P-032 **Effect of preparation method and surfactant type on cephalexin microspheres**

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INTRODUCTION AND OBJECTIVES

In our previous study (Chaisri 2009), we tried to prepare cephalexin (CPX)-loaded PLGA microspheres for dairy mastitis by double emulsion solvent evaporation technique. However, the loading efficiency of CPX in the microspheres obtained was rather low (<19%). In the W/O/W emulsion process, the type of surface-active substance added is of importance to create a stable emulsion that in turn will affect the drug loading efficiency (Nihant 1994, Yang 2001). Previous reports have been demonstrated that surfactants in the primary emulsion improve drug-loading efficiency (Rojas 1999). However, no reports have been published so far in which the effect of surfactants with different characteristics added to the primary emulsion on size and entrapment of CPX in PLGA microspheres. The aim of the present work was to evaluate the effect of the preparation method and surfactant type on the properties of the CPX microspheres emphasizing on enhancement of drug loading efficiency. According to this purpose, microspheres characteristic such as size, morphology of microspheres, as well as drug-loading efficiency were investigated. Moreover, physical characteristics, e.g., crystallinity and thermal behavior of drug in the microspheres as well as the antimicrobial activity of a selected microsphere formulation were also investigated.

MATERIALS AND METHODS

Preparation of CPX Microspheres Method A: CPX solution was emulsified in 5 mL of 5% w/v PLGA solution in chloroform/acetone by using Polytron® to form the primary W1/O emulsion. This W1/O emulsion was then poured into 2% PVA in distilled water and homogenized in Polytron®. The resulting W1/O/W2 emulsion was stirred at 700 rpm for 18 h at room temperature. The effect of pH of internal water phase and amount of drug loaded were also studied.
Method B: CPX solution was emulsified to 2.5 mL of 10% w/v PLGA in dichloromethane by using ultrasonic probe to yield a stable W1/O emulsion. This W1/O was added a 1% PVA aqueous solution and further emulsified by Polytron®. The organic solvent was allowed to evaporate at 40°C. Different types and concentrations of surfactant were added in primary water phase to study the influence of surfactant on microsphere characteristic

Characterization of microspheres The particle size was determined by using particle sizing systems AccuSizer Model 780. The supernatant after microspheres formation was analyzed for drug loading by HPLC. The morphology of the microspheres was investigated by scanning electron microscopy (SEM). The X-ray diffraction (XRD) patterns and thermal behavior of CPX, physical mixtures of CPX/PLGA, CPX-loaded and unloaded microspheres were obtained by using a Siemen D500 X-ray diffractometer (BrukerAXS, Madison, USA) and Perkin Elmer DSC7 (Norwolk, CT, USA), respectively.

Microbiological Tests Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by using a broth dilution technique against selected strains of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

RESULTS AND DISCUSSION

Effect of Preparation Method on Particle Size and CPX Loading Efficiency Microspheres prepared with water as an internal phase yielded particles with a size of 3.2±0.2 μm and a CPX loading efficiency of 20.6%. Replacement of the internal phase with 0.5 M in order to increase the CPX solubility resulted in microspheres with the same size and loading efficiency. Using preparation method A, the loading efficiency and particle size were not affected by the amount of drug dissolved in the 0.5 M HCl inner phase. As a consequence the loading capacity of the particles prepared with 50 mg of drug was about 10 times higher than those prepared with 5 mg drug. The loading efficiency and size of the microspheres prepared with method B were substantially higher than those of the particles prepared with method A. The mean diameters of particles prepared with method B are bigger than that of the particles prepared with method A. Because of the high encapsulation efficiency, Method B was selected to study the effect of surfactants on CPX microspheres.

Influence of Surfactants on Particle Size and CPX Loading Efficiency The mean size of the microspheres was not affected by the type and concentration of surfactant used for the primary emulsion and varied between 24 and 27 μm. Our results (Fig. 1) demonstrate that surfactants played an important role on CPX encapsulation. The results revealed that surfactants played an important role on CPX encapsulation. In the absence of surfactant, the drug encapsulation was 58.7%.Our results indicate that Tween 80 is the most suitable surfactant for preparing stable CPX primary emulsions and at a surfactant concentration of 0.03% yielded CPX-loaded microspheres with thehighest drug entrapment

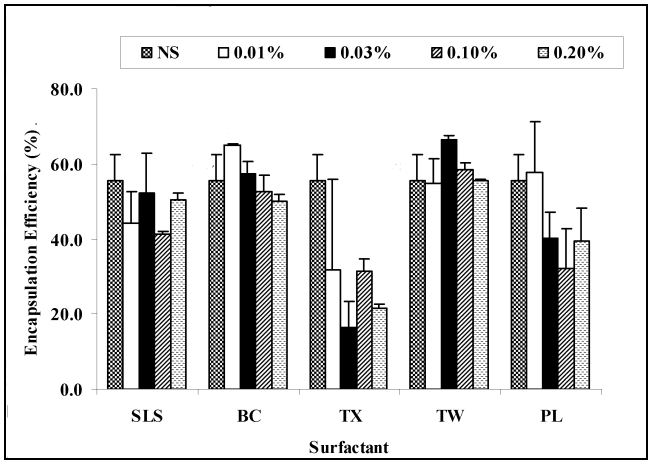

Figure 1 : Encapsulation efficiency of CPX microspheres with different concentration of surfactant

Morphological Characterization The obtained microspheres had a spherical shape and smooth surface, with some small pores.

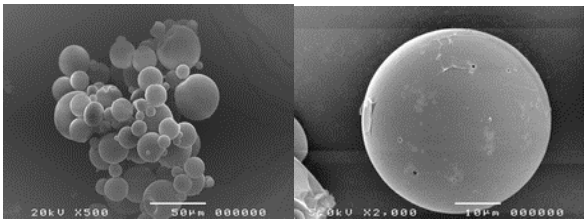

Figure 2 : SEM of CPX microspheres

X-Ray Diffraction Studies The XRD diffractogram of unloaded microspheres showed a halo pattern confirming that PLGA is amorphous. The XRD of CPX-loaded PLGA exhibited a halopattern similar to that of unloaded particles, suggesting that CPX is entrapped in the microspheres in an amorphous state.

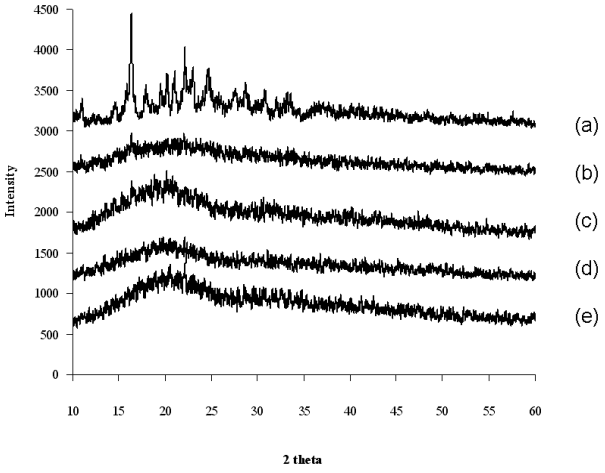

Figure 3 : X-ray diffraction patterns

Thermal Behavior of the Microspheres The thermogram of CPX microspheres, the glass transition temperature of this system was observed at a higher temperature (42–44°C) than that of PLGA. This increase in Tg indicates that CPX and PLGA interact with each other leading to a decrease in mobility of the PLGA chains. In fact, CPX acts as an anti-plasticizer for PLGA. Obviously, this interaction also prevented crystallization of CPX in the polymer matrix as discussed in previous section on XRD analysis of the CPX microspheres.

Antimicrobial Activity of the Microspheres MIC and MBC values of free drug and drug loaded microspheres are shown in Tables 1 and 2, respectively. The activity in the loaded microspheres was found to be similar or even better than that of the free drug.

Table 1 : MIC of free CPX and CPX microsphères

Bacterial Strains	MIC (µg/mL)		
	Free CPX	CPX* Microspheres	CPX** Microspheres
S.aureus	4	128	5.3±0.9
E.Coli	32	512	21.3±3.5

Table 2 : MBC of free CPX and CPX microsphères

Bacterial Strains	MBC (µg/mL)		
	Free CPX	CPX* Microspheres	CPX** Microspheres
S. aureus	2,048	2,048	85.0±13.8
E. Coli	2,048	4,096	170.0±27.6

*µg of microsphere; **µg of CPX

CONCLUSIONS

In this paper, we demonstrated the effect of preparation method as well as the type of surfactant used in the inner water phase of W/O/W emulsion on the physicochemical properties of CPX microspheres. It was found that the size and drug loading efficiency were dependent on method preparation. It was found that CPX was dispersed in its amorphous state in the microspheres. Finally it was shown that the CPX-loaded PLGA microspheres had excellent anti-microbiological activities.

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