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Propranolol HCl release profiles from ethyl cellulose based microparticle blends

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Abstract

The purpose of this study was to investigate the effect of dispersion time interval (DTI) and formulation of second primary emulsion on ethyl cellulose-based microparticle blends containing the same drug (propranolol HCl [Pro]). Microparticle blends were formulated with W/O/W solvent evaporation method. The first Pro emulsion (W/O) and second Pro emulsion (W/O) were dispersed in an external aqueous phase, with DTI of 0 and 60 min. The morphology of microparticle blends were characterized by optical microscopy and scanning electron microscopy (SEM). The particle size mean of the 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. Results showed that the resulting microparticle blends obtained by solvent evaporation method were spherical and two populations. FBRM data showed that the size of microparticle blends prepared with DTI of 60 min and stirring time 4 h was larger than the microparticle blends with DTI of 0 min. The encapsulation efficiency (EE) was about 76.53% to 78.81% for propranolol HCl in microparticle blends containing the same drug. In vitro drug release in phosphate buffer (pH 7.4) after 28 days showed that the propranolol HCl release from microparticle blends containing the same drug with DTI 60 min (54.05%) was slower than microparticle blends with DTI 0 min (73.28%). This phenomenon attributable to the interaction of second primary emulsion (Pro) with hard particles from first primary emulsion (Pro), whereby the second primary emulsion (Pro) had blocked and coated pores on the surface of hard particle from first primary emulsion. The novel microparticle blends containing drugs of the same solubility offer a high potential for controlled release drug delivery systems.

Key words: microparticles blend, propranolol HCl, ethylcellulose, FBRM, solvent evaporation method

1. 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 (Bodmeier and McGinity, 1988; Freitas et al., 2005; Li et al., 2008; O’Donell and McGinity, 1997). 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 (Pérez et al., 2003; Yamakawa et al., 1992; Yang et al., 2000a). 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 (Herrmann and Bodmeier, 1995; Pérez et al., 2000; Yang et al., 2000b and 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. Non biodegradable polymers with good biocompatibility are also used as drug carriers, such as ethyl cellulose (degradable but non biodegradable). EC is a derivative of cellulose in which some of the hydroxyl groups on the repeating anhydroglucose units are modified into ethyl ether groups, largely called as non-ionic ethyl ether of cellulose. EC has extensively been used for microencapsulation due to its many versatile properties such as water insoluble but soluble in many organic solvents such as alcohol, ether, ketone and ester; biocompatible and compatible with many celluloses, resin and almost all plasticizers; stable against light, heat, oxygen and wetness and chemicals; non-toxic; etc (Murtaza, 2012). EC is used for microencapsulation of various pharmaceuticals to stabilize them against active interactions, hydrolysis and oxidation. It also is employed as a matrix and/or coating agent to impart sustained release characteristics.

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. (2000) have incorporated a lipophilic and a hydrophilic drug simultaneously within biodegradable, poly (ε-caprolactone)-based microparticles by solvent evaporation methods. In another study, 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. Combination products also known as fixed dose combinations are combinations of two or more active drugs produced in a single dosage forms. They provide the advantages of combination therapy while useful to improve adherence and can simplify procurement, storage and distribution of medicines. Fixed dose combination drugs are an important approach to addressing the management of both chronic and acute diseases.

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 ethyl cellulose 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 microparticle blends formation during solvent evaporation process, FBRM can be used to provide in situ/on-line particle characterization in a wide range of applications (Dowding et al., 2001; 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; Dowding et al., 2001; Kail et al., 2009; Ruf et al., 2000; Vay et al., 2012; 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 emulsion/oil phase on ethyl cellulose based microparticle blends contained the same drug (propranolol HCl) which prepared by solvent evaporation method.

2. Materials and methods

2.1. Materials

All materials were of at least reagent grade and used as received: ethyl cellulose (Ethocel® Standard 4 Premium, Colorcon Ltd, Kent, UK); polyvinyl alcohol (PVA,
Mowiol® 40–88, Kuraray Europe GmbH, Frankfurt, Germany); propranolol HCl, sodium chloride, sodium hydroxide, potassium dihydrogen phosphate and dichlormethane (Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

2.2. Methods

2.2.1. Microparticle preparation

2.2.1.1. Microparticle containing propranolol HCl

Drug loaded microparticles based on ethyl cellulose were prepared using a water-in-oil-in-water (W/O/W) solvent evaporation method. The drug loaded systems contained either one drug only (propranolol HCl).

For the W/O/W method, 43 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 3 ml dichlormethane containing 300 mg of ethyl cellulose. 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 (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) 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.

2.2.1.2. Microparticle blends

The first and second primary emulsion were prepared by W/O/W method. A first primary emulsion containing propranolol HCl was prepared as follows: 43 mg propranolol HCl were dissolved in 0.25 g purified deionized water. Propranolol HCl aqueous solution was emulsified by probe sonication for 30 s under ice-cooling into 3 ml dichlormethane containing 300 mg of ethyl cellulose. Four different second primary emulsion were prepared: (1) The same formulation as the first primary emulsion, (2) 3 ml dichlormethane containing 300 mg of ethyl cellulose, but with a drug loading twice as high as the first primary emulsion, (3) 3 ml dichlormethane containing 300 mg of ethyl cellulose and (4) 3 ml dichlormethane. The first primary emulsion containing propranolol HCl and one of the second primary emulsion phases 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 above process.

2.2.2. 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 concentration in the obtained aqueous solution was determined by UV-spectrophotometry at wavelengths of 289 nm (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 %.

2.2.3. 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 (Fig. 1). 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).
2.2.4. 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).

2.2.5. 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 °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 concentration was detected UV spectrophotometrically at wavelengths of 289 nm (n = 3) (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany).

3. Results and discussion

3.1. Morphology and particle size/distribution of microparticle blends

The surface morphology of the microparticles was observed by scanning electron microscopy (SEM). The microparticle blends contained the same drug (propranolol HCl and propranolol HCl) revealed that the microparticles were spherical and not aggregated (Fig. 2) with diameters of 104.26 μm to 127.64 μm. For the microparticle blends prepared with second primary emulsion consisted of EC and dichloromethane or propranolol HCl, EC and dichloromethane (with DTI 60 min) appeared two population of microparticles. These microparticles show with pores and rough surface (Fig 2c and Fig. 2d). Whereas microparticle which prepared with DTI 0 min (Fig. 2b) produced microparticles with pores. Micropores were observed on the microparticles surface that it was propranolol HCl loaded EC microparticles. 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; Grattard et al., 2002; Pérez et al., 2000, 2003; Siepmann et al., 2004; Yeo and Park, 2004).
Fig. 2. SEM pictures of ethyl cellulose microparticles blend with varying dispersion time interval between primary emulsion 1 and primary emulsion 2 [(a) Pro (W/O/W); (b) Pro (W/O/W) and Pro (W/O/W), DTI: 0 min; (c) Pro (W/O/W) and Pro (W/O/W), DTI: 60 min; (e) Pro (W/O/W) and EC 4 cp (W/O/W), DTI: 60 min; (e) Pro (W/O/W) and dichloromethane, DTI: 60 min]

Microparticle blends contained the same drug (propranolol HCl), the particle size mean of microparticles blend with DTI 60 min was larger than the microparticles blend
with DTI 0 min after stirring 4 h (Fig. 3). When second oil phase consisted of just dichloromethane (with DTI 60 min), the particle size mean of microparticles blend was smaller than the microparticles normal (Fig. 3b). Whereas, the particle size mean of microparticles blend was larger than the others when the second primary emulsion contain EC and dichloromethane (Fig. 3b).

Fig. 3. Effects of dispersion time interval (a) and the primary emulsion formulation 2 (b) on particle size mean of ethyl cellulose based microparticle blends obtained by the FBRM method during the solvent evaporation process. (Primary emulsion 2 is added at time = 60 min)

It was observed from FBRM data that addition of second primary emulsion after stirring 60 min into single external aqueous phase affected the particle size. Addition of dichloromethane as second primary oil phase dissolved polymer on the surface of first hard particles, so produce particles in smaller size. While, addition of second primary emulsion consisted of EC and dichloromethane or EC, propranolol HCl and dichloromethane (with DTI 60 min and stirring time 4 h) had been increased the particle size mean. They contributed in enhancement of particle size, so produce particles in larger size. All batches of microparticles show different particle size distributions profile (Fig. 4). As expected the larger particles size mean gave longer particle size distribution, and a reduced count rate due to the decreased number of particles. This phenomenon is applicable to all microparticle blends. The number of particles for microparticle blends was higher than for normal microparticles, it is due to higher volume of EC solution.
Fig. 4. Effects of dispersion time interval (a) and the primary emulsion formulation 2 (b) on particle size distribution obtained by the FBRM method for all batches of ethyl cellulose based microparticle blends (stirring time = 4 h)
3.2. Entrapment efficiency within microparticle blends

The encapsulation efficiency (EE) was about 76.53% to 78.81% for propranolol HCl in microparticle blends containing the same drug (Table 1).

Table 1. Formulation, drug entrapments and particle size mean of microparticles (whole size)

<table>
<thead>
<tr>
<th>Emulsion 1</th>
<th>Emulsion 2</th>
<th>Dispersion time interval (minute)</th>
<th>Actual drug loading (%) (± SD)</th>
<th>Encapsulation efficiency (%) (± SD)</th>
<th>Particle size mean (µm) (± SD)</th>
</tr>
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<tbody>
<tr>
<td>Pro&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>9.62 (± 0.35)</td>
<td>76.96 (± 2.83)</td>
<td>108.38 (± 4.07)</td>
</tr>
<tr>
<td>Pro&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Pro&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>9.69 (± 0.31)</td>
<td>77.52 (± 2.47)</td>
<td>104.26 (± 3.16)</td>
</tr>
<tr>
<td>Pro&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Pro&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60</td>
<td>9.82 (± 0.26)</td>
<td>78.56 (± 2.11)</td>
<td>116.05 (± 5.37)</td>
</tr>
<tr>
<td>Pro&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No drug</td>
<td>60</td>
<td>9.85 (± 0.38)</td>
<td>78.81 (± 3.04)</td>
<td>127.64 (± 6.71)</td>
</tr>
<tr>
<td>Pro&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No drug</td>
<td>60</td>
<td>5.12 (± 0.29)</td>
<td>76.53 (± 2.32)</td>
<td>125.16 (± 6.08)</td>
</tr>
<tr>
<td>Pro&lt;sup&gt;a&lt;/sup&gt;</td>
<td>dichloromethane</td>
<td>60</td>
<td>9.54 (± 0.38)</td>
<td>76.32 (± 3.04)</td>
<td>97.02 (± 4.25)</td>
</tr>
</tbody>
</table>

Pro<sup>a</sup>: consisted of propranolol HCl (DL 12.5%), ethyl cellulose and dichloromethane
Pro<sup>b</sup>: consisted of propranolol HCl (DL 25%), ethyl cellulose and dichloromethane
No drug: consisted of ethyl cellulose and dichloromethane

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. This leakage process is believed to happen mainly during the first minutes of emulsification since the polymer precipitates rapidly thereby decreasing leakage (Alhaman and Basit, 2011; Freiberg and Zhu, 2004; Hsu and Lin, 2005; Pérez et al., 2000, 2003). 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., 2000, 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.
3.3. Release of drugs

3.3.1. Release of propranolol HCl from microparticle blends

The release of propranolol HCl from EC microparticle blends in pH 7.4 phosphate buffer showed a difference in release rate (Fig. 5). Different release rates of propranolol HCl which were given by microparticle blends containing the same drug which were prepared by the W/O/W method (primary emulsion 1) and W/O/W method (primary emulsion 2) (Fig. 5). The cumulative percent of propranolol HCl released from each microparticle blends (with range of actual drug loading (ADL) ≈ 4.92% to 8.76%) at pH 7.4 after 28 days is in the range of 28.95% to 73.28% (Fig. 5). The propranolol HCl release from microparticle blends with dispersion time interval 0 min (73.28%) was faster than 60 min (54.05%) (Fig. 5a). It is apparent from the data reported in Fig. 5 that the type of dispersion time interval and composition of formulation second primary emulsion affected the percent of propranolol HCl released. Porosity of the microparticles influenced drug release mechanism. This could lead to a more rapid release of propranolol HCl. The mechanism for release of propranolol HCl from ethyl cellulose matrix is diffusion through water-filled pores (Chiao and Price, 1994; Freiberg and Zhu, 2004; Grattard et al., 2002; Yeo and Park, 2004).
Fig. 5. Effects of dispersion time interval (a) and the primary emulsion formulation 2 (b) on propranolol HCl release from ethyl cellulose microparticle blends (phosphat buffer, pH 7.4, 37 °C, 75 rpm).

Propranolol HCl release profile from each microparticle blends indicated there is interaction between first primary emulsion and second primary emulsion during preparation process of microparticle blends. The second primary emulsion had blocked and coated pores on the surface of hard particle from first primary emulsion (Fig. 6b. a1,b1). This hypothesis was supported by cross section of the microparticles blends (DTI
60 min) whereby the internal structure appeared reducing in the number of pores (Fig. 6b, a3,b3).

Fig. 6. SEM pictures of ethyl cellulose microparticle blends with varying dispersion time interval between primary emulsion 1 and primary emulsion 2 (higher magnification and cross-section). (a) a.1-2. Pro (W/O/W), and b.1-2. Pro (W/O/W) and Pro (W/O/W), DTI: 0 min. and (b) a.1-4. Pro (W/O/W) and Pro (W/O/W), DTI: 60 min; and b.1-4. Pro (W/O/W) and EC 4 cp (W/O/W), DTI: 60 min

4. Conclusion

The novel microparticle blends containing drugs of the same solubility (e.g. propranolol HCl and propranolol HCl) offer a high potential for controlled release drug delivery systems. Microparticle blends (with DTI 60 min) containing drugs of the same
solubility gave propranolol HCl release was slower than propranolol HCl release from microparticle blends (with DTI 0 min) and microparticles normal. FBRM studies showed that particle size of microparticle from first primary emulsion (Pro) was smaller than particle size of microparticle after addition second primary emulsion (Pro) (with DTI 60 min). It was caused second primary emulsion (Pro) 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 pores and rough surface. This phenomenon might be attributable to the interaction of second primary emulsion with hard particles from first primary emulsion, whereby the second primary emulsion had blocked and coated pores on the surface of hard particle from first primary emulsion.

Type of dispersion time interval and formulation type of second primary emulsion in preparation process of microparticle blends influenced the physical properties of the microparticle blends.

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