meglumine and water as solvent which shall not be part of final product.

**Packing Profile:**

Dosage form to be packed in HDPE Bottle pack of 100s.

***6.7.1 Components of the Developed Drug Product:***

The quantitative composition ( per tablet and % W/W), compandial status and function of each component used in the developed drug product is provided in table 23-6.

| Formula ingredients | Specification | Function(s) | Quantity (mg/tablet) | Quantity (% w/w) |
|---|---|---|---|---|
| **Binding Stage :** | | | | |
| Lornoxicam | USP | API | 16.00 | 8.89 |
| PVP K 30 | NF | Binder | 5.00 | 2.78 |
| Lactose Monohydrate | USP NF | Filler | 54.25 | 30.14 |
| Micro crystalline Cellulose * | USP NF | Filler | 54.25 | 30.14 |
| Meglumin | USP NF | pH modifier - Alkalizing agent | 25.50 | 14.17 |
| NaOH | USP NF | pH modifier - Alkalizing agent | 3.00 | 1.67 |
| Distilled Water** | IH | Processing Solvent | Q.s. | Processing Solvent |
| | | | 158.00 | 87.78 |
| **Addition of extra-granular Hydrophilic Matrix forming agent** | | | | |
| HPMC K100M CR | USP NF | Matrix Forming agent | 8.50 | 4.72 |
| HPMC K100LV | USP NF | Matrix Forming agent | 8.50 | 4.72 |

| Formula ingredients | Specification | Function(s) | Quantity (mg/tablet) | Quantity (% w/w) |
|---|---|---|---|---|
| Total weight of core tablet | | | 175.00 | 97.22 |
| **Lubrication Stage :** | | | | |
| Mg. Stearate | USP NF | Glidant | 2.00 | 1.11 |
| Talc/Purified Talc | USP NF | Antiadherent | 2.00 | 1.11 |
| Colloidal silicon dioxide | USP NF | Lubricant | 1.00 | 0.56 |
| **Total weight of core tablet** | | | **180.00** | **100.00** |

**Table 23 - 6 Optimised Composition of Lornoxicam tablet (mg/tablet)**

* Quantity is compensated depending on the potency of Lornoxicam to maintain tablet weight constant.** Used as processing solvent, does not remain in the final product.

### *6.7.2 Coating process*

Ratio of solids used in coating solution is Critical Process parameter and it has direct impact on drug release table 24-6 mentions binding /coating solution composition.

| **Binding Composition 389.50 gm (Total)** | |
|---|---|
| **Ratio within Solid content** | |
| Sodium Hidroxide | 32.32 % w/w |
| PVP K 30 | 10.10 % w/w |
| Meglumin | 51.51 % w/w |
| Lornoxicam | 6.06 % w/w |
| **Ratio of Water : Solid** | |
| Water | 82.90 % w/w |
| Solid | 17.10 % w/w |

**Table 24 - 6 Optimised Coating/Binding Solution composition**

### *6.7.3 Critical Quality Attributes: (CQAs)*

1. Blend Uniformity
2. Uniformity of weight
3. Assay
4. Dissolution
5. Uniformity of Dosage unit
6. Stability

### *6.7.4 Critical Process Parameters (CPPs)*

1. Spray Rate / Paristaltic Pump RPM
2. Product Temperature:
3. Air volume
4. Spray rate
5. Atomization air pressure
6. Compression ( Speed, Compression force)

## 6.8 Conclusion:

Robust, 16 mg of Lornoxicam formulation is developed which gives drug release independent of agitation intensity and dissolution volume, retarding the drug release through hydrophilic matrix formulation. The optimised formulation will subjected to stability studies and *in-vivo* studies.

6.9 References:


- Yassin el-said hamza, and mona hassan aburahma Pharmaceutical Development and Technology, 2010; 15(2): 139–153 Research article "Design and in vitro evaluation of novel sustained Release Matrix tablets for lornoxicam based on the combination Of hydrophilic matrix formers and basic pH modifiers"
- D. Sandeep kumar, K. Shashidhar Reddy, Amit kumar tiwari And Suddhasattya Dey Vol.1, Issue-4, Oct-Dec 2010 International Journal of Pharma and Bio Sciences. "Design and evaluation of buccal patches of Lornoxicam"
- AAPS PharmSciTech Nov, 2009 Research Article " Design and *In Vitro* Evaluation of Novel Sustained-Release Double-Layer Tablets of Lornoxicam: Utility of Cyclodextrin and Xanthan Gum Combination Yassin El-Said Hamza and Mona Hassan Aburahma
- Pruss TP, Slroissnig H, Radhofer-Welte S, et al. Overview of the pharmacological properties, pharmacokinetics and animal safety assessment of lornoxicam. ostgrad Med J 1990; 66 (4): 18-21.
- Young Hoon Kim, Hye Young Ji, Eun-Seok Park , Soo Wan Chae , and Hye Suk Lee Arch Pharm Res Vol 30, No 7, 905-910, 2007 "Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric Determination of Lornoxicam in Human Plasma"
- Sameer Sheaikh, A.V. Chandewar Asian Journal of Biomedical and Pharmaceutical Sciences 3(17) 2013, 14-19 "Oral Osmotic System for Delivery of Lornoxicam: Development and *In Vitro* Charecterization"
- Mahrous O. Ahmed* and Abdullah A. Al-Badr Chapter 6 " Lornoxicam"
- Prasad Byrav D S, B Medhi, A Prakash, S Patyar, S Wadhwa IJPMR 2009; 20 (1):27-31 Lornoxicam: a Newer NSAID
- Y. Gao, J. J. Zhang, W. M. Fan, B. J. Ren, Q. N. Ping "Solubilization study of lornoxicam for parenteral formulation" School of Pharmacy, China Pharmaceutical University, China, Zhenyuan Quality Drug Development Laboratory, China
- D. M. Bramhane, N. S. Saindane, P. R. Vavia. J Incl Phenom Macrocycl Chem (2011) 69:453–460 "Inclusion complexation of weakly acidic NSAID with b-cyclodextrin: selection of arginine, an amino acid, as a novel ternary component"

- Young Hoon Kim, Hye Young Ji, Eun-Seok Park 1, Soo-Wan Chae 2, and Hye Suk Lee Arch Pharm Res Vol 30, No 7, 905-910, 2007 "Liquid Chromatography-Electrospray Ionization Tandem MassSpectrometric Determination of Lornoxicam in Human Plasma"
- F. Langenbucher & J. Mysicka Br. J. clin. Pharmac. (1985), 19, 151S-162S In vitro and in vivo deconvolution assessment of drug release kinetics from oxprenolol Oros preparations
- Yassin El-Said Hamza and Mona Hassan Aburahma AAPS Pharm Sci Tech, Vol. 10, No. 4, December 2009 "Design and In Vitro Evaluation of Novel Sustained-Release Double-Layer Tablets of Lornoxicam: Utility of Cyclodextrin and Xanthan Gum Combination".

- Hall PE, Derry S, Moore RA, McQuay HJ "Single dose oral lornoxicam for acute postoperative pain in adults (Review)" The Cochrane collaborations Wiley Publishers

- Mona Hassan Aburahma, and Yassin El-Said Hamza *Pharmaceutical Development and Technology*, 2010, 1–15 Novel sustained-release fast-disintegrating multi-unit compressed tablets of lornoxicam containing Eudragit RS coated chitosan-alginate beads

- Yassin El-Said Hamza and Mona Hassan Aburahma *Drug Development and Industrial Pharmacy*, 2010; 36(3): 337–349 "Innovation of novel sustained release compression-coated tablets for lornoxicam : formulation and in vitro investigations"

- US patent : 6599529 B1 "Modified release Multiple unit compositions of the " Non steroidal anti inflammatory drug substances ( NSAIDS)"

- European patents EP 1017370B1 "Modified Release Multiple-Units Compositions Of Non-Steroid Anti-Inflammatory Drug Substances (Nsaids)"
- US patent US 2006/0024365 A1 "NOVEL DOSAGE FROM"

## 7.0 STABILITY STUDIES

Stability of a drug has been defined as the ability of a particular formulation, in a special container, to remain within its physical, chemical, therapeutic and toxicological specifications. The period of stability of a pharmaceutical preparation may be defined as the time from the date of manufacture of the formulation until its chemical or biological activity is lost below 90 % of labeled potency and its physical characteristic have not changed appreciably or deleteriously

The stability studies were carried out in accordance with the ICH guidelines for stability. The samples were subjected to stability in HDPE bottles with induction seal. The samples were subjected to stability at 40$^{o}$C/75%RH (accelerated stability) for 1, 2, 3, 6months and at 25$^{o}$C/60%RH (controlled room temperature) at 3, 6, 9, 12months as mentioned in table 1-7. The parameters studied are the drug content (Assay), average weight, dissolution studies, description and water by KF.

| Condition | 1Month | 2Month | 3Month | 6Month | 9Month | 12Month |
|---|---|---|---|---|---|---|
| 40$^{o}$C/75%RH | √ | √ | √ | √ | -- | -- |
| 25$^{o}$C/60%RH | -- | -- | √ | √ | √ | √ |

**Table 1 - 7 List of stability Stations**

### 7.1 Materials and Methods

Accelerated and long term stability studies were carried out on the optimized formulations. To access the stability, optimized formulations were stored at 40 ± 2°C /75 ± 5% RH for 1, 2, 3, 6months and at 25 ± 2°C /60 ± 5% RH for 3, 6, 9, 12months and were observed for physical change (appearance), drug content, dissolution, average weight and water by KF. The method of stability studies was in conformity with the recommendations in WHO document pertaining to stability testing of products intended for global market (Mathews, 1999) and ICH guidelines. The samples were withdrawn periodically and evaluated for physical change (appearance), drug content, dissolution, average weight and water by KF. (Mathews, 1999; Verma et al., 2003)

### 7.2 Pack Style

The selected formulations were packed in 60cc HDPE bottle with induction seal. The packed formulations were stored upto 6 months in ICH certified stability chambers (40 °C / 75 % RH) and upto 12months (25 °C / 60 % RH).

### 7.3 Evaluation of the Samples

The samples were withdrawn at specified intervals and evaluated for the appearance, drug content, average weight, dissolution and water by KF. Drug content, Dissolution was determined by specific methods such as UV/HPLC methods given in chapter 3(for butorphanol) and chapter5 (for lornoxicam) for the respective drug.

### 7.4 In-house Specifications and Limits

For determining the physical changes, drug content, water by KF, average weight and dissolution in-house specifications were generated as mentioned below in table 2-7 and 3-7.

| **Description** | **(%) Assay** | **Avereage weight (mg)** | **% drug dissolved** | | | | | **Water Content** |
|---|---|---|---|---|---|---|---|---|
| | | | **2 Hr** | **4 Hr** | **8 Hr** | **14 Hr** | **24 Hr** | |
| White colored, circular, biconvex, film coated tablet, plain on both side | 95.00 to 105.00 % | 245 ± 3% | NMT 15% | 10-30% | 30-55% | 50-80% | NLT 80% | NMT 5% |
| **Complies** | 101.62 | 242.60 | 9.1 | 20.3 | 36.2 | 58.8 | 91.3 | 2.9 |

**Table 2 - 7 Initial results Butorphanol Tartrate**

| **Description** | **(%) Assay** | **Avereage weight (mg)** | **% Drug Dissolved** | | | | **Water Content** |
|---|---|---|---|---|---|---|---|
| | | | **2 Hr** | **4 Hr** | **8 Hr** | **12 Hr** | |
| Yellow colored, circular, biconvex, film coated tablet, plain on both side | 95.00 to 105 % | 180 ± 3% | NMT 40% | 40 - 60% | 70-80% | NLT 85% | NMT 5% |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Complies** | 100.2 | 181.83mg | 29.7 | 49.70 | 77.20 | 95.40 | 2.4 |

**Table 3 - 7 Initial results of Lornoxicam**

### 7.5 Results

**STABILITY DATA OF BUTORPHANOL TARTARATE EXTENDED RELEASE TABLETS 40°C/75%RH:**

| Stability condition | Specifications | Description | (%) Assay | Avereage weight | % drug dissolved | | | | | Water Content | F2 (With Respect to initial) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2 Hr | 4 Hr | 8 Hr | 14 Hr | 24 Hr | | |
| | Pack Style | | 95.00 to 105.00% | 245 ± 3% | NMT 15% | 10-30% | 30 to 55 | 50 to 80 | NLT 80 | NMT 5% | |
| **Initial** | HDPE Bottle | White colored, circular, biconvex, film coated tablet, plain on both side | 101.62 | 242.60mg | 9.1 | 20.3 | 36.2 | 58.8 | 91.3 | 2.9 | Not Applicable |
| **1M 40°C/75%RH** | HDPE Bottle | Complies | 100.92 | 246.70mg | 8.1 | 24.5 | 34.9 | 59.6 | 95.1 | 2.8 | 80 |
| **2M 40°C/75%RH** | HDPE Bottle | Complies | 100.56 | 241.32mg | 12.3 | 21.2 | 38.8 | 56.7 | 92.4 | 3.2 | 81 |
| **3M 40°C/75%RH** | HDPE Bottle | Complies | 99.98 | 240.61 | 10.2 | 20.1 | 37.7 | 59.1 | 90.3 | 3.4 | 90 |
| **6M 40°C/75%RH** | HDPE Bottle | Complies | 101.2 | 243.91 | 11.4 | 24.5 | 34.2 | 56.2 | 89.2 | 3.1 | 77 |

**Table 4 - 7 STABILITY DATA OF BUTORPHANOL TARTARATE EXTENDED RELEASE TABLETS 40°C/75%RH**

| Storage Condition | Specifications | Description | (%) Assay | Avereage weight | % drug dissolved | | | | | Water Content | F2 (With Respect to initial) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2 Hr | 4 hr | 8 Hr | 14 Hr | 24 Hr | | |
| | Pack Style | | 95.00 to 105.00% | 245 ± 3% | NMT 15% | NMT 5% | 30 to 55 | 50 to 80 | NLT 80 | NMT 5% | |
| **3M 25°C/60%RH** | HDPE Bottle | Complies | 99.92 | 247.82 | 7.1 | 22.4 | 37.3 | 58.2 | 93.2 | 3.2 | 87 |
| **6M 25°C/60%RH** | HDPE Bottle | Complies | 100.22 | 241.6 | 10.2 | 23.7 | 40.4 | 59.1 | 90.5 | 2.8 | 76 |
| **9M 25°C/60%RH** | HDPE Bottle | Complies | 99.1 | 243.47 | 8.2 | 22.8 | 39.5 | 59.7 | 87.8 | 3.0 | 79 |
| **12M 25°C/60%RH** | HDPE Bottle | Complies | 99.62 | 244.58 | 9.4 | 22.3 | 37.4 | 56.2 | 90.5 | 3.1 | 87 |

**Table 5 - 7 STABILITY DATA OF BUTORPHANOL TARTARATE EXTENDED RELEASE TABLETS 25°C/60%RH**

Table 4-7 and 5-7 respectively shows stability data for BT up to 6M 40°C/75%RH and 12M 25°C/60%RH. Figure 1-7 and 2-7 respectively shows stability data up to 12M 25°C/60%RH and 6M 40°C/75%RH.

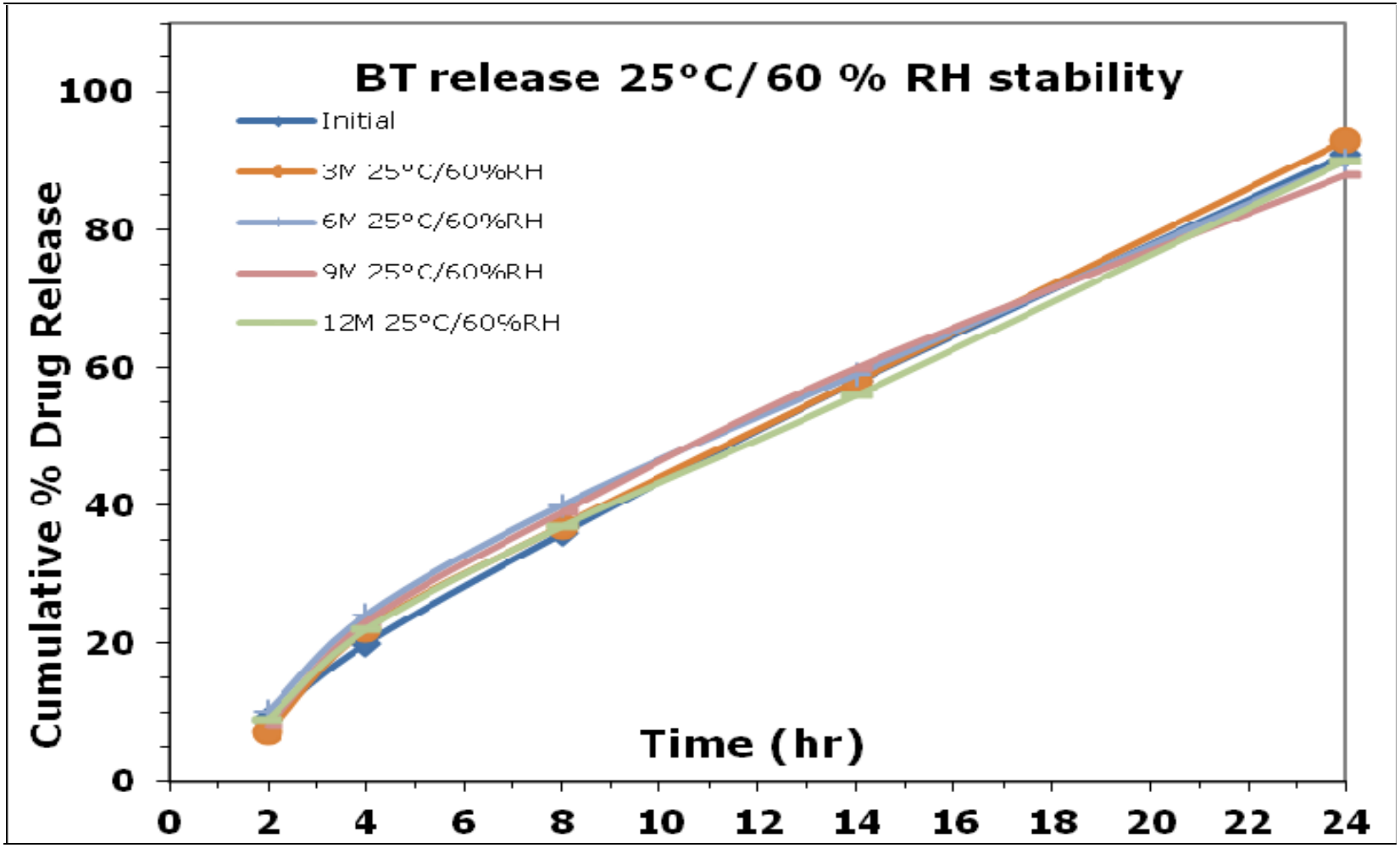



**Figure 1 - 7 STABILITY GRAPH OF BUTORPHANOL TARTARATE ER TABLETS 25°C/60%RH**

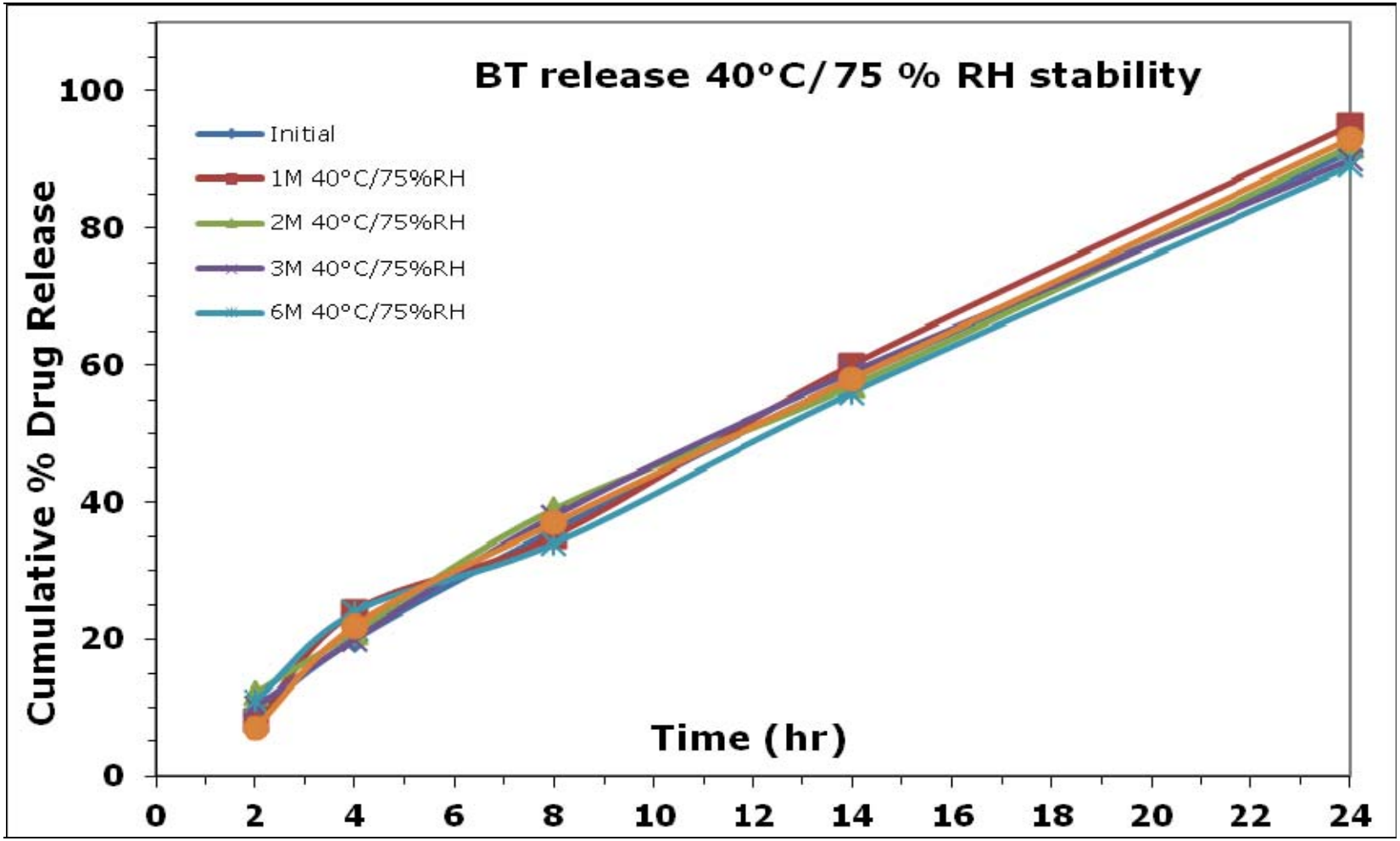



**Figure 2 - 7 STABILITY GRAPH OF BUTORPHANOL TARTARATE ER TABLETS 40°C/75%RH**

**STABILITY DATA OF LORNOXICAM EXTENDED RELEASE TABLETS:**

| Stability condition | Specifications | Description | (%) Assay | Avereage weight | % Drug Dissolved | | | | Water Content | F2 (With Respect to initial) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2 Hr | 4 Hr | 8 Hr | 12 Hr | | |
| | Pack Style | | 95.00 to 105 % | 180 ± 3% | NMT 40% | 40 to 60% | 70-80% | NLT 85 | NMT 5% | |
| **Initial** | HDPE Bottle | Yellow colored, circular, biconvex, film coated tablet, plain on both side | 100.2 | 181.83mg | 29.7 | 49.70 | 77.20 | 95.40 | 2.4 | |
| **1M 40°C/75%RH** | HDPE Bottle | Complies | 99.8 | 180.64mg | 32.1 | 53.2 | 75.3 | 92.8 | 2.7 | 80 |
| **2M 40°C/75%RH** | HDPE Bottle | Complies | 99.4 | 182.9mg | 26.4 | 50.9 | 78.1 | 94.2 | 2.6 | 81 |
| **3M 40°C/75%RH** | HDPE Bottle | Complies | 100.9 | 178.6mh | 28.6 | 54.3 | 75.2 | 95.1 | 2.4 | 80 |
| **6M 40°C/75%RH** | HDPE Bottle | Complies | 99.8 | 183.62mg | 29.7 | 52.1 | 76.1 | 92.2 | 3.2 | 84 |

**Table 6 -7 STABILITY DATA OF LORNOXICAM ER TABLETS 40°C/75%RH**

| Stability condition | Specifications | Description | (%) Assay | Avereage weight | % Drug Dissolved | | | | Water Content | F2 (With Respect to initial) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2 Hr | 4 Hr | 8 Hr | 12 Hr | | |
| | Pack Style | | 95.00 to 105 % | 180 ± 3% | NMT 40% | 40 to 60% | 70-80% | NLT 85 | NMT 5% | |
| **3M 25°C/60%RH** | HDPE Bottle | Complies | 100.6 | 182.87mg | 28.1 | 48.1 | 74.3 | 96.2 | 2.5 | 82 |
| **6M 25°C/60%RH** | HDPE Bottle | Complies | 99.5 | 180.94mg | 31.7 | 52.1 | 77.7 | 94.5 | 2.6 | 86 |
| **9M 25°C/60%RH** | HDPE Bottle | Complies | 98.9 | 184.35mg | 30.3 | 52.8 | 76.5 | 92.3 | 3.0 | 81 |
| **12M 25°C/60%RH** | HDPE Bottle | Complies | 99.6 | 185.82mg | 34.1 | 54.4 | 76.9 | 94.4 | 2.9 | 76 |

**Table 7 - 7 STABILITY DATA OF LORNOXICAM ER TABLETS 25°C/60%RH**

Table 6-7 and 6-7 respectively shows stability data up to for LOR 6M 40°C/75%RH and 12M 25°C/60%RH. Figure 3-7 and 4-7 respectively shows stability data up to 12M 25°C/60%RH and 6M 40°C/75%RH.

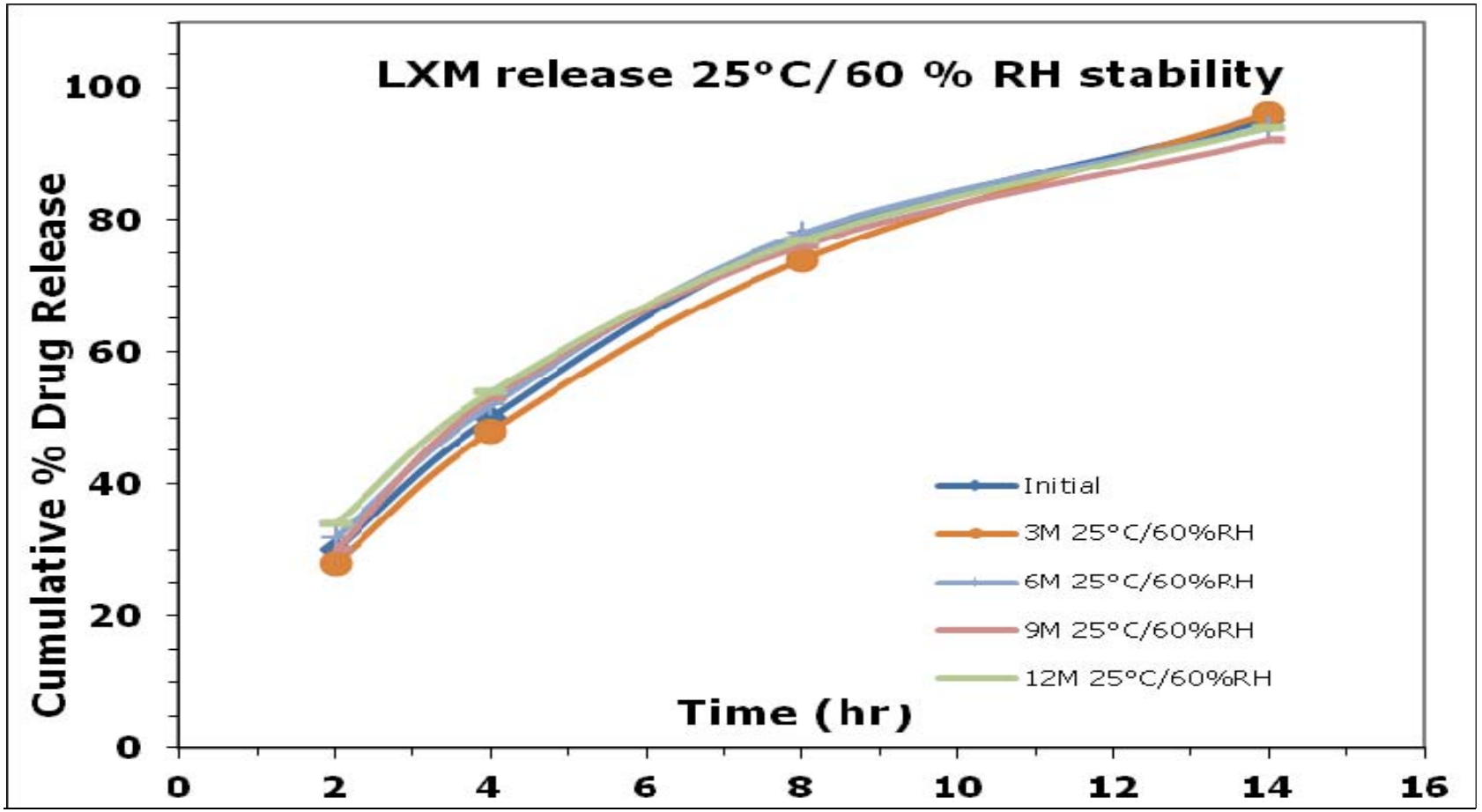



**Figure 3 - 7 STABILITY GRAPH OF LORNOXICAM ER TABLETS 25°C/60%RH**

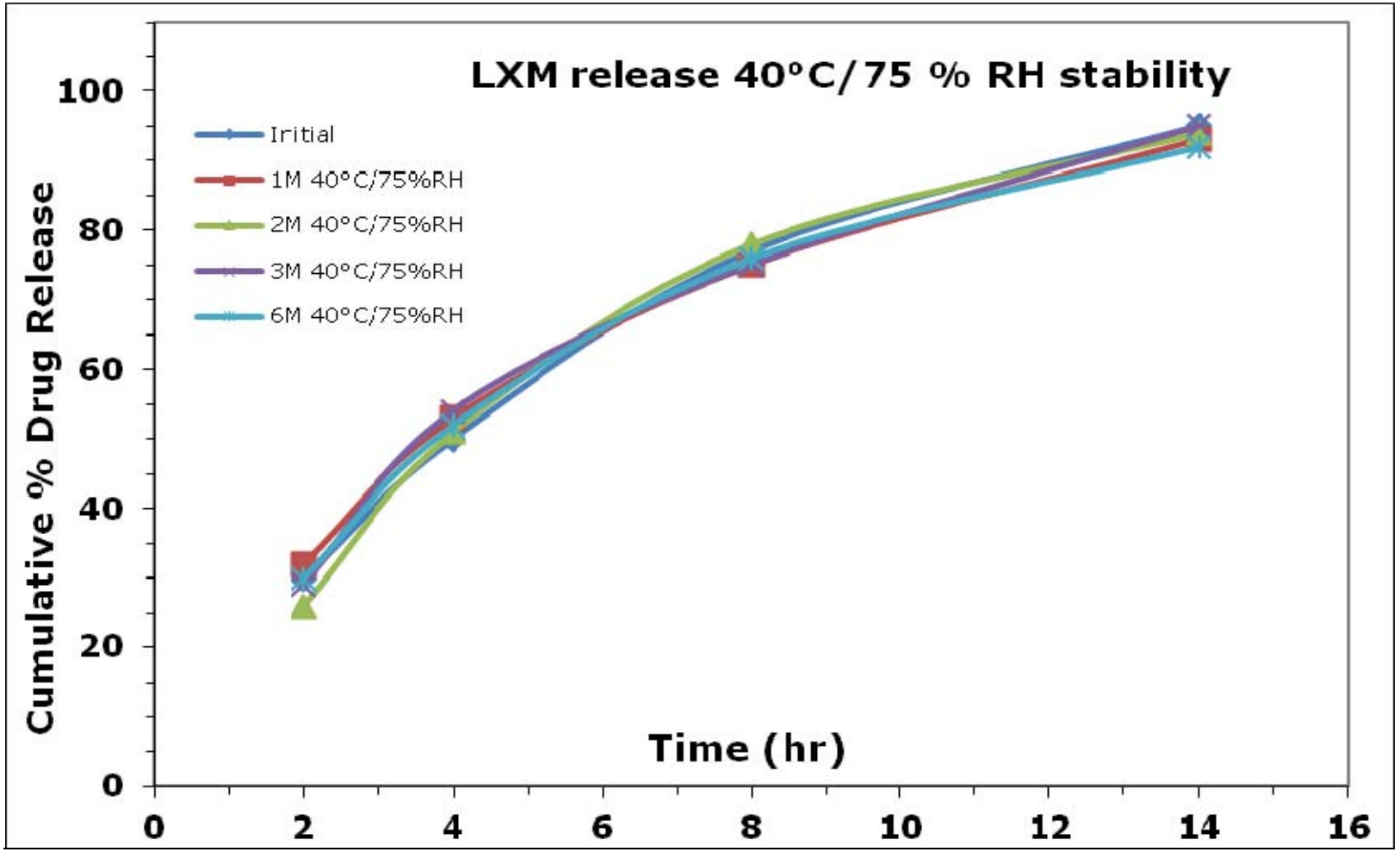



**Figure 4 - 7 STABILITY GRAPH OF LORNOXICAM ER TABLETS 40°C/75%RH**

### 7.6 Discussion

During and after the completion of the stability studies, all the studied parameters like description, assay, water by KF, average weight were found to be within the predefined limits. It was also observed that the drug release studies showed no significant change in the dissolution (with respect to the initial dissolution). The graphs of the dissolution studies are depicted in the figure No 1-7,2-7,3-7,4-7. The dissolution studies were compared using the similarity factor (f2). The f2 values > 50 clearly indicate a similar dissolution profile as compared to the initial drug release.

### 7.7 Conclusion:

It can be concluded from the results of accelerated and long term stability studies that the optimized formulations were found to be stable and consistent in terms of all the studied parameters. Henceforth, the optimized formulations could provide a minimum shelf life of one year

### 7.8 References:


- "Developing Solid Oral Dosage FormsPharmaceutical : Theory and Practice" Elsevier Publication 2009 Edited by David LeBlond "Statistical Design and Analysis of Long-term Stability Studies for Drug Products"
- ICH Q1C. (1997).Stability Testing for New Dosage Forms, May.
- ICH Q1D. (2003). Bracketing and Matrixing Designs for StabilityTesting of New Drug Substances and Products, January.
- ICH Q1E. (2004). Evaluation of Stability Data, June.

# 8.0 IN-VIVO PHARMACOKINETIC STUDIES

## 8.1 Methods

### 8.1.1 Animals

All animal experiments conducted were approved by the Social Justice and Empowerment Committee for the purpose of control and supervision on animals and experiments, Ministry of Government of India. Wistar rat weighing between **250-300 gm** were selected for PK studies.

### 8.1.2 Dosing Procedure

Mini Tabs were dosed intact to the rat by the following procedure. Rats were partially anesthetised using chloroform. Rats were restrained by grasping the scruff of the neck with one hand and the rear with the other hand. Rat's tail was wrapped around small finger to secure the lower portion of the rat. The minitab was placed in the center of the mouth using the holder follow the roof of the mouth to the opening of the oesophagus. The rat's head was tilted back with the shaft of the sample holder. This straightens the oesophagus and makes insertion of the makes the insertion of easier. After dosing, rats were dose with a few ml of water as this further facilitate movement of the minitab into the stomach.

### 8.1.3 Blood Sampling Procedure

Collection Site: **Tail Vein**

The acceptable quantity and frequency of blood sampling was determined by the circulating blood volume and the red blood cell (RBC) turnover rate. Excessive blood collection may result in hypovolemic shock, physiological stress and even death of the animal.

Because it was necessary to take multiple samples, smaller blood volumes i.e. 300 µl were drawn 5 time. Without fluid replacement, the maximum blood volume which can be safely removed for a one time sample is 10% of the total blood volume or 5.5-7 ml/kg. For a 300 g rat, this was equivalent to 1.7-2.1 ml. For a 300 g rat if subcutaneous fluid replacement is done then collection volume can be increased equivalent to 2.5-3.2 ml.

Animal recovery : If sampling done every 2 weeks, up to 10% of the total blood volume may be drawn or 5.5-7 ml/kg (4). For a 300 g rat, this is equivalent to about 1.7-2.1 ml every 2 weeks. Approximate Blood Sample Volumes for Body Weights are tabulated in table 1-8.

| **Body weight (g)** | **Circulating Blood Volume (ml) (CBV)** | **10% CBV (ml) every 2 wks†** |
|---|---|---|
| 250 | 13.75 – 17.50 | 1.4 – 1.8 |
| 300 | 16.50 – 21.00 | 1.7 – 2.1 |

**Table 1 - 8 Approximate Blood Sample Volumes for Body Weights**

### 8.1.4 Sampling Procedure

- Tail vain sampling is recommended for collecting a large volume of blood sample (up to 2ml /withdrawal)
- Animal was restrained properly.
- The tail was not rubbed from the base to the tip as it may result in leukocytosis. If the vein was not visible, the tail is dipped into warm water (40°C).
- Local aesthetic cream was applied on the surface of the tail 30 min before the experiment.
- A 23G needle inserted into the blood vessel and blood is collected using a a syringe with a needle. In case of difficulties, 0.5 to 1 cm of surface of the skin is cut open and blood is collected with a syringe with a needle.
- Having completed blood collection, silver nitrate ointment was applied to stop the bleeding.
- Each sample was immediately placed in an apendrop tube containing potassium EDTA equivalent to 2 mg/ml and refrigerated.
- Blood sample were frozen 20 ºC until analysed.
- Plasma Sample obtained by centrifugation of blood samples at 4000 RPM.
- Butorphanol Tartrate equivalent to 1 ng / µl of plasma was spiked to make the released drug quantifiable.

**8.2 Study Design**

Two treatments, four Periods, cross-over bioavailability study design. Blood sample were collected at 0Hr, 2Hr, 4Hr, 8Hr, 12 Hr after dosing after giving 20 day washout same rats were dosed again and blood samples were collected at 14Hr, 18Hr, 21Hr, 24Hr, 48 Hr after dosing. Same procedure was followed for BT extended release formulation and BT solution. And LOR extended release formulation and LOR IR formulation. In-vivo Study design for both formulation is tabulated in table 2-8.

| **Blood Sampling Time Points After 0Hr, 2Hr, 4Hr, 8Hr, 12 Hr after dosing** | | **Blood Sampling Time Points After 14Hr, 18Hr, 21Hr, 24Hr, 48 Hr after dosing** | |
|---|---|---|---|
| **Cohort I** | **Cohort II** | **Cohort I** | **Cohort II** |
| Rat 1 – T | Rat 7 – R | Rat 1 – T | Rat 7 – R |
| Rat 2 – T | Rat 8 – R | Rat 2 – T | Rat 8 – R |
| Rat 3 – T | Rat 9 – R | Rat 3 – T | Rat 9 – R |
| Rat 4 – T | Rat 10 – R | Rat 4 – T | Rat 10 – R |
| Rat 5 – T | Rat 11 – R | Rat 5 – T | Rat 11 – R |
| Rat 6 – T | Rat 12 – R | Rat 6 – T | Rat 12 – R |
| **Blood Sampling Time Points After 0Hr, 2Hr, 4Hr, 8Hr, 12 Hr after dosing** | | **Blood Sampling Time Points After 14Hr, 18Hr, 21Hr, 24Hr, 48 Hr after dosing** | |
| **Cohort I** | **Cohort II** | **Cohort I** | **Cohort II** |
| Rat 1 – R | Rat 7 – T | Rat 1 – R | Rat 7 – T |
| Rat 2 – R | Rat 8 – T | Rat 2 – R | Rat 8 – T |
| Rat 3 – R | Rat 9 – T | Rat 3 – R | Rat 9 – T |
| Rat 4 – R | Rat 10 – T | Rat 4 – R | Rat 10 – T |
| Rat 5 – R | Rat 11 – T | Rat 5 – R | Rat 11 – T |
| Rat 6 – R | Rat 12 – T | Rat 6 – R | Rat 12 – T |

**Table 2 - 8 In-vivo Study design**

### 8.3 Statistical Analysis

All data are reported as mean ± SD (standard deviation) and the difference between the groups were tested using Student's t-test at the level of $P<0.05$. Non-Compartmental analysis of plasma data after extravascular input was evaluated using PK Solver Software Ver. 2.0 with Linear Trapezoidal method. The pharmacokinetic parameters of Butorphanol tartrate in rat after oral administration of drug solution and ER formulation were recorded.

### 8.4 Preparation of Equivalent formulation for Rat Model

Rat LD 50 of Butorphanol Tartrate is 315 mg/kg dose proportional

Dose proportional formulation was prepared for ingestion in to rat model except thickness of the coating needed to increase from 6 % to 9% to have similar release profile as that of the original formulation. The whole process was similar to that of the original formulation.

| **Formula ingredients** | **Quantity (mg/tablet)** | **Quantity (% W/W)** |
|---|---|---|
| **Dry Mixing** | | |
| Butorphanol tartrate | 0.51 | 3.99 |
| Sodium Chloride | 5.12 | 39.85 |
| Lactose* | 5.12 | 39.85 |
| PVP K 30 | 0.61 | 4.78 |
| Isopropyl Alcohol** | Processing Solvent | Processing Solvent |
| | **11.38** | 88.48 |
| Lubrication | | |
| Mg. Stearate | 0.15 | 1.20 |
| Talc/Purified Talc | 0.15 | 1.20 |
| Colloidal silicon dioxide | 0.10 | 0.80 |
| **Total weight of un coated core tablet** | **11.79** | 91.66 |
| **Coating 9 % Weight Gain** | | |
| Cellulose CA398-10 | 0.54 | 4.17 |
| PEG 400 | 0.40 | 3.08 |
| D – Sorbirol | 0.14 | 1.08 |
| Purifies Water ** | Processing Solvent | Processing Solvent |
| Acetone** | Processing Solvent | Processing Solvent |
| **Total weight of coated tablet after coating** | **13** | **100.00** |

**Table 3 - 8 Composition of Butorphanol tartrate for In-vivo studies.**

**Tablet Size:**

**Diameter** **:** 3 mm

**Thickness** **: 1.8 mm**

**Dissolution** **:**

**Volume: 250 ml**

**Dissolution medium : SGF for 2 Hrs followed by SIF**

| Time (hr) | SGF 2 Hrs + SIF |
|---|---|
| **0** | 0.00 |
| 1 | 4.18 |
| 2 | 9.23 |
| 4 | 20.48 |
| 6 | 31.62 |
| 8 | 39.13 |
| 10 | 47.21 |
| 14 | 62.58 |
| 17 | 72.19 |
| 21 | 82.17 |
| 24 | 93.58 |

**Comparison of rat and Human Extended release formulation:**

| | | |
|---|---|---|
| **Similarity Factor** | **F2** | **89.01** |

Similarity factor confirms the developed formulation and formulation for In-vivo studies have similar behaviour.

**8.5 Pharmacokinetic Studies of Butorphanol tartrate formulation**

The extended release formulation and immediate release solution of Butorphanol Tartrate were evaluated for pharmacokinetic studies in rat for 48 hrs after oral administration. The results of pharmacokinetic for formulations are tabulated.

The extended release 510 mcg formulation and immediate release solution 170 mcg triplicate at of 0 hr, 9 hr and 17 Hr administration of Butorphanol Tartrate were evaluated for similarity in pharmacokinetic studies in rat for 48 hrs after oral administration. The results of pharmacokinetic for formulations are tabulated.

| **Subject No** | **Test** | | | **Referance** | | |
|---|---|---|---|---|---|---|
| | **($C_{max}$)** | **($AUC_{inf}$)** | **(AUC)** | **($C_{max}$)** | **($AUC_{inf}$)** | **(AUC)** |
| 1 | 1.81 | 30.12 | 29.12 | 1.56 | 31.32 | 31.32 |
| 2 | 1.67 | 28.72 | 28.72 | 1.67 | 32.56 | 32.3 |
| 3 | 1.73 | 28.52 | 28.32 | 1.52 | 31.92 | 31.92 |
| 4 | 1.68 | 30.19 | 30.05 | 1.63 | 30.87 | 30.87 |
| 5 | 1.67 | 29.22 | 29.52 | 1.43 | 31.39 | 31.3 |
| 6 | 1.71 | 28.62 | 28.02 | 1.69 | 32.98 | 32.08 |
| 7 | 1.69 | 27.42 | 27.6 | 1.54 | 31.99 | 31.79 |
| 8 | 1.64 | 29.23 | 29.03 | 1.65 | 31.02 | 31.02 |
| 9 | 1.63 | 27.39 | 28.94 | 1.52 | 30.9 | 30.9 |
| 10 | 1.79 | 28.21 | 27.14 | 1.79 | 31.32 | 31.32 |
| 11 | 1.64 | 27.03 | 28.43 | 1.5 | 31.42 | 31.42 |
| 12 | 1.63 | 30.12 | 29.12 | 1.54 | 31.64 | 31.64 |
| N | 12 | 12 | 12 | 12 | 12 | 12 |
| **Mean** | 1.69 | 28.73 | 28.67 | 1.59 | 31.61 | 31.49 |
| **SD** | 0.060 | 1.096 | 0.816 | 0.100 | 0.652 | 0.463 |
| **CV %** | 3.54 | 3.81 | 2.85 | 6.33 | 2.06 | 1.47 |
| **Geo. Mean** | 1.690 | 28.713 | 28.657 | 1.584 | 31.605 | 31.487 |

**Table 4 - 8 Individual Cmax, AUC and AUC inf data for Test and reference formulation of Butorphanol tartrate**

Individual $C_{max}$, AUC and AUC $_{inf}$ data for Test and reference formulation of Butorphanol tartrateare tabulated in table 4 – 8.

| | Ratio ( % Ref) | CI 90 Lower | CI 90 Higher |
|---|---|---|---|
| $C_{max}$ | 1.07 | 1.03 | 1.10 |
| | | | |
| AUC | 0.91 | 0.89 | 0.93 |
| | | | |
| $AUC_{Inf}$ | 0.91 | 0.89 | 0.93 |

**Table 5 - 8 Summary of Pharmacokinetic Parameters for butorphanol tartrate**

| Pharmacokinetic Parameter | BT (XR Formulation) | (Solution) |
|---|---|---|
| $T_{max}$ (hr) | 12 | 21 |
| $C_{max}$ (ng/ml) | 1.69 | 1.59 |
| $AUC_{(0\rightarrow t)}$ ng/ml*h | 28.67 | 31.49 |
| $AUC_{(0\rightarrow \infty)}$ ng/ml*h | 28.73 | 31.61 |
| $T_{1/2}$ (hrs) | 4.62 | 4.23 |

**Table 6 - 8 Pharmacokinetic parameters of Butorphanol tartrate formulations**

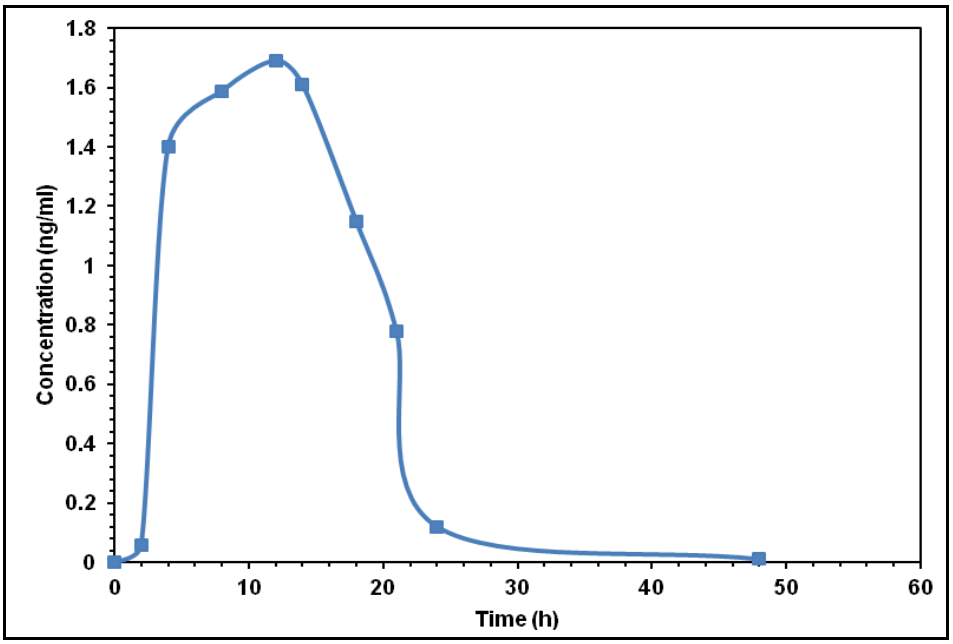



**Figure 1 - 8 Plasma Concentration vs Time profile for Butorphanol Tartrate Extended release formulation**

Figure 1-8 shows Plasma Concentration vs Time profile for Butorphanol Tartrate Extended release formulation. Figure 2 - 8 shows Plasma Concentration vs Time profile on log normal scale for Butorphanol Tartrate Extended release formulation

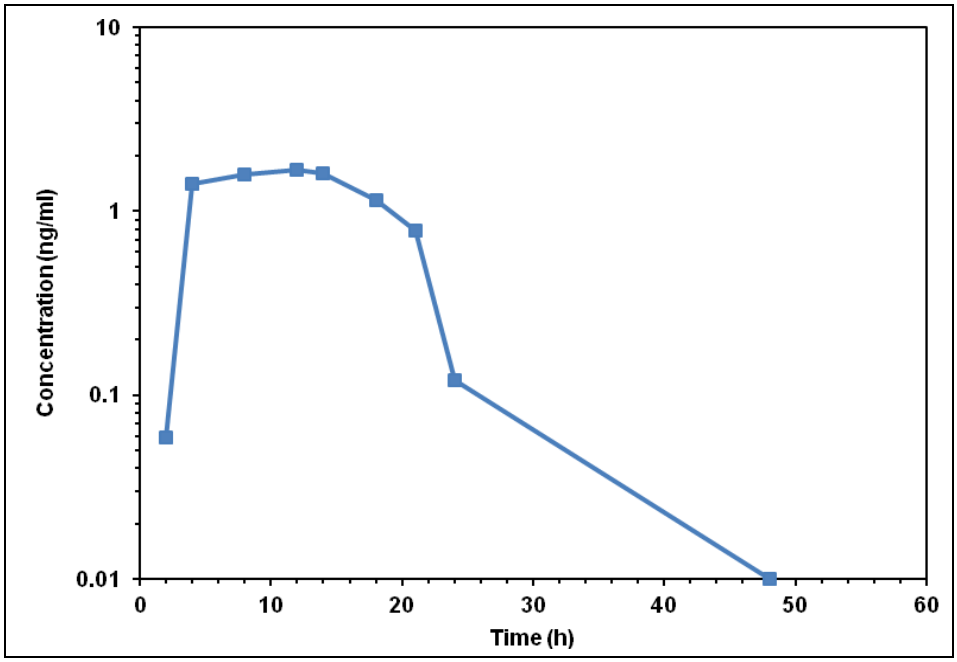



**Figure 2 - 8 Plasma Concentration vs Time profile on log normal scale for Butorphanol Tartrate Extended release formulation**

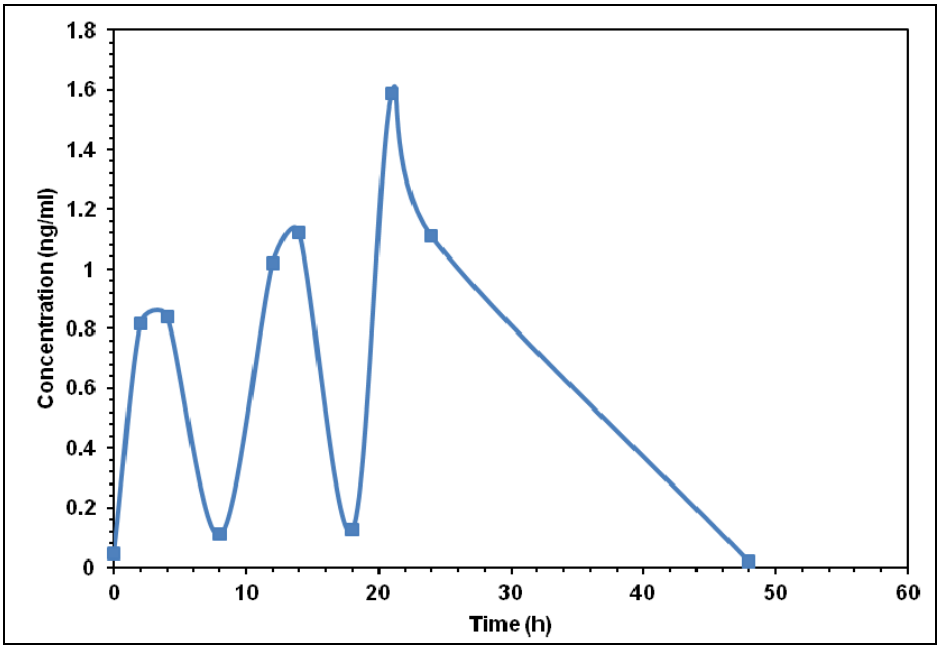



**Figure 3 - 8 Plasma Concentration vs Time profile for Butorphanol Tartrate Immediate release solution formulation**

Figure 3 - 8 shows plasma Concentration vs Time profile for Butorphanol Tartrate Immediate release solution formulation, Figure 4 - 8 Plasma Concentration vs Time profile log normal scale for Butorphanol Tartrate Immediate release solution formulation

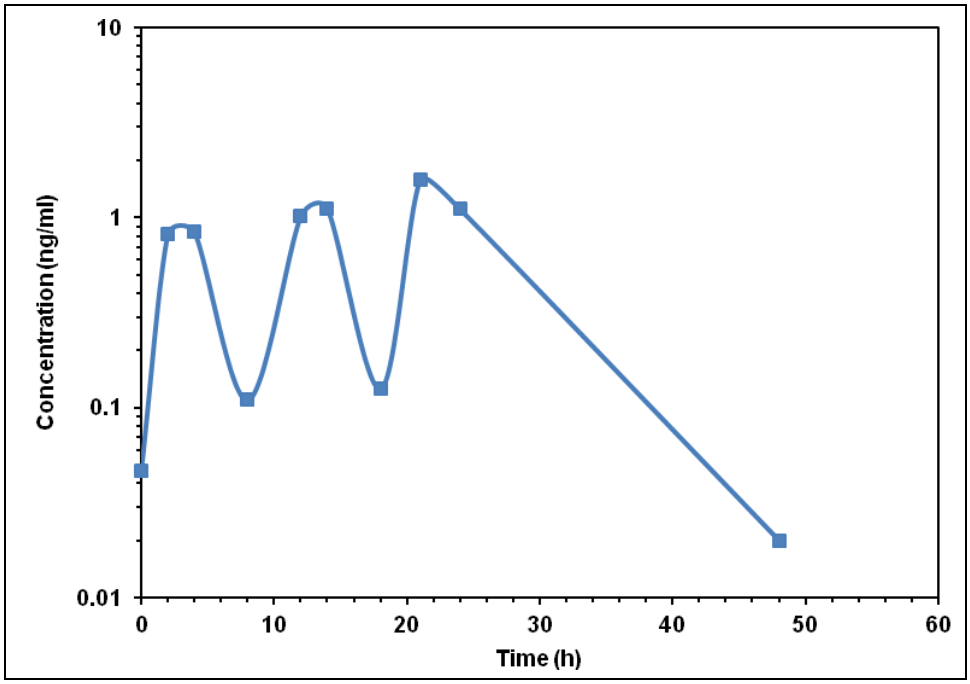



**Figure 4 - 8 Plasma Concentration vs Time profile log normal scale for Butorphanol Tartrate Immediate release solution formulation**

Constant blood level of Butorphanol tartrate was observed following administration of extended release formulation 510 mcg formulation and typical saw tooth pattern was observed following immediate release solution 170 mcg triplicate at of 0 hr, 9 hr and 17 Hr administration of Butorphanol Tartrate indicative of maintaining the therapeutic concentration after oral administration.

# 8.6 LORNOXICAM

## 8.7 Methods

### 8.7.1 Animals

All animal experiments conducted were approved by the Social Justice and Empowerment Committee for the purpose of control and supervision on animals and experiments, Ministry of Government of India. Wistar rat weighing between **250-300 gm** were selected for PK studies.

### 8.7.2 Dosing Procedure

Mini Tabs were dosed intact to the rat by the following procedure. Rat were partially anesthetised using chloroform. Rat were restrained by grasping the scruff of the neck with one hand and the rear with the other hand. Rat's tail was wraped around small finger to secure the lower portion of the rat. The minitab was placed in the center of the mouth using the holder follow the roof of the mouth to the opening of the esophagus. The rat's head was tilted back with the shaft of the sample holder. This straightens the esophagus and makes insertion of the makes the insertion of easier. After dosing, rat were dose with a few ml of water as this further facilitate movement of the minitab into the stomach.

### 8.7.3 Blood Sampling Procedure

Collection Site: **Tail Vein**

The acceptable quantity and frequency of blood sampling was determined by the circulating blood volume and the red blood cell (RBC) turnover rate. Excessive blood collection may result in hypovolemic shock, physiological stress and even death of the animal.

Because it was necessary to take multiple samples, smaller blood volumes i.e. 300 µl were drawn 5 time. Without fluid replacement, the maximum blood volume which can be safely removed for a one time sample is 10% of the total blood volume or 5.5-7 ml/kg. For a 300 g

rat, this was equivalent to 1.7-2.1 ml. For a 300 g rat if subcutaneous fluid replacement is done then collection volume can be increased equivalent to 2.5-3.2 ml.

Animal recovery : If sampling done every 2 weeks, up to 10% of the total blood volume may be drawn or 5.5-7 ml/kg (4). For a 300 g rat, this is equivalent to about 1.7-2.1 ml every 2 weeks.

| **Rat Body weight (g)** | **Circulating Blood Volume (ml) (CBV)** | **10% CBV (ml) every 2 wks†** |
|---|---|---|
| 250 | 13.75 – 17.50 | 1.4 – 1.8 |
| 300 | 16.50 – 21.00 | 1.7 – 2.1 |

**Table 7- 8 approximate Blood sample volumes for rat body weight**

Table 7 -8 shows approximate Blood Sample Volumes that for normal rat with normal body weights

### 8.7.4 Sampling Procedure:

- Tail vain sampling is recommended for collecting a large volume of blood sample (up to 2ml /withdrawal)
- Animal was restrained.
- The tail was not rubbed from the base to the tip as it may result in leukocytosis. If the vein was not visible, the tail is dipped into warm water (40°C).
- Local aesthetic cream was applied on the surface of the tail 30 min before the experiment.
- A 23G needle inserted into the blood vessel and blood is collected using a a syringe with a needle. In case of difficulties, 0.5 to 1 cm of surface of the skin is cut open and blood is collected with a syringe with a needle.
- Having completed blood collection, silver nitrate ointment was applied to stop the bleeding.
- Each sample was immediately placed in potassium EDTA and refrigerated.
- Plasma Sample obtained by centrifugation of blood samples.
- Blood sample were frozen 20 ºC until analysed.

**8.8 Study Design**

Two treatment, four Period, cross-over bioavailability study design under fasting condition. Design as mentioned in table 8-8. Blood sample were collected at 0Hr, 2Hr, 4Hr, 8Hr, 12 Hr after dosing after giving 20 day washout same rats were dosed again and blood samples were collected at 14Hr, 18Hr, 21Hr, 24Hr, 48 Hr after dosing. Same procedure was followed for BT extended release formulation and BT solution. And LOR extended release formulation and LOR IR formulation.

| **Blood Sampling Time Points After 0Hr, 2Hr, 4Hr, 8Hr, 12 Hr after dosing** | | **Blood Sampling Time Points After 14Hr, 18Hr, 21Hr, 24Hr, 48 Hr after dosing** | |
|---|---|---|---|
| **Cohort I** | **Cohort II** | **Cohort I** | **Cohort II** |
| Rat 1 – T | Rat 7 – R | Rat 1 – T | Rat 7 – R |
| Rat 2 – T | Rat 8 – R | Rat 2 – T | Rat 8 – R |
| Rat 3 – T | Rat 9 – R | Rat 3 – T | Rat 9 – R |
| Rat 4 – T | Rat 10 – R | Rat 4 – T | Rat 10 – R |
| Rat 5 – T | Rat 11 – R | Rat 5 – T | Rat 11 – R |
| Rat 6 – T | Rat 12 – R | Rat 6 – T | Rat 12 – R |
| **Blood Sampling Time Points After 0Hr, 2Hr, 4Hr, 8Hr, 12 Hr after dosing** | | **Blood Sampling Time Points After 14Hr, 18Hr, 21Hr, 24Hr, 48 Hr after dosing** | |
| **Cohort I** | **Cohort II** | **Cohort I** | **Cohort II** |
| Rat 1 – R | Rat 7 – T | Rat 1 – R | Rat 7 – T |
| Rat 2 – R | Rat 8 – T | Rat 2 – R | Rat 8 – T |
| Rat 3 – R | Rat 9 – T | Rat 3 – R | Rat 9 – T |
| Rat 4 – R | Rat 10 – T | Rat 4 – R | Rat 10 – T |
| Rat 5 – R | Rat 11 – T | Rat 5 – R | Rat 11 – T |
| Rat 6 – R | Rat 12 – T | Rat 6 – R | Rat 12 – T |

**Table 8 - 8 Study design for lornoxicam formulation**

### 8.9 Statistical Analysis

All data are reported as mean ± SD (standard deviation) and the difference between the groups were tested using Student's t-test at the level of $P<0.05$. Non-Compartmental analysis of plasma data after extravascular input was evaluated using PK Solver Software Ver. 2.0 with Linear Trapezoidal method. The pharmacokinetic parameter of Lornoxicam in rat after oral administration of ER formulation were recorded and compared with that of the pharmacokinetic parameter after administration of Lornoxicam Immediate Release formulation.

### 8.10 Preparation of Equivalent formulation for Rat Model

Rat LD 50 of Butorphanol Tartrate is 315 mg/kg dose proportional

| **Extended release formulation** | **Quantity (mg/tablet)** | **Quantity (% W/W)** |
|---|---|---|
| Lornoxicam | 1.14 | 8.89 |
| HPMC K100M CR | 0.61 | 4.72 |
| HPMC K100LV | 0.61 | 4.72 |
| PVP k 30 | 0.36 | 2.78 |
| Lactose Monohydrate | 3.88 | 30.14 |
| Micro crystalline Cellulose | 3.88 | 30.14 |
| Meglumin | 1.82 | 14.17 |
| NaOH | 0.21 | 1.67 |
| Mg. Stearate | 0.14 | 1.11 |
| Purified Talc | 0.14 | 1.11 |
| Aerosil 200 | 0.07 | 0.56 |
| | 12.86 | 100 |

**Table 9 -8 Lornoxicam formulation for In-vivo studies.**

| **Immediate Release formulation of lornoxicam** | **Quantity (mg/tablet)** | **Quantity(% W/W)** |
|---|---|---|
| Lornoxicam | 0.57 | 4.38 |
| Micro crystalline Cellulose | 12.08 | 92.92 |

| | | |
|---|---|---|
| Mg. Stearate | 0.14 | 1.08 |
| Purified Talc | 0.14 | 1.08 |
| Aerosil 200 | 0.07 | 0.54 |
| | 13 | 100 |

**Table 10 - 8 Lornoxicam IR formulation for In-vivo studies**

Tablet 9-8 and 10-8 respectively show XR and IR formulation of lornoxicam for In-vivo studies.

**Specifications of Core Tablet**

**Diameter** **:** 3 mm

**Thickness** **: 1.9 mm**

**Dissolution** **:**

**Volume: 250 ml**

**Dissolution medium: SIF**

| Time (hr) | PBS 4.5 |
|---|---|
| 0 | 0.00 |
| 1 | 11.27 |
| 2 | 28.40 |
| 4 | 47.21 |
| 6 | 61.75 |
| 8 | 75.18 |
| 12 | 92.85 |
| 14 | 98.48 |
| 16 | 101.59 |

**Table 11 - 8 Release profile of XR formulation for in-vivo studies.**

**Comparison of Extended release Lornoxicam rat and Human formulation:**

| Similarity Factor | F2 | 84.01 |
|---|---|---|

**Similarity Factor:** As the name specifies, it stresses on the comparison of closeness of two comparative formulations.

$f2= 50\times\log \{[1+ (1/n) \Sigma_{t=1}^{n} (R_t-T_t)^2]^{-0.5} \times 100\}$

### 8.11 Pharmacokinetic Studies of Lornoxicam formulation

The extended release formulation and immediate release formulation of lornoxicam were compared & evaluated for pharmacokinetic studies in rat for 48 hrs after oral administration. The results of pharmacokinetic for formulations are tabulated.

The extended release 1.14 mg formulation and immediate release formulation 570 mcg duplicate at of 0 hr and 11 Hr administration of Lornoxicam were evaluated for similarity in pharmacokinetic studies in rat for 48 hrs after oral administration. The results of pharmacokinetic for formulations are tabulated.

| **Subject No** | **Test** | | | **Reference** | | |
|---|---|---|---|---|---|---|
| | **($C_{max}$)** | **($AUC_{inf}$)** | **(AUC)** | **($C_{max}$)** | **($AUC_{inf}$)** | **(AUC)** |
| 1 | 513 | 7554 | 7023 | 570 | 7819 | 7539 |
| 2 | 465 | 7845 | 7836 | 583 | 7987 | 7877 |
| 3 | 475 | 7577 | 7309 | 594 | 7645 | 7613 |
| 4 | 456 | 7534 | 7535 | 538 | 7874 | 7873 |
| 5 | 502 | 7772 | 7723 | 561 | 7834 | 7834 |
| 6 | 476 | 7774 | 7751 | 579 | 7802 | 7712 |
| 7 | 487 | 7128 | 7034 | 593 | 7696 | 7396 |
| 8 | 462 | 7364 | 7390 | 556 | 7459 | 7459 |
| 9 | 493 | 7391 | 7356 | 586 | 7813 | 7813 |
| 10 | 461 | 7688 | 7369 | 553 | 7535 | 7535 |
| 11 | 472 | 7428 | 7123 | 579 | 7758 | 7648 |
| 12 | 498 | 7804 | 7783 | 548 | 7884 | 7834 |
| N | 12 | 12 | 12 | 12 | 12 | 12 |
| **Mean** | 480.00 | 7571.58 | 7436.00 | 570.00 | 7758.83 | 7677.75 |
| **SD** | 18.355 | 217.067 | 290.936 | 18.503 | 151.347 | 170.042 |
| **CV %** | 3.82 | 2.87 | 3.91 | 3.25 | 1.95 | 2.21 |
| **Geo. Mean** | 479.681 | 7568.703 | 7430.775 | 569.723 | 7757.469 | 7676.017 |

**Table 12 - 8 Individual Cmax, AUC and AUC inf data for Test and reference formulation of Lornoxicam**

Table 12-8 shows Individual $C_{max}$, AUC and $AUC_{inf}$ data for Test and referene Lornoxicam formulation

| | **Ratio ( % Ref)** | **CI 90 Lower** | **CI 90 Higher** |
|---|---|---|---|
| **$C_{max}$** | 0.84 | 0.82 | 0.86 |
| | | | |
| **AUC** | 0.97 | 0.95 | 0.98 |
| | | | |
| **$AUC_{Inf}$** | 0.98 | 0.96 | 0.99 |

**Table 13 - 8 Summary of Pharmacokinetic Parameters**

Table 13-8 ND 14- 8 shows Summary of Pharmacokinetic Parameters obtained for lornoxicam after administration in rat model.

| **Pharmacokinetic Parameter** | **LOR (XR Formulation)** | **LOR (IR Formulation)** |
|---|---|---|
| $T_{max}$ (hr) | 08 | 14 |
| $C_{max}$ (ng/ml) | 480 | 570 |
| $AUC_{(0\rightarrow t)}$ ng/ml*h | 7436 | 7677 |
| $AUC_{(0\rightarrow \infty)}$ ng/ml*h | 7571 | 7758 |
| $T_{1/2}$ (hrs) | 7.22 | 6.24 |

**Table 14 - 8 Summary of Pharmacokinetic Parameters**

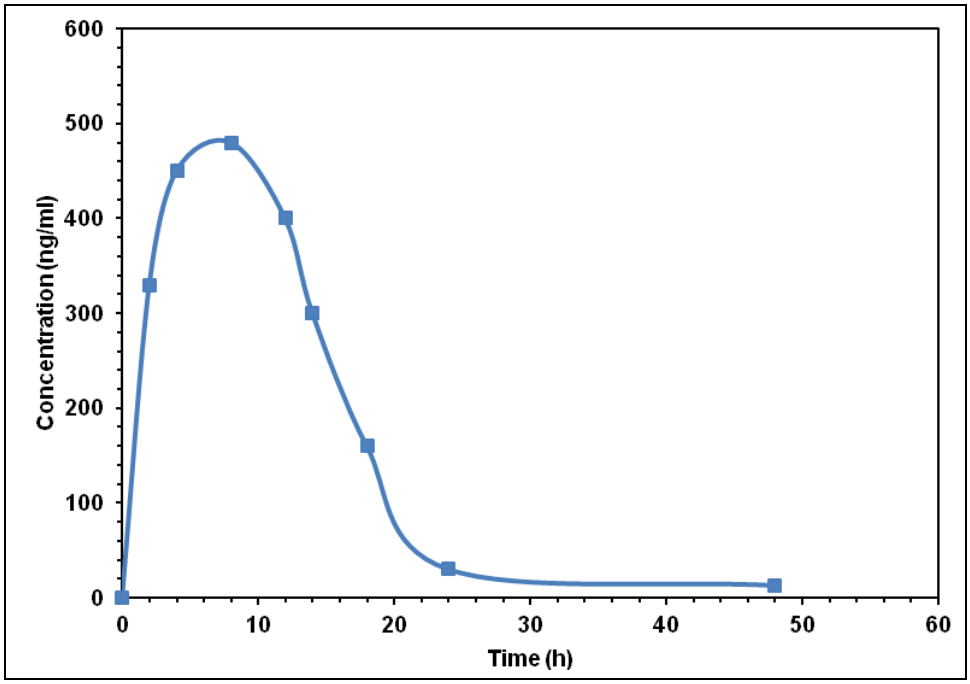



**Figure 5 - 8 Plasma Concentration vs Time profile for Lornoxicam Extended release formulation**

Figure 5 - 8 shows plasma Concentration vs Time profile for Lornoxicam Extended release formulation

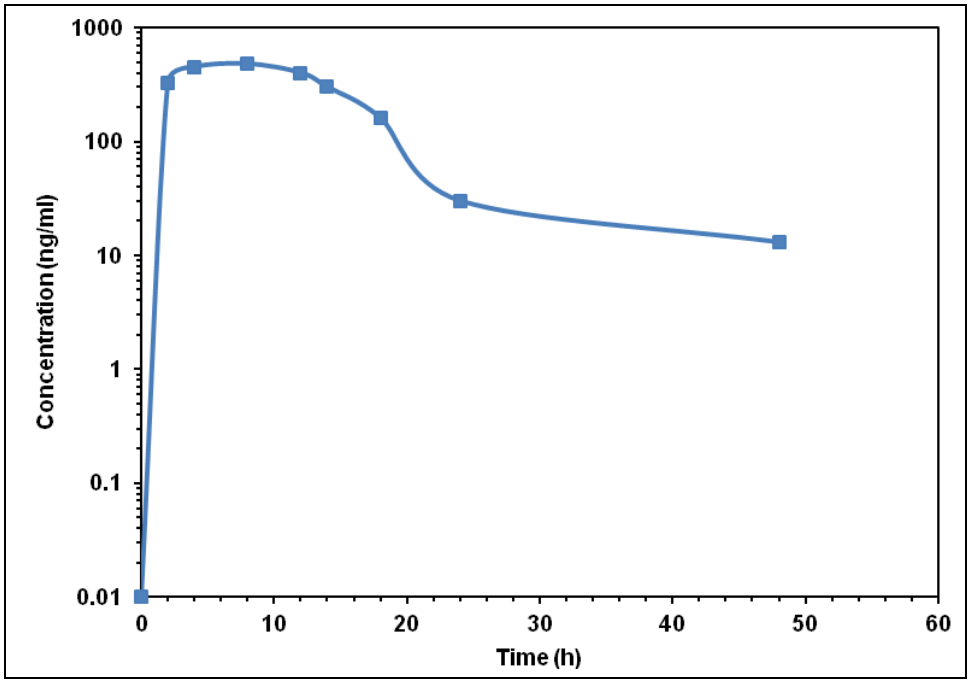



**Figure 3 - 8 Plasma Concentration vs Time profile on log normal scale for Lornoxicam Extended release formulation**

Figure 4 - 8 shows plasma Concentration vs Time profile on log normal scale for Lornoxicam Extended release formulation

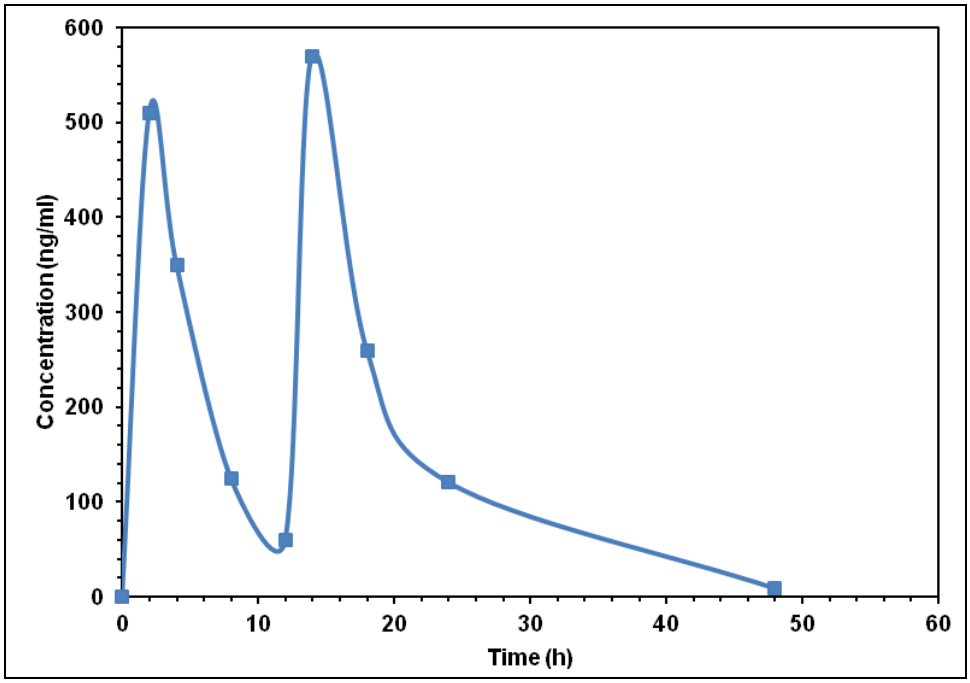



**Figure 75 - 8 Plasma Concentration vs Time profile for Lornoxicam Immediate release solution formulation**

Figure 7 - 8 shows plasma Concentration vs Time profile for Lornoxicam Immediate release solution formulation.

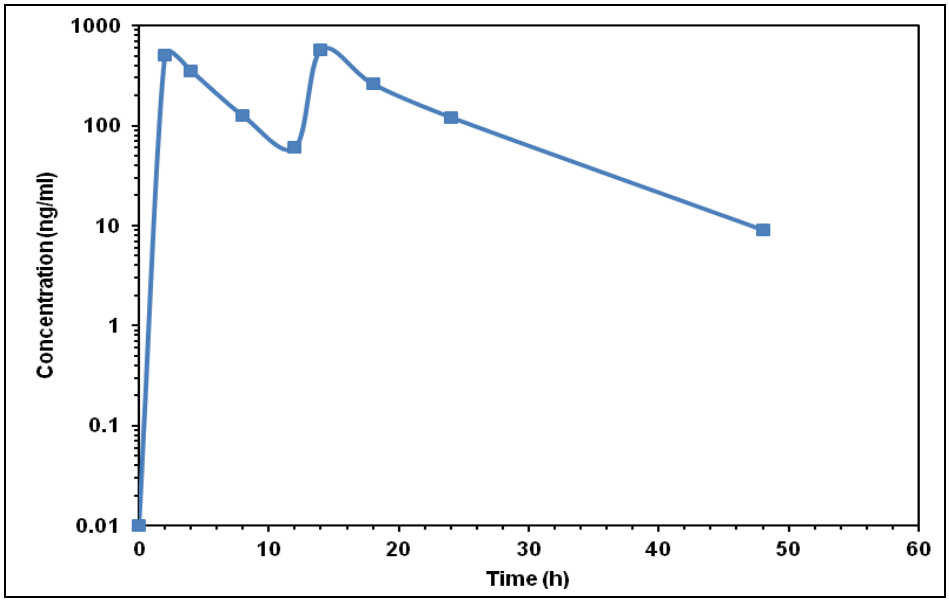



**Figure 8 - 8 Plasma Concentration vs Time profile log normal scale for Lornoxicam Immediate release solution formulation**

Figure 8 - 8Plasma Concentration vs Time profile log normal scale for Lornoxicam Immediate release solution formulation

**8.12 Conclusions**

To conclude, equivalent pharmacokinetic parameters of butorphanol tartrate observed following administration of extended release formulation 510 mcg formulations compared to immediate release 170 mcg triplicates. Additionally typical saw tooth pattern observed following immediate release 170 mcg triplicate at of 0 hr, 9 hr and 17 Hr administration of Butorphanol Tartrate.

Equivalent pharmacokinetic parameters of Lornoxicam observed following administration of extended release formulation 1.14 mg formulation compared to immediate release 570 mcg duplicate. Additionally typical saw tooth pattern observed following immediate release 570 mcg duplicate at of 0 hr and 10 Hr administration of Lornoxicam.

Extended released formulations of Butorphanol tartrate and Lornoxicam and are bio-available as compared to the immediate release dosage form. The ratios of AUC0-t, $C_{max}$, and AUC0-$_{inf}$ from Extended release formulation to Immediate release formulation both the drug at 90 % confidence interval fall within 80-125.

**8.13 References**


- www.fda.gov
- Mitruka BM, Rawnsley HM. 1981. Clinical, biochemical and hematological reference values in normal experimental animals and normal humans. New York: Masson Publishing; 413 p.
- McGuill MW, Rowan AN. 1989. Biological effects of blood loss: implications for sampling volumes and techniques. *ILAR News* 31:5-20
- Harkness JE, Wagner JE. 1989. Biology and husbandry. In: Harkness JE, Wagner JE, editors. The biology and medicine of rabbits and rodents, 3rd ed. Philadelphia: Lea & Febiger; 372 p.
- Br. J. clin. Pharmac. (1985), 19, 151S-162S In vitro and in vivo deconvolution assessment of drug release kinetics from oxprenolol Oros preparations
  F. LANGENBUCHER & J. MYSICKA Pharmaceutical Development, Ciba-Geigy Ltd, Basle, Switzerland.
- Balaji Agorama, Walter S. Woltosza, Michael B. Bolgera,b , Advanced Drug Delivery Reviews 50 (2001) S41–S67 Predicting the impact of physiological and biochemical processes on oral drug bioavailability.
- Vogel HG, editor. Drug discovery and evaluation: Pharmacological assays. 2 nd ed. Berlin: Springer; 2002.
- Therapeutic Review Butorphanol Rodney Schnellbacher, DVM Journal of Exotic Pet Medicine, Vol 19, No 2 (April), 2010: pp 192–195.
- S. Radhofer-Weltea, P. Dittrich Journal of Chromatography B, 707 (1998) 151–159 Determination of the novel non-steroidal anti-inflammatory drug lornoxicam and its main metabolite in plasma and synovial fluid.

- http://www.jpharmacol.com/article.asp?issn=0976500X;year=2010;volume=1;issue=2;spage=87;epage=93;aulast=Parasuraman ( Assessed On 21, July 2011)

- Effect of intranasally (in) administered butorpranol Tartrate in tbe rat tail flick test. S.n. Suberg, J.m. Libera*. Preclinical cns research. Bristol Myers co., wallingford, ct 06492, USA. Poster No.298 Tues Room No.3 Board 58.

- Rodney Schnellbacher, DVM Journal of Exotic Pet Medicine, Vol 19, No 2 (April), 2010: pp 192–195 Therapeutic Review "Butorphanol"

- Stability Indicating HPLC Methods for drug analysis Second edition Quanyun A Xu, Lawrence A trissel Pharmacutical Press London UK " BUTORPHANOL" 85

## 9.0 SUMMARY & CONCLUSION

The objective here was to provide an extended release pharmaceutical composition of lornoxicam and butorphanol tartrate, which upon ingestion results in blood plasma levels having pharmacological effect for an extended period of time equivalent to the multiple dosing of the immediate release formulations. It is needed to produce a pharmaceutical composition which releases drugs in predetermined manner. The use of high viscosity grade hydrophilic and the hydrophobic polymers to produce extended or controlled release pharmaceutical composition is very well known. For extending the release, the tablet comprising the drug also comprises of high viscosity grade hydrophilic polymer. In some cases the core is coated with hydrophobic coating membrane with hydrophilic pore formers incorporated in it. On contact with gastric fluid, fluid enters the tablet core and results into the hydration of the polymer which also controls the release of the drug. In case of coated system fluid on contact with osmotically active core exerts osmotic pressure and because of positive pressure in the system drug gets release in constant fashion from the osmotic Control of the rate of release benefits therapy by producing constant blood plasma levels of the active ingredient and by decreasing the frequency of administration, thereby improving the patient compliance to the dosage regimen.

Butorphanol was developed as osmotic drug delivery system to have constant drug release irrespective of food effect, agitation intensity and pH. Lactose, mannitol and sodium chloride and combination thereof were verified as core components besides it was also considered that Butorphanol tartrate being water soluble salt it may exert some osmotic pressure. UV-Visible Spectrophotometric analytical method for characterization of drug for Assay (%) Content Uniformity, in different dissolution media was developed. And HPLC analytical methods for characterization of Controlled porosity osmotic pump of Butorphanol tartrate in different media and animal plasma was developed.

Drug excipient compatibility study was done at an early stage of proposed drug product development by mixing drug with Lactose and mannitol in Drug to Excipient ratio of 1:5 W/W and Other excipients in Drug to Excipient ratio of 5:1 proportion and exposed to 40°C /75 % RH temperatures for 4 weeks to accelerate drug degradation. The blend exposed to

stress conditions was compared with their respective initial blend stored at controlled condition by physical observation. The samples are then characterized for the drug content, which were determined quantitatively using analytical method. Selected excipients were compatible with Butorphanol tartrate and do not produce any potential degradation products. During differential scanning colorimetric (DSC) evaluation no specific interaction between the drug and excipients used in the formulation were observed.

For development of the formulation target release profile which can produce effective plasma concentration for extended period of time was predicted using Wagner nelson de-convolution.

Core tablets were prepared by wet granulation the powdered blend was characterized for Assay, Blend uniformity, LOD, Bulk Density, Tapped Density.

Granules were compressed on 8 Station rotary tablet compression machine from general machinery company. Compressed core was characterized for Description, Uniformity of Weight (mg), Hardness (kg/cm2), Friability (% wt loss), Thickness (mm), Diameter (mm), Disintegration Time (min.), % drug Dissolution, % drug Assay and % drug Content Uniformity. During core finalizing Lactose, mannitol and sodium chloride separately and in combination with each other in equal ratio were used as osmogen and its effect on drug release was determined keeping coating & compression parameters constant.

Compressed core were coated on 6" perforated coating machine. Cellulose Acetate 398-10 was used as coating polymer which will be forming semi-permeable membrane. PEG 400 was incorporated as water soluble plasticizer and D-sorbitol as pore forming agent. Water and acetone were used as a solvent. Solid content in the coating formula has immense impact on uniformity of coating. Some trial of coating was done on dummy tablet to have idea of solid content in the coating solution and based on literature after 4% solid content was confirmed.

Machine parameters like Fluid nozzle diameter (mm), Spray pan size (Inch), Baffles (Nos.), Inlet air CFM, Outlet air CFM, Inlet air temperature (°C), Out let air temperature (°C), Pre-warm tablet bed (°C), Tablet surface bed temp (°C) and Atomizing air pressure ( kg/cm2) were fixed on the basis of previous experience and Gun-to-bed distance, Spray Rate / Peristaltic Pump RPM, Perforated Coating pan Speed, Tablet Surface bed Temperature,

Atomization air pressure, Spray rate and air volume for optimum spray pattern were fixed on dummy tablet. Effect of osmogen and combination thereof in the core on drug release, Effect of level of pore former in the coating membranes and Effect of coating thickness on drug release (Weight gain) was determined. Optimised formulation was characterized for Description, Uniformity of Weight (mg), Thickness (mm), Diameter (mm), Assay (%) Content Uniformity (%) and degradation. The stability studies were carried out in accordance with the ICH guidelines for stability. The samples were subjected to stability in HDPE bottles with induction seal. The samples were subjected to stability at 40° C/75 % RH (accelerated stability) for 1, 2, 3, 6months and at 25° C/60 % RH (controlled room temperature) at 3, 6, 9, 12months. The parameters studied are the drug content (Assay), average weight, dissolution studies, description and water by KF. During and after the completion of the stability studies, all the studied parameters like description, assay, water by KF, average weight were found to be within the predefined limits. It was also observed that the drug release studies showed no significant change in the dissolution (with respect to the initial dissolution). The dissolution studies were compared using the similarity factor (f2). The f2 values > 50 clearly indicate a similar dissolution profile as compared to the initial drug release. Surface electron microscopy of the exhausted cell after coating was done to confirm the drug release through pore formed through osmotic mechanism. In order to study the effect of alcohol on drug release or to verify weather alcohol leads to dose dumping 4 % V/V, 20 % V/V and 40 %V/V alcohol was added in the release media and drug release was verified against control ( without alcohol).

Mini tab dose proportionate formulation with similarity factor 89.01 with optimised formula made and the pharmacokinetic parameters verified after oral delivery in rat model with N = 12 Wistar rat weighing between **250-300 gm** were selected for PK studies. The extended release 510 mcg formulation and immediate release solution 170 mcg triplicate at of 0 hr, 9 hr and 17 Hr administration of Butorphanol Tartrate were evaluated for similarity in pharmacokinetic studies in rat for 48 hrs after oral administration. Pharmacokinetic parameters lice $C_{max}$, $T_{max}$, AUC (t) and AUC $_{0-> inf}$ were determined and compared by ratio of % ref, Lower and higher confidence interval.

Non-Compartmental analysis of plasma data after extravascular input was evaluated using PK Solver Software Ver. 2.0 with Linear Trapezoidal method.

Significant improvement in plasma butorphanol concentration was observed following administration of extended release formulation 510 mcg formulation compared to typical saw tooth pattern was observed following immediate release solution 170 mcg triplicate at of 0 hr, 8.5 hr and 17 Hr administration of Butorphanol Tartrate.

The major problem associated with the formulation and effectiveness of the lornoxicam is its variable oral absorption due to insufficient aqueous solubility at gastrointestinal pH, thus making solubility the rate-determining step in the gastric absorption to solve this issue micronized lornoxicam was used and pH modifier Meglumine and NaOH were added pH modifier and alkalizing agent in the lornocxicam formulation. The use of high viscosity grade hydrophilic and the hydrophobic polymers to produce extended or controlled release pharmaceutical composition is very well known and was used to have extended effect.

Lornoxicam was developed as oral matrix drug delivery system to have constant drug release. Reduce the dosing frequency and increase the patient compliance. UV-Visible Spectrophotometric analytical method for characterization of drug for Assay (%) Content Uniformity, in different dissolution media was developed. And HPLC analytical methods for characterization of oral matrix tablet of Lornoxicam in release media and animal plasma was developed.

Drug excipient compatibility study was done at an early stage of proposed drug product development by mixing drug with Excipient and exposed to 40°C /75 % RH temperatures for 4 weeks to accelerate drug degradation. The blend exposed to stress conditions was compared with their respective initial blend stored at controlled condition by physical observation. The samples are then characterized for the drug content, which were determined quantitatively using analytical method. Selected excipients were compatible with Lornoxicam and do not produce any potential degradation products. During differential scanning colorimetric (DSC) evaluation no specific interaction between the drug and excipients used in the formulation were observed.

For development of the formulation target release profile which can produce effective plasma concentration for extended period of time was predicted from immediate release plasma data using Wagner nelson de-convolution.

Tablets were prepared by wet granulation the powdered blend was characterized for Assay, Blend uniformity, LOD, Bulk Density, Tapped Density.

Granules were compressed on 8 Station rotary tablet compression machine from general machinery company. Compressed core was characterized for Description, Uniformity of Weight (mg), Hardness (kg/cm2), Friability (% wt loss), Thickness (mm), Diameter (mm), Disintegration Time (min.), % drug Dissolution, % drug Assay and % drug Content Uniformity. Lornoxicam's poor aqueous solubility can lead to absorption rate limiting step and thus delay in onset action. Solubility being an important parameter for absorption of water insoluble drugs it is a key rate-limiting step so incorporating an alkylating agent Sodium bicarbonate in the formulation during finalizing concentration of meglumine and Sodium hydroxide were used as alkylating agent in addition of dissolution another objective is to have drug release over extended period of time so matrix forming polymers i.e. HPMC of different viscosity and polyox were used in the initial formulation.

For that drug was dissolved in solution of NaOH & meglumine in water and using that solution as a for binder followed dry mix of Lactose Monihydrate & MCC in wruster coater. The above granules further mixed with extra granular matrix forming agent followed by addition of lubrication, antiadherant & glident.

Palm Glatt Wuruster coating machine was used for making granules. Machine parameters like Fluid nozzle (mm), Inlet air temperature (°C), Out let air temperature (°C), Product Temp (°C), Atomizing air pressure ( kg/cm2), Inlet Opening ,Spray rate (g/min), Peristaltic Pump RPM, Purging Time were fixed on the basis of previous experience and Gun-to-bed distance, Spray Rate / Paristaltic Pump RPM, Perforated Coating pan Speed, Tablet Surface bed Temperature, Atomization air pressure, Spray rate and air volume for optimum spray pattern were fixed on dummy tablet. Optimised formulation was characterized for Description, Uniformity of Weight (mg), Thickness (mm), Diameter (mm), Assay (%) Content Uniformity (%) and Degradation. The stability studies were carried out in accordance with the ICH guidelines for stability. The samples were subjected to stability in HDPE bottles with

induction seal. The samples were subjected to stability at 40° C/75 % RH (accelerated stability) for 1, 2, 3, 6months and at 25° C/60 % RH (controlled room temperature) at 3, 6, 9, 12months. The parameters studied are the drug content (Assay), average weight, dissolution studies, description and water by KF. During and after the completion of the stability studies , all the studied parameters like description, assay, water by KF, average weight were found to be within the predefined limits. It was also observed that the drug release studies showed no significant change in the dissolution (with respect to the initial dissolution). The dissolution studies were compared using the similarity factor (f2). The f2 values > 50 clearly indicate a similar dissolution profile as compared to the initial drug release. Surface electron microscopy of the exhausted cell after coating was done to confirm the drug release through pore formed through osmotic mechanism. In order to study the effect of alcohol on drug release or to verify weather alcohol leads to dose dumping 4 % V/V, 20 % V/V and 40 %V/V alcohol was added in the release media and drug release was verified against control ( without alcohol).

Mini tab dose proportionate formulation with similarity factor >50 with optimised formula made and the pharmacokinetic parameters verified after oral delivery in rat model with N = 12 Wistar rat weighing between **250-300 gm** were selected for PK studies. The extended release 1.14 mg formulation and immediate release solution 507 mcg duplicate at of 0 hr, and 12 Hr administration of lornoxicam were evaluated for similarity in pharmacokinetic studies in rat for 48 hrs after oral administration. Pharmacokinetic parameters lice $C_{max}$, $T_{max}$, AUC (t) and AUC $_{0\text{-}> \inf}$ were determined and compared by ratio of % reference, Lower and higher confidence interval.

Non-Compartmental analysis of plasma data after extra vascular input was evaluated using PK Solver Software Ver. 2.0 with Linear Trapezoidal method.

Significant improvement in plasma Lornoxicam concentration was observed following administration of extended release formulation 1.14 mg formulation compared to typical saw tooth pattern was observed following immediate release solution 507 mcg duplicate at of 0 hr, and 12 Hr administration of Lornoxicam.

**Conclusion**

The scope of this development is to extended release formulation of analgesic drugs. Butorphanol tartrate and Lornoxicam are drugs with shorter half-life.

Butorphanol is not available in market as oral formulation due to higher first pass metabolism which is addressed by developing Controlled porosity osmotic Pump tablet. It is verified that excipients used for developing formulation do not have any incompatibility with the drug. Developed formulation releases the drug independent of pH of the release media and agitation intensity. Formulation is optimised for core composition, percentage weight gain during coating (thickness of the coating membrane) and concentration of pore-former in the coating. Surface characteristic observation of exhausted shell after coating through SEM analysis confirms formation of micropores in the coating during dissolution. Cellulose acetate serves as an excellent semipermeable membrane and drug release is driven by osmotic mechanism also confirmed though observation of CPOP tablet before and after dissolution. Formulation is subjected to ethanol induced dose dumping study which confirmed no possibility of alcohol induced dose dumping.

Prepared formulation is characterised for description, uniformity of weight (mg), thickness (mm), diameter (mm), assay (%) content uniformity (%), degradation and stability studies. Developed formulation is stable as per ICH guidelines at 40° C/75 % RH (accelerated stability) for 1, 2, 3, 6 months and at 25° C/60 % RH (controlled room temperature) at 3, 6, 9, 12 months.

Dose proportionate formulation with established similarity with developed formulation is proven to be bio equivalent in-vivo in rat model same one third dose of immediate relase solution give three time orally. Pharmacokinetic parameters like $C_{max}$, $T_{max}$, AUC (t) and AUC $_{0\text{->}\ inf}$ are determined and compared by ratio of % reference, Lower and higher confidence interval. Non-Compartmental analysis of plasma data after extravascular input is evaluated using PK Solver Software Ver. 2.0 with Linear Trapezoidal method confirming developed extended release formulation of butorphanol Tartrate.

Once daily formulation of lornoxicam is not available in market it is available as immediate release oral formulation twice daily. It is drug with shorter half life with very less solubility which is addressed by dissolving drug in solution of alkalizing agent sodium hydroxide and meglumine and using that solution as binding solution. It is verified that excipients used for developing formulation do not have any incompatibility with the drug. Formulation is subjected to ethanol induced dose dumping study which confirmed no possibility of alcohol induced dose dumping.

Prepared formulation is characterised for description, uniformity of weight (mg), thickness (mm), diameter (mm), assay (%) content uniformity (%), degradation and stability studies. Developed formulation is stable as per ICH guidelines at 40° C/75 % RH (accelerated stability) for 1, 2, 3, 6 months and at 25° C/60 % RH (controlled room temperature) at 3, 6, 9, 12 months.

Dose proportionate formulation with established similarity with developed formulation is proven to be bio equivalent in-vivo in rat model same half dose of immediate release formulation given twice orally. Pharmacokinetic parameters like $C_{max}$, $T_{max}$, AUC (t) and AUC $_{0->\ inf}$ are determined and compared by ratio of % reference, Lower and higher confidence interval. Non-Compartmental analysis of plasma data after extravascular input is evaluated using PK Solver Software Ver. 2.0 with Linear Trapezoidal method confirming developed extended release formulation of Lornoxicam.