

# Preparation and Characterization of Cephalexin Loaded PLGA Microspheres

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**Abstract:** The aim of this study was to evaluate the effects of emulsion type and process parameters on the properties of CPX-loaded PLGA microspheres in order to obtain delivery systems suitable for the treatment of dairy mastitis. The microsphere size was analyzed by photon correlation spectrophotometry. Determination of the drug loading was achieved by HPLC. It was found that CPX-loaded PLGA microspheres prepared using a w/o/w double emulsion technology were slightly larger (~ 3-5  $\mu\text{m}$ ) but much higher in drug content (~ 18 % w/w) than those obtained using o/w single emulsion preparation technology (average size was 2  $\mu\text{m}$ , encapsulation efficiency was less than 2 %). It was also demonstrated that stirring during emulsification and a change in both the internal and external phase of the emulsion, affected the size and the drug entrapment efficiency of the microspheres obtained. A 60/40 v/v mixture of chloroform and acetone was found to be the best organic solvent system for creating the primary emulsion. To obtain a high yield (>90%) of microspheres with a desirable size and high drug entrapment efficacy, a stirring rate of 8,000-10,000 rpm gave the best results. It is concluded CPX-loaded PLGA microspheres with suitable characteristics for the treatment of cows with dairy mastitis can be prepared by a w/o/w double emulsion preparation method.

**Keywords:** Microsphere, cephalexin, PLGA, emulsion solvent evaporation, PVA.

## INTRODUCTION

Microspheres of biodegradable polymers have been widely studied as drug delivery systems [1-2]. Besides their ability to improve the delivery of drugs to the target site, they have been reported to control drug release, reduce drug-associated adverse effects, protect the compound from inactivation before reaching its site of action, increase the intracellular penetration and enhance the pharmacological activity [3]. A great variety of both natural and synthetic biodegradable polymers such as chitosan, gelatin, polylactic-co-glycolic acid, polyalkylcyanoacrylate, polymethylmethacrylate, polylactic acid and polycaprolactone are used for the preparation of drug loaded microspheres [4-6]. 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 [7]. Several methods, including phase separation or coacervation [8], emulsification diffusion [9-10], spray-drying [11], and emulsion-solvent evaporation techniques [12] have been used to obtain PLGA microspheres. With the emulsion-solvent evaporation technique, numerous hydrophilic drug substances including proteins and peptides have been encapsulated into PLGA nano- and microparticles using w/o/w double emulsion methods and the mechanism of drug release from these particles has been thoroughly studied [13-14]. It has been reported that the drug release from PLGA microspheres is due to polymer biodegradation, which involves a bulk erosion process [15] and diffusion through the (porous) matrix [16].

Mastitis is economically the most important disease of dairy cattle [17]. Although various preventive measures and management practices are available, there is still a great need for effective therapeutics to treat mastitis. Among general routes of therapeutic drug administration, the intramammary route is the most convenient option for the treatment of dairy animal mastitis. The therapeutic effect of drugs via this route, however, is always limited because of diminished ability to reach the target site of infection, namely mammalian cell and mammary macrophage, even when a high potent drug is used. It has been shown in many studies that polymeric microspheres can target a drug to its site of action. Because of the attractive features of PLGA microspheres we therefore aimed in the present study to develop a microparticle formulation for the treatment of mastitis using cephalexin (CPX) as the therapeutic drug.

Cephalexine (CPX) is a member of the first generation of cephalosporins that possesses antibacterial activity against both gram-positive and gram-negative bacteria. Further, this drug is widely applied for treatment of bacterial infections both in humans and animals. It is also effective in the treatment of group A beta-hemolytic streptococcal throat infections [18]. Finally, it has been shown to be highly efficient against various pathogenic microorganisms in dairy cow mastitis [19]. Unfortunately, CPX frequently fails in treating mastitis. This is likely due to an intracellular localization of pathogenic bacteria in the epithelial cell lining of the udder and mammary macrophages. These bacteria are especially difficult to eradicate because they fight for their survival using several ingenious mechanisms. Actually, cephalosporins are poorly taken up by phagocytes [20]. As a result, a sufficiently high concentration of the drug is not reached in the infected macrophage, the target site of inter-

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est. It can be foreseen that if the drug is encapsulated in microspheres ranging in size between 2 and 8  $\mu\text{m}$ , it could potentially be taken up by phagocytes and therefore offer therapeutic value for treatment of dairy mastitis [21-22]. Therefore, the utilization of CPX-loaded PLGA microspheres within the optimum size range could achieve pathogenic bacterial inhibition in mastitis via an intramammary route. There are many techniques to prepare drug loaded PLGA microspheres. The emulsion solvent evaporation technique is the preferred method to prepare CPX-loaded PLGA microspheres due to the physico-chemical properties because CPX ( $\log P = 0.076 \pm 0.025$ ) [23] is slightly soluble in water (approximately 10 mg/ml). Depending on the desired size for high therapeutic efficiency, the parameters affecting the formation of CPX-loaded PLGA microspheres should be studied. The aim of the present work was to investigate the factors influencing the formation of CPX-microspheres and their physicochemical properties such as size, morphology, zeta potential, and drug entrapment efficiency. The o/w single emulsion and w/o/w double emulsion techniques were studied in order to prepare microspheres within the optimum size range and drug loading suitable for treatment of mastitis.

## 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-loaded PLGA microspheres was based on emulsion solvent evaporation technique described by Bodmeier and McGinity [24] and Iwata and McGinity [25] with some modifications. For the single emulsion technique, 25 mg of PLGA was dissolved in 1-3 ml of chloroform in order to study the effect of internal phase volume on the microsphere characteristics. Then 5 mg of CPX (particle size of the drug crystal was around 30  $\mu\text{m}$ ) was dispersed in the polymer solution. The mixture was poured into 10 ml of 2% w/v PVA aqueous solution and emulsified with a high speed homogenizer (Polytron®) at 10,000 rpm for 1 min. The organic solvent was allowed to evaporate by stirring the mixture at 700 rpm for 18 h under ambient temperature. When the effect of PLGA concentration on particle characteristics was studied, various amounts of PLGA (25 mg to 250 mg) were dissolved in 3 ml of chloroform.

For the double emulsion technique, 5 mg of CPX was dissolved in 0.5 ml of deionized water and the drug solution

was added to 5 ml of 5% w/v PLGA in chloroform and emulsified with a high speed homogenizer (Polytron®) at 10,000 rpm for 1 min to yield a w/o emulsion. Next, the w/o primary emulsion was added to 10 ml of a 2% PVA aqueous solution and further emulsified for 1 min at a stress-mixing speed at 10,000 rpm. The organic solvent was allowed to evaporate by the same manner as for the o/w single emulsion technique. In order to study the effect of mechanical stirring on microsphere characteristics, various rpm's were used at a fixed stirring time of 1 min. When the effect of the external phase on microsphere characteristics was studied, 5 ml of PLGA solution was added to 10-50 ml of a 2% PVA aqueous solution and an emulsion was prepared at a fixed stirring rate of 10,000 rpm for both emulsions. A mixture of chloroform and acetone (volume ratio was 3:2) was used to dissolve PLGA in all cases, except when the effect of solvent system was studied. The obtained microparticles were collected by centrifugation at 15,000 rpm for 10 min and washed twice with deionized water. After separation, the microparticles were resuspended in a suitable amount of deionized water. These suspensions were then used for particle characterization.

### Microparticle Characterization

#### *Morphology and Dispersibility*

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.

#### *Determination of Drug Encapsulation Efficiency*

The amount of encapsulated CPX in the microspheres was calculated by the difference between the amount of CPX added to the microsphere forming solution and the measured non-entrapped CPX remaining in the external phase after microsphere formation. After formation, the microsphere suspension was centrifuged for 10 min at 15,000 rpm and the supernatant was analyzed for the non-entrapped CPX by HPLC with UV detection at 260 nm. The chromatographic method was carried out isocratically. The mobile phase consisted of 1.25% acetic acid in water methanol (75:25) and the flow rate was set at 1 ml/min. Separation was achieved by using an Inersil® C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ) analytical column connected to an Inersil® C18 (50 mm x 4.6 mm, 5  $\mu\text{m}$ ) guard column. The column temperature was 40 °C. Calibration curves were obtained over concentration ranges of 0.004 mg/ml to 0.5 mg/ml.

## RESULTS AND DISCUSSION

### Microspheres Prepared by the o/w Emulsion Method

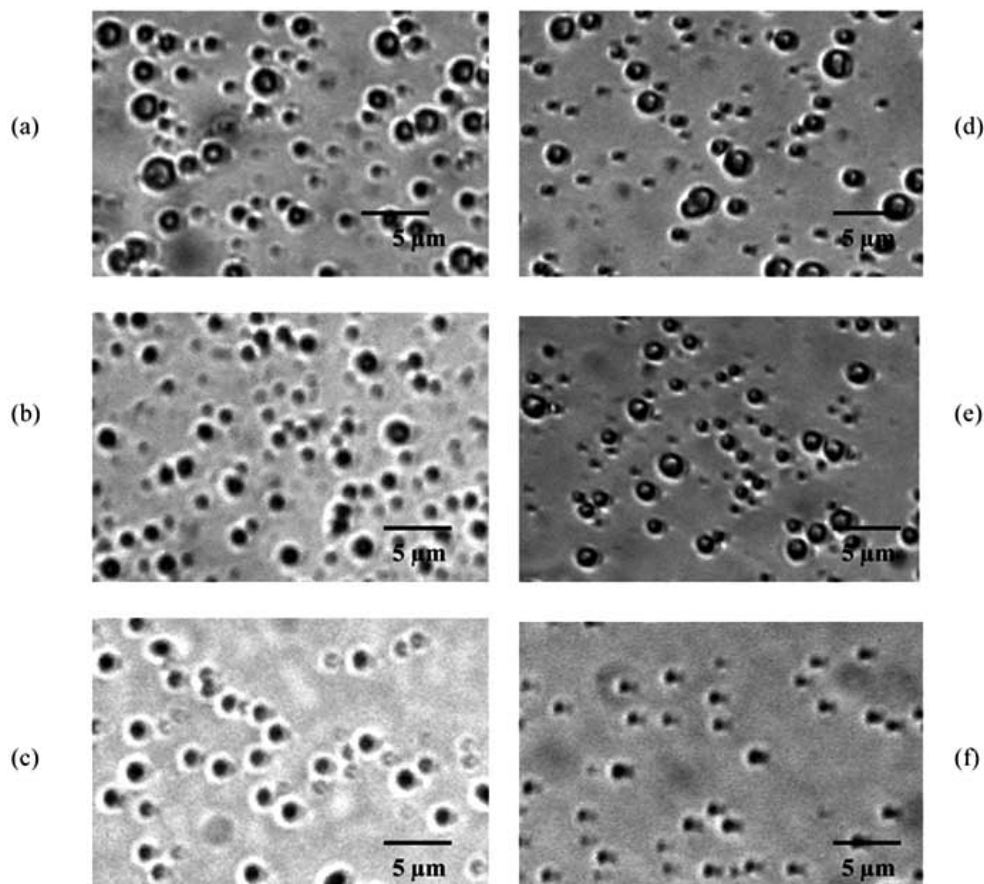
#### *Effect of Internal Phase Volume and PLGA Concentration in the Internal Phase on Size of CPX-Loaded PLGA Microspheres*

Various formulation factors were reported to have a key role on the size distribution of PLGA microspheres [26]. In our study, we first prepared CPX-loaded PLGA microspheres by an o/w single emulsion technique using CPX dispersed in PLGA solution as the internal organic phase and an aqueous PVA solution as the external phase. The effect of the volume of the internal phase and PLGA concentration on the size of the obtained microparticles was studied. Microscopy showed that the morphology of CPX-loaded PLGA particles was spherical (Fig. 1). It was found that an increase in the internal phase volume from 1 to 3 ml resulted in a decrease in microsphere mean size from  $2.32 \pm 0.43$  to  $1.38 \pm 0.12$   $\mu\text{m}$  as shown in Table 1. This result suggests that the viscosity of the internal phase which in turn is dependent on the polymer concentration is an effective factor determining the droplet size of the emulsion in the external aqueous phase [9]. The concentration of the polymer solution might also affect the size of the formed microspheres. Table 2 shows that when the PLGA concentration in chloroform increased from 4.2 to 33.3 mg/ml the particle size of the resulting microspheres increased from  $1.26 \pm 0.12$   $\mu\text{m}$  to  $2.45 \pm 0.09$   $\mu\text{m}$  ( $n=3$ ). These results are in agreement with the pre-

vious work reported by Mainardes *et al.* [27]. Likely, a low PLGA concentration resulted in a low viscosity of the polymer solution which in turn resulted in smaller emulsion droplets in the aqueous phase. In turn, increasing the PLGA content in the internal phase caused an increase in viscosity. The high viscosity of the internal phase might resist against the shear forces of high speed homogenizer (Polytron®) during emulsification. Hence, the formation of small droplets was difficult and only a coarse emulsion was formed leading to bigger particles during the solvent elimination process.

#### *CPX Loading Efficiency Obtained from Single Emulsion Evaporation Technique*

The CPX entrapment efficiency was calculated as a percentage of the ratio of drug entrapped in the microspheres to the initial amount of drug added to the system. Table 1 and Table 2 give the results. It was found that the CPX entrapment efficiency was rather low ( $< 2.2$  %). CPX is slightly soluble in water (10 mg/ml) and insoluble in the organic phase in which the PLGA was dissolved. As a result, CPX dispersed in the polymer solution will be extracted by the external aqueous phase. However, taken together, the entrapment efficiency of CPX using the single emulsion preparation was too low for practical applications and that CPX was found back almost quantitatively ( $\sim 98$ %) in the PVA phase. The results suggest that the single emulsion technique is not suitable for preparation of CPX-loaded PLGA microspheres. Therefore, an alternative method, namely the double



**Fig. (1).** Photomicrographs of CPX-microspheres taken immediately after their preparation by the single emulsion technique with variation of the internal phase volume of 1 ml (a), 2 ml (b), 3 ml (c), and PLGA concentration of 33.3 mg/ml (d), 16.7 mg/ml (e), and 4.2 mg/ml (f)

**Table 1.** The Effect of the Internal Phase Volume on the Particle Size of CPX-Loaded PLGA Microspheres (Drug/Polymer Ratio=1:5). The Results are Shown as the Average  $\pm$  SD (n=3)

Internal phase volume (ml)	Cumulative undersize distribution (%)			Mean diameter ( $\mu\text{m}$ )
	10%	50%	90%	
1	1.50 $\pm$ 0.05	2.19 $\pm$ 0.40	3.34 $\pm$ 1.00	2.32 $\pm$ 0.43
2	1.32 $\pm$ 0.11	1.76 $\pm$ 0.04	2.43 $\pm$ 0.09	1.83 $\pm$ 0.02
3	0.76 $\pm$ 0.02	1.29 $\pm$ 0.11	2.13 $\pm$ 0.23	1.38 $\pm$ 0.12

**Table 2.** The Effect of PLGA Concentration in the Internal Phase on the Particle Size and Drug Entrapment Efficiency of CPX-Loaded PLGA Microspheres

PLGA concentration (mg/ml)	Mean diameter ( $\mu\text{m}$ ) n=3	Entrapment efficiency (%)
4.2	1.26 $\pm$ 0.12	0.90 $\pm$ 0.23
8.3	1.38 $\pm$ 0.12	0.97 $\pm$ 0.14
12.5	1.83 $\pm$ 0.02	1.11 $\pm$ 0.17
16.7	1.91 $\pm$ 0.01	1.62 $\pm$ 0.23
25	2.32 $\pm$ 0.43	2.21 $\pm$ 0.60
33.3	2.45 $\pm$ 0.09	1.81 $\pm$ 0.01

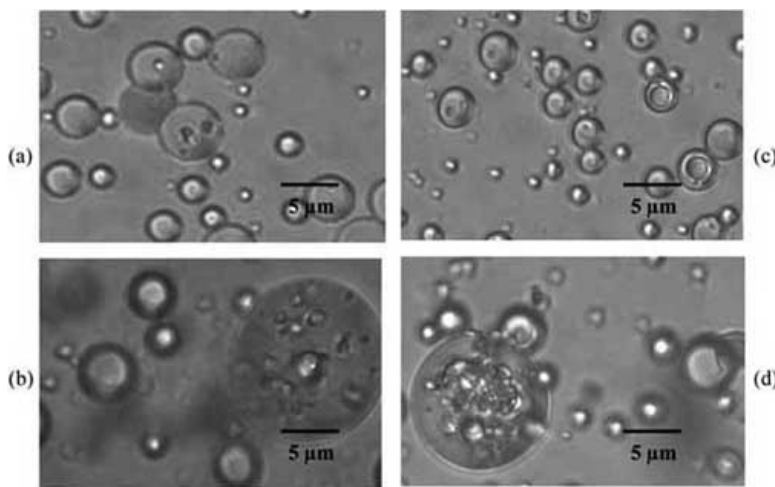
emulsion solvent evaporation method, was investigated for the preparation of CPX-PLGA microspheres with a high loading efficiency.

#### Microspheres Prepared by a w/o/w Double Emulsion Technique

##### *Effect of Solvent Type on Size of CPX-Loaded PLGA Microspheres*

The preparation of CPX-loaded PLGA microspheres by a double emulsion technique was investigated since the drug entrapped by the o/w single emulsion technique was very

low. CPX is slightly soluble in water (5 mg/0.5 ml) whereas it is essentially insoluble in PLGA solvents (like chloroform and dichloromethane, etc.) and explains why the encapsulation efficiency in PLGA microspheres is low using an o/w emulsion solvent evaporation method. Consequently, dissolving CPX in an aqueous phase that is subsequently dispersed in the polymer solution might be a better way to achieve a better drug entrapment efficiency. The w/o/w type of double emulsion was therefore investigated for the preparation of CPX loaded microspheres using chloroform, dichloromethane, ethyl acetate or a mixture of these solvents and acetone dissolve PLGA. Microscopic investigations



**Fig. (2).** Photomicrograph of microspheres taken immediately after their preparation by the double emulsion technique with variation of solvent system; chloroform (a), dichloromethane (b), chloroform and acetone (3:2) (c), dichloromethane and acetone (3:2) (5% w/v PLGA solution, 10 ml of 2% PVA solution)

showed that the CPX-microspheres obtained with the various solvents were spherically shaped as shown in Fig. (2). The mean size of the microspheres obtained with the different solvent systems is shown in Table 3. 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 [28, 29]. In our study, addition of acetone to chloroform or dichloromethane also decreased the size of CPX-loaded PLGA 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.

**Table 3. The Effect of Solvent System on Particle Size of CPX-Loaded PLGA Microspheres (Drug /Polymer Ratio= 1:50 with 5 ml of Total Volume of the Solvents Used in the Preparation)**

Solvent system	Particle size ( $\mu\text{m}$ )
Chloroform	4.24 $\pm$ 0.25
Chloroform : Acetone (3:2)	3.01 $\pm$ 0.27
Dichloromethane	4.41 $\pm$ 0.85
Dichloromethane : Acetone (3:2)	4.33 $\pm$ 0.60
Ethyl acetate	> 100
Ethyl acetate : Acetone (3:2)	> 100

#### **Effect of Solvent Ratio and Stirring Rate on Size of CPX-Loaded PLGA Microspheres**

The data presented in Table 4 show that a mixture of chloroform and acetone (3:2) resulted in the formation of CPX-loaded (18% encapsulation efficiency) PLGA microspheres with a small size. However, the solvent ratio might affect the size of the microspheres. To study this, three ratios of these two solvents (4:1, 3:2, and 2:3 respectively) were used to prepare PLGA microspheres. The results in Table 4 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.

**Table 4. The Effect of Solvent Ratio on Particle Size and Drug Entrapment Efficiency of CPX-Loaded PLGA Microspheres (Drug /Polymer Ratio= 1:50 with 5 ml of Total Volume of the Solvents Used in the Preparation)**

Chloroform/acetone Ratio	Mean diameter ( $\mu\text{m}$ )	Entrapment efficiency (%)
4 : 1	3.30 $\pm$ 1.09	16.3 $\pm$ 3.9
3 : 2	3.18 $\pm$ 1.43	18.3 $\pm$ 1.2
2 : 3	3.37 $\pm$ 1.42	17.3 $\pm$ 1.5

The microspheres of Table 5 were prepared at fixed stirring speed of 10,000 rpm for both the primary and secondary emulsion. The effect of stirring speed of both the primary and secondary emulsion on particle size of CPX-loaded PLGA microspheres was studied in more detail. The study was done using a solvent ratio chloroform/acetone of 3:2. The results shown in Table 5 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 5 also gives the encapsulation efficiencies. This table shows that with decreasing stirring rate the encapsulation efficiency tends to increase. A probable 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 [30].

#### **Effect of Volume of the External Aqueous System on CPX-Loaded PLGA Microspheres**

In the experiments described above we used 10 ml of a 2% w/v solution of PVA as the external aqueous system of the w/o/w double emulsion for the preparation of CPX-loaded PLGA microspheres. Particles were also prepared at larger volumes of the external phase (Table 6). An increase of volume of this solution from 10 ml to 50 ml caused a slight increase in size of the microspheres from 3.2 to 4.8  $\mu\text{m}$ . A probable explanation is that with increasing the external volume, the shear forces to break the emulsion droplets become smaller yielding larger emulsified droplets which in turn results in larger PLGA microspheres. Table 6 also shows that the drug encapsulation efficiency decreased significantly (from ~18 to ~5 %) when the volume of the external aqueous phase increased from 10 ml to 50 ml. This was expected since a greater volume of the aqueous external phase allows that more drugs can be dissolved in this phase.

The zeta potential of particles plays an important role on their stability. A higher value of zeta potential indicates a lower chance of particle aggregation. It was found that CPX-loaded PLGA microspheres prepared with different volumes of the external phase ranging from 50 to 10 ml produced particles with slightly different zeta potentials (ranging from -7 to -10 mV, Table 6).

#### **Effect of Molecular Weight of PVA on Characteristics of CPX-Loaded PLGA Microspheres**

PVA is a water-soluble synthetic polymer and a macromolecular emulsifier widely used for the fabrication of polymeric microspheres. PVA consist both hydrophilic (hy-

**Table 5. The Effect of Stirring on Particle Size and Drug Entrapment Efficiency of CPX-Loaded PLGA Microspheres (5% w/v Polymer Solution, 10 ml of 2% PVA Solution)**

Stirring (rpm)		Mean diameter ( $\mu\text{m}$ )	Entrapment efficiency (%)
1 <sup>st</sup> emulsion	2 <sup>nd</sup> emulsion		
10,000	10,000	3.18 $\pm$ 1.23	18.3 $\pm$ 1.2
8,000	8,000	4.95 $\pm$ 0.06	18.6 $\pm$ 0.3
8,000	4,000	16.50 $\pm$ 2.60	19.1 $\pm$ 0.5

**Table 6. The Effect of External Aqueous Phase Volume on CPX-Loaded PLGA Microspheres (2% PVA Solution)**

PVA volume (ml)	Mean diameter ( $\mu\text{m}$ )	Zeta potential (mV)	Entrapment efficiency (%)
10	3.18 $\pm$ 1.43	-10.5 $\pm$ 0.6	18.3 $\pm$ 1.2
20	3.45 $\pm$ 0.89	-9.9 $\pm$ 0.6	9.9 $\pm$ 0.9
40	3.60 $\pm$ 0.22	-7.8 $\pm$ 2.3	6.5 $\pm$ 0.8
50	4.77 $\pm$ 0.54	-7.6 $\pm$ 2.9	4.8 $\pm$ 0.8

droxyl-rich domains of the polymer) and hydrophobic (domains rich in residual acetyl groups) segments. Because of these properties PVA increases the viscosity of the aqueous solution and adsorbs onto the water-oil droplet interface. Hence, it has a good emulsifying and stabilizing function for emulsion droplets. In this study, the effect of molecular weight of PVA used as emulsifier on external aqueous phase was evaluated. The w/o/w emulsion was prepared in the same manner as mentioned above at a 2 % PVA concentration and the speed of homogenization of the primary and secondary emulsion was fixed at 8,000 rpm. Table 7 shows that there was a decrease in particle size from 4.95 to 3.17  $\mu\text{m}$  when the molecular weight of PVA was increased from 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 [9, 27].

Table 7 also shows that the CPX entrapment efficiency was not dependent on the PVA molecular weight. According to the desired size and the stability of CPX-loaded PLGA microspheres 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.

## CONCLUSION

This paper shows that CPX-loaded PLGA microspheres can be prepared using emulsion solvent evaporation techniques. The microspheres prepared from w/o/w double emulsion were slightly larger than those prepared using o/w single emulsion method but yielded a higher drug entrapment. It was found that many factors including solvent type, solvent composition ratio, volume of internal and external phases, PLGA content, stirring rate and the molecular weight of the PVA affected the physicochemical properties of CPX-loaded PLGA microspheres. The microspheres with desired size and entrapment efficiency could be obtained from suitable internal volume (5 ml) of 5% PLGA in 3:2 mixture of chloroform-acetone and external volume (10 ml) of 2% PVA (MW 100,000) at the preparation stirring range of 10,000 rpm. However, the entrapment of CPX is only 18%. Therefore, further investigations should be focused on improving the entrapment efficiency.

**Table 7. The Effect of Molecular Weight of PVA on Size and Drug Entrapment Efficiency of CPX-Loaded PLGA Microspheres (5% Polymer Solution with 10 ml of 2% PVA Solution)**

MW of PVA	Cumulative undersize distribution (%)			Mean diameter ( $\mu\text{m}$ )	Entrapment efficiency (%)
	10%	50%	90%		
15,000	1.46 $\pm$ 0.01	4.28 $\pm$ 0.05	9.69 $\pm$ 0.01	4.95 $\pm$ 0.06	18.6 $\pm$ 0.3
30,000-70,000	2.00 $\pm$ 0.74	3.66 $\pm$ 0.09	5.48 $\pm$ 0.14	3.70 $\pm$ 0.23	18.6 $\pm$ 0.5
100,000	1.61 $\pm$ 0.33	3.10 $\pm$ 0.13	4.82 $\pm$ 0.75	3.17 $\pm$ 0.16	19.1 $\pm$ 0.5

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## ABBREVIATIONS

CPX	=	Cephalexin
PLGA	=	Poly(lactide coglycolic acid)
PVA	=	Poly(vinyl alcohol)
HPLC	=	High performance liquid chromatography
RPM	=	Round per minute
w/o/w	=	Water in oil in water
o/w	=	Oil in water

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