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PREPARATION AND CHARACTERIZATION OF POLY(Є-CAPROLACTONE) MICROPARTICLE BLENDS CONTAINING PROPRANOLOL HCl AND CARBAMAZEPINE

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Abstract

The purpose of this study was to investigate physicochemical properties of poly(ϵ-caprolactone) microparticle blends containing drugs with different solubilities (Propranolol HCl [Pro] and carbamazepine [CBZ]). Microparticle blends were formulated with W/O/W emulsion for Pro and O/W emulsion for CBZ. The Pro emulsion (W/O) and CBZ oil phase (O) were dispersed in an external aqueous phase (W), with dispersion time interval (DTI) of 0 and 60 min. Morphology of microparticle blends were characterized by scanning electron microscopy (SEM). Particle size mean of emulsion droplets/hardened microparticles were monitored by focused beam reflectance measurement (FBRM). Encapsulation efficiency (EE) and in vitro drug release in phosphate buffer (pH 7.4) were also investigated. The results showed that the resulting microparticle blends obtained by solvent evaporation method were spherical and having two populations. FBRM data showed that the size of microparticle blends prepared with DTI 60 min and stirring time 4 h was larger than those with DTI 0 min. The encapsulation efficiency were 62.05% to 66.38% for Pro and 70.56% to 73.85% for CBZ in microparticle blends. Drug release in phosphate buffer after 28 days showed that the Pro release (33%) was slower than CBZ release (60%) from microparticle blends with DTI 60 min. This phenomenon was attributed to the interaction of oil phase (CBZ) with hard particles from primary emulsion (Pro), whereby the oil phase had blocked and coated pores on the surface of hard particle from primary emulsion.

Key words: microparticle blends, propranolol HCl, carbamazepine, poly(ϵ-caprolactone), FBRM, solvent evaporation method

INTRODUCTION

Microparticles are widely used in different applications such as the controlled release of drugs, cosmetics and chemical reagents. Several methods are potentially useful for the preparation of microparticles in the field of controlled drug delivery. One of the most common methods for preparing microparticles is the solvent evaporation method (Li et al., 2008). The control of the microparticle preparation processes is essential to produce a desired mean size of the microparticles, size distribution and morphology of the microparticles. The solubility properties of the drugs of the microparticles are important parameters when selecting the emulsion phases for a microparticles preparation process. A low solubility of the drugs in the continuous phase is required for obtaining a high yield. Microparticles can encapsulate many types of drugs including small molecules, proteins, and nucleic acids. Depending on the solubility of the drug, simple or multiple emulsion techniques like oil-in-water (O/W) or water-

in-oil-in-water (W/O/W) methods are used (Yang et al., 2001). The microparticle preparation method is a governing factor in the encapsulation and release of drugs. In addition, a complicated array of factors including the type of polymer, the polymer molecular weight, the copolymer composition, the nature of any excipients added to the microparticle formulation (e.g., for stabilization of the drugs), porosity, and the microparticle size can have a strong impact on the delivery rates (Yang et al., 2001).

Polymers have been used as a main tool to control the drug release rate from the formulations. Polymers can bind the particles of a solid dosage form. Pharmaceutical polymers are widely used to achieve taste masking; controlled release (e.g., extended, pulsatile, and targeted), enhanced stability, and improved bioavailability. Biodegradable and non biodegradable polymers with good biocompatibility are also used as drug carriers, such as polycaprolactone, PLGA and ethyl cellulose (degradable but non biodegradable).

In most studies reported so far, only one drug was entrapped within controlled release microparticles at a time. Only few attempts have been made on the co-encapsulation of two drugs, especially if the latter exhibits significantly different solubility behavior. Pérez et al. (2003) have successfully incorporated the hydrophilic drug propranolol HCl and/or the lipophilic drug nifedipine separately as well as simultaneously within non-degradable, ammonio methacrylate copolymers(Eudragit RS:RL 4:1 blends) based microparticles. They were prepared with an oil-in-water (O/W) and a water-in-oil-in-water (W/O/W) solvent evaporation method. Whereas, Nippe and General (2012) have developed a combination of lipophilic steroidal drugs ethinyl estradiol and drospirenone poly(lactic-co-glycolic acid) (PLGA) microparticles.

Microparticle blends containing two drugs with different solubility have not been reported yet. In the present study, the solvent evaporation method was used to incorporate a lipophilic and a hydrophilic drug within polycaprolactone based microparticle blends. The hydrophilic drug propranolol HCl and the lipophilic drug carbamazepine were used as model drugs. Accurate particle size analysis during solvent evaporation process is a key to study microparticle blends formation from oil-in-water (O/W) and water-in-oil-in-water (W/O/W) methods. For more information about micropaticle blends formation during solvent evaporation process, FBRM can be used to provide in situ/on-line particle characterization in a wide range of applications (Kail et al., 2009; Vay et al., 2012; Wu et al., 2011; Zidan et al., 2010). The great advantage of this technique is that data is acquired on-line and in real time to give particle size data and population trends of particles in suspension, emulsion etc. (Boxall et al., 2010; Wu et al., 2011; Zidan et al., 2010).

The purpose of this study was to investigate effect of dispersion time interval (DTI) and formulation of second primary oil phase on poly(ϵ-caprolactone) based microparticle blends contained drugs with different solubility (Propranolol HCl and carbamazepine) which prepared by solvent evaporation method.

RESEARCH METHOD

Materials

All materials were of at least reagent grade and used as received: poly (ε-caprolactone) (Mw. 10000) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany); polyvinyl alcohol (PVA, Mowiol® 40–88, Kuraray Europe GmbH, Frankfurt, Germany); propranolol HCl, carbamazepine, sodium chloride, sodium hydroxide, potassium dihydrogen phosphate and dichloromethane (Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

Methods

Preparation of Microparticle containing propranolol HCl or carbamazepine

Drug loaded microparticles based on poly (ε-caprolactone) were prepared using an oil-in-water (O/W) and a water-in-oil-in-water (W/O/W) solvent evaporation method. The drug loaded systems contained either one drug only (propranolol HCl or carbamazepine). For the

O/W method, 300 mg of poly (ε-caprolactone) were dissolved in 4 ml dichloromethane. 60 mg carbamazepine were dissolved within this organic phase. The organic phase was then emulsified into 800 ml aqueous PVA solution (0.25% w/v) containing 0.5 M NaCl and NaOH at pH 12. The emulsion was stirred for 4 h at 500 rpm with a propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) to allow microparticle hardening.

For the W/O/W method, 60 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was first emulsified by probe sonication (Sonoplus® HD 250, Bandelin Electronic GmbH & Co. KG, Berlin, Germany) for 30 s under ice-cooling into 4 ml dichloromethane containing 300 mg of poly (ε-caprolactone). This first emulsion (W/O) was then dispersed into 800 ml aqueous PVA solution (0.25% w/v) containing 0.5 M NaCl and NaOH at pH 12. A W/O/W emulsion was formed by extensive stirring with a propeller stirrer for 4 h at 500 rpm to allow microparticle hardening. In all cases, after 4 h the microparticles were separated from the external aqueous phase by wet sieving (stainless steel test sieves ISO 3310 - 40, 70, 100 and 160 μm) followed by washing with 200 ml deionized water, desiccator-drying for 24 h and storage in a desiccator.

Preparation of Microparticle blends containing propranolol HCl and carbamazepine

The first primary emulsion containing propranolol HCl (W/O/W) and second primary oil phase containing carbamazepine (O/W). For the W/O/W method, 60 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was first emulsified by probe sonication for 30 s under ice-cooling into 4 ml dichloromethane containing 300 mg of poly (ε-caprolactone). This gave the first primary emulsion containing propranolol HCl. For the O/W method, 300 mg of poly (ε-caprolactone) were dissolved in 4 ml dichloromethane. 60 mg carbamazepine were then dissolved in this organic phase. This process produced the second primary oil phase containing carbamazepine. Following, the first primary emulsion containing propranolol HCl and the second primary oil phase containing carbamazepine were dispersed in an external aqueous phase (800 ml aqueous PVA solution [0.25% w/v] containing 0.5 M NaCl and NaOH at pH 12), with dispersion time intervals (DTI) of 0 and 60 min, and stirred for 4 h at 500 rpm with a propeller stirrer to allow microparticle hardening. The subsequent process steps were similar to the preparation of microparticle containing single drug process.

Determination of the actual drug loading and encapsulation efficiency

Microparticles (10 mg) were extracted in 1 ml methanol, followed by agitation in a horizontal shaker (IKA HS 501 digital horizontal Shaker, Janke & Kunkel GmbH & Co. KG IKA Labortechnik, Staufen, Germany) for 2 h (n = 3). 0.1 ml of methanol extract was diluted in 10 ml of pH 7.4 phosphate buffer. The polymer was separated from aqueous solution by filtration using filter paper (Whatman®, GE Healthcare UK Limited, Buckinghamshire, UK). Propranolol HCl and/or carbamazepine concentration in the obtained aqueous solution was determined by UV-spectrophotometry at wavelengths of 289 nm and 285 nm, respectively (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany). The actual drug loading and encapsulation efficiency were calculated as follows:

Actual drug loading (%) = (drug mass in microparticles/mass of microparticles) x 100 %

Encapsulation efficiency (%) = (actual drug loading/theoretical drug loading) x 100 %.

For microparticle blends, the amounts of incorporated propranolol HCl and carbamazepine were determined UV-spectrophotometrically by simultaneously measuring at wavelengths of 227 and 285 nm. The subsequent process steps were similar to the above process.

Particle size analysis

Particle size mean and size distribution of the microparticles were measured by focused beam reflectance measurement. FBRM probe (Lasentec® FBRM D600T, Mettler Toledo AutoChem, Inc., Redmond, WA, USA) was immersed and positioned in the emulsification vessel (WO/W and O/W emulsions mentioned above) to ensure good flow against the probe window and hence allowing a representative sample of the particle system to be measured. The measurement range of the FBRM D600T probe is 0.25 - 4000 µm. In these experiments, FBRM measurements were performed every 10 seconds, during a period of 4 h. All batches were measured in triplicate. The size information was extracted through the iC FBRM® 4.0 software (Mettler Toledo AutoChem, Inc., Redmond, WA, USA).

Microparticle characterization

Optical microscopy

Microparticles were spread on microscope slides and observed with an optical light microscope (Axiotrop 50, Carl Zeiss AG, Jena, Germany) equipped with an image analysis system (INTEQ Informationstechnik GmbH, Berlin, Germany) consisting of a digital camera (type MC1) and the EasyMeasure® software (version 1.4.1).

Scanning electron microscopy

The external and internal morphology of microparticles was analysed by scanning electron microscopy (SEM). For surface imaging, the microparticles were fixed on a sample holder with double-sided tape. To investigate the inner structure, the particles were spread on transparent tape and then cut with a razor blade. All samples were coated under argon atmosphere with gold to a thickness of 8 nm in a high-vacuum (SCD 040, Bal-Tec GmbH, Witten, Germany). Samples were then analysed on the scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

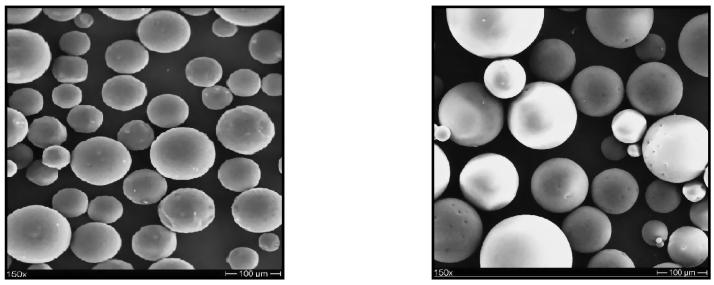
In vitro drug release studies

10 mg microparticles/microparticle blends (particle size: < 70 µm) were placed in 10 ml pH 7.4 phosphate buffer (USP XXIV) and shaken at 37 ^{o}C in a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany) at 75 rpm. At predetermined time points, 1 ml samples were withdrawn and replaced with 1 ml fresh medium each 7 days, filtered and analyzed. Propranolol HCl and/or carbamazepine concentration was detected UV spectrophotometrically at wavelengths of 289 nm and 285 nm, respectively (n = 3) (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany).
For microparticle blends, the concentration of propranolol HCl and carbamazepine were determined UV-spectrophotometrically by simultaneously measuring at wavelengths of 227 and 285 nm (n = 3).

RESULT AND DISCUSSION

Morphology and particle size/distribution of microparticle blends

The surface morphology of the microparticles was observed by scanning electron microscopy (SEM). The surface analysis of drug-loaded microparticle blends with different drug solubility prepared by the WO/W (Pro) and O/W (CBZ) reveal that the microparticles were spherical and not aggregated (Fig. 1) with a diameter range of 73 µm to 81 µm. Microparticle blends containing both, propranolol HCl and carbamazepine, prepared by the WO/W (Pro) and O/W (CBZ) methods with DTI 60 min appeared in two population of microparticles, smooth and rough surface (Fig. 1a). While microparticle which prepared with DTI 0 min produced microparticles with pores and smooth surface (Fig. 1b). Micropores were observed on the microparticles surface that it was propranolol HCl loaded poly (ε-caprolactone) microparticles. No pores were observed on the microparticles surface that it was carbamazepine loaded poly (ε-caprolactone) microparticles.



a **b**

Fig. 1. SEM pictures of poly (ε-caprolactone) microparticles blend with dispersion time interval of (a) 60 min and (b) 0 min between Pro (W/O/W) and CBZ (O/W)

The preparation conditions substantially affected the morphology and porosity of the microparticles. In W/O/W method, the microparticles revealed a porous inner structure caused by the inner aqueous phase. The aqueous droplets are precursors of pores and are the result of phase separation occurring in the organic phase during the hardening of the microparticles (Freiberg and Zhu, 2004).

For microparticle blends contained different drugs with different solubility (propranolol HCl and carbamazepine), the size of microparticle blends prepared by W/O/W (propranolol HCl) and O/W (carbamazepine) methods (with DTI 60 min and stirring time 4 h) was larger than the microparticle blends (with DTI 0 min) and microparticles normal (Fig. 2). Based on FBRM data the addition of second primary oil phase contained poly (ε-caprolactone), carbamazepine and dichloromethane (with DTI 60 min) contributed in enhancement of particle size.

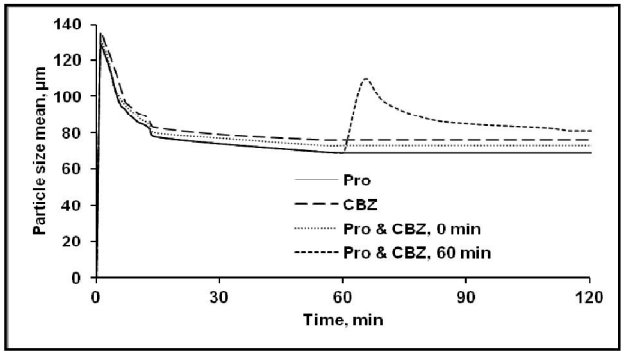


Fig. 2. Particle size mean of poly (ε-caprolactone) based microparticle blends obtained by the FBRM method (before and after primary oil phase addition) during the solvent evaporation process (primary oil phase is added at time = 60 min)

Entrapment efficiency within microparticle blends

Encapsulation efficiency (EE) was about 62.05% to 66.38% for propranolol HCl and 70.56% to 73.85% for carbamazepine in microparticle blends.

The difference observed in the EE of the two drugs in the microparticle blends can be explained with the different solubilities of the drugs in the aqueous continuous phase used for the two encapsulation techniques. The high solubility of the propranolol HCl in the external aqueous phase and its high volume compared to that of the internal aqueous phase (W/O/W technique) caused the leakage of the drug into the continuous phase. However, after the

precipitation of the polymer, the propranolol HCl, due to its hydrophilic nature, still tends to diffuse through the polymeric matrix into the external aqueous phase. Beside that, the degree of ionization of the drug and the pH of the external aqueous phase are critical for the entrapment of ionizable drugs such as propranolol HCl (Pérez et al., 2003). Increasing the pH of the external phase above the pKa of the propranolol HCl results in a decrease of its solubility and, consequently, in an increase of its entrapment in the microparticles.

Release of propranolol HCl and carbamazepine from microparticle blends

Different release rate were observed for propranolol HCl and carbamazepine from poly(ε-caprolactone) microparticle blends in pH 7.4 phosphate buffer (Fig. 3). The propranolol HCl release from microparticle normal and microparticle blends (with DTI 0 min), were faster than carbamazepine release (Fig. 3). Whereas, propranolol HCl release (33%) was slower than carbamazepine release (60%) from poly(ε-caprolactone) microparticle blends (with DTI 60 min) (Fig. 3). Fig. 3 shows that the cumulative percent of propranolol HCl and carbamazepine released from each microparticle blends (the range of ADL Pro ≈ 9.32% to 9.86% and ADL CBZ ≈ 10.69% to 10.97%) at pH 7.4 after 28 days are in the range of 33% to 69% (propranolol HCl) and 41% to 60% (carbamazepine).

In all cases, the resulting release rate(s) of the incorporated drug(s) was/were found to be controlled over periods of at least 28 days. Furthermore, the release of carbamazepine was generally slower than that of propranolol HCl which can most probably be attributed to the much lower solubility of carbamazepine compared to propranolol HCl in the release medium (0.2 mg/ml vs. 250 mg/ml), resulting in lower concentration gradients, the driving forces for diffusion.

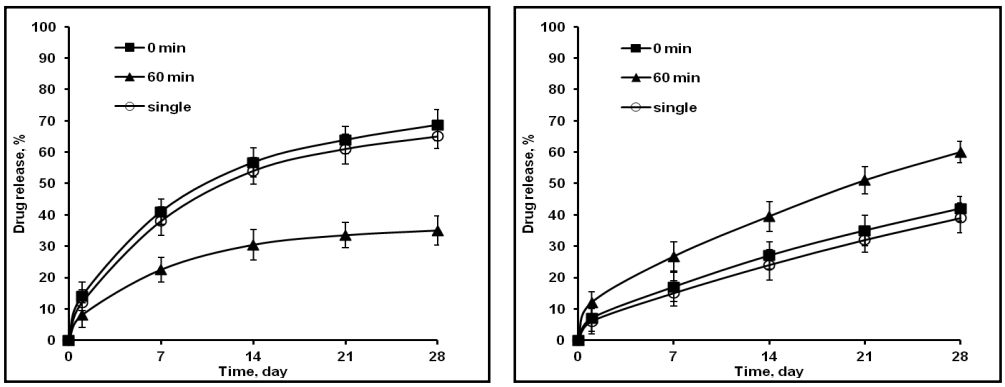
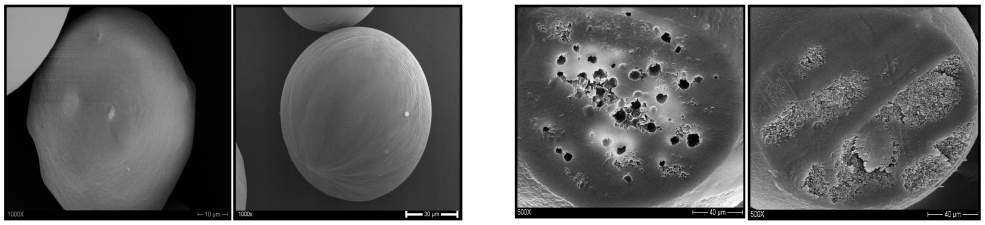


Fig. 3. Effects of dispersion time interval on propranolol HCl and carbamazepine release from polycaprolactone microparticle blends (phosphate buffer, pH 7.4, 37 ^{o}C, 75 rpm). [(a) Propranolol HCl and (b) Carbamazepine]

The increase of carbamazepine release from microparticle blend with the W/O/W (Pro) and O/W (CBZ) (DTI 60 min) could be due to the incorporation of carbamazepine on surface of propranolol HCl loaded microparticle. This may have reduced propranolol HCl migration to the surface of the microparticles, and its leakage in the dissolution medium as compared to the microparticles prepared with W/O/W method where the propranolol HCl is either molecularly dispersed or amorphous in the matrix. In addition, the porous membrane observed in the case of the microparticles prepared with W/O/W method favoured a fast release of the hydrophilic propranolol HCl. The incomplete release of carbamazepine from microparticles may be the result of the hydrophobic nature of the drug and its very low water solubility.

On the contrary, the release of propranolol HCl was significantly slowed down in the case of the microparticle blends (DTI 60 min) compared to that of the microparticles normal.

Only 33% of propranolol HCl was released from microparticle blends prepared by W/O/W (Pro) and O/W (CBZ) methods with DTI 60 min. It has to be emphasized that the propranolol HCl was inside of microparticle and carbamazepine was on outer surface of microparticle. Thus only the drug located close to the outer surface could be initially released. The release of surface associated drug creates water-filled channels that allow subsequent diffusion of the drugs located inside the microparticles. A major mechanism for release of propranolol HCl and carbamazepine are diffusion through water-filled pores.



a **b**

Fig. 4. SEM pictures of polycaprolactone microparticle blends with dispersion time interval of 60 min between primary emulsion and primary oil phase (a. higher magnification and b. cross-section).

Based on release data for each microparticle blends, it can be assumed that there is interaction between first primary emulsion (propranolol HCl) and second primary oil phase (carbamazepine) during preparation process of microparticle blends. FBRM data about particle size mean before and after addition of second primary oil phase into single external aqueous phase (Fig. 2) and surface morphology of microparticles blend (Fig. 4a) have indicated it. In addition, cross section of the microparticles revealed a porous inner structure and absence of pores (Fig. 4b). For microparticle blends which were prepared with DTI 60 min the internal structure appeared reducing in the number of pores and size of pores (Fig. 4b). This phenomenon might be attributable to the interaction of second primary oil phase (CBZ) with hard particles from first primary emulsion (Pro), whereby the second primary oil phase (CBZ) had blocked and coated pores on the surface of hard particle from first primary emulsion. This hypothesa supported by optical microscopy pictures. It indicates that the emulsification stage first W/O/W (Pro) and second (CBZ/dye) resulting in two kind of microparticle blends (Fig. 5). This picture showed microparticle with black plaque on the surface and black microparticles.

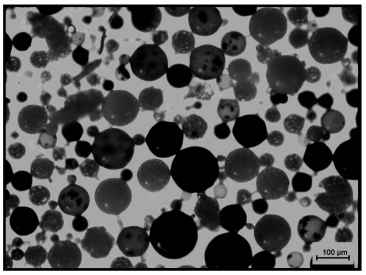


Fig. 5. Optical microscopy pictures of polycaprolactone microparticle blends with dispersion time interval of 60 min. [Microparticles containing dye (black)]

CONCLUSION AND SUGGESTION

The novel microparticle blends containing drugs of different solubility (e.g. propranolol HCl and carbamazepine) offer a high potential for controlled release drug delivery systems. For

microparticle blends (with DTI 60 min) containing drugs of different solubility gave propranolol HCl release was slower than carbamazepine release. FBRM studies showed that particle size of microparticle from first primary emulsion (Pro) was smaller than particle size of microparticle after addition second primary oil phase (CBZ) (with DTI 60 min). It was caused second primary oil phase (CBZ) interacted with microparticles from first primary emulsion (Pro). Optical and scanning electron microscopy revealed that microparticle blends (DTI 60 min) were spherical and had two populations. These microparticle blends consisted of microparticles with smooth and rough surface. This phenomenon might be attributable to the interaction of second primary oil phase with hard particles from first primary emulsion, whereby the second primary oil phase had blocked and coated pores on the surface of hard particle from first primary emulsion. Type of dispersion time interval of microparticle blends influenced the physical properties of the microparticle blends.

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