ORAL BIOAVAILABILITY ENHANCEMENT OF POORLY SOLUBLE AND POORLY PERMEABLE DRUGS USING SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEMS AND THE EFFECT OF PIPERINE

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by

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This thesis is dedicated to …..

My family members for their love and encouragement

and

To the Almighty

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LIST OF ABBREVIATION & SYMBOLS

ºC	=	Degree centigrade
ACC	=	Accuracy
ANOVA	=	Analysis of variance
ATP	=	Adenosine triphosphate
AUC	=	Area under curve
BBB	=	Blood Brain Barrier
BCS	=	Biopharmaceutic Classification system
BCRP	=	Breast Cancer Resistant Protein
cAMP	=	Cyclic adenosine monophosphate
C_{max}	=	Maximum plasma drug concentration
cm	=	Centimeter
cm/sec	=	Centimeter per second
CNT	=	Concentrative Nucleoside Transporter
cP	=	Centipoise
CV	=	Coefficient of variation
CYP	=	Cytochrome P
CYP3A	=	Cytochrome P3A
df	=	Degree of freedom
DLS	=	Dynamic light scattering
EDTA	=	Ethylene Diamine Tetraacetic Acid
ENT	=	Equilibrate Nucleoside Transporter
ER_{max}	=	Maximum urinary excretion rate
FDA	=	Food and Drug Administration
FM	=	Flippase Model
GI	=	Gastrointestinal
hCNT	=	human Concentrative Nucleoside Transporter
hENT	=	Human Equilibrate Nucleoside Transporter
HLB	=	Hydrophilic–Lipophilic Balance
hOCT	=	human Organic Cation Transporter
hOCTN	=	human Carnitine and Organic Cation Transporter
HP	=	Hydroxypropyl
HPLC	=	High Performance Liquid Chromatography

HPMC	=	Hydroxypropyl methyl cellulose
hPepT	=	human Peptide Transporter
hPHT	=	human Peptide/Histidine Transporter
hr	=	Hour
HSD	=	Honestly Significant Difference
HVC	=	Hydrophobic Vacuum Cleaner
ID	=	Internal diameter
I.S.	=	Internal Standard
kDa	=	Kilo Daltons
K_e	=	Elimination rate constant
LC	=	Liquid Crystals
LCT	=	Long Chain Triglycerides
LFCS	=	Lipid Formulation Classification System
LOD	=	Limit Of Detection
LOQ	=	Limit Of Quantification
LRP	=	Lung Resistant Protein
M	=	Molar
MCT	=	Monocarboxylic acid Co-Transporter
ME	=	Microemulsion
mg	=	Milligram
min	=	Minute
MRP	=	Multi Drug Resistant Protein
nm	=	Nanometer
OAT	=	Organic Anion Transporter in human
Oatp	=	Organic Anion Transporter in rodents
OCT	=	Organic Cation Transporter
OCTN	=	Carnitine and Organic Cation Transporter
O/W	=	Oil in Water
P_{eff}	=	Effective permeability coefficient
PCS	=	Photon Correlation Spectroscopy
PEG	=	Polyethylene glycol
PepT	=	Peptide Transporter
P-gp	=	P-glycoprotein

PHT	=	Peptide/Histidine Transporters
POT	=	Proton/Oligopeptide Transporter
PT	=	Peptide Transporter
RH	=	Relative humidity
rOCT	=	Rat Organic Cation Transporter
rOCTN	=	rat Carnitine and Organic Cation Transporter
RPM	=	Rotation Per Minute
RSD	=	Relative Standard Deviation
SD	=	Standard deviation
sec	=	Second
SEDDS	=	Self-Emulsifying Drug Delivery Systems
SMEDDS	=	Self-Microemulsifying Drug Delivery Systems
T_{max}	=	Time taken to reach maximum plasma concentration
$t_{½}$	=	Half life
TEM	=	Transmission electron microscopy
TPGS	=	D-Alpha-Tocopheryl Polyethylene Glycol Succinate 1000
UDP glucuronyl transferase	=	Uridine 5'-diphospho glucuronyl transferase
USFDA	=	United States Food and Drug Administration
v/v	=	Volume by volume
w/w	=	Weight by weight
Ml	=	Milliliter
µg/ml	=	Microgram per milliliter
µl	=	Microliter
Mm	=	Millimeter
mg / kg	=	Milligram/kilogram

LIST OF APPENDICES

LIST OF PUBLICATION & PRESENTATIONS

1 Mallikarjun Chitneni, Srujana Janagama, Peh K. Khiang and Darwis Yusrida. (2009). Bioavailability enhancement of sulpiride by self-microemulsifying drug delivery system. Lat Am J Pharm (Article In press).

2 Mallikarjun Chitneni, Peh Kok Khiang, Yusrida Darwis. (2008). Preparation and in vitro evaluation of self-microemulsifying drug delivery system (SMEDDS) contaning sulpiride. *Asean scientific conference in pharmaceutical technology,* 1st-3rd June 2008, The Bayview Beach Resort, Penang, Malaysia.

3 Mallikarjun Chitneni, Jawad Akram, Peh Kok Khiang & Yusrida Darwis. (2008). Enhancing peroral bioavailability of sulpiride using self-microemulsifying drug delivery system. *3rd International conference on postgraduate education (ICPE-3 '08),* 16th- 17th December 2008, The Gurney Resort Hotel & Residences, Penang, Malaysia.

4 Mallikarjun Chitneni, Peh Kok Khiang and Yusrida Darwis. (2009). Bioavailability enhancement of norfloxacin by self-microemulsifying drug delivery system. *Malaysian J Pharm,* 1, S42.

5 Mallikarjun Chitneni, Kok Khiang Peh and Yusrida Darwis. (2009). Enhancement in the intestinal permeability of sulpiride by using self-microemulsifying drug delivery system. *Symposium of USM fellowship holders 2009*, 14th- 15th November 2009, Universiti Sains Malaysia, Penang, Malaysia.

6 Mallikarjun Chitneni, Peh Kok Khiang, Yusrida Darwis. (2009). Oral bioavailability enhancement of poorly soluble and poorly permeable drugs by self microemulsifying drug delivery system. Universiti Sains Malaysia, Penang, Malaysia.

PENINGKATAN BIOKEPEROLEHAN ORAL DRUG-DRUG SUSAH LARUT DAN SUSAH TELAP MELALUI SISTEM PENGHANTARAN DRUG "PENGEMULSIAN-MIKROKENDIRI" DAN KESAN PIPERINE

ABSTRAK

Terdapat lebih kurang 40% molekul drug baru yang ditemui dan sebahagian melekul drug yang terdapat dalam pasaran, mempunyai bioperolehan yang rendah disebabkan oleh keterlarutan dan/atau ketelapan yang rendah. Kajian ini bertujuan menghasilkan suatu sistem penghantaran drug "pengemulsian-mikrokendiri" (SMEDDS) dengan menggunakan dua drug BCS Kelas IV, iaitu sulpiride dan norfloxacin. Sulpiride ialah substrat P-gp dan norfloxacin ialah substrat MRP2, BCRP dan suatu pam efluks yang tidak diketahui. Daripada kajian keterlarutan drug-drug dalam pelbagai minyak, surfaktan dan ko-surfaktan, asid oleik, Tween 80 dan propilena glikol, dipilih untuk menyediakan formulasi SMEDDS. Dua formulasi dipilih untuk kajian seterusnya berdasarkan pada saiz titisan dan keterlarutan. Formulasi pertama terdiri daripada 4.76 % b/b asid oleik, 63.49% b/b Tween 80 dan 31.75% b/b propilena glikol, dengan diameter purata titisan 9.27 nm dan keterlarutan 22 mg/1000 mg untuk sulpiride, manakala 9.57 nm dan 17.33 mg/1000 mg untuk norfloxacin masing-masing. Formulasi yang satu lagi terdiri daripada 17.71% b/b asid oleik, 55.14% b/b Tween 80 dan 27.15% b/b propilena glikol, dengan diameter purata titisan 85 nm dan keterlarutan drug 32 mg/1000 mg sulpiride, manakala 92 nm dan 27.53 mg/1000 mg untuk norfloxacin. Kaedah-kaedah isokratik HPLC-fluoresens yang ringkas, spesifik dan sensitif telah dibangunkan dan divalidasikan untuk menentukan kepekatan sulpiride dan norfloxacin dalam perfusat usus kecil tikus dan plasma arnab. Eksperimen ketelapan *in situ* dijalankan atas tiga bahagian usus kecil, duodenum, jejunum dan ileum tikus dengan teknik "single-pass perfusion". Formulasi SMEDDS

dan larutan misel menunjukkan peningkatan signifikan dalam koefisi ketelapan berkesan drug melalui ketiga-tiga bahagian usus kecil, dibandingkan dengan larutan drug untuk kedua-dua drug. Tidak terdapat perbezaan signifikan nilai-nilai koefisi ketelapan berkesan antara formulasi SMEDDS dan larutan misel untuk kedua-dua drug. Apabila kesan saiz titisan atas ketelapan jejunum dikaji, didapati tidak ada perbezaan signifikan untuk nilai-nilai koefisi ketelapan dengan saiz titisan kurang daripada 100 nm. Daripada kajian bioperolehan *in vivo* arnab, didapati pengurangan signifikan T_{max} dan peningkatan signifikan C_{max} dan AUC formulasi SMEDDS apabila dibandingkan dengan Dogmatil®/Norfloxin®. Sebaliknya, kadar dan amaun sulpiride/norfloxacin diserap tidak dipengaruhi dengan signifikan, apabila saiz titisan kurang daripada 100 nm dibandingkan. Apabila kesan perasa piperine atas bioperolehan oral ampaian sulpiride/norfloxacin dan formulasi SMEDDS dinilai, didapati pengambilan bersama kedua-dua drug dengan piperine, dalam ampaian dan formulasi SMEDDS meningkatkan bioperolehan kedua-dua drug secara signifikan. Pengambilan bersama piperine dengan formulasi SMEDDS meningkatkan lagi bioperolehan drug. Maka, SMEDDS boleh digunakan untuk meningatkan biperolehan drug-drug yang susah larut dan susah telap.

ORAL BIOAVAILABILITY ENHANCEMENT OF POORLY SOLUBLE AND POORLY PERMEABLE DRUGS USING SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEMS AND THE EFFECT OF PIPERINE

ABSTRACT

There are approximately 40% of new drug molecules discovered and some drug molecules that are available in the market, having poor bioavailability due to poor solubility and/or poor permeability. The present study aimed at formulating a self-microemulsifying drug delivery system (SMEDDS) using two BCS class IV drugs, namely sulpiride and norfloxacin. Sulpiride is a P-gp substrate and norfloxacin is a substrate of MRP2, BCRP and an unknown efflux pump. From the solubility studies of drugs in various oils, surfactants and co-surfactants, oleic acid, Tween 80 and propylene glycol were chosen to prepare SMEDDS formulations. Two formulations were chosen for further study based on droplet size and solubility. The first formulation consisted of 4.76% w/w of oleic acid, 63.49% w/w of Tween 80 and 31.75% w/w of propylene glycol, with a mean droplet diameter of 9.27 nm and drug solubility of 22 mg/1000 mg for sulpiride, 9.57 nm and 17.33 mg/1000 mg for norfloxacin respectively. The other formulation consisted of 17.71% w/w of oleic acid, 55.14% w/w of Tween 80 and 27.15% w/w of propylene glycol, with a mean droplet diameter of 85 nm and drug solubility of 32 mg/1000 mg for sulpiride, 92 nm and 27.53 mg/1000 mg for norfloxacin. Isocratic HPLC- fluorescence methods were developed and validated for the determination of sulpiride and norfloxacin in rat intestinal perfusates and rabbit plasma separately. The *in situ* permeability experiment was performed on three intestinal segments, duodenum, jejunum and ileum in rats using single-pass perfusion technique. The SMEDDS formulation and micellar solution exhibited significant increase in the effective permeability

coefficient of the drug across all the three intestinal segments compared with drug solution for the two drugs. There was no significant difference in the effective permeability coefficients values between SMEDDS formulation and micellar solution for both drugs. When the effect of droplet sizes on jejunum permeability was studied, it was found that there was no significant difference in permeability coefficients with droplet sizes of less than 100 nm. From the *in vivo* bioavailability study performed in rabbits, it was found that there was a significant decrease in the T_{max} and significant increase in the C_{max} and AUC of the SMEDDS formulations when compared with Dogmatil®/Norfloxin® respectively. On the other hand, the rate and extent of absorption of sulpiride/norfloxacin were not significantly affected, when the droplet sizes of less than 100 nm were compared. When the effect of dietary spice piperine on the oral bioavailability of sulpiride/norfloxacin suspension and SMEDDS formulations was evaluated, it was found that concomitant administration of both the drugs with piperine in suspension and SMEDDS formulations significantly enhanced the oral bioavailability of these two drugs. Concomitant administration of piperine with SMEDDS formulation further increased the bioavailability of the drugs. Hence, SMEDDS can be used to increase the bioavailability of poorly soluble and poorly permeable drugs.

Chapter 1
Introduction

1.1 Oral drug delivery

The predominant way to deliver drugs to the systemic circulation to generate pharmacological and clinical effects is the oral route. The oral route is easily accessible with minimum discomfort to patients, in comparison with other routes of drug administration. The design and composition of the pharmaceutical dosage form as well as the physico-chemical properties of the drug itself affect the *in vivo* performance and hence the therapeutic outcome (Petri and Lennernas, 2003).

The rate and extent of absorption of drugs following oral administration is governed by several factors. As the drug passes down the gastrointestinal tract, part of the dose may not be available for absorption owing to poor aqueous solubility, limited membrane permeability, and/or chemical or biological degradation. Drug molecules absorbed into the intestinal membranes can then be further subject to intestinal and/or hepatic first pass elimination before reaching the systemic circulation. A thorough understanding of the quantitative contributions of the two processes, solubility and permeability, during absorption is important for enhancing the oral bioavailability of drugs.

1.2 Biopharmaceutic Classification System (BCS)

In view of the importance of solubility and permeability on the oral bioavailability of drugs, USFDA has introduced the Biopharmaceutic Classification System (BCS), a scientific framework for classifying drug substances based on their dissolution rate, aqueous solubility, and intestinal permeability (Amidon *et al.,* 1995; Yu *et al.,* 2002).

According to BCS, drug substances are classified as follows (The Biopharmaceutic Classification System Guidance, USFDA):

Class I - High Permeability, High Solubility

Class II - High Permeability, Low Solubility

Class III - Low Permeability, High Solubility

Class IV - Low Permeability, Low Solubility

The class boundaries are set based on the following criteria:-

- A drug substance is considered HIGHLY SOLUBLE when the highest dose strength is soluble in < 250 ml water over a pH range of 1 to 7.5.
- A drug substance is considered HIGHLY PERMEABLE when the extent of absorption in human is determined to be > 90% of an administered dose, based on mass-balance or in comparison with an intravenous reference dose.
- A drug product is considered to be RAPIDLY DISSOLVING when > 85% of the labeled amount of drug substance dissolves within 30 min using USP apparatus I or II in a volume of < 900 ml buffer solutions (Yu *et al.,* 2002).

Class I drugs behave like an oral solution having fast dissolution and rapid bioavailability. Since the dissolution and absorption of these drugs is very fast, bioavailability and bioequivalence studies are not necessary for the products that contain these drugs. The drugs belonging to this class are good candidates for controlled drug delivery if they have suitable pharmacokinetic and pharmacodynamic attributes.

Drugs belonging to Class II have low solubility and high permeability. Hence, the dissolution rate becomes the governing parameter for bioavailability. The drugs

belonging to this class may exhibit poor oral bioavailability. Various formulation techniques have been reported to enhance the dissolution rate and the bioavailability of this class of drug molecules.

For drugs belonging to Class III, permeation through the intestinal membranes forms the rate-determining step for bioavailability. The drug release from the dosage form and the dissolution rate do not influence the bioavailability of such drugs. Generally, these drugs exhibit low oral bioavailability. Different permeability enhancement techniques have been developed to enhance the bioavailability of this class of drugs. Drugs belonging to this class exhibit poor and variable bioavailability.

The oral bioavailability of Class IV is governed by solubility and permeability. These drugs are not generally suitable for oral administration and special drug delivery technologies are required to make them suitable for oral administration. A combination of techniques used for Class II and Class III drugs can be used to enhance the solubility and permeability and hence the bioavailability of these drugs (Ku, 2008).

1.3 Techniques to enhance solubility or dissolution rate of poorly soluble drugs

Drugs with poor aqueous solubility (BCS Class II and IV) show *in vivo* performance limitations, such as incomplete or erratic absorption and poor bioavailability. The effectiveness can vary from patient to patient, and there can be a strong effect of food on drug absorption. To overcome the *in vivo* performance limitations with BCS class II and class IV drugs, various formulation techniques have been developed to

enhance the solubility and dissolution rate of these drugs and their oral bioavailability. These formulation techniques are elaborated below.

1.3.1 Adjustment of pH and use of co-solvent

For drug molecules that are ionizable, changing the pH of the system may be the simplest and most effective means of increasing aqueous solubility. Under the proper conditions, the solubility of an ionizable drug can increase exponentially by adjusting the pH of the solution. A drug that can be efficiently solubilized by pH control should be either weak acid or a weak base. There is little effect of pH on unionizable substances (Tong, 2008). Unionizable, hydrophobic substances can have improved solubility by changing the dielectric constant of the solvent by the use of co-solvents rather than the pH of the solvent. Water miscible solvents with intermediate dielectric constant values, like propylene glycol, polyethylene glycols, glycerin and alcohol are generally used as co-solvents in the preparation of oral dosage forms of poorly soluble drug substances (Seedher and Bhatia, 2003).

1.3.2 Salt form of the drug

Salts of acidic and basic drugs have, in general, higher solubilities than their corresponding acid or base forms. Salt formation to increase aqueous solubility is the most preferred approach for the development of liquid formulations for parenteral administration (Sweetana and Akers, 1996). For solid dosage forms, Nelson (1957, 1958) demonstrated that dissolution rates of salt forms of several weakly acidic compounds under gastrointestinal pH conditions were higher than those of their respective free acid forms. He attributed the higher dissolution rate of a salt to its higher solubility (relative to the free acid form) in the aqueous diffusion layer surrounding the solid. There was a significant increase in the rate and extent of

absorption of novobiocin and tolbutamide in salt form compared to their respective base (Furesz, 1958; Nelson *et al.,* 1962). This method is not applicable to extremely water-insoluble drugs. Salts may precipitate out in the gastrointestinal fluid after oral administration, into their free acid and base forms. For extremely insoluble drugs, the precipitates may not redissolve rapidly due to their very low aqueous solubilities (Serajuddin, 2007).

1.3.3 Particle size reduction

Particle size reduction can enhance the dissolution rate of drugs. By reducing the particle size, the increased surface area increases the dissolution of the drug. Conventional methods of particle size reduction, such as comminution and spray drying, rely upon mechanical stress to disaggregate the active compound. Micronization is used to increase the specific surface area for dissolution. Micronization of drugs by conventional methods is carried out by milling techniques using jet mill, rotor stator and colloid mills. Spray drying is a commonly used particle size reduction method. In this method, hot nitrogen gas is used to dry the liquid feed of the drug. The liquid feed may be a solution, colloidal dispersion or suspension of the drug. The basic principle involved in the process of spray drying is the liquid feed is passed through a nozzle. Spray drying of the salicylic acid dispersed in acacia solutions resulted in as much as a 50% improvement in the solubility of poorly water soluble salicylic acid (Kawashima *et al.,* 1975).

The micronization of drug powders to sizes between 1 and 10 μm to increase the surface area, and thus the dissolution velocity, is not sufficient to overcome

bioavailability problems of many very poorly soluble drugs. A consequent step was to move from micronization to nanonization (Keck and Muller, 2006).

Nanonization is one of the most promising techniques to improve the oral bioavailability of poorly soluble drugs by increasing the surface area and saturation solubility via reduction of the particle size to less than 1μm. Such size reduction cannot be achieved by the conventional milling techniques. The various technologies that have emerged to decrease the particle size to nanosize include pearl milling (Nano Crystals®) ((Bhupendra *et al.,* 2007; Junghanns and Muller, 2008), homogenization techniques (IDD-P™ technology) (Junghanns and Muller, 2008), DissoCubes® technology (Junghanns and Muller, 2008), Nanopure® technology (Bushrab and Muller, 2003), supercritical fluid technology (Byrappa *et al.,* 2008), spray freezing into liquid (Vaughn *et al.,* 2006) and evaporative precipitation into aqueous solution (Vaughn *et al.,* 2005).

1.3.4 Polymorphic modification (polymorphs)

Polymorphism is the ability of a compound to crystallize in more than one crystalline form. Different polymorphs of drugs are chemically identical, but they exhibit different physicochemical properties and biological activities including solubility, melting point, density, texture, stability and bioavailability. With regard to bioavailability, it is preferable to change drug from stable crystal forms into metastable or amorphous forms. Nonetheless, there have been numerous reports demonstrating the influence of polymorphic and crystalline form on dissolution rate and/or oral bioavailability. Metastable forms of phenobarbital, spironolactone and carbamazepine provided enhanced dissolution behaviour compared to the other

polymorphs of the respective drugs (Kato *et al.,* 1984; Salole and Al-Sarraj, 1985; Kobayashi *et al.,* 2000). Singhal and Curatolo (2004) reviewed a number of examples showing differences in pharmacokinetic profiles in human subjects relating to batch to batch variations in the polymorphic forms of carbamazepine and oxytetracycline. Although the utilisation of metastable polymorphs offers improved dissolution and oral bioavailability, concern still exists with respect to conversion of these materials to more stable crystalline forms during processing and storage. It is therefore preferable to develop the most thermodynamically stable polymorph of the drug to assure reproducible bioavailability of the product over its shelf-life under a variety of real-world storage conditions. For instance, ritonavir is the active ingredient in Norvir®, a protease inhibitor used to treat HIV/AIDS. It was launched by Abbott Laboratories in 1996, as an amorphous semisolid dispersion consisting of medium chain triglycerides, polyoxyl 35 castor oil, citric acid, ethanol, polyglycolyzed glycerides, polysorbate 80, propylene glycol and 100 mg of ritonavir. The dissolution and the oral bioavailability were decreased due to crystallization of amorphous ritonavir into an insoluble crystal form during storage. This polymorph (form II) was 50% less soluble than the original form in the market, and caused the drug to fail its regulatory dissolution specifications. Finally, the drug was relaunched with the form II polymorph in a soft gelatin formulation that required refrigeration (Datta and Grant, 2004). Therefore, it is important to note that the selection of a polymorph of a drug should balance between solubility and stability to maintain its potency over the shelf-life period.

Generally, the anhydrous form of a drug has a greater solubility than the hydrates. This is because the hydrates are already in interaction with water and therefore have

less energy for crystal breakup in comparison to the anhydrates (i.e. thermodynamically higher energy state) for further interaction with water. On the other hand, the organic (nonaqueous) solvates have greater solubility than the nonsolvates. Glibenclamide has been isolated as pentanol and toluene solvates, and these solvates exhibit higher solubility and dissolution rate than two non-solvated polymorphs (Suleiman and Najib, 1989).

1.3.5 Complexation

Cyclodextrins (CD) and their derivatives have been employed as complexing agents for enhancement of solubility, dissolution rate and bioavailability of drugs. Cyclodextrin inclusion is a molecular phenomenon in which usually only one guest molecule interacts with the cavity of a cyclodextrin molecule to become entrapped and form a stable association. The internal surface of cavity is hydrophobic and external is hydrophilic. Molecules or functional groups of molecules which are less hydrophilic than water can be included in the cyclodextrin cavity in the presence of water. In order to become complex, the "guest molecules" should fit into the cyclodextrin cavity. The cavity size as well as possible chemical modifications determine the affinity of cyclodextrins to the various molecules. Three naturally occurring CDs are α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin, are available. Various other cyclodextrins with better properties have been developed, for instance hydroxypropyl β-cyclodextrin. It was found that cyclodextrins increased paclitaxel solubility by 950 folds (Singla *et al.,* 2002). Complex formation of norfloxacin (Guyot *et al.,* 1995), clofibrate (Anguiano-Igea *et al.,* 1996), taxol (Dordunoo and Burt, 1996), cyclosporine A (Ran *et al.,* 2001) and rofecoxib (Baboota *et al.,* 2005) with cyclodextrins improved the solubility and dissolution rate of these drugs.

1.3.6 Solid dispersions

Solid dispersions are a eutectic mixture of drugs with water-soluble carriers formed by the melting of their physical mixtures (Serajuddin, 1999). Sekiguchi and Obi (1961) reported that the drug was present in a eutectic mixture in a microcrystalline state. Later, Goldberg *et al.* (1996) reported that drugs present in solid dispersions might not necessarily be present in microcrystalline state as certain fraction of the drug might be molecularly dispersed in the matrix, thereby forming a solid solution. In either case, once the solid dispersion was exposed to aqueous media and the carrier dissolved, the drug was released as very fine, colloidal particles. Because of greatly enhanced surface area obtained, the dissolution rate and the bioavailability of poorly water-soluble drugs were expected to be high. Solid dispersions are generally prepared either by melt technique or solvent evaporation technique.

Although the solid dispersion technology has been developed in the early 1960's, their commercial use has been very limited, primarily because of some limitations. These include method of preparation, reproducibility of its physicochemical properties, its formulation into dosage forms, the scale-up of manufacturing processes, and the physical and chemical stabilities of drug and vehicle. Only two products, griseofulvin-in polyethylene glycol solid dispersion (Gris-PEG®, Novartis) and nabilone-in-povidone solid dispersion (Cesamet®, Lilly) were marketed after three decades following the initial work of Sekiguchi and Obi in 1961 (Serajuddin, 1999).

1.3.7 Lipid based drug delivery systems

Lipid-based delivery systems range from simple oil solutions to complex mixtures of oils, surfactants and co-surfactants/co-solvents. The latter mixtures are typically self-dispersing systems often referred to as self-emulsifying drug delivery systems (SEDDS) or self-microemulsifying drug delivery systems (SMEDDS) (Pouton, 2006).

Lipid Formulation Classification System (LFCS) was introduced as a working model in 2000 (Pouton, 2000), and an additional 'type' of formulation was included in 2006 (Pouton, 2006). The main purpose of the LFCS is to enable *in vivo* studies to be interpreted more readily. The LFCS is shown in Table 1.1, with advantages and disadvantages of each system (Pouton and Porter, 2008).

There are a few marketed preparations based on lipid based drug delivery. Many of the marketed lipid based products are Type III systems, but this group is particularly diverse as a result of the wide variation in the proportions of oily and water-soluble materials used. Considering this issue, this group has been further divided into Type IIIA and Type IIIB to distinguish between formulations which contain a significant proportion of oils (greater than 20% of oil) (Type IIIA) and those which are predominantly water-soluble substances (less than 20% of oil) (Type IIIB) (Pouton, 2000; Pouton, 2006).

Table 1.1: The Lipid Formulation Classification System: characteristic features, advantages and disadvantages of the four essential types of 'lipid' formulations (Pouton and Porter, 2008).

Formulation type	Materials	Characteristics	Advantages	Disadvantages
Type I	Oils without surfactants (e.g. tri, di-and monoglycerides)	Non-dispersing, requires digestion	Simple; excellent capsule compatibility	Formulation has poor solvent capacity unless drug is highly lipophilic
Type II	Oils and water-insoluble surfactants	SEDDS formed without water-soluble components	Unlikely to lose solvent capacity on dispersion	Turbid o/w dispersion (particle size 0.25-2 μm)
Type III	Oils, surfactants, co-solvents	SEDDS/SMEDDS formed with water-soluble components	Clear or almost clear dispersion; drug absorption without digestion	Possible loss of solvent capacity on dispersion; less easily digested
Type IV	Water-soluble surfactants and co-solvents (no oils)	Formulation disperses typically to form a micellar solution	Formulation has good solvent capacity for many drugs	Likely loss of solvent capacity on dispersion; may not be digestible

1.3.7 (a) Formulation of Type I systems

This formulation system is without a surfactant and contains only lipophilic oils which have little or no water solubility. Typically, they contain vegetable oils which are rapidly digested and completely absorbed from the intestine. As these systems are prepared without the incorporation of surfactants, they depend on digestion to facilitate colloidal dispersion by solubilization of digestion products in mixed micelles. Both long chain triglycerides (LCT) and medium chain triglycerides (MCT) are digested by pancreatic enzymes when they enter the gastrointestinal tract (Porter *et al.,* 2007). The in *vitro* lipolysis experiments revealed that there are differences in the processing of the digestion products, which are likely to have effects on the fate of drugs in the intestinal lumen. Bile produced is not generally required for digestion of MCT, whereas bile is very essential for digestion of LCT. MCT of C8 and C10 fatty acids and monoglycerides can exist as separate dispersed or dissolved phases (MacGregor *et al.,* 1997). The LCT digestion products remain extensively solubilised within mixed bile salt–lecithin micelles until they are absorbed. The swollen mixed micelles formed by LCT digestion products are good solubilising systems for many drugs (MacGregor *et al.,* 1997; Kossena *et al.,* 2003; Kossena *et al.,* 2004). Generally, the digestive products of MCT are absorbed through systemic circulation whereas those of the LCT through lymphatic system (Porter *et al.,* 2007).

1.3.7 (b) Formulation of Type II systems

These formulations are formulated using oils and water insoluble surfactants. Typical formulations are mixtures of MCT oil and polysorbate 85 (Pouton, 1997) or mixtures of MCT oil and Tagat TO® (surfactant) (Wakerly *et al.,* 1986). Self-emulsifying systems are formed when the surfactant concentration exceeds 25%w/w, the

optimum concentration range being 30–40% surfactant. Above 50% of surfactant, these systems emulsify slowly due to the formation of viscous liquid crystalline phases at the oil-water interface (Pouton, 2006). A Type II system was evaluated in dog (Charman *et al.,* 1992) but since then these systems received limited attention and no marketed products have emerged.

1.3.7 (c) Formulation of Type III systems

Sandimmun Neoral® (Cyclosporine A) is a Type IIIA formulation, a SEDDS/SMEDDS formulation containing water-soluble surfactant and a significant mass of lipid components (Pouton, 2006). These formulations have the potential to disperse quickly to form fine submicron dispersions, often fine enough to form transparent dispersions. The Type IIIB formulation, which contains less than 20% of total weight of oil and high amount of water soluble substances, may fail to maintain the drug in a solubilized state. Nevertheless, acyclovir (Patel and Sawant, 2007) and tacrolimus (Borhade *et al.,* 2008) SMEDDS formulations were stable for more than 24 hr after dilutions, when prepared as type IIIB formulation.

1.3.7 (d) Formulation of Type IV systems

These systems contain pure surfactants or mixtures of surfactants and cosolvents. It is generally accepted that formulation of poorly water-soluble drugs in pure cosolvents is likely to result in precipitation of the drug. The only advantage that could be gained is the possibility that the drug precipitates as a suspension of very fine crystalline or amorphous particles. Reliability is likely to be a problem with this strategy. Formulation of the drug in pure water-soluble surfactant makes more sense with regard to the aim of avoiding precipitation, since loss of solvent capacity is less

significant. There are two problems with using pure surfactants. The first is that surfactants often take a considerable time to dissolve, due to the formation of viscous liquid crystalline phases at the surfactant-water interface. The second is that pure surfactants can be irritant and poorly tolerated in the gastrointestinal tract. The amprenavir capsule formulation (Agenerase®, GSK) is a type IV formulation, a blend of tocopheryl polyethylene glycol 1000 succinate, PEG 400 and propylene glycol (Pouton, 2006; Pouton and Porter, 2008).

1.4 Permeability

The gastrointestinal (GI) tract morphology varies greatly from relatively no folding in the esophagus to high degrees of folding in the small intestine (Tortora and Grabowski, 1993). The villous epithelium of the small intestine acts as a primary barrier to GI absorption of drugs. The cells that mediate drug absorption across the intestinal villi are the polarized columnar enterocytes, which are eminent by the presence of apical membrane microvilli. The villi and the microvilli provide a significant increase in the intestinal absorptive surface area (Tortora and Grabowski, 1993). Nevertheless, the compound's physicochemical properties will dictate the pathway and extent of absorption.

Paracellular and transcellular diffusion are the two routes of GI permeation (Adson *et al.,* 1995). Paracellular permeation occurs by the diffusion of dissolved solute between cells through the tight junctions and tortuous pathway in the intercellular spacing (Adson *et al.,* 1995; Knipp *et al.,* 1997). The paracellular pathway is generally more restrictive. There are several physicochemical characteristics of a drug molecule that would favor paracellular diffusion including charge,

hydrophilicity, shape/conformation, size, and molecular weight (Adson *et al.,* 1995; Knipp *et al.,* 1997).

The transcellular route is comprised of several potential parallel pathways for drug permeation including passive transcellular diffusion, ion channels, facilitated diffusion, active transport, and endocytosis (Oh and Amidon, 1999).

In the past, the pH-partition hypothesis, first postulated in the mid to early 1900s, was used for predicting the absorption and/or disposition of a drug across biological membranes based on the octanol to aqueous partition coefficients as a function of ionization of the drug molecule (Jacobs, 1940). However, this method was inconsistent as many drugs deviated from the hypothesis (Higuchi and Davis, 1970). To overcome this problem, "The Rule of Five" was proposed by Lipinski *et al.* (1997; 2000; 2001) to estimate the permeability of compounds *in silico* based on molecular descriptors at the early stages of drug discovery. Lipinski's Rule of Five states: "poor absorption or permeability is more likely when there are more than 5 *H-bond donors*, 10 *H-bond acceptors*, the *molecular weight is greater than 500* and the *calculated log P is greater than 5*." (Lipinski *et al.,* 1997). The main drawback of these techniques was that they never consider the role of drug transporters, in the overall absorption process. The Caco2 cell lines and *in situ* single-pass perfusion method were developed to overcome the problems faced with *in silico* techniques. However, all these techniques also have advantages and disadvantages.

The complex nature of the intestine and the process of absorption as a whole relentlessly make the research regarding absorption process more challenging. The

lack of comprehensive predictive absorption methodologies is better understood when one considers the numerous roles elucidated for different drug transporters in mediating transcellular influx and efflux of xenobiotics. There are numerous classes of transporter proteins that have been identified, each with different and sometimes overlapping, substrate specificity, capacity and affinity, as well as specific tissue, cellular and temporal expression patterns. Transporter proteins are integral proteins of the membranes that function *via* either a facilitated diffusion, or active, energy-dependent mechanisms to mediate transcellular flux of xenobiotics and nutrients (Oh and Amidon, 1999). A compound's physicochemical properties greatly influence its interactions with transporters and lipophilic character (i.e., partitioning) plays a major role in determining these interactions (Stewart *et al.,* 1997; Ekins *et al.,* 2000; Kimura *et al.,* 2002; Van DeWaterbeemd, 2002; Harrison *et al.,* 2004; Kassel, 2004; Sun *et al.,* 2004; Ekins *et al.,* 2005). Enzymes of the Cytochrome P3A (CYP3A) family are predominant phase I drug metabolizing species found in human, accounting for approximately 30% of hepatic CYP (Shimada *et al.,* 1994) and greater than 70% of small intestine CYP (Watkins *et al.,* 1987; Zhang *et al.,* 1999). So, along with efflux pumps the CYP3A is also a significant factor that needs to be addressed. The various influx and efflux transporters are presented in Fig 1.1.

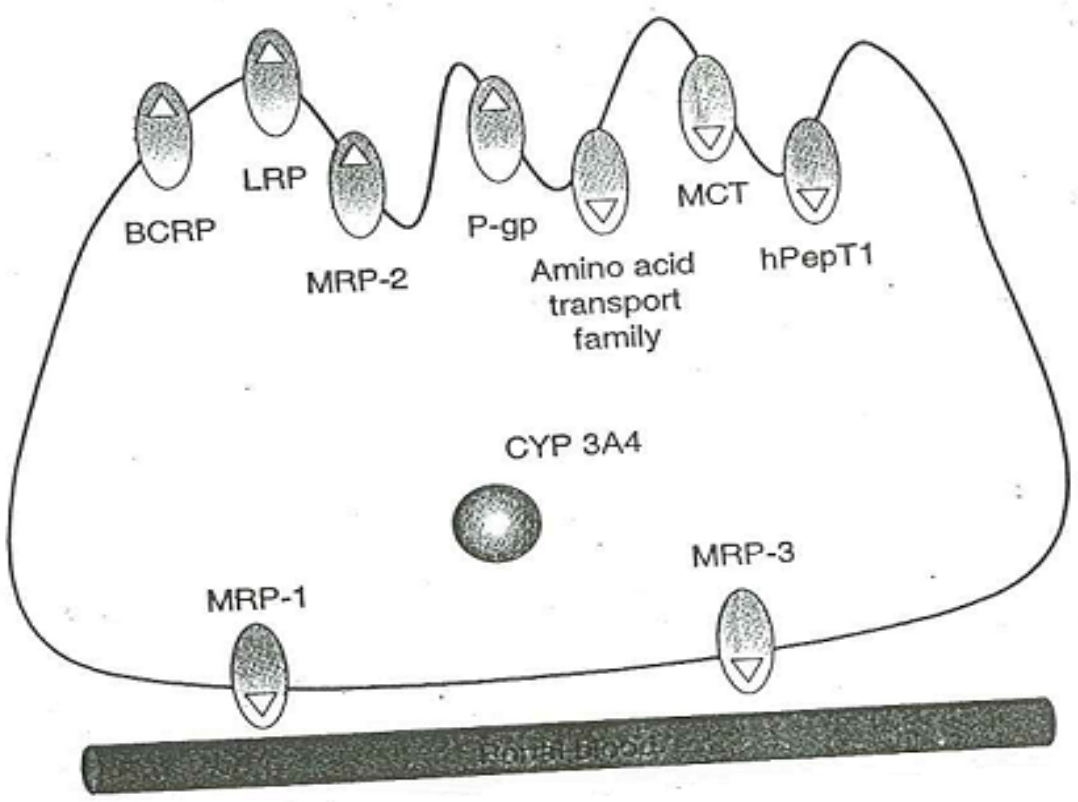

Fig 1.1: Few of the influx and efflux drug transporters that affect the intestinal transport of drugs and their metabolites across intestinal epithelial membranes in humans. P-gp: P-glycoprotein; BCRP: Breast Cancer Resistant Protein; LRP: Lung Resistant Protein; MRP: Multi Drug Resistant Protein family; hPepT1: oligopepetide carrier for di and tripeptides; MCT: Monocarboxylic acid Co-Transporter. CYP3A4: Cytochrome P3A4 enzyme. (Adapted from Petri and Lennernas, 2003).

1.4.1 ATP Binding Cassette transporters

Several members of the ATP Binding Cassette (ABC) transporter protein have been reported to impart multidrug resistance by their ability to efflux xenobiotics from the cytoplasm and across the cellular membrane in an energy-dependent, polarized manner. A common characteristic shared by ABC transporters is the presence of nucleotide binding domain(s), which enable these integral membrane proteins to hydrolyze ATP to drive efflux (Higgins, 1991). Forty nine human ABC transporter isoforms have been identified. These again were separated into seven distinct subfamilies based on their sequence homology (ABCA to ABCG). Among these subfamilies, the ABCB and ABCC subfamilies contain the most widely investigated transporters influencing human intestinal absorption. Specifically, P-glycoprotein (ABCB1, P-gp) and multidrug resistance associated proteins (ABCC, MRP) have not only been shown to be expressed along the GI tract, but due to their cellular localizations and broad substrate specificities, appear to be the primary efflux pumps determining xenobiotic absorption. The ABCG2 isoform, also known as the breast cancer resistance protein (BCRP), has also been demonstrated to affect the intestinal absorption of various therapeutic agents.

1.4.1 (a) P-glycoprotein (P-gp; ABCB1)

P-gp is a 170-180 kDa, ATP-dependent transmembrane glycoprotein, which is formed by the post translational glycosylation of a 140 kDa pro-P-gp protein (Kramer *et al.,* 1995). P-gp is comprised of four major domains; two membrane-bound domains, each with six transmembrane segments and two cytosolic ATP binding motifs, that bind and hydrolyze ATP (Leveille-Webster and Arias, 1995).

Xenobiotics bind to separate sites on P-gp, indicating that different drugs can independently regulate P-gp function (Leveille-Webster and Arias, 1995).

P-gp is localized to the apical brush-border membrane of the intestinal epithelium (Thiebaut *et al.,* 1987). Due to the localized expression of P-gp at the apical side of brush border cells (Terao *et al.,* 1996), P-gp limits the absorption of compounds by directly effluxing them back into the intestinal lumen. The level of P-gp expression increases from proximal to distal regions of the intestine (Mouly and Paine, 2003). P-gp expression has also been reported in kidney, adrenal gland, liver, colon, apical membrane of the placenta, T lymphocytes and natural killer cells, blood-tissue barrier and lungs (Fojo *et al.,* 1987; Sugawara *et al.,* 1988; Thiebaut *et al.,* 1989; Chaudhary and Roninson, 1991; Drenou *et al.,* 1993; Gatmaitan and Arias, 1993). Low-level P-gp is also expressed in prostate, skin, spleen, heart, skeletal muscle, stomach, and ovary (Fojo *et al.,* 1987; Gatmaitan and Arias, 1993).

Two hypotheses have been postulated, namely, the "hydrophobic vacuum cleaner" (HVC) and the "flippase model" (FM), to explain the mechanism by which P-gp actively effluxes xenobiotics. The HVC model suggests P-gp clears substrates before they enter the cytoplasm (Higgins and Gottesman, 1992; Gottesman and Pastan, 1993). Alternatively, the FM proposes that P-gp interacts with the xenobiotics as they enter through the lipid membrane and "flips" the drug from the inner leaflet to the outer leaflet and back into the extracellular media. Some of the P-gp substrates that have been identified are sulpiride (Baluom *et al.,* 2001), digoxin (Anderle *et al.,* 2004), and paclitaxel (Yang et *al.,* 2004**).** In contrast, some of the inhibitors of P-gp include quinidine, verapamil, cyclosporine A (Watanbe *et al.,* 2004) and a few

pharmaceutical excipients like Tween 80, Cremophor EL, and polyethylene glycols (Hugger *et al.,* 2002).

1.4.1 (b) Multidrug Resistance-Associated Protein Family (MRP; ABCC)

The human *MRP* gene encodes an MRP polypeptide with an apparent mass of 170 kDa, which is post translationally converted to a 190 kDa form by the addition of N-linked complex oligosaccharides (Almquist *et al.,* 1995). Nine members within the MRP family have been identified, named as MRP1 to MRP6 (ABCC1 to ABCC6) and MRP7 to MRP9 (ABCC10 to ABCC12) (Belinsky *et al.,* 1998; Bera *et al.,* 2001; Kubota *et al.,* 2001; Bera *et al.,* 2002; Kruh and Belinsky, 2003).

MRP1 is expressed on the basolateral side of the membrane; therefore its substrates are transported towards basolateral side of the epithelium (Evers *et al.,* 1996). MRP2 is expressed on apical side of the membranes (Evers *et al.,* 1996). MRP2 is found in hepatocytes, in the luminal membrane of the small intestine and proximal tubules of the kidney (Schaub *et al.,* 1997; Schaub *et al.,* 1999; Mottino *et al.,* 2000; Van Aubel *et al.,* 2000). In human jejunum, MRP2 are amongst the highest expressed of all tested ABC transporters. MRP3 is expressed in liver, small and large intestine, adrenal gland, and to a lower extent in pancreas and kidney (Kool *et al.,* 1997; Kiuchi *et al.,* 1998; Scheffer *et al.,* 2002). Lee *et al.* (2000) reported that MRP4 is localized to the human basolateral membrane of prostate. In contrast, Van Aubel *et al.* (2002) found human and rat MRP4 primarily in the apical side of brush border membrane of proximal tubular cells in the kidney. MRP4 is also expressed in several other tissues including jejunum, brain, lung, and gall bladder (Kool *et al.,* 1997; Zhang *et al.,* 2000; Taipalensuu *et al.,* 2001; Van Aubel *et al.,* 2002). The highest

expression of MRP5 is found in basolateral membranes of brain and skeletal muscle (McAleer *et al.,* 1999). MRP5 is also present in basolateral erythrocyte membranes, colon, liver and kidney (Kool *et al.,* 1997; McAleer *et al.,* 1999; Jedlitschky *et al.,* 2000; Zhang *et al.,* 2000). MRP6 is highly expressed in the kidney and liver, with low expression in several other tissues, like duodenum, colon, brain, and salivary gland (Zhang *et al.,* 2000). Not much is known about MRP7, MRP8, and MRP9, although they are gaining increasing attention due to their involvement in conveying multidrug resistance (Bera *et al.* 2002; Kruh and Belinsky, 2003; Hopper-Borge *et al.,* 2004; Chen *et al.,* 2005).

A few substrates of MRP1 are anthracyclines, vinca alkaloids, and camptothecin (Cole *et al.,* 1994; Konig *et al.,* 1999; Kruh and Belinsky, 2003). Inhibitors of MRP1 are verapamil and its analogs (Cole *et al.,* 1994).

The glucuronide conjugates of bilirubin, estradiol, acetaminophen, grepafloxacin, triiodo-L-thyronine, and SN-38 are few of the MRP2 substrates identified (Suzuki and Sugiyama, 1998; Suzuki and Sugiyama, 1999). MRP3 also has the capacity to transport organic anionic drugs and glucuronate-conjugated drugs, as well as a wide range of bile salts such as glycocholate, taurolithocholate-3-sulfate, and taurochenodeoxycholate-3-sulfate (Hirohashi *et al.,* 2000; Zeng *et al.,* 2000; Zelcer *et al.,* 2001). Substrates for MRP4 include folic acid, folinic acid (Chen *et al.,* 2002) and thiopurines (Wielinga *et al.,* 2002). MRP5 has an affinity for nucleotide-based substrates including anticancer thiopurine and thioguanine drugs (Wijnholds *et al.,* 2000; Wielinga *et al.,* 2002; Reid *et al.,* 2003). Organic anion such as benzbromarone and sulfinpyrazone inhibit MRP5 (Wijnholds *et al.,* 2000). MRP6 is

involved in the transport of agents such as etoposide, doxorubicin, and cisplatin in Chinese hamster ovary cells (Belinsky *et al.,* 2002), while MRP7 transports docotaxel and 17β-estradiol-(17β-D-glucuronide) (Hopper-Borge *et al.,* 2004). MRP8 has been reported to mediate the efflux of cyclic nucleotides (Guo *et al.,* 2003).

1.4.1 (c) Breast Cancer Resistance Protein (BCRP; ABCG2)

Human BCRP encodes a 655 amino acid ABC protein, containing a single N-terminal ATP binding cassette, followed by 6 putative transmembrane segments. Based on structural and sequence homology, BCRP belongs to the ABCG gene family, containing amongst others the Drosophila *white*, *brown*, and *scarlet* protein genes, the human *white* homologue *ABCG*1, and the more recently identified genes *ABCG5* and *ABCG*8. *BCRP* was therefore renamed *ABCG*2 (Berge *et al.,* 2000; Lee *et al.,* 2001).

BCRP is expressed in the apical side of small intestine, colon, liver, placenta and ovary (Scheffer *et al.,* 2000; Maliepaard *et al.,* 2001). Some of the substrates of this pump include methotrexate (Sarkadi *et al.,* 2004), camptothecins (Sarkadi *et al.,* 2004), and estrone 3-sulfate (Leslie *et al.,* 2005), while the inhibitors are tamoxifen and novobiocin (Staud and Pavek, 2005).

1.4.1 (d) Proton/Oligopeptide Transporters (POT; SLC15A)

Peptides like agents are widely used in the treatment of many disorders including AIDS, hypertension, and cancer. To date peptide transporters that are reported include the Peptide Transporters 1 and 2, PepT1 (SLC15A1) and PepT2 (SLC15A2);

the Peptide/Histidine Transporters 1 and 2, PHT1 (SLC15A4) and PHT2 (SLC15A3); and the Intestinal Peptide Transporter PT1 (CDH17) (Fei *et al.,* 1998; Meredith and Boyd, 2000).

PepT1 protein was found to be expressed in the human small intestine (Liang *et al.,* 1995; Herrera-Ruiz *et al.,* 2001) and is localized on the apical plasma membrane of enterocytes in rats (Ogihara *et al.,* 1999).

Two human peptide/histidine (hPHT) transporters have been recognized (Botka *et al.,* 2000; Knipp and Herrera-Ruiz, 2003). Both hPHT1 and hPHT2 have reported to be expressed along the entire GI tract, especially in the small intestine and colon (Herrera-Ruiz *et al.,* 2001). Some of the substrates of peptide transporters are cefatrizine, cefepine, cefixime, cloxacillin, cyclacillin, valaciclovir, and valganciclovir while the inhibitors are latamoxef, enalaprilate and fosinoprilate (Bhardwaj *et al.,* 2006).

1.4.1 (e) Organic Anion Transporters (OAT, SLC22A; OATP, SLCO)

It has been reported that intestinal absorption of various ionized drugs is mediated by the organic anion (OA) or organic cation (OC) transporter systems (Katsura and Inui, 2003; Sai and Tsuji, 2004; Steffansen *et al.,* 2004).

The organic anion transporters are classified as organic anion transporters (OATs), organic anion transporting polypeptides (rodents: Oatps; human: OATPs) and multiple drug resistance-associated proteins (MRPs) (Hagenbuch and Meier, 2003; Van Montfoort *et al.,* 2003; Koepsell and Endou, 2004).

OATP/Oatp members mediate the transport of organic anions and other compounds in a Na^+-independent manner (Tirona and Kim, 2003; Hagenbuch and Meier, 2003; Van Montfoort *et al.,* 2003). Glutathione (GSH) plays a significant role in rat Oatp1 and Oatp2 (Li *et al.,* 1998; Li *et al.,* 2000) substrate transport. A proton-coupled transport mechanism has also been suggested for OATP/Oatp isoforms (Kobayashi *et al.,* 2003). OATP/Oatp family members (e.g., OATP-B, OATP-D, and OATP-E) are expressed in blood-brain barrier (BBB), lung, heart, kidney, placenta, and intestine (Hagenbuch and Meier, 2003; Kim, 2003; Van Montfoort *et al.,* 2003).

1.4.1 (f) Organic Cation Transporters (OCT, OCTN; SLC22A)

Generally most of the organic cations are polar and positively charged at physiological pH, membrane bound transporters are essential to enhance their intestinal permeability. Organic cation transporter 1 (OCT1) was the first of the OC family transporter that was identified (Grundemann *et al.,* 1994). Subsequently, other organic cation transporters (OCT2–3), carnitine and organic cation transporters (OCTN1–3) have been identified (Koepsell *et al.,* 2003; Koepsell and Endou, 2004; You, 2004). Tetraethylammonium and *N*-methylquinine, acyclovir, ganciclovir, metformin and phenformin, memantine and quinidine are few of the drugs that are reported to be transported by various organic cationic transporters (Koepsell *et al.,* 2003; Koepsell and Endou, 2004; You, 2004).

OCTs transport organic cations and other compounds in an electrogenic manner, for the rat isoforms rOCT1, rOCT2, and rOCT3 (Busch *et al.,* 1996; Nagel *et al.,* 1997; Kekuda *et al.,* 1998; Okuda *et al.,* 1999), and for the human transporters hOCT1 and hOCT2 (Gorboulev *et al.,* 1997; Busch *et al.,* 1998). In addition, OCTs mediated

transport is independent from Na^+ and H^+ ions (Busch *et al.,* 1996; Gorboulev *et al.,* 1997; Kekuda *et al.,* 1998). Driving force for substrate transport is provided by the substrate concentration gradient and the membrane potential (Busch *et al.,* 1996; Gorboulev *et al.,* 1997; Busch *et al.,* 1998; Kekuda *et al.,* 1998; Okuda *et al.,* 1999). OCT isoforms are mainly expressed in the liver and kidney, and to a less extent, in the heart, skeletal muscle, placenta, and small intestine. hOCT1 is mainly expressed in the liver, whereas hOCT2 is mainly found in the kidney (Gorboulev *et al.,* 1997). hOCTN1 and hOCTN2 are both expressed abundantly in the kidney, skeletal muscle, placenta, prostate, and heart (Tamai *et al.,* 1997; Tamai *et al.,* 1998; Wu *et al.,* 1999), with hOCTN2 also being expressed at low level in the liver (Tamai *et al.,* 1998). rOCTN1 is present principally in the liver (Wu *et al.,* 2000).

1.4.1 (g) Nucleoside Transporters (CNT, SLC28A; ENT, SLC29A)

The cellular transport of nucleosides is mediated by two distinct families of transporter proteins: the high affinity, concentrative nucleoside transporters (CNT; SLC28) and the low affinity, equilibrate nucleoside transporters (ENT; SLC29). The SLC28 family is sodium dependent and works through an active transport mechanism, while the SLC29 family functions by a facilitated diffusion mechanism. The human SLC28 family consists of three subtypes of sodium-dependent, concentrative nucleoside transporters, hCNT1 (SLC28A1), hCNT2 (SLC28A2); also termed SPNT for sodium-dependent purine nucleoside transporter, and hCNT3 (SLC28A3). The human SLC29 transporter family contains four members, hENT1 (SLC29A1), hENT2 (SLC29A2), hENT3 (SLC29A3), and hENT4 (SLC29A4) (Gray *et al.,* 2004).