CHAPTER 3. RESULTS AND DISCUSSION

1. Enteric microparticles

The solubility and permeability of a drug are key determinants of its oral bioavailability. Various formulation strategies have been investigated to improve the solubility/rate of dissolution and hence the oral bioavailability of lipophilic drugs. These strategies include a reduction of drug particle size, the use of different polymorphic/amorphic drug forms, complexation (e.g., cyclodextrins), the use of cosolvents, solubilization by surfactants and the formation of solid drug solutions/dispersions (Pinnamaneni et al., 2002).

Lipophilic drugs have also been encapsulated in various polymeric carriers in the form of nano-/microparticles (Arangoa et al., 2001; Roger et al., 2003; Leroux et al., 1995). Enteric polymers, which are practically insoluble in water and acidic solutions but soluble in buffer solution with a pH above 5.5-7.0, are promising carriers for a variety of reasons. They protect the gastric mucosa from drug irritation and prevent drug degradation in the stomach by enzymes or acidic fluids (Amorim and Ferreira , 2001), they can deliver the drug to a particular region of the intestine or colon (Lamprecht et al., 2004), they can enhance the bioavailability by increasing the wettability and dissolution rate of the drug (Nazzal et al., 2002) and they can stabilize the drug within the matrix (Leroux et al., 1996; De Jaeghere et al., 2000; 2001).

Various methods for the preparation of solid dispersions or micro-/nanoparticles using enteric polymers have been developed including solvent evaporation (Nazzal et al., 2002), coprecipitation (Kislalioglu et al., 1991), emulsification-evaporation (Lee et al., 1999), emulsification-diffusion (De Jaeghere et al., 2000; 2001) and salting-out methods (Leroux et al., 1995; 1996). Each approach has its benefits and drawbacks (Galindo-Rodriguez et al., 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 has to be ground and sieved. Scale-up and physical and chemical instability are the 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). Coprecipitates 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 low polymer solution concentrations is necessary to obtain small particles and avoid large aggregates (Fessi et al., 1989).

For the emulsification-evaporation method, a drug/polymer solution in a water-immiscible solvent (e.g. dichloromethane, chloroform, ethyl acetate) 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 nano-/microparticles. 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 diffusion of the solvent from the O/W emulsion to form nano-/microparticles (De Jaeghere et al., 2001).

In the salting-out process, an organic solution of drug/polymer 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 nano-/microparticles. 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 micro-/nanoparticles in order to improve the dissolution rate and oral bioavailability of lipophilic drugs (De Jaeghere et al., 2000; 2001; Paradkar et al., 2004; Dollo et al., 2003).

Generally, coacervation method is used to coat acidic or enzyme sensitive compounds with enteric polymers (e.g. cellulose acetate phthalate, cellulose acetate butyrate, hydroxypropyl methylcellulose phthalate). In the simple coacervation process, a non-solvent for polymer was added to a polymer solution containing drug particles. With the removal of the associated solvent from the polymer rich phase into the non-solvent, the molecules of polymer tend to deposit and aggregate around drug particles forming coacervate. For example, a cellulose acetate phthalate solution in acetone containing a dispersed enzyme power is emulsified into liquid paraffin with a suitable emulsifier. Acetone is evaporated by raising the temperature to 25°C, and the filtered

microcapsules are washed with benzene to remove residual liquid paraffin (Kitajima et al., 1973). Another cellulose derivative enteric polymer, HPMCP, is dissolved in methylene chloride into which the enzyme particles are dispersed. This suspension is then emulsified into ethylene glycol to form an O/W emulsion. Stirring is maintained until the methylene chloride has evaporated, producing the coating by phase separation of the polymer. The microcapsules are obtained after removal from the ethylene glycol and washing with water (Fukushima et al., 1975). Due to the complicated process and using toxic solvent (e.g. methylene chloride), a simple and toxic solvent-free coacervation method is desired.

Aggregation or clustering of micropartilces during coacervation preparation is troublesome problem commonly encountered in simple and complex coacervation. The rapid rise in apparent viscosity of polymer rich region (gelatin-acacia) causes undesirable cohesion and aggregation of microparticles. This could be lessened by using a shock-preventing agent (e.g. carboxymethylcellulose, sodium carboxymethylstarch, pectic acid) or cationic surfactants during the isolation and drying stages of complex coacervation. The surfactant is strongly adsorbed at the interface between the coacervate and its surrounding medium, reducing the interfacial tension and leading to reduced tendency of the particles to aggregate (Deasy, 1984). Poly (1-vinyl-2-pyrrolidone) is used as a stabilizer to form heparin/gelatin microcapsules by a complex coacervation using a spray-drying technique (Mei and Burgess, 1997).

The objectives of this study were to develop a coacervation method to formulate enteric microparticles for lipophilic drugs. The non-solvent (water) containing various hydrophilic polymers as stabilizers was added into the enteric polymer solution with lipophilic drugs. The parameters influencing the formation of enteric microparticles such as polymer type, concentration and pH of aqueous phase, type of enteric polymer, type of organic solvent and the mechanism of encapsulation of lipophilic drugs with different functional groups were investigated.

1.1. Formation of drug-free enteric microparticles

Enteric microparticles were prepared by a coacervation method, whereby an aqueous polymer solution was added to an organic enteric polymer solution (Fig. 1.1). Water was a nonsolvent for the enteric polymer causing the phase separation and the formation of coacervate droplets, which hardened into microparticles upon further addition of the aqueous phase. The hydrophilic polymer in the aqueous phase acted as a

stabilizer for the coacervate droplets, preventing coalescence. The effect of the following parameters on the formation of enteric microparticles was investigated: type, concentration and pH of aqueous polymer solution, type of enteric polymer and organic solvent and type of lipophilic drug. The mechanism of encapsulation of lipophilic drugs into the enteric microparticles by this coacervation method was further investigated.

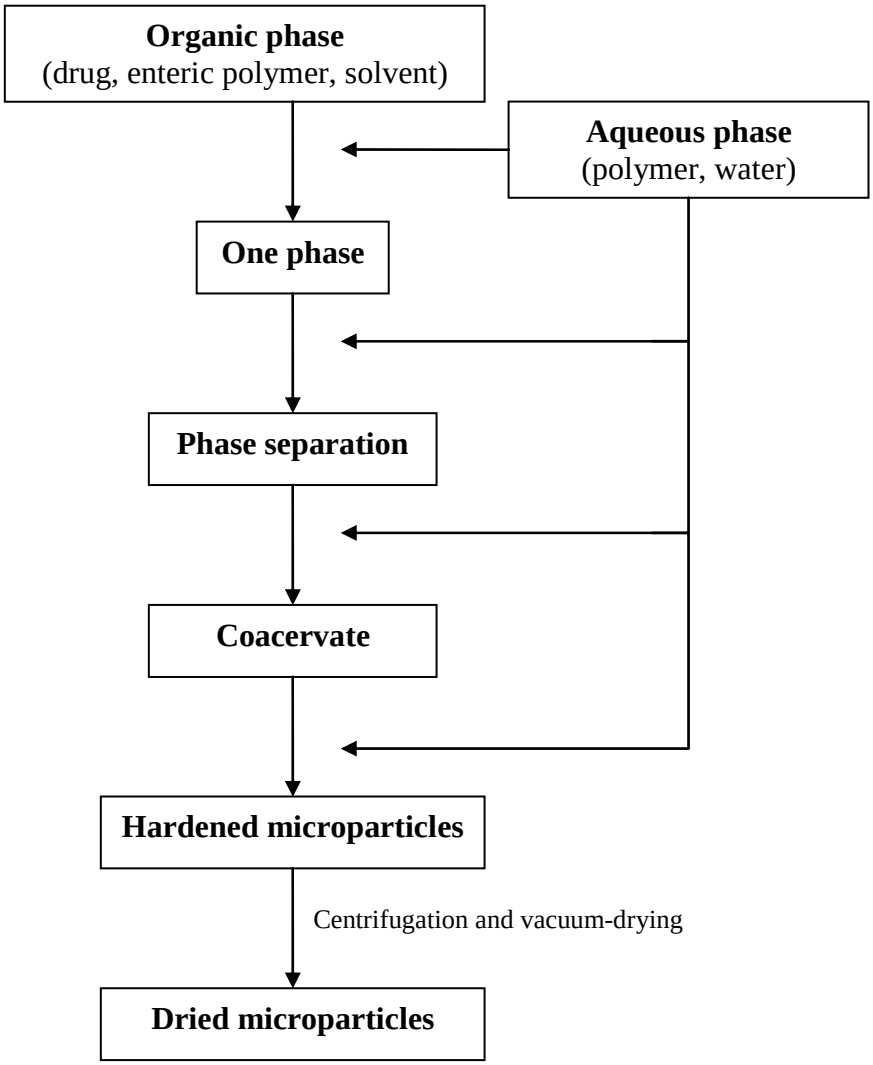


Figure 1.1 Diagram of the coacervation method to prepare enteric microparticles.

1.1.1. Aqueous phase

The order of addition of the organic enteric and aqueous polymer solutions was very important for the successful preparation of the microparticles. Large polymer precipitates formed when the organic enteric polymer solution was added into the aqueous polymer solution. A small amount of enteric polymer solution was in contact with a large excess of aqueous polymer solution (nonsolvent), resulting in rapid organic solvent diffusion and polymer concentration/precipitation because of the complete miscibility of the polymer solvent (ethanol) and the aqueous phase. The use of water-immiscible organic solvents would allow the formation of microparticles by emulsification of the polymer solution in the aqueous phase (solvent evaporation method) (Lee et al, 1999). However, unwanted toxic organic solvents such as dichloromethane (ICH class 2) are used in the solvent evaporation method. In this study, the goal was to form microparticles with less toxic organic solvents such as ethanol, ispropanol and acetone (ICH class 3).

Next, the order of addition was reversed; the aqueous phase was added to the enteric polymer solution. The choice of aqueous phase strongly affected the successful microparticle formation. For example, the addition of water or an aqueous solution of 1% (w/v) $CaCl_2$ and 0.25% w/v Tween 20 (surfactant) (Zaghloul et al., 2001A; 2001B) to the ethanolic Eudragit® L100-55 solution resulted in the formation of aggregates at room temperature. The coacervate droplets were not stable towards coalescence and aggregated. Various hydrophilic polymers were investigated as polymeric stabilizers for the coacervate droplets and the formation of hardened microparticles (Table 1.1). Microparticles were successfully formed with HPMC, HPC and 5% w/w Poloxamer 407 solutions (Table 1.1, Fig. 1.2). Large aggregates formed with HEC, PVA and 2% w/w Poloxamer 407. The organic phase was completely miscible with 10% w/w Poloxamer 407, no coacervation or polymer precipitation occurred.

The hydrophilic polymer had to be soluble in the newly formed ethanol/water mixture in order to stabilize the coacervate droplets. HPMC, HPC and Poloxamer 407 were soluble, while HEC and PVA were insoluble thus not acting as polymeric stabilizers and leading to aggregates.

Table 1: Effect of aqueous polymer phase on the formation of Eudragit® L100-55 Microparticles

Aqueous phase (%, w/w)	Viscosity (mPas)	Miscible with $EtOH/H_2O$ (80/20 v/v)[1]	Particle formation[2]
Water	-	+	-
$CaCl_2$ (1) and Tween 20 (0.25)	-	+	-
HPMC (0.75)	156 ± 8	+	+
HPMC (1)	410 ± 8	+	+
HPMC (1.5)	1528 ± 32	+	+
Poloxamer 407 (2)	3 ± 4	+	-
Poloxamer 407 (5)	3 ± 4	+	+
Poloxamer 407 (10)	8 ± 8	+	solution
HPC (1)	285 ± 8	+	-
HPC (3)	6420 ± 484	+	+
HEC (1)	492 ± 12	-	-
HEC (3)	9855 ± 161	-	-
PVA (10)	1577 ± 33	-	-

[1] + miscible /- not miscible

[2]+ particle formation / - no particle formation

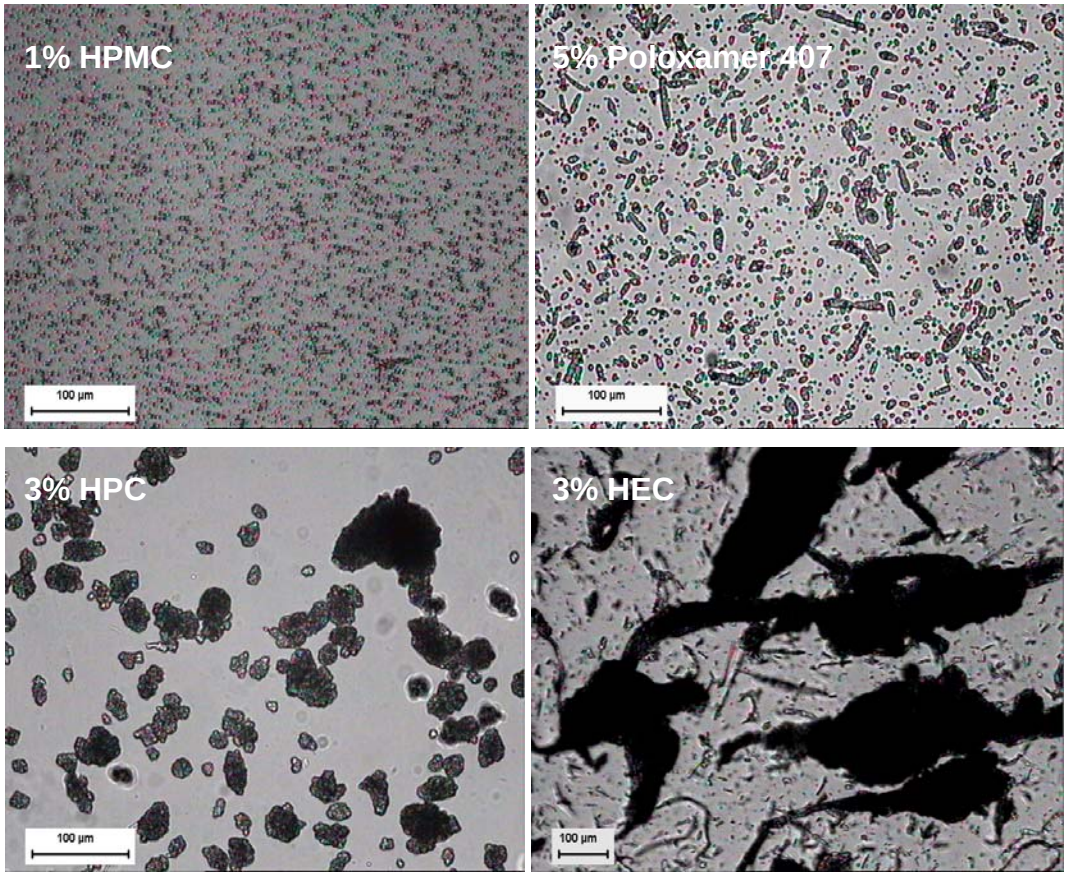
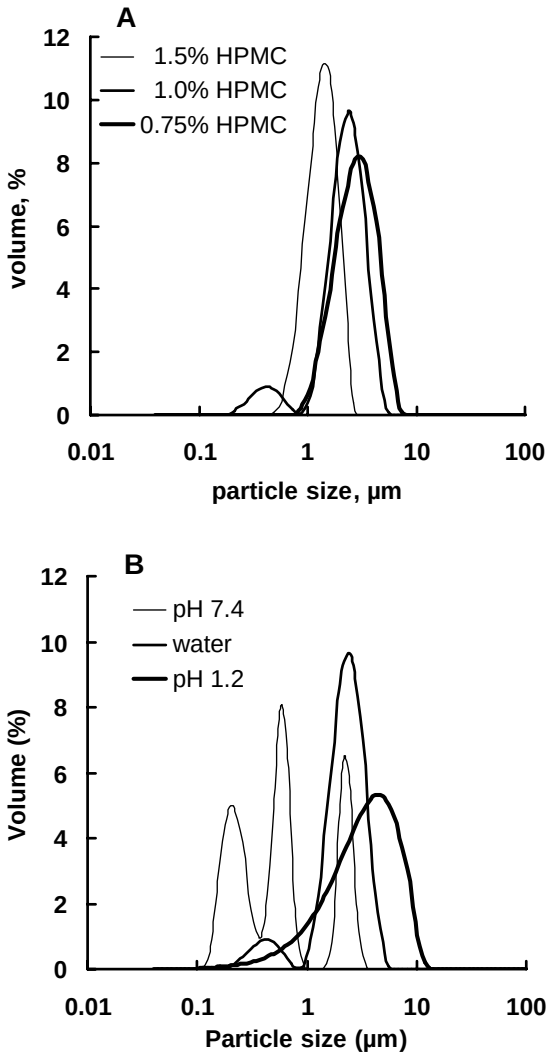


Figure 1.2 Photographs of Eudragit® L100-55 microparticles/precipitates formed with different aqueous polymer phases: 1% w/w HPMC, 5% Poloxamer 407, 3% HPC and 3% HEC.

1.1.2. Concentration and pH of HPMC solution and type of enteric polymer

HPMC was most effective in forming Eudragit® L100-55 microparticles by acting as a stabilizer and thickening agent to prevent coalescence of the coacervate droplets. The particle size decreased with increasing HPMC concentration due to the increase of stabilizing and thickening effects of HPMC (Fig. 1.3 A).



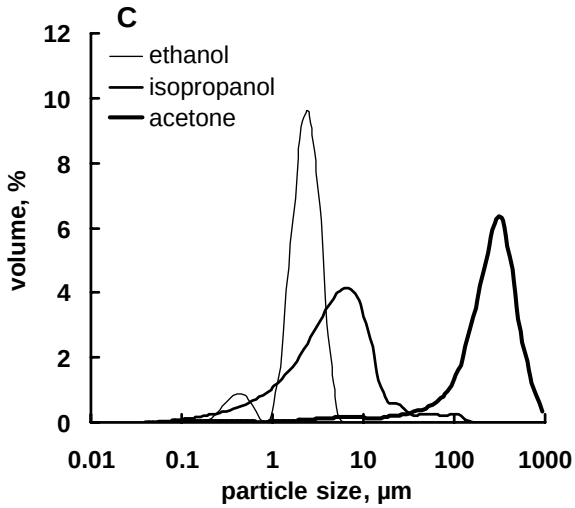


Figure 1.3 Effect of (A) HPMC concentration in water, (B) pH of the HPMC (1.0%) solution and (C) type of organic solvent on the size distribution of Eudragit® L100-55 microparticles.

The effect of pH of the HPMC solution and the type of enteric polymer on the formation of enteric microparticles were evaluated by adding a 1% w/w HPMC solution dissolved in 0.1 N HCl (pH 1.2), water or phosphate buffer (pH 7.4) to different enteric polymer solutions (Table 1.2). Eudragit® L100-55 and L100 formed particles at all pH-values, while the cellulose ester cellulose acetate phthalate (CAP) did not form microparticles irrespective of the pH of the HPMC solution. Microparticles were obtained with Eudragit® S100, HPMCP HP-55S and HPMCAS at pH 7.4, but lumps formed at pH 1.2 or in water (Table 1.2, Fig. 1.4).

Table 2: Effect of enteric polymer (20% w/w) and pH of HPMC solution (1% w/w) on the formation of enteric microparticles

Enteric polymer	Solvent	COOH No[1]	Microparticle formation[2]		
			pH 1.2	water	pH 7.4
Eudragit® L100-55	96% EtOH	537	+	+	+
	isopropanol		+	+	+
	acetone		+	+	+
Eudragit® L100	96% EtOH	537	+	+	+
Eudragit® S100	96% EtOH	349	-	-	+
HPMCP HP-55S	80% EtOH	181-235	-	-	+
HPMCAS-MF	80% EtOH	99-138	-	-	+
CAP	acetone/EtOH 1:1	238	-	-	-

[1] Number of carboxyl groups per 100,000 Dalton (based on the structure of each unit)
[2] + particle formation, - no particle formation

Addition of HPMC-pH 7.4 solution to the enteric polymer (Eudragit® L100-55) solution led to a slower phase separation and polymer precipitation when compared to HPMC solution in water or low pH (Table 1.3). The particle size decreased with increasing pH of the aqueous phase (Fig. 1.3 B, Fig. 1.4). This was caused by the partial neutralization of the carboxyl groups at the higher pH, resulting in an electrostatically induced stabilizing effect of the microparticles. The lower microparticle yield at pH 7.4 (Eudragit® L100-55: 46.7%; HPMCP: 91.3%) when compared to 0.1 N HCl (Eudragit® L100-55: 92.7%; HPMCP: 104%) was attributed to the formation of a colloidal polymer dispersion which was difficult to collect by centrifugation and possibly also because of polymer dissolution. Eudragit® L100 or S100 powders are redispersed in an alkaline solution to form stable aqueous latex-type dispersions for enteric coating purposes through partial neutralization of the polymers (Degussa, Röhm Pharma Polymers).

Table 3: Amount of HPMC solution added to 10 g Eudragit® L100-55 (20% w/w) solution in different solvents, or to different enteric polymers dissolved in ethanol (20% w/w), or to drug solutions in ethanol resulting in polymer phase separation or drug precipitation.

Enteric polymer or drug solutions	1% HPMC solution		
	pH 1.2	water	pH 7.4
20% Eudragit® L100-55			
ethanol	7.4	8.6	9.5
isopropanol	-	6.9	-
acetone	-	5.2	-
20% enteric polymer in ethanol			
HPMCP 55S	-	4.3	-
HPMCAS	-	6.7	-
Eudragit® S100	-	4.5	-
Eudragit® L100	-	8.8	-
drug in ethanol			
10% carbamazepine	-	27.2	-
10% indomethacin	-	13.1	-
10% cyclosporine A	-	13.4	-
10% ibuprofen	-	16.0	-
20% ibuprofen	-	13.6	-
30% ibuprofen	-	12.2	-

The content of carboxyl groups of the enteric polymers strongly affected the formation and particle size of the enteric microparticles. The number of carboxyl groups in each polymer was calculated based on the molecular weight of one polymer unit

containing one carboxyl group, for example, one methacrylic acid-ethyl acrylate unit of Eudragit® L100-55 (186 dalton) containing one carboxyl group. Eudragit® L100-55 (having a higher content of carboxyl groups) formed microparticles, even in low pH medium (Table 1.2) and required the addition of more aqueous HPMC solution for polymer phase separation than the other enteric polymers (Table 1.3). In contrast, large aggregates formed with the enteric polymers with less carboxyl groups, Eudragit® S100, HPMCP HP-55S and HPMCAS-MF at pH 1.2 and in water. Microparticles formed in pH 7.4, where partial neutralization and thus stabilization occured (Table 1.2).

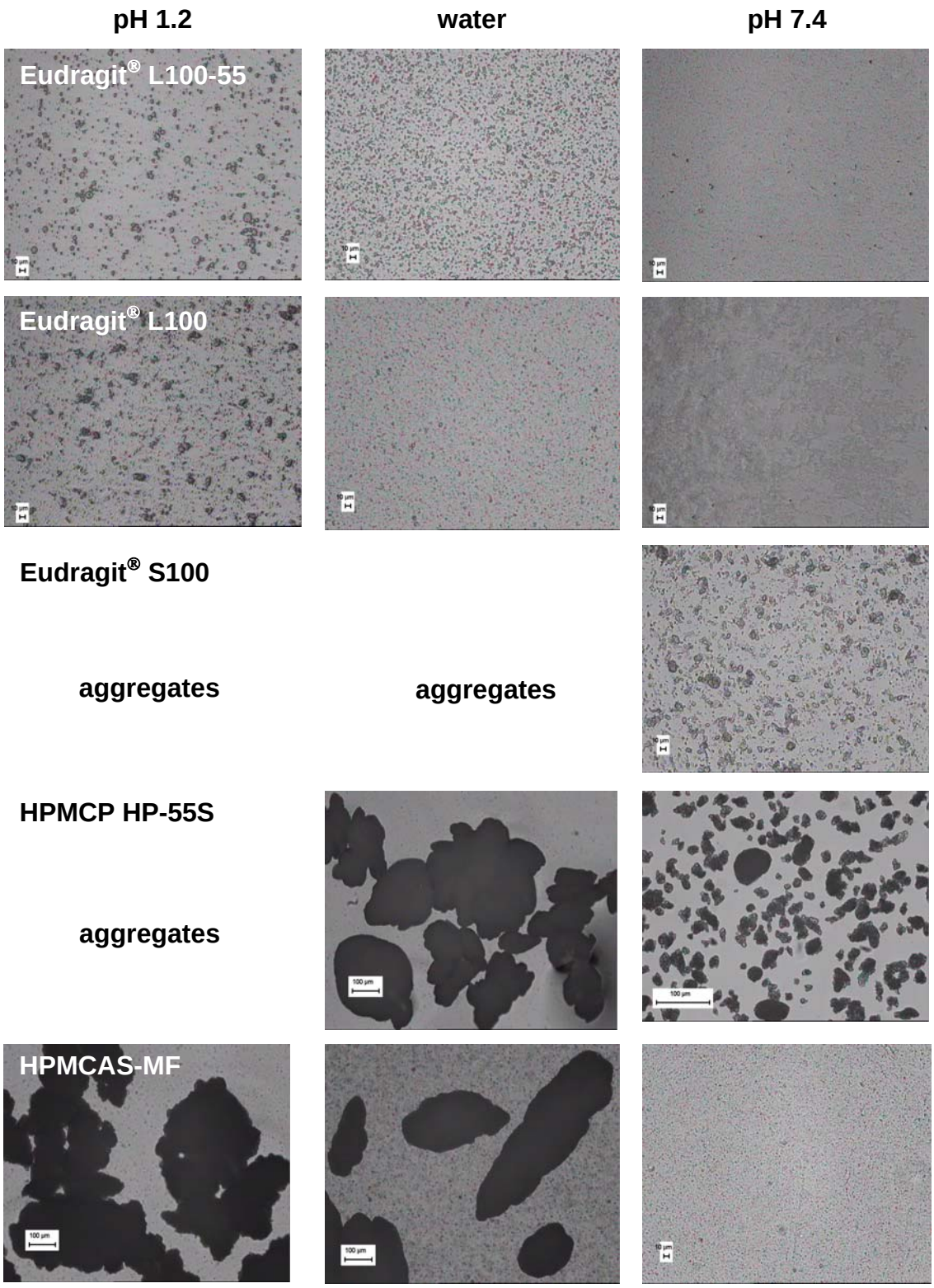


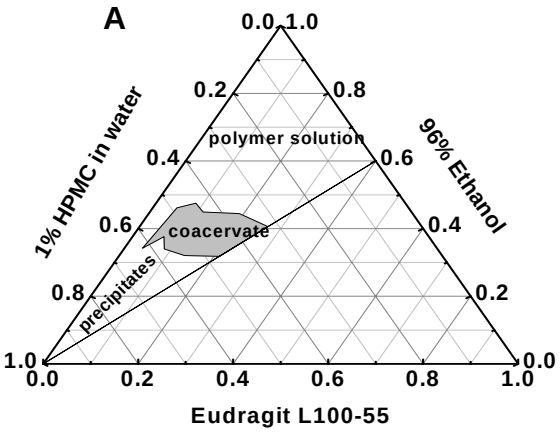
Figure 1.4 Photographs of enteric microparticles/precipitates prepared with different enteric polymers (Eudragit® L100-55, Eudragit® L100, Eudragit® S100, HPMCP HP-55S, HPMCAS-MF) and with aqueous HPMC solutions of different pH.

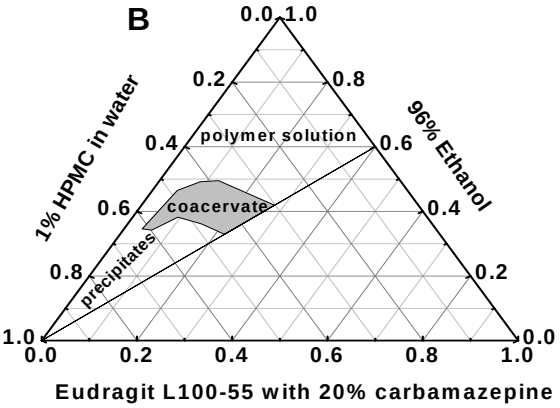
1.1.3. Effect of organic solvent

Acetone, ethanol and isopropanol, which are ICH class 3 solvents (CDER, Guidance for Industry Q3C Impurities) with a low risk to human health, were evaluated as solvents for the enteric polymers. The size of the enteric microparticles was strongly affected by the organic solvents and decreased in the following order: acetone > isopropanol > ethanol (Fig. 1.3 C). Ethanol, with strong hydrogen bond capability and high Hildebrand solubility parameter (26.0 $MPa^{1/2}$), is a good solvent for Eudragit® L100-55 thus forming small coacervate droplets and no precipitates (Barton, 1983). However, acetone (moderate hydrogen bond capability, Hildebrand solubility parameter 20.0 $MPa^{1/2}$), is a poor solvent for the enteric polymer (Galindo-Rodriguez et al., 2004), resulting in a rapid phase separation and polymer precipitation, subsequently forming large particles upon addition of the aqueous polymer solution (Table 1.3).

1.1.4. Phase diagrams for the characterization of the phase separation of enteric polymers

The phase separation and subsequent precipitation of the enteric polymers were achieved by dropwise addition of 1% w/w HPMC aqueous solution to ethanolic solutions of the enteric polymers (Eudragit® L100-55, HPMCP) with/without carbamazepine (20% w/w). A phase diagram of the enteric polymer-ethanol 96% v/v (solvent)-1% w/w HPMC aqueous solution (non-solvent) revealed the coacervation regions (Fig. 1.5). The phase separation and polymer precipitation occurred in a narrow ethanol concentration (phase separation, 42-47%; precipitation, 32-37%) (Fig. 1.5). The faster phase separation and precipitation of HPMCP than Eudragit® L100-55 was attributed to the higher lipophilicity of HPMCP having less carboxyl groups (Fig. 1.5 C, Table 1.2).





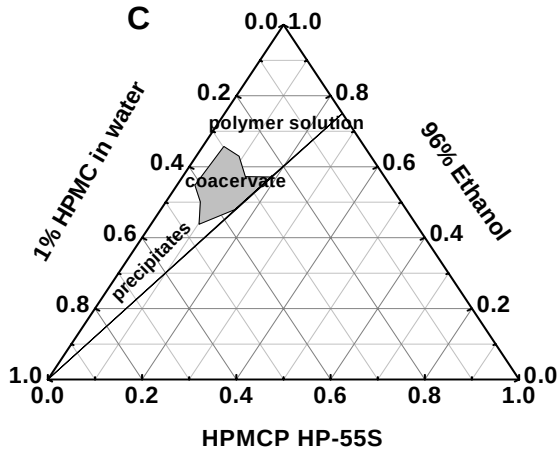
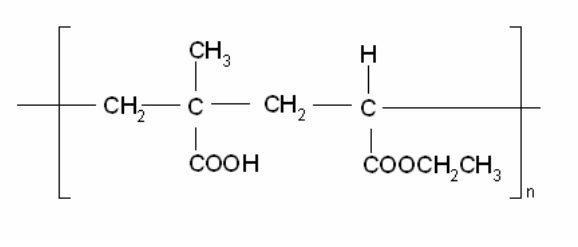


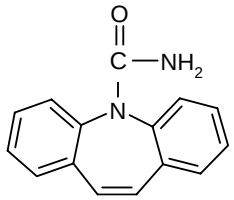
Figure 1.5 Phase diagrams for the simple coacervation of (A) Eudragit® L100-55, (B) Eudragit® L100-55 with 20% w/w carbamazepine and (C) HPMCP in ethanol induced with 1% w/w HPMC aqueous solution.

1.2. Drug-loaded enteric microparticles

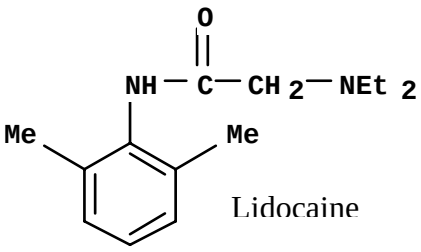
Enteric polymers containing carboxyl groups have the potential to form hydrogen-bond with drugs which have proton-donor or acceptor groups. This interaction might prevent instability (crystallization) of solid dispersion upon storage. In order to investigate the compatibility/miscibility of drugs within enteric polymers, three kinds of lipophilic drugs (Fig. 1.6), including amide-containing drugs (carbamazepine, cyclosporin A, lidocaine), carboxyl-containing drugs (indomethacin, ibuprofen) and hydroxyl-containing drug (estradiol) were evaluated by dissolving in enteric polymer (Eudragit® L100-55) solution and casting into films. The physical states of drugs in freshly prepared and 18 months stored films were observed under a polarized light microscope in an attempt to evaluate the compatibility of drugs and polymers and physical stability of drugs in the corresponding films (Table 1.4).



Eudragit® L100-55

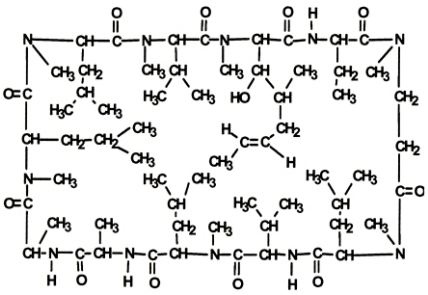


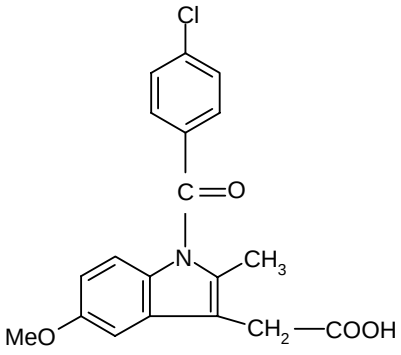
Carbamazepine



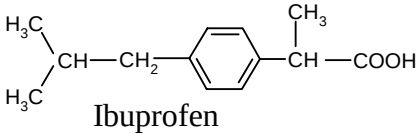
Lidocaine

Cyclosporin A

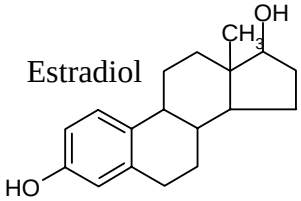




Indomethacin



Ibuprofen



Estradiol

Figure 1.6 Structures of Eudragit® L100-55 and model drugs.

Table 1.4: Physical states of drugs in freshly prepared films and and stored Eudragit® L100-55 films.

Drugs	Freshly prepared films			Films stored 1.5 year		
	10%	20%	30%	10%	20%	30%
Carbamazepine	+	+	+	+	+	+
Lidocaine	+	+	+	+	+	+
Cyclosporine A	+	+	+	+	+	+
Ibuprofen	+	+	-	+		
Indomethacin	-					

+: clear films. -: turbid films

Casting film method is one alternative to develop solid solution/dispersion of lipophilic drugs with related polymers. However, the disadvantages of these methods such as incomplete evaporation of residual solvents, soft and sticky masses and difficulties of pulverization and sieving, limited the application of this method to industrial scale. The purpose of casting film here is to investigate the compatibility, solubility and stability of drugs within enteric polymer films. The amide-containing drugs (carbamazepine, lidocaine and cyclosporin A) could dissolve in Eudragit® L100-55 to 30% (w/w, based on solid mass) by forming transparent films. They dispersed in polymer films in molecular states and stable for 18 months by the absence of crystallization of drugs. However, ibuprofen was soluble in Eudragit® L100-55 less than 30% w/w. Indomethacin was insoluble in this polymer as indicated by the turbid films.

Various lipophilic drugs (10% w/w based on polymer and drug) were dissolved in the organic Eudragit® L100-55 solution and tested for their influence on the formation of drug-loaded enteric microparticles and their encapsulation behavior. Carbamazepine, cyclosporine A, lidocaine and ibuprofen were successfully encapsulated in the enteric microparticles without crystals being visible in the external liquid phase (Fig. 1.7). The encapsulation efficiency of carbamazepine was 90 %. Scanning electron micrographs revealed spherical carbamazepine- and cyclosporine A-loaded microparticles with smooth surfaces (Fig. 1.8). In contrast, indomethacin and estradiol crystallized outside the polymeric microparticles (Fig. 1.7).

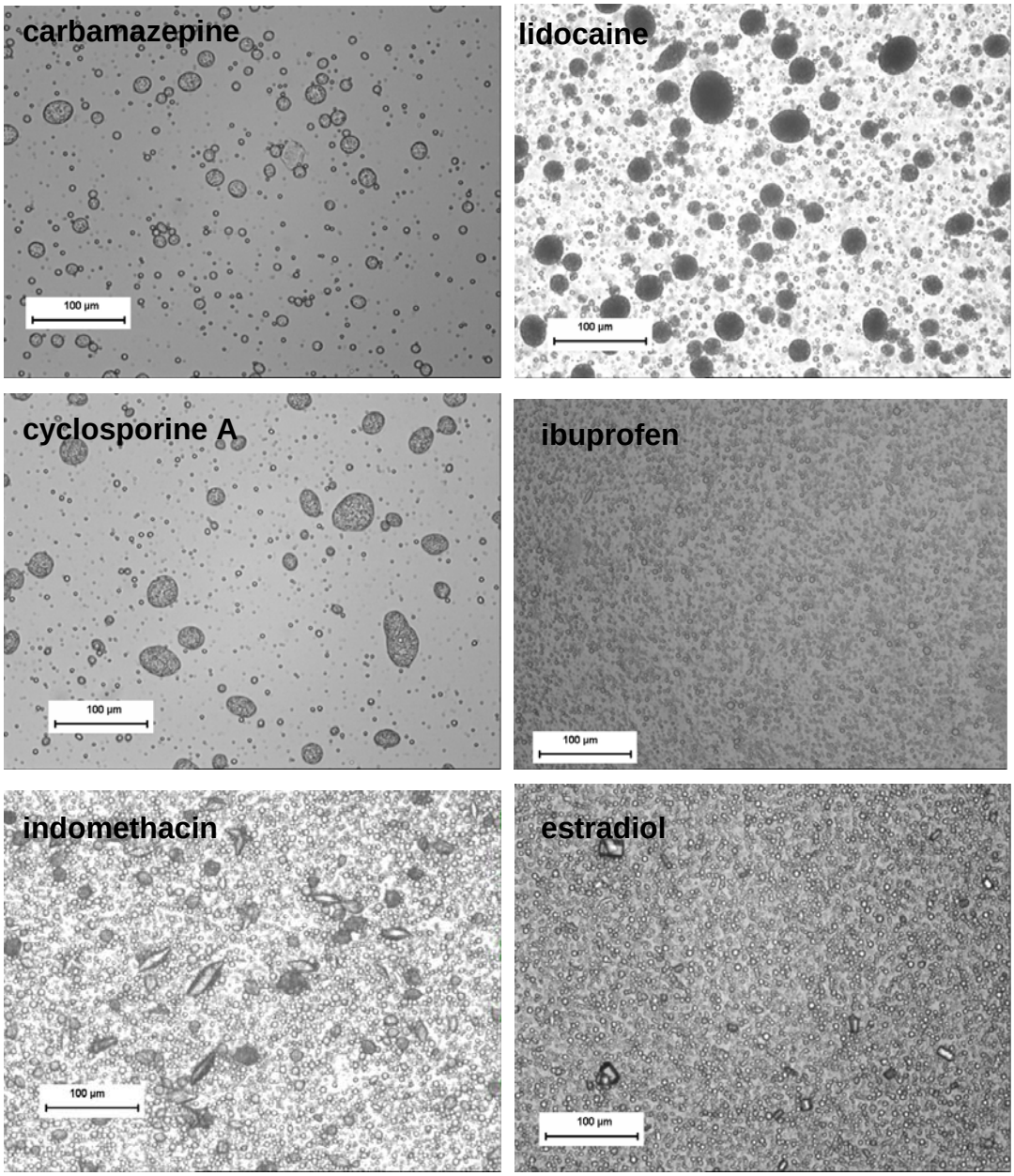


Figure 1.7 Photographs of drug-loaded (10% w/w) Eudragit® L100-55 microparticles.

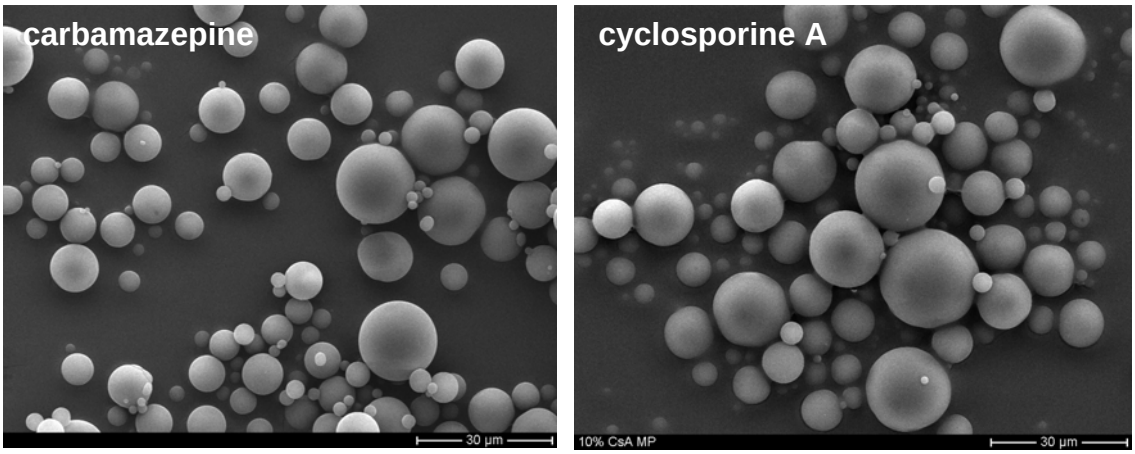


Figure 1.8 Scanning electron micrographs of 10% w/w carbamazepine- and 10% w/w cyclosporine A-loaded Eudragit® L100-55 microparticles.

In DSC study, the heating of a mixture of Eudragit® L100-55 and carbamazepine resulted in a loss of the thermal signal indicating the melting of the crystalline drug in the enteric polymer (Fig. 1.9 A). Obviously this change indicated the interaction of the amide-containing drug and the carboxyl-containing polymer. The physical state of carbamazepine in enteric microparticles could not be identified by DSC method. X-ray analyses were therefore performed to analyze the physical state of both polymer and drug in particle formulations immediately after production and after 5 months of storage at room temperature. The non-crystalline state of carbamazepine in Eudragit® L100-55 microparticles at 20% w/w drug loading was illustrated, where physical mixture showed the typical drug crystalline peaks (Fig. 1.10). In this study, ibuprofen could be encapsulated in Eudragit® L100-55 microparticles in a non-crystalline state upto 10% w/w (Fig. 1.9 B). The extra drug crystallized when drug loading was over 10% w/w. Indomethacin completely crystallized inside the polymer matrix (Fig. 1.9 C).

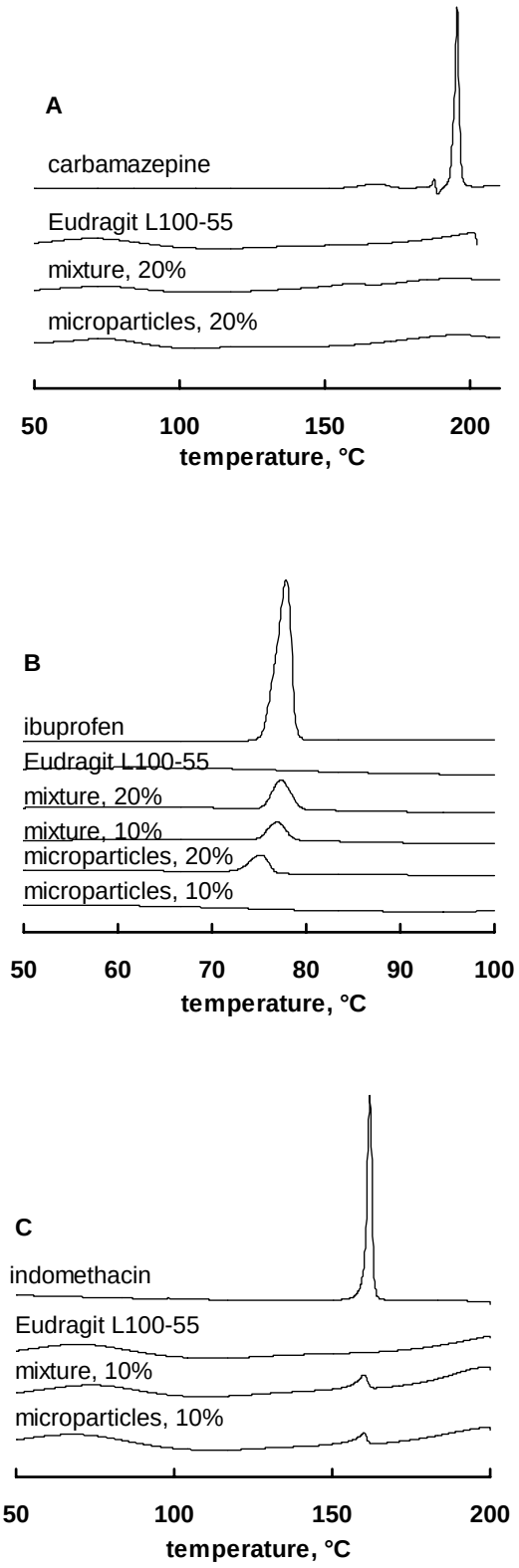


Figure 1.9 DSC curves of drugs, Eudragit® L100-55, physical mixture of drug and Eudragit® L100-55 and drug-loaded Eudragit® L100-55 microparticles of (A) carbamazepine, (B) ibuprofen and (C) indomethacin.

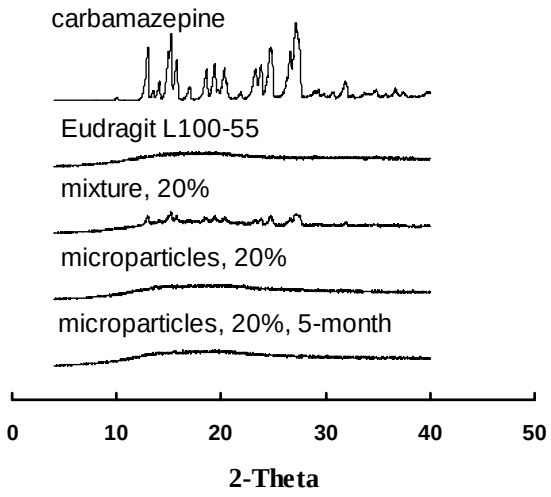


Figure 1.10 X-ray diffraction patterns of carbamazepine, Eudragit® L100-55, physical mixture, freshly prepared microparticles and microparticles stored for 5 months (20% w/w drug loading).

The aqueous HPMC solution, which induced coacervation of the enteric polymers, was also a nonsolvent for the lipophilic drugs. Upon addition of the HPMC solution, Eudragit® L100-55 came first out of solution at lower amounts of aqueous HPMC solution than the drugs, thus indicating the lower solubility of the polymer in the solvent/nonsolvent mixture (Table 1.3, Fig. 1.11). Depending on the solubility of the drugs within the enteric polymers, they were either dissolved in the enteric microparticles or they crystallized inside or outside the polymeric matrix in the aqueous phase. A simple qualitative test for drug/polymer compatibility is the casting of drug-containing films. Transparent films reflect drug dissolved in the polymer matrix, while turbid films indicate undissolved (crystallized or amorphous) drug. Carbamazepine, cyclosporine A and lidocaine (30% w/w) formed transparent Eudragit® L100-55 films indicating dissolved drug. The drugs did not recrystallize within 1.5 years of storage. Ibuprofen formed transparent films up to 20% w/w, but not at 30% w/w drug loading, while indomethacin and estradiol formed crystals already at 10% w/w drug loading, indicating a low drug/polymer compatibility (low drug solubility in the polymer).

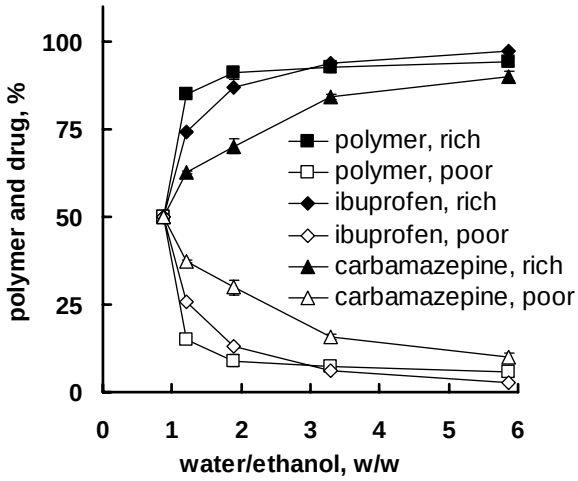


Figure 1.11 Amount of Eudragit® L100-55, ibuprofen and carbamazepine in polymer-rich/-poor regions.

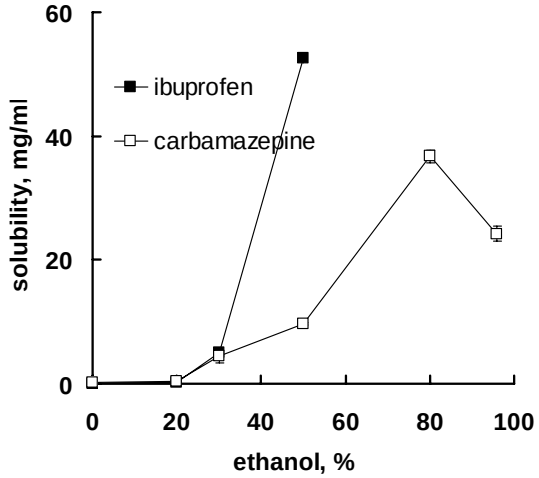


Figure 1.12 Solubility of ibuprofen and carbamazepine in water/ethanol mixtures at room temperature.

In order to investigate the mechanism of drug encapsulation within the enteric microparticles, the amount of Eudragit® L100-55, carbamazepine or ibuprofen (20% w/w) in both polymer-rich/-poor regions were determined during the coacervation process (Fig. 1.11). The enteric polymer formed coacervates prior to the drug precipitation. The two drugs preferentially stayed in the polymer-rich phase in the initial stage. With the addition of 1% w/w HPMC solution, the solubility of the drugs further decreased in the polymer-poor phase (Fig. 1.12), resulting in a drug partitioning into the polymer-rich phase. Carbamazepine with its high solubility in the enteric polymer was encapsulated in a non-crystalline state (possibly in a molecular state), but ibuprofen

partially crystallized due to its limited solubility in the enteric polymeric matrix (Fig. 1.13). Interestingly, the particle size of carbamazepine microparticles was larger than of ibuprofen particles.

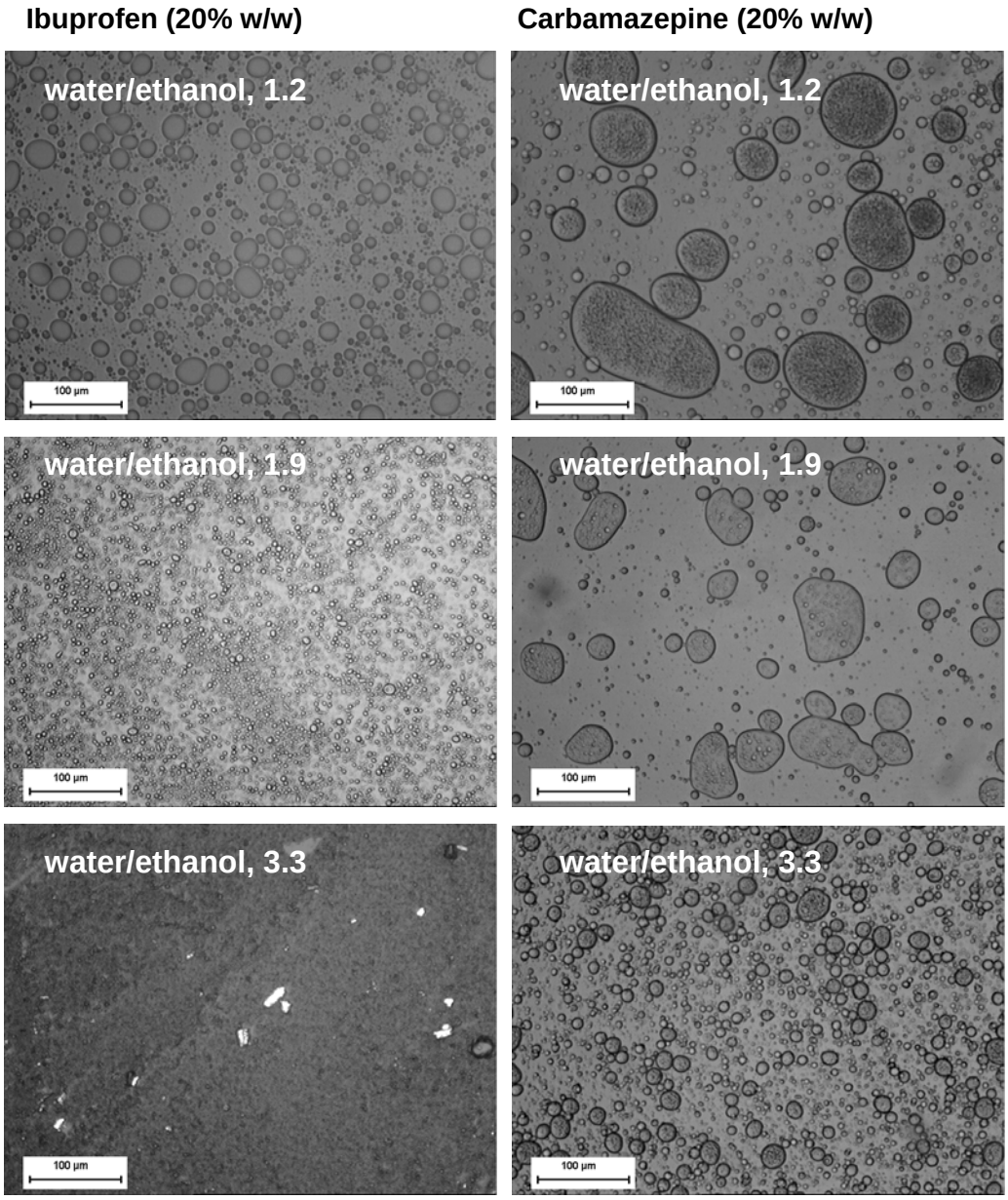


Figure 1.13 Photographs of coacervate droplets and hardened enteric microparticles containing ibuprofen and carbamazepine (20% w/w) as a function of water/ethanol ratio.

As reported, ibuprofen melting with Eudragit® E 100 but not with Eudragit® L100 is attributed to the ionic interaction of the cationic polymer (E 100) with the anionic drug (Petereit and Weisbrod, 1999). Hydrogen bonds between amide and carboxyl groups are reported stronger than that between carboxyl groups (Horisawa et al., 2000).

In this study, the amide-containing drugs (carbamazepine, lidocaine, cyclosporine A) possibly formed stronger hydrogen bonds with Eudragit® L100-55 than the carboxyl- (indomethacin, ibuprofen) and hydroxyl-containing (estradiol) drugs. The adhesive force of drug/polymer was therefore stronger than cohesive forces of drug/drug and polymer/polymer, which contributed to the successful encapsulation and stabilization of the drugs in the enteric microparticles in non-crystalline states.

1.3. Characterization of drug-loaded enteric microparticles

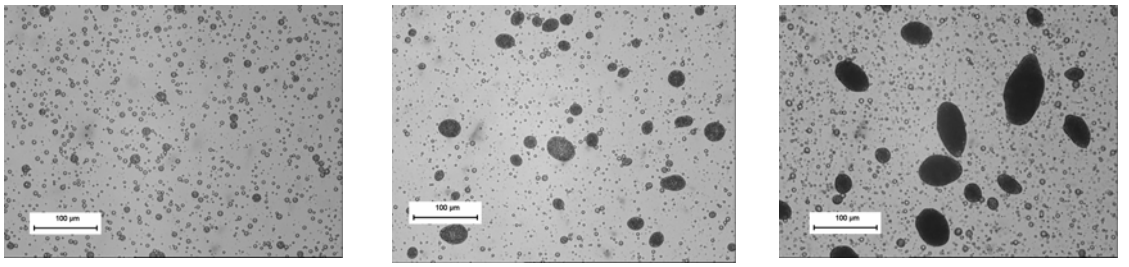
The cyclic polypeptide cyclosporin A (CsA) is a potent immunosuppressive agent used to inhibit organ rejection in transplanted patients and for the treatment of autoimmune disorders (Fahr, 1993). The oral bioavailability is approximately 30% from a Sandimmune® oral formulation with wide inter- and intrasubject variability (Lindholm et al., 1988). Different reasons have been suggested for the low and erratic oral bioavailability. These include a poor solubility in water (27.67µg/ml, 25°C) (Ismailos et al., 1991), a low permeability through the intestinal membrane, poor dissolution characteristics, extensive metabolism by cytochrome P-450 3A4 both in liver and gut, effect of P-glycoprotein mediated drug efflux and the influences of intake of food and concomitant medications. Some reports suggest an absorption window for CsA in the upper small intestine (Drewe et al., 1992). This is particularly important since CsA is highly lipophilic. Thus, it is essential to develop a formulation with quantitative dissolution in the upper part of the GIT. Solid dispersions composed of cyclosporin A, surfactant (HCO-60) and enteric polymers were developed and HPMCP HP-55 preparations gave the highest plasma CsA level and the highest lymphatic availability than Sandimmune® and other compositions (Takada et al., 1989).

In the present study, cyclosporin A-loaded enteric microparticles of were prepared by the novel coacervation method. Various processes and formulation variables have been investigated with this coacervation method with regard to the optimization of microparticle formation and drug entrapment. The obtained microparticles are evaluated in vitro through particle size distribution, morphology, encapsulation efficiency, yield, wettability and dissolution testing. In vivo bioavailability is evaluated using rabbit as animal model and physical mixture as the reference by determining the plasma level of carbamazepine and its active metabolite, carbamazepine-10,11-epoxide.

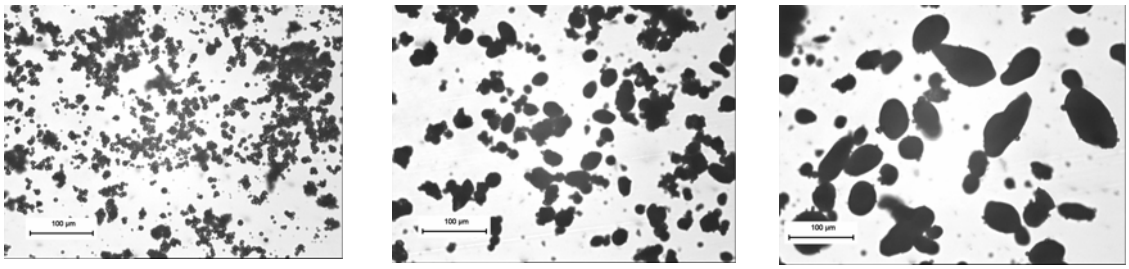
1.3.1. Cyclosporin A-loaded enteric microparticles

5, 10 and 15% (w/w) of CsA-loaded enteric micropartilces were prepared (Fig. 1.14, Fig. 1.15). No significant difference of particle size was observed visually between the wet and dry states at the same drug loading. The increase of particle size with increase of drug loading might attribute to the enhanced hydrophobicity of internal drug/polymer complex, which also revealed the interaction of drug and polymer. SEM pictures showed that 10% w/w CsA-loaded microparticles were oval-like and had a dense structure (Fig. 1.16).

The size of CsA-loaded enteric microparticles could be reduced by using homogenizer at 8000 rpm instead of magnetic stirring with addition of aqueous polymer solution to CsA/polymer solution (Fig. 1.15).



5% CsA wet | 10% CsA wet | 15% CsA wet



5% CsA dry | 10% CsA dry | 15% CsA dry

Figure 1.14 Photographs of cyclosporine A-loaded enteric microparticles with 5, 10 and 15% w/w drug loading in wet and dry states.

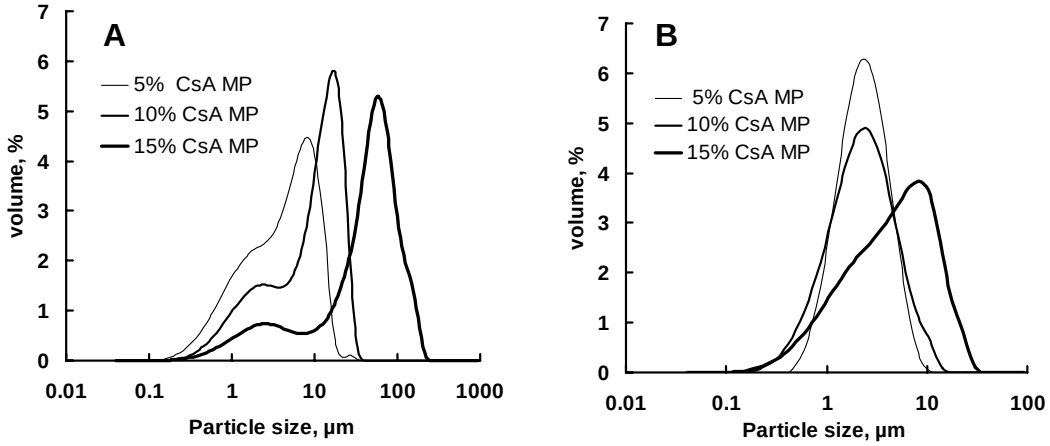


Figure 1.15 Effect of CsA loading and agitation method (A) magnetic stirring and (B) homogenizing at 8000 rpm on the size distribution of CsA-loaded microparticle.

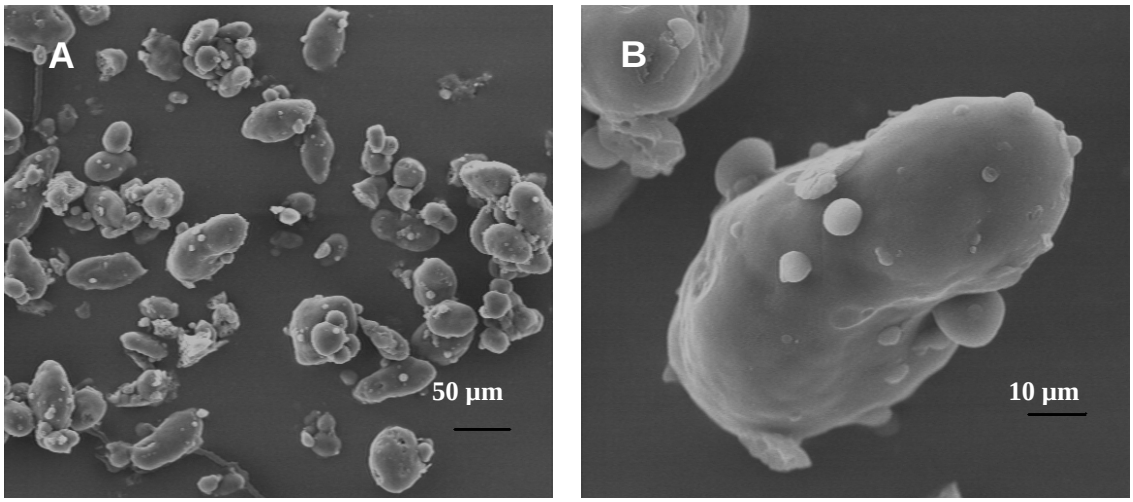


Figure 1.16 Scanning electron micrographs of the (A) group and (B) surface of the 10% w/w CsA-loaded Eudragit® L100-55 microparticles.

Both encapsulation efficiency and yield were over 92% (w/w) irrespective of the drug loadings, which were particularly satisfying on a lab-scale (Table 1.5).

Table 1.5: Encapsulation efficiency and yield of CsA-loaded enteric microparticles.

Formulations	Encapsulation efficiency (%)	Yield (%)
5% CsA MP	94.9 ± 1.7	93.9 ± 1.1
10% CsA MP	95.4 ± 2.9	92.8 ± 0.9
15% CsA MP	93.2 ± 0.3	93.8 ± 1.5

The contact angle of 10% w/w CsA-loaded enteric microparticles (20 ± 3°) against pH 7.4 phosphate buffer was lower than pure drug (71 ± 3°) and physical blends with polymer (60 ± 3°). With CsA loading increasing from 5 to 30% (w/w), the contact angles of CsA loaded films increased from 43° to 59°.

Owing to the poor water solubility of cyclosporin A (27.67 µg/ml), there were technical difficulties in evaluating in-vitro dissolution. The use of surfactants or organic solvents in dissolution testing has been proposed to circumvent such problems (Saarinen-Savolainen et al., 1997; Gander et al., 1985). However, these conditions might not reflect the in-vivo drug release. Therefore, in vitro dissolution test of CsA-loaded enteric microparticles was not performed.

CsA-loaded enteric microparticles (5, 10 and 15%, w/w) containing 10 mg CsA were dispersed in 5 ml pH 6.8 phosphate buffer to investigate the solubility of the drug. Samples were withdrawn at 2 h, 4 d and 8 d, and centrifugated at 17,000 rpm for 30 min

then filtrated through 0.2 µm filters. The drug in obtained solutions/suspensions was measured by HPLC.

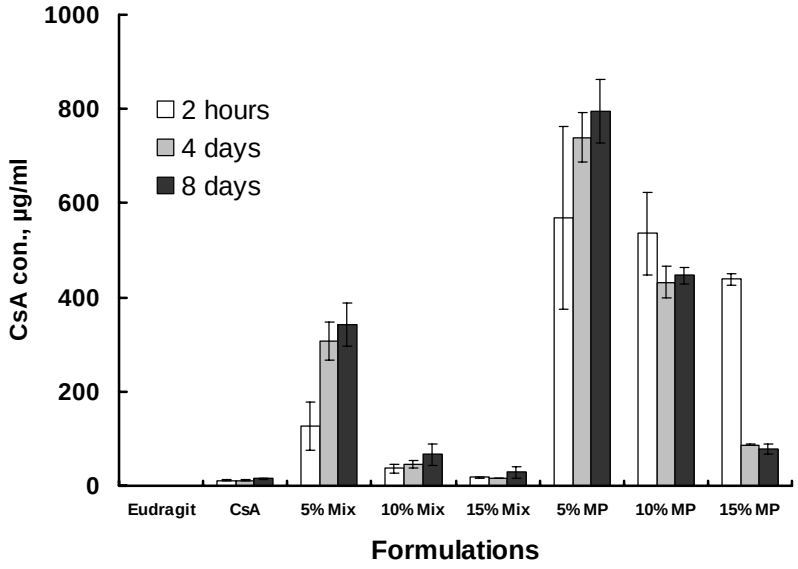


Figure 1.17 Dissolution of cyclosporin A in different formulations.

Colloidal suspensions of CsA were obtained after dissolving the microparticles in phosphate buffer even after the centrifugation and filtration. The lower the CsA loading, the more turbid were the suspensions. It can not conclude that the solubility of CsA was significant enhanced by incorporation into enteric polymer matrix (Fig. 1.17). However, the achieved colloidal systems might also enhance the bioavailability of CsA by oral administration due to the enhanced surface areas of drug to the GIT. Eudragit® L100-55 could enhance the solubility of CsA in aqueous phase reflected from the higher CsA concentrations of different physical mixtures than CsA powder (Fig. 1.17).

1.3.2. Carbamazepine-loaded enteric microparticles

Carbamazepine is an effective antiepileptic drug characterized by a slow and an irregular gastrointestinal absorption. The drug has an experimental log P value of 2.45 and is practically insoluble in water (113 µg/ml, 25°C). Variations in the dissolution rates and the absorption also occurred between its different crystalline forms. In this study, this BCS class II drug (Kasim et al., 2003) was encapsulated in an enteric polymer Eudragit® L100-55 by a toxic solvent-free coacervation method, whereby an aqueous polymeric stabilizer solution was added to an organic enteric polymer solution containing carbamazepine. Various processes and formulation variables have been investigated with this coacervation method with regard to the optimization of

microparticle formation and drug entrapment (Dong, 2005). The obtained microparticles are evaluated in vitro through particle size distribution, morphology, encapsulation efficiency, yield, wettability and dissolution testing. In vivo bioavailability is evaluated using rabbit as animal model and physical mixture as the reference by determining the plasma level of carbamazepine and its major active metabolite, carbamazepine-10,11-epoxide.

1.3.2.1. In vitro characterization

Carbamazepine-loaded enteric microparticles were prepared by a coacervation method, whereby an aqueous polymeric solution was added to an organic carbamazepine/Eudragit® L100-55 solution (Dong, 2005). Water is a nonsolvent for the drug and the enteric polymer causing phase separation and the formation of coacervate droplets, which hardened into microparticles upon further addition of the aqueous phase. The hydrophilic polymer HPMC in the aqueous phase acted as a stabilizer for the coacervate droplets, preventing coalescence.

The average particle size was in the lower µm range and increased with increasing drug loading (Table 1.6, Fig. 1.18). This was most likely due to the formation of hydrogen bond (H-bond) between amide of carbamazepine and carboxylic acid of the enteric polymer, which prevented the interaction of the enteric polymer with water/ethanol. Thus, increasing the lipophilicity of the drug/polymer complex by increasing drug loadings led to a quicker drug/polymer precipitation and bigger particles. The carbamazepine-loaded enteric microparticles were spherical and had smooth surface without visible drug crystals, which suggested that carbamazepine was molecularly entrapped (dissolved) in the polymeric matrix (Fig. 1.19).

Table 1.6: Effect of drug loading and stirring time on the encapsulation efficiency, microparticle yield and particle size of carbamazepine-loaded enteric microparticles

Theoretical drug loading (%)	Encapsulation efficiency (%)		Yield (%)	Particle size (µm)
	10 min	24 h		
5	85.4 ± 0.5	89.4 ± 0.2	90.1 ± 1.1	3.6
10	90.0 ± 2.0	94.9 ± 0.5	92.5 ± 1.3	6.6
15	88.7 ± 1.6	93.2 ± 0.3	90.4 ± 0.2	12.6
20	91.2 ± 0.6	93.4 ± 1.1	92.2 ± 0.4	34.8

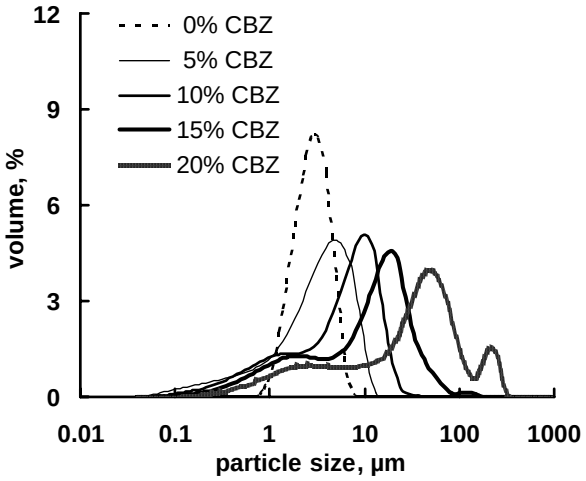


Figure 1.18 Effect of carbamazepine loading on the particle size distribution of drug-loaded enteric microparticles.

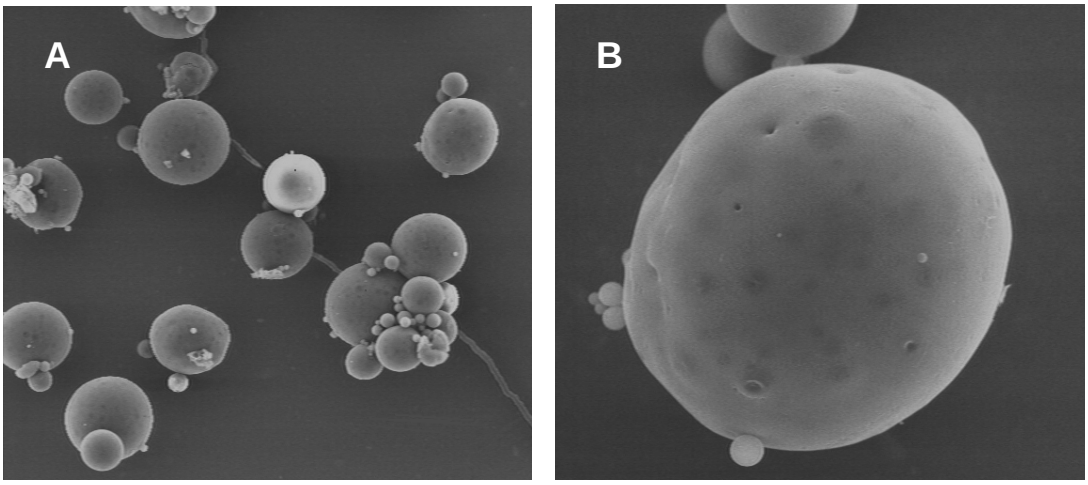


Figure 1.19 Scanning electron micrographs of 20% w/w carbamazepine-loaded enteric microparticles (A) group (B) surface.

The yield of microparticles was over 90% and the encapsulation efficiencies were over 85% w/w irrespective of the drug loading (Table 1.6). Interestingly, the encapsulation efficiencies were 2 to 5% higher when the stirring time of the microparticles prior to separation from the liquid phase was extended from 10 min to 24 h. Initially, carbamezepine and Eudragit® L100-55 are both dissolved in ethanol. Upon addition of the aqueous HPMC solution, coacervation of the enteric polymer was induced and liquid-filled coacervate droplets formed.

Because of the high drug encapsulation efficiencies, carbamazepine apparently located itself preferentially within the coacervate droplets and not in the external

ethanol/aqueous HPMC solution phase. This uptake in the coacervate droplets might be time-dependent thus explaining the increased drug entrapment at the longer stirring time. In addition, the solubility of the drug in the aqueous phase decreased during stirring because of the evaporation of ethanol, thus also promoting the carbamazepine partitioning into the microparticles. In order to clarify this point, carbamazepine powder was added either in the polymer solution and then forming coacervate or in the preformed coacervate of Eudragit® L100-55 at different water/ethanol ratios. At ratio of 1.2, around 65% w/w drug partitioned into polymer rich phase with the same partition coefficient (3.1) as drug initially dissolved in polymer solution (Fig. 1.20). However, a slower partition of the drug into polymer rich phase at higher water/ethanol ratio (1.9) and lower partition coefficient (5.2) than that of drug dissolved in polymer solution (7.5) were attributed to the formation of relatively hardened microparticles. The partition of drug into the polymer-rich phase indicates that carbamazepine has an affinity for the polymer as expressed by a high solubility in excess of 30 % in Eudragit® L100-55. The affinity may be from the interaction between the amide-containing drug and carboxyl-containing enteric polymers.

The absence of carbamazepine crystal peaks by X-ray analysis indicated that carbamazepine was either dissolved in the Eudragit® L100-55 or (less likely) dispersed in amorphous form (Fig. 1.10). The spectrum did not change during a 5-month storage period, indicating good physical stability. This is particularly advantageous with a view to physical instabilities frequently reported with solid dispersions (Khalil et al., 1978; Vila Jato et al., 1984). Other amide-containing lipophilic drugs such as HIV-1 protease inhibitors (CGP 57813, CGP 70726) were also encapsulated in enteric polymers in a non-crystalline state (De Jaeghere et al., 2000; Leroux et al., 1995, 1996).

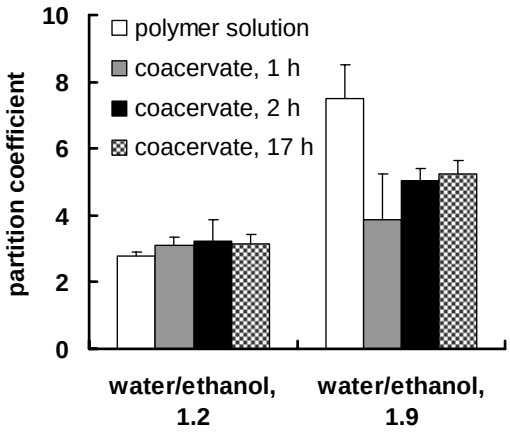
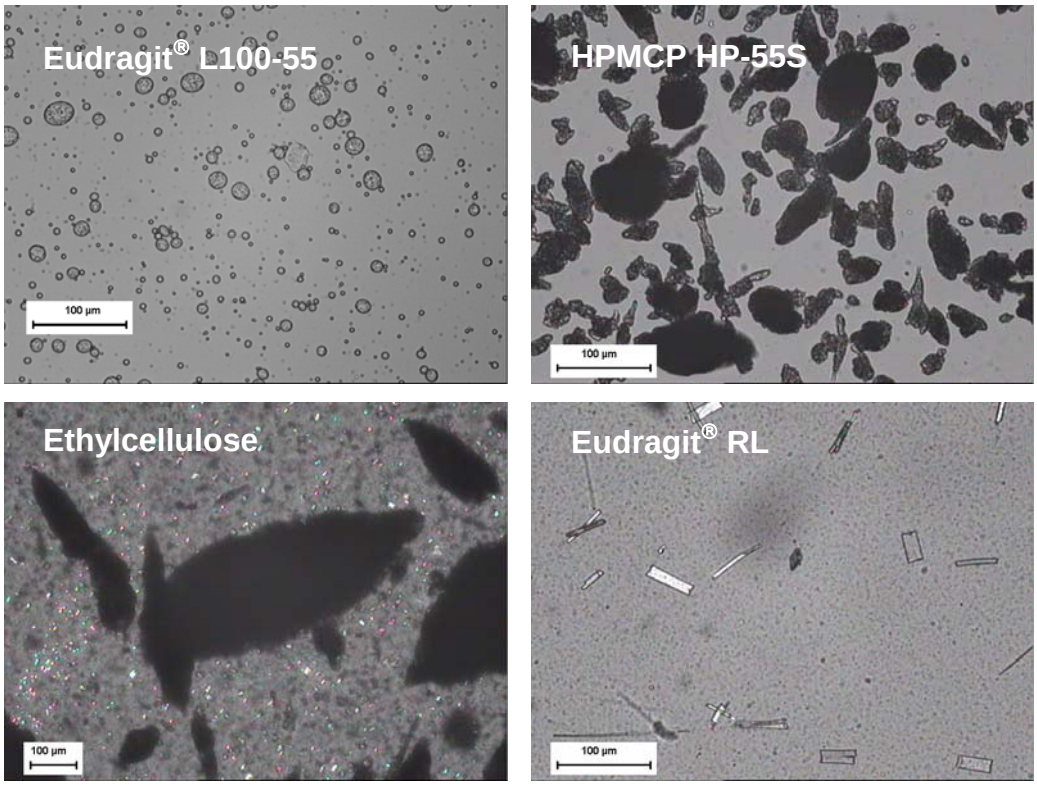


Figure 1.20 Partition coefficient of carbamazepine between polymer rich/poor regions with addition of drug initially in the polymer solution and forming coacervate or in the preformed coacervates.

The successful encapsulation of carbamazepine (10% w/w) in another enteric polymer (HPMCP HP-55S), but crystallized outside the non-enteric polymers ethylcellulose and Eudragit® RL also supported the existence of the interaction through H-bond (Fig. 1.21). A higher drug loading of carbamazepine in Eudragit® L100-55 (20% w/w) than HPMCP HP-55S (15% w/w) was due to the higher content of carboxylic acid of Eudragit® L100-55 (537/100,000 dalton) than HPMCP HP-55S (181-235/100,000 dalton) (Fig. 1.21). The content of carboxyl groups in enteric polymer (100,000 dalton) was calculated based on the molecular weight of each unit which containing one carboxylic acid (Table 1.2).

10% w/w carbamazepine



20% w/w carbamazepine

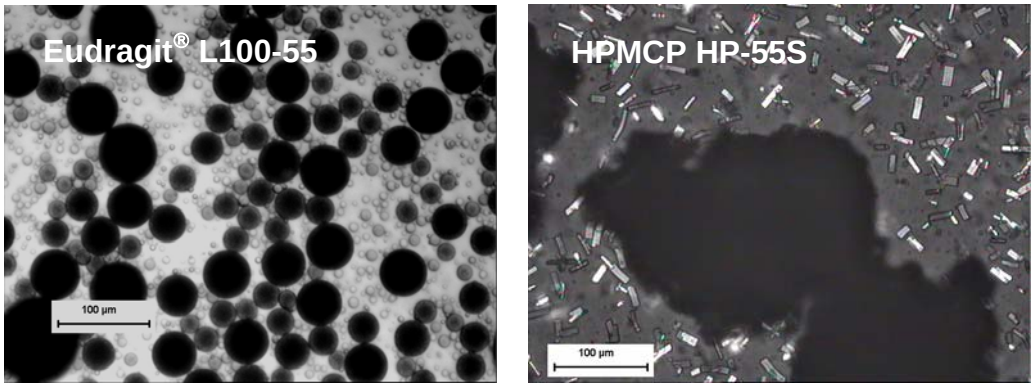


Figure 1.21 Effect of polymer type: enteric polymer (Eudragit® L100-55, HPMCP HP-55S) and non-enteric polymer (ethylcellulose and Eudragit® RL) on the encapsulation of carbamazepine.

The dissolution studies were performed under sink conditions in 0.1 N HCl and pH 6.8 phosphate buffer. The micronized carbamazepine crystals showed a slower dissolution profile with a high standard deviation in both media. As expected, the drug release from enteric microparticles was slower at low pH because of the insolubility of the enteric polymer Eudragit® L100-55 in 0.1 N HCl than in pH 6.8, where the polymer

dissolved (Fig. 1.22). The drug release decreased with increasing drug loading in 0.1 N HCl. This decrease in release was probably caused by an overall increased lipophilicity of the drug/polymer combination at higher drug loadings. In addition, the size of the microparticles inreased with increasing drug loading, thus also contributing to the slower release. Unexpectedly, the opposite trend was seen in pH 6.8 phosphate buffer. This could be explained with the aggregation of the microparticles upon contact with phosphate buffer, which was caused by a partial dissolution/gelling of the enteric polymer. Further polymer and drug dissolution was then slowed down. The aggregation tendency was much more pronounced with the smaller microparticles, which had a lower drug loading. In contrast, the microparticles were individually dispersed in 0.1 N HCl because of the insolubility of the polymer. From an in-vivo point of view, it is speculated that the microparticles would be finely dispersed in gastric fluid and then individually transported in the upper intestine, where they would dissolve rapidly. The in vitro aggreagation of the microparticles in pH 6.8 buffer thus probably would not occur in vivo.

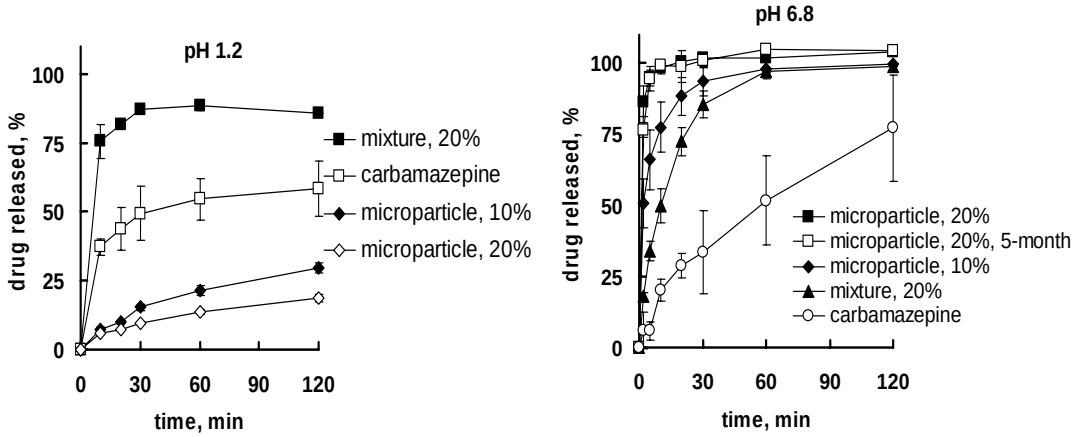


Figure 1.22 Carbamazepine dissolution/release profiles of Eudragit® L100-55 microparticles, microparticles stored for 5 months, of a physical mixture, and of carbamazepine in pH 1.2 0.1 N HCl and pH 6.8 phosphate buffer under sink conditions.

The greatly enhanced dissolution rate of carbamazepine from the microparticles compared to micronized drug and or a physical mixture (Fig. 1.22) can be attributed to its physical state in the enteric matrix. The drug is dissolved (moleculary dispersed) in the enteric matrix and thus immediately in solution upon dissolution of the enteric polymer. In addition, the microparticles had better wetting than the pure drug, the

polymer or the physical mixture as indicated by a lower contact angle (Table 1.7). The lower contact angle of the microparticles when compared to the pure polymer powder could possible be caused by the surface-adsorbed polymeric stabilizer HPMC. The faster dissolution of carbamazepine from the physical mixture compared to the pure drug was mainly due to the fine dispersion of the drug particles in the polymer particles, which separated the drug particles and thus resulted in better wetting and prevention of aggregation tendency. The unchanged release profile of microparticles after 5 months storage indicated that carbamazepine was physically stable in the enteric matrix which was confirmed by X-ray study (Fig. 1.22, Fig. 1.10).

Table 1.7: Contact angles of different compressed powders with pH 6.8 phosphate buffer (n = 6)

Compacts	Contact angle (°)
Eudragit® L100-55	35 ± 2
Carbamazepine	52 ± 4
Physical mixture (20% w/w drug)	38 ± 3
Microparticles (20% w/w drug)	16 ± 3

Next, the release study was performed under non-sink conditions at a drug amount 12 times in excess of its solubility (126.7 µg/ml). Carbamazepine was rapidly released in pH 6.8 phosphate buffer from the drug-loaded enteric microparticles because of the small particle size and instant dissolution of the enteric polymer. A supersaturated solution formed intially and was then followed by drug crystallization (Fig. 1.23, Fig. 1.24). In vivo, the BCS Class II drug with low solubility and high permeability could be rapidly absorbed from the supersaturated solution, thus avoiding the problem of drug crystallization. Needle-shaped crystals precipitated out of the supersaturated solution (Fig. 1.24); the concentration of dissolved drug then approached the concentration, which obtained during release studies with physical mixtures of drug and Eudragit® L100-55. The concentration of drug (about 300 µg/ml) was higher than its solubility in pH 6.8 phosphate buffer (126.7 µg/ml), indicating that aqueous solution of Eudragit® L100-55 increasing the solubility of carbamazepine. The increase in solubility of carbamazepine by Eudragit® L100-55 can probably be explained by the formation of soluble complexes between the enteric polymer and the lipophilic drug. Rod-shaped crystals were obtained with the physical mixture after 24 h because of Ostwald ripening (Fig. 1.24).

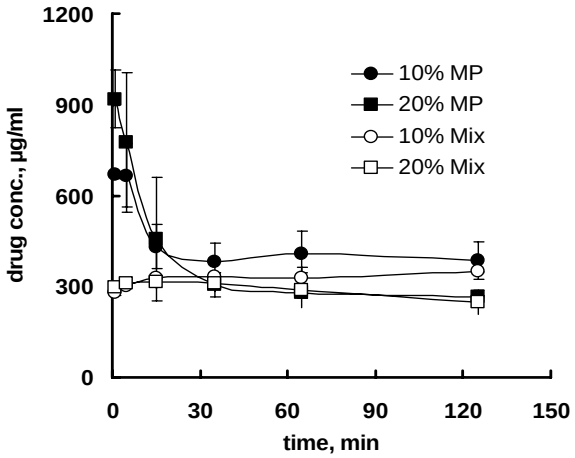


Figure 1.23 Dissolution profiles of 10% and 20% w/w carbamazepine-loaded enteric microparticles and physical mixtures in pH 6.8 phosphate buffer under non-sink conditions.

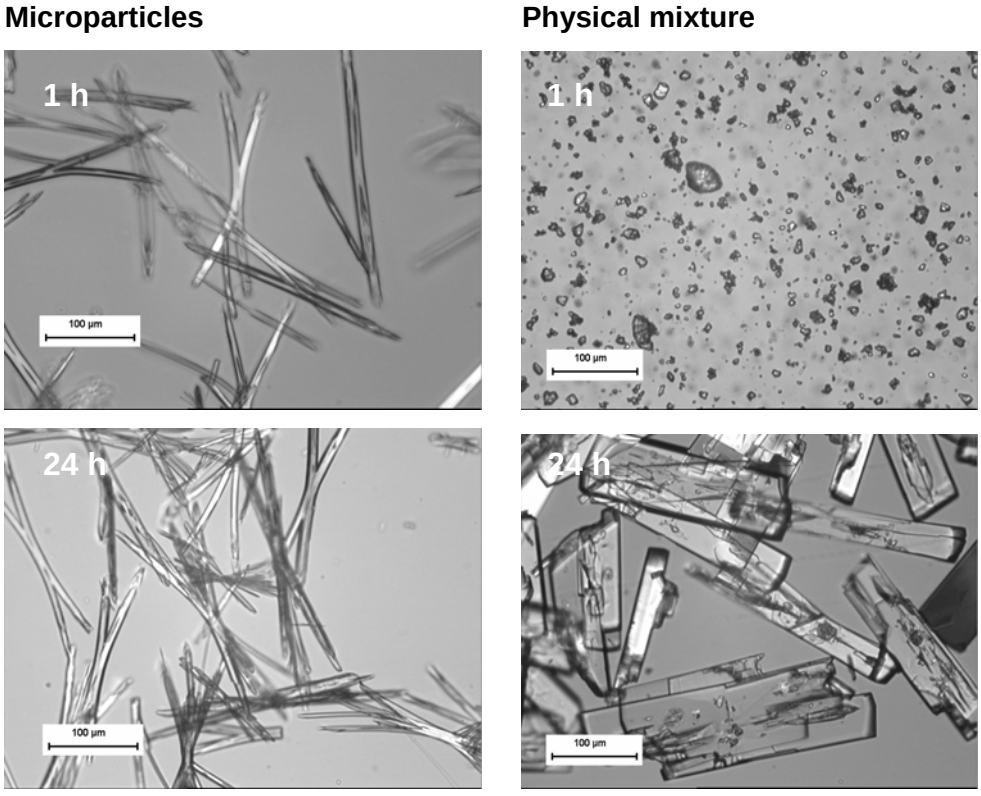


Figure 1.24 Carbamazepine crystal formation from 20% w/w carbamazepine-loaded enteric microparticles and a physical mixture after 1 h and 24 h in pH 6.8 phosphate buffer under non-sink conditions.

1.3.2.2. In vivo study

A HPLC method was used to determine of carbamazepine and carbamazepine-10,11–epoxide simultaneously in plasma by using phenytoin as an internal standard (Nagasawa et al., 2002). The retention times of carbamazepine-10,11–epoxide, phenytoin and carbamazepine were 4.1, 5.5 and 8.1 min, respectively, with the excellent resolution of carbamazepine-10,11–epoxide/phenytoin (Rs = 2.1) and phenytoin/ carbamazepine (Rs = 3.7). The calibration curves for carbamazepine (0.1-5.0 µg/ml) and carbamazepine-10,11–epoxide (0.5-7.5 µg/ml) showed good linearity ($r^2 > 0.999$). Acetonitrile was used to precipitate plasma proteins. No other contamination interfered with the measurements of drug and its metabolite (Fig. 1.25).

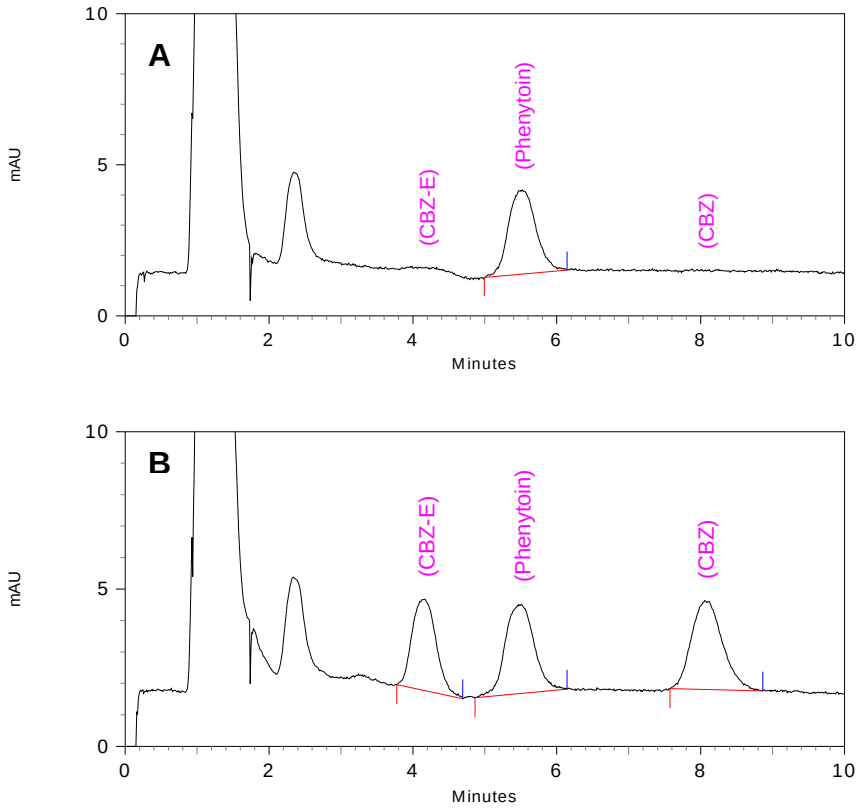


Figure 1.25 HPLC chromatograms of rabbit plasma samples of (A) predose and (B) 8 h postdose after the oral administration of a single dose of carbamazepine-loaded enteric microparticles.

The superior performance of the enteric microparticles with regard to a rapid drug release was also seen in vivo. The administration of carbamazepine-loaded microparticles resulted in an almost 5-fold enhancement in relative bioavailability when compared to the physical mixture for the reasons discussed above (Fig. 1.26, Fig. 1.27 Table 1.8).

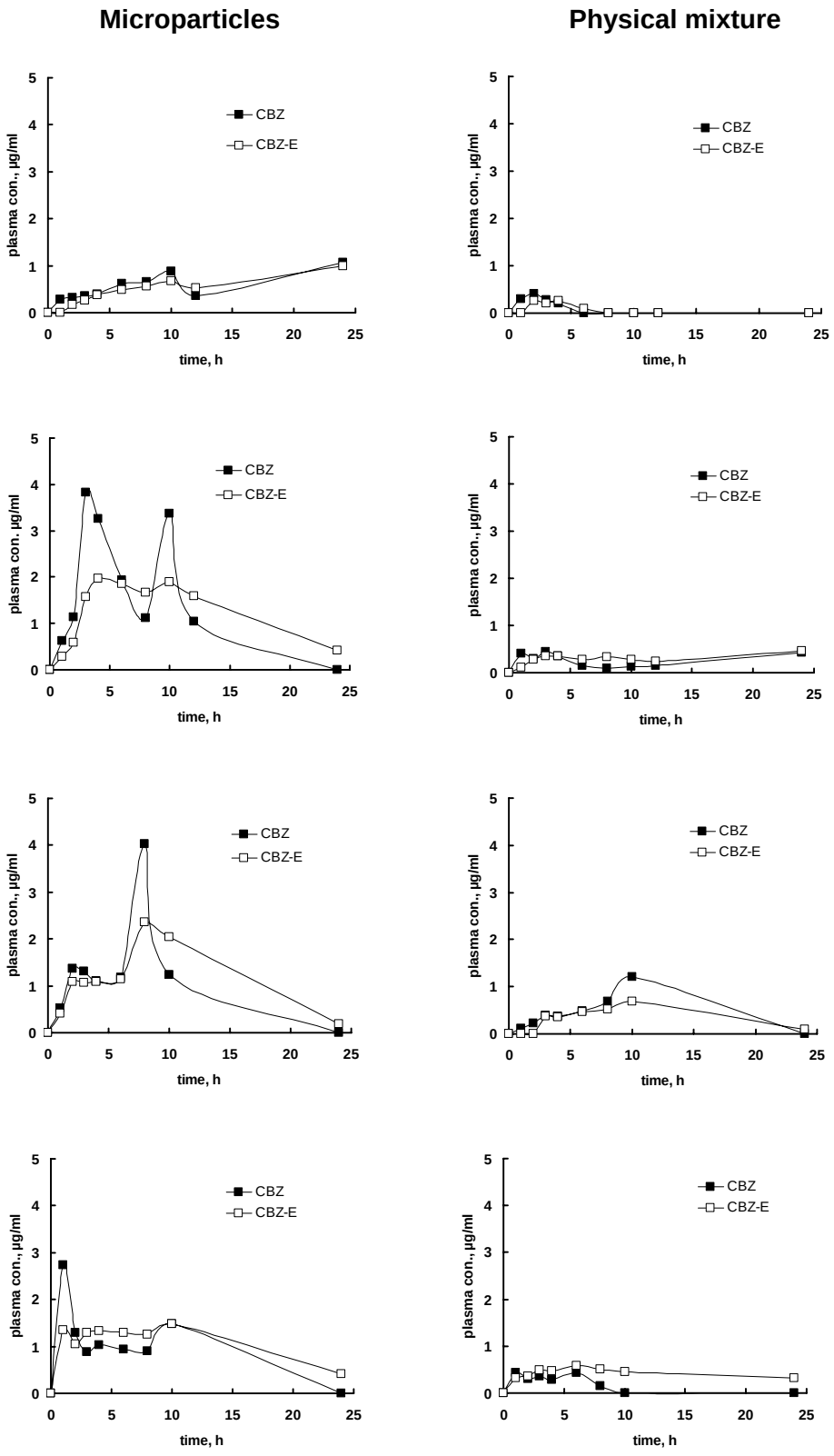


Figure 1.26 Plasma levels of carbamazepine (CBZ) and carbamazepine-10,11–epoxide (CBZ-E) in individual rabbits following the oral administration of 20% w/w carbamazepine-loaded enteric microparticles or a corresponding physical mixture.

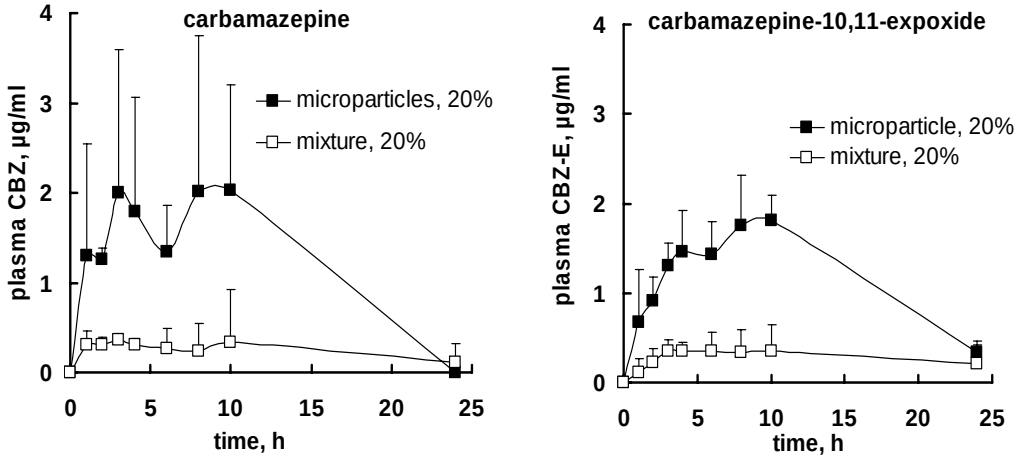


Figure 1.27 Plasma levels of carbamazepine and carbamazepine-10,11–epoxide in rabbits following the oral administration of 20% w/w carbamazepine-loaded enteric microparticles or the corresponding physical mixture.

Table 1.8: Pharmacokinetics parameters of carbamazepine and carbamazepine-10,11-expoxide after oral administration of 20% w/w drug-loaded enteric microparticles and of a physical mixture

Parameter	Carbamazepine		Carbamazepine-10,11-expoxide	
	Microparticles	Phys. mixture	Microparticles	Phys. mixture
C_{max}, µg/ml	1.74 ± 1.12	0.33 ± 0.59	1.52 ± 0.61	0.36 ± 0.29
t_{max}, h	10	10	10	10
$AUC_{0\rightarrow 24h}$, µg.h/ml	27.2 ± 3.6*	5.8 ± 4.7*	29.5 ± 2.0**	6.8 ± 3.4**

* P = 0.006; ** P = 0.005 (student t-test).

Two absorption peaks were seen in the plasma curves (Fig. 1.26, Fig.1.27). The ratio of the drug in bile and plasma are constant at 0.62, which indicated no enterohepatic circulation (Terhaag et al., 1978). The two peaks were probably caused by the pH-dependent solubility of the enteric polymer in the gastrointestinal-fluids. Carbamazpine diffused from intact microparticles in gastric fluid (low pH) possibly leading to the first peak. The remaining drug was then rapidly released upon dissolution of the polymer in intestinal fluids (high pH) leading to the second absorption peak.

A fast degradation of carbamazepine in vivo shown by the high $AUC_{0\rightarrow 24h}$ of carbamazepine-10,11–epoxide remained unclear. It might be attributed to the fast dissolution and absorption of carbamazepine in the upper GI-tract, thus resulting in a higher hepatic first pass effect (El-Zein et al., 1998).

Because of the similar experimental conditions adopted, the summation of $AUC_{0\rightarrow 24h}$ of carbamazepine and carbamazepine-10,11–epoxide of the present

CBZ/Eudragit® L100-55 microparticles (56.7 µg.h/ml) are comparable with CBZ/HP-β-cyclodextran complex (83.1 µg.h/ml), CBZ/phospholipids (L-α-dimyristoyl phosphatidyl glycerol, DMPG) coprecipitates (59.7 µg.h/ml), CBZ/PEG 6000 solid dispersion (50.8 µg.h/ml) and commercial Tegretol® suspension (43.9 µg.h/ml) as reported previously (El-Zein et al., 1998). Although the difference could not be estimated accurately, it is likely that the bioavailability of carbamazepine-loaded enteric microparticles is higher than the commercial suspension and similar to CBZ/DMPG coprecipitates and CBZ/PEG 6000 solid dispersions.

2. In-situ forming microparticles

2.1. Parenteral in-situ forming microparticles

In-situ forming microparticles (ISM) consisted of an internal phase (drug-containing polymer solutions or suspensions) and a continuous phase (oil and viscosity enhancer and emulsifier). The two phases were separately stored in two-chambered syringes and mixed through a connector before parenteral administration. It is crucial to reduce the size and increase the wettability of drug particles in the internal phase to minimize the initial release and modulate the following drug release.

Heparin, a polydispersed glycosaminoglycan, is a powerful anticoagulant indicated for the prevention of deep venous thrombosis and pulmonary embolism in high-risk patients. The development of parenteral controlled release formulation is highly desirable, not only to improve patient convenience and compliance, but also to allow for the use of heparin for other clinical indications. In the present study, heparin ISM was developed, methods such as ball milling, wetting and co-lyophilization in reducing heparin particles were evaluated and subsequent drug releases were compared.

Ball milling. Heparin (10% w/w) was micronized either alone or with RG 502H powder by ball-milling under a liquid nitrogen environment. The resultant heparin powder was suspended in a solution of 40% w/w RG 502H in *N*-methyl-2-pyrrolidone (NMP). The blend of heparin and RG 502H was dispersed in NMP until the polymer dissolved. Heparin particles were inhomogeneously suspended in the polymer solutions with big particles (> 10 μm) and aggregates of small particles (Fig. 2.1 A, B) possibly caused by amorphous state and poor wettability of the drug particles. A high initial drug release in pH 7.4 phosphate buffer was observed from the resulting ISM (Fig. 2.2). This burst release was mainly from the big heparin particles or particles embedded on the surface of the polymeric matrix. Ball milling was therefore not efficient in pulverizing heparin, and the obtained drug particles also tended to aggregate due to the poor wettability in polymer solutions.

Wetting method. Heparin aqueous solution containing 10% v/v ethanol as wetting agent was added to RG 502H powder. The blend was dried and suspended in NMP. A homogenous rod-like heparin suspension was observed after solubilization of the polymer (Fig. 2.1 C). The small size of heparin particles led to a relatively low initial release (Fig. 2.2).

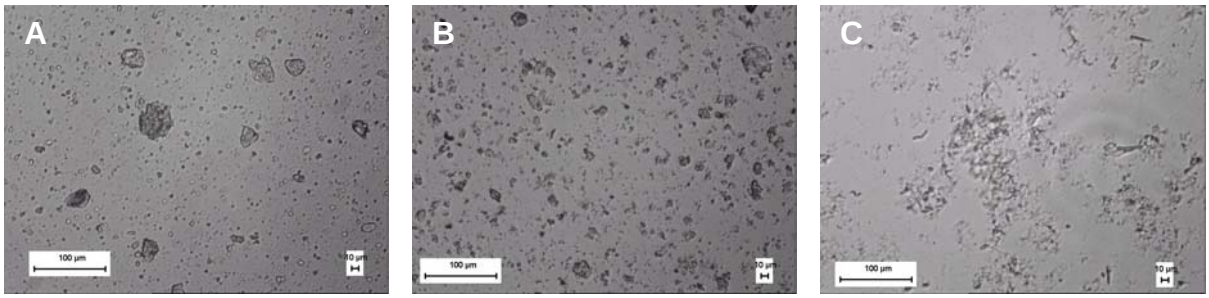


Figure 2.1 Photographs of micronized heparin by (A) ball-milling of drug, (B) ball-milling of blend of drug and RG 502H powder, and (C) wetting method in a solution of 40% (w/w) RG 502H in NMP.

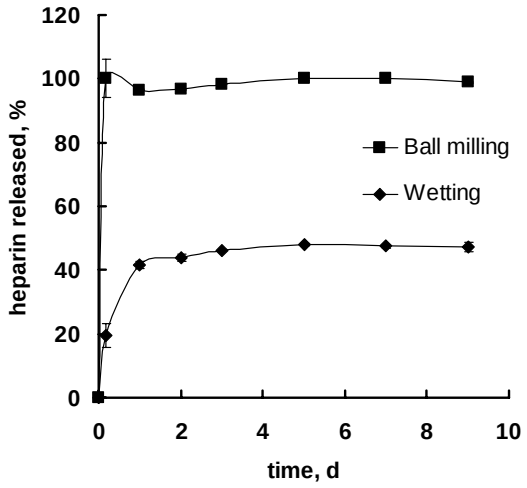


Figure 2.2 Release profile of heparin-loaded in-situ forming microparticles by micronizing heparin with ball milling and wetting method [internal phase: 10% w/w heparin and 40% w/w RG 502H in NMP; external phase: sesame oil with 2% Span 80; phase ratio: 1/2].

Co-lyophilization method. Heparin and amphiphilic polymers (i.e. Poloxamer 188, Poloxamer 407, PEG 1500, polyvinyl pyrrolidone (PVP 17 and PVP 12)) in mass ratio of 2/1, 1/1 and 2/3 were dissolved in water and lyophilized. A phase separation was induced by freezing-condensation, which provided a solid emulsion comprised of spherical heparin particles dispersed in a continuous amphiphilic polymer phase (Morita et al., 2001). Upon dispersing the solid emulsions in polymer solutions (40% of RG 502H in NMP, or 20% of RG 502H in TEC), the heparin particles were uniformly

suspended in the polymer solutions after the dissolution of the continuous amphiphilic polymer phase (Fig. 2.3).

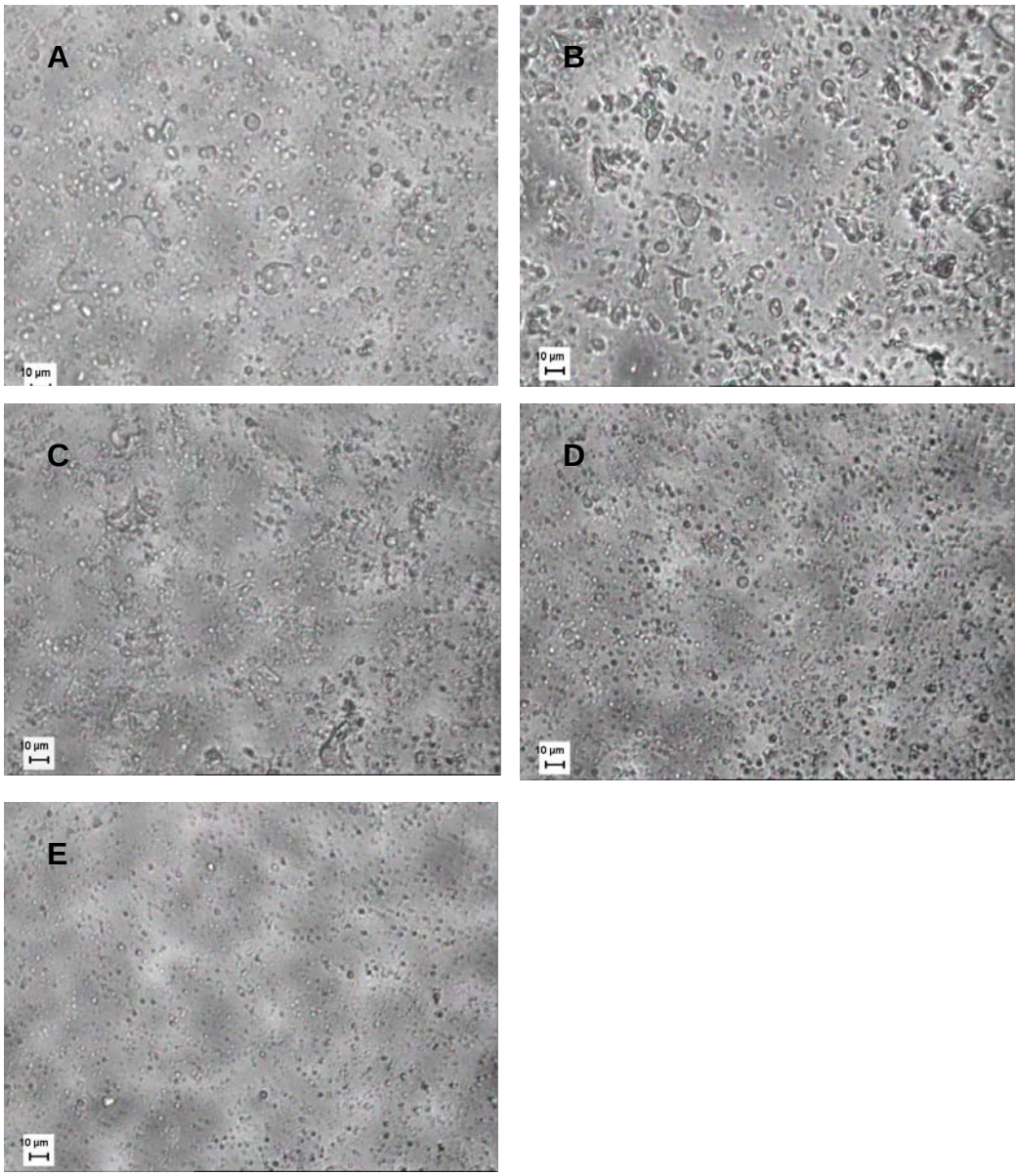


Figure 2.3 Photographs of heparin suspensions after dispersing co-lyophilizates of the drug with (A) Poloxamer 188, (B) Poloxamer 407, (C) PEG 1500 in 40% of RG 502H NMP solutions, (D) PVP 17 and (E) PVP 12 in 20% of RG 502H TEC solutions [heparin/amphiphilic polymer, 1/1].

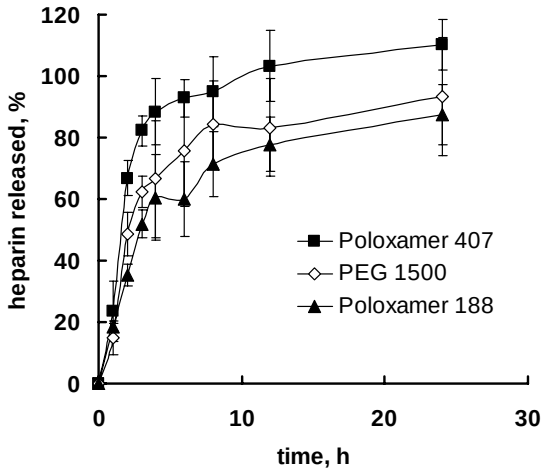


Figure 2.4 In vitro release profiles of heparin-loaded in-situ forming microparticles prepared with various kinds of amphiphilic polymer [internal phase: 10% heparin, 10% amphiphilic polymers, and 40% RG 502H in NMP; external phase: sesame oil with 2% Span 80; phase ratio: 1/2] (n=3).

Amphiphilic polymers such as Poloxamer 188, Poloxamer 407, PEG 1500, PVP 17 and PVP 12 were evaluated as adjuvants in the micronization of heparin by co-lyophilization phase separation method. At mass ratio of 1:1, heparin microparticles less than 10 µm in diameter were obtained by using PVP 17 and PVP 12 as colyophilate agents (Fig. 2.3 D, E). Irregular and inhomogeneous heparin particles (some particles > 10 µm) was achieved in the size order Poloxamer 407 > Poloxamer 188 > PEG 1500 (Fig. 2.3 A, B, C), which led to fast releases (> 80% in 24 h) (Fig. 2.4). The biggest heparin particles with Poloxamer 407 resulted in the fastest release. Other contributions to these fast releases might be using the water miscible solvent (NMP), low viscous polymer solution (40% RG 502H in NMP) and amphiphilic polymers acting as channeling or wicking agents.

A remarkable change of drug particle size was found by varying the ratio of heparin/PVP 17. From 1:1 to 2:3, a slight decrease of heparin particle size was observed visually with increase of PVP 17 (Fig. 2.5 B, C). However, big rod-like heparin particles were resulted at 2:1 (Fig. 2.5 A). In this ratio, the amount of PVP 17 was not sufficient as the continuous phase, subsequently big heparin particles were obtained. Heparin release rates were proportional to the drug particle size in polymer solutions (Fig. 2.5, Fig. 2.6). The release of heparin was slightly affected by the type of PVP, a faster and constant release was observed with PVP 17 than with PVP 12 at ratio of 1:1

due to relatively bigger heparin particles by using PVP 17 as co-lyophilate agent (Fig. 2.5 B, D).

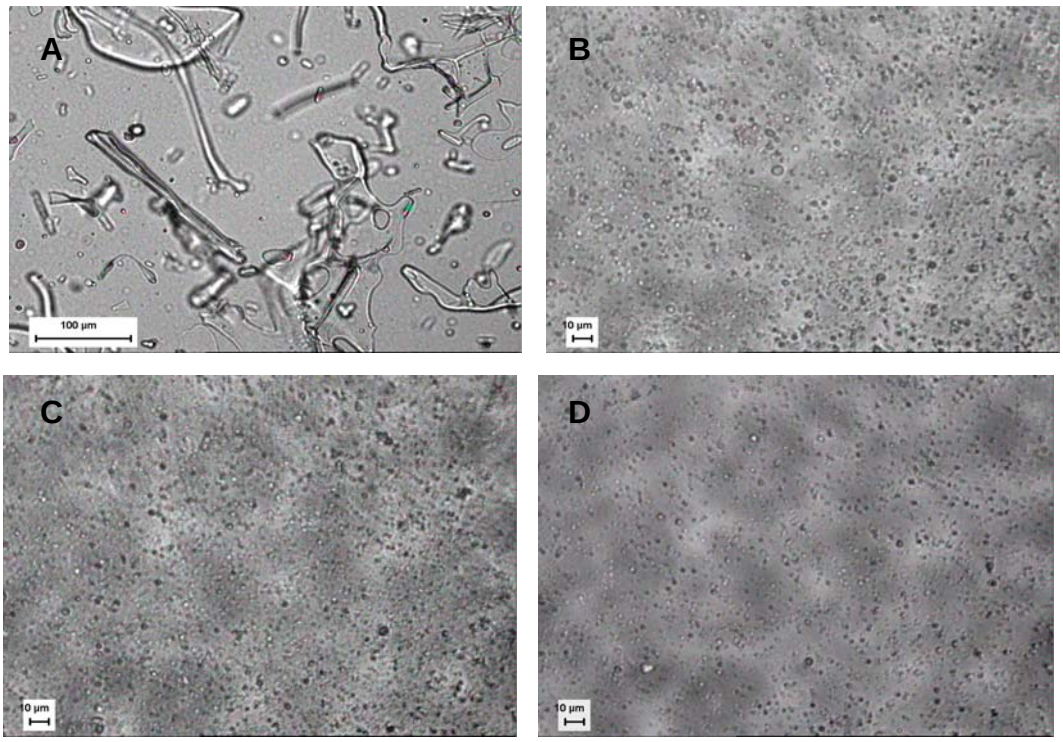


Figure 2.5 Influence of heparin/PVP 17 ratio of (A) 2:1, (B) 1:1, (C) 2:3 and heparin/PVP 12 (D) 1:1 on heparin particle size in a solution of 20% of RG 502H in triethyl citrate.

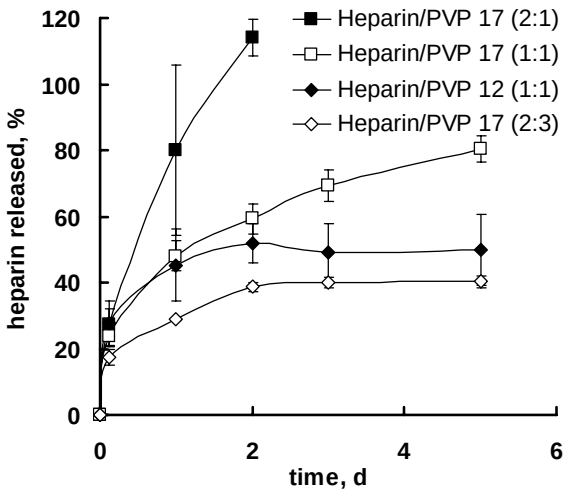


Figure 2.6 Effect of heparin/PVP ratio on drug release from in-situ forming microparticles [internal phase: 10% heparin and 10% PVP in 20% RG 502H TEC solution; external phase: sesame oil containing 2% Span 80; phase ratio: 1/2] (n=3).

A constant heparin release for 5 days by using heparin/PVP 17 (1:1) was obtained (Fig. 2.6). This release profile was predominantly diffusion-controlled because erosion of RG 502H (PLGA) does not start in 5 days (Von Burkersroda et al., 2002). Several factors contributing to this constant release pattern included (i) employment of low water soluble triethyl citrate (7% water solubility) as solvent, (ii) small and non-aggregated heparin particles in polymer solution and (iii) PVP 17 acting as leaching agent. In some European countries (e.g. Germany and Austria), low-molecular povidone types with a k-value of up to 18 are approved for injection (Kollidon, 2001). They possess an excellent solubility in water and in other solvents and affinity to hydrophilic and hydrophobic surfaces which lead them to be used as colyophilate agents, wetting agents and leaching agents of heparin.

Due to the incomplete release of heparin from ISM, sesame oil or blank ISM formulation was added to the heparin solutions to validate the absorption of heparin with sesame oil or RG 502H. After 10 days storage in horizontal shaker (80 rpm, 37°C), the concentration of heparin in the different media was determined and recoveries were calculated (Fig. 2.7). Heparin was not absorbed by sesame oil or blank ISM formulation in phosphate buffer and stable in the storage period. This revealed that no heparin lost or absorbed on the surface of polymer microparticles once released into phosphate buffer.

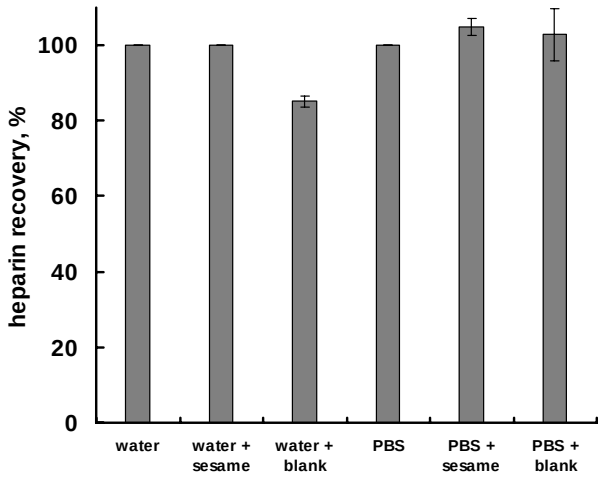


Figure 2.7 Recovery of heparin in water and phosphate buffer in the presence of sesame oil and blank ISM formulation after 10 days at 37°C.

As a conclusion, heparin-loaded ISM (O/O) was developed. Approaches to reduce heparin particle size were evaluated, including ball milling, wetting and co-lyophilization phase separation. Heparin particles were successfully micronized by the co-lyophilization phase separation method. Polyvinyl pyrrolidones (PVP 17 and PVP 12) were the most efficient co-lyophilate agent in reducing heparin particle size. Small heparin particles were homogenously dispersed in a polymer solution and a constant heparin release for 5 days was resulted.

2.2. Oral in-situ forming microparticles

Micro-/nanoparticles had been extensively investigated to entrap macromolecules and lipophilic drugs to promote their oral bioavailability (Andrianov and Payne, 1998). However, the complicated manufacturing procedure was the major limitation. It is desired to develop a simple method to form microparticles. In the present study, based on the concept of parenteral in-situ forming microparticles, oral in-situ forming microparticles were formulated and evaluated.

2.2.1. Selection of excipients

Polymers. RG 502H (PLGA) was chosen for oral application. Its low molecular weight, low inherent viscosity and uncapped carboxyl group led to a rapid water uptake and hydrolysis. Subsequently, it provided a relatively fast drug release than PLA or other high molecular weight grade of PLGA. Eudragit® RS and RL, polycationic

polymers, were used alone or blending with PLGA. The positively charged characteristics of these polymers potentially promoted the interaction of resultant microparticles with the negatively charged intestinal epithelium cells, accordingly enhancing the bioadhesion and bioavailability of macromolecule drugs loaded microparticles (Jiao et al., 2002B).

Solvents. The most frequently utilized solvents for oral administration are propylene glycol, glycerol, polyethylene glycol 400 (PEG 400) and ethanol (Strickley, 2004). Eudragit® RS/RL and Eudragit® L100-55 are soluble in ethanol. PLGA is soluble but unstable in PEG 400. Triacetin and triethyl citrate (TEC), the commonly used plasticizers for coating, are alternative solvents for PLGA.

Dimethyl sulfoxide (DMSO) is one of the least toxic organic chemicals known. It is a natural substance derived from wood pulp and exists in nature. It could be used as the solvent for PLGA and Eudragit® as well.

External oil. The oils used in commercially available oral formulations include the long-chain triglycerides (peanut oil, corn oil, soybean oil, sesame oil, olive oil, peppermint oil, hydrogenated vegetable oils, hydrogenated soybean oil) and the medium chain triglycerides (MCT) derived from coconut and palm seed oils. Other water-insoluble excipients include beeswax, vitamin E and oleic acid (Strickley, 2004). The preliminary studies showed that unstable emulsions were obtained by using liquid oils as external phase. A quick phase separation occurred after the emulsification. Therefore, a semilsolid external phase was desired to prevent the phase separation and to ensure the stability of the resultant emulsions.

2.2.2. Oral ISM using PLGA

Oral ISM was prepared by emulsifying an internal phase of PLGA (RG 502H, 20% w/w) in triacetin or mixture of triacetin and PEG 400 to an external phase (Gelucire 35/10 or Gelucire 44/14) at 60°C under homogenizing at 8,000 rpm (Table 2.1). The resulting O/O emulsions were stored in a refrigerator or ice bath in semisolid state (Fig. 2.8 A).

By using PEG 400/triacetin (1:1), an agglomeration of polymeric particles was observed upon dispersing the obtained semisolid emulsions in 0.1 N HCl (Table 2.1, formulation C). Gelucire 35/10 as external oil phase led to bigger particle size compared to Gelucire 44/14 (Table 2.1, formulation B), this type of Gelucire can not form a

dispersion instantly upon contact with 0.1 N HCl, therefore internal droplets agglomerated with water diffusion.

Table 2.1: Effect of solvent and Gelucire on the formation of microparticles.

Formulations	A (%)	B (%)	C (%)
Internal phase			
RG 502H	8	8	8
Triacetin	32	32	16
PEG 400	-	-	16
External phase			
Gelucire 44/14	60	-	60
Gelucire 35/10	-	60	-
Particle size (0.1 N HCl, µm)	30.6 ± 9.0	96.8 ± 34.7	agglomeration

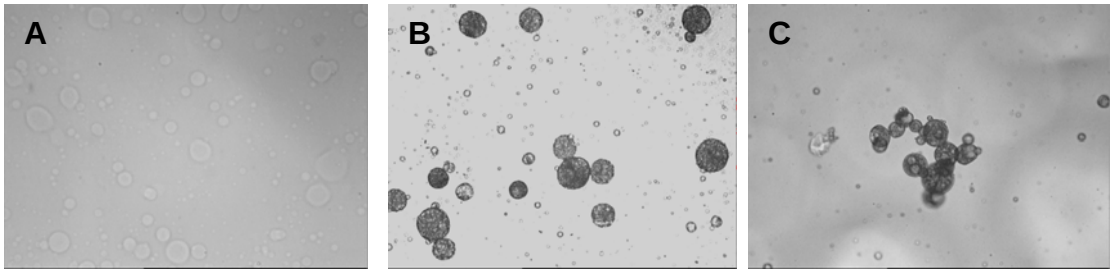


Figure 2.8 Dispersion and agglomeration of PLGA microparticles after addition of blank ISM in 0.1 N HCl at 37°C, (A) ISM, (B) individual MP and (C) agglomerated MP. [Internal phase: 20% RG 502H in triacetin; external phase: Gelucire 44/14; phase ratio: 1/2].

Gelucire 44/14 is amphiphilic in character having a melting point at 44°C (42.5-47.5°C) and a HLB value of 14. It is derived from the reaction of hydrogenated palm kernel oil with PEG 1500 and forms a fine dispersion in contact with GI-fluid at 37°C. These characteristics met the requirement as an external phase of ISM for oral administration, whereby a quick dispersion of the internal droplets was subsequently hardened into microparticles after solvent diffusion into water phase (Table 2.1, formulation A). However, an agglomerated PLGA mass was formed due to the incomplete hardening (Fig. 2.8 B, C) possibly due to a slow solvent diffusion into the aqueous phase. Therefore, triacetin was replaced by a more polar solvent triethyl citrate (TEC). As reported, incorporation of Tween 80 in polymer solution facilitated the maintenance of the PLGA microglobule spherical shape and allowed efficient extraction

of the vehicles in water. This resulted in hardening of PLGA microglobules into discreet and deaggregated microspheres (Jain et al., 2000).

Primary study showed that incorporation of Tween 80 in the internal phase did not prevent the agglomeration trend. As previously reported, a positively charged self-emulsifying oil formulation (SEOF) for improving oral bioavailability of progesterone are developed and characterized containing a cationic lipid, oleylamine (OA). The positive charge of the formulation is attributed to the localization of the cationic lipid at the oil/water interface of the diluted SEOFS (Gershanik and Benita, 1996). Therefore, in this study, 5% w/w oleylamine was incorporated in the internal phase to produce positively charged microparticles to prevent agglomeration by electrostatic repulsion and improve the interaction of the microparticles with negatively charged intestinal M cells. The composition of formulations was shown in Table 2.2.

Table 2.2: Composition of ISM formulations by using different type of PLGA and incorporation of Tween 80 and oleylamine.

Formulations	A (%)	B (%)
Internal phase		
PLGA	20 (RG 502H)	20 (RG 503)
Tween 80	5	5
Oleylamine	5	5
Triethyl citrate	70	70
External phase	Gelucire 44/14	Gelucire 44/14
Phase ratio	1:2	1:2

By addition of 5% of Tween 80 and 5% of oleylamine in the internal polymer solution, small particles (< 5μm) were obtained using RG 502H. The average particle size was 1.18 μm measured by laser diffraction. A dramatic increase of particle size was found using RG 503 (53.76 μm) (Fig. 2.9). This can be attributed to a higher inherent viscosity and hydrophobicity of RG 503 compared to RG 502H. The particle surface zeta potential was pH-dependent, a positive charge in 0.1 N HCl (Fig. 2.10 A) and a negative charge in pH 7.4 phosphate buffer (Fig. 2.10 B). At pH 1.2, the carboxylic acid groups of RG 502H were protonated, the cationic lipid (oleylamine) located on the surface of particles leading to the positive charge properties. At pH 7.4, the deprotonated carboxyl groups resulted in negative charge surface of microparticles. However, the different zeta potential of RG 502H and RG 503 in phosphate buffer

might be due to the particle size effect or less residual oleylamine remained on the surface of RG 503 microparticles.

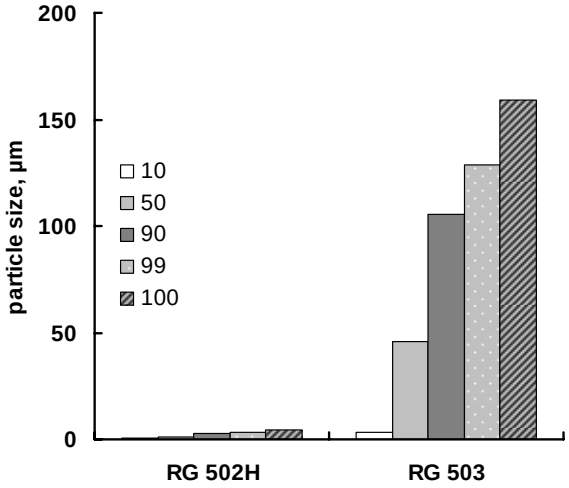


Figure 2.9 Effect of PLGA type on the particle size after dispersing blank formulations in 0.1 N HCl at 37°C [Internal phase: 20% of RG 502H (or RG 503) in TEC containing 5% of Tween 80 and 5% of oleylamine; external phase: Gelucire 44/14; phase ratio: 1/2].

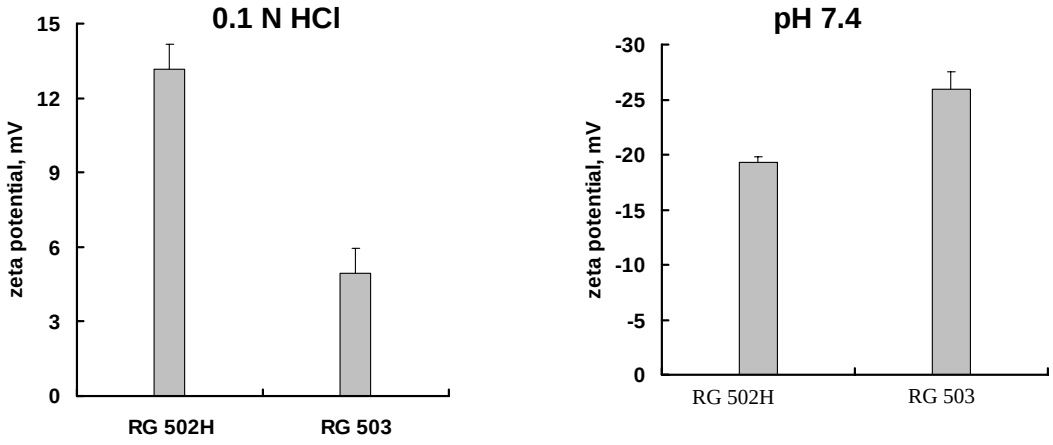


Figure 2.10 Effect of PLGA type and dispersion media on the surface zeta potential of resultant particles [internal phase: 20% of RG 502H (or RG 503) in TEC containing 5% of Tween 80 and 5% of oleylamine; external phase: Gelucire 44/14; phase ratio: 1/2].

No remarkable difference of the average particle size and the zeta potential of the solidified microparticles in 0.1 N HCl was found by increasing oleylamine from 3 to 10% (Fig. 2.11). The amount of oleylamine entrapped in the solidified microparticles

might be limited, and the excess portion diffused in aqueous phase and did not play a role in the formation of microparticles. Considering the irritation of oleylamine, it is optimal to use it as less as possible. A slight increase of particle size was observed by increasing polymer concentration from 20% to 30% w/w (Fig. 2.12).

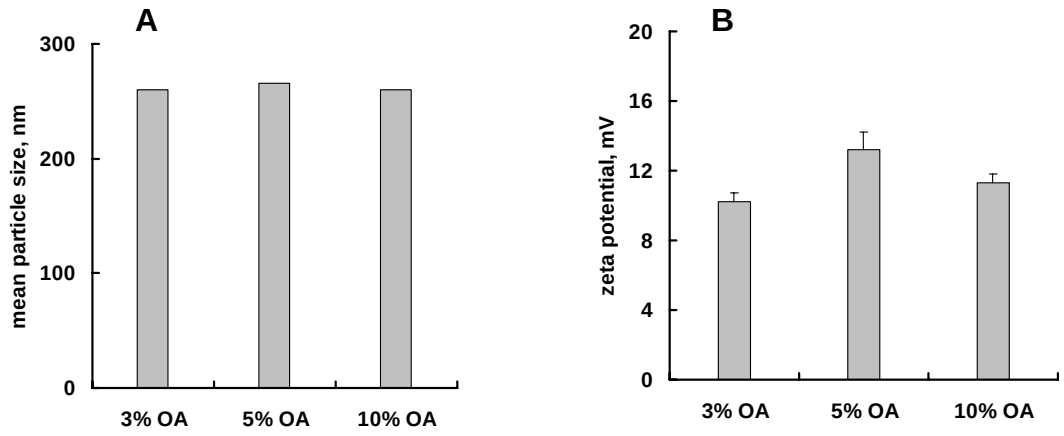


Figure 2.11 Effect of oleylamine (OA) concentration in the internal polymer solution on (A) the mean particle size and (B) surface zeta potential after dispersing the ISM in 0.1 N HCl measured by PCS [Internal phase: 20% of RG 502H in TEC containing 3-10% of oleylamine; external phase: Gelucire 44/14; phase ratio: 1/2].

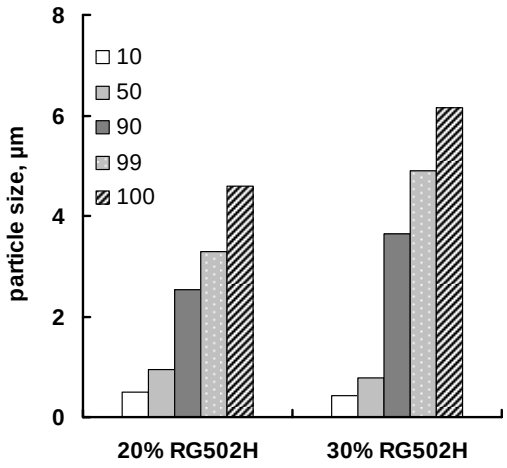


Figure 2.12 Effect of RG 502H concentration on particle size distribution after dispersing ISM in 0.1 N HCl at 37°C [Internal phase: 20% and 30% w/w of RG 502H in TEC containing 5% w/w Tween 80 and 5% w/w oleylamine; external phase: Gelucire 44/14; phase ratio: 1/2].

In summary, a semisolid ISM was developed and evaluated by dispersing in 0.1 N HCl and pH 7.4 phosphate buffer. The size of solidified PLGA (RG 502H) microparticles was reduced by incorporation of Tween 80 and oleylamine in the internal polymer phase, with size less than 5 µm. Therefore, this system might be potentially used as a carrier for poorly water soluble drugs or macromolecule drugs.

2.2.3. Oral ISM using Eudragit® RS/RL

Another approach to obtain positively charged microparticles was to use polycationic polymers (Eudragit® RS or RL). After dispersing an oral ISM in 0.1 N HCl solution composed of 30% Eudragit® RS/RL in EtOH as internal polymer phase and Gelucire 44/14 as external phase, the obtained particles were in nano range (200-300 nm, Fig. 2.13 A) with positively charged surface in both 0.1 N HCl and pH 7.4 phosphate buffer (Fig. 2.13 B). Phase ratio of polymer solution to Gelucire 44/14 greatly affected the mean particle size but not on surface charge, the lower ratio of the polymer solution the smaller was the particle size (Fig. 2.13 A). The relatively larger particles with Eudragit® RL than Eudragit® RS was possibly because of the high permeability of Eudragit® RL to water than RS. It was not clear that surface charge of Eudragit® RL particles was lower than Eudragit® RS particles. Eudragit® RL possesses more ammoniomethacrylate units (8.85-11.96%) on dry substance than Eudragit® RS (4.48-6.77%) (Fig. 2.13 B).

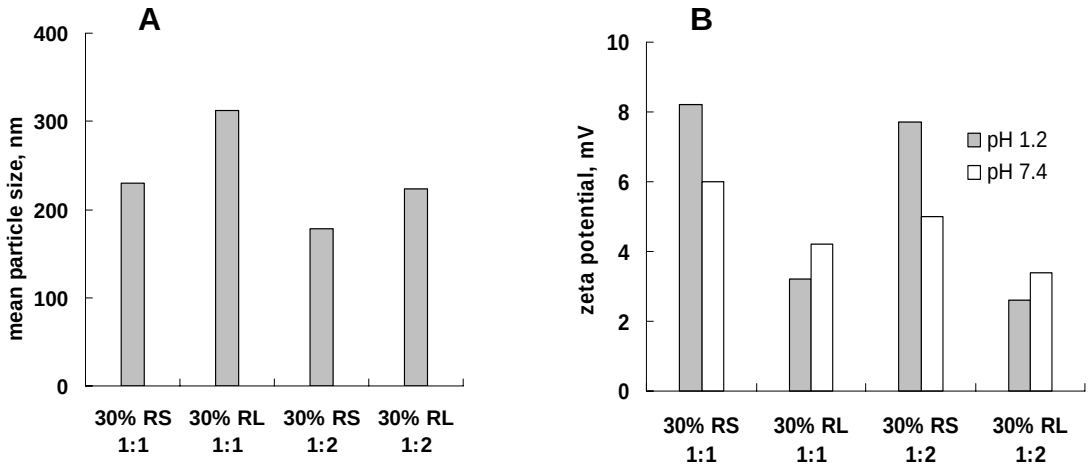


Figure 2.13 Effect of polymer type and phase ratio on (A) mean particle size and (B) surface zeta potential in different media.

Two lipophilic drugs, indomethacin and carbamazepine (10% w/w loading), were dissolved in 30% Eudragit® RS/RL (1:1) ethanolic solution and emulsified into Gelucire 44/14 at a ratio of 1:2. The resulting ISM displayed fast drug release profiles in pH 7.4 phosphate buffer (Fig. 2.14). These quick releases resulted mainly from the fast solvent and drug diffusion from polymeric nanoparticles. Incomplete release profiles were found with indomethacin loaded ISM (78% released) and carbamazepine blank formulation. In the case of indomethacin, the electrostatic interaction of drug (negative charge after deprotonation of the carboxylic group) with positively charged polymers might contribute to the incomplete drug release profile.

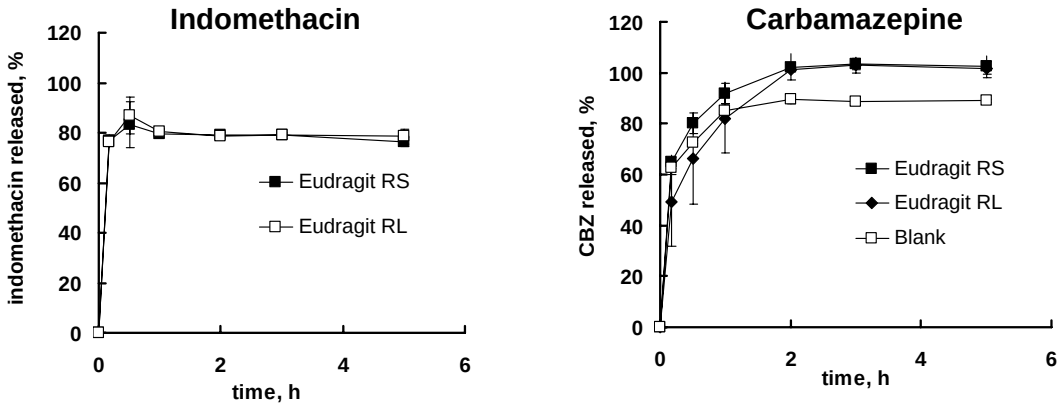


Figure 2.14 Lipophilic drugs indomethacin and carbamazepine release profiles in oral ISM systems [internal phase: 30% w/w Eudragit® RS/RL containing 10% w/w drug; external phase: Gelucire 44/14; phase ratio: 1/2].

2.2.4. Oral ISM using blend of PLGA and Eudragit® RS/RL

Polymer blends combine the advantages of two polymers: one biodegradable polymer giving protection from acidic and enzyme environment and the other contributing positive charge to enhance the interaction of particles and epithelium cells. By varying the blend ratio, the particle size, the surface charge and the drug release may be effectively adjusted. As reported, polymeric micro-/nanoparticles containing unfractionated heparin were developed with polycationic polymethacrylate (Eudragit® RS and RL) alone or blend with biodegradable polymers (poly-ε-caprolactone, (PCL) and PLGA) (Jiao et al., 2001; 2002A). A 2-fold increase in the activated partial thromboplastin time (APTT) was observed with heparin-loaded microparticles

confirming the release of integrity heparin from microparticles as well as its absorption from the GIT, leading to increase in bioavailability (43% to 48%) (Jiao et al., 2002B). In the present study, an oral heparin-loaded ISM delivery system was developed using blend of polycationic polymethacrylate (Eudragit® RS and RL) and PLGA (RG 502H) in ratios of 2:1, 1:1 and 1:2. The internal microglobules after emulsifying into external Gelucire 44/14 were shown (Fig. 2.15). The higher ratio of Eudragit® RS/RL, the smaller was the droplets owing to low viscosity of Eudragit® RS/RL solution than RG 502H.

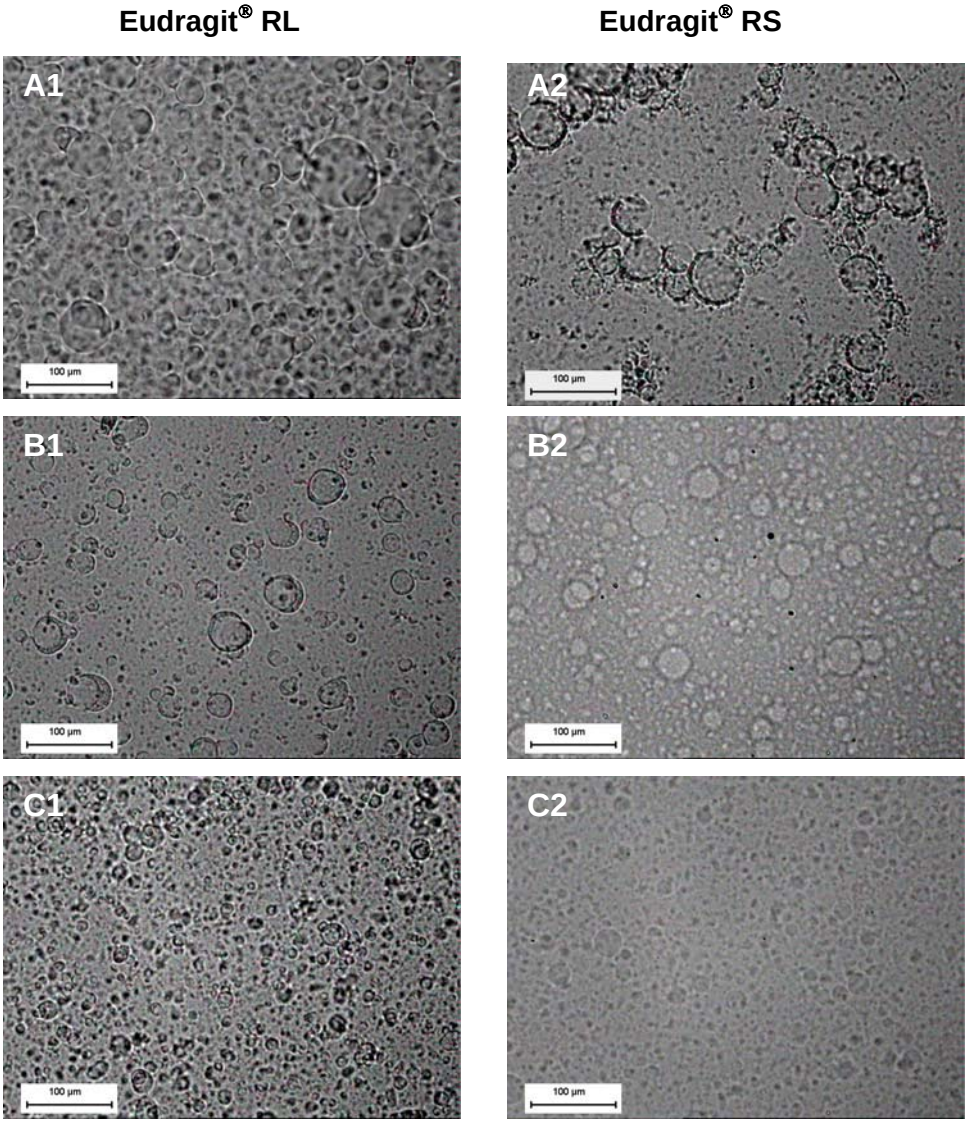


Figure 2.15 Photographs of blank ISM with ratio of RG 502H to Eudragit® RL or RS at (A) 2:1, (B) 1:1 and (C) 1:2.

A remarkable difference of heparin release using blends of RG 502H and Eudragit® RL at different ratios was observed (Fig. 2.16 A) in contrast to blends of RG 502H and Eudragit® RS (Fig. 2.16 B). The higher content of Eudragit® RL the slower drug release because Eudragit® RL possessed more ammoniomethacrylate units (8.9-12.0%) than Eudragit® RS (4.5-6.8%).

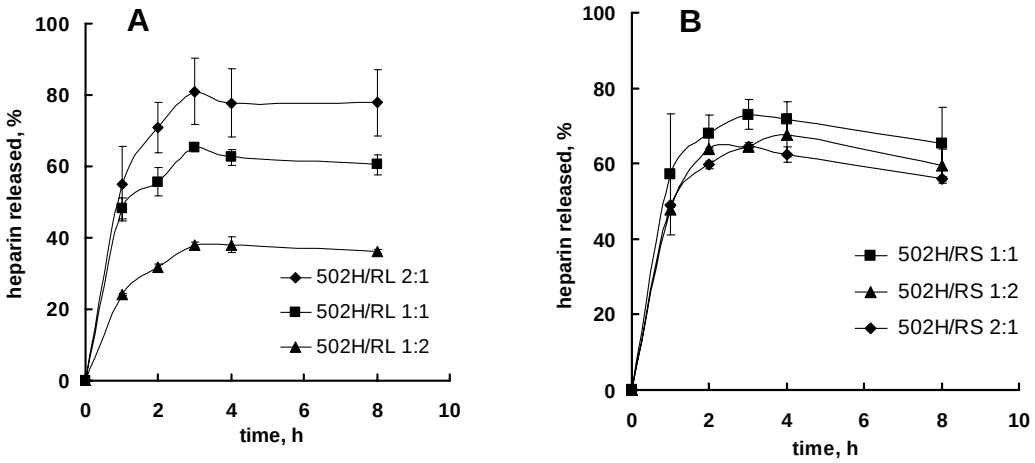


Figure 2.16 Heparin release profiles from ISM using blends of RG 502H and (A) Eudragit® RL and (B) Eudragit® RS at different ratio in pH 7.4 phosphate buffer [Internal phase: heparin (10%, w/w) in 30% of polymer blend in DMSO; external phase: Gelucire 44/14; phase ratio 1/2].

3. Stability of PLGA in in-situ forming systems

In-situ forming biodegradable drug delivery systems represent an attractive alternative to biodegradable implants and microparticles. These liquid formulations form solid/semisolid depots after sc or im injection by solvent diffusion or temperature change. Thermoplastic pastes, in-situ cross-linking implant, in-situ polymer precipitation and thermally induced gelling systems have been used to prepare in-situ forming parenteral drug depots (Packhaeuser et al., 2004; Hatefi and Amsden, 2002). In-situ forming systems have the following advantages compared to the preformed biodegradable implants and microparticles: (i) ease of administration and (ii) less complicated fabrication and less stressful manufacturing conditions for sensitive drug molecules.

In-situ forming implant- and microparticles (ISM)-systems based on biodegradable polymers polylactides (PLA) or poly(lactide-co-glycolides) (PLGA) have received considerable attention over the past few years (Chandrashekar and Udupa, 1996; Dunn et al., 2004; Bodmeier, 1997; Kranz and Bodmeier, 1998). The in-situ forming implants are formed from drug-containing PLGA solutions in a biocompatible solvent (e.g., *N*-methyl-2-pyrrolidone). The polymer solutions form implants after s.c. or i.m. injection in contact with aqueous body fluids through precipitation of the polymer (Dunn and English, 2003; Chern and Zingerman, 2004; Brodbeck et al., 2004). Until now, two commercial products (Atridox® and Eligard®) have been developed by Atrix Laboratories with this technique.

Disadvantages associated with in-situ forming implant such as high injection force, local irritation at the injection site, variability in the solidification rate, irregular shape of the implants formed depending on the cavity into which the formulation is introduced, undesired initial bursts and the potential solvent toxicity have to be addressed. A novel in-situ forming microparticle system has been developed to overcome some of these problems (Bodmeier, 1997; Kranz and Bodmeier, 1998; Kranz et al., 2001; Jain et al., 2000; Bhagwatwar et al., 2003). The ISM-system consists of an internal phase (drug-containing polymer solutions or suspensions) and an external phase (oil or aqueous solutions with an emulsifier). The two phases are stored seprately in two syringes and mixed through a connector before administration. The initial release, myotoxicity and viscosity are significantly reduced with the ISM-system compared to the in-situ implant system (Kranz et al., 2001).

The degradation properties of PLGA have been extensively studied and reviewed in several publications (Holland et al., 1986; Gopferich, 1996). Studies on the degradation have shown that polymer intrinsic properties (molecular weight, chemical structure, hydrophobicity and crystallinity), release medium properties (pH, temperature, ionic strength, solvent and presence of biocatalysts or microorganisms), formulation properties (particle size, presence of zinc carbonate, plasticizer and tertiary amine based drugs) and sterilization significantly affect the degradation rate of the polyesters.

The stability of PLGA and drug in in-situ forming systems during their preparation and storage has not been investigated in detail (Wang et al., 2003). Various solvents, drugs and excipients were used in in-situ systems and polymer solutions have been heated to accelerate the dissolution of PLGA or other excipients (Jain et al., 2000; Bhagwatwar et al., 2003).

The objective of this study was to investigate the stability of PLGA and leuprolide acetate in in-situ systems and, in particular, in various biocompatible solvents and oily and aqueous solutions. The effects of storage time and temperature, water content of polymer solutions, biocompatible solvent, presence of leuprolide acetate, various oils and aqueous phases on the stability of PLGA were investigated. Due to the potential instability of the polymer and drug in organic solvents, solid PLGA sponges were developed by dissolving PLGA and drugs (leuprolide acetate, lidocaine) in acetic acid or dioxane and then lyophilized. The sponges could be reconstituted in 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 studied.

3.1. Polymer organic solution

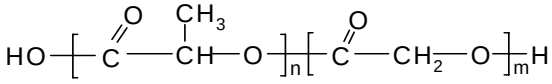
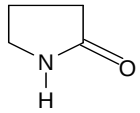
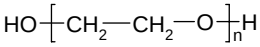
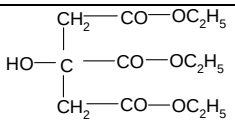
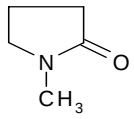
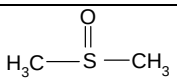
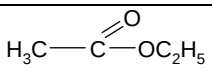
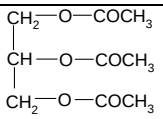
The chemical stability of PLGA and of the drug is a major concern during the development of in-situ forming biodegradable drug delivery systems. With in-situ implant systems, PLGA is dissolved in a biocompatible organic solvent (e.g. NMP) and the drug is either dissolved or suspended in the same solvent or stored separately from the polymer solution in another syringe. With in-situ forming microparticle systems, an additional external oil or aqueous phase is either in contact with the PLGA solution (O/O- of O/W-emulsion) or stored separately. Therefore, the stability of PLGA and drug in the organic solvents and in oily or aqueous suspensions has to be investigated in

order to optimize preparation processes and storage conditions for in-situ forming systems. Lyophilized PLGA sponges, which are reconstituted in the organic solvent to form liquid injectable PLGA systems just before administration, were developed as an alternative to the liquid PLGA systems to store the polymer and the drug in a dry state.

3.1.1. Selection of organic solvents for in-situ PLGA systems

Biocompatible solvents (Table 3.1), such as polyethylene glycol 400 (PEG 400), 2-pyrrolidone, triethyl citrate, *N*-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO), triacetin and ethyl acetate, have been investigated as solvents for PLGA in-situ forming systems (Dunn and English, 2003; Brodbeck et al., 2000; 2004; Kranz et al., 2001). NMP, DMSO and PEG 400 have been used in commercial injectable products for human use (Strickley, 2004) and 2-pyrrolidone in veterinary injectable products (EMEA/MRL/457/98-final, 1998). Triacetin and triethyl citrate are used in oral dosage forms and are generally recognized as safe (FDA GRAS list). Triacetin has been considered as a potential parenteral nutrient (Bailey et al., 1991). Ethyl acetate is an ICH class 3 solvent (FDA Guidance for Industry Q3C Impurities: Residual Solvents). ICH class 3 solvent may be regarded as less toxic and of low risk to human health and includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. It shows low in vitro and in vivo myotoxicity (Im-Emsap, 2002).

Table 3.1: Structures of RG 503H and organic solvents.

RG 503H		
Polar protic solvents	2-pyrrolidone (2-P)	
	Polyethylene glycol (PEG 400)	
	Triethyl citrate (TEC)	
Polar aprotic solvents	N-methyl-2-pyrrolidone (NMP)	
	Dimethyl sulfoxide (DMSO)	
	Ethyl acetate (EA)	
	Triacetin (TA)	

3.1.2. Water uptake of organic PLGA solvents during storage at 75 % relative humidity

PLGA degrades by hydrolysis. A low water content in the PLGA delivery system is therefore of utmost importance for a good storage stability. The water uptake of the organic solvents was determined during storage at elevated humidity for 2 weeks and was in the following order: DMSO (33.7%) > NMP (26.5%) > 2-pyrrolidone (23.7%)> PEG 400 (15.2%) > triacetin ≅ triethyl citrate (1.5%) (Fig. 3.1). This rank order correlated with the dielectric constant of the solvents (DMSO 48, NMP 32, 2-pyrrolidone 28, and triacetin 7; less polar solvents such as triacetin or triethyl citrate had a much lower water uptake. An excellent moisture-protective packaging is therefore of utmost importance for good storage stability of the PLGA solutions. Commercial in-situ forming implant systems (Eligard®) are therefore double pouched in aluminium foils with a desiccant bag and heat-sealed under nitrogen (Ravivarapu et al., 2000).

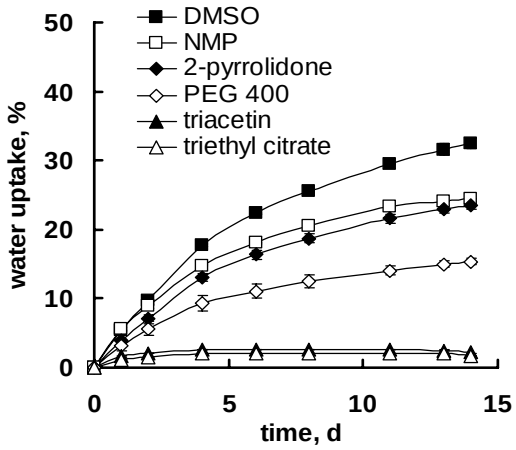


Figure 3.1 Water uptake of solvents during open storage at room temperature/75% r.h. for 2 weeks.

3.1.3. Effect of solvent type and storage temperature on PLGA stability

The degradation rate of PLGA in solutions in different organic solvents with a similar water content (0.10-0.36%) (Table 3.2) was in the following order: PEG 400 > 2-pyrrolidone > triethyl citrate > DMSO > NMP > triacetin (Fig. 3.2, Table 3.3). A slower degradation occurred in polar aprotic solvents (DMSO, NMP, triacetin) than in polar protic solvents (PEG 400, 2-pyrrolidone, triethyl citrate). The polar protic solvents are capable of donating protons and possibly forming hydrogen bonds with PLGA, consequently the ester bonds might be exposed to water in the solvents and the hydrolysis was enhanced by the high water accessibility. In contrast, the ester bonds of the polymer in polar aprotic solvent might be shielded inside the polymer chains and prevented the access of water. The fastest degradation of PLGA in PEG 400 might be due to both the high water content (0.36%) and a stronger hydrogen bond capacity of the solvent.

Table 3.2: Water content of organic solvents, PLGA RG 503H solutions (20% w/w) and PLGA RG 503H.

	Solvent (%)	Polymer solution (%)
PEG 400	0.14 ± 0.01	0.36 ± 0.06
NMP	0.07 ± 0.01	0.18 ± 0.01
2-pyrrolidone	0.06 ± 0.01	0.17 ± 0.01
Triethyl citrate	0.08 ± 0.01	0.14 ± 0.02
Ethyl acetate	0.06 ± 0.01	0.16 ± 0.06
DMSO	0.04 ± 0.01	0.12 ± 0.01
Triacetin	0.01 ± 0.01	0.10 ± 0.01
RG 503H	0.52 ± 0.01	

PLGA was stable in the solid state and in most organic solvents (except 2-pyrrolidone and PEG 400) at 4°C. The PLGA powder and solutions in triacetin, DMSO and NMP still had acceptable stability at 25°C (Fig. 3.2). As expected, higher storage temperatures increased the degradation rate. The effect of temperature on the degradation rate is generally represented by the Arrhenius equation: $k = Ae^{-Ea/RT}$, where k is the specific reaction rate, A is a constant, E_a the activation energy, R the gas constant and T the absolute temperature. The degradation rate (k) of PLGA for different polymer solutions was determined from the slope of ln%Mw vs. time (Table 3.3), this parameter allowed a good ranking of the solvents. Interestingly, the polar aprotic solvents retarded the degradation at storage temperatures of 40°C and 60°C when compared to the original PLGA powder. The PLGA powder was close to/above the glass transition temperature and therefore in the rubbery state, where degradation is accelerated when compared to the glassy state.

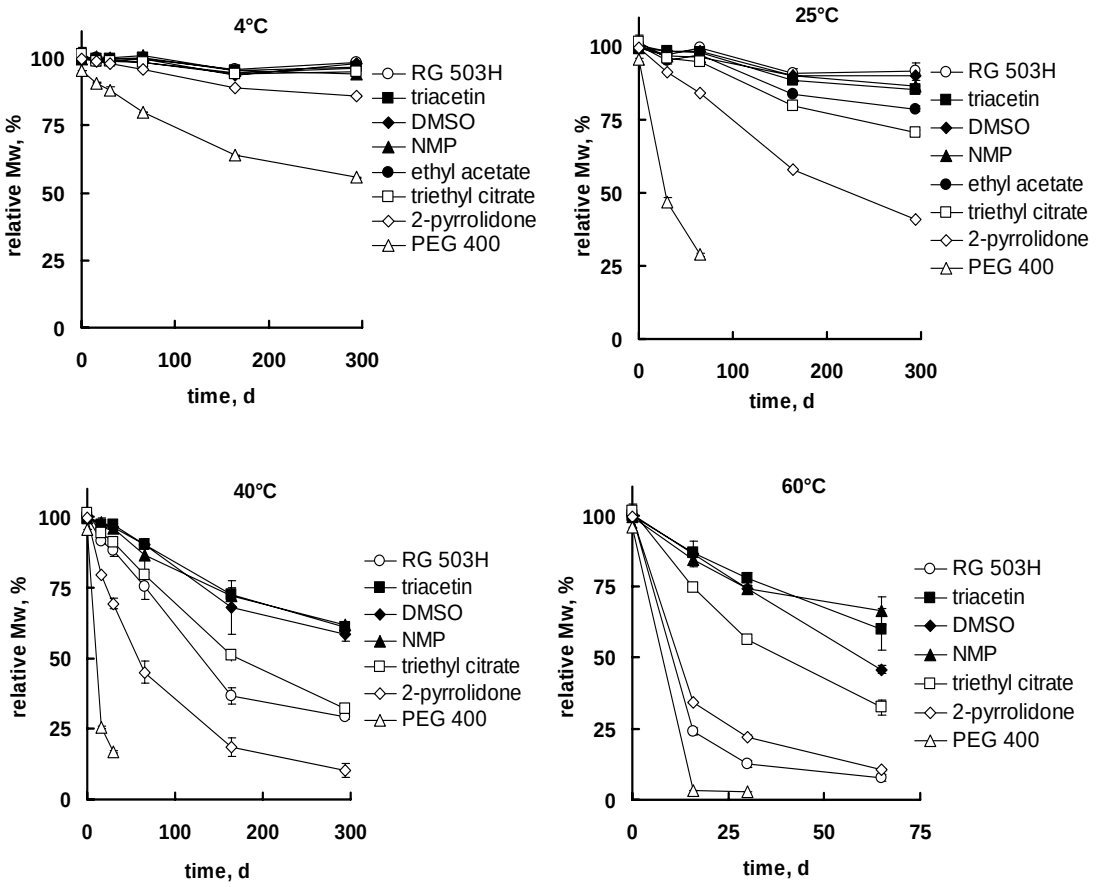


Figure 3.2 Effect of organic solvent on PLGA stability in solution at 4°C, 25°C, 40°C and 60°C.

Generally, polymers degrade either through unzipping (cleavage of the last unit at the end of the chain), or through random scission (cleavage of a bond randomly along the chain). The polydispersity index (PI) (Mw/Mn) is used as an indicator for the mode of PLGA degradation (Hasirci et al., 2001). In this study, a nearly unchanged PI at 4°C and 25°C (1.7-1.9) revealed that random chain scission was the preferred chain cleavage mechanism in most cases. Unzipping was probably the predominant degradation pathway of PLGA in 2-pyrrolidone (40°C) as indicated by the increase of PI from 1.8 to 2.2 (Fig. 3.3).

Table 3.3: PLGA RG 503H degradation rate constant ($\times 10^{-3}$, 1/days) after storage in different organic solvents in the absence and presence of 10% w/w leuprolide at different temperatures.

Solvents	No drug				10% leuprolide		
	4°C	25°C	40°C	60°C	4°C	25°C	40°C
RG 503H powder	-0.2	-0.5	-4.8	-69.6	-	-	-
Triacetin	-0.3	-0.7	-2.0	-7.9	-0.7	-3.4	-9.8
NMP	-0.3	-0.6	-2.0	-9.9	-1	-7.9	-22.1
DMSO	-0.4	-0.6	-2.4	-12.3	-3.2	-6.3	-16.8
Ethyl acetate	-0.4	-1.0	-3.4	-	-0.9	-6.8	-19.6
Triethyl citrate	-0.4	-1.4	-4.2	-17.5	-0.9	-4.7	-12.9
2-pyrrolidone	-0.7	-3.3	-10.0	-32.5	-1.6	-12.2	-22
PEG 400	-2.4	-18.0	-58.0	-214.6	-5.3	-69.5	-117

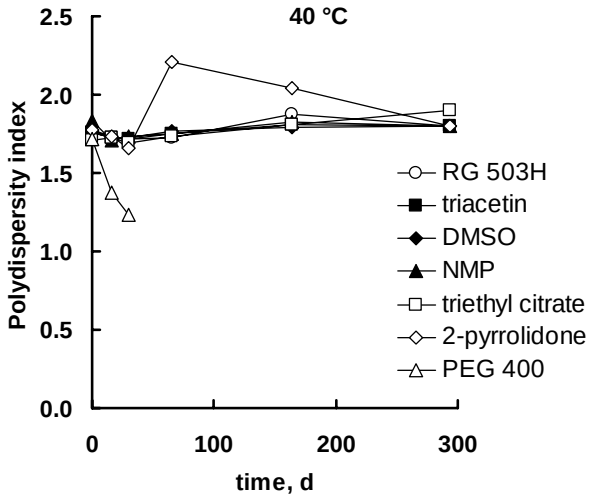


Figure 3.3 Polydispersity index of PLGA in different organic solutions at 40°C.

3.1.4. Effect of water content on PLGA stability in solution

In order to investigate the effect of water content on the PLGA degradation, PLGA solutions were spiked with different amount of water (0.1, 0.7, 1.2% w/w based on polymer solution) and stored at different temperatures for 45 days. The degradation of PLGA increased with increasing water content in NMP, 2-pyrrolidone, triacetin and triethyl citrate (Fig. 3.4). The effect became more evident by increasing temperature. In 2-pyrrolidone, the degradation was rapid and dominated by the storage temperature irrespective of the water content in the investigated range.

Controlled storage conditions with respect to water content and temperature are therefore very important for PLGA solutions used in in-situ forming drug delivery systems.

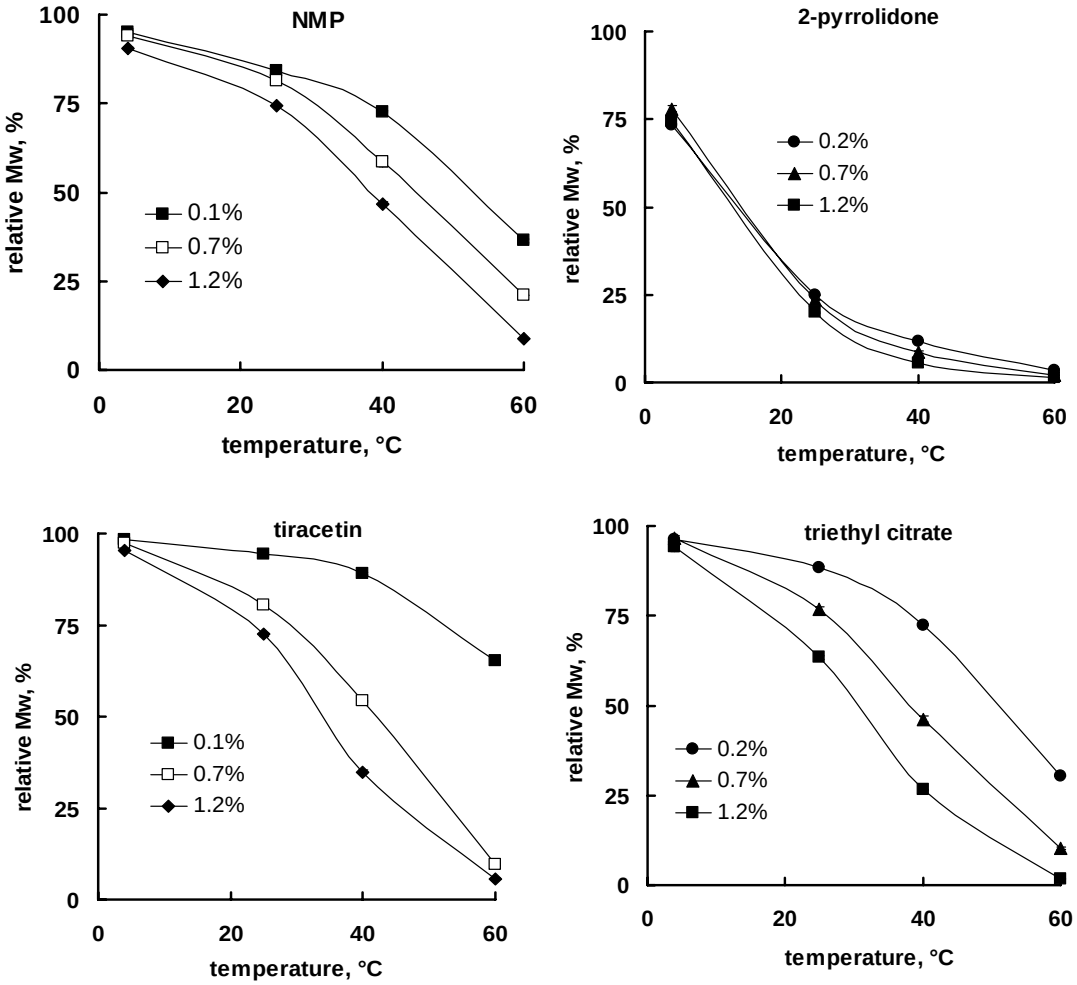


Figure 3.4 Effect of spiking of polymer solutions with different amounts of water on the PLGA degradation at 4°C, 25°C, 40°C and 60°C (after 45 d storage).

3.1.5. Effect of leuprolide acetate on PLGA stability in solution

A significantly faster degradation of PLGA in solutions was observed in the presence of leuprolide acetate (Fig. 3.5, Table 3.3), which has been also reported(Ravivarap et al., 2000). Leuprolide acetate had a water content of 4.85% w/w, resulting in 0.10% w/w additional water in the leuprolide acetate-containing PLGA solutions.

PLGA had similar degradation rates in drug-free NMP and triacetin solutions, but a faster degradatdion in leuprolide-containing NMP than in leuprolide-containing triacetin solutions (Table 3.3). Leuprolide was soluble in NMP, DMSO, 2-pyrrolidone, PEG 400 and insoluble and thus suspended in triacetin, triethyl citrate and ethyl acetate (2% w/w drug based on polymer solution). The faster PLGA degradation in NMP than in triacetin could therefore be explained with the physical state of the leuprolide, being dissolved in NMP but dispersed in triacetin solutions. Dissolved leuprolide released its bound water completely and possibly could also enhance the polymer degradation when compared to leuprolide dispersed in organic solvents. Peptides containing primary amino groups accelerate polyester hydrolysis (Lin et al., 1994). For example, cyclosporine A catalyses PLGA degradation (Chacon et al., 1999). Therefore, the acceleration of PLGA hydrolysis in the presence of leuprolide might be also due to interactions with polymer.

Leuprolide acetate should therefore be stored separately from the PLGA solutions, when it is soluble in the PLGA solutions. Dispersed leuprolide acetate might result in stable PLGA/drug dispersions with minimzed water content and when stored at 4°C. However, physical stability and chemical stability issues of the drug still have to be addressed.

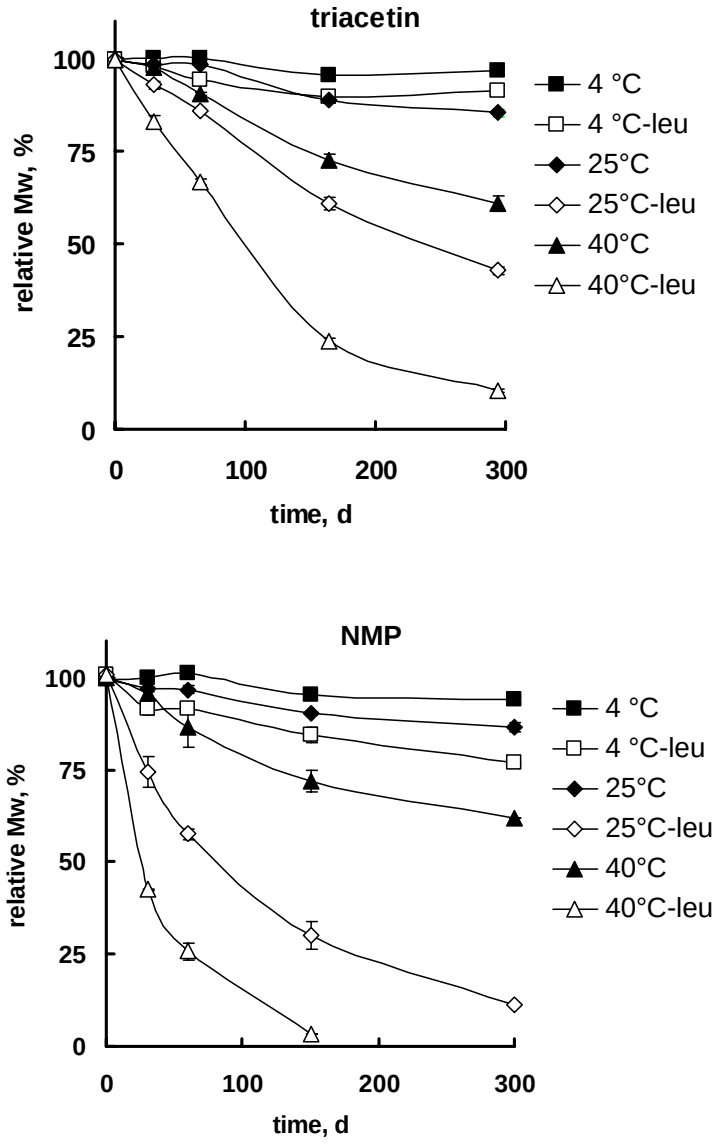


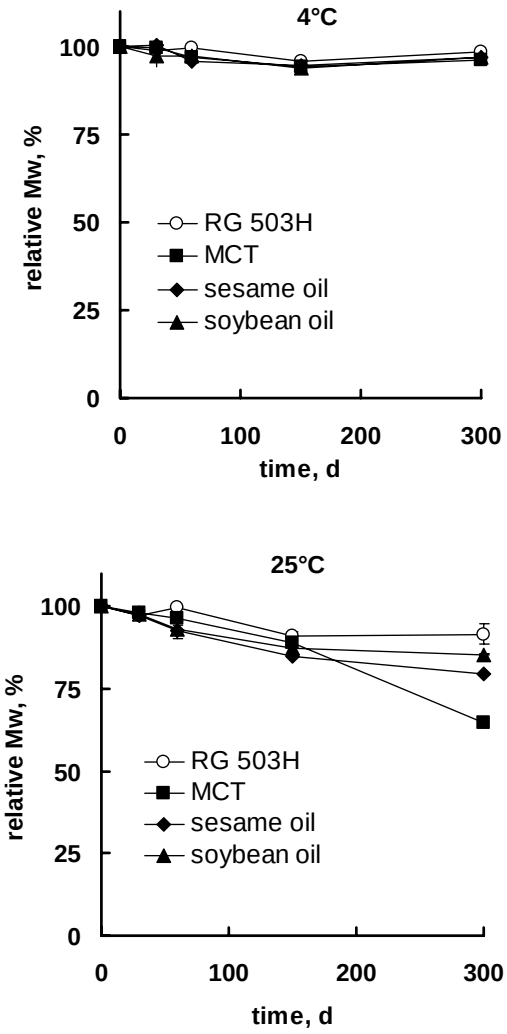
Figure 3.5 Effect of storage temperature and presence of leuprolide acetate (leu) on the stability of PLGA in *N*-methyl-2-pyrolidone (NMP) and triacetin solutions.

3.2. Stability of PLGA in oils and aqueous phases used for in-situ forming microparticles

In-situ forming microparticles (ISM) are based on an emulsion of a PLGA solution in an external oil (O/O-ISM) or aqueous phase (O/W-ISM). The stability of PLGA was therefore also investigated in contact with these external phases.

Oils/lipids have been used in micrencapsulation methods for the preparation of PLGA micro-/nanoparticles (Fessi et al., 1989; Santos Magalhaes et al., 1995; Urata et

al., 1999; Govender et al., 1999; Aliza et al., 2002). However, their influence on the stability of PLGA has not been investigated. In order to evaluate the stability of PLGA in ISM O/O-emulsions, PLGA powder was suspended in various oils (medium chain triglycerides, sesame oil, soybean oil), which are frequently used in parenteral preparations, and were stored at different temperatures. PLGA had the same stability in the oils and in the solid state at 4°C and degraded only slightly faster in the oils at 25°C (Fig. 3.6). Medium chain triglycerides retarded the degradation in contrast to the other two oils and also to the PLGA powder at 40°C. This might be caused by the lower water content of MCT (0.031% w/w) when compared to the other oils (0.043% w/w) and the higher purity of the oil. No interaction between the oils and the polymer was observed as indicated by an unchanged T_g of approx. 47°C (DSC-data not shown). Therefore, the inclusion of oils in ISM-formulations has no negative effect on the PLGA stability.



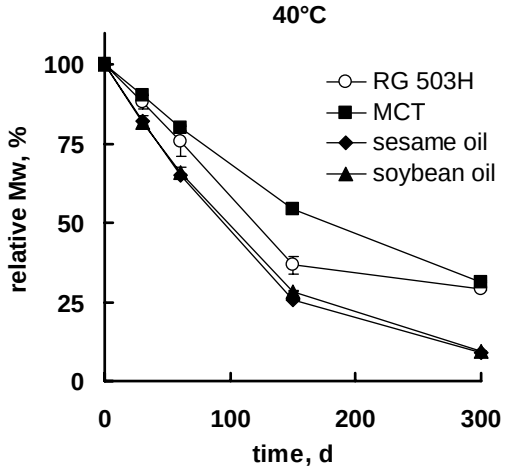


Figure 3.6 Effect of oils on the stability of PLGA at 4°C, 25°C and 40°C.

Table 3.4: Glass transition temperature (T_g) of RG 503H in the absence or presence of oils and aqueous phases (n = 2).

Materials	T_g, °C
RG 503H	46.2 ± 0.7
RG 503H + sesame oil	47.4 ± 0.0
RG 503H + soybean oil	47.7 ± 0.2
RG 503H + MCT	47.4 ± 0.0
RG 503H + saturated NaCl	38.5 ± 0.2
RG 503H + Water	-

O/W-ISM are formed by emulsifying an internal PLGA solution into an external aqueous phase. The stability of PLGA suspended in water and a saturated aqueous sodium chloride solution (lower water activity) was therefore investigated. PLGA was quite stable in both aqueous media after 5 months storage at 4°C, but degraded at 25°C (Fig. 3.7). The degradation in water could be reduced, but not prevented by the use of a saturated salt solution.

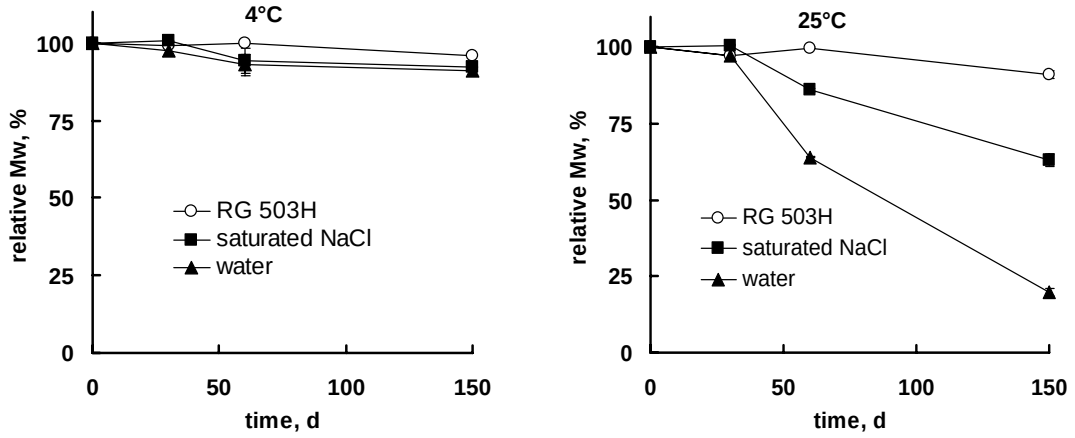


Figure 3.7 Degradation profiles of PLGA in water and saturated sodium chloride solution at 4°C and 25°C.

As previously reported, PLGA degradation is independent of ionic strength of medium in the range of 0.15-0.60 M (Schmitt et al., 1994). However, in the present study, a slower degradation occurred in the saturated sodium chloride solution than in water (Fig. 3.7). The saturated sodium chloride solution with high ionic strength might minimize the amount of bound water in polymer phase. Bound water in polymer phase acts as plasticizer to reduce the T_g of the polymer. The less reduction of T_g of the polymer in sodium chloride solution than in water indicated the low bound water (Table 3.4). In addition, saturated sodium chloride solution might reduce bulk water in the polymer phase by increasing the surface tension (or salting out effect) leading to worse wetting of the polymer.

Thus, considering only the PLGA stability, an organic PLGA phase or solid PLGA powder might be stored in contact with oils up to room temperature and with aqueous phases at 4°C.

3.4. Stability of PLGA sponges prepared by lyophilization of PLGA solutions

In-situ PLGA implants are formed from drug-containing PLGA solutions in a biocompatible solvent. For stability reasons, the drug is usually kept separate from the polymer solution in the final dosage form and the PLGA solution is stored at 4°C (Ravivarapu et al., 2000). The preparation of the polymer solution just prior to injection from solid PLGA would circumvent the low temperature storage and possibly also the separate storage of drug and PLGA. For this reason, PLGA sponges, which can be

reconstituted rapidly in an injectable solution, were prepared by lyophilization of drug-containing PLGA solution.

Dioxane (ICH Class 2) and acetic acid (ICH class 3) dissolve PLGA and can be removed by lyophilization. However, residual solvents might affect the degradation of PLGA in the lyophilzed sponges. PLGA sponges prepared from dioxane solutions had the same degradation as the original PLGA powder at 4°C and 25°C (residual solvent: 0.41% w/w) (Fig. 3.8). In contrast, residual acetic acid (0.52% w/w) facilitated the degradation, possibly by its catalysing effect, at 25°C. At 40°C, the original PLGA powder degraded even faster than the PLGA-sponges. Water present in the PLGA powder (0.52% w/w) was probably removed/reduced during the lyophilization. The faster polymer degradation at 25°C than at 40°C with acetic acid as solvent was the result of evaporation of acetic acid at 40°C. The secondary drying step during lyophilization thus has to be optimized in order to minimize the water content and the organic solvent.

Next, the effect of drug (leuprolide acetate and lidocaine base) on the PLGA stability in lyophilized sponges was investigated (Fig. 3.8). Leuprolide acetate did not affect the PLGA stability negatively and thus does not have to be stored separately from the polymer as it has to with polymer solutions. However, lidocaine significantly enhanced the polymer degradation. The faster degradation could be attributed to the plasticizing effect and the basic character of lidocaine. Lidocaine decreased the T_g of PLGA from 46.2°C to 35.1°C, thus enhancing chain mobility and degradation. Plasticized PLA films showed a faster degradation compared to plasticizer-free films (Kranz et al., 2000). In addition, the basic character of lidocaine (pKa 7.9) accelerated PLGA degradation (Wakiyama et al., 1981). Stabilizers should be included with basic drugs when entrapped in PLGA matrix (O´Donnell and McGinity, 1998).

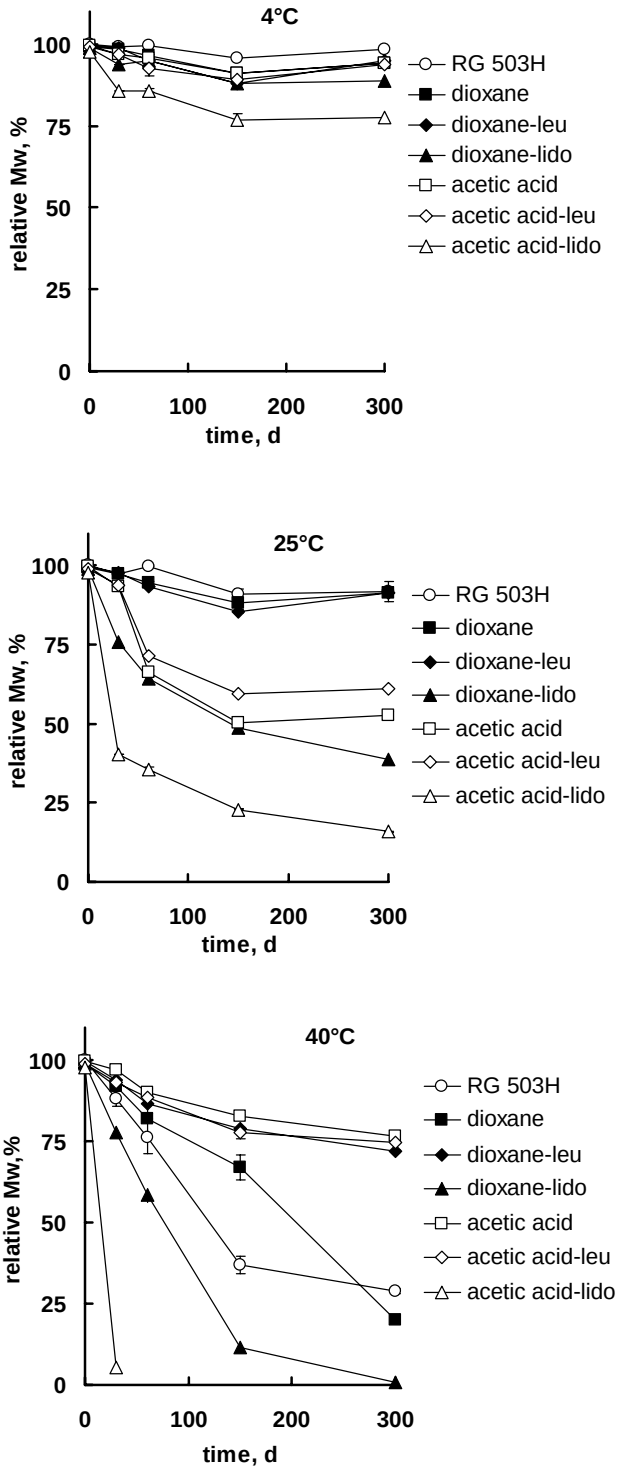


Figure 3.8 Effect of organic solvent used for lyophilization and drug on the stability of PLGA in lyophilized sponges at 4, 25, and 40°C (leu: leuprolide; lido: lidocaine).

3.5. Stability of leuprolide acetate

Besides the stability of PLGA, the chemical stability of the drug is of utmost important. Leuprolide acetate was stable for 150 days (no aggregation and degradation peaks were detected by HPLC), when suspended in PLGA solutions (triacetin, triethyl citrate, ethyl acetate) and oils and in lyophilized PLGA-sponges. However, it was unstable when dissolved in PLGA solutions (NMP, DMSO, 2-pyrrolidone, PEG 400) at 25°C and 40°C with degradation rates in the following order: 2-pyrrolidone > DMSO > NMP > PEG 400 (Fig. 3.9). The drug was reported to be very stable in pure DMSO at 37°C (Stevenson et al., 1999), but was unstable in DMSO solution containing PLGA. Aggregation was the predominant pathway of leuprolide acetate degradation (Fig. 3.10). This was consistent with previous reports on the peptide stability in DMSO (Hall et al., 1999; Stevenson et al., 1999).

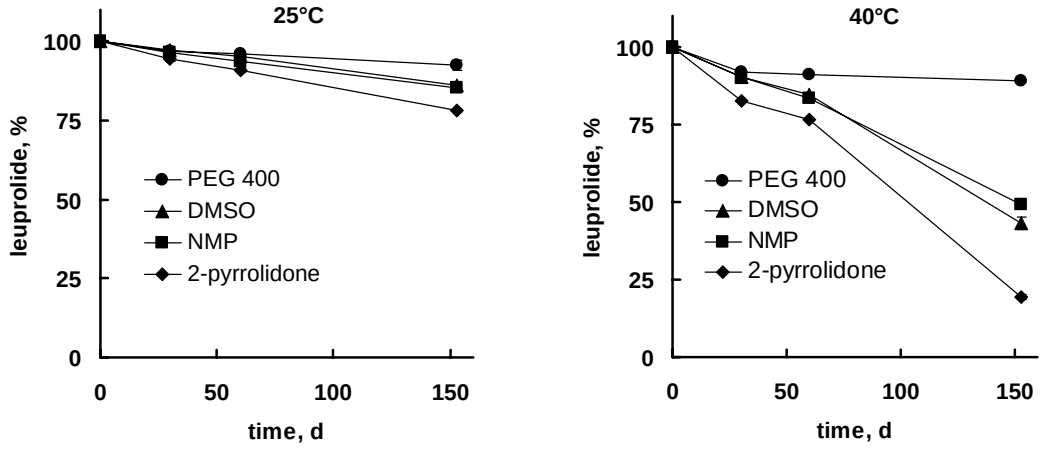


Figure 3.9 Stability of leuprolide acetate in PLGA solutions in different organic solvents at 25°C and 40°C.

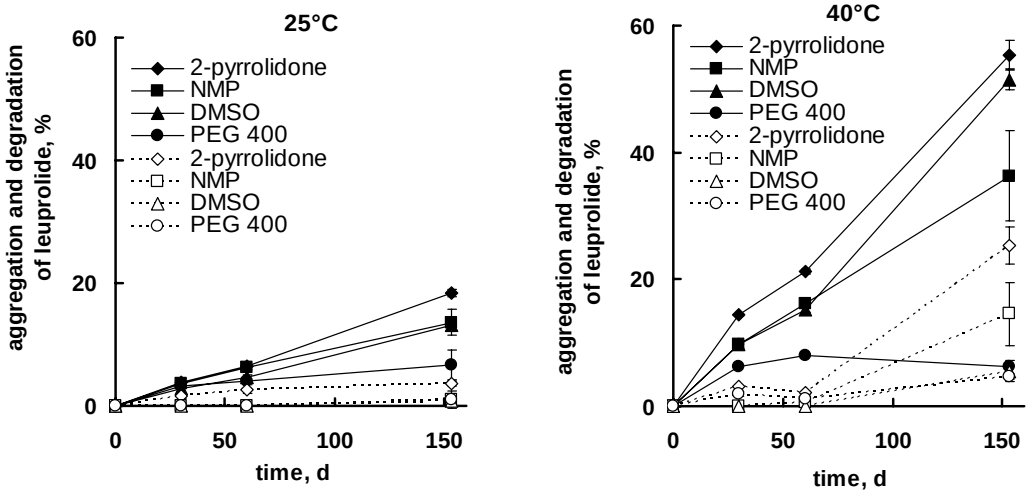


Figure 3.10 Aggregation (full line) and degradation (dashed line) of leuprolide acetate in PLGA solutions in organic solvents at 25°C and 40°C.