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Synopsis

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Introduction

The term ‘soft matter’ unites broad class of physical states ranging from colloids to micelles and vesicular system (1). The properties of these systems are intermediate to those of solids and liquids. The malleability of vesicular system or the phase volume fraction in emulsion system depends on the amount of ingredients used for the development of optimized formulation. The major challenge in development of such formulations is drug loading and size (2). Because this finally defines the cost of the formulation and effectiveness. Considering these two industrially defined challenges, the rationale of the current project was defined for the poorly water-soluble drugs.

STATEMENT OF THE PROBLEM AND RESEARCH ENVISAGED

To facilitate effective absorption after oral administration, solubility and permeability of drug in gastrointestinal (GIT) fluid is desirable (3). Approximately 70% of discovery compounds and 40% of pipeline candidates having poor water solubility or low luminal solubility have slow dissolution rate (4). These often limits their development as useful medicine. Additionally, poor solubility is also associated with high intra- and inter-subject variability, potential reduced clinical efficacy and lack of dose proportionality (5).

Lipid based drug delivery systems (LBDDS) are one of the several approaches that have been explored for improvement of oral bioavailability of poorly water-soluble drugs (6). Lipids have the capability to improve absorption of co-administered poorly water-soluble drugs by enhancing solubilization and dissolution in the GIT fluids (7). LBDDS range from liquid system, solid systems and vesicular to particulate drug delivery systems (8). The formulation approaches explored here are:

- Lipid based emulsion:
  Self Microemulsifying Drug Delivery System (SMEDDS)
- Lipid as vesicular system:
  Niosomes

SMEDDS are isotropic mixture of oil, surfactant and co-surfactant, when taken orally emulsify under gentle condition in the GIT to form emulsion (9). Typical size of the emulsion droplet ranges between 10 – 200 nm. Hydrophobic drugs can be loaded in the oil. NIOSOMES are colloidal particles in which concentric bilayer made up of amphiphiles surrounds aqueous core (10). The term "Nios" means non-ionic Surfactants. Non-ionic surfactants impart superior benefits for stability, compatibility and toxicity profile compared to charged surfactants. Generally, use of SMEDDS or Niosomes leads to improved bioavailability, enabling reduction in dose, selective targeting of drug from the absorption window and protection of drug from unreceptive GIT environment.

The size of LBDDS lesser than 200 nm is desirable and optimal for lymphatic uptake. Particles of larger than 100 nm size shows preferential uptake by lymphatic route only but at a slower rate. So size is an important parameter for lymphatic targeting of LBDDS formulation.

Iloperidone (ILO), anti-psychotic drug and Vardenafil (VAR), used in erectile dysfunction are drug of choice for the treatment of their respective disease. But the problem of low oral bioavailability due to high first pass metabolism and low solubility in GIT fluid hampers their effectiveness. These issues of drugs can be solved by novel drug delivery system by solubilizing the drug in lipid or its encapsulation in lipid bilayer. These molecular cargos when delivered orally will enhance its bioavailability by avoiding first pass metabolism.

AIM and OBJECTIVES

Overall:

The overall hypothesis of current research is exploration of the potential of different Lipid Based Drug Delivery Systems for enhancement of oral bioavailability of Vardenafil HCl trihydrate and Iloperidone.

Specific:

- Development of nano sized lipid based formulation - SMEDDS and Niosomes of Vardenafil HCl Trihydrate and Iloperidone

- Optimization of formulations using Design of Experiment and Soft Computing Techniques
- Development of control space in the explorable space by specifying tolerance interval limits
- Effect of formulation variables on *In-vitro*, *Ex-vivo* and *In-vivo* performance of the formulation

DRUG CANDIDATE SELECTION

Vardenafil HCl trihydrate

Erectile Dysfunction (ED) or impotence is a major sexual problem affecting a major population, especially geriatrics (11,12). Prevalence of ED has been reported to be 52% with complete ED ranging from 5 to 15% in men aged 40-70 yrs (13). However, its prevalence varies from 2% in men aged < 40 years to 86% in men aged >80 yrs (14). ED is a medical condition that has significant impact on self-esteem, interpersonal relationships and quality of life (15). Incomplete erection or inability to maintain erection during sexual intercourse is known as ED (16). The stigma and embarrassment of having ED symptoms may even lead to denial of problem. Due to this man himself does not identify as erectile dysfunction sufferer and due to performance issues in marital life, cases of depression are becoming a part of snowball sampling (17). Advances in understanding of the physiologic basis to ED over the past decade have led to important new therapeutic approaches to treat ED (18). Injectable medications, pumps, surgery or oral phosphodiesterase – 5 (PDE – 5) inhibitors are being prescribed (19). However, self - injection therapy in which the drug is injected to the base of penis makes self-administration quite challenging. This has very serious side effects such as priapism, penile pain etc (20). For other therapies men has to endure varying degrees of discomfort. This has led to increased use of PDE-5 inhibitors as they provide the most physiologic assisted erection in which no device is required, however, stimulation is necessary for erection and detumescence occurs when a stimulus has been removed (21).

Vardenafil hydrochloride tri-hydrate **(VAR)** is a potent and highly selective inhibitor of cGMP specific PDE-5, which belongs to BCS class II (22). The chemical class of the drug is benzenesulfonamide. VAR has been proven to be safe and effective treatment for ED. It is the most potent and specific of the three commercially available PDE-5 inhibitors. The drug is generally well tolerated, with a favorable safety profile. However, the drug is poorly absorbed following oral administration. The absolute oral bioavailability of the drug is only **15%** due to high first pass metabolism (23). Hence it is necessary to develop a patient compliant formulation that will help to overcome the above limitations and enhance bioavailability.

Iloperidone

The polygenetic disorder of schizophrenia is a highly complex disorder showing disorganized symptoms and cognitive dysfunction (24). Schizophrenia, characterized by profound disruptions in thinking, affecting language, perception and the sense of self, is a severe mental disorder which typically begins in late adolescence or early adulthood (25). Although recent progress has been achieved in the identification of aberrant genetic events and signaling pathways, the rate of schizophrenic episodes still increases remarkably and they continue to be the cause of a disproportionate level of suicide and mortality across individuals worldwide (26). Urbanization has been repeatedly associated with increased incidence of schizophrenia. As there is no surgical procedure to arrest the progression of disease, P.O. administration of drug is the only option left for the treatment. Consequently, a crucial challenge is to deliver therapeutic agents in effective concentration to the D2 and 5-HT2 receptors where it provides antagonistic action. As most of the new atypical antipsychotics are BCS class II drugs, dissolution becomes the rate determining step for absorption (27). This often leads to BID to QID administration of the molecule to maintain the minimum effective concentration in plasma, which drops – off the patient compliance in the therapy. The potential way to ward off this rate limiting step of mass transfer from the solid surface to liquid phase is to hasten it by solubilising drug in lipid or oil prior which can enhance solubilisation in gut and permeation through the mucosal membrane (28).

Iloperidone **(ILO)**, D2 and 5 HT2 receptor antagonist, is a drug of choice for treatment of schizophrenia because of reduced liability to extrapyramidal side effects. Treatment of schizophrenia with second generation antipsychotic drug Iloperidone is USFDA approved regimen (29).

But its low solubility in water (0.012 mg/mL) restricts its effectiveness after oral administration. Significant first pass metabolism leads to poor absolute bioavailability (30). The absolute oral bioavailability of Iloperidone is only **36%**. Hence it is necessary to develop a formulation which will help to overcome the above limitations, thereby providing patient compliant formulation.

Experimental

ANALYTICAL TECHNIQUES

1. UV-Visible spectrophotometry

For both the selected molecules, ILO and VAR, UV-Visible spectrophotometric technique was developed in buffer pH 6.8, Methanol and Acetonitrile, and validated according to ICH guideline Q2 R1 (31). The technique developed in pH 6.8 buffer was used for *in-vitro* drug release. The Acetonitrile and Methanol technique was used for drug content and assay.

2. HPLC

The HPLC technique for both the drug was used for *ex-vivo* permeation study. For VAR, the mobile phase was acetonitrile: 0.01 M KH2PO4 Buffer :: 65: 35 pH adjusted to 6.5 with KOH for C18 column. At 220 λmax, for 1 mL/min flow rate the retention time was found to be 4.56 min. For ILO, the mobile phase was Acetonitrile: 2.5 mM ammonium formate pH adjusted to 3.5 with 95% Formic acid for C18 column. At 230 λmax, for 0.55 mL/min flow rate the R.T. was found to be 6.57.

3. LC-MS

The *in-vivo* pharmacokinetic drug release was studied by LC-MS. Both the drugs having high plasma protein binding were separated using Solid Phase Extraction (SPE) (32). The

rat plasma was separated from the blood at 3000 rpm for 5 min. The plasma was treated by SPE technique to prepare analyte for LC-MS. The analyte samples so prepared were quantified by LC-MS.

4. Gas Chromatography (GC)

To determine the residual solvents chloroform and methanol concentration, the GC method was used (33). This method was used during Niosomes preparation. For niosomes preparation while using thin film hydration, the ingredients were dissolved in methanol: chloroform combination. These solvents are Class 2 solvent as per ICH guidelines. The residual limit for both the solvents are 2000 and 40 ppm respectively. Based on the quantification of the residual solvent, the drying time for thin film hydration was determined.

PRE-FORMULATION STUDIES

DSC, FTIR and PXRD were used for drug identification and characterization. Based on the FTIR study, the compatibility between drug – excipient was determined. The selection of excipients for SMEDDS system was carried out based on compatibility and solubility of drug in oil/surfactant/co-surfactant (34). For Niosomes, compatibility and % Entrapment efficiency were taken into consideration (35).

SMEDDS - FORMULATION DEVELOPMENT and CHARACTERIZATION

For preparation of SMEDDS of both the drugs, screening of oils, surfactants and co-surfactants was carried out (34). Based on the solubility of **ILO** in different oils, oleic acid and Capmul MCM C8 were selected. On the basis of emulsifying capacity, Cremophor EL and Transcutol HP were selected as surfactant and co-surfactant respectively. The composition of ILO SMEDDS was optimized using Design of Experiment (DoE) based I-optimal mixture design and soft computing technique, Artificial Neural Network (ANN). Size was kept as response for optimization algorithm (36). Comparing both techniques for response - size, ANN was found to be having high prediction and low error value with high R value. For **VAR** SMEDDS, Capmul MCM C8 as oil, Cremophor EL as surfactant and PEG 400 as co-surfactant were selected. When emulsification ability of Cremophor EL

was compared with Tween 20, there was no significant difference. So, to compare the efficacy of formulation containing either tween 20 or Cremophor EL, the VAR SMEDDS formulation was prepared with both the surfactants. Based on the phase ternary diagram, the preliminary ratio of Oil: S_{mix} was identified. To further optimize the formulation i.e. to find control space in the explorable space, the DoE based approach was used (37).

The optimized formulation of SMEDDS were characterized for size measurement. The size measured using Dynamic Light Scattering (DLS) was compared with Small Angle Neutron Scattering (SANS) (38). Based on the size obtained from both the techniques, it was found that the hydrodynamic diameter (117 nm) measured by DLS was significantly larger than the diameter obtained by SANS (10.6 nm). Comparing the Tween 20 and Cremophor EL based hydrodynamic diameter for VAR SMEDDS, it was found that Tween 20 based formulation gave larger size. The higher R_h (hydrodynamic radius) for Tween 20 may be attributed to its viscosity strain (drag) at surface which is correlated to mobility and volume based size using Stokes-Einstein relation. When the static radius, R, was measured using SANS technique, the radius for both the samples was not significantly different (7.2 nm for Tween-20 and 10.2 nm for Cremophor- EL) (39).

The samples of SMEDDS were further characterized for Appearance, Transparency, Cryo-TEM, Zeta potential, Cloud point, Content Uniformity, Assay, viscosity, *in-vitro* lipolysis and *in-vitro* dissolution (40).

The Cryo-TEM, SANS and DLS were compared to identify actual radius, static radius and hydrodynamic radius.

NIOSOMES - FORMULATION DEVELOPMENT and CHARACTERIZATION

Different surfactants such as Span series and Tween series were used along with cholesterol to formulate niosomes by thin film hydration technique (35). The % Entrapment and Size were kept as response for selection of surfactant. For both the drugs, Span 60 was found to provide highest % Entrapment (10). Comparing Tween and Span series, it was found that surfactant with higher HLB values were found unsuitable to form niosomes. The Tween series when used it showed precipitation in some batches. In Span series, when the chain length increased, the % Entrapment was found to be increased. This behavior was due to

when molecular weight of surfactant increased, it made vesicles which were less leaky and stable to osmotic gradient. The % Entrapment was found to be dependent on the molar ratio used for drug: lipid and internal lipid ratio (Cholesterol: Span 60). Both ILO niosomes and VAR niosomes were optimized by DoE approach using D-optimal mixture design.

To prepare niosomes, cholesterol, Span 60 and drug were dissolved in Methanol: Chloroform::1:2. The thin film was formed at 50°C. After complete evaporation of solvent, the film was re-hydrated with water. This formed multi-lamellar vesicles. After probe sonication, the size was reduced (41).

The formulation was quantified for residual solvents (33). Based on that, the drying time to form thin film was determined. Based on the % Entrapment, the hydration time and sonication time was determined. The annealing time was given between two cycles of sonication which lead to formation of uni-lamellar vesicles with smaller size.

Niosomes were characterized for size by DLS and SANS. As SANS measures the size accurately for lesser than 100 nm, the static radius, R for niosomes vesicle could not be measured. It gave size of the bilayer thickness. This can be used to determine whether the drug is in between bilayer or the vesicle core comparing R of placebo niosomes with drug loaded niosomes.

The Cryo-SEM, Zeta potential, %Entrapment, assay and *in-vitro* release studies were carried out for niosomes.

STABILITY STUDIES

The developed formulations were evaluated for stability under ambient temperature for real time stability study and accelerated condition. The samples evaluated for appearance, size and drug release study did not show any significant difference from the actual values indicating the stability of samples.

CELL LINE STUDIES

In-vitro cell line studies were carried out using CACO 2 cell line. Cell viability study (MTT study), Cell uptake by FACS (quantitative), Cell uptake by confocal microscopy (qualitative) and cell permeability study were carried out (42). The toxicity profile comparison between pure drug and developed formulation by MTT showed that, the

formulations were non-toxic in nature. The formulation in which drug was replaced with coumarin 6 was used for the study. The cell uptake by confocal microscopy, when compared for the formulation with pure drug, was found that at the end of incubation period, the formulation treated cells showed more fluorescence as the penetration of surfactant based formulation is high. Its evidence was also obtained from FACS study wherein higher number of cells showed fluorescence for formulation treatment (43).

EX-VIVO STUDIES

The small intestine removed from the Sprague Dawley rat, washed with PBS to remove lumen content, was used for permeation study (44). The amount permeated across the intestinal membrane was more from formulation when compared with pure drug suspension. This may be attributed to the excipients used for formulation development. The surfactants used for SMEDDS and Niosomes leads to reversible opening of tight junction of the intestinal barrier (45).

IN-VIVO STUDIES

Pharmacokinetic and pharmacodynamic studies were carried out as per the approved protocol by IAEC, Faculty of Pharmacy, The M S University of Baroda. The rats or mice used herein were grouped in number of six for every practical.

To compare the bioavailability of the SMEDDS formulation with pure drug, the formulation and drug suspension containing equivalent amount of drug were administered orally to Sprague Dawley rats. The blood was withdrawn and plasma was separated. As both the drugs ILO and VAR have high protein binding, instead of protein precipitation, Solid phase Extraction (SPE) technique was carried out. For conditioning of Oasis HLB (make: Waters) cartridge, pH was raised for ILO to increase the retention as it is basic in nature. Whereas for VAR, pH was decreased below the pKa for retention. The elution was carried out using methanolic solvent having opposite pH value which was used for retention. The analyte sample so obtained was quantified by LC-MS technique.

The pathway of LBDDS absorption is lymphatic route. To confirm whether ILO and VAR absorption from SMEDDS occurs from the lymphatic route or not, the pathway was

blocked using cycloheximide inhibitor model (46). After oral administration of SMEDDS to Sprague – Dawley rats, the same methodology as described above was carried out and drug concentration was measured.
When these pharmacokinetic models were compared, it was found that SMEDDS increased drug bioavailability as compared to suspension. When SMEDDS result was compared with cycloheximide + SMEDDS, it was found that the drug absorption decreased after lymphatic absorption inhibition by cycloheximide.

For Niosomes formulation, the concentration after niosomes administration was compared with pure drug. The bioavailability increased with niosomes but C_{max} was less with respect to SMEDDS. The vesicular nature of the system sustained the drug release from niosomes which may have decreased C_{max}.

Coumarin 6 based formulation was administered orally to Sprague Dawley rats and the intestine tissue was removed for cryo-sectioning (47). The tissue was embedded in OCT media and cut into 7 µm thickness which was stained for nucleus visibility by DAPI dye. The confocal imaging of the tissue showed villi of the intestine and the absorption through the intestine was clearly visible as indicated by green fluorescence of Coumarin dye.

For ILO based formulation, pharmacodynamic studies were carried out using MK-801 induced psychosis model in mice (48). The mice were administered MK-801 by i.p. route. Forced swimming, rota rod and elevated plus maze types of behavioral tests were carried out. The brain removed from humanely euthanized mice were homogenized for ILO and Dopamine measurement by LC-MS and Nitric Oxide (NO) level by Griess's method (49). The results showed improvement in psychosis condition for treated mice when compared with model control mice.

For VAR based formulation, Streptozotocin (STZ) induced Diabetes Mellitus model in rats was used (50,51). The rats were kept under close monitoring after i.p. administration of STZ. The body weight was decreased and polyuria was observed with fruity smell. The animals were given access to food and water ad libitum. The glucose measurement before

and after administration of STZ indicated, diabetic condition of rats. This was further confirmed by measuring glycosylated Hb content. The rats which were eligible diabetic models were used and grouped accordingly.

After treatment period of 21 days, the rats were euthanized humanely. Penis and testes were isolated for histopathology study by Hematoxylin – Eosin (HE) staining and Masson trichome (MT) staining (52,53). Testosterone level and serum NO level was measured for each animal. After the scarification, the sperm count for every animal was carried out. The results showed that there was change in seminiferous tissue of testis and corpus cavernous of penis.

Conclusions

ILO and VAR based SMEDDS and Niosomes of nano size as confirmed by DLS, SANS and Cryo-TEM were successfully developed. These formulations were optimized by Design of Experiment approach and were compared with soft computing based ANN technique. The developed formulations were found to be non-toxic in CACO 2 cell line studies up to 100 µg/mL concentration as the surfactant concentration used in both the vesicular carrier systems niosomes and SMEDDS was chosen as per GRAS limit. Zeta potential confirmed the stability of the developed formulation. The higher Cmax and AUC as observed for *in-vivo* drug release indicated the increased relative bioavailability for both drugs. *In-vitro* lipolysis for SMEDDS also indicated that drug precipitation did not occur after digestion which was confirmed by PXRD. The *in-vivo* drug release in presence of cycloheximide confirmed lymphatic route of drug absorption for SMEDDS. The cryosection of intestine tissue after Coumarin 6 based formulation administration showed the drug absorption path form the intestinal villi. The pharmacodynamic study for developed formulation in MK 801 induced psychosis in mice and STZ induced ED in rats model proved the increased efficacy of developed formulation as indicated by measurement of various parameters.

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