Preparation of cephalexin microspheres by double emulsion technique

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INTRODUCTION

Microspheres of biodegradable polymers have been widely studied as drug delivery systems [1]. A great variety of both natural and synthetic biodegradable polymers such as chitosan, polylactic-co-glycolic acid, polymethylmethacrylate, polylactic acid and polycaprolactone have been used for the preparation of drug loaded microspheres [2]. In particular, polylactic-co-glycolic acid (PLGA) has received tremendous interest for the development of controlled drug delivery systems due to its excellent biocompatibility and biodegradability. Several methods, including phase separation, coacervation, emulsification diffusion, spray-drying and emulsion-solvent evaporation techniques have been used to obtain PLGA microspheres. Cephalexine (CPX) is a member of the first generation of cephalosporins. In vitro study has shown its high inhibition against various pathogenic microorganisms in dairy cow mastitis [3]. However, it shows poor efficiency in the treatment of this disease. It is considered that a sufficiently high concentration of the drug is not reached in the infected macrophage, the target site of interest. It can be foreseen that if the drug is encapsulated in microspheres with a suitable small size, the drug-loaded particle can potentially be taken up by phagocytes and therefore offer therapeutic value for treatment of dairy mastitis [4]. The aim of the present work was to prepare and investigate the factors influencing the formation of CPX microspheres and their physicochemical properties. The w/o/w double emulsion technique was used in order to prepare microspheres within the optimum size range and drug loading.

EXPERIMENTAL

Materials

Poly (dl-lactide-co-glycolide) (PLGA), with a copolymer ratio of dl-lactide/glycolide of 60/40 and an inherent viscosity of 0.5 dl/g was purchased from PURAC Biochem (Gorichem, The Netherlands). Polyvinyl alcohol (PVA) with 86-89% hydrolysis degree and molecular mass range of 15,000–100,000 g/mol was obtained from Fluka (Buchs, Switzerland). Cephalexin monohydrate (CPX) was a generous gift from Siam Pharmaceutical (Bangkok, Thailand). Chloroform was purchased from Fisher Chemicals (Loughborough, UK). Dichloromethane, acetone, ethyl acetate, glacial acetic acid and methanol were purchased from Labscan (Dublin, Ireland). These reagents were of analytical grade except methanol that was HPLC grade.

Method of preparation

The preparation method of the CPX microspheres was based on emulsion solvent evaporation technique described by Bodmeier and McGinity [5] with some modifications. An exact amount of CPX was dissolved in deionized water and the drug solution was added to 5% w/v PLGA in chloroform and emulsified with a high speed homogenizer (Polytron®) at 10,000 rpm to yield a w/o emulsion. The w/o primary emulsion was added to 2% PVA aqueous solution and then emulsified at a stress-mixing speed at 10,000 rpm to yield a w/o/w emulsion.

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Morphology study

The morphology of the microparticles was examined by a light microscope (Olympus®) with digital image capabilities. One drop of the freshly prepared microsphere suspension was poured onto a slide and sealed with a cover glass. With the highest magnitude of amplification, the morphology, size uniformity, and aggregation or coalescence of the microspheres were studied. The images were captured using a personal computer running on built-in software.

Particle size and zeta potential measurement

The particle size of the prepared microparticles was determined by using a Cilas® 1064 laser diffraction analyzer, yielding the mean size and size distribution. Only the samples with the aimed size range were measured for zeta potential using a Zetasizer® Nano ZS analyzer at a scattering angle of 173 ° at a temperature of 25 °C.

RESULTS AND DISCUSSION

Effect of solvent type on size of CPX microspheres

Microscopic investigations showed that the CPX microspheres obtained with the various solvents were spherically shaped as shown in Fig. 1. The mean size of the microspheres obtained with the different solvent systems is shown in Table 1. The particles size of the microspheres obtained by using chloroform or dichloromethane was much smaller than those obtained using ethyl acetate. It was reported previously that using acetone as a co-solvent decreased the particle size [6]. In our study, addition of acetone to chloroform or dichloromethane also decreased the size of CPX microspheres. Acetone is water-miscible while chloroform or dichloromethane are water-immiscible. Acetone is miscible with chloroform as well as dichloromethane. Consequently, the addition of acetone to chloroform or dichloromethane increases water solubility of the halogenated solvents resulting in a rapid extraction of the solvent by the external aqueous phase. Due to the rapid solvent extraction, an interfacial turbulence occurs between the organic polymer phase and the external water phase leading to the formation of small particles.

Effect of solvent ratio and stirring rate on size of CPX microspheres

The results on effect of different solvent ratio show that within the experimental error the sizes of the formed particles are independent of the chloroform/acetone ratio in the solvent mixture of PLGA as shown in Table 2. The effect of stirring speed of both the primary and secondary emulsion on particle size of CPX microspheres was studies in more detail. The study was done using a solvent ratio chloroform/acetone of 3:2. The results shown in Table 3 reveal that a decreasing stirring rate caused an increase in particle size of the microspheres. This was in line with expectations since a reduction in stirring from 10,000 rpm to 4,000 rpm caused a concomitant reduction of breaking energy, resulting in larger emulsion droplets and thus in larger PLGA particles. Table 3 also gives the encapsulation efficiencies. This table shows that with decreasing stirring rate the encapsulation efficiency tends to increase. A probably explanation is that the surface area of the large particles is lower which led to less transport of the cephalexin into the external aqueous phase.

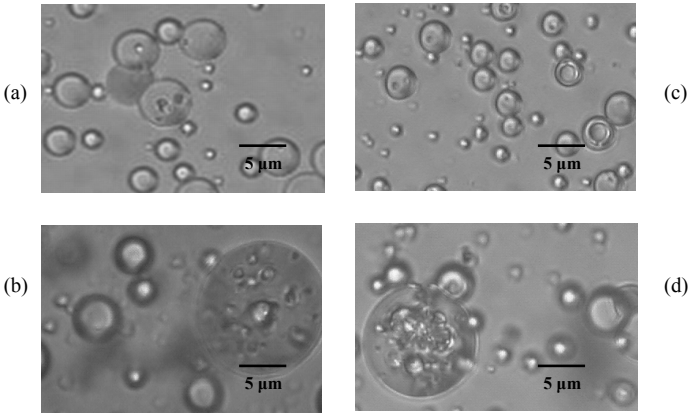

Fig. 1. Photomicrograph of microspheres obtained from various solvent systems; chloroform (a), dichloromethane (b), chloroform and acetone (3:2) (c), dichloromethane and acetone (3:2)

Table 1. The effect of solvent system on particle size of CPX microspheres

Solvent system	Particle size (µm)
Chloroform	4.2 ± 0.3
Chloroform : Acetone (3:2)	3.0 ± 0.3
Dichloromethane	4.4 ± 0.9
Dichloromethane : Acetone (3:2)	4.3 ± 0.6
Ethyl acetate	> 100
Ethyl acetate : Acetone (3:2)	> 100

Table 2. The effect of solvent ratio on particle size and drug entrapment efficiency of CPX microspheres

Chloroform/acetone ratio	Mean diameter (µm)	Entrapment efficiency (%)
4 : 1	3.30 ± 1.09	16.3 ± 3.9
3 : 2	3.18 ± 1.43	18.3 ± 1.2
2 : 3	3.37 ± 1.42	17.3 ± 1.5

Table 3. The effect of stirring on size and drug entrapment efficiency of CPX microspheres

Stirring (rpm)	Mean diameter (µm)	Entrapment efficiency (%)
10,000	3.18 ± 1.23	18.3 ± 1.2
8,000	4.95 ± 0.06	18.6 ± 0.3
4,000	16.50 ± 2.60	19.1 ± 0.5

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Effect of molecular weight of PVA on characteristics of CPX microspheres

Table 4 shows that there was a decrease in particle size from 4.9 to 3.2 µm when the molecular weight of PVA was increased form 15,000 to 100,000 g/mol. A likely explanation is that a higher molecular weight PVA yielded a higher viscosity of the solution. This viscous solution could better stabilize the emulsion droplet against coalescence, resulting in a smaller particle size. The result also shows that the CPX entrapment efficiency was not dependent on the PVA molecular weight. According to the desired size in order to be potentially taken up by the infected macrophage in dairy mastitis, PVA with a molecular weight of 100,000 g/mol was considered to be the most suitable and showed highest percentage of drug encapsulation in this study.

Table 4. Effect of PVA molecular weight on CPX microspheres

MW of PVA	Mean diameter (µm)	Entrapment efficiency (%)
15,000	4.9±0.1	18.6±0.3
30,000-70,000	3.7±0.2	18.6±0.5
100,000	3.2±0.2	19.1± 0.5

CONCLUSION

It was found that many factors including solvent type, solvent composition ratio, PLGA content, stirring rate and the molecular weight of PVA affected the physicochemical properties of CPX-loaded PLGA microspheres. The microspheres with desired size and entrapment efficiency were obtained from 5% PLGA in 3:2 mixture of chloroform-acetone and 2% PVA (MW 100,000) at the preparation stirring range of 10,000 rpm [7].

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