CHAPTER 1. INTRODUCTION

This study was divided into three major sections.

The aim of section 1 was to develop and characterize enteric microparticles loaded with lipophilic drugs produced by a coacervation method. Conventional and alternative pharmaceutical methods to improve oral bioavailability of lipophilic drugs are compared. The advantages and disadvantages of each method are presented.

The aim of the section 2 was to develop and evaluate in-situ forming micropaticles for parenteral and oral administrations. The current state of research concerning parenteral in-situ forming implant (ISI) and in-situ forming microparticles (ISM), conventional oral micro-/nanoparticles, oral in-situ gel, oral ISM and heparin oral delivery systems is reviewed.

The aim of the section 3 was to investigate the stability of PLGA in in-situ forming microparticles. The application of PLGA in sustained release systems and the PLGA based commercially available products are summarized. PLGA degradation profiles and influence factors are also presented.

Finally, the objectives of these works are laid out.

1. Lipophilic drugs

Up to 40% of lipophilic drug candidates fail to reach market although exhibiting potential pharmacodynamic activities (Radtke, 2001; Lipinski, 2002). Meanwhile, some lipophilic drugs on the market have to be administered at high doses. Thereby, various formulation strategies have been investigated to improve the solubility and the rate of dissolution and hence the oral bioavailability of lipophilic drugs. These strategies include the solubilization and surfactants, the use of different polymorphic/amorphic drug forms, the reduction of drug particle size, the complexation (e.g., cyclodextrins) and the formation of solid drug solutions/dispersions (Pinnamaneni et al., 2002; Leuner and Dressman, 2000; Ran et al., 2001; Chen et al., 2002; Rogers et al., 2002). In the following, an overview of the different alternative strategies is given.

1.1. Solubilization and surfactants

One approach to increase the bioavailability of lipophilic drugs is the solubilization of the drugs by means of pH adjustment, cosolvent, microemulsification, self-emulsification, micelles, liposomes and emulsions (Strickley, 2004). Each has its advantages and limitations.

pH adjustment. pH adjustment is the simplest and most commonly used method to increase water solubility of ionizable compounds. However, this salt formation is infeasible for unionized compounds. The formed salts may also converse to respective acid or base forms in gastrointestinal-tract (GIT).

Colsovent. Colsolvents are the mixtures of miscible solvents often used to solubilize lipophilic drugs. The solubilizing excipients used in commercially available oral and injectable formulations are listed in Table 1. Currently, the water-soluble organic solvents are polyethylene glycol 400 (PEG 400), ethanol, propylene glycol, and glycerin. For example, Procardia® (nifidipine) was developed by Pfizer contains glycerin, peppermint oil, PEG 400 and sodium saccharin in soft gelatin capsules. The water-insoluble solvents include long-chain triglycerides (i.e. peanut oil, corn oil, soybean oil, sesame oil, olive oil, peppermint oil, hydrogenated vegetable oil and hydrogenated soybean oil), medium-chain triglycerides (Miglyol 812), beeswax, d-α-tocopherol (vitamin E) and oleic acid. Progesterone is a water-insoluble steroid and is solubilized in peanut oil (Prometrium®).

Table 1: Solubilizing excipients used in commercially available oral and injectable formulations.

Water-soluble	Water-insoluble	Surfactants
Dimethylacetamide (DMA)	Beeswax	Polyoxyl 35 castor oil (Cremophor EL)
Dimethyl sulfoxide (DMSO)	Oleic acid	Polyoxyl 40 hydrogenated castor oil (Cremophor RH 40)
Ethanol	Soy fatty acids	Polyoxyl 60 hydrogenated castor oil (Cremophor RH 60)
Glycerin	*d*-α-tocopherol (Vitamin E)	Polysorbate 20 (Tween 20)
N-methyl-2-pyrrolidone (NMP)	Corn oil mono-di-tridiglycerides	Polysorbate 80 (Tween 80)
PEG 300	Medium chain (C_8/C_{10}) mono- and diglycerides	*d*-α-tocopheryl polyethylene glycol 1000 succinate (TPGS)
PEG 400	Long-chain triglycerides	Solutol HS-15
Poloxamer 407	Castor oil	Sorbitan monooleate (Span 20)
Propylene glycol	Corn oil	PEG 300 caprylic/capric glycerides (Softigen 767)
Hydroxypropyl-β-cyclodextrin	Cottonseed oil	PEG 400 caprylic/capric glycerides (Labrasol)
Sulfobutylether-β-cyclodextrin (Captisol®)	Olive oil	PEG 300 oleic glycerides (Labrafil M-1944CS)
α-cyclodextrin	Peanut oil	PEG 300 linoleic glycerides (Labrafil M-2125CS)
Phospholipids	Peppermint oil	Polyoxyl 8 stearate (PEG 400 monosterate)
Hydrogenated soy phosphatidylcholine (HSPC)	Safflower oil	Polyoxyl 40 stearate (PEG 1750 monosterate)
Distearoylphosphatidylglycerol (DSPG)	Sesame oil	Peppermint oil
L-α-dimyristoylphosphatidyl-choline (DMPC)	Soybean oil	
L-α-dimyristoylphosphatidyl-glycerol (DMPG)	Hydrogenated soybean oil	
	Hydrogenated vegetable oils	
	Medium-chain triglycerides	
	Caprylic/capric triglycerides derived from coconut oil or palm see oil	

(Strickley, 2004)

Microemulsion. Microemulsion is a thermodynamically stable isotropical dispersion composed of a polar solvent, an oil, a surfactant and a cosurfactant. The formation of microemulsions is spontaneous and does not involve the input of external energy. One theory considers negative interfacial tension while another considers swollen micelles. The surfactant and the cosurfactant alternate each other forming a mixed film at the interface contributing to the stability of the microemulsion. Microemulsions are potential drug delivery systems for poorly water soluble drugs due to their ability to solubilize the drugs in the oil phase, thus increasing their dissolution rate (Kawakami et al., 2002). Even if the microemulsions are diluted after oral administration below the critical micelles concentration (CMC), the resultant drug precipitates have a fine particle size allowing enhanced absorption (Lieberman et al., 1988).

Self-emulsification. In the absence of external phase (water), the mixture of oil, surfactant, cosurfactant, one or more hydrophilic solvents and cosolvent forms a transparent isotropic solution that is known as the self-emulsifying drug delivery system (SEDDS). This forms fine O/W emulsions or microemulsions spontaneously upon dilution in the aqueous phase and is used for improving lipophilic drug dissolution and

absorption (Gershanik and Benita, 1998; 2000). The self-emulsification process is specific to the nature of the oil/surfactant pair, surfactant concentration, oil/surfactant ratio and temperature at which self-emulsification occurs. The ease of emulsification could be associated with the ease of water penetrating into the various liquid crystalline or gel phases formed on the surface of the droplet. A few parameters have been proposed to characterize the self-emulsifying performance including the rate of emulsification, the emulsion size distribution and the charge of resulting droplets. Among them, emulsion droplet size is considered to be a decisive factor in self-emulsification/dispersion performance, since it determines the rate and extent of drug release and absorption (Shah et al., 1994; Tarr et al., 1989). In addition, positively charged emulsion droplets could be obtained by incorporation of a small amount of cationic lipid (oleylamine) into such system (Gershanik and Benita, 1996; 1998). The oral bioavailability of progesterone was significantly enhanced in rats by forming positively charged emulsion in comparison to the corresponding negatively charged formulation (Gershanik and Benita, 1996).

One of the advantages of SEDDS in relation to scale-up and manufacture is that they form spontaneously upon mixing their components under mild agitation and they are thermodynamically stable. The drawbacks of this system include chemical instabilities of drugs and high surfactant concentrations. The large quantity of surfactant in self-emulsifying formulations (30-60%) irritates GIT. Consequently, the safety aspect of the surfactant vehicle had to be considered. Moreover, volatile cosolvents in the conventional self-emulsifying formulations are known to migrate into the shells of soft or hard gelatin capsules, resulting in the precipitation of the lipophilic drugs. As an example of self-emulsification, Neoral® is composed of ethanol, corn oil-mono-di-triglycerides, Cremophor RH 40 and propylene glycol. It exhibits less variability and better drug uptake compared to Sandimmune®.

1.2. Modification of polymorphs

Polymorphs are different crystalline forms of a drug that may have different physicochemical properties and biological activities, such as shelf-life, melting point, vapor pressure, solubility, morphology, density and bioavailability (Kobayashi et al., 2000; Kachi et al., 1998). Metastable forms are associated with higher energy with increased surface area, subsequently solubility, bioavailability and efficacy (Vippagunta et al., 2001; Kachi et al., 1998). With regard to bioavailability, it is preferable to change

drug from crystal forms into metastable or amorphous forms. However, the possibility of a conversion of the high energy amorphous or metastable polymorph into a low energy crystal form having low solubility can not be ruled out during manufacture and storage. It is 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 (Pharmacy Today, 1999). Finally, the drug was relaunched with the form II polymorph in a soft gelatin formulation that required refrigeration. 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.

1.3. Reduction in particle size

Micro-/nanonization is one of the most promising approaches to improve the bioavailability of lipophilic drugs by an increase in surface area and saturation solubility via reduction of the particle size to less than 1 μm (Merisko Liversidge, 2003). Such size reduction cannot be achieved by the conventional milling techniques. Patented engineering processes have come up based on the principles of pearl milling (NanoCrystals®), high-pressure homogenization (DissoCubes®), solution enhanced dispersion by supercritical fluids (SEDS), rapid expansion from supercritical to aqueous solution (RESAS), spray freezing into liquid (SFL) and evaporative precipitation into aqueous solution (EPAS) (Hu et al., 2004).

Pearl milling. NanoCrystals® involves filling an aqueous suspension of drug into a pearl mill containing glass or zirconium oxide pearls as milling media. The drug microparticles are ground to nanoparticles (< 400 nm) in between the moving milling pearls over a few days. The milling efficiency is dependent on the properties of the drug, the medium and the stabilizer. Rapamune®, an immune suppressant agent, is the

first FDA approved nanoparticle drug using NanoCrystals® technology developed by Elan Drug Delivery. Emend® is another product containing 80 or 125 mg aprepitant formulated by this technique. The limitation of the pearl milling process is the introduction of contamination to the product from the grinding material, batch-to-batch variations and the risk of microbiological problems after milling in an aqueous environment for a few days.

High pressure homogenization. DissoCubes® manufacture involves dispersing a drug powder in an aqueous surfactant solution and passing through a high pressure homogenizer, subsequently nanosuspensions are obtained. The cavitation force experienced is sufficient to disintegrate drug from microparticles to nanoparticles. The particle size is dependent on the hardness of the drug substance, the processing pressure and the number of cycles applied. The possible interesting features of nanosuspensions are (Müller et al., 2001):

- Increase in saturation solubility and dissolution rate of drug
- Increase in adhesive nature, thus resulting in enhanced bioavailability
- Increase the amorphous fraction in the particles, leading to a potential change in the crystalline structure and higher solubility
- Possibility of surface modification of nanosuspensions for site specific delivery
- Possibility of large-scale production, the prerequisite for the introduction of a delivery system to the market.

However, only brittle drug candidates might be broken up into nanoparticles by this technique. A few points have to be considered, such as chemical instability of fragile drugs under the harsh production conditions, Ostwald ripening in long-term storage, toxicity of surfactants, redispersibility of the dried powder, batch-to-batch variation in crystallinity level and finally the difficulty of quality control and the stability of the partially amorphous nanosuspensions.

Solution enhanced dispersion by the supercritical fluids (SEDS). The SEDS process was developed and patented by the University of Bradford (Hanna and York, 1998). The use of a coaxial nozzle provides a means whereby the drug in the organic solvent solution mixes with the compressed fluid CO_2 (antisolvent) in the mixing chamber of the nozzle prior to dispersion, and flows into a particle-formation vessel via a restricted orifice. Such nozzle achieves solution breakup through the impaction of the solution by a higher velocity fluid. The high velocity fluid creates high frictional surface forces, causing the solution to disintegrate into droplets. A wide range of materials has

been prepared as carriers of microparticles and nanoparticles using the SEDS process (York, 1999; Hanna and York, 1998). A key step in the formation of nanoparticles is to enhance the mass transfer rate between the droplets and the antisolvent before the droplets coalesce to form bigger droplets. In another study, a significant decrease in the particle size is achieved by using the ultrasonic nozzle-based supercritical antisolvent process (Subramaniam et al., 1997A; 1997B).

Rapid expansion from supercritical to aqueous solution (RESAS). This process induces rapid nucleation of the supercritical fluid dissolved drugs and surfactants resulting in particle formation with a desirable size distribution in a very short time. The surfactants in the supercritical fluid stabilize the newly formed small particles and suppress any tendency of particle agglomeration or particle growth when spraying this solution (drug + surfactant + CO_2) into an aqueous solution containing a second surface modifier (Young et al., 2000; Pace et al., 2001). The low solubility of poorly water soluble drugs and surfactants in supercritical CO_2 and the high pressure required for these processes restrict the utility of this technology in pharmaceutical industry.

Spray freezing into liquid (SFL). The SFL technology was developed and patented by the University of Texas at Austin in 2003 and commercialized by the Dow Chemical Company. This technique involves atomizing an aqueous, organic, aqueous-organic cosolvent solution, aqueous-organic emulsion or suspension containing a drug and pharmaceutical excipients directly into a compressed gas (i.e. CO_2, helium, propane, ethane), or the cryogenic liquids (i.e. nitrogen, argon, or hydrofluoroethers). The frozen particles are then lyophilized to obtain dry and free-flowing micronized powders (Williams et al., 2003). Using of acetonitrile as the solvent increased the drug loading and decreased the drying time for lyophilization. The dissolution rate was remarkably enhanced from the SFL powder contained amorphous nanostructured aggregates with high surface area and excellent wettability (Rogers et al., 2002; 2003; Hu et al., 2002; 2003).

Evaporative precipitation into aqueous solution (EPAS). The EPAS process utilizes rapid phase separation to nucleate and grow nanoparticles and microparticles of lipophilic drugs. The drug is first dissolved in a low boiling point organic solvent. This solution is pumped through a tube where it is heated under pressure to a temperature above the solvent's boiling point and then sprayed through a fine atomizing nozzle into a heated aqueous solution. Surfactants are added to the organic solution and the aqueous

solution to optimize particle formation and stabilization. In EPAS, the surfactant migrates to the drug-water interface during particle formation, and the hydrophilic segment is oriented towards the aqueous continuous phase (Chen et al., 2002). The hydrophilic stabilizer on the surface inhibits crystallization of the growing particles and therefore facilitates dissolution rates.

1.4. Complexation

Cyclodextrins and their derivatives have been employed as complexing agents to increase water solubility, dissolution rate and bioavailability of lipophilic drugs for oral or parenteral delivery (Koester et al., 2004; Choi et al., 2003; Sridevi et al., 2003). The solubility enhancement factors of pancratistatin, hydrocortisone, and paclitaxel are 7.5, 72.7 and 99000 by forming complexes with cyclodextrin derivatives (Loftsson and Brewster, 1996). The lower the aqueous solubility of the pure drug, the greater the relative solubility enhancement obtained through cyclodextrin complexation. Pharmaceutical applications of cyclodextins in drug solubilization and stabilization (Loftsson and Brewster, 1996), in vivo drug delivery (Rajewski et al., 1996), toxicological issues and safety evaluation (Irie and Uekama, 1997) and mechanisms of cyclodextrins modifying drug release from polymeric drug delivery systems (Bibby et al., 2000) have been previously reviewed. There are at least 23 commercially available cyclodextrin-based pharmaceutical products on the market and more cyclodextrin-based products awaiting regulatory approval (Table 2) (CD Cydex, 2003).

Table 2: Cyclodextrin based commercially available products.

cyclodextrin/Drug	Route of dosing	Market	Trade names
SE7-β-CD (CAPTISOL)			
Zipradidone	IM	Europe, USA	Zeldox, Geodon
Voriconazole	IV	Europe, USA	Vfend
α-CD			
PG1, Alprostadil	IV	Europe, Japan, USA	Prostanding, Prostavasin, Edex
OP-1206	Oral	Japan	Opalmon
Cefotiam hexetil HCl	Oral	Japan	Pansporin T
β-CD			
Piroxicam	Oral, Rectal	Europe	Brexin, Cycladol, Brexidol
PGE2	Buccal	Japan	Prostarmon, E
Benexate	Oral	Japan	Ulgut, Lonmiel
Iodine	Topical	Japan	Mena-Galgle
Dexamethasone Glyteer	Dermal	Japan	Glymesason
Nitroglycerin	Buccal	Japan	Nitropen
Nimesulide	Oral	Europe	Nimedex, Mesulid Fast
Tiaprofenic acid	Oral	Europe	Surgamyl
Omeprazole	Oral	Europe	Ombeta
ME 1207 Cephalosporin	Oral	Japan	Meiact
γ-CD (None)			
HP-β-CD (Encapsin)			
Itraconazole	Oral, IV	Europe, USA	Sporanox
Cisapride	Rectal	Europe	Prepusid
Mitomycin	IV	USA	Mitozytrex

Cyclodextrins are a group of cyclic oligosaccharides obtained from enzymatic degradation of starch. The three major cylcodextins α-, β-, and γ- (CD) are composed of six, seven, and eight D-(+)-glucopyranose units. These agents have a torus structure with primary and secondary hydroxyl groups orientated outwards. Consequently, cyclodextrins have a hydrophilic exterior and a hydrophobic internal cavity. This cavity enables cyclodextrins to complex 'guest' drug molecules and hence alters the properties of the drugs such as solubility, stability, bioavailability and toxicity profiles (Szejtli, 1988, 1990; Albers and Müller, 1995; Loftsson and Brewster, 1996; Rajewski and Stella, 1996; Thompson, 1997). The forces driving complexation were attributed to (i) the exclusion of high energy water from the cavity, (ii) the release of ring strain particularly in the case of α-CD, (iii) van der Waals interactions, and (iv) hydrogen and hydrophobic bindings (van Helden, 1992; Ross and Rekharsky, 1996). β-CD, the most widely used native cyclodextrins, is limited in its pharmaceutical application by its low aqueous solubility (1.85 g/100 ml, 25°C), toxicity profile and low aqueous solubility of

the formed complexes. Accordingly, derivatives such as hydroxypropyl-β-CD (HP-β-CD; Enapsin®) and sulphobutylether-β-CD (SE-β-CD; Captisol®) have been developed to produce more water-soluble and less toxic entities.

1.5. Solid solutions/dispersions

Solid dispersion was firstly introduced to overcome the low bioavailability of lipophilic drugs by forming of eutectic mixtures of drugs with water-soluble carriers (Sekiguchi and Obi, 1961). It was defined as the dispersion of one or more active ingredients in an inert carrier matrix in solid-state prepared by melting (fusion), solvent or melting-solvent method (Chiou and Riegelman, 1971). More than 500 papers have been published on the subject and various materials are employed as drug carriers (Leuner and Dressman, 2000). Despite an active research interest, the number of marketed products arising from this approach is disappointing mainly caused by the physical and chemical instability and scale-up problems (Franco et al., 2001; Serajuddin, 1999; Craig, 2002). Only two commercial products, a griseofulvin in polyethylene glycol 8000 solid dispersion (Gris-PEG, Novartis) and a nabilone in povidone solid dispersion (Cesamet, Lilly) were marketed during the last four decades following the initial work of Sekiguchi and Obi.

1.5.1. Production methods

Solid solutions/dispersions are generally produced either by a solvent method, whereby the drug and carrier are dissolved in a common solvent and then the solvent is evaporated under vacuum (coevaporate), freeze-drying (Betageri and Makarla, 1995), spray-drying (Lo and Law, 1996) and spray–freezing into liquid (Rogers et al., 2002; Hu et al., 2002); or by a melting method, whereby drug-carrier mixtures are co-melted and cooled. An important prerequisite to manufacture solid solutions/dispersions by the hot melt method are the miscibility of the drugs and the carriers in the melt forms. Another limitation to the melt method is the thermo-instability of the drugs and carriers. However, with the development of new techniques such as hot melt extrusion (Hülsmann et al., 2000) and hot spin melting (Dittgen et al., 1995), the second limitation associated with the melting method was partially solved. For solvent-based methods, the ecological and subsequent economic problems associated with the use of toxic organic solvents became more and more problematic. Therefore, hot melt extrusion is the current method of choice for preparation of solid dispersions. Briefly, the blend of drug and carrier is processed with a twin-screw extruder of the same type used in the polymer

industry. The blend is simultaneously melted, homogenized and then extruded and shaped as tablets, granules, pellets, sheets, sticks or powder. An important advantage of the hot melt extrusion method is that the blend is only subjected to an elevated temperature for about 1 min, which enables drugs or carriers that are thermolabile to be processed.

1.5.2. Carriers

Many water soluble excipients were employed as carriers of solid solutions/dispersions. Among them, polyethylene glycols (PEG, Mw 1500-20000) were the most commonly used due to their good solubility in water and in many organic solvents, low melting points (under 65°C), ability to solubilize some compounds and improvement of compound wettability. The marketed Gris-PEG is the solid dispersion of griseofulvin in PEG 8000. The others carriers include polyvinyl pyrrolidone (PVP), polyvinylalcohol (PVA), polyvinylpyrrolidone polyvinylacetate copolymer (PVP-PVA), hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), urea, Poloxamer 407, sugars, emulsifiers (SDS, Tween 80) and organic acids (succinic acid and citric acid). Because of the rapid dissolution of the water-soluble carriers than the drugs, drug-rich layers were formed over the surfaces of dissolving plugs, which prevented further dissolution of drug from solid dispersions. Therefore, surface-active or self-emulsifying agents including bile salts, lecithin, lipid mixtures, Gelucire 44/14 (Hülsmann et al., 2000) and Vitamin E TPGS NF (Khoo et al., 2000) were used as additional additives, acting as dispersing or emulsifying carriers for the liberated drug to prevent the formation of any water-insoluble surface layer. In addition, the release behaviors of many drugs are also improved by using water-insoluble polymers such as crospovidone (Hirasawa et al., 2003; 2004) and enteric polymers such as hydroxypropyl methylcellulose phthalate (HPMCP), cellulose acetate phthalate (CAP), Eudragit® L100 and S100 (Takada et al., 1989) and Eudragit® E (Horisawa et al, 2000; Jung et al., 1999).

1.5.3. Challenges

Although a great interest in solid dispersion in the past four decades, the commercial utilization is very limited. Problems of solid dispersion involve (i) method of preparation, (ii) reproducibility of its physicochemical properties, (iii) formulation into dosage forms, (iv) scale-up of manufacturing processes and (v) physical and chemical stability of drugs and vehicles.

Method of preparation. High melting temperature may chemically decompose drugs and carriers.

No report addresses how much residual solvent is present in solid dispersions when different solvents, carriers or drying techniques are used.

Reproducibility of physicochemical properties. Various investigators observe that heating rate, maximum temperature used, holding time at a high temperature, cooling method and rate, method of pulverization and particle size distribution may influence the properties of solid dispersions prepared by the melting method. In addition, the nature of solvent used, ratios of drug/solvent or carrier/solvent, solvent evaporation method and rate may significantly affect the physicochemical properties of solid dispersions formed.

Dosage form development. Very few reports address the difficulty of pulverization and sieving of the solid dispersion, which are usually soft and tacky, poor flow and mixing properties. Thus, poor compressibility, drug-carrier incompatibility and poor stability of the related dosage forms are resulted.

Scale-up of manufacturing processes. Most of solid dispersions reported in literatures are prepared in lab-scale. The scale-up of the preparation methods can be very challenging. The physicochemical properties and stability of solid dispersions may be affected by scale-up because heating and cooling rates of solid dispersion in large-scale differ from small-scale. It is also not practical and high-cost to evaporate hundreds and even thousands of liters of organic solvents to prepare solid dispersion for kilogram quantities of drugs. Removal of residual toxic organic solvent may be difficult because the solid dispersions are usually amorphous and may exist in viscous and waxy forms.

Stability. The physical instability of solid dispersions due to crystallization of amorphous drugs is the subject of most published reports (Khalil and Mortada, 1978; Vila Jato, et al., 1984). In a solid dispersion prepared by the melt method, a certain fraction of the drug may remain molecularly dispersed depending on its solubility in the carrier. The excess drug existing may greatly depend on the manufacture method. It may form a supersaturated solution, separate out as an amorphous phase or crystallize out. The supersaturated and amorphous forms may, in turn, crystallize out on aging. Certain carriers may also exist in thermodynamically unstable states in solid dispersions and undergo changes with time. As reported, polyvinyl pyrrolidone acts as stabilizer in the solid dispersion by retarding crystallization of drug at a low humidity. Hydrogen

bonds between the drug and PVP restrain drug crystallization (Taylor and Zografi, 1997).

1.6. Enteric microparticles

As the use of enteric polymer to prepare microparticles is the major focus of the present work, an overview on the current state of the art is given a special section (1.6)

1.6.1. Enteric polymers

Enteric polymers are mainly applied as enteric-coatings to conventional solid dosage forms such as tablets, capsules or pellets, which are designed to be insoluble in gastric fluid and soluble in intestinal fluid (Table 3). They consist of a long-chain polymer with ionizable carboxyl groups. In low-pH environment, the acidic groups are protonized and the polymer is lipophilic. A dramatic change in pH occurs when the dosage form is emptied into the duodenum. Neutralization of the acidic groups and, therefore, increased solubility occurs in the intestinal tract as shown in the simplified equation:

$$R\text{-}COOH + OH^- \leftrightarrow R\text{-}COO^- + H_2O$$

Table 3: Range of pH values in the gastrointestinal tract.

Area	pH
Stomach	2-5
Duodenum	6.0
Jejunum	6.5
Ileum	7.0
Colon	6.5-7.0

(Lehmann, 2001)

Enteric polymers should have an apparent pKa between 4 and 6. Based on Henderson-Hasselbach equation:

$pH - pK_a = \log$ [concentration (ionized)/concentration (nonionized)]

When the pH exceeds the pKa by two units, the percentage of ionized acid groups approaches 99%. Ionization causes charge repulsion within the polymer leading to stretching of the polymer chain, water penetration into the dosage form and disintegration in the intestines. The dissolution of the enteric coatings depends on the intrinsic solubility and pKa of the polymer and medium properties (Ozturk et al., 1988).

Advantages of the enteric-coating of a drug product include (i) protecting the gastric mucosa from drug irritation, (ii) preventing drug degradation in the stomach by

enzymes or acidic fluids, (iii) delivering the drug rapidly to a particular region such as the upper part of the small intestine or colon, (iv) delivering proteins and therapeutic peptides (Chourasia and Jain, 2003) and (v) providing sustained release of the drug by forming a matrix tablet, or in a wet granulation process to form particulates or beads which exhibit controlled-release characteristics in GIT. For example, modified release of theophylline matrix tablets are produced by compressing material from spray-dried theophylline and enteric polymers (e.g., cellulose acetate phthalate). Both enteric release and sustained release can be achieved. The addition of fumaric acid further slows down the drug release in buffer (McGinity, 1997).

A variety of enteric polymers are available and threshold pH of the polymers is shown in Table 4.

Table 4: Threshold pH of commonly used enteric polymers.

Polymer	Threshold pH	$M_w \times 10^{-3}$	$M_n \times 10^{-3}$	M_w/M_n	Manufacture
Eudragit® L100	6.0	135			Röhm
Eudragit® S100	7.0	135			Röhm
Eudragit® L100-55	5.5	250			Röhm
Eudragit FS 30D	7.0	220			Röhm
PVAP	5.0				Colorcon
HPMCP: HP-50	5.0	78	24	3.3	Shin-Etsu
HP-55	5.5	84	21	4.1	Shin-Etsu
HP-55S	5.5	132	36	4.0	Shin-Etsu
HPMCAS: AS-L	5.5	93	46	2.0	Shin-Etsu
AS-M	6.0	80	44	1.8	Shin-Etsu
AS-H	6.8	55	33	1.7	Shin-Etsu
CAT	5.0				Eastman
CAP	6.2				Eastman

1.6.2. Enteric polymers as carriers for lipophilic drugs

Enteric polymers have been successfully used to increase the dissolution of lipophilic drugs (griseofulvin and spironolactone) (Hasegawa et al., 1985). The drugs are either in crystalline state (CoQ_{10}) (Nazzal et al., 2002) or non-crystalline state in solid dispersions [HIV-1 protease inhibitors (CGP70726, CGP57813), lipophilic compound (RRR01)] (De Jaeghere et al., 2000; 2001; Leroux et al., 1995), and exhibits enhanced absorption than aqueous suspensions. The improvement of bioavailability might be due to the combination of following properties including: (i) the high specific surface area of the release system, (ii) the promotion of dissolution rate of the drug in a non-crystalline state, and (iii) the rapid release of the drug close to its expected absorption window (Leroux et al., 1995; 1996). The difference of bioavailability

between nanoparticles and microparticles is not due to the size effect (De Jaeghere et al., 2000) but the enhanced wettability because of residual polyvinyl alcohol (PVA) on the surface of nanoparticles (De Jaeghere et al., 2001).

Drugs. Currently, the lipophilic drugs have been processed using enteric polymers as carriers including griseofulvin and spironolactone (Hasegawa et al., 1985), coenzyme Q_{10} (Nazzal et al., 2002), naproxen (Zaghloul et al., 2001A; 2001B), erythromycin (Morishita et al., 1991), cyclosporine A (Takada et al., 1989), ibuprofen (Kislalioglu et al., 1991) and HIV-1 protease inhibitors (Leroux et al., 1995; 1996; De Jaeghere et al., 2000).

Polymers. Eudragit® L100-55 is the most commonly used carrier for poorly water soluble drugs (Nazzal et al., 2002; Zaghloul et al., 2001A; 2001B; Kislalioglu et al., 1991; Leroux et al., 1995; De Jaeghere et al., 2000; 2001). Other enteric polymers include Eudragit® L100, Eudragit® S100, HPMCP HP-55 and cellulose acetate phthalate (CAP) (Takada et al., 1989; Kislalioglu et al., 1991; Leroux et al., 1995).

Methods. Various methods for the preparation of solid dispersion or micro-/nanoparticles based on enteric polymers have been developed including solvent evaporation (Nazzal et al., 2002), coprecipitation (Zaghloul et al., 2001A; 2001B; Kislalioglu et al., 1991), emulsification-evaporation (Lee et al., 1999), emulsification-diffusion (De Jaeghere, et al., 2000; 2001), and salting-out method (Leroux et al., 1995; 1996). Each approach has its benefits and drawbacks (Galindo-Rodriguez, 2004).

Solvent evaporation is a common method to prepare solid solutions/dispersions by dissolving drug and carrier in a solvent and then evaporating the solvent. The resultant solid mass is ground and sieved. Scale-up and physical and chemical instability are major problems. The limitations of this method have been reviewed previously (Serajuddin, 1999).

Coprecipitation has been studied extensively as a means of increasing the dissolution of lipophilic drugs such as griseofulvin, ketoprofen, sulphathiazide, spirinolactone, tolbutamide and nifedipine (Lerk, 1989). Nanoprecipitates are prepared by transferring a solution of drug/polymer in a water-miscible solvent into an aqueous solution containing a stabilizer. The coprecipitates are formed instantaneously by rapid solvent diffusion. The use of polymer solution with low concentrations is necessary to obtain small particles and avoid large aggregates (Fessi et al., 1989).

In the emulsification-evaporation method, a drug/polymer solution in a water-immiscible solvent (e.g. dichloromethane, chloroform) is emulsified into an aqueous

solution containing an emulsifier. The subsequent evaporation of the solvent from the O/W emulsion results in the formation of micro-/nanoparticles. The emulsification-diffusion method is similar to the emulsification-evaporation method, but uses a partially water-soluble solvent (e.g. benzyl alcohol). A large amount of water is needed to induce the diffusion of the solvent from the O/W emulsion to form micro-/nanoparticles (De Jaeghere et al., 2000).

In the salting-out process, an organic solution of polymer and drug is emulsified into an aqueous phase containing an electrolyte (e.g., $MgCl_2$) and a stabilizer (e.g., polyvinyl alcohol). Sufficient water is subsequently added to the O/W emulsion to induce the diffusion of the organic solvent, leading to polymer precipitation and formation of micro-/nanoparticles. A complicated purification stage is necessary to eliminate the high amounts of emulsifying agent and electrolyte (Leroux et al., 1996; Galindo-Rodriguez et al., 2004).

Spray drying of drug-polymer solutions is another alternative to prepare microparticles in order to improve the dissolution rate and oral bioavailability of lipophilic drugs (Dollo et al., 2003; Paradkar et al., 2004 ; De Jaeghere, et al., 2000; 2001).

2. In-situ forming drug delivery systems

2.1. Parenteral in-situ forming systems

The preparation of biodegradable implants and microparticles is complicated and involves multiple step processes and formulation parameters to be controlled (Jalil and Nixon, 1990). Additional issues are scale-up and costs. As an alternative to solid implant and microparticles, in-situ forming systerms have been developed. The systems have the following advantages: (i) ease of administration and (ii) less complicated fabrication and less stressful manufacturing conditions for sensitive drug molecules. Operating expenses for the production of in-situ forming application are marginal, thus lowering investment and manufacturing costs. Various strategies have been developed to prepare in-situ forming parenteral drug depots, such as thermoplastic pastes, in-situ cross-linking implant, in-situ polymer precipitation and thermally induced gelling systems,. The potential benefits and drawbacks of each method have been reviewed (Packhaeuser et al., 2004; Hatefi and Amsden, 2002).

Thermoplastic pastes. Drugs are incorporated into a molten polymer by mixing without the application of solvents. This semisolid mixture can be injected as a melt and

form a depot upon cooling to body temperature. The requirement for the polymer includes low melting temperature (25-65°C) and low intrinsic viscosity (0.05-0.8 dl/g). Poly(ortho esters) (AP Pharma) have emerged as a polymer class for thermoplastic pastes due to their (i) low melting temperature (25-45°C), (ii) surface erosion, (iii) good biocompatibility, (iv) avoidance of organic solvents in the formulations and (v) promising in the context of periodontal treatment (Schwach-Abdellaoui et al., 2001; 2002).

In-situ cross-linked polymer systems. DepoGel™, poly(ethylene glycol) based copolymer containing multiple thiol (-SH) groups cross-linked by vinylsufones, contains more than 90% water, shows good biocompatibility and release properties without initial drug burst. However, there is little data available as of yet and the non-degradability of the polymer represents a drawback.

Thermally induced gelation. ReGel® drug delivery system is composed of proprietary thermosensitive, biodegradable polymers/hydrogels which are solutions at administration temperature and become insoluble gels at the injection site. Briefly, drug and triblock copolymer (PLGA-PEG-PLGA) are dissolved in pH 7.4 phosphate buffer. This formulation is easily administered through small-gauge needles or needle-free injectors. The system is completely biocompatible and biodegradable and degrades into products which are well known to be easily metabolized and cleared. Drug release is controlled through a combination of diffusion and degradation of the polymer. OncoGel™ is a novel formulation of paclitaxel utilizing ReGel®, MacroMed's proprietary drug delivery system by dissolving 23% (w/w) ABA-triblock copolymer in pH 7.4 phosphate buffer. It contains paclitaxel (6 mg/g) for intratumoral injection and provides a continuous drug release over 6 weeks. For protein candidates, some initial protein release is difficult to avoid. Further investigations with regard to the stability of proteins in the aqueous polymer solutions, the shelf-life of the formulations and in vivo study are ongoing.

In the following sections, special emphasis would be placed on the in-situ polymer precipitation technique by using PLGA as the carrier.

2.1.1 In-situ forming implant (ISI)

The disadvantages of preformed biodegradable microparticles include (i) the need for reconstitution before injection, (ii) the inability to remove the dose once injected and (iii) relatively complicated manufacturing procedures to produce a sterile, stable and

reproducible product. Therefore, in-situ forming implant was introduced by Dunn and co-workers (Dunn et al., 1990) and receives considerable attention over the past years, especially by using PLGA as carriers (Tipton and Fujita, 1991; Shah et al., 1993; Ravivarapu et al., 2000; Brodbeck et al., 1999; Wang et al., 2003; Graham et al., 1999). The in-situ implants are formed from drug-containing PLGA in a biocompatible solvent. The polymer solutions form implants after s.c. or i.m. injection and contact with aqueous body fluids through precipitation of the polymer. The advantages these delivery systems include ease of manufacture and application, localized delivery for a sit-specific action, prolonged drug action, improved patient compliance and comfort and compatibility with biological systems. A series of patents were generated at Atrix Laboratories (Dunn et al., 1991; 1994; 2002; 2003) and similar systems are also developed by Merck and Alza (Chern et al., 2004; Brodbeck et al., 2000; 2004). Until now, two commercial products (Atridox® and Eligard®) are developed by Atrix by using this technique.

To control the burst effect, a few factors have been examined: polymer molecular weight (Lambert and Peck, 1995), polymer concentration (Radomsky et al., 1993), type of solvent (Lambert and Peck, 1995) and solvent mixture (Brodbeck et al., 1998; 2000; 2004), and the presence of other excipients (e.g. mannitol, surfactant or oil) (Shah et al., 1993). Among these factors, solvent type and polymer concentration are the critical determinants to modulate drug release.

To meet the requirements of parenteral application, the solvents have to be biocompatible and non-toxic. Meanwhile, the polymers should show good solubility and stability in the solvents. The viscosity of the resulting solution must be low enough to allow application through needles. Thus the search for suitable organic solvents for parenteral use has become a key issue in the development of in-situ forming implant. A broad range of solvents has been suggested, of these NMP (*N*-methyl-2-pyrrolidone) (Ravivarapu et al., 2000; Brodbeck et al., 1999; Lambert and Peck, 1995), DMSO (Dimethyl sulfoxide) (Lambert and Peck, 1995), 2-pyrrolidone (Chandrashekar et al., 1996), glycofurol (Eliaz et al., 2001), triacetin (Brodbeck et al., 1998; 1999; 2000; 2004; Shah et al., 1993), triethyl citrate (TEC) (Shah et al., 1993; Brodbeck et al., 1998), ethyl benzoate and benzyl benzoate (Brodbeck et al., 1999; Wang et al., 2003), PEG 400 (Jain et al, 2000) and glycerol formal (Chern and Zingerman, 2004) were the most thoroughly investigated. NMP, DMSO, PEG 400, and glycofurol are water-soluble solvents and have been successfully used in commercially injectable products

(Strickley, 2004; Kibb et al., 1994). 2-pyrrolidone is a versatile drug solubilizer widely used in veterinary injectables with the maximum dose of 40 mg/kg (EMEA, 1998). Glycerol formal is also used in a veterinary parenteral product in the market (Ivomec S 0.27%^{TM}) for subcutaneous injection in young pigs.

Due to the low water miscibility of triacetin (7%), TEC (7%) and benzyl benzoate (insol. in water), a single or a blend with a water miscible solvent could be used to dissolve polymer to minimize the initial release and conveniently modify the duration of drug release. For example, in the case of PLGA and human growth hormone, benzyl benzoate may provide one month or longer release than ethyl benzoate (one week) (Brodbeck et al., 2000). Moreover, benzyl benzoate is thought to be less irritating. Triacetin and triethyl citrate are generally applied in oral pharmaceutical formulations and or food additives generally recognized as safe (FDA´s GRAS list). Triacetin is also considered as a potentially parenteral nutrient (Bailey, et al., 1991). Ethyl acetate is an ICH Class 3 solvent (FDA Guidance for Industry Q3C Impurities: Residual Solvents) and FDA´s GRAS listed regarded as less toxic and of lower risk to human health.

2.1.2. In-situ forming microparticle (ISM)

Disadvantages associated with in-situ forming implant need to be overcome such as high injection force, local irritation at the injection site, variability in the rates of solidification, irregular shape of the implants formed depending on the cavity into which the formulation is introduced, undesirable high initial bursts of the drug and the potential solvent toxicity. As an alternative to in-situ implant, a novel in-situ forming microparticle system (ISM) has been developed by Bodmeier (Bodmeier, 1997; Kranz and Bodmeier, 1998) and several patents emerged (Bodmeier Roland ‘Multiphasensystem’, WO 98/55100 A1; EP 996426 A1; DE 19724784 A1). These ISM-systems consist of an internal phase (drug-containing polymer solutions or suspensions) and a continuous phase (aqueous solutions with a surfactant, oil phase with viscosity enhancer and emulsifier). The two phases are separately stored in dual-chambered syringes and mixed through a connector before administration. The excipients used in these systems, such as polymer (PLGA, PLA), oil (peanut oil), solvent (NMP, DMSO, PEG 400), viscosity enhancer (aluminum monostearate) and emulsifier (Span 80), have been approved for parenteral administration. The initial release, myotoxicity and viscosity are significantly reduced in the presence of the oil

phase (Kranz et al., 2001). These lead to the ISM as an attractive alternative for parenteral controlled drug delivery systems.

A similar ISM system comprised of a dispersion of an organic solution of PLGA and drug in a continuous oil phase is also developed (Jain et al., 2000A; 2000B). PLGA is firstly dissolved in triacetin by heating at 65°C and a solution of drug and PEG 400 is added, followed by an addition of Tween 80. This mixture (oil phase I) is added to a mixture of Miglyol 812 and Span 80 (oil phase II) dropwise with continuous homogenization to form the premicrospheres (microglobules). Upon injection into the body, water penetrates into the system while the solvent diffuses out leading to hardening of the microglobules into solid microspheres. The limitations of this system include poor drug loading (< 0.02%, w/w), high burst effect, uncertainty of long-term formulation stability and drug stability. In addition, accelerating dissolution of PLGA in triacetin by heating up to 65°C may destabilize the polymer.

Based on the above concepts and investigations, a ready-to use gelled polymer O/O dispersion is developed and used potentially for other administration routes (Bhagwatwar et al., 2003). The process for making such a dispersion comprises the steps of (i) dissolving a polymer and/or drug in a biocompatible solvent at an elevated temperature to form a drug/polymer solution, (ii) preparing a second oil phase solution of a biocompatible oil (sesame oil) and a biocompatible emulsifier (sorbitan monostearate, sorbitan monopalmitate) at an elevated temperature, (iii) mixing the polymer solution with oil phase solution at an elevated temperature and subsequently cooling to refrigeration temperature. The solidification of sorbitan monostearate or sorbitan monopalmitate leads to a gel-like emulsion at a low temperature. Sorbitan monopalmitate has been approved for intramuscular injection (FDA, CDER, inactive ingredient guide, 1996, pp. 139), however sorbitan monostearate is not. The biodegradable thermalsensitive polymers (PLGA) and fragile drugs may undergo degradation due to the stress conditions such as elevated temperature and high speed homogenization. Meanwhile, incorporation of drug in polymer solutions may potentially accelerate the drug or polymer (PLGA) degradations, this is probably the reason that dual-syringe system has been adopted for Eligard® and Atridox® by Atrix Laboratories. At last, long-term physical stability of this system is not provided.

Most of the drugs incorporated in ISI and ISM are soluble in the solvent or cosolvent as previously reported. It is a challenge to incorporate solvent-insoluble drugs in ISI or ISM. The particle size of drug plays an important role on the initial burst and

following drug release. Furthermore, the long-term stabilities of drugs and polymers in ISM need to be investigated.

2.2. Oral in-situ forming systems

2.2.1. Conventional oral micro-/nanoparticles

Micro-/nanoparticles have been extensively investigated to entrap macromolecule drugs (such as peptides, proteins, vaccines, and polysaccharides) and lipophilic drugs to promote their oral bioavailability. For lipophilic drugs, water soluble polymers, water insoluble polymers or enteric polymers are used as carriers (Rogers et al., 2002; Jaeghere et al., 2000). Great interests have been put on the encapsulation of vaccine antigens in polymeric carriers (Andrianov and Payne, 1998) especially using biodegradable microparticles (poly (lactide-co-glycolide)) for induction of mucosal and systemic immune responses by oral immunization (Maloy et al., 1994; Desai et al., 1996; McClean et al., 1998).

The mechanism of uptake of microparticles is particle size dependent (Desai et al., 1997). In general, microparticles less than 5 μm in diameter are more efficiently taken up by M cells. The efficiency of uptake of 100 nm size particles by the rat intestinal tissue is 15-250 folds higher compared to larger size microparticles (1 and 10 μm) (Desai et al., 1996). Peyer's patches are the predominant sites for particle uptake and have 2-200 folds higher uptake of particles than the non-patch tissue. Histological evaluation of the tissue sections demonstrates that 100 nm particles diffused throughout the submucosal layers while the larger size micro-/nanoparticles are predominantly localized in the epithelial lining of the tissue. After taken up by antigen-presenting cells (M cells) in Peyer's patches, the microparticles slowly degrade in vivo and release entrapped antigens. Therefore, microparticles have considerable potential as a controlled release antigen delivery system for the induction of long-term immune responses at mucosal surfaces (Challacombe et al., 1992). However, the affinity of PLA particles for intestinal epithelia and gut associated lymphoid tissue (GALT) needs to be enhanced to improve oral bioavailability of macromolecules (McClean et al., 1998).

Besides the effect of particle size, the physicochemical surface properties of microparticles are also crucial to their uptake by M cells. The important surface properties are the ionic state (positive or negative), charge density and hydrophobic/hydrophilic balance. These can determine the efficiency of interaction with the M cells and the translocation of the microparticles (Andrianov and Payne, 1998).

2.2.2. Oral in-situ forming gel

In-situ gelling formulations as vehicles for oral sustained drug delivery have been developed and evaluated in vitro and in vivo by using derivatives from natural origins such as enzyme-degraded xyloglucan, gellan gum, sodium alginate, pectin (Miyazaki et al., 1999; 2001; 2003; Kubo et al., 2003; 2004; Kawaski et al., 1999) and chitosan-glyceryl monooleate (GMO) (Ganguly and Dash, 2004).

Xyloglucan, a polysaccharide derived from tamarind seeds, is composed of a (1-4)-β-D-glucan backbone chain which has (1-6)-α-D-xylose branches that are partially substituted by (1-2)-β-D-galactoxylose. The resultant product (44% of galactose removal) exhibits thermally reversible gelation between 22°C to 27°C in diluted aqueous solution (1-2%, w/w). After oral administration of a chilled solution containing drug and 1% xyloglucan, a constant drug plasma concentration is noted and maintained over a period of 6-7 h. It has been demonstrated that similar (paracetamol, cimetidine) or 3-fold (indomethacin) in vivo bioavailability as commercial suspensions are obtained with the thermally reversible xyloglucan gels as vehicles for oral drug delivery (Miyazaki et al., 2003; Kawasaki et al., 1999). Xyloglucan gels are widely applied in drug delivery since gelation of the solutions of this polysaccharide does not require the presence of H^+ ions as in gellan and alginate formulations. Moreover, their utilization is not restricted by the nature of the drug. Xyloglucan is non-toxic and has an advantage over other synthetic in-situ gelling materials such as poloxamers (gelation occurring at 15-25% w/v), of gelation at much lower concentration (1-2%, w/v).

Gellan gum is an anionic deacetylated exocellular polysaccharide secreted by pseudomonas elodea, with a tetrasaccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucose residues. It has the characteristic property of temperature-dependent and cation-induced gelation. This gelation involves the formation of double helical junction zones followed by aggregation of the double-helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water. The formulation adopted was a gellan solution containing calcium chloride (as a source of Ca^{2+}), and sodium citrate, which complexes the free Ca^{2+} ions and releases them only in the acidic environment of the stomach. In this way, the formulation remains in liquid form until it reaches the stomach, where gelation of gellan gum is instantaneous. The bioavailability of theophilline from the gellan gels increases 4-5 folds in rats and 3 folds in rabbits compared to a commercial sustained release liquid dosage form (Miyazaki et al., 1999).

Sodium alginate is widely used in pharmaceutical formulation. Gelation of dilute solutions of sodium alginate occurs on addition of di- and trivalent metal ions by a co-operative process involving consecutive guluronic residues in the α-L-guluronic acid blocks of the alginate chain. Two strategies for ensuring gelation of alginate formulations, one is achieved by the separated oral administration of a calcium salt solution immediately following that of the sodium alginate solution (Katayama et al., 1999), the other involves a supply of complexed calcium ions that are released in the acidic environment of the stomach similar to that described above for the gellan gum solutions (Kubo et al., 2003; Miyazaki et al., 2001).

Pectins are family of polysaccharides in which the polymer backbone mainly comprises α-(1→4)-D-galacturonic acid residues. Low methoxy pectins readily form gels in aqueous solution in the presence of free calcium ions, which cross-link the galacturonic acid chains in a manner described by the 'egg-box' model (Dumitriu et al., 1996). The procedure to achieve gelation is similar to gellan and sodium alginate outlined above.

The SABER® delivery system is a potential parenteral delivery platform that can successfully deliver therapeutic levels of a wide spectrum of drugs from a few days to months from a single injection. This delivery system is a three or four component technology consisting of sucrose acetate isobutyrate (SAIB), a pharmaceutically acceptable solvent and one or more additives (Franklin et al., 2002). SAIB is a very hydrophobic, fully esterified sucrose derivative with a nominal ratio of six isobutyrates to two acetates, which exists as a very viscous liquid. The drug to be delivered utilizing the SABER® system is dissolved or dispersed in the SAIB/solvent solution for subsequent injection subcutaneously or intramuscularly. Upon injection, the SABER® system forms a high viscosity depot from which the drug is slowly delivered. However, this system has the potential to be used for sustained release of orally administered active by incorporation into soft gelatin capsules (Carraway et al., 1999; Sullivan et al., 1998).

2.2.3. Oral in-situ forming microparticles

As described in section 2.1.2, most of the developed in-situ forming microparticle systems are for parenteral administration. The system developed (Bodmeier, 1997; Kranz and Bodmeier, 1998) may be not suitable for oral administration because the emulsion has to be prepared shortly before administration. The other system developed

by Jain (Jain et al., 2000a; 2000b) has the limitations of low drug loading, high burst and instability on long-term storage. Although Bhagwatwar claims that a ready-for-injection, stable, gelled polymer droplet-in-oil dispersion has developed and stabilized by incorporating sorbitan monostearate or sorbitan monopalmitate in external oil phases, the further details are not provided such as particle size and size distribution, aggregation of resultant particles and particle surface properties (Bhagwatwar et al., 2003).

A successful in-situ forming microparticle system for oral administration should possess the following profiles:

- Stable emulsion in semisolid state at room temperature.
- Immediate dispersing of external oil phase in gastric fluid to obtain small droplets or microparticles.
- Low initial release.
- Small size (< 5μm) and proper physicochemical surface, such as charge properties, charge density, hydrophobic/hydrophilic balance and bioadhesive property.

2.3. Heparin oral delivery systems

Heparin, a polydispersed glycosaminoglycan, is a powerful anticoagulant indicated for the prevention of deep venous thrombosis and pulmonary embolism in high-risk patients. Currently, heparin is administered parenterally because its oral bioavailability is negligible mainly due to its big molecular size (Mw avg. = 15 kDa) and highly negative charge (Fig.1.1).

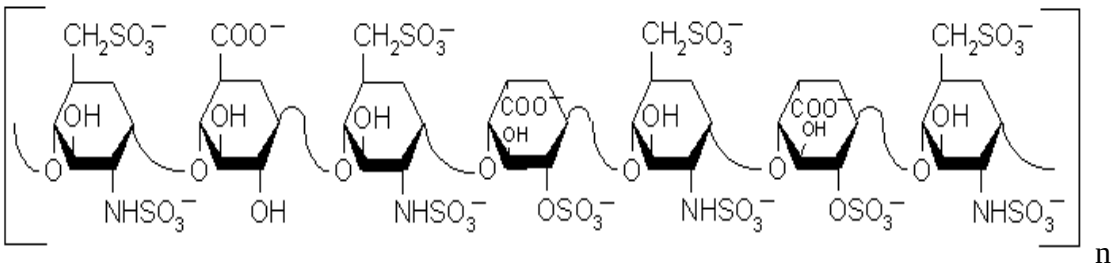

Figure 1.1 Structure of heparin molecule.

The development of oral formulation of heparin is highly desirable, not only to improve patient convenience and compliance, but also allow for other clinical indications. Recent studies have shown that sodium N-[8-(2-hydroxybenzoyl)amino]

caprylate (SNAC) and other N-acylated amino acids developed by Emisphere Technologies enable oral heparin absorption, which is in phase III clinical trials (Malkov et al., 2002; Leone-Bay et al., 1998; Pineo et al., 2001). SNAC interacts with heparin forming more lipophilic non-covalent complex, which potentially diffuses passively across cell membranes and cross the intestinal epithelium via the transcellular pathway, dissociates later upon dilution in the bloodstream. SNAC appears non-toxic, but causes nausea at high doses in humans due to bad tasting. It is also not convenient for patients to drink 15 ml solution containing 2.25 g SNAC every 8 hours. Thereby, a solid dosage form is developed using sodium-N-amino decanoate (SNAD) as carrier with an enhanced oral bioavailability to 38% relative to the subcutaneous injection. The studies conducted have shown SNAD to be four times more efficient than SNAC for oral heparin delivery (Rogers and Eroschenko-Styer, 2001). It is obvious to note that SNAC and SNAD are good delivery agents to promote the oral absorption of heparin. However, the biological activity does not last much longer than heparin administered parenterally (Baughman et al., 1998).

Other works describe as well the absorption of unfractionated heparin (UH) and low molecular weight heparin (LMWH) in GIT by using polymeric permeation enhancer (Thanou M., et al., 2001A; 2001B), bile acids or salts (Ziv et al., 1983) and forming conjugates (Lee et al., 2000; 2001). Co-administration of LMWH with Carbopol® 934P (poly(acrylate) derivative) results in enhanced anti-Xa levels, which are sustained for 6 h after delivery. The mechanism of the absorption enhancement is probably a combination of the mucoadhesive properties of Carbopol® 934P and its influence on intercellular tight junctions to allow for paracellular drug permeation (Thanou et al., 2001). Complexes of heparin with either spermine or lysine (Morton et al., 1981), bile acids alone (Guarini and Ferrari, 1985), monoolein (Taniguchi et al., 1980), or monoolein mixed micelles combined with sodium glycocholate and/or taurocholate (Muranishi et al., 1977) are formulated and administered either orally or intraduodenally in rats. Among these formulations, both monoolein mixed micelles and bile salts demonstrate an enhancement on the intestinal absorption of heparin. Conjugates of heparin and deoxycholic acid (DOCA) are synthesized to enhance the heparin absorption in gastrointestinal tract. The absorption of heparin-cholesterol, heparin-palmitic acid, and heparin-lauric acid conjugates in the GIT is lower than that of heparin-DOCA. They do not cause any damage to the microvilli and the cell layer (Lee et al., 2000). The bioavailability of LMWH (6 kDa)-DOCA at the 20 mg/kg dosage is

calculated to be 7.8%. Two possibilities are proposed to explain the results: one is the increased hydrophobic property of heparin-DOCA, and the other is the interaction between the coupled DOCA and bile receptors in the ileum (Lee et al., 2001).

Considering the lack of oral absorption of heparin, the reason is mainly due to its anionic charge profile. Therefore, polymeric micro-/nanoparticles containing unfractionated heparin are developed with polycationic polymethacrylate (Eudragit® RS and RL) alone or blended with biodegradable polymers (poly-ε-caprolactone, PLGA) (Jiao et al., 2001; 2002A). A 2-fold increase in APTT (activated partial thromboplastin time) is observed with heparin-loaded microparticles confirming the release of heparin from microparticles without loss of activity as well as its absorption from GIT, leading to dramatic increases in bioavailability (43 and 48%) (Jiao et al., 2002B). Similar results in APTT and anti-Xa levels are observed after oral administration of heparin entrapped nanoparticles with 23% bioavailability (Jiao et al., 2002C). The major advantages of these heparin delivery systems are (i) safety of the excipients employed and (ii) low dose (similar to the intravenous route). The mechanisms governing heparin absorption with micro-/nanoparticles are still hypothetical and include (i) uptake via a paracellular pathway, (ii) intracellular uptake and transport via the epithelial cells of the intestinal mucosa, (iii) lymphatic uptake via the M cells and the Peyer's patches. With respect to the microparticles prepared with polycationic polymers, they are supposed to coat the gastrointestinal mucosa thus increasing the contact surface area with the intestine and the heparin concentration gradient rather than permeation through the intestinal wall or their uptake by Peyer's patches.

3. PLGA stability in in-situ forming systems

3.1. Application of PLGA

Poly (lactic-acid) (PLA) and poly (D,L-lactide-co-glycolide) (PLGA) display important advantages of biocompatibility, predictability of biodegradation kinetics, ease of fabrication and regulatory approval (Lewis, 1990). Extensive studies have been carried out to develop parenteral controlled release delivery systems of proteins (Sinha and Trehan, 2003; van de Weert et al., 2000; Cleland J.L., et al., 1997), peptides (Okada, 1997; Woo, 2001), local anaesthetics (Le, 1997; Wakayama, 1982) and orally controlled delivery (Andrianov and Payne, 1998) of vaccines (Delgada et al., 1999), heparin (Jiao et al., 2002) and other orally inactive drugs (Mandal et al., 2002).

Currently, a number of FDA-approved products in the market utilize PLA and PLGA as excipients to achieve sustained release of the active ingredient (Table 5). The total annual sales for these products exceed $2.5 billion, with Lupron Depot as the leading product. There are more than 20 companies worldwide working in the area of biodegradable parenteral polymeric drug delivery. In addition, several biotechnology companies (such as Amgen, Genentech, and Isis Therapeutics) have formulation groups that are studying the use of lactide-based polymers for the reformulation of their products. Future demand for these polymers will continue to grow, as parenteral chemotherapeutics as well as modern biotechnology drugs (proteins & antibodies) become targets for reformulation and life-cycle management.

Table 5: PLA/PLGA-based commercially available products.

Product	Drug	Dosage form	Administration	Manufacturer
Atridox®	Doxycycline hyclate	Liquid	Subgingival	Atrix Laboratories
Decapeptyl®	Triptorelin	Microsphere	I.M.	Ferring
Eligard®	Leuprolide	Liquid	S.C.	Atrix
Enantone® Depot	Leuprolide	Microspheres	I.M.	Takeda
Lupron Depot®	Leuprolide	Microspheres	I.M.	TAP
Nutropin Depot®	Somatropin	Microspheres	S.C.	Genentech
Plenaxis	Abarelix	Microspheres	I.M.	Praecis
Profact® Depot	Buserelin	Implant	S.C.	Aventis Pharma
Sandostatin LAR®	Octreotide	Microspheres	Intragluteal	Novartis
Trelstar® Depot	Triptorelin	Microsphere	I.M.	Pharmacia Upjohn
Zoladex®	Goserelin	Implant	S.C.	AstraZeneca

There are four established suppliers of GMP-grade PLA and PLGA: Purac (Trade name: Purasorb); Birmingham Polymers, a subsidiary of Durect Corporation (Trade name: Lactel); Boehringer Ingelheim (Trade Name: Resomer), and Alkermes (Trade name: Medisorb). Other newer suppliers include Absorbable Polymer Technologies (US), and smaller manufacturers catering to the local niche markets worldwide.

In summary, PLA and PLGA represent non-traditional excipients in the pharmaceutical industry. While the present demand for these polymers is limited, a significant growth potential exists, primarily driven by the developments and advances in parenteral drug sector.

3.2. PLGA degradation and erosion

To successfully deliver drugs for a desired period, it is essential to understand drug stability as well as the degradation profiles of the polymers. Polyesters such as PLA and PLGA are biodegradable and biocompatible synthetic polymers which degrade in vivo to lactic acid ($C_3H_6O_3$) and glycolic acid ($C_2H_4O_3$) which are subsequently

metabolized and eliminated as CO_2 and H_2O via the Krebs cycle (Reed and Gilding, 1981). These polymers degrade by simple hydrolysis of the ester linkages in the polymer backbone by hydrolytic attack of water molecules a process which is both acid and base catalyzed. The degradation properties of PLGA have been extensively studied and are reviewed (Holland et al., 1986; Vert et al., 1992; 1994; Gopferich, 1996). Studies on the degradation have shown that polymer properties such as morphology, copolymer composition (Park, 1995), tacticity, molecular weight, crystallinity, glass transition temperature and end group; release medium properties such as pH, temperature, ionic strength, solvent and presence of biocatalysts or microorganisms (Cai et al., 2001); formulation properties such as particle size (Grizzi et al., 1995; Dunne et al., 2000), presence of zinc carbonate (Tracy et al., 1999), plasticizer (Kranz et al., 2000), tertiary amine based drugs (Maulding et al., 1986; Wakayama et al., 1982) and sterilizations (Athanasiou et al., 1996; Montanari et al., 1998; Mohr et al., 1999), significantly affect the degradation rate of the polyesters.

Polymeric structures degrade either through unzipping, the breakage of the last unit at the end of the chain, or through random scission, cleavage of a bond random along the chain. A number of studies point out to the random scission route for PLGA degradation (Cha and Pitt, 1990). However, others indicate that the hydrolytic scission of ester bonds tend to preferential target the linkages between glycolide/glycolide (G-G) and glycolide/lactide (G-L) partially due to the steric effects of the voluminous alkyl group of lactide which hinders the attack of water. Under harsh conditions (low pH, high temperature or electromagnetic radiation), the degradation path appears to change from the random scission to unzipping as indicated by the increasing of polydispersity (Hasirci et al., 2001).

A theoretical model shows that all degradable polymers could undergo surface erosion or bulk erosion depending on the diffusivity of water inside the matrix, the degradation rate of the polymer's functional groups and matrix dimensions (von Burkersroda et al., 2002). Below a critical dimension, a polymer matrix will always undergo bulk erosion. While above it, surface erosion occurs. In the case of PLGA, the critical dimension is 7.4 cm. A bulk erosion mechanism is therefore considered as the main degradation pathway for PLA and PLGA based products: random chain scission on the linkage of ester bonds in the polymer backbone proceeds homogenously throughout the device. Further studies indicate that massive devices of PDLA, PLA and PLGA degrade via a heterogeneous mechanism, i.e. the degradation proceeds more

rapidly in the center than on the surface. This is attributed to the autocatalytic action of the carboxylic acid end groups of degrading products which are trapped in the matrix (Park, 1995).

3.3. PLGA stability

Despite the successful application of ISI and the development of ISM, the stability of PLGA and drug in ISI and ISM during preparation and storage is so far not investigated in detail. To understand the factors on the stability of PLGA is essential in achieving stable formulations and desirable release performances. Therefore, in this study, the stability of PLGA and leuprolide acetate was studied in ISI and ISM systems, namely, in biocompatible solvents and oily and aqueous suspensions. The effects of storage time and temperature, water content of polymer solutions, biocompatible solvent, and presence of leuprolide acetate, oils with different chain length and aqueous phase with different ionic strength on the stability of PLGA were investigated. Due to the potential instability of the polymer and drug in organic solutions, sponges were developed by dissolving PLGA and drugs (leuprolide acetate, lidocaine) in acetic acid or dioxane and then lyophilized. The sponges could be reconstituted by a solvent and formulated into in-situ forming systems before parenteral administration. The influence of residual solvent and encapsulated drug on the stability of PLGA in the sponges was studies.

4. Research objectives

This study is divided into three major parts, the research objectives are as follows:

4.1. Development of enteric microparticles

(i) Development of a novel coacervation method to formulate enteric microparticles to encapsulate lipophilic drugs for the improvement of the rate of dissolution.

(ii) In vitro and in vivo evaluation of enteric microparticles or the poorly soluble drug carbamazepine

4.2. Development of in-situ forming microparticles (ISM)

(i) Development and characterization of parenteral ISM of heparin for a one week delivery period.

(ii) Development of a stable semisolid in-situ forming microparticle system to promote the oral bioavailability of lipophilic and macromolecule drugs.

4.3. PLGA stability in in-situ forming systems

The stability of biodegradable polymer PLGA and leuprolide acetate in in-situ forming drug delivery systems, namely, in organic solvents, oily and aqueous suspensions and lyophilized sponges was investigated.