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Preparation and characterisation of ethylcellulose microparticles containing propolis

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ABSTRACT

Ethylcellulose microparticles containing propolis ethanolic extract (PE) were prepared by the emulsification and solvent evaporation method. Three ratios of ethylcellulose to PE dry residue value (DR) were tested (1:0.25, 1:4 and 1:10). Moreover, polysorbate 80 was used as emulsifier in the external phase (1.0 or 1.5% w/w). Regular particle morphology without amorphous and/or sticking characteristics was achieved only when an ethylcellulose:DR ratio of 1:0.25 and 1.0% polysorbate 80 were used. Microparticles had a mean diameter of 85.83 μm. The entrapment efficiency for propolis of the microparticles was 62.99 ± 0.52%. These ethylcellulose microparticles containing propolis would be useful for developing propolis aqueous dosage forms without the strong and unpleasant taste, aromatic odour and high ethanol concentration of PE.

Keywords: Brazilian propolis; ethylcellulose; emulsification and solvent evaporation; microparticle characterisation; optimisation.

INTRODUCTION

Over the past few decades in pharmaceutical research, many biodegradable microparticle systems have been studied as potential drug carriers (Bruschi et al., 2003; Hasan et al., 2007).

Biodegradable and biocompatible materials such as cellulose derivatives have been investigated in microencapsulation processes (Yamada et al., 2001; Heng et al., 2005; Homar et al., 2007). Ethylcellulose is a semisynthetic material (Finch, 1990) whose main attractive features are its biocompatibility and degradation to non-toxic and readily excreted products (Chandra & Rustgi, 1998). Moreover, being a water-insoluble polymer and wall-forming material, ethylcellulose is very useful for the preparation of microparticle drug-delivery systems (Jones & Pearce, 1995; Hasan et al., 2007). In such microcapsules, it is possible to make solid bodies from oils, to control odour or taste, to protect drugs from moisture or oxidation, to alter solubility, to delay volatilization, to prevent incompatibilities, and to modify/control drug delivery (Tirkkonen et al., 1995; Zhang et al., 2000; Bruschi et al., 2006).

Propolis (bee glue) is a strongly adhesive resinous bee-hive product collected by honeybees from leaf buds and cracks in the bark of various plants (Burdock, 1998). Bees masticate this resin, salivary enzymes are added (mainly β-glucosidase), and the partially digested material is mixed with beeswax and used in the hives to exclude draughts, to protect against external invaders and to mummify their carcasses (Pietta et al., 2002; Bruschi et al., 2006). Its complex chemical composition typically consists of waxes, resins, water, inorganics, phenolics and essential oils, the exact composition of the propolis depending on the source plant(s) (Burdock, 1998). It has been widely used in traditional medicine for hundreds of years, at least since 300 BC (Ghisalberti, 1979), and has been reported to possess antimicrobial (Koo et al., 2000; Sforcin et al., 2000; Banskota et al., 2001; Santos et al., 2002; Kartal et al., 2003; Melliou & Chinou, 2004; Bruschi et al., 2006), fungicidal (Ota et al., 2001; Murad et al., 2002; Sawaya et al., 2002; Bruschi et al., 2006; Oliveira et al., 2006; Dias et al., 2007), antiviral (Ghisalberti, 1979; Marcucci, 1995; Kujumgiev et al., 1999), antiulcer (Burdock, 1998), immunostimulating (Burdock, 1998), hypotensive (Marcucci, 1995; Burdock, 1998), anti-inflammatory (Burdock, 1998; Song et al., 2002a; 2002b), antioxidant (Isla et al., 2001; Nagai et al., 2001), and cytostatic (Banskota et al., 2001; 2002) activities.

Alone or incorporated in another dosage form, propolis ethanolic extract is commonly used in therapy (Burdock, 1998; Dias et al., 2007). Some disadvantages of propolis ethanolic extracts are the strong and unpleasant taste, aromatic odour and high ethanol concentration. These characteristics cause problems in packing, transport and incorporation into another dosage form. Patient compliance to the treatment is also prejudiced. Bruschi et al. (2003; 2006) showed that it is possible to produce gelatin microparticles containing propolis, without the strong and unpleasant taste, aromatic odour or high ethanol concentration. However, these microparticles are water

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soluble and cannot be added to some aqueous formulations (Bruschi et al., 2004; 2007).

The aim of the present study was to develop a method to prepare propolis microparticles with ethylcellulose as the wall-forming polymer and to characterize the propolis microparticles produced.

MATERIAL AND METHODS

Materials

Propolis was collected at the experimental farm of State University of Maringá (UEM), Paraná State, Brazil. Standard premium ethylcellulose NF 20 (Dow, Brazil) and polysorbate 80 (Synth, Brazil) were used without further purification. Acetone (Merck; analytical grade), ethyl acetate (Merck; analytical grade), methanol (Merck; analytical grade), acetic acid (Merck; analytical grade), aluminium chloride (Merck; analytical grade) and ethyl alcohol (96 ºGL; pharmaceutical grade) were also used.

Preparation and characterisation of the propolis extract

Propolis extract (PE) was prepared with a propolis/ethanol ratio of 30/70 (w/w) by turbo extraction, filtered through filter paper and made up to the initial weight with ethanol (Bruschi et al., 2002). Exactly 10 g of PE was weighed and concentrated on a water bath (100 ºC) with occasional shaking. The concentrated material was dried on the Ohaus–MB 200 infrared analytical balance (Pine Brook, NJ, USA) at 110 °C and the final weight was designated the dry residue value (DR). Three replicates were carried out to estimate the inherent variability of each determination.

The total flavonoids content of PE was obtained by a technique described earlier (Bruschi et al., 2003). Equal volumes (3.0mL) of distilled water, acetone, and PE were mixed in a separating funnel. This mixture was extracted three times with 15 mL of ethyl acetate. These extracts were made up to 50.0 mL with ethyl acetate, (S1). Exactly 1mL of aluminium chloride ethanolic solution (2% w/v) was added to 10mL of S1, followed by methanolic solution of acetic acid (5% v/v) to a total volume of 25.0 mL (MS). In parallel, 10.0 mL of S1 was made up to 25.0 mL with MS alone, as a compensatory solution. After 30 min, the solutions were analysed in a Shimadzu UV-1650PC spectrophotometer (Tokyo, Japan) at $\lambda = 425$ nm. Three replicates were carried out to estimate the inherent variability of the determination and the total flavonoids content of PE was calculated in grams of quercetin (specific absorptivity: 500) extracted from 100 g of dried propolis.

Preparation of propolis microparticles

Propolis microparticles (PM) were prepared by emulsification and solvent evaporation (Finch, 1990; Quintanar-Guerrero et al., 1998). In brief, ethylcellulose (0.25 g) was dissolved in ethyl acetate with stirring (Fisatom hot plate/stirrer, Brazil) and PE was dispersed into this solution in three ethylcellulose:DR ratios (1:0.25, 1:4 and 1:10 w/w). This drug/polymer organic dispersion was emulsified by mixing at 800 rpm, with a Heidolf R1RZ overhead stirrer provided with a paddle rotor, into an aqueous external phase containing polysorbate 80 (1.0 or 1.5% v/v) at room temperature (18-20 ºC). The organic phase was added to the aqueous phase at a constant rate, until the final volume of the two phases was 70 mL, the volume ratio (organic:aqueous) then being 1:2.5. Stirring of the resultant O/W emulsion was continued until the ethyl acetate had evaporated. The microparticles were collected by filtration, washed with deionised water and freeze-dried (freeze dryer Christ Alpha 1-4, Germany). The microparticles were kept dry until further analysis.

Evaluation of preparation methods

To analyze these experiments, two properties were assessed in the product: macroscopic characteristics and residual moisture content. Residual moisture was measured with an Ohaus–MB 200 infrared analytical balance on a 3.0 g sample at 110 ºC. Three replicates were carried out to estimate the inherent variability of the analysis.

Scanning electron microscopic study

PM were coated with gold/palladium under argon atmosphere and examined under a scanning electron microscope (SHIMADZU—SS550, Tokyo, Japan). The scanning electron photomicrographs (SEM) were analysed.

Particle size analysis and distribution

The samples of propolis microparticles (PM) were subjected to particle size analysis with an optical microscope (Carl Zeiss, Germany) and the Carl Zeiss *AxioVision Image Analysis System*. Particles were placed on glass slides and their size was determined by measuring Feret's diameter. Two thousand microparticles were measured and the particle size distribution was estimated.

Assay for propolis entrapment efficiency

The amount of propolis in the PM was determined by a spectrophotometric technique (Bruschi et al., 2006). Three grams of PM, 3.0 mL of acetone, and 3.0 mL of distilled water were mixed in a separating funnel. This mixture was extracted three times with 15 mL of ethyl acetate. Ethyl acetate was then added to 50.0 mL to give M1. Exactly 1.0 mL of aluminium chloride ethanolic solution (2%, w/v) was added to 10.0 mL of M1 and methanolic solution of acetic acid (5%, v/v) was added to a total of 25.0 mL, giving M2. In parallel, methanolic solution of acetic acid (5%, v/v) was added to 10.0 mL of M1, to a total of 25.0 mL, to be used as a compensatory solution. After 30 min, M2 was analysed

by Shimadzu UV-1650PC spectrophotometer (Tokyo, Japan) at λ = 425 nm. Three replicates were carried out to estimate the inherent variability of the determination and the total flavonoids content contained in PM was calculated in grams of quercetin (specific absorptivity = 500) obtained from 100 g of dried PM. The entrapment efficiency of the PM for propolis was calculated by comparing the flavonoid content of the PE with that of the PM.

RESULTS

Characterisation of the PE

Propolis extract dry residue (DR) was 18.43 ± 0.17% with 0.92% relative standard deviation (RSD). Total flavonoids content was 2.60 ± 0.06%, with 2.24% RSD (Table 1).

Evaluation of preparation methods

The higher emulsifier concentration (1.5%) in the external phase and the ethylcellulose:DR ratios of 1:4 and 1:10 did not yield good structures. These structures displayed amorphous and/or sticking characteristics and the products could not be collected after washing or freeze-dried. Only the PM prepared with an ethylcellulose:DR ratio of 1:0.25 and 1.0% polysorbate 80 in the external phase yielded good results (Figure 1). The moisture content in this experiment was 0.98% (Table 1).

Scanning electron microscopy

Photomicrographs of the PM are shown in Figure 2. Most of the particles were small and spherical and had a uniform surface. The PM tended to form clusters.

Table 1. Characteristics of propolis extract (PE) and ethylcellulose microparticles containing propolis (PM)

Parameters	PE	PM
Dry residue (%)	18.43 ± 0.17	N/A
Moisture content (%)	N/A	0.98 ± 0.00
Total flavonoids content (%)	2.60 ± 0.06	0.49 ± 0.00
Drug trapping efficiency (%)	N/A	62.99 ± 0.52

N/A (not applicable)

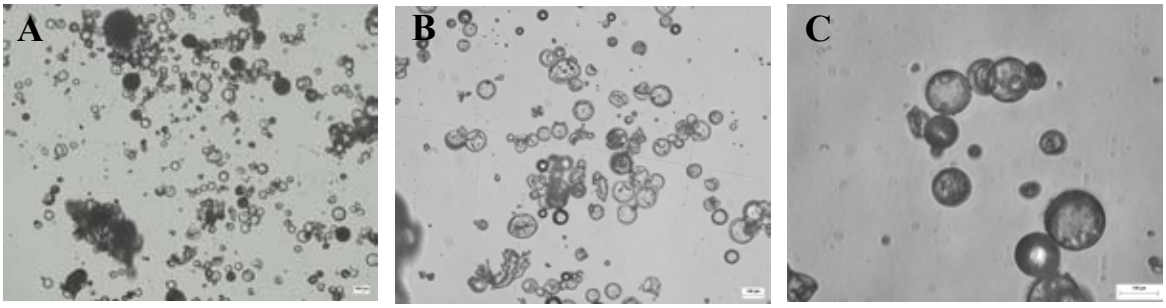

Figure 1. Optical photomicrographs of propolis microparticles (PM), showing the outer topology of PM formed in the experiment with an ethylcellulose:DR ratio of 1:0.25 and 1.0% polysorbate 80: (A) original magnification 100X; (B) original magnification 200X; and (C) original magnification 400X.

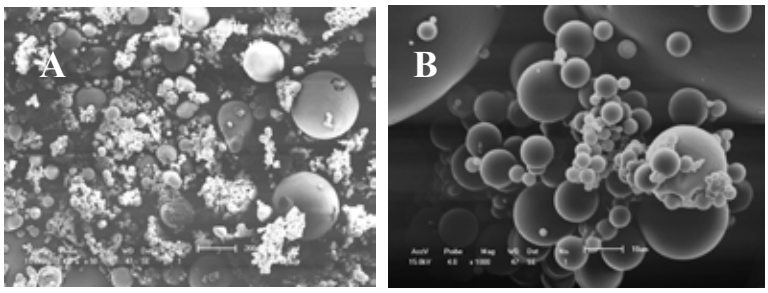

Figure 2. SEM images of propolis microparticles showing the outer topology: (A) original magnification 50x; (B) original magnification 1000x.

Particle size analysis and distribution

PM were prepared by the method described above, with an ethylcellulose:DR ratio of 1:0.25 and 1.0% polysorbate 80 in the external phase. A typical size distribution graph is presented in Figure 3. The microparticles had a mean diameter of 85.83 µm, confirming that the structures produced were microparticles.

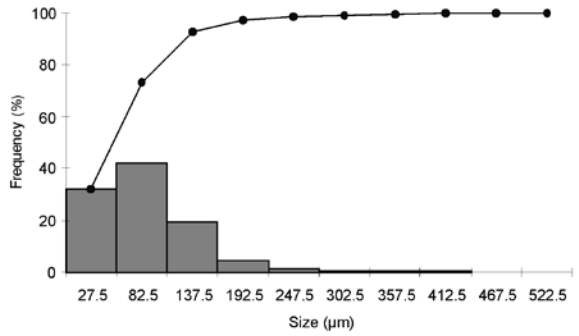

Figure 3. Size distribution of ethylcellulose microparticles containing propolis: size frequency distribution (bars) and size cumulative frequency distribution (line). The particle size class interval is 55 µm.

Entrapment efficiency of propolis in microparticles

To calculate the entrapment efficiency of the PM for propolis, the flavonoids in PE and PM were quantified by spectrophotometry. The results are displayed in Table 1.

DISCUSSION

In this study, ethylcellulose microparticles containing propolis (PM) were prepared by the emulsification and solvent evaporation process, in which the organic solvent is lost from the surface of the droplets while they are emulsified in the aqueous external phase. There is a subsequent increase in the concentration of polymer until a critical point is reached where the concentration of the polymer exceeds its solubility in the organic phase. The polymer then precipitates to produce microparticles (Bodmeier & McGinity, 1988).

Two factors were examined in this study, polymer concentration and concentration of emulsifier (polysorbate 80) in the external phase. PM prepared with an ethylcellulose:DR ratio of 1:0.25 and 1.0% polysorbate 80 in the external phase yielded good results (Figure 1). The effect of the emulsifier concentration on the physical appearance and on the drug loading of the microparticles prepared by the solvent evaporation process has been reported previously: increasing the concentration of emulsifier in the aqueous phase led to low yield and reduction of the drug content of these microparticles (Benita et al., 1984; Cavalier et al., 1986; Jones & Pearce, 1995). The best results were probably due to a good combination of the drug:polymer ratio and emulsifier concentration (Jones & Pearce, 1995; Bruschi et al., 2003).

Moreover, the PM were small, spherical and had a uniform surface. These PM characteristics are the result of the combination of drug:polymer ratio, emulsifier concentration and stirring method. They are in agreement with Palmieri et al. (1996), Amiet-Charpentier et al. (1998), Billon et al. (2000) and Bruschi et al. (2006).

The entrapment efficiency of the PM for propolis is a parameter of fundamental importance to the process used. Failure to achieve acceptable loadings may preclude the use of the method for economic reasons. To ensure an efficient process it is essential that the core drug material is retained within the polymer phase until the solid microparticle is produced (Bodmeier & McGinity, 1988). The drug used in this study, propolis extract, is a complex mixture of propolis components dispersed in ethanol. Some of these substances are insoluble in water and exhibit properties that help the structuring of the microparticles (Bruschi et al., 2003). Therefore, the entrapment efficiency observed is due to the low degree of partitioning of PE from the organic phase to the aqueous phase, prior to solidification of the polymer droplets. These observations confirm that drug solubility in the external phase is an important determinant in the entrapment of drugs (Jones & Pearce, 1995).

The present results indicate that the preparation of ethylcellulose microparticles containing propolis by the emulsion hardening process is a feasible and simple method. The characteristics and morphology of microparticles could be further improved by a proper selection of ethylcellulose and polysorbate 80 proportions. Thus, these results would be useful for the development of a propolis aqueous dosage form without the strong and unpleasant taste, aromatic odour and high ethanol concentration of the propolis extract.

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RESUMO

Preparação e caracterização de micropartículas de etilcelulose contendo propolis

Micropartículas de etilcelulose contendo extrato de propolis (EP) foram preparadas através do método de emulsificação e evaporação do solvente. A proporção de etilcelulose e de resíduo seco do EP (RS) foi avaliado em três razões (1:0,25; 1:4 e 1:10). Além disso, polissorbato 80 foi utilizado com emulsificante na fase externa (1,0

ou 1,5% p/p). Partículas de morfologia regular sem características de material amorfo ou com aderência foram obtidas quando a razão de etilcelulose:RS de 1:0,25 e 1,0% de polissorbato 80 foram utilizados. As micropartículas apresentaram diâmetro médio de 85,83 µm. A eficiência de encapsulação da própolis nas micropartículas foi de 62,99 ± 0.52%. Essas micropartículas de etilcelulose contendo propolis podem ser úteis para o desenvolvimento de formas farmacêuticas aquosas com propolis sem o sabor forte e desagradável do EP, sem o odor aromático e a alta concentração de etanol.
Palavras-chave: própolis brasileira; etilcelulose; emulsificação e evaporação do solvente; caracterização de micropartículas; otimização.

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