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In vitro and in vivo evaluation of carbamazepine-loaded enteric microparticles

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Abstract

The objective of the study was to prepare and evaluate carbamazepine-loaded enteric microparticles produced by a novel coacervation method. An aqueous polymeric stabilizer solution was added to an organic carbamazepine/Eudragit® L100-55 solution. Water, which is a non-solvent for the drug and the enteric polymer, caused phase separation and the formation of coacervate droplets. These droplets hardened into microparticles upon further addition of the aqueous phase. The microparticles were characterized with respect to particle size distribution, morphology, encapsulation efficiency, yield, physical state and physical stability of the drug, wettability, in vitro release and in vivo bioavailability. Microparticles with a smooth surface and dense structure were obtained with high encapsulation efficiency (>85%) and yield (>90%). The drug was in a non-crystalline state in the matrix and physically stable for 5 months at room temperature. Under sink conditions, the drug dissolution rate from the microparticles was significantly enhanced compared to the physical mixture and to the pure drug; the release profile of the microparticles was stable after 5 months. Under non-sink conditions, an unstable supersaturated solution of carbamazepine was obtained from microparticles with the subesequent formation of needle-shaped crystals. The high surface area and good wettability of the microparticles, the non-crystalline state of the drug in the matrix and the fast dissolution rate contributed to a significantly enhanced oral bioavailability from the microparticles when compared to the physical mixture.

Keywords: Carbamazepine; Coacervation; Dissolution rate; Enteric polymer; Microencapsulation

1. Introduction

Biopharmaceutics Classification System (BCS) Class II drugs exhibit low solubility and high permeability characteristics. Their oral absorption is mostly governed by in vivo dissolution; the solubility and the dissolution rate are therefore key determinants for the oral bioavailability of these drugs.

Although salt formation, solubilization, particle size reduction and solid solution/dispersion technologies have commonly been used to increase the dissolution rate of poorly soluble drugs, there are practical limitations of these techniques. Salt formation is not feasible for neutral compounds. Salts are potentially converted back into aggregates of their respective free acid or base forms in the gastrointestinal tract. The solubilization of drugs in organic solvents or in aqueous media with surfactants or cosolvents leads to liquid formulations with potential stability problems and precipitation within the gastrointestinal tract. Although particle size reduction is commonly used to increase the dissolution rate, there is a practical limit with regard to size. The use of very fine powders in a dosage form may also be problematic because of handling difficulties and poor wettability (Serajuddin, 1999).

Enteric polymers, which are insoluble in water and acidic solutions but soluble in aqueous media with a pH above 5.5–7, are promising carriers for lipophilic drugs. The improvement of bioavailability was attributed to the (1) high specific surface area of the particles, (2) non-crystalline state of the drug and (3) rapid release of the drug close to its absorption window (Leroux et al., 1995, 1996).

Various lipophilic drugs have been processed with enteric polymers as carriers including griseofulvin and spironolactone (Hasegawa et al., 1985), coenzyme Q_{10} (Nazzal et al., 2002), naproxen (Zaghloul et al., 2001a,b), erythromycin

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(Morishita et al., 1991), cyclosporine A (Takada et al., 1989), ibuprofen (Kislalioglu et al., 1991) and HIV-1 protease inhibitors (De Jaeghere et al., 2000; Leroux et al., 1995, 1996). Enteric polymers used include Eudragit® L100-55 (poly(methacrylic acid-co-ethyl acrylate) 1:1), Eudragit® L100 (poly(methacrylic acid-co-methyl methacrylate) 1:1), Eudragit® S100 (poly(methacrylic acid-co-methyl methacrylate) 1:2), hydroxypropyl methylcellulose phthalate and cellulose acetate phthalate. The methods for the preparation of solid dispersions or nano-/microparticles based on enteric polymers include solvent evaporation (Nazzal et al., 2002), emulsification–diffusion and spray-drying (De Jaeghere et al., 2000, 2001), salting-out (Leroux et al., 1995, 1996), coprecipitation (Zaghloul et al., 2001a,b) and emulsification–evaporation (Lee et al., 1999). Each approach has its benefits and limitations. The major problems are the use of toxic solvent (e.g. methylene chloride, methanol) or of complicated processes.

Carbamazepine, a BCS Class II drug (Kasim et al., 2003), is an effective antiepileptic drug characterized by a slow and irregular gastrointestinal absorption (Bertilsson, 1978). The drug has an experimental log P value of 2.45 and is practically insoluble in water (113 μg/ml, 25 °C) (Sethia and Squillante, 2002). Variations in the dissolution rates and absorption also occurred between its different crystalline forms. In this study, carbamazepine 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 recently investigated with this coacervation method with regard to the optimization of microparticle formation and drug entrapment (Dong and Bodmeier, 2006). The microparticles were evaluated in vitro through particle size distribution, morphology, encapsulation efficiency, yield, wettability and dissolution testing. In vivo bioavailability was evaluated using rabbits as animal model by determining the plasma level of carbamazepine and its major active metabolite, carbamazepine-10, 11-epoxide.

2. Materials and methods

2.1. Materials

Micronized carbamazepine (CBZ) (BASF AG, Ludwigshafen, Germany), carbamazepine-10,11-epoxide (CBZ-E), phenytoin (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), Eudragit® L100-55 (poly(methacrylic acid-co-ethyl acrylate) 1:1), Eudragit® RL PO (poly(ethyl acrylate-co-methyl methacrylate-co-trimethyl-ammonioethyl methacrylate chloride) 1:2:0.2) (Degussa AG, Darmstadt, Germany), ethylcellulose (Ethocel® Standard 7) (Dow Chemical, Midland, MI, USA), hydroxypropyl methylcellulose (HPMC, Methocel® K15 M) (Colorcon Ltd., Orpington, UK), ethanol 96% (v/v), isopropanol (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), acetonitrile, methanol (Carl Roth GmbH, Karlsruhe, Germany).

2.2. Preparation of carbamazepine-loaded microparticles

The carbamazepine-loaded enteric microparticles were prepared by a coacervation method (Dong and Bodmeier, 2006). Briefly, 15 g HPMC aqueous solution (1%, w/w) was added dropwise to 10 g ethanolic solution of Eudragit® L100–55 (20%, w/w based on polymer and solvent) and of carbamazepine (5–20%, w/w, based on drug and polymer) under magnetic stirring (Janke & Kunkel GmbH, Staufen, Germany) at 500 rpm within 5 min. The viscous microparticle suspension was diluted with 50 ml deionized water and the microparticles were collected by centrifugation (3000 rpm, 10 min) (Biofuge 22R, Heraeus Sepatech GmbH, Osterode, Germany), vacuum-dried (18 h) (Heraeus Holding GmbH, Hanau, Germany) and stored in desiccators.

2.3. In vitro characterization of the microparticles

2.3.1. Particle size analysis

The size of the microparticles was determined by laser diffractometry (LD) (Coulter LS 230, Beckman Coulter GmbH, Krefeld, Germany) and calculated on the basis of the volume distribution.

2.3.2. Scanning electron microscopy

The microparticles were spread and fixed on a holder with double-sided tape and coated with gold–palladium under an argon atmosphere (SCD 040, Balzers Union, Balzers, Lichtenstein). The surface morphology of 20% (w/w) carbamazepine-loaded microparticles was examined with a scanning electron microscope (SEM) (Zeiss DSM 950, Carl Zeiss, Jena, Germany).

2.3.3. Encapsulation efficiency and microparticle yield

The drug loading was determined by dissolving 10 mg microparticles in 100 ml pH 6.8 phosphate buffer and UV-spectrophotometric analysis of the solution at 287 nm (UV-2101 PC, Shimadzu Scientific Instrument, Columbia, MD, USA) ($n = 3$). The enteric polymers did not interfere at this wavelength. The encapsulation efficiency was the ratio of the actual and theoretical drug loading, expressed on a percentage basis. The microparticle yield was the ratio between the amount of microparticles recovered and the weight of drug and polymer used for the preparation of the microparticles, expressed on a percentage basis.

2.3.4. Partition of carbamazepine between polymer rich/poor regions in coacervate

The drug partition coefficient between polymer-rich and -poor regions was determined to clarify the mechanism of encapsulation of carbamazepine within enteric microparticles. Blank coacervate particles were first prepared by addition of 15 g 1% (w/w) HPMC solution to 10 g ethanolic solution of Eudragit® L100-55 (20%, w/w) under magnetic stirring at 800 rpm. 0.22 g (or 0.11 g) carbamazepine was either dissolved in the polymer solution and formed coacervate or in the preformed coacervate. The complete dissolution of the drug was ensured by observation

under polarized light microscope (Carl Zeiss Jena GmbH, Jena, Germany). The drug content in both regions was determined after 1, 2 and 17 h stirring (200 rpm) at room temperature by separating the two regions by centrifugation (13000 rpm, 20 min; Biofuge 13/Haemo, Heraeus Instruments, Osterode, Germany), by then dissolving the two regions (0.2 g) in 0.1N NaOH and determining the drug content by UV-spectrophotometric analysis at 287 nm. The partition coefficient was the ratio of drug concentrations between polymer-rich and -poor regions.

2.3.5. In vitro drug release

2.3.5.1. Sink conditions. The in vitro dissolution/release of carbamazepine as pure drug, physical mixture and microparticles (20%, w/w, loading) was determined with a USP XXV rotating paddle method (900 ml 0.1N HCl or pH 6.8 phosphate buffer; 50 rpm; 37 °C; $n = 3$) (VanKel® 700, Vankel Industries, Edison, NJ, USA). The solubility of carbamazepine in pH 6.8 phosphate buffer is 126.7 μg/ml. A sample equivalent to 10 mg drug (<10% of drug solubility in 900 ml medium) was used to maintain sink conditions. At predetermined time intervals, samples were withdrawn (3 ml, not replaced) and assayed UV-spectrophotometrically at 287 nm.

2.3.5.2. Non-sink conditions. Carbamazepine-loaded enteric microparticles (equivalent to 15 mg drug) and physical mixtures of the same composition were dispersed in 10 g pH 6.8 phosphate buffer filled in 20 ml plastic syringes (B. Braun Melsungen AG, Melsungen, Germany), coupled with a filter (0.2 μm, Schleicher & Schuell MicroScience GmBH, Dassel, Germany) and shaken in a horizontal shaker (37 °C, 80 rpm, GFT, Gesellschaft Für Labortechnik GmbH, Berlin, Germany). 0.5 g samples were filtered through the filter at predetermined time points and were properly diluted with pH 6.8 phosphate buffer before UV measurement. Drug crystals in the release medium were observed under a polarized light microscope (Carl Zeiss Jena GmbH, Jena, Germany) after 1 and 24 h incubation.

2.3.6. Wettability

Compacts (diameter: 13 mm; weight: 500 mg) of Eudragit® L100-55 powder, carbamazepine, a physical mixture of 20% (w/w) carbamazepine and Eudragit® L100-55 and of 20% (w/w) drug-loaded microparticles were prepared manually with a hydraulic press (SPECAC Ltd., Orpington, England) at a compression force of 1200 kg. A drop of pH 6.8 phosphate buffer (KH_2PO_4 102.1 mM, Na_2HPO_4 97.9 mM) was placed onto the surface of the compacts from a microsyringe and observed under a microscope. The contact angle was measured after 1 min using a goniometer (Krüss GmbH, Hamburg, Germany).

2.3.7. Stability of the microparticles

Carbamazepine-loaded enteric microparticles were stored for 5 months in a desiccator at room temperature. The microparticles were characterized by powder X-ray diffraction (Philips Industrial, Electro-acoustic Systems Division, Almelo, The Netherlands) and by a release study in pH 6.8 phosphate buffer to evaluate the physical stability of the drug/microparticles.

2.4. In vivo study

2.4.1. Animal experiments

Male adult New Zealand rabbits (2.69 ± 0.06 kg, $n = 8$) were randomly divided into two groups to investigate 20% (w/w) carbamazepine-loaded enteric microparticles (group A) and a physical mixture (group B). The rabbits were fasted but had free access to water overnight. Each rabbit orally received a dose equivalent to 100 mg carbamazepine filled in the hard gelatin capsules followed by 10 ml water. After dose administration, the rabbits were kept in cages and had free access to food and water after 6 h. Serial blood samples (1.0 ml) were withdrawn from the marginal ear vein into a vial containing sodium citrate (50 μl) at predose (−30 min) and post-doses (1, 2, 3, 4, 6, 8, 10 and 24 h), which were gently mixed and centrifuged at 5000 rpm for 10 min (Biofuge 22R, Heraeus Sepatech GmbH, Osterode, Germany) within 1 h of collection. The obtained plasma samples were stored at −20 °C until analysis.

2.4.2. Assay of drug and its metabolite in plasma

Carbamazepine and its major active metabolite carbamazepine-10,11-epoxide were determined in the blood plasma by HPLC (Nagasawa et al., 2002). 0.8 ml acetonitrile containing phenytoin (1 μg/ml) as internal standard was added to 0.2 ml plasma to precipitate proteins. After 30 s vortexing, the mixture was centrifuged at 17,000 rpm for 10 min (Biofuge 22R, Heraeus Sepatech GmbH, Osterode, Germany). Twenty microliter supernatant was injected into a HPLC apparatus (SCL-10A vp, Shimadzu Co., Tokyo, Japan) equipped with a diode array detector (SPD-M10Avp, Shimadzu Co., Tokyo, Japan). The HPLC analysis conditions were as follows: C_{18} column, 150 mm × 4.6 mm i.d., Eurospher-100 (Knauer GmbH, Berlin, Germany); mobile phase, acetonitrile–methanol pH 7.0 phosphate buffer (0.33 mM) (18:18:64, v/v); column temperature, 40 °C; flow rate, 1.0 ml/min; wavelength, 210 nm.

2.4.3. Pharmacokinetic analysis

The maximum plasma level (C_{max}) and the time to reach C_{max} (t_{max}) of the drug and its metabolite were obtained directly from the actual observed data. The area under the curve from 0 to 24 h ($AUC_{0\rightarrow 24\,h}$) was calculated by means of linear trapezoidal rule.

3. Results and discussion

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 and Bodmeier, 2006). Water is a non-solvent 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; Fig. 1). This was most likely due to the formation of hydrogen bond (H-bond) between amide of carbamazepine and carboxylic

Table 1
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 (%)		Microparticle 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

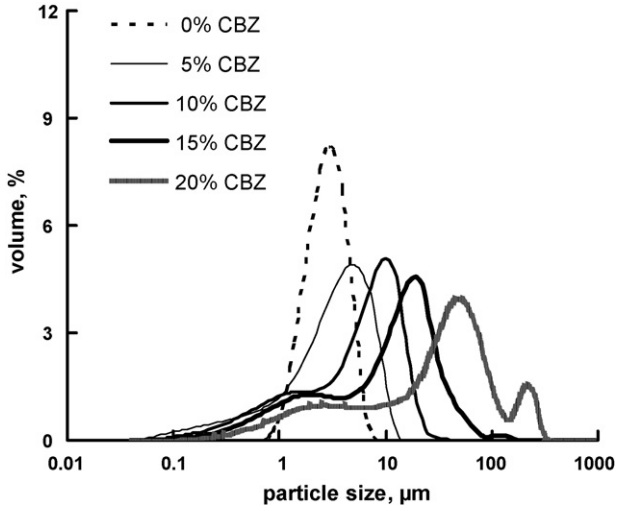

Fig. 1. Effect of carbamazepine loading on the particle size distribution of drug-loaded enteric microparticles.

acid of the enteric polymer. The increased lipophilicity of the drug/polymer complex and high drug loadings led to a faster drug/polymer precipitation and larger particles. The carbamazepine-loaded enteric microparticles were spherical and had a smooth surface without visible drug crystals, which suggested that carbamazepine was molecularly entrapped (dissolved) in the polymeric matrix (Fig. 2).

The yield of microparticles was over 90% and the encapsulation efficiency over 85% (w/w) irrespective of the drug loading (Table 1). Interestingly, the encapsulation efficiencies were 2–5% 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

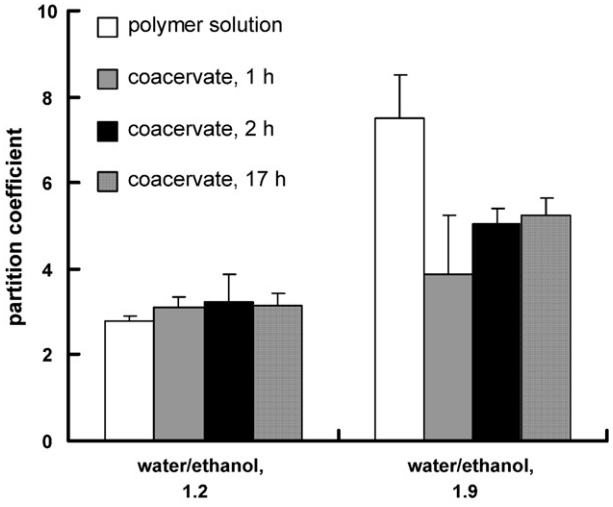

Fig. 3. Partition coefficient of carbamazepine between polymer-rich/-poor regions with addition of the drug to the polymer solution prior to forming coacervates or to the preformed coacervates.

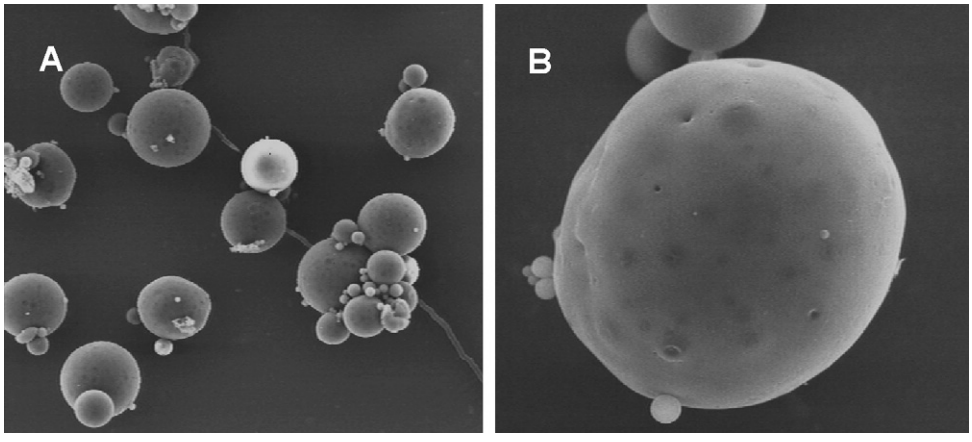

Fig. 2. Scanning electron micrographs of 20% (w/w) carbamazepine-loaded enteric microparticles (A) group (B) surface.

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 carbamazepine partitioning into the microparticles.

In order to clarify this point, carbamazepine powder was added either in the polymer solution prior to formation of the coacervate or in the preformed coacervate of Eudragit® L100-55 at different water/ethanol ratios. At a ratio of 1.2, around 65% (w/w) drug partitioned into the polymer-rich phase with the same partition coefficient (3.1) as drug initially dissolved in the polymer solution (Fig. 3). However, the slower partitioning of the drug into the polymer-rich phase at a higher water/ethanol ratio (1.9) and the lower partition coefficient (5.2) than that of the drug dissolved in polymer solution (7.5) were attributed to the formation of hardened microparticles. The partitioning of the drug into the polymer-rich phase indicated that carbamazepine had an affinity for the polymer as expressed by a high drug solubility in excess of 30% in Eudragit® L100-55 (Dong and Bodmeier, 2006). The affinity may be from the interaction between the amide-containing drug and the carboxyl goup-containing enteric polymer.

The absence of carbamazepine crystal peaks (as shown by X-ray analysis) indicated that carbamazepine was either dissolved in the Eudragit® L100-55 or (less likely) dispersed in amorphous form (Dong, 2005). The spectrum did not change during a 5-month storage period, indicating good physical stability. This is particularly advantageous because physical instabilities are frequently reported with solid dispersions (Khalil and Mortada, 1978; Vila Jato et al., 1984). Other amide-containing

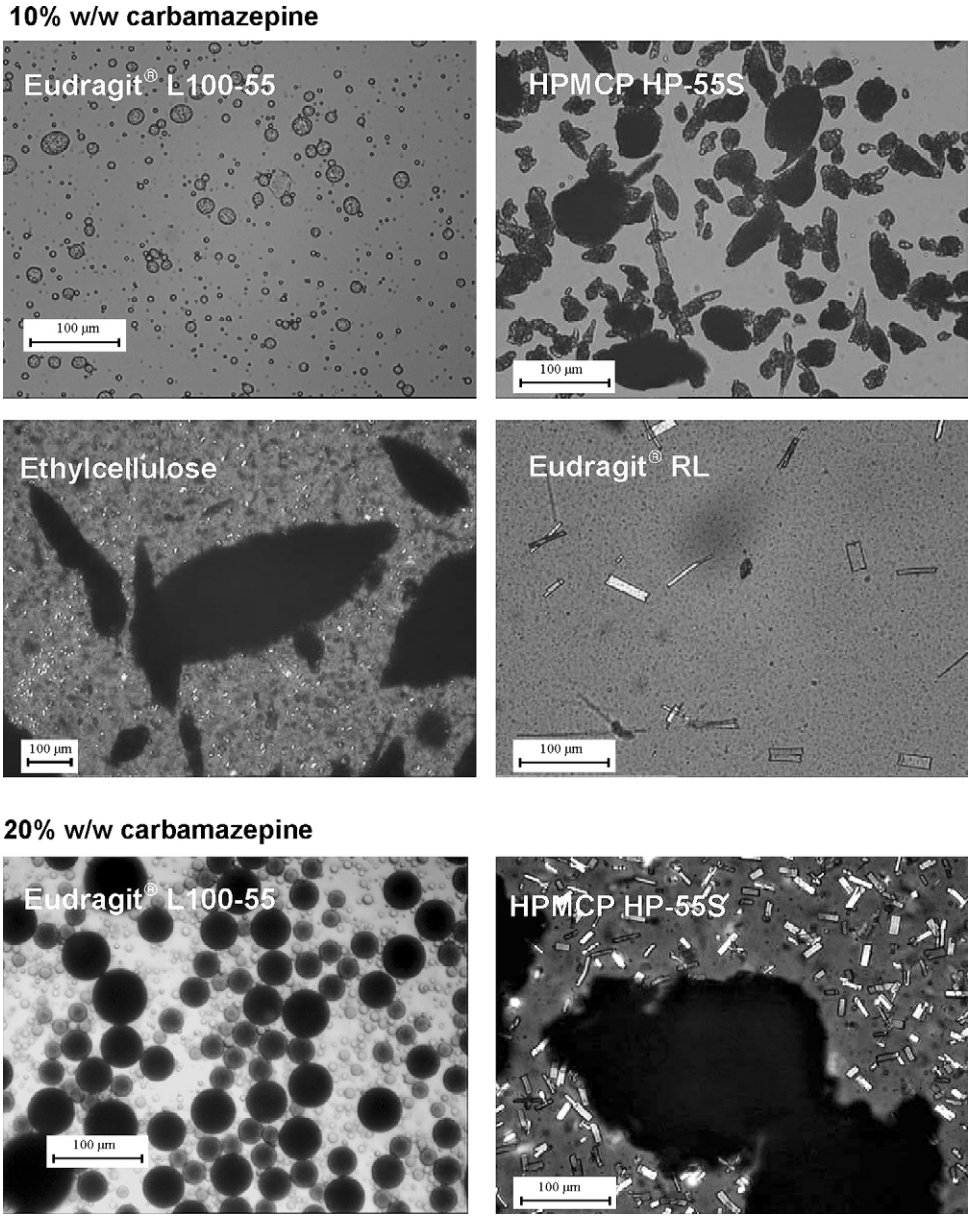

Fig. 4. Effect of polymer type on the encapsulation of carbamazepine: enteric polymers (Eudragit® L100-55, HPMCP HP-55S), non-enteric polymers (ethylcellulose and Eudragit® RL).

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).

The successful encapsulation of carbamazepine (10%, w/w) in another enteric polymer (HPMCP HP-55S), but the crystallization outside the non-enteric polymers ethylcellulose and Eudragit® RL also supported the existence of interactions through H-bonds (Fig. 4). 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 Da) than of HPMCP HP-55S (181–235/100,000 Da) (Fig. 4). The content of carboxyl groups in enteric polymer (100,000 Da) was calculated based on the molecular weight of each unit which containing one carboxylic acid.

Dissolution studies were performed under sink conditions in 0.1N HCl and pH 6.8 phosphate buffer. The micronized carbamazepine crystals resulted in 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 0.1N HCl when compared to pH 6.8, where the polymer dissolved (Fig. 5). The drug release decreased with increasing drug loading in 0.1N HCl. This decrease was probably caused by an overall increased lipophilicity of the drug/polymer combination at higher drug loadings. In addition, the size of the microparticles increased 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 0.1N 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 be individually transported in the upper intestine, where they would dissolve rapidly. The in vitro aggregation of the microparticles in pH 6.8 buffer thus probably would not occur in vivo.

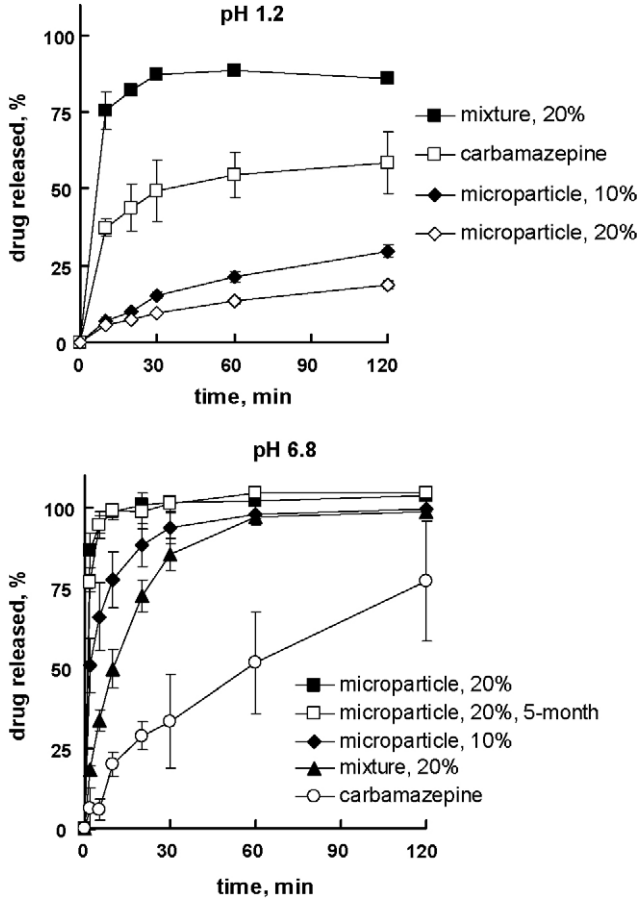

Fig. 5. Carbamazepine dissolution/release profiles of Eudragit® L100-55 microparticles, microparticles stored for 5 months, of a physical mixture, and of carbamazepine in 0.1N HCl and pH 6.8 phosphate buffer under sink conditions.

The greatly enhanced dissolution rate of carbamazepine from the microparticles compared to the micronized drug and the physical mixture (Fig. 5) can be attributed to its physical state in the enteric matrix. The drug is dissolved (molecularly dispersed) in the enteric matrix and thus immediately in solution upon dissolution of the enteric polymer. In addition, the microparticles were wetted better than the pure drug, the polymer or the physical mixture as indicated by a lower contact angle (Table 2). 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 than of the pure drug was mainly due to the fine dispersion of the drug particles and the polymer particles, which separated the drug particles and thus resulted in better wetting and prevention of aggregation. The unchanged release profile of microparticles after 5 months storage indicated that carbamazepine was physically stable in the enteric matrix. This was confirmed by X-ray study (Dong, 2005).

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 released rapidly 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 initially followed by drug crystallization (Figs. 6 and 7). Needle-shaped crystals precipitated out of the supersaturated solution (Fig. 7); the concentration of dissolved drug then approached the con-

Table 2
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% drug)	38 ± 3
Microparticles (20% drug)	16 ± 3

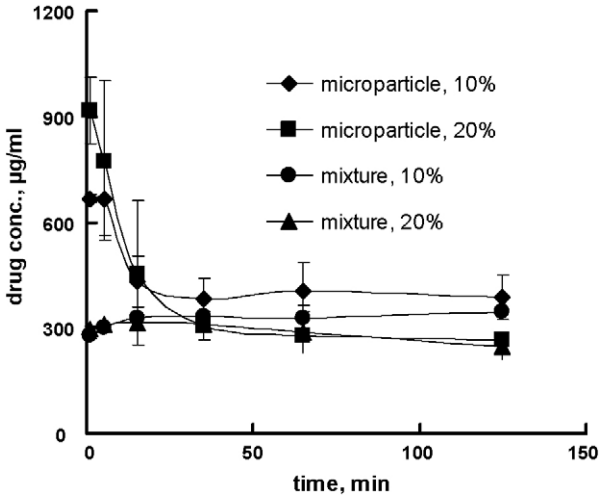

Fig. 6. Dissolution profiles of 10% and 20% (w/w) carbamazepine-loaded enteric microparticles and physical mixtures in pH 6.8 phosphate buffer under non-sink conditions.

centration, 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 an 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 (Fig. 7). 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.

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 five-fold enhancement in relative bioavailability when compared to the physical mixture for the reasons discussed above (Fig. 8; Table 3).

Two absorption peaks were seen in the plasma curves (Fig. 8). 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 $AUC_{0\rightarrow 24\,h}$ 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).

The summation of the $AUC_{0\rightarrow 24\,h}$ of carbamazepine and of the $AUC_{0\rightarrow 24\,h}$ of carbamazepine-10,11-epoxide of the present CBZ/Eudragit® L100-55 microparticles (56.7 μg h/ml) were comparable to rabbit data obtained with CBZ/HP-β-

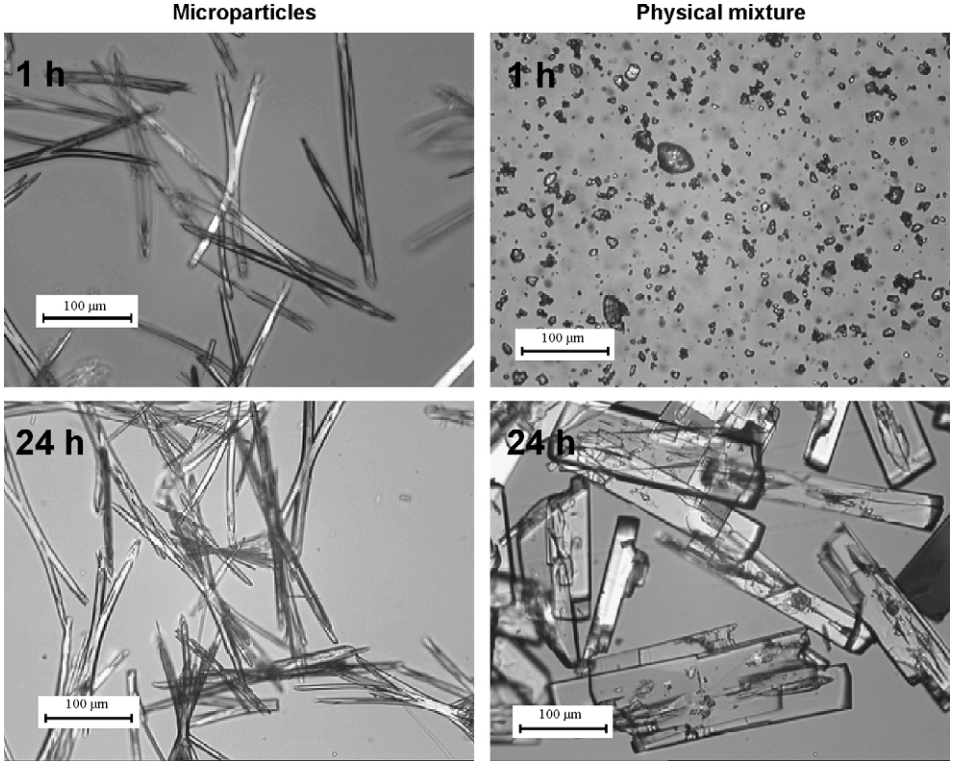

Fig. 7. Carbamazepine crystal formation from 20% (w/w) carbamazepine-loaded enteric microparticles and a physical mixture after 1 h and 24 h incubation in pH 6.8 phosphate buffer under non-sink conditions.

Table 3
Pharmacokinetics parameters of carbamazepine and carbamazepine-10,11-epoxide after oral administration of 20% (w/w) drug-loaded enteric microparticles and of a physical mixture

Parameter	Carbamazepine		Carbamazepine-10,11-epoxide	
	Microparticles	Physical mixture	Microparticles	Physical mixture
C_{max} (μg/ml)	1.7 ± 1.1	0.3 ± 0.6	1.5 ± 0.6	0.4 ± 0.3
t_{max} (h)	10	10	10	10
$AUC_{0\rightarrow 24\,h}$ (μg h/ml)	27.2 ± 3.6*	5.8 ± 4.7*	29.5 ± 2.0**	6.8 ± 3.4**

* $P = 0.006$ (Student t-test).
** $P = 0.005$ (Student t-test).

cyclodextrin 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).

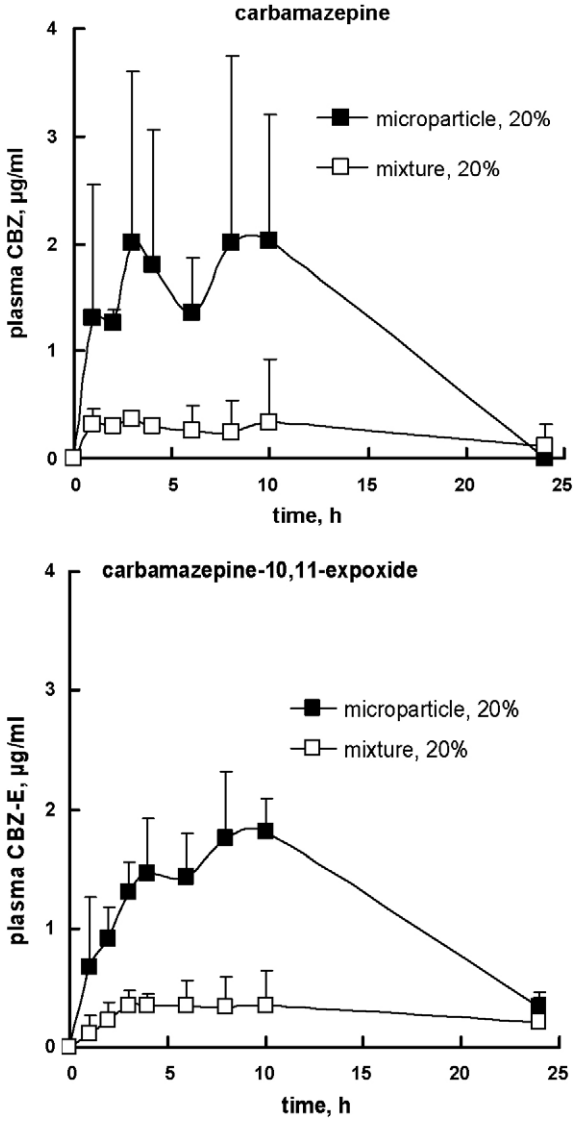

Fig. 8. Plasma levels of carbamazepine and carbamazepine-10,11-epoxide in rabbits following the oral administration of 20% (w/w) carbamazepine-loaded enteric microparticles or the corresponding physical mixture.

4. Conclusions

Carbamazepine, an amide-containing lipophlic drug, was successfully encapsulated in the enteric micropartilces by a novel toxic solvent-free coacervation method. The drug was encapsulated in the carrier in a non-crystalline state and physically stable for 5 months. A remarkable improvement of dissolution rate and wettability of the microparticles was achieved. This led to a significant enhancement of bioavailability of the drug from the microparticles compared to the drug/enteric polymer physical mixture.

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