1.0. Introduction

Generally, a physician prescribes drugs on the basis of the physiognomies of the medications and on the panorama that reliable and reproducible clinical effects will result. However, variability in drug response among patients is common, often leading to challenges in optimizing a dosage regimen for an individual patient (1). There are various routes by which drugs are commonly administered out of which oral route is strongly favored because of its suitability and comparatively economic and high level of patient compliance. Conversely, the oral dosage forms have to disintegrate, dissolved in gastrointestinal fluids, absorb across intestinal epithelia and metabolized in the liver, before they can reach their specific site of action. Due to such long pathway there are number of factors responsible for the low and variable oral bioavailability including release of active ingredient from the formulation, dissolution rate, stability of the drug candidate in the gastrointestinal (GI) atmosphere, permeability and metabolism in gut wall and liver. It is very well recognized that biological barriers such as hepatic as well as intestinal drug metabolizing enzymes (DMEs) and efflux drug transporters (EDTs), act as concierges of drugs and limit the systemic drug availability (2-4).

Solubility and permeability are very important parameter for a drug molecule to reach at site of action. Adequate levels of drug solubility and permeability is required and determines the amount of drug available for absorption and therapeutic effect (5). Several terms such as absorption, permeability and bioavailability need to understand to know how drug molecules are reaching the systemic circulation. Oral drug absorption is referred to as drug transfer across the apical membrane of an enterocyte, because the apical membrane is considered to be the rate limiting step for permeation of a membrane.

While permeability is a general term describing how readily the drug is transferred through a membrane. The Bioavailability (F) is defined as the fraction of the dose that reaches the systemic circulation and can be described according to Eq. 1.1,

$$F = f_a * (1 - E_g) * (1 - E_h) \qquad (1.1)$$

Where f_a is the fraction absorbed over the intestinal epithelia, E_g is the gut wall extraction and E_h is the hepatic extraction.

1.1. Major factors influencing bioavailability of drugs

In cases where the bioavailability was lower than 90%, the influence of the following mentioned factors has to be taken into account. There are several factors (Figure 1.1) which influence the permeability/bioavailability of the compounds, such as

1. Biopharmaceutical factors/ Factors related to the drug and to the dosage form

2. Pharmacokinetic factors/ Factors related to patient

3. Physiological factors related to Absorption,

- Gastrointestinal barrier
- Gastrointestinal (GI) pH
- Gastrointestinal motility and emptying
- Vascularity and Blood flow
- Instability of drugs in the GI tract
- Donnan Effect
- Drug interaction and Complexation
- Malabsorption

4. Drug Dispositioning effect and variation in plasma level

- Presystemic metabolism

- Partition in the body fat
- Metabolism and biotransformation
- Excretion

5. Factors related to patients

- Age
- Sex
- Body mass Index
- Diseased condition
- Genetic make up

Several drugs after oral administration transit to an insoluble form at physiological pH, which effectively slows the absorption rate. Dissolution rate and poor aqueous solubility are the foremost issues which effects oral delivery of numerous drugs. Furthermore, now a days around 40% of new chemical entities discovered exhibits poor aqueous solubility (6, 7). These drugs are generally belong to biopharmaceutical classification system [BCS] Class II or IV often shows low bioavailability and hefty variations in subject pharmacokinetics which results in to dearth of dose proportionality (7). Drugs degradation by the high acid content and digestive enzymes is also a vital defy in oral drug delivery.

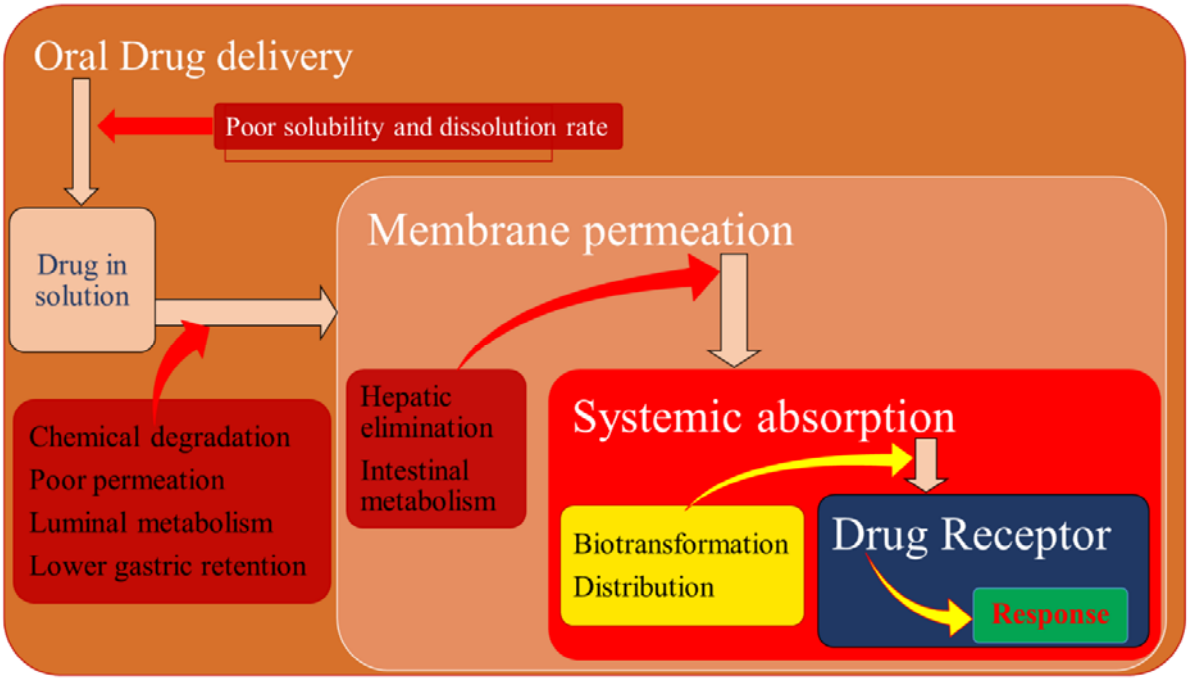

Figure 1.1 Factors influencing bioavailability of drugs in oral drug delivery

1.2. Strategies to improve bioavailability of drugs

As described earlier, oral delivery remains the most favorable and preferred route for drug administration. Currently more than 60% of drugs are marketed as oral products. These drugs cannot be effectively delivered by the oral route of administration in their original form due to above mentioned factors. Overcoming these barriers is currently one of the most challenging goals in oral drug delivery (8-10). Several strategies have been employed to improve the bioavailability of drugs after oral administration. Following are some of the strategies may be applied alone or in combination to provide a solution to the problem of poor bioavailability. Various strategies contemporary used to enhance oral bioavailabilty has been depicted in Figure 1.2.

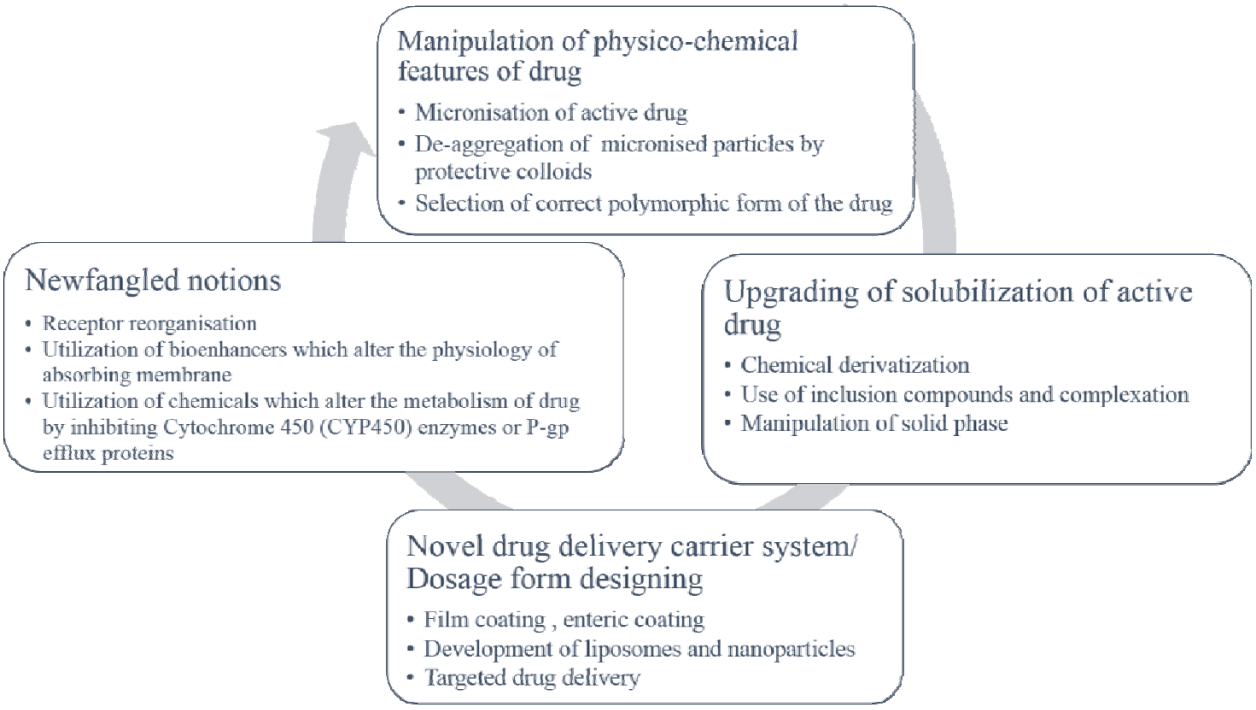

Figure 1.2 Various Strategies to improve bioavailability of drugs

1. Lipid based strategies

- Lipid solutions and suspensions
- Micro emulsions
- Self-emulsifying drug delivery system (SEDDS)
- Self-microemulsifying drug delivery system (SMEDDS)
- Self-double emulsifying drug delivery system (SDEDDS)
- Solid lipid nanoparticles (SLN)
- Nanostructured lipid carriers (NLC)
- Lipid – drug conjugate (LDC)
- Liposomes
- Nano emulsions

2. Intestinal transport improvement by drug nano carriers

- Mucoadhesive nanoparticles
- Permeation enhancer nanoparticles
- Coated nanoparticles
- Mucolytic nanoparticles

3. Different dosage form designing

- Gastro retentive systems for the enhancement of site-specific absorption
- Spray freeze drying
- Chitosan derivatives

4. Chemical derivatization of Moiety

- Prodrug

5. Utilization of chemicals

- Bile salts
- Saponins
- Straight chain fatty acids
- Natural bioenhancers

Although some of these approaches have been demonstrated to be successful in laboratory scale research, they still present challenges in terms of long-term safety and reproducibility in the clinical situation. Even various sophisticated novel drug delivery approaches have been attempted to improve bioavailability, which has less market potential due to its high cost of manufacturing and lack of stability.

1.2.1 Lipid based strategies

Oral lipid-based products entered the market in 1981 and presently having well commercially availability in oral formulations. Lipid-based formulations group having very different physical appearance, ranging from simple triglyceride vehicles to more sophisticated formulations such as solutions, suspensions, emulsions, self-emulsifying systems and micro-emulsions. They represent one of the most popular approaches to overcome the absorption barriers and to improve the bioavailability. These systems contain a wide range of oils, surfactants and co-solvents.

1.2.1.1. Lipid solutions and suspensions

The simple lipid-based formulations encompass one excipient such as oleic acid, corn oil, peanut oil, sesame oil, medium chain triglycerides or medium chain mono and diglycerides. Many of the over-the-counter sold products contain polyethylene glycol as the solubilizing excipients. Table 1.1 illustrates some over the counter or some marketed formulations of lipid solutions. Therefore, it is better to formulate a drug in an oily liquid and achieve good absorption which leads to enhanced bioavailability.

Table 1.1 Marketed formulations of lipid solutions

Manufacturer	**Active Pharmaceutical ingredient**
AstraZeneka	Clomethiazole
Solvay Pharmaceuticals	Dronabinol
Mochida Pharmaceuticals	Ethyl icosapentate

1.2.1.2. Micro emulsions

Micro emulsion concept was introduced by Hoar and Schulman in 1943. They prepared first micro emulsions by dispersing oil in an aqueous surfactant solution with addition of alcohol as a co-surfactant, leading to a transparent, stable formulation. Some Patents of micro emulsions is shown in Table 1.2. The existence of this theoretical structure was later confirmed by use of various technologies (11).

Table 1.2 Patents on micro emulsions

Patent No	Title of Patent
US5023271	Pharmaceutical microemulsions
US5055303	Solid controlled release Bioadherent Emulsions
US5744155	Bioadhesive emulsion preparations for enhanced drug delivery
US5925626	Hyaluronic acid fractions having pharmaceutical activity, and pharmaceutical composition containing the same

1.2.1.3. Self-emulsifying drug delivery system (SEDDS)

SEDDS are mixtures of oils and surfactants, ideally isotropic and sometimes containing co-solvents which emulsify spontaneously to produce fine oil-in-water emulsions when introduced into aqueous phase under gentle agitation. The most widespread approach for enhancing the bioavailability is the incorporation of the active lipophilic component into inert lipid vehicles, surfactant dispersions. They have also been formulated using medium chain tri-glyceride oils and non-ionic surfactants, the latter being less toxic. Upon peroral

administration, these systems form fine emulsions (or micro-emulsions) in gastro-intestinal tract (GIT) with mild agitation provided by gastric mobility. Some examples of marketed formulations of SEDDS are listed in Table 4 which have potential advantages of enhanced oral bioavailability enabling reduction in dose, more consistent temporal profiles of drug absorption, selective targeting of drug(s) toward specific absorption window in GIT and protection of drug(s) from the hostile environment in gut.

1.2.1.4. Self-microemulsifying drug delivery system (SMEDDS)

The absorption of poorly permeable drugs can be enhanced by using self-microemulsifying drug delivery systems. In current decade a lot of research has been conducted on developing self-micro emulsifying drug delivery systems (SMEDDS). Generally these systems are isotropic mixtures of oils, surfactants and co-solvents /co-surfactants. Once administered in to the GI system, they are diluted with gastrointestinal fluid and the gastric motility provides the agitation for the formation of a fine oil-in-water (o/w) micro emulsion (SMEDDS). The major difference between a SEDDS and SMEDDS is that the former when diluted results in a droplet size between 100 and 300 nm and the later results in a droplet size of less than 50 nm (12).

1.2.1.5. Self-double emulsifying drug delivery system (SDEDDS)

The self-double emulsifying drug delivery systems (SDEDDS) can be used for enhancing oral bioavailability of drugs with high solubility and low permeability, but their industrial application is inadequate because of low stability. These are Water-in-oil-in-water (w/o/w) double emulsions complex systems consisting of aqueous droplets dispersed within larger oil droplets. The internal aqueous droplets encapsulated by the oil membrane can be seen as a storage chamber for hydrophilic drugs. This structure can

safeguard the drug dissolved in the internal aqueous phase and have shown great promise for enhancing oral bioavailability of compounds. Similar to SEDDS, SDEDDS can be extemporaneously emulsified in the mixed aqueous gastrointestinal environment. But the formed emulsions are water in- oil-in-water (w/o/w) double emulsions not o/w emulsions, and drugs are encapsulated in the internal water phase of the double emulsions. Compared to conventional thermodynamically unstable emulsions, SDEEDS are stable formulation system. In addition SDEDDS can be filled directly into soft or hard gelatin capsules which are easy for administration and storage (13).

1.2.1.6. Solid-lipid nanoparticle (SLN)

Solid lipid nanoparticles (SLN) are an alternative carrier system to colloidal carriers such as - emulsions, liposomes and polymeric micro and nanoparticles. It was firstly introduced in 1991. Nanoparticles prepared from solid lipids are enticing major consideration as novel colloidal drug carrier for intravenous route. SLN are colloidal carriers ranging from 50 to 1000nm, which are composed of physiological lipid, dispersed in water or in aqueous surfactant solution. In order to overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by a solid lipid, which eventually transformed into solid lipid nanoparticles. SLN gives unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals (14). Reduced size and narrow size distribution provides biological opportunities for site-specific drug delivery. SLNs show controlled release of active drug over a long period. SLN are easy to produce in industrial scale by hot dispersion method having no toxic metabolite and economical as compared to other

dosage forms. SLNs have been reported to be useful as drug carriers to treat neoplasms. Tumor targeting has been achieved with SLNs loaded with drugs such as methotrexate and camptothecin. Tamoxifen an anticancer drug is incorporated in SLN to prolong release of drug after *i.v.* administration. Mitoxantrone-loaded SLN local injections were formulated to reduce the toxicity and improve the safety and bioavailability of drug. Efficacy of doxorubicin (Dox) has been reported to be enhanced by incorporation in SLNs. In the methodology the Dox was complex with soybean-oil-based anionic polymer and dispersed together with a lipid in water to form Dox-loaded solid lipid nanoparticles. The system has enhanced its efficacy and reduced breast cancer cells.

1.2.1.7. Nanostructured lipid carriers

The application of NLC as a drug delivery system is enhanced by eliminating the use of organic solvents in the preparation stage and using the hot high-pressure homogenization technique. Polysorbate20 and polysorbate80 are non-ionic surfactants commonly used as excipients and emulsifiers in medications for parenteral administration. In 2006 Souto and Müller reported that among the nanostructured lipid carriers that contain solid lipids together with liquid oils are Miglyol®, α-tocopherol etc. (15).

1.2.1.8. Lipid–drug conjugates (LDC)

LDC nanoparticles having drug loading capacity up to 33% overcome the loading issues with SLN. An insoluble drug- lipid conjugate bulk is first prepared either by salt formation or by covalent linking. This LDC is further processed with an aqueous surfactant solution such as tween to a nanoparticle formulation, using high pressure homogenization. Such matrices may have potential application in brain targeting of hydrophilic drug in serious protozoal infections. Increase in bioavailability is achieved by

LDC with the advantage of control and targeted drug release. LDCs are easy to validate, scale up and sterilize (16).

1.2.1.9. Nano emulsions

Nano-emulsions can be another outstanding vehicles for oral delivery of poorly permeable drugs since they can be manufactured from both kinds of excipients that have solubilising or permeation enhancing properties. Oral nano emulsions have a droplet size of less than 150 nm. These are mainly o/w type. As compare to conventional emulsions, they promote enhanced gastrointestinal absorption and reduce individual inequality for drugs. Moreover nano emulsions convinced degree of protection against degradation or may progress difficult organoleptic properties of the actives. Some nano-emulsions tend to self-emulsify in aqueous media, which makes them remarkable for oral formulations. Pluronics® are class of non-ionic surfactants which are very well known for their very low toxicity. The Pluronics®, also known as poloxamersare triblock copolymers of poly (oxy ethylene)–poly (oxy propylene)–poly (oxy ethylene). It has been demonstrated that Pluronics® influence the carrier mediated transport of drugs depending on their structural composition. This effect might be advantageous for the treatment of drug resistant tumors as well as to enhance the oral bioavailability of actives. Nano emulsion system based on different Pluronics® have been found, that can be used to stabilize lipophilic drugs. It has been demonstrated that formulations have influence on intestinal permeation of both transcellularly and paracellularly transported drugs. They can, therefore find their use for actives with either low permeability or low solubility or a combination of both problems. Furthermore since the Pluronics® are known to obstruct p-gp-mediated drug efflux, they might also be used for formulation of actively effluxed drugs like many cytostatics. Since

cytotoxicity is not perceived with these formulations, they can find their use in early formulations (17).

1.2.2. Intestinal transport improvement by drug nano carriers

1.2.2.1. Mucoadhesive nanoparticles

Generally, after oral administration of nanoparticles, the bulk of the nanoparticles transit through the GI tract is over a short time, leading to inadequate release of the contents, thereby precluding the realization of a high drug concentration in the GI tract, leading to low oral bioavailability and poor efficacy. In last few years, the mucoadhesive properties of some materials have been exposed for the strategy to improve the residence time of nanocarriers in the GI tract. It is done by improving the minimizing direct transit and fecal elimination (18, 19). Other than these advantages, there are chances of entrapment in the slackly devotee mucus layer and subsequently defenseless for rapid mucociliary clearance before slow penetration into firmly adherent mucus layer. So, functioning attempts are required to overcome these shortcomings by exploiting the benefits of surface modification of nanoparticles or using specific pH-responsive formulations. Polymer such as CS, PLA, PLGA, poly(sebacic acid) and PAA shown to have mucoadhesive properties and can be employed in the preparation of mucoadhesive nanoparticles (20).

1.2.2.2. Permeation enhancer nanoparticles

Lack of adequate absorption by mucoadhesive nanoparticles prompted researches into the development of concocted muco-inert mucus-penetrating nanoparticles with the use of carriers that can penetrate across the mucus layer. These carriers can release drug in the locality of the epithelium cells. They take the advantages of penetration enhancer

materials in different ways, including the possibility of mucus rheology, reducing mucus viscosity, and enhancing the fluidity of the membrane by interaction with lipids or proteins in the epithelial cells. This leads to the perturbation of intercellular lipids and intracellular barriers disturbance, inhibiting the effect on various existent enzymes within mucus layer, interfering with tight junction components (in particular desmosomes), and increasing the thermodynamic activity of the drugs altering their partition coefficient and interference with the calcium ions. Surfactants, chelators and fatty acids are the most important permeation enhancers used in nanostructures (21, 22).

1.2.2.3. Coated nanoparticles

In surface modification coating nanoparticle approach has also been used. In this strategy nanoparticle surface coating with uncharged hydrophilic materials such as PEG has been done which leads to reduced hydrophobicity, increased zeta potential, and consequent improved stability in the mucus, as well as enhanced transport across the mucus (23). It may result in larger particles; decreased epithelial transport or other unidentified mechanisms without altering mucus structure. Despite that, larger particles may help for higher drug encapsulation and slower drug release, allowing to enhance therapeutic efficacy (24, 25). Improving the rate and amount of transport by overcoming friction forces predicted by the Stokes–Einstein equation, as well as enhancing encapsulation efficiency should be taken in mind during this approach. As, it is observed that due to the variation in thicknesses of mucus in different regions of GI tract and according to the aims of each study, nanoparticles with different characteristics need to be developed.

1.2.2.4. Mucolytic nanoparticles

In this phenomenon, oral nano particulate delivery is achieved by unsettling the barrier properties of the mucus lining. Mucinex®) is a commonly used mucolytic agent, can create a mucus-free surface that eases the penetration and enhances nanoparticles access to underlying epithelial cells. N-acetyl-L-cysteine (NAC) shows a significant reduction in the cross-linking of mucin fibers by cutting disulfide bonds and reducing mucus thickness and rheology. Therefore, the use of such agents along with targeting moieties to improve the intestinal absorption of nanoparticles may be considered. Although, few studies have been reported that bacterial translocation was significantly enhanced by the removal of mucus barrier which indicates the importance of mucus role in protection of deep seated epithelial cells from contamination. Also, mucus discouragement may lead to damage of the epithelial cells due to encountering to enzymes, acids or other GI destructive secreted substances. Till date several mucolytic agents are established with the aim to help nanoparticle mucosal transport such as pilocarpine, nacystelyn, thymosin-4 and gelsolin (20, 26-28).

1.2.3. Different dosage form designing

1.2.3.1. Gastro retentive systems for the enhancement of site-specific absorption

Drugs such as antacids, misoprostol and antibiotics have been designed using gastroretentive systems to enhance local action in the stomach (29). Gastroretentive systems employed in pharmaceutical formulations for drugs that are preferentially absorbed in the stomach may be bioadhesive, swelling and expanding (30) floatable (31, 32), delayed gastric emptying (33), high density. commercially available gastroretentive formulations are Valrelease®, a diazepam floating capsule; Madopar®, a combination

formulation of benserazide and levodopa; Topalkan®, an aluminium-magnesium antacid, liquid Gaviscon®; a floating liquid alginate preparation; and Almagate Flot-Coat®, an antacid preparation. However, it is relevant to memo that an effective gastroretentive drug delivery system must be able to withstand the peristaltic forces, constrictions, grinding and churning mechanisms in order to resist premature gastric emptying. Novel gastroretentive drug delivery systems combined with a timed release mechanism holds the promise of a future once-a-day regimen for a wide range of drugs. However, as promising as gastroretentive drug delivery system appears, it must be pointed out that it is not suitable for drugs that may cause gastric irritation or those unstable in acidic medium.

1.2.3.2. Spray freeze drying

Spray freeze drying (SFD) an also important technique for enhancement of permeability of poorly permeable drugs. The SFD processed formulation and commercial available generally exhibit large inter-animal variability in oral bioavailability. Literature suggest that improvement in both dissolution and intestinal permeability by the SFD processed system is precarious to reduce the considerable inter individual inconsistency in oral absorption commonly observed with class III and IV compounds.

1.2.3.3. Chitosan derivatives

Chitosan itself is a non-toxic polymer having a number of applications in drug delivery including that of absorption of hydrophilic macromolecular drugs. Chitosan, when protonated, is capable to increase the paracellular permeability of peptide drugs across mucosal epithelia. Chitosan derivatives have been assessed to overcome chitosan's incomplete solubility and effectiveness as absorption enhancer at neutral pH values such as those found in the intestinal tract. Monocarboxy methylated chitosan (MCC) is poly

ampholytic with anionic macromolecules at neutral pH values. MCC appears to be less potent compared to the quaternized derivative. Nevertheless MCC is found to increase the permeation and absorption of low molecular weight heparin (LMWH; an anionic polysaccharide) across intestinal epithelia. Neither chitosan derivatives aggravates damage of the cell membrane, nor therefore do they not alter the variability of intestinal epithelia cells (34).

1.2.4. Chemical derivatization of Moiety

1.2.4.1. Prodrug

To enhance the drug absorption and bioavailability chemical modification of drugs to produce prodrugs and more permeable analogues has been widely studied as a useful approach. Various ampicillin derivatives are one of the well-known examples of increasing the lipophilicity of agents to enhance absorption of a polar drug by prodrug strategy (35). Ampicillin due to its hydrophilic nature is only 30 - 40% absorbed from the gastrointestinal tract. By esterification of carboxyl group of ampicillin the produgs of ampicillin such as pivampicilline, bacampicilln and talampicillin were synthesized. These produgs were more lipophilic than the parent compound following oral administration and they showed higher bioavailability in comparison with ampicillin. To improve the bioavailability of BCS Class 3 drug acyclovir a prodrug named as gancyclovir has also been available in the market.

1.2.5. Utilization of chemicals

1.2.5.1. Bile salts

Bile, which contains glycine and taurine conjugates of cholic acid and chenideoxycholic acid, emulsifies dietary fat and accelerates lipolysis and transport of lipid products

through the unstirred water layer of the intestinal mucosa by micellar solubilisation. The bile salts which escape from active reabsorption in the ileum are metebolised to secondary bile salts deoxycholic acid & lithocholic acid by the bacterial flora. The diminishing order of hydrophilicity is as follows taurine conjugates > glycine conjugates > free bile salts. Polarity increases with the number of hydroxyl groups. Bile salts are capable to bind calcium, their binding properties decreasing with increasing hydrophilicity. Although mechanism is yet to be not clear but it may be carried out by effects on the mucous layer and on paracellular and transcellular absorption routes. They have been reported to affect the intestinal glycocalyx structureand to diminish gastric and intestinal mucous. A transcellular absorption enhancing effect is suggested by the phospholipid disordering action of unconjugated and conjugated bile salts (36).

1.2.5.2. Saponins

Saponins are glycosides of vegetable source with surface tension reducing properties and haemolytic action. They are capable of precipitating sterols and exert intestinal and transdermal absorption promoting properties. It is imaginable that the absorption supporting properties of saponins are mediated by their surfactants like properties. On the other hand, a transcellular promoting effect may also be caused by interaction with the membrane stabilizer cholesterol. This shows that saponins exhibit absorption promoting activity at relatively low concentrations. However; also for these compounds the issue of safety vs. efficacy requires further investigation.

1.2.5.3. Straight chain fatty acids

The medium chain fatty acids including capric acid (C10), lauric acid (C12) and long chain fatty acids, such as oleic acid (C18) have been shown to increase the permeability

of a series of hydrophilic drugs by dilating the tight junction and/or changing the cytoskeleton of the intestinal epithelial cells without prominent cytotoxicity. One of the foremost advantages of these excipients is the comfort of incorporating into the conventional oral dosage forms without the need for complex or expensive formulation technique.

1.2.5.4. Natural bioenhancers

The bioenhancers, act as competitive, uncompetitive, non-competitive, mixed or irreversible inhibitors of EDTs and DMEs. The bioenhancers can be a ligand of P-gp, CYP450 or ligand of both. Bioenhancers increases drug bioavailability by increasing the drug diffusion and decrease in hepatic and intestinal metabolism. Some of structural features of bioenhancers that have been reported as P-gp inhibitors includes hydrophobic character of the molecule, especially those comprising two co-planar aromatic rings, a positively charged nitrogen group, or a carbonyl group. The bioenhancers can be co-administered with compounds such as aminoacridines, antibiotics, antiestrogens, benzazepines, cephalosporines, colchicine, cyclic peptides, dibenzazepines, epipodophyllotoxins, flavones, imidazole, isoquinolines, macrolides, opioids, phenylalkylamines, phenothiazines, piperazines, piperidines, polyethylene glycols, pyridines, pyridones, pyrimidines, pyrrolidines, quinazolines, quinolines, quinones, rauwolfia alkaloids, retinoids, salicylates, sorbitans, steroids, unsaturated fatty acids and vinca alkaloids.

1.3. Gastrointestinal permeability and metabolism a major concern

Deprived gastrointestinal permeability and metabolism are also one of the major factors and considerably affects oral bioavailability of many drugs. Small intestine

has been progressively recognized as a significant site for first-pass metabolism. Efflux proteins and cytochrome P450 isoenzymes (37-41), are abundantly expressed in small intestine and contribute in deprived bioavailability of several drugs (42-44). CYP3A4 is the most important in the CYP family which is responsible for metabolism of many drugs (45). Transporters in small intestine are the chief contributor to poor absorption and low bioavailability. From ABC transporter family, four members are localized in membrane of enterocytes and are supposed to form a barrier to intestinal absorption of drugs. These four transporters are P-gp, MRP2, BCRP, and MRP4 (46). Their expression level differs between different sections of the intestine. In general, MXR, MRP2 and P-gp are expressed at high level in the small intestine and considered as the rate limiting barrier to oral drug absorption. P-gp is the most thoroughly characterized ABC transporter concerning the role in limiting intestinal absorption. The most direct evidence has come from the numerous in vivo studies utilizing P-gp knock-out mice. One of the example is drug paclitaxel and its area under curve [AUC] was shown to be 6-fold greater in the P-gp-/- mice than the wild type [P- gp+/+] mice (47). It was also observed that greater increase of paclitaxel AUC in mice when the P-gp function was blocked with P-gp inhibitor (48). Although the expression levels of both the MRP2 and MXR are higher in the small intestine than the expression of Pgp, there are much fewer data available on their role in drug absorption.

A few examples, however, have been published: MRP2 has been shown to limit absorption of a phenylimidazo [4,5-b]pyridine [PhIP] derivative, a food derived carcinogen (49), and MXR has been shown to limit absorption of topotecan in Mdr1a/1b [-/-] mice (50).

1.4. Drug Metabolizing Enzymes (DMEs)

There are several pathways of pre-systemic drug loss following oral administration. Metabolism by CYP enzymes at the intestinal wall and in the liver are the major contributors of drug loss during the absorption pathway. In particular, due to high abundance of CYP and the tactical location of liver, whereby the drug absorbed through the gut wall is first taken to the liver, the hepatic CYP enzymes are the leading major components of first-pass effect. While hepatic metabolism is generally considered as the major contributing site to first-pass metabolism but recent research has also indicated that intestinal metabolism also plays as important or even more significant role than liver metabolism. Intestinal and hepatic metabolism appears to be regulated independently and there is no correlation in their contents and activity. There has been a strong interest in delineating relative contribution of intestinal or hepatic metabolism to the overall first-pass effect and oral bioavailability of drugs (51). About 40% of CYP enzymes have been shown to be genetically polymorphic. Three main phenotypes are extensive metabolisers (EM), poor metabolisers (PM), and intermediate metabolisers (IM) (52). Now as the patients which are EM will metabolise the drug more extensively, resulting in lower plasma concentration and consequently, poor or no drug effect. Patients that are PM, by contrast, will metabolise the drug less extensively, resulting in higher plasma concentrations that might lead to drug toxicity (53). CYP3A, CYP2C, and CYP1A2 are the major constitute of the human liver CYP family (54). In intestine major constituent are CYP3A and CYP2C9 accounting for 80 and 15%, respectively, of total immune quantified (55). In terms of the number of substrate drugs, the most prominent isoenzyme is CYP3A4, which can metabolize more than 60% of drugs presently in use.

1.5. Efflux Drug Transports (EDTs)

Efflux of drugs is recognized as the expulsion of the drug molecules across the cellular membrane from the cells via a clinically significant systematic transportation system such as P-glycoprotein (P-gp), breast cancer resistant protein (BCRP), cytoplasmic transport, multidrug resistant associated protein (MRP), flurochrome efflux, methotrexate efflux (folates) etc (56, 57). ABC proteins family members are present in all living organisms and is one of the largest protein families. The preserved structure and function of these transporters suggests an important role. The phenomena of drugs resistance by ABC proteins has been known from 38 years (58). Multi drug resistance [MDR] in humans are due to MDR-associated protein and breast cancer resistance protein and P-glycoprotein (59). As shown in Figure 1.3, these transporters are localized in the apical and basolateral membrane of intestinal enterocytes and function as organism defense mechanism by driving drugs out of cell and back to the intestinal lumen (60-62). Table 1.3 exhibit the different transporters with their drug substrates. In all of this P-gp has been found to play a major role in the eviction of drug molecule (63). P-gp is a phosphorylated glycoprotein having an ostensible molecular weight of 170 kDa. It fits in ABC transporters. It is vigor reliant on drug efflux pump and is a kind of ATPase (64). It is uttered as a single chain containing two homologous portions of equal length, each containing six transmembrane domains and two ATP binding regions separated by a flexible linker polypeptide region between the Walker A and B motifs.

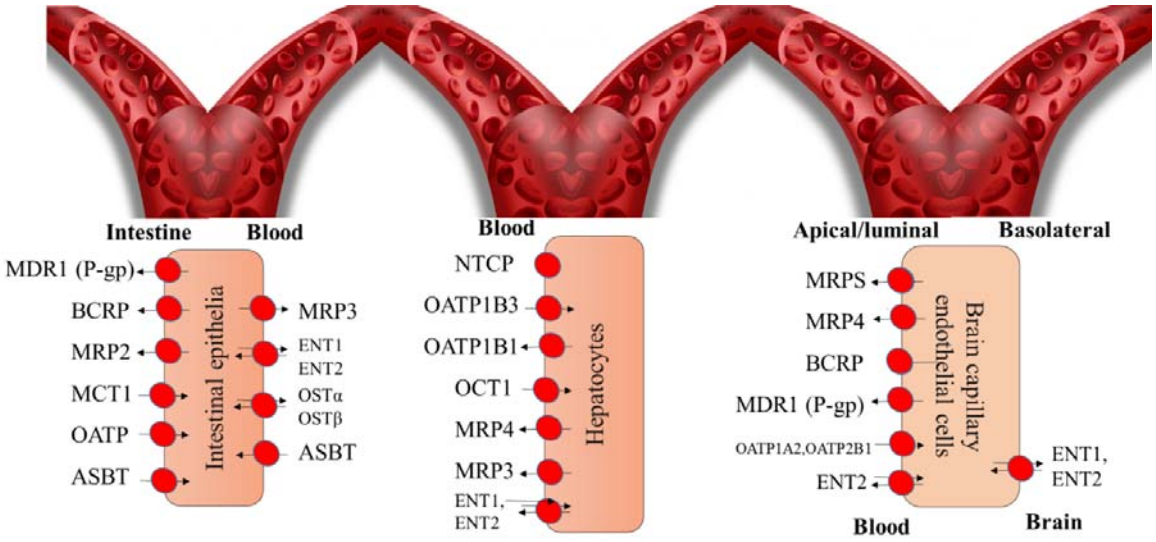

Figure 1.3 Transporters are localized in the apical and basolateral membrane

Table 1.3 Different transporter protein with their drug substrate

Transporter protein	Drug Substrates
P-gp/MDR1	Actinomycin D, cerivastatin, colchicine, cyclosporine A, daunorubicin, digoxin, docetaxel, doxorubicin, erythromycin, etoposide, fexofenadine, imatinib, indinavir, irinotecan, ivermectin, lapatinib, loperamide, losartan, nelfinavir, oseltamivir, paclitaxel, quinidine, ritonavir, saquinavir, sparfloxacin, tamoxifen, terfenadine, topotecan, verapamil, vinblastine, vincristine.
BCRP	Abacavir, ciprofloxacin, dantrolene, dipyridamole, enrofloxacin, erlotinib, etoposide, furosemide, gefitinib, genistein, glyburide, grepafloxacin, hydrochlorothiazide, imatinib, irinotecan, lamivudine, lapatinib, methotrexate, mitoxantrone, prazosin, rosuvastatin, tamoxifen, triamterene, zidovudine.
MRP1	Daunorubicin, doxorubicin, epirubicin, grepafloxacin, methotrexate, vincristine.
MRP2	Indinavir, methotrexate, ritonavir, saquinavir, vinblastine.

MRP3	Etoposide, methotrexate.
MRP4	Ceftizoxime, topotecan.
PepT1	Ampicillin, bestatin, captoril, cephalexin, enalapril, fosinopril, oseltamivir, valciclovir.
OATP 1A2	Fexofenadine, levofloxacin, methotrexate, ouabain, rosuvastatin, saquinavir.
OATP 2B1	Atorvastatin, bosentan, fluvastatin, glyburide, pitavastatin, pravastatin, montelukast
MCT1	Arbaclofen placarbil, carindacillin, gabapentin enacarbil, ketoprofen, naproxen, phenethicillin, rosuvastatin.
SMVT	Gabapentin enacarbil
OCTN1	Quinidine, verapamil.
OCTN2	Cephaloridine, imatinib, ipratropium, tiotropium, quinidine, verapamil, propicillin.
CNT1	Cytarabine, gemcitabine, zidovudine.
CNT2	Clofarabine, fluorouridine, ribavirin.
ENT1	Cladribine, clofarabine, cytarabine, gemcitabine, ribavirin.
ENT2	Clofarabine, gemcitabine, zidovudine.

As from the Immuno histochemical exploration, through monoclonal antibody, the occurrence of P-gp has been verified in an extensive assortment of tissues chiefly in capillary endothelial cells, columnar epithelial cells of lower gastrointestinal tract (GIT) and in the proximal tubule of kidney at apacial side. Due to discerning spreading of P-gp at the harbor of drug entrance and exit it shows a vigorous physiological role in absorption, distribution and excretion of drugs. In a comprehensive way P-gp labored as a biochemical barricade for the entrance of drugs and as a vacuity cleaner to banish them from liver etc. and eventually results in segregation from the systemic circulation (70-72). Numerous epitomes has been proposed in past to explicate P-gp mechanism by which

they expelled drug candidates. The widespread model proposed was not able to clearly explain the mechanism but the most reasonable mechanism was suggested by Gottesman and Pastan in 1993. As for the functioning of P-gp the binding and hydrolysis of ATP were found to be critical. The explanation about the catalytic cycle of P-gp by Sauna et al., enlarges the prospects for the discovery of the P-gp inhibitors. In this cycle initially both the ATP and drug bind to the protein at their respective binding sites. Here the nucleotide hydrolyses to ADP, produces potency for the extrusion of drug and after that the release of ADP from binding site ends the first part of catalytic cycle following the conformational change that reduces affinity. Furthermore, second catalytic series is initiated by the hydrolysis for another molecule of ATP which leads to the release of energy which is utilized to reorient the protein to its innate conformation. Consecutive liberation of ADP ends up the additional catalytic cycle which takes P-gp back to the original state and from where it again binds to both substrate and nucleotide to initiate the next cycle (68-71).

1.6. Tactics used to inhibit EDTs and DMEs

There are number of strategies which has been used to inhibit EDTs and DMEs such as:

- Synthetic approaches
    - ❖ Small molecule inhibitors
        - First Generation Molecules
        - Second Generation Molecules
        - Third Generation Molecules
    - ❖ Novel drug delivery system (NDDS) and synthetic excipients
- Natural Bioenhancers

1.6.1. Synthetic approaches

1.6.1.1. Small molecule inhibitors [SMIs]

These molecules were developed on the basis of the screening of available compounds including parent molecule optimizations and chemical synthesis using combinational chemistry approaches. These molecules are further classified in to three categories first, second and third generation inhibitors.

1.6.1.1.1. First generation inhibitors

These molecules are non-selective and less potent inhibitors. These agents are primarily developed for other indications but later in trials observed to be having property of P-gp inhibitors e.g. verapamil, cyclosporine, quinidine and reserpine. As these agents are very less potent so a very high dose of these agents required for inhibition of P-gp which upsurge them to toxic level due to which many of these molecules has been failed in clinical trials (72).

1.6.1.1.2. Second-generation inhibitors

In the interest of development of P-gp inhibitors and issues comes out with first generation inhibitors a new class called as second-generation inhibitors were introduce with some structural modification in to the first generation inhibitors. As in a case chirality of the verapamil was alter to attain the improved pharmacological profile so dexverapamil was used. This R-isomer of verapamil does not show any cardiac activity. However, due to the chiral optimization, these second-generation ended up as they were found to be CYP3A4 substrates which lead to their metabolism (73).

1.6.1.1.3. Third generation inhibitors

After failure of first and second-generation inhibitors a new modern technique has been involved in development of molecules for P-gp inhibition i.e quantitative structure-activity relationship [QSAR] application to high throughput screening techniques [HTS] and combinational chemistry methods. The compounds develop using these techniques are extremely specific, having lesser interactions with CYP3A4. As this generation was believe to be the most promising, but still due to toxicity reports are not used frequently. Compounds synthesized with mentioned strategy include VX-710 (biricodar, a cyclopropyldibenzosuberane modulator, developed by Eli Lilly Inc.) and GF 120918 (elacridar, an acridonecarboxamide derivative, developed by GlaxoSmithKline) (74).

1.6.2. Novel drug delivery system (NDDS) and synthetic excipients

A flawless compound which can be used as P-gp and CYP inhibitor is that which is non-toxic and has no pharmacological action of its own. During the exploration of these inhibitors, several pharmaceutical inert additives and excipients were studied in the past to study their P-gp and CYP inhibition activity and to recognize their potential in increasing the drug permeability across the lipid membrane. Several pharmaceutical agents of synthetic origin like surfactants, polymers etc. were found to have the P-gp inhibitory action. In a study conducted by wasan et al. it has been reported that efflux of Rh123 remains unaffected across the cell monolayer when treated in the absorptive direction but the EDTs activity was reduced after treatment with Peceol®, Gelucire® 44/14 and positive control,100μM verapamil. Additionally, it has been also enlightened that reduction in P-gp efflux activity was due to reduction in protein expression after treatment with Peceol® and Gelucire® 44/14. In this study it was concluded that 0.25%

(v/v) and 0.5% (v/v) concentrations of Peceol® reduces P-gp protein levels to 62.4% and 68.4% of the control respectively. While, treatments with Gelucire® 44/14 in 0.01% (w/v) and 0.02% (w/v) concentration reduced P-gp protein levels to 64.5% and 51.8% respectively. These consequences of synthetic excipients enlighten a newer approach that contributes a step ahead in the enhancement of bioavailability for drugs by formulating with such synthetic lipid based excipients (75).

As we all know nowadays, there is a huge increase in the research area of novel drug delivery systems [NDDS] such as microspheres, nanoparticles and liposomes. These systems have inherent P-gp circumventing property (76). In this a P-gp carrier was saturated by the stealth particles which reversed P-gp efflux and concentration of drug increases across the membrane (77). The escaping of P-gp efflux action in polymeric conjugates and mixed micelles has been observed as they has been transported via receptor mediated endocytosis. Additionally to this their degradation products can block P-gp by direct interaction and inhibition mechanism (78, 79). The surfactant polymer nanoparticles undergo endocytic vesicular transport (80) and overcome P-gp mediated efflux.

The combination of these above two approaches P-gp inhibiting excipients and NDDS can create more potent inhibitors and can create a great opportunity for the betterment to health care system. In NDDS system liposome are mostly preferred. These are made up of bilayer or multilayers that contain phospholipids and cholesterol enveloping hydrophilic aqueous region. In this system both kind of drugs like lipophilic and hydrophilic can be encapsulated within nano-carrier and made available for absorption at intestinal membrane surface. Anionic liposomes composed of anionic phospholipids such

as cardiolipin and phosphatidylserine enhances cellular absorption and cellular toxicity as compared to free drugs when administered. These lipids directly interact with P-gp pump and inhibit the P-gp pump resulting in higher cellular absorption of incorporated drugs. If liposomes penetrate into the cell, it will release the drug intracellularly to overcome P-gp efflux system. The study conducted by Parmentier et al. shows an ideal example of combination of NDDS and synthetic excipients. In this study, it has been revealed that oral delivery of proteins or other drug substances, which have a low oral bioavailability due to gastro-intestinal degradation and low permeation can be improvised by formulating a liposomes containing both the stabilizing agent glycerylcaldityl tetraether (GCTE) and synthetic excipients (as a bioenhancer). In this study, it has been clearly shown that GCTE improves stability of liposomes against sodium taurocholate and especially it can reduce the destabilising effect of synthetic excipients (bioenhancers) in the liposomal membrane. Various approaches other than these has been summarized in Table 1.4 (81-87).

Table 1.4 Various NDDS approaches to bypass EDTs

Approach's	Action
Stimuli Responsive Liposomes	These types of Liposomes trigger the release based on response in terms of pH change, Temperature change or any other stimuli.
Liposomes and EDTs inhibitor combination	These will inhibit P-gp and successfully deliver our desired drug candidate
Gene therapy approaches resistance	Can be used to bypass EDTS or to prevent drug
Anionic Liposomes	Contain Anionic Phospholipid Cardiolipin or Phosphatidylserine that may inhibit P-gp by interaction with membrane lipids.

1.6.3. Natural bioenhancers

The bioenhancers, act as competitive, uncompetitive, non-competitive, mixed or irreversible inhibitors of EDTs and DMEs. The bioenhancers can be a ligand of P-gp, CYP450 or ligand of both. Bioenhancers increases drug bioavailability by increasing the drug diffusion and decrease in hepatic and intestinal metabolism. Some of structural features of bioenhancers that have been reported as P-gp inhibitors includes hydrophobic character of the molecule, especially those comprising two co-planar aromatic rings, a positively charged nitrogen group, or a carbonyl group. The bioenhancers can be co-administered with compounds such as aminoacridines, antibiotics, antiestrogens, benzazepines, cephalosporines, colchicine, cyclic peptides, dibenzazepines, epipodophyllotoxins, flavones, imidazole, isoquinolines, macrolides, opioids, phenylalkylamines, phenothiazines, piperazines, piperidines, polyethylene glycols, pyridines, pyridones, pyrimidines, pyrrolidines, quinazolines, quinolines, quinones, rauwolfia alkaloids, retinoids, salicylates, sorbitans, steroids, unsaturated fatty acids and vinca alkaloids.

Bioenhancers whenever used in an apt concentration, decrease the activity of P-gp, especially P-gp drug transport back into the intestinal lumen will be reduced. The appropriate concentration should include amounts necessary to increase integrated systemic concentrations over time of the drug used in conjunction with the bioenhancer. The concentration of bioenhancer required to produce a sufficient inhibition of P-gp drug transport varies with the delivery vehicle used for the bioenhancer and the drug. The luminal concentration of the bioenhancer should be related to the drug and bioenhancer relative affinities for P-gp. As the affinity of drug for P-gp increases, the required

concentration of the appropriate bioenhancer will increase. Most bioenhancers of commercial application will decrease P-gp drug transport by at least 10%, more preferably by at least 50%, and even more preferably by at least 75%.

Drug biotransformation is also reduced by natural bioenhancer by inhibiting CYP3A activity in gut epithelial cells which leads to a total increase in drug bioavailability in the systemic circulation. Scarcer drug molecules will be metabolized by phase I enzymes in the gut and will not be available for phase II conjugation enzymes. This leads to an increase in the concentration of untransformed drug passing from gut into the blood and into other tissues in the body. Reduction of drug biotransformation or increased drug absorption will decrease variability of oral bioavailability, to some degree, because the increase in bioavailability will begin to approach the theoretical maximum of 100% oral bioavailability. The rise in bioavailability will be generally larger in subjects with lower oral bioavailability, which results in the reduction of inter-individual and intra-individual variation. Addition of bioenhancer will reduce inter-individual and intra-individual variation of systemic concentrations of a drug or compound. The decay in biotransformation by inhibition of CYP in other tissues, will also results in increased drug bioavailability. The major advantage of targeting a bioenhancer to the gut, however, is that it allows the use of lower systemic concentrations of bioenhancer compared to inhibitors that target CYP3A in the liver. After oral administration of a bioenhancer, concentrations will be highest at the luminal surface of the gut epithelia if they have not been diluted by systemic fluids of the body. Luminal concentrations that are greater as compared to blood concentrations will permit preferential inhibition of CYP3A in gut instead of the liver. Bioenhancers that preferentially inhibit gut CYP3A will also be a

particularly effective means of increasing drug bioavailability while minimizing the effects of greater concentrations of bioenhancers in tissues (other than the gut).

1.6.3.1. Mechanism of Action

There are several mechanism by which natural bioenhancers acting. They increase bioavailability of drugs by acting on drug metabolism process while they increase bioavailability of nutraceuticals by acting on gastrointestinal tract to enhance absorption. Different mechanisms of action assumed for natural bioenhancers are shown in Figure 1.4.

1.6.3.1.1. Piperine

Piperine interaction with enzymatic drug biotransformation reaction in hepatic tissue has been studied in both *in vitro* and *in vivo*. DMEs inhibited by piperine as shown in Figure 1.5 are AHH, Uridine diphosphate- (UDP-) glucuronyl transferase, Ethylmorphine-N-demethylase, 7-Ethoxycoumarin-O-deethylase, 3-Hydroxy-benzo (a) pyrene glucuronidation (88, 89), UDP-glucose dehydrogenase (UDP-GDH) 5-lipoxygenase, Cyclooxygenase-1 (90, 91) Cytochrome P450 (92). Several studies demonstrated that piperine is a nonspecific inhibitor of DMEs.

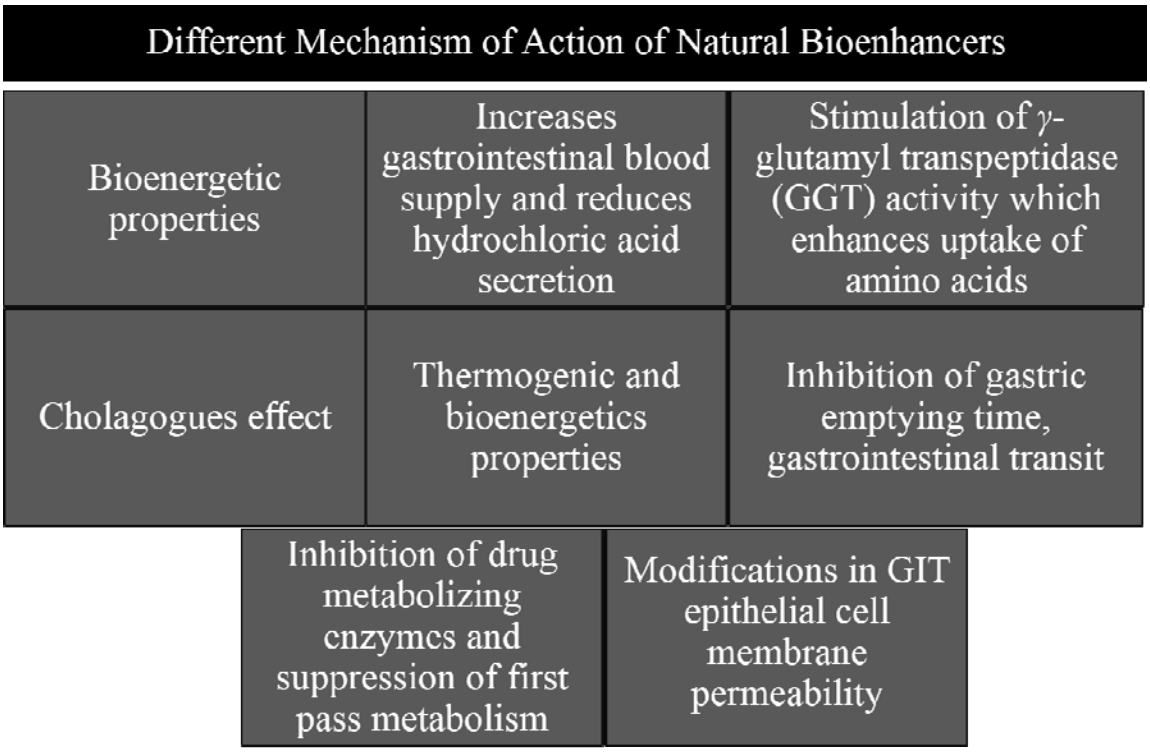

Figure 1.4 Different Mechanism of actions of natural bioenhancers

Co-administration of piperine strongly inhibited the hepatic AHH and Uridine diphosphate-(UDP-) glucuronyltransferase (UGT). The utmost inhibition of AHH observed within an hour. Piperine modified the rate of glucuronidation by lowering the endogenous UDP-glucuronic acid (UDP-GA) content and also by inhibiting the transferase activity. Allameh et al. (93) previously reported that piperine in rat tissues improves bioavailability of aflatoxin B_1. Nevertheless, few investigational findings also indicate piperine's ability to increase the bioavailability of drugs, like rifampicin (94, 95), isoniazid (96), and diclofenac sodium (97). Co-administration of piperine has also been reported to increase the blood levels and efficacy of many of drugs such as spartein, sulfadiazine, tetracycline, theophylline and propranolol in humans. A list of drugs bioenhanced by pieperine has been represented in Table 1.5.

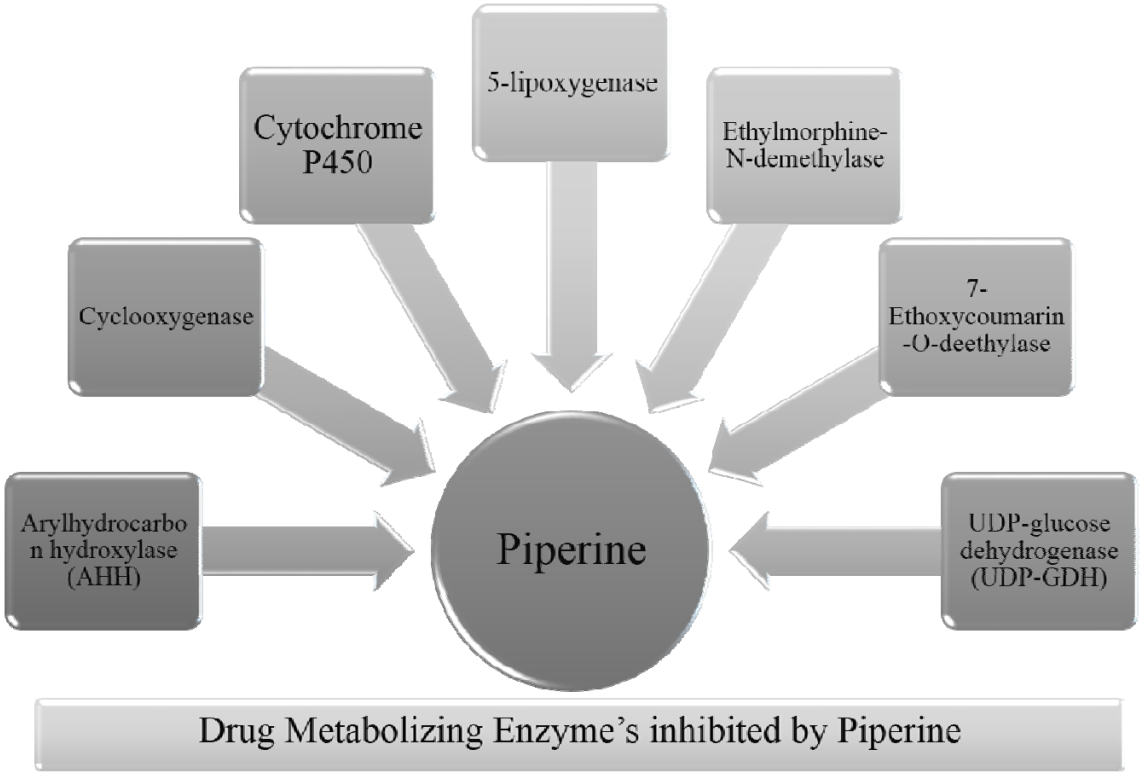

Figure 1.5 Drug Metabolizing enzymes inhibited by Piperine

In addition to these a study on pharmacokinetics profile of phenytoin with the co-administration of piperine to healthy volunteers has also been reported. In this study, five volunteers received either a single oral dose of phenytoin alone and in combination with multiple doses of piperine (20 mg × 7 days) followed by an oral dose of phenytoin. Blood samples were collected up to 48 hr after the drug administration and analyzed for phenytoin by the enzyme-multiplied immunoassay technique. The results were compiled and reported that a single daily dose of piperine for 7 days decreased the $t_{1/2\alpha}$ ($P < 0.05$), prolonged the $t_{1/2}$ ($P < 0.01$), and produced a higher Area under curve (AUC) ($P < 0.05$) in comparison to control pheytoin. From this study, It has been concluded that piperine also on multiple-dose administration improves the pharmacokinetic profile of the antiepileptic (98).

Table 1.5 Drugs bioavailability bioenhanced by Piperine

Drug	Dose of Piperine	Study model
Curcumin	20mg/kg	Rats
Carbamazepine	20mg/kg	Human Volunteers
Resveratrol	10mg/kg	Mice
Fexofenadine	10 or 20mg/kg	Rats
Gatifloxacin	15mg/kg	Layer Birds
Phenytoin	20mg/kg x 7days	Human Volunteers
Ampcillin and norloxacin	20mg/kg	Rabbits
Nimuslide	10mg/kg	Mice
Nevirapine	20mg for 7 days	Human Volunteers
Atenolol	10mg/kg	Rats
Losartan Potassium	10mg/kg	Rats
Nutraceuticals bioenhancer by piperine: Vitamin B_1, Vitamin B_2, Lysine, isoleucine, Boswellic acid, Ginsenosides etc.		

1.6.1.3.2. Naringin

Naringin is a major flavonoid glycoside found in grapefruit, apples, onions and tea. It exerts a variety of pharmacological effects such as antioxidant activity, antiulcer activity, antiallergic activity and anticancer activity, and blood lipid lowering. Naringin has also been reported as a CYP3A4 inhibitor as well as a P-glycoprotein modulator (99, 100). In a comparison study on diltiazem in rats, the control was diltiazem alone and test was diltiazem was dose to rats treated with naringin, from the results obtained it was observed that C_{max} and AUC of diltiazem increased by approx. 2-fold in rats pretreated with naringin. Although, there was no significant change in t_{max} and terminal $t_{1/2}$ of diltiazem. Subsequently, absolute bioavailability (AB) and relative bioavailability (RB) values of

diltiazem in the presence of naringin were significantly higher ($P < 0.05$) than those from control group. As also Metabolite parent AUC ratio in the presence of naringin decreased by 30% compared to the control, suggesting that naringin could be effective to inhibit the metabolism of diltiazem. In conclusion, the concomitant use of naringin significantly enhanced the oral exposure of diltiazem in rats (101). A list of drugs bioenhanced by pieperine has been represented in Table 1.6.

Table 1.6 Drugs bioavailability bioenhanced by Naringin

Drug	Dose of Naringin	Study model
Paclitaxel	3.3 mg/kg and 10mg/kg (pretreated 30min)	Rats
Verapamil	1.5, 7.5 and 15mg/kg (pretreated 30min)	Rabbits

In another study for paclitaxel rats were pretreated for thirty min with oral naringin at the dose level of 3.3 and 10 mg/kg before intravenous (3 mg/kg) administration of paclitaxel. After intravenous administration of paclitaxel, the AUC was significantly rises (40.8% and 49.1% for naringin doses of 3.3 and 10 mg/kg, resp.), and Cl_B was significantly slower (29.0% and 33.0% decrease, resp.) than controls. The significantly greater AUC was due to an inhibition of metabolism of paclitaxel via CYP3A1/2 by oral naringin (102). Naringin effect on the pharmacokinetics profile of verapamil was also studied using rabbit as animal model. The pharmacokinetic parameters were evaluated after administering verapamil (9 mg/kg) orally as a control to rabbits and verapamil 9 mg/kg to the pretreated rabbits with naringin at different dose levels (1.5, 7.5, and 15 mg/kg). Naringin pretreatment significantly altered the pharmacokinetic parameters of verapamil as compared to the control group C_{max}, and AUC of verapamil were found to be

significantly high in rabbits pretreated with naringin. However, no significant change has been observed in t_{max} and $t_{1/2}$ of verapamil. Consequently, pretreatment of naringin significantly increased the AB of verapamil significantly in a dose-dependent manner and elevated the RB of verapamil by 1.26–1.69 fold. In conclusion, pretreatment or co-administration of naringin could enhance the oral bioavailability of drugs and can be used to reduce the issue of low and variable bioavailability (103).

1.6.1.3.3. Cow urine distillate

As literature suggests, cow urine distillate is more prominent as bioenhancer than cow urine specially referred to increase the effectiveness of anti-microbial, antifungal, and anticancer drugs (104). The bioenhancing activity is due to increase in the absorption of drugs across the cell membrane. Cow urine has antitoxic activity against the cadmium chloride toxicity and it can be used as a bioenhancer of zinc. In a study, mature male mice exposed to cadmium chloride only showed 0% fertility rate. However, the animals exposed to cadmium chloride with cow urine distillate and zinc sulfate showed 90% fertility rate with 100% viability and lactation indices. Fertility index was also found to be 88% in group treated with cadmium chloride + cow urine distillate. Thus, these results indicate that cow urine distillate works as an antitoxic against the cadmium chloride toxicity and it can be used as a bioenhancer of zinc (105). Cow urine distillate increased the activity of rifampicin by about five to seven times against *Escherichia coli* and three to eleven times against Gram-positive bacteria. It probably acts by enhancing the transport of antibiotics across the membrane of GIT. The enhancement in transport is approximately two to seven times (106). Cow urine distillate enhanced the gonadotropin releasing hormone conjugate on the reproductive hormones and estrous cycle of female

mice (107). Cow urine distillate significantly enhanced the effect of gonadotropin releasing hormone on the gonadosomatic indices, sperm motility, sperm count, and sperm morphology, especially in 90 and 120 day treated groups ($P < 0.05$) in male mice. Cow urine distillate enhanced these effects because of its immune modulatory properties (108).

1.6.1.3.4. Glycyrrhizin

It is a glycoside obtained from roots and stolon of Liquorice (Glycyrrhiza glabra). It has expectorant action to treat bronchitis and can also reduce inflammation, allergies, asthma, gastritis, peptic ulcers, rheumatism, and sore throat. It helps the liver to detoxify drugs and is used for treatment of liver disease. It strengthens the immune system, stimulates the adrenal gland and is diuretic and laxative. Glycyrrhizin is fifty times sweeter than sugar. Primary uses include treatment for peptic ulcers and stomach ailments, respiratory, and intestinal passages. Glycyrrhizin exhibits activities like antihepatotoxic activity (109, 110), anti-inflammatory activity (111, 112), anticancer activity (113), and antiviral activity (114, 115). Cell division inhibitory action of anticancer agent Taxol (paclitaxel) in the animal cell culture experiments using cancerous cell line MCF-7 was found to be increased in the presence of glycyrrhizin. The anticancerous activity of Taxol in terms of inhibiting the growth and multiplication of MCF-7 cancer cells was markedly enhanced by almost five fold and cancerous cells growth inhibited by Taxol (0.01 μg/mL) in presence of glycyrrhizin (1μg/mL) was even higher than the treatment with Taxol (0.05 μg/mL) alone (116, 117).

1.6.1.3.5. Cuminum cyminum

The foremost components of cuminum cyminum oil are cumin aldehyde and β-pinene (118). The bioenhancer chemical constituent present in cumin is 3', 5-dihydroxyflavone-

7-O-β-D-galactouronide-4'-β-O-Dglucopyranoside. The attribute of C. cyminum of bioavailability enhancement could be due to permeation enhancement effect. C. cyminum exhibits activities like estrogenic activity (119) anticancer activity (120), anti-microbial activity (121), antitussive effect (122), antioxidant activity (123), and antifungal activity (124). The various dose of its fractions responsible for the bioavailability enhancement activity ranged from 0.5 to 25mg/kg body weight. The dosage level of the composition comprising C. cyminum extract is in the range of 10–30 mg/kg body weight and composition comprising bioactive fraction is in the range of 2–20 mg/kg body weight. The composition contain C. cyminum extract which provides bioavailability up to 335%. Both the Polar and nonpolar extracts of C. cyminum and piperine increased bioavailability up to 435% (125).

1.6.1.3.6. Allicin

Allicin is an allyl sulfur containing compound obtained from Garlic (Allium sativum). It exhibits activities like antiplatelet activity (126), antioxidant activity (127, 128), antibacterial activity (129, 130), anticancer activity (131), Immunomodulating effect (132), antidiabetic activity (133, 134), antiparasitic activity (135), antifungal activity (136-138), antioxidant and anti-inflammatory activity (139) and antiviral activity (140). Cu^{2+} showed a dose-dependent fungicidal activity against saccharomyces cerevisiae cells. Its lethal effect was extremely enhanced in presence of allicin. The fungicidal activity of Cu2+ was unaffected or rather attenuated by other sulfur containing compounds such as N-acetyl-cysteine, lcysteine, or dithiothreitol. Ca^{2+} could absolutely protect against the lethal effect of Cu^{2+} itself, but showed no protection against the fungicidal activity of Cu^{2+}. Cu^{2+} accelerated an endogenous generation of reactive oxygen species (ROS) in S.

cerevisiae cells at a lethal concentration, but such intracellular oxidative stress induction was not observed during cell death progression upon treatment with Cu^{2+} and allicin. A surfactant, sodium-lauroyl sarcosinate (SLS) enhanced the solubilization of a few proteins including alkyl hydroperoxide reductase1 (AHP1) in intact cells, accounting for the absence of this protein in the extract from allicin treated cells. Allicin treated cells were rendered extremely sensitive to the subsequent Cu^{2+} treatment as in the case of sls treated cells. Allicin treated cells and sls treated cells similarly showed an increased sensitivity to exogenously added tert-butyl hydroperoxide (t-BOOH), organic peroxide that is detoxified by the action of AHP1. This study suggests the influences of allicin on the mode of cell surface localization or the related function of AHP1 as a defense against phospholipid peroxidation by the external action of Cu2+ (141).

1.6.1.3.7. Quercetin

Quercetin is a plant derived flavonoid found in fruits, vegetables, leaves, and grains. It exhibited activities including antioxidant, radical scavenging, anti-inflammatory, antiatherosclerotic, anticancer, and antiviral effects (142). It is a potent inhibitor of CYP3A4 and a modulator of P-glycoprotein (143, 144). It has been shown to decrease bioavailability of cyclosporin in pigs and rats (145). Wang et al. (146) reported that co-administration of quercetin with digoxin leads to lethal effects in pigs. The effect of quercetin on the bioavailability of paclitaxel was studied after oral administration in rats. A list of drugs bioenhanced by pieperine has been represented in Table 1.7.

Table 1.7 Drugs bioavailability enhanced by Quercetin

Drug	Dose of Quercetin	Study model
Paclitaxel and prodrug	2,10,20 mg/kg	Rats
Verapamil and norverapamil	5 and 15mg/kg	Rabbits
Diltiazem	2,10,20 mg/kg	Rabbits
Tamoxifen	2.5, 7.5 and 15 mg/kg	Rats
Fexofenadine	500mg for 7 days	Healthy volunteers
Etoposides	1, 5 and 15mg/kg	Rats
Green tea extract		Rats

Paclitaxel (40mg/kg) and prodrug (280 mg/kg, 40 mg/kg as the paclitaxel) were administered orally to rats pretreated with quercetin (2, 10, 20 mg/kg). The pharmacokinetic parameters indicated that quercetin increases bioavailability of paclitaxel in rats. The plasma concentrations of paclitaxel pretreated with quercetin were increased significantly ($P < 0.01$ for paclitaxel; $P < 0.05$ for prodrug) compared to the control. The areas under the plasma concentration-time curve (AUC) and the peak concentrations (Cmax) of paclitaxel pretreated with quercetin were significantly higher ($P < 0.01$) than the control. The half-life ($t_{(1/2)}$) and mean residence times were significantly ($P < 0.05$) longer compared to the control. The absolute bioavailability (AB %) of paclitaxel pretreated with quercetin was significantly higher ($P < 0.01$) than the control. The AUC of paclitaxel after administration of the prodrug to rats pretreated with quercetin was significantly ($P < 0.05$) higher than the prodrug control. The relative bioavailability of paclitaxel after administration of the prodrug to rats pretreated with quercetin was one to two fold higher than the prodrug control (147). In a study, pharmacokinetic profile of verapamil and norverapamil were also compared after the oral

administration of verapamil (10mg/kg) to rabbits in the presence and absence of quercetin (5.0 and 15 mg/kg). While co-administration of quercetin concurrently was not effective to enhance the oral bioavailability of verapamil, but as in the pretreatment of quercetin thirty min before administration of verapamil significantly altered the pharmacokinetics of verapamil. Compared with the control group, the Cmax and AUC of verapamil increased approximately 2-fold in the rabbits pretreated with 15 mg/kg quercetin. There was no significant change in t_{max} and $t_{1/2}$ of verapamil in the presence of quercetin. Consequently, absolute and RB values of verapamil in the rabbits pretreated with quercetin were significantly higher ($P < 0.05$) than those from the control group. In conclusion, pretreatment of quercetin significantly enhanced the oral exposure of verapamil (148).

In a study, effect of quercetin at different dose level (2, 10, 20 mg/kg) pretreatment on the bioavailability of diltiazem (15mg/kg) was studied in rabbits after oral administration. The plasma concentrations of diltiazem in the rabbits pretreated with quercetin were increased significantly ($P <0.05$, at 2mg/kg; $P < 0.01$, at 10 and 20mg/kg) compared with the control, but the plasma concentrations of diltiazem co-administered with quercetin were not significant. The AUC and Cmax of the diltiazem in the rabbits pretreated with quercetin were significantly higher ($P < 0.05$, at 2mg/kg; $P < 0.01$, at 10 and 20mg/kg) than the control. The AB of diltiazem in the rabbits pretreated with quercetin was significantly ($P < 0.05$ at 2mg/kg, $P < 0.01$ at 10 and 20 mg/kg) higher (9.10–12.81%) than the control (4.64%). The bioavailability of diltiazem in the rabbits pretreated with quercetin is increased significantly but not in the case of co-administration of quercetin in rabbits. The pharmacokinetic parameters of tamoxifen in plasma were determined after

oral administration of tamoxifen (10mg/kg) with or without quercetinat different dose levels (2.5, 7.5 and 15 mg/kg). The co-administration of quercetin (2.5 and 7.5mg/kg) significantly ($P < 0.05$) increased the Ka, Cmax, and AUC of tamoxifen. The AB of tamoxifen with 2.5 and 7.5 mg/kg quercetin ranged from 18.0 to 24.1%, which was significantly higher than the control group, 15.0% ($P < 0.05$). The RB of tamoxifen co-administered with quercetin was approximately 2 times higher than the control group. The co-administration of quercetin caused no significant changes in the terminal t1/2 and tmax of tamoxifen. The enhanced bioavailability of tamoxifen as a result of its co-administration with quercetin might be due to the effect of quercetin promoting the intestinal absorption and reducing the first-pass metabolism of tamoxifen (149). Short term effect of quercetin on the pharmacokinetics of fexofenadine was studied in 12 healthy volunteers. Subjects were treated daily for 7 days with 500 mg quercetin or placebo three times a day. On day 7, a single dose of 60 mg fexofenadine was administered orally. Effects on AUC, Cmax, t1/2, Kel, and ClR are shown in after administration of fexofenadine and in combination with quercetin. The results showed that short term use of quercetin elevated the plasma concentrations of fexofenadine in humans (150). The effect of quercetin on the pharmacokinetics of etoposide was studied in rats. Etoposide was administered to rats orally (9mg/kg) or intravenous (3 mg/kg) without or with quercetin (1, 5, or 15 mg/kg). The presence of quercetin significantly (5 mg/kg, $P < 0.05$; 15 mg/kg, $P < 0.01$) increased the AUC of orally administered etoposide from 43.0–53.2%. The presence of quercetin significantly (5 mg/kg, $P < 0.05$; 15 mg/kg, $P < 0.01$) increased the AB of etoposide to 12.7 or 13.6%. The results from the study shows that bioavailability of etoposide was enhanced by quercetin (151).

Epigallocatechin gallate (EGCG) is a main anticancer component in green tea. The pharmacokinetic parameters after oral administration of green tea extract (GTE) and GTE + quercetin (Q). In rat, supplementations of GTE and GTE +Q raised the plasma Cmax were 55.29 ± 1.70 and 94.44 ± 1.59 ng/mL, respectively. The corresponding t1/2 elimination was 2.04 ± 0.2h and 2.28 ± 0.049 h. The AUC 0−24 h was 510.16 ± 9.88 ng·h/mL and 794.08 ± 15.27 ng·h/mL ($P \leq 0.05$), respectively. The results showed that quercetin increased bioavailability of EGCG in rat.

1.7. *In vitro* models for prediction of intestinal absorption

A wide range of predicting absorption models has been developed. In this section we have mainly focused on *in vitro* models. In the past, the lipophilicity measurement was a commonly used method for the prediction of membrane permeability, having a drawback of unreliable absorption prediction. Therefore, to overcome this, various *in vitro* models have been developed. The main rational behind these *in vitro* permeability assays is to screen and predict oral absorption potential of new formulations for intestinal drug delivery. *In vitro* models are valuable and provide an opportunity to predict the interactions between the GI cells and the nanoparticles and thus, providing a better understanding on drugs is interacting with intestinal cells. In terms of nanoparticulate oral drug delivery systems, *in vitro* models are still at an early stage and far from a routine absorption evaluation method; however, there are a lot of attempts to improve the applicability of these models. In recent years, two introduced prevalent permeability assays are cell-based (especially Caco-2 cells) and PAMPA. Using cell culture models to mimic the intestinal absorption of drugs is the most common *in vitro* method for

prediction of drug bioavailability. These two tests are complementary tests and should be used in parallel to evaluate both passive and active permeability of drugs (152, 153).

1.7.1. Parallel artificial membrane permeability assay (PAMPA)

It was introduced as a support to the usual cellular models which have gathered a significant interest in the pharmaceutical industry (154, 155). It is basically a non-cell permeability model designed to evaluate passive transcellular permeability of drug formulations. It lacks in the paracellular spaces and active transporters. Assay is usually carried out in 96-well filter plates to measure the ability of drugs or other compounds to diffuse from a donor to an acceptor compartment separated by an artificial lipid membrane of lecithin prepared in an inert organic solvent like dodecane. One of the advantageous properties of these models is the ability to determine the effect of pH on the permeability and absorption of nanoparticles by adjusting the pH of the solutions used in the analysis. There is also an excellent correlation between the data of PAMPA and human intestinal absorption of passively absorbed compounds. Another advantage of PAMPA is less time and labour intensive compared to cell culture or *in vivo* models, while the predictability is similar. PAMPA is also automation compatible, inexpensive and straightforward (156). In PAMPA model, for improving *in vitro* and *in vivo* correlation of nano particulate for oral delivery, it is also essential to optimize the incubation time, pH, lipid mixture and lipid concentration due to their influence on enhancing the assay's ability to predict the absorption of nanoparticles. By optimizing the PAMPA model, many reports have shown the application of this model for examining the absorption of drug-loaded nanocarriers.

1.7.2. Caco-2 Cells

Over the last decades, human colorectal carcinoma Caco-2 cells have been particularly employed and well-studied for estimating drug permeability across the lining of the small intestine cells (157, 158). Caco-2 cells are golden choice for intestinal absorption studies. However, it is acknowledged that Caco-2 cells differ from enterocytes and the *in vivo* physiological environment in various means, therefore, the data achieved from this model need to be interpreted with caution and is not always in agreement with *in vivo* observations. The first difference is derivation of Caco-2 cells from cancerous cells of the human colon which results in different activated biochemical pathways. Despite all inconsistencies, owing to the reliability, ease of experiment and small quantity needed to the tested compound, this model can be considered as the most widely valuable *in vitro* intestinal model (159-161).

1.7.3. HT-29 and HT-29/Caco-2 Co-culture

HT-29 cell line was developed from human colon carcinoma cells. Currently, there are variable subtypes of HT-29 intestinal model are available and applicable for some specific studies. For example, HT-29GlucH multilayer cells are characterized with high proportion of goblet cells capable of mucin secretion with the aim to simplify studies related to the effect of mucus layer on drug transport. Another subtype is a highly reliable intestinal model HT-29MTXE12 monolayer cells with desirable mucus thickness and tight junctions in their structure, representing a well in vivo correlated model to predict the effect of mucus on nanoparticle absorption studies. In comparison to Caco-2 cells, HT-29 models are leakier, allowing hydrophilic compounds to pass easier through paracellular pathway. To combine positive effects of these models, they can be co-

cultured to provide a rational biophysical model of the intestine. In the co-culture, HT29-MTX cell lines which are adapted from parental HT-29 using methotrexate (MTX) are usually employed to yield spontaneous mucin production. This system resembles the small intestine more closely than Caco-2 and HT-29 cells alone by producing mucus and decreasing overall tightness resistance values (lower P-gp activity), resulting in enhanced paracellular permeability. This co-cultured model is suitable to determine the extent of the nanoparticles transport by both passive paracellular and carrier-mediated mechanisms (162-164).

1.7.4. Madin-Darby canine kidney (MDCK Cells)

In an appropriate culture medium, similar to Caco-2 cells, MDCK differentiate into columnar epithelial cells containing tight junctions. Few years ago, this model was used to assess the membrane permeability of therapeutics. Despite basic differences in the nature of MDCK and Caco-2 cells, there is a good similarity for passive absorption in these two models; however, there is different levels of transporters expression. Three-day cultivation time of MDCK cells is an advantage toward Caco-2 cells, considering decreased labour intensive and contamination. MDCK cells are developed to study permeation mechanisms in distal renal epithelia as well as cell growth regulation. In light of its suitability for absorption assessments, many researchers point out the necessity of more experiments to confirm this fact (165, 166).

1.7.5. 2/4/A1 cell line

2/4/A1 monolayer cells are basically originated from the fetal rat intestine to mimic more closely passive paracellular permeability of the human small intestine. Due to the similar paracellular pore radius of about 9.0 ± 0.2 Å with human intestine and lower

transepithelial resistance (TEER) compared to Caco-2 cells, representing an ideal cell line for poorly permeable compounds. Other benefits of this model include the presence of brush-border membrane enzymes, transporter proteins and TJs (167).

1.7.6. T-84

T-84 Cell lines are perepared from pulmonary metastasis of human colon carcinoma cells. These cells are currently known as the best available model for investigating colon specific nanoparticles. These are highly polarized monolayers with few microvilli in apical membrane and considerably high TER deducing the presence of well differentiated tight junctions (168).

1.7.7. Lewis lung carcinoma-porcine kidney cells (LLC-PK1)

Lewis lung carcinoma-porcine kidney cells are an alternative to Caco-2 for permeability assessment (transcellular and paracellular passive absorption). They expresses higher levels of CYP3A4, suggesting a desirable model to study the effect of metabolism and transport in the cell line. Recently, researchers have proposed the suitability of transfected cell lines with influx transporters including OAT and human peptide transporter (hPepT1) or efflux transporters such as multidrug resistance-associated protein 1 and multidrug resistance associated protein 2 (MDP1 and MRP2) (169). These cell lines render efficient models for *in vitro* permeability assessment to elucidate the role of carrier mediated processes (170).

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