Solid dispersions: Formulation, characterisation, permeability and genomic evaluation

Muhammad Sheraz Akbar Khan

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Poor water solubility is characterised by low dissolution rate and consequently reduced bioavailability. Formulation of solid dispersion of the drug has attracted considerable interest as a means of improving dissolution process of a range of poorly water soluble drugs. This current study investigates the formulation of solid dispersion for a range of poorly water soluble drugs with varying physicochemical properties including paracetamol, sulphamethoxazole, phenacetin, indomethacin, chloramphenicol, phenylbutazone and succinylsulphathiazole.

Solid dispersions were prepared using various drugs to polymer ratios. PEG 8000 was selected as a carrier in the solid dispersions. The study revealed that inclusion of drug within the polymeric matrix, ratio of drug to polymer and physicochemical properties of the drug molecules enhance the dissolution rate. Characterisations of the solid dispersions were performed using DSC, FTIR and SEM. These studies revealed that all seven drugs were present in the amorphous form within the solid dispersions and there was a lack of interaction between the PEG 8000 and drug. Stability studies for solid dispersions showed that all seven drugs studied were unstable at accelerated conditions (40°C±2°C/75%RH±5%RH) whereas, they were found to be stable for 12 months at room conditions.

Permeability of indomethacin, phenacetin, phenylbutazone and paracetamol were higher for solid dispersions as compared to drug alone across Caco-2 cell monolayers. From the cell uptake studies it was shown that PEG 8000 enhanced rhodamine123 uptake which suggested that PEG 8000 may increase the permeability of these drugs in solid dispersions. Gene expression profiles analyzing the expression changes in the ABC and solute carrier transporter during permeability studies.ABCA10, ABCB4, ABCC12, SLC12A6, MCT13, SLC22A12 and SLC6A6 gene expression were increased by indomethacin alone whereas solid dispersion of indomethacin resulted in a slight increase in expression. ABCC12 and SAMC gene expression was increased in case of paracetamol alone but slightly increased when exposed to solid dispersion of paracetamol.

Key words: PEG 8000, dissolution, stability studies, Caco-2, microarray.

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Abbreviations used in this study

2X	2times
18S	18 Svedberg
28S	28 Svedberg
@	At
%	Percentage
Ω	Ohm
µL	Microlitre
µg/mL	Microgram per millitre
λmax	Wavelength maximum
aa-dUTP	5-(3-Aminoallyl)-2'-Deoxyuridine-5'-Triphosphate
ABC-transporter	ATP-binding cassette transporter
ABC-me	ABC-mitochondrial erythroid
ACE-inhibitors	Angiotensin-converting enzyme inhibitors
ATP	Adenosine triphosphate
A2E	*N*-retinylidene-*N*-retinyl-ethanolamine
ß-lactam antibiotics	Beta lactam antibiotics
BBB	Blood-brain barrier
BCS	Biopharmaceutics Classification System
BRB	Blood retinal barrier
BSA	Albumin from Bovine serum
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate

CDER	Center for Drug Evaluation and Research
cDNA	Complementary deoxyribonucleic acid
cm^{-1}	A reciprocal centimeter
cells/ cm^2	Cells per square centimeter
cm/s	Centimeter per second
CYP P450	Cytochrome P450
cm^2	Square centimeter
Da	daltons
DAPI	4',6-Diamidino-2-phenylindole dihydrochloride
DMEM	Dulbecco's modified Eagle's medium
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
EDTA	Ethylenediaminetetraacetic acid
e.g.	exempli gratia ('for sake of an example')
etc	et cetera
FBS	Fetal bovine serum
FDA	Food and drug administration
FDR	False discovery rate
FTIR	Fourier transform infrared spectroscopy
GABA	γ-amino butyric acid
GSH	Glutathione
GIT	Gastro-intestinal tract
g/mol	Gram per moles

ΔG	Free energy change
h	Hour
hrs	Hours
HBSS	Hank's balanced salt solution
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hNET	Human norepinephrine transporter
HPLC	High performance liquid chromatography
Hyper DSC	Hyper differential scanning calorimetry
ΔH	Binding enthalpy
H_2O	Water
ICH	International Conference on Harmonization
i.e	id est
IND	Indomethacin
IR spectra	Infrared spectra
ITC	Isothermal titration calorimetry
L	Litre
K	Binding constant
KATP	ATP-sensitive potassium
KCC3	K^+ Cl^- cotransporters
kDa	Kilodaltons
m	Meter
mA	milliampere
mAU	milli-absorbance unit

MCT	Monocarboxylate cotansporter
MDR	Multidrug resistance
mRNA	Messenger ribonucleic acid
MRP	Multidrug resistance protein
min	minute
mins	minutes
mL	millilitre
mM	milliMolar
mPa.S	milli pascal second
ms	millisecond
mTP	mitochondrial targeting presequence
N	Stoichiometry
NaOH	Sodium hydroxide
NBF	Nucleotide binding folds
NEAA	Non-essential amino acids
nm	nanometer
n=3	Triplicate
Papp	Permeability coefficients
PARA	Paracetamol
PBS	Phosphate-buffered saline
PCA	Principle component analysis
PCR	Polymerase chain reaction
PEG	Polyethylene glycol

PEGs	Polyethylene glycols
PEG 300	Polyethylene glycol molecular weight 300
PEG 600	Polyethylene glycol molecular weight 6000
PEG 8000	Polyethylene glycol molecular weight 8000
P-gp	Phosphoglycoprotein
pH	Potential of hydrogen
PKC	Paroxysmal kinesigenic choreoathetosis
PM	Physical mixture
PMT	Photo-multiplier tube
PVP	Polyvinylpyrrolidone
RH (r.h)	Relative Humidity
rpm	Revolutions per minute
RNA	Ribonucleic acid
RPE	Retinal pigment epithelial cells
rRNA	Ribosomal Ribonucleic acid
R^2	Coefficient of Determination
s (sec)	Second
SAM	Significant analysis of microarrays
SAMC	S-adenosylmethionine carrier
SD-PARA	Solid dispersion of paracetamol
SD-IND	Solid dispersion of indomethacin
SDS	Sodium dodecyl sulfate
SD	Solid dispersion

S.D	Standard deviation
SEM	Scanning electron microscopy
SLC transporters	Solute carrier transporters
SSC	Saline-sodium citrate
SUR2	Sulsonyl urea receptor
ΔS	Entropy change
TAE	Tris-acetate-EDTA
TAPL	Transporter associated with antigen processing (TAP)-like
TC-Plate	Tissue culture plate
TEER	Transepithelial electrical resistance
TGA	Thermo gravimetric analysis
TM	Transmembrane
UGT/ UDP-glucuronosyltransferase	Uridine 5'-diphospho-glucuronosyltransferase
WHO	World Health Organization
w/w	weight by weight

Chapter 1

Introduction

1.1 Physiology of the gastro-intestinal tract (GIT)

The gastro-intestinal tract consists of esophagus which is 25 cm in length, followed by the stomach. The volume of the stomach changes with the change in the amount of food present in it, whereas its length is approximately 25 cm. The volume of an empty stomach is approximately 50 mL yet its volume can become as much as 4 L when it is dilated by food (Curtis and Barnes, 1994). The stomach is divided into three main parts: the fundus, the body and the pylorus region. The curved uppermost part of stomach is fundus which pushes gastric contents towards pylorus region by its slow continuous contractions. Body is the largest part of the stomach which acts as a reservoir and stores the indigested food and liquids for a certain time period. The lowest part of the stomach is pylorus region; it prevents larger particles of food from entering into the small intestine. There are millions of deep gastric pits in the smooth lining of the stomach, through which the gastric juice produced by the gastric glands enters the stomach. The gastric juice consists of water, hydrochloric acid, pepsin and intrinsic factors. The environment inside the stomach is kept extremely acidic (pH 1-2.5) through hydrochloric acid. It kills the bacteria that are present in food and it is also vital for the activation and optimal activity of pepsin. The breakdown of proteins is carried out through pepsinogen, an inactive enzyme of pepsin. Vitamin B12 is absorbed in the small intestine by intrinsic factor which is a glycoprotein.

The extreme acidity and the presence of pepsin; which is capable of digesting the stomach itself, provides the most rough conditions in the digestive tract to which mucosa of the stomach is exposed. Thick alkaline mucus is secreted by the stomach which

prevents stomach mucosa from digestion. The thick mucus layer is responsible for limiting the absorption inside the stomach despite its large epithelial surface. The underlying tissue layers are kept protected by tight junctions in the epithelial cells of the mucosa from the gastric juice of the stomach. Damaged epithelial cells are shed and replaced quickly, with the whole stomach epithelium being replaced every 3-6 days. The partially digested food then reaches the small intestine, the longest part of the gastro-intestinal tract. It is divided into three main parts. The first 20-30 cm of the small intestine is called duodenum (pH 5.5-6) which have deeply folded mucous membrane in its thick wall, containing duodenal digestive gland and Brunner's glands. An alkaline secretion free from enzymes is produced by the Brunner's glands to neutralise the hydrochloric acid present in the gastric juice. This causes a change in the pH of chyme (the food/gastric fluid matter) that enters the small intestine.

The jejunum (pH 6.0-7.0) and the ileum (pH 7.0-7.5) are the continuation of the duodenum and constitute the remaining part of the small intestine. Although the jejunum has a thicker wall than either the ileum or duodenum, small intestine cannot be differentiated completely as its parts are not demarcated but are distinct in properties. The small intestine is approximately 2 m long. The small intestine is highly specialised for the purpose of absorbing nutrients. Along with the advantage of being greater in length, small intestine also has three main structural features which increase surface area and therefore, enhance the uptake of nutrients.

The plicae circulares are large circular folds that increase the surface area. Villi are finger like projections that also contributes to the increase in surface area. Villi have tiny projections on their surface called microvilli which increase the surface area that further enhances the absorption process.

The gastric emptying differs with the amount of food digested (Davis et al., 1988) and with the type of preparation (Christensen et al., 1985). It has been reported that it takes around 3±1 hrs for a dosage form to reach the large intestine (Davis et al., 1986) though another work has reported that the mean small intestine transit time for a radiotelemetry capsule was 5.7±2 hrs (Evans et al., 1988).

The large intestine is much shorter than the small intestine as its length is approximately 1.5 m but its diameter is quite larger as compared to the small intestine. In the large intestine very little digestion and absorption of food is carried out as most of it has already taken place in the small intestine. Bacterial fauna present in the large intestine produces water, electrolytes and vitamins that are to some degree absorbed over the 12 – 24 hrs time period for which the faecal matter remains in the large intestine.

1.2 Biopharmaceutics Classification System (BCS)

The Biopharmaceutics Classification System (BCS) is a scientific method in which drugs are classified according to the solubility in water related to their dose at three different pHs and intestinal permeability (Amidon et al., 1995). The BCS system divides the drug substances into following four classes;

Class 1 (high solubility–high permeability)

Class 2 (low solubility–high permeability)

Class 3 (high solubility–low permeability)

Class 4 (low solubility–low permeability)

However, the dissolution rate is used as a criterion for the classification of rapid release oral dosage forms. The BCS can be used in association with the dissolution of a product to monitor the three basic properties that are the determining factors in the rate and extent of drug absorption from immediate release solid oral dosage forms: dissolution rate, solubility and permeability. The BCS has been successfully used for extended release solid dosage forms besides for immediate release forms and it is regarded as the basic instrument in the development of drugs over the last few years (Lennernas and Abrahamsson, 2005; Wei et al., 2008; Ku, 2008; Grudzien et al., 2009).

1.3 Dissolution rate

According to modified Noyes-Whitney equation (equation 1.1) the dissolution rate can be improved by increasing the surface area available for dissolution, decreasing particle size and/or increasing wettability, decreasing the boundary surface thickness, ensuring sink conditions for dissolution and increasing the apparent saturation solubility (Noyes and Whitney, 1897; Nernst, 1904).

$dC/dt = (AD[Cs - C])/h \rightarrow$ (equation 1.1)

Where,

dC/dt is the rate of dissolution,

A is the surface area available for dissolution,

D is the diffusion coefficient of the compound,

Cs is the solubility of the compound in the dissolution medium,

C is the concentration of drug in the medium at time t and

h is the thickness of the diffusion boundary layer adjacent to the surface of the dissolving compound.

1.4 Solubilisation

Solubilisation is the use of an inert substance to enhance the solubility of a drug. There are different ways in which the solubility and dissolution of a drug can be increased by using adsorbents (Alsaidan et al., 1998; Bogdanova et al., 2007), surfactant (Krasowska, 1980) or co-solvents (Etman and Nada, 1999).

1.5 Micronisation

It has been shown that the dissolution rate of a variety of drugs can be significantly increased and also the apparent equilibrium solubility can be improved by using micronisation. As predicted by the modified Noyes-Whitney equation (equation 1.1) the dissolution rate is associated with the particle size (Maillols et al., 1982). The therapeutic dose of griseofulvin has been reduced to half by micronisation process (Atkinson et al., 1962; Kabasakalian et al., 1970). It has been proved that the bioavailability can be increased through ultra-micronised dispersions of griseofulvin (Straughn et al., 1980). The problem with very small particles is that they cause poor wettability and their handling is also very problematic (Serajuddin, 1999).

1.6 Complexation

Sparingly soluble drugs have been shown to display improved dissolution by the formation of a complex with water soluble complexation agents. The most widely used complexation agents are cyclodextrins. Cyclodextrin complexes are formed by the binding of the hydrophobic core of the agent with the hydrophobic region of the drug (Florence and Attwood, 1988). The characteristic feature of these complexes is that they are reversible; hence facilitating the absorption by releasing the drug from the complex during dissolution as it is soluble in the fluids of the gastro-intestinal tract. Pitha and Pitha (1985) explained that the solubility of the sparingly soluble drugs can be improved by the application of cyclodexrin complexes. Aqueous solution of cyclodextrins was used to increase the solubility of a variety of steroids.

1.7 Chemical modification (Prodrug)

Chemical modification can render a drug into a more soluble prodrug (Albert, 1958), where the prodrug is defined as "a bio-reversible chemical derivative of an active parent drug" (Taylor, 1996). It includes salt forms and complexes which can be easily disassociated from the drug (Anderson, 1985). Diclofenac, for instance, is generally formulated as a sodium and potassium salt whereas, dissolution is greater from potassium salt because potassium salt has very high dissociation rate. The basic objective is to increase the solubility either by introducing an ionisable group or by reducing the lattice enthalpy of a drug (Amidon, 1981).

Salt formation, by its nature requires a drug to be basic or acidic; therefore it is unsuitable for neutral drugs such as griseofulvin (Berge et al., 1977). The possibility of the salt form of a drug to aggregate in the gastro-intestinal tract demolishes any increase in the dissolution rate that was desired to have been attained by this process (Serajuddin, 1999). Numerous drugs along with griseofulvin have been modified into prodrug to enhance their solubility but this does not improve their bioavailability although they have been shown to have the ability to degrade at the intestinal lumen, therefore, a complete knowledge of the kinetics and mechanisms of prodrug degradation is necessary to make their use effective. The factors responsible for this are incomplete conversion, simultaneous breakdown into inactive derivatives and elimination from the body before complete conversion has occurred. To decrease the crystal lattice enthalpy digoxin prodrugs and complexes with hydroquinone are produced and the proof of their effectiveness is that a digoxin prodrug product is available in the United States (Acylanid®) (Higuchi and Ikeda, 1974; Stella, 1975).

1.8 Introduction to solid dispersions

Enhancement of bioavailability of hydrophobic drugs is one of the major challenges in drug development. Of the plethora of pharmaceutical technologies available to address this issue, solid dispersion is one of the useful methods for the dispersion of the drug into an inert, hydrophilic polymer matrix (Chiou and Reigelman, 1971; Serajuddin, 1999). Solid dispersions in water-soluble carriers have attracted considerable interest as a means of improving the dissolution rate, and hence possibly bioavailability of a range of hydrophobic drugs. Although a large number of studies have been published but the

mechanisms underpinning the observed enhancement of the rate of drug release are not yet understood (Sjokvist-Sears and Craig, 1992; Friedrich et al., 2006).

The use of solid dispersions as an effective source of improving the dissolution rate of poorly soluble drugs has been well studied and demonstrated (Chiou and Reigelman, 1971; Corrigan, 1985; Ford, 1986; Craig, 1990). The poorly water soluble drugs are characterised by insufficient bioavailability (low dissolution rates) and absorption in the gastro-intestinal tract. Different methods have been used to increase the dissolution and bioavailability of poorly water soluble drugs including micronisation (Atkinson et al., 1962), the use of surfactants (Khalafallah et al., 1975), and the formation of solid dispersions (Sekiguchi and Obi, 1961). Solid dispersions display an enhanced solubility of drug because of the conversion of the drug's crystal lattice into an amorphous form, particle size reduction and increased wettability by the hydrophilic polymer. Therefore, the same pharmacological results can be obtained from a reduced amount of drug given to the patient.

1.8.1 First generation solid dispersions

It has been shown by Sekiguchi and Obi in 1961 (Sekiguchi and Obi, 1964) that the formulation of eutectic mixtures improved the rate of drug release which in turn increases the bioavailability of poorly water soluble drugs. Solid dispersions systems were developed by Levy (1963) and Kanig (1964), who made solid solutions by using molecular dispersions instead of using eutectic mixtures, with mannitol as carrier. These improvements were due to faster carrier dissolution, releasing particles of drug. These

dispersions prepared using crystalline carriers were described as first generation of solid dispersions. Urea (Sekiguchi and Obi, 1964; Goldberg, et al., 1966b) and sugars (Kanig, 1964) were the first crystalline carriers to be used in dispersions. The major drawback of first generation solid dispersion is that they form crystalline solid dispersions, which being thermodynamically more stable did not release the drug as quickly as amorphous ones.

1.8.2 Second generation solid dispersions

It was noticed in the late sixties (Simonelli et al., 1969; Chiou and Riegelman, 1969), that solid dispersions with drug in the crystalline state are not as effective as amorphous because they are thermodynamically stable (Simonelli et al., 1969; Vippagunta et al., 2006; Urbanetz, 2006). Therefore, second generations of solid dispersions were introduced having amorphous carriers instead of crystalline. Formerly, the drugs were molecularly dispersed in amorphous carriers which are usually polymers in random pattern (Vilhelmsen et al., 2005).

1.8.3 Third generation solid dispersions

Third generation of solid dispersions appeared as the dissolution profile could be increased by using carriers having surface activity and self-emulsifying characteristics. These contain surfactant carriers or a mixture of amorphous polymers and a surfactant as carrier. The third generation solid dispersions stabilise the solid dispersions, increase the bioavailability of the poorly soluble drugs and reduce recrystallisation of drug. The use of

surfactants such as poloxamer 407 as carriers resulted in high polymorphic purity and improved vivo bioavailability (Majerik et al., 2007).

1.8.4 Manufacturing methods for solid dispersions

Melting and solvent evaporation methods are the two major processes of preparing solid dispersions. Sekiguchi and Obi (1961) were the first to use melting method. The method depends on melting either the carrier or the drug or both. One component is melted and the second is dissolved in it. The solution is then cooled to prepare a solid dispersion. Goldberg and co-workers applied temperatures more than 100 °C to prepare paracetamol-urea and chloramphenicol-urea dispersions (Goldberg et al., 1966a; Goldberg et al., 1966b). The solvent evaporation method consists of solubilisation of the drug and carrier in a volatile solvent that is later evaporated (Lloyd et al., 1999; Hasegawa et al., 2005; Rodier et al., 2005).

1.8.5 Proposed mechanisms for drug release from solid dispersions

Different factors influence the enhancement of dissolution rate of solid dispersions. The use of increased amount of urea enhances the dissolution rate of drug as was shown in a study with 20% chloramphenicol and 80% urea (Goldberg et al., 1965). This was due to the reduction in particle size. However, it was later found that the dissolution rate could be improved without any change in the particle size (Sjokvist-Sears and Nystrom, 1988). Non surface active carrier can enhance the wettability of a drug (Chiou and Reigelman, 1971) by reducing the contact angle and thus causing an increase in the surface area available for dissolution. A drug can be retained in the solution by inhibiting its

precipitation with the addition of a polymer (Simoneli et al., 1976; Hilton and Summers, 1986; Usui et al., 1997). The drug dissolves back into the solution, after precipitating out as metastable polymorph as this form is more soluble than the original polymorph of the drug, as highlighted in a study with indomethacin (Ford and Rubinstein, 1978; Hilton and Summers, 1986).

Carrier-controlled or drug-controlled dissolution mechanisms were first proposed by Craig (2002) in which the drug release depends either on the carrier or the drug itself. This method is based on the models proposed by Higuchi et al. (1965) and Higuchi (1967). The dissolution surface is non-disintegrating and the dissolution of both parts is diffusion controlled. The dissolution is controlled through a drug rich dissolving surface, formed only if the drug makes the larger component (Corrigan, 1985). In high polymer loading there is insufficient drug to support the drug controlling layer formed at the dissolving surface. This causes the drug to disperse within the polymer resulting in a carrier-controlled drug release process. In high drug loading solid dispersions, the dissolution rate of the drug can be measured, by considering the polymer as faster dissolving component.

Hence, the dissolution of the drug is controlled by polymer dissolution if the drug forms the minor component in the solid dispersion. The carrier-controlled dissolution was further supported by another study investigating the incorporation of ten drugs into PEG 6000 solid dispersions where identical dissolution rates were reported (Dubois and Ford, 1985). A linear relationship was shown when the dissolution rate was plotted against the

drug content. Carrier-controlled dissolution works up to a limited concentration depending upon the drug (0-2% w/w for phenylbutazone and 0-15% w/w for paracetamol) as is evident from the differences in the linear relationships for various drugs.

Currently, there is no mechanism that can predict the behavior of a drug in solid dispersion, as various factors are pivotal in deciding drug release. Extensive work is required in order to fully understand the association of the carrier and drug in dispersion.

1.8.6 Characterisation of solid dispersions

There are different techniques that have been used to characterise solid dispersions. Among these the most important methods are thermoanalytical, fourier transform infra-red spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA) and measurement of release rate of the drug. These methods can be employed efficiently to differentiate between solid solutions (molecularly dispersed drug) and solid dispersions in which drug is only partly molecularly dispersed and physical mixtures of drug and carrier. It is difficult to differentiate accurately between molecularly dispersed and non dispersed systems because of the complex composition of these preparations.

Differential scanning calorimetry (DSC) is the most reliable thermoanalytical technique. Processes in which energy is either required or produced can be quantitatively observed with the help of DSC**.** It is a thermal process to find out the heat flow and temperature

related with substance transitions as a function of time and temperature. With the help of DSC we can find melting temperatures as well as monitor and study the thermal behavior of various substances. Interactions between drugs and polymer are generally said to have cause the changes in the exothermic and endothermic peaks (Ribeiro et al., 2005; Borges et al., 2005).

FTIR spectroscopic imaging is regarded as more beneficial than other methods because it takes into account the specific absorbance of molecular vibrations in the sample for quality assessment of biomedical materials. Hence, dyes or various labelling methods are not necessary for seeing chemical components within the sample. It has opened a new horizon of information about structure, conformation and dynamics of various molecular components (Mantsch and Chapman, 1996). It can be applied to follow changes in bonding between functional groups.

SEM has been a process for ultrastructural analysis in the pharmaceutical industry. The characteristic properties of drug crystals like particle size and morphological surface can be known by the preparation method and chemical composition (Ramadan and Tawashi, 1990). Additionally, the shape and granulometric properties of the powder particles can be explained through the range of parameters automatically obtained by connecting SEM with an image processor. As this method is automatic and gives precise measurements, it is time saving as well as reliable, therefore, it gives valid conclusions with smaller number of observations (Paraira et al., 1994).

Isothermal titration calorimeter (ITC) is a powerful technique with high precision that can quickly and directly explain the complete thermodynamic profiles of interaction in a single experiment (Haq et al., 2000; Leavitt et al., 2001). The heat absorbed or generated during binding can be measured by an ITC experiment. It involves the addition of one binding component (titrant) into the other binding component (titrate) over time using single or multiple injections. The absorbed or generated heat is measured as a change in temperature or as the change in power needed to maintain temperature between the sample and a reference cell. Based on the information of the cell volume and the concentration of the reactants the energy is changed into a binding enthalpy. The enthalpy measured includes the heat of binding between titrant and titrate, also any heat sources related with the reaction due to solvent effects, conformational changes and heats of dilution. It involves a very careful preparation of solutions to obtain thermodynamic parameters that accurately show the concern results.

TGA is a powerful technique for studying the changes in weight of a sample when heated, cooled or held at constant temperature. Its main application is to characterise samples with regard to their composition. Thus, the determinations of the moisture content in a solid dispersion can be determined by using TGA.

1.9 Polyethylene glycol (PEG)

Polyethylene glycols (PEGs) are polymers of ethylene oxide, having molecular weight usually falling in the range 200-30000 daltons (Da). PEGs with molecular weights of 1500-20000 Da can be used efficiently in the formation of solid dispersions. PEG

polymers are widely used and employed in different formulations because of low melting point, low toxicity, compatibility with drugs and hydrophilic nature (Chiou et al., 1971). The viscosity of PEG increases with the increase in molecular weight. PEGs are fluid at molecular weights of up to 600 Da, while in the range of 800-1500 Da they are vaseline-like, and tend to be waxy from 2000 to 6000 Da and those with molecular weights of 20000 Da and above form hard, brittle crystals at room temperature. Their ability to solubilise in many organic solvents makes PEGs highly useful for the formation of solid dispersions. The melting point of different molecular weight of PEGs lies under 65 °C (Price, 1994). The low melting point of the polymer is an ideal feature for the formulation of solid dispersions. Additionally, other favourable characteristics that make PEGs more suitable for solid dispersions include their ability to solubilise some compounds (Betageri and Makarla, 1995) and also to improve compound wettability. Aspirin, which is a soluble drug, showed an increase in the dissolution rate when formulated as a solid dispersion in PEG 6000 (Asker and Whitworth, 1975). PEG 8000 formulation displayed enhanced dissolution rates as compared to drug alone (Perng et al., 1998). PEGs with low molecular weight are more likely to cause slightly greater toxicity as compared to those having higher molecular weight (Price, 1994).

In PEGs with molecular weights falling between the ranges of 4000–6000 Da, hygroscopy is not a problem despite the fact that they possess higher aqueous solubility and have melting points above 50 ºC. Formulation of a pharmaceutically acceptable product is difficult if a PEG having very low molecular weight is used as it may result in a product with a sticky consistency (Shah et al., 1995). PEGs with higher molecular

weight are employed to improve the process: PEG 8000 (Perng et al., 1998) and 10000 (Khan and Zhu, 1998) have been shown to increase dissolution rates of the products than the pure drug.

PEG 6000 solid dispersions were used with 14 different drugs revealing that carrier plays a vital role in the formulation of solid dispersions successfully (Dubois and Ford, 1985). Dubois and Ford showed that the carrier governs the release rate and not the properties of the drug, if the dug is found in a low drug/carrier ratio (<2% in the case of phenylbutazone, up to 15% in the case of paracetamol). Further studies suggested that there is an inverse relationship between release rate and the chain length of PEG (Ford et al., 1986). Similar results were obtained in a study for etoposide (Shah et al., 1995) and griseofulvin (Chiou and Riegelman, 1969). However, the release of glyburide PEG 4000 solid dispersion was shown to be slower than an identical solid dispersion in PEG 6000 exhibiting contradictory behavior (Betageri and Makarla, 1995). The capability of PEG 6000 to dissolve increased amounts of drug than PEG 4000 provides the best possible explanation for improved release, causing more drugs to become molecularly dispersed. Additionally, PEG 6000 promotes the dissolution of the carrier by avoiding precipitation of the drug due to its higher viscosity.

PEG molecular weight effects the release rate as is illustrated by the detailed study of phenylbutazone/PEG solid dispersions (Ford et al., 1986).When a low percentage of the drug was used (0.5-2%), the release followed the rank order 1500> 4000> 6000> 20000 at percentages of 3 and 4% the rank order was PEG 1500> 4000> 20000> 6000 and at a

5% loading the order was 20000> 4000> 1500> 6000. The authors deduced from this data that the release is dependent on the extent of the formation of a molecular dispersion, as the rank order is associated with the crystallinity of the solid dispersion. However, chloramphenicol/PEG solid dispersions displayed contradictory results, for which the rank order of release was PEG 6000> 4000> 12000> 20000 (Kassem et al., 1979). The molecular weight of the PEG did not affect the release rate in some cases. For instance, 10% dispersions of naproxen in PEG 4000, 6000 and 20000 displayed identical release rate, as was shown by Mura et al. (1999).

In addition to this, certain problems may arise in subsequent formulation of the solid dispersions into an acceptable dosage form. It becomes very difficult and impossible to manufacture a tablet dosage form if the resulting dispersion is too soft. The reason for this is that the molecular weight of the PEG used is very low or the drug has a plasticising effect on the PEG (Shah et al., 1995). PEG 8000 was selected as a carrier for this study because it has excellent solubility in aqueous medium and low melting point. PEGs show lack of toxicity and immunogenicity; favorable kinetics as well as tissue distribution in the body (Zaplisky and Harris, 1997).

1.10 Dissolution of Polymer

In order to study the dissolution behavior of polymers, different methods and techniques can be employed including differential refractometry, optical microscopy, florescence and gravimetry (Miller-Chou and Koeing, 2003).

The method of differential refractometry works on the principle that as the concentration of the polymer is increased within the solution, the refractive index changes; this is one of the older methods to study polymer dissolution. This technique is sensitive to change in concentration and hence can also be used for detecting any increase that may occur in the polymer concentration. However, there are certain drawbacks of this method as it is only applicable to high polymer concentration and cannot be used for small polymer concentration due to low sensitivity (Miller-Chou and Koeing, 2003). Additionally, it is only applicable to polymer solution alone as the addition of any other substance (drug) will affect the refractive index.

The optical microscopy method provides direct method for observing the polymer dissolution within a given solution. Therefore, it is the most practical technique to study polymer dissolution. The basic limitation is that no quantitative information can be obtained and thus to know the polymer dissolution profile is not possible.

The use of laser inferometry makes it possible to measure the dissolution rates of a polymer (Rodriguez et al., 1985). In this process monochromatic light is passed through a polymer film placed between two mirror lenses. Through the interference lines from the monochromatic light, information about the dissolution of the polymer can be obtained. The main drawback of this technique is that it can only be applied with the transparent film of polymer. In this method the dissolution profiles of the polymer cannot be obtained.

Gravimetric method can be used to follow the dissolution of a polymer. This method involves the preparation of pellets of polymer followed by performing dissolution tests with these pellets. Pellets are weighed periodically at predetermined intervals by stopping the dissolution run. The major shortcoming of this method is the involvement of compression that is required in preparing the pellets. These forces are most likely to destroy the structure of solid dispersion.

Microviscometry has been successfully applied in the polymer dissolution studies of solid dispersions using PVP (Esnaashari et al., 2005). The advantage of this technique is that samples can be taken during dissolution process (both drug and polymer concentration can be determined concurrently) and only a small sample is required. An instrument called Anton Parr microviscometer has the capability of measuring accurately viscosities as low as 0.3 mPa.S.

1.11 Mathematical models

Different mathematical techniques may be used to treat dissolution data (Shah et al., 1998). To determine and study the release mechanism of solid dispersions, the release data were fitted to the Korsemeyer–Peppas equation which is mostly used to describe drug release from polymeric systems (solid dispersions) for binary systems and when the release mechanism is not known or when more than one release mechanism is involved (Korsmeyer et al., 1983). The best fit mathematical model for binary systems ranked in the following order of Korsemeyer–Peppas > Higuchi @ first-order > Hixson-Crowell cube root law zero-order (Ahuja et al., 2007).

1.12 Stability of Solid dispersions

One of the challenges is assuring product stability during drug development in the pharmaceutical industry. Stability refers to the ability of a product to withstand any degradation in the storage time allowed. It is important that a solid dispersion should be stable throughout the shelf life of the product as is the case for any other pharmaceutical product. On storage neither should its dissolution profile nor should any physicochemical properties change.

Indomethacin PEG 6000 dispersions were used for carrying out the earliest stability studies. The dissolution profile exhibited an alteration in drug dissolution when it was stored at temperatures between 25 °C and 45 °C at 71% RH. Crystallisation of indomethacin was said to have taken place because the colour of the tablets became less yellow with time (amorphous indomethacin is yellow, crystalline indomethacin is off-white) which resulted in a decrease in the dissolution profile (Ford and Rubinstein, 1980). A slightest change such as this colour change would raise concerns for any licensed pharmaceutical product. In another study, PEG dispersions of temazepam and triamterene were assessed for their stability profile and various physicochemical parameters including dissolution profile studies were investigated as stability markers (Dordunoo et al., 1997).

In a recent paper seven drugs were studied to determine the influence of functional group on stability of the formulations. Four of the seven drugs studied consisted of carboxylic acid groups; BAY 12-9566, naproxen, ketoprofen and indomethacin, whereas, one had

hydroxyl groups (testosterone), one an amide (phenacetin) and the last consisted of a no proton donating group (progesterone). It was concluded that upon storage, the presence of interacting groups (such as carboxylic acid, hydroxyl) were stable possibly due to inhibition of reverse crystallisation by the interacting functional groups (Gupta et al., 2002).

1.13 Physicochemical properties of drugs

Table 1.1 illustrates the physicochemical properties of seven drugs used as a model drugs as solid dispersions in the current study.

Table 1.1. Physicochemical properties of the drugs used in the current study (drug bank).

Drug	Molecular Weight	Melting Point	H_2O Solubility	LogP
Paracetamol	151.163g/mol	169-170.5°C	14mg/L	0.917
Sulphamethoxazole	253.279g/mol	167°C	610mg/L	2.447
Phenacetin	179.216g/mol	134-136°C	763mg/L	1.667
Indomethacin	357.787g/mol	158°C	0.937mg/L	3.655
Chloramphenicol	323.129g/mol	150.5°C	2500mg/L	1.476
Phenylbutazone	308.374g/mol	105°C	47.5mg/L	4.214
Succinylsulphathiazole	355.38g/mole	186-188ºC	-	-

1.13.1 Paracetamol

Paracetamol is an acylated aromatic amide (Sweetman, 2002). The structure of paracetamol is shown in Figure 1.1. Paracetamol has analgesic and antipyretic properties and weak anti-inflammatory activity. Von Mering introduced it for the first time as an

antipyretic/analgesic in 1893. It has been used in home medication as an analgesic for over 30 years. Paracetamol is used in the symptomatic treatment of moderate pain and fever (Sweetman, 2004).

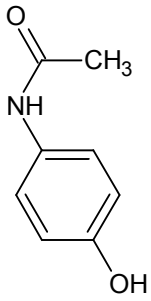

Figure1.1. Structure of Paracetamol

1.13.2 Sulphamethoxazole

Sulphamethoxazole is a sulfonamide drug. The structure of sulphamethoxazole is shown in Figure 1.2. It is a class 2 drug and it has relatively low solubility and high permeability (Sweetman, 2002). It is extensively applied in the treatment of bacterial and protozoal infections.

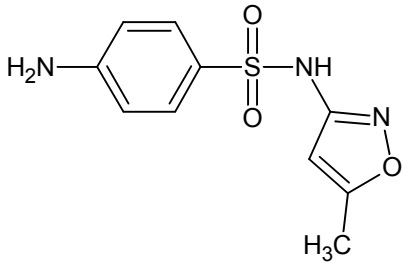

Figure 1.2. Structure of Sulphamethoxazole

1.13.3 Phenacetin

Phenacetin (structure shown in figure 1.3) was introduced for the first time in therapy in 1887. Analgesic mixtures were prepared by using phenacetin with other drug. For a long time it was employed as an analgesic and fever-reducing drug in both human and veterinary medicine.

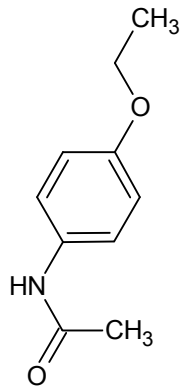

Figure 1.3. Structure of Phenacetin

1.13.4 Indomethacin

Indomethacin is poorly water soluble drug (Hancock and Parks, 2000). It is a member of the non-steroidal anti-inflammatory drugs. It is used for treating pain/swelling involved in osteoarthritis, rheumatoid arthritis and headaches (Sweetman, 2005). It is a class 2 drug (Lobenberg and Amidon, 2000). Due to its hydrophobic nature, it often demonstrates low absorption and low bioavailability; so enhancement in dissolution rate and solubility are important factors for development of drug preparations (Hirasawa et al., 2003). The structure of indomethacin is shown in Figure 1.4.

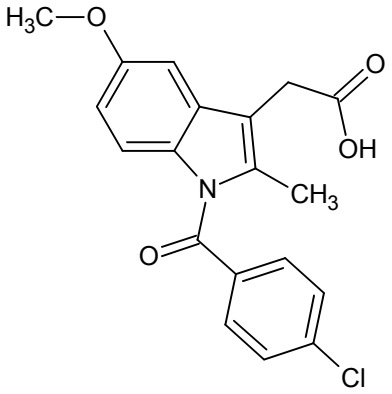

Figure 1.4 Structure of Indomethacin

1.13.5 Chloramphenicol

Chloramphenicol (structure shown in figure 1.5) is a bacteriostatic antibiotic originally derived from the bacterium Streptomyces venezuelae, isolated by David Gottlieb, and introduced into clinical practice in 1949. It was the first antibiotic to be manufactured synthetically on large scale. A vast range of microorganisms can be effectively treated by chloramphenicol; it is active against Gram positive bacteria, Gram negative bacteria and anaerobes (Ambrose, 1984; Kramer, 1984; Sweetman, 2002).

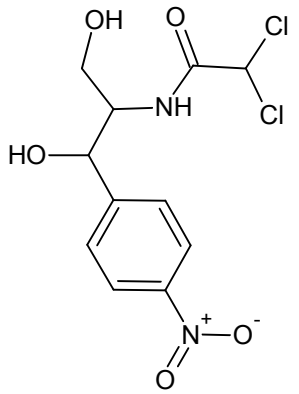

Figure 1.5. Structure of Chloramphenicol

1.13.6 Phenylbutazone

Phenylbutazone (structure shown in figure 1.6) is a non-steriodal anti-inflammatory drug used in the treatment of pain and arthritis. It is mostly used in horses as an analgesic and antipyretic.

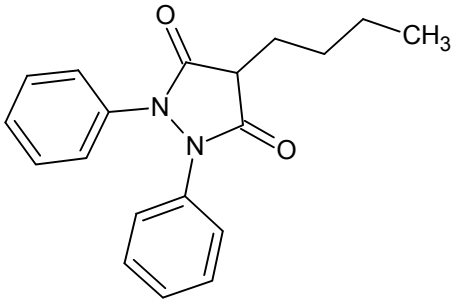

Figure 1.6. Structure of Phenylbutazone

1.13.7 Succinylsulphathiazole

Succinylsulphathiazole is useful as an intestinal antiseptic. The structure of succinylsulphathiazole is shown in figure 1.7. It has a melting point of 186-188 ºC.

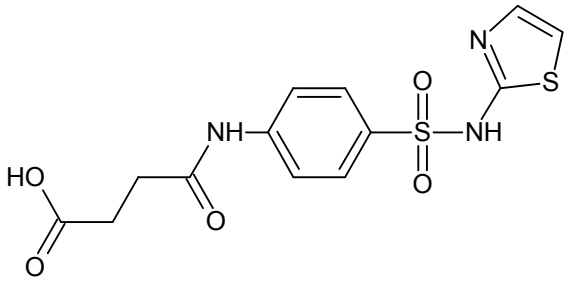

Figure 1.7. Structure of Succinylsulphathiazole

1.14 Permeability and Caco-2 cells

Permeability is the general term that explains the mechanism by which a drug moves and passes across a membrane. The physicochemical properties of a drug, such as its pH, charge, size, lipophilicity and polar surface area influence its permeability (Rowland & Tozer, 1995; Lipinski et al., 2001). The unionized molecules of the drug pass through the intestinal barrier more easily showing that the intestinal pH plays an important role in determining the permeability of the drug (Shore et al., 1957).

Among the various processes involved in the oral drug absorption, the determining factor is the drug permeability to the intestinal membrane in the absorption of the drug. The intestinal permeability of the drug can be determined by applying a variety of *in vivo, in situ* and *in vitro* methods. Along with determining the mechanism of drug absorption, the basic purpose of these studies is to predict oral drug absorption in humans. Cultured epithelial cell lines, most specifically Caco-2 cells, have drawn the focus of the pharmaceutical industry for the last decade. Assays to screen the compounds for evaluating their pharmacokinetic properties, like absorption, metabolism, etc are facing a challenge as it is highly difficult to keep pace with drug development pipeline because of the tremendously large number of compounds that are produced and analysed as potential pharmaceuticals. In vitro model membrane the Caco-2 cell monolayers have been recognised as vital for the rapid screening of the intestinal drug absorption.

Formation of the monolayers from the Caco-2 cells occurs by their spontaneous differentiation into mature cells. Caco-2 cells acquire many characteristic properties of

the absorptive intestinal cells during culture, such as microvilli, enzymes and different carrier mediated transport systems for sugars, amino acids and various drugs (Blais et al., 1987; Hidalgo et al., 1989; Hidalgo and Borchardt, 1990; Hilgers et al., 1990; Dantzig and Bergin, 1990; Chen et al., 1994), despite their origin from colon carcinoma. The transcellular and paracellular movements of drugs through the epithelial layer can be differentiated by the attachment of the adjacent cells via tight junctions formed at the apical sides of the monolayers (Tanaka et al., 1995).

There exists a strong correlation between drug permeability in Caco-2 monolayers and that of intestinal membrane *in vivo*. It has been suggested that oral drug absorption in humans can be detected by the permeability of the drug to Caco-2 monolayers. (Artursson and Karlsson, 1991; Rubas et al., 1993; Stewart et al., 1995; Chong et al., 1996; Lennernas et al., 1996; Yamashita et al., 1997). However, numerous inconsistencies have been reported by these studies that are likely to be present in the experimental conditions including change in pH, shaking rate and solubilising agents in the medium. These conditions can significantly influence the calculated permeability of tested drugs in *in vitro* studies. As the permeability of even monolayers can be affected by the cell culture conditions, it is necessary to develop stable techniques of *in vitro* transport study to have a much reliable prediction of *in vivo* drug absorption.

1.15 Scope of Solid dispersion

Griseofulvin in polyethylene glycol 8000 solid dispersion (Gris-PEG, Novartis) and a nabilone in PVP solid dispersion (Cesamet, Lilly) are the only commercial products that

have been produced commercially and marketed in the last four decades following the initial work of Sekiguchi and Obi (1961), despite the fact that a great deal of work has been done as much as almost 500 papers have been published on this subject and different drug carriers have been tested (Leuner and Dressman, 2000). Chemical or physical instability and scale-up problems are the main factors responsible for the lack in commercial turn around (Serajuddin, 1999; Franco et al., 2001; Craig, 2002).

1.16 Microarray

Microarray is the most widely used method for studying gene expression in many organisms. It has led to large-scale gene discovery and is effectively used for the expression of great number of genes at the same time. Microarray consist of chemically coated glass slides that act as a support onto which DNA segments are arranged and attached on it in a fixed regular pattern. These DNA segments are then covered with a labelled nucleic acid sample. The gene products are labelled either using a fluorescent tag or a radioactive tag, and are washed over the array after being labelled. The DNA segments present on the support hybridise with their complementary sequences of the nucleic acid in the sample. This hybridisation can be measured and analysed with the help of labelling. Laser scanner (for fluorescence) or a phosphorimager (for radioactive materials) are used for detecting hybridisation signals, producing digital images (Chen et al., 1997). Microarray technology is developing rapidly (Blohm and Guiseppi-Elie, 2001; Hughes and Shoemaker, 2001) since microarrays were first reported in the literature in 1995 (Schena et al., 1995). Microarray can also be called as DNA chips, gene chips or DNA arrays. On an array thousands of DNA spots are present and there are numerous

identical DNA molecules found in each spot of lengths varying from 25 to hundreds of nucleotides.

The three main steps that constitute the process of expression analysis include: (1) array fabrication; (2) probe preparation and hybridisation; and (3) data collection, normalisation and analysis.

To study gene expression various techniques have been employed. Microarrays consisting of either oligonucleotides or cDNA fragments are considered to be the most practical method for analysing and studying multiple samples (Schena et al., 1995; Chee et al., 1996).

Biological processes including pathway analysis of specific genes can be effectively characterised using microarrays. Highly accurate maps and sequenced databases for diverse species have made the positional cloning projects extremely easy to carry out. The availability of this extensive data has been proven to be very helpful in further study of genome function.

1.17 Microarray applications

The foremost advantage of DNA microarray technology is its versatility to study the transcript levels of expression of thousands of genes which can be measured in parallel. The high capacity of cDNA microarrays system was first shown by Schena & coworkers to examine the expression of 45 Arabidopsis genes in parallel (Schena et al., 1995). The

cDNA microarray has been observed to exhibit a number of applications in various organisms including plant (Schena, 1996), yeast (Shalon et al., 1996; DeRisi et al., 1997) and human beings. Microarray was also employed to discover novel disease-related genes and investigate complex diseases. For example, inserting human chromosome 6 could reverse the tumorigenic properties of human melanoma cell line UACC-903 as observed by DeRisi et al. (1996). Rheumatoid arthritis and inflammatory bowel disease were examined for their distinctive gene expression by Heller and coworkers (1997). It was established that microarray could be effectively used to identify disease-related genes and study the diseases. The challenge of treating chronic diseases effectively was solved to a greater extend as this method provided an opportunity for drug development and hence improving disease therapies. The function of a particular gene can be ascertained by knowing its expression pattern. A target can be implicated in any pathway or disease without any difficulty by utilising the knowledge of sequence homology to a known gene family along with the selective gene expression.

The alterations in the gene expression caused by a drug can also be detected by the DNA microarray method. It has been shown by Pietu et al. (1996) that the quantitative hybridisation of a high density cDNA array resulted in the expression of novel gene transcript in human muscle cells. The cathepsin K is a novel cysteine protease that is selectively expressed in osteoclasts. This resulted in the development of drugs to inhibit cathepsin K. The gene expression sequence of a particular disease can be detected by the simultaneous measurement of thousands of genes. Microarray technology has also been applied to study ‘‘toxicogenomics’’, investigating the different responses shown by

different individual towards a particular toxic substance due to differences in individual patterns of gene expression (Lettieri, 2006; Ferrer-Dufol and Menao-Guillen, 2009).

DNA microarray was also used to identify the target effects of secondary drugs and also in drug validation studies by Marton (1998). Its effective use in gene discovery, gene expression, and mapping are ample proof of the fact that cDNA microarray technology has developed very rapidly. The correlation of the gene sequences and clinical medicine for both human beings and animals can be obtained by it. The applications of DNA microarray are of great importance in both molecular biology research and clinical diagnostics.

1.18 Aims of the study

In order to enhance the solubility of poorly water soluble drugs, solid dispersions are carried out as it is a highly effective way of increasing the dissolution rate of a drug and thus increasing its solubility. Most drugs used in this study are poorly water soluble and belong to class 2 according to BCS. Phosphate buffer saline (PBS) is used due to its inert nature and exhibits no interaction either with the drug or polymer. PBS having pH value of 7.4 was used as it is similar to the pH condition in the small intestine. Therefore, for all solid dispersions studied the pH during dissolution studies was kept simulate to that in intestinal pH condition.