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Preparation of Biodegradable Microspheres Containing Water-Soluble Drug, β-lactam Antibiotic

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Poly (*l*-lactic acid) (PLLA) microspheres loaded with ampicillin sodium (AMP-Na), β-lactam antibiotic, were prepared by a w/o/w multiple emulsion-solvent evaporation method. The amounts of each component in three phases (inner water phase, organic phase, and outer water phase) were carefully examined in the preparation of PLLA microspheres. The stirring rate, another preparation parameter, was also investigated for study on the effect of mechanical stress on the drug loading and morphology of PLLA microspheres. Most of the preparation parameters had a great influence on the drug loading, surface morphology and size distribution of PLLA microspheres. PLLA microspheres with 15.89 w/w% drug loading were subjected to the *in vitro* release experiment. The release of ampicillin sodium was constant at a rate of 1.68 μg/ml/day per 1 mg of microspheres for 18 days after a 4 days initial burst effect.

Key words : Poly (*l*-lactic acid), Microspheres, (w/o/w) Emulsion, Solvent evaporation, Ampicillin sodium, Antibiotics

INTRODUCTION

Considerable attention has been paid to polyesters such as poly (lactic acid) and poly (lactide-co-glycolide) since 1973 as the carriers of biodegradable and biocompatible drug delivery systems (Swarbrick, 1990). Features such as biocompatibility, predictability of biodegradation kinetics, ease of fabrication, and regulatory approval in commercial suture applications have been attractive points for investigators. An additional merit for these polyesters was available versatility. These polymers could be tailored by various preparation factors, such as monomer stereochemistry, comonomer ratio, polymer chain linearity and molecular weight.

Early research efforts were focused on the homopolymers of lactic acid rather than on the lactic-glycolic copolymers. According to the chirality of lactic acid, Poly (lactic acid) homopolymers is classified into three polymers that could be synthesized: Poly (*d*-lactic acid) (PDLA), Poly (*d,l*-lactic acid) (PDLLA), Poly (*l*-lactic acid) (PLLA). The PLLA is preferred in bioapplications, because PLLA could be degraded into *l*-lactic acid and eliminated from the body (Park *et al.*, 1993).

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Many kinds of methods have been used when microspheres have been prepared with lactide/glycolide polymers. Among them, the solvent evaporation method was the most commonly used technique in drug delivery system for hydrophilic drugs (Spenlehauer *et al.*, 1986, Bodmeier *et al.*, 1987, Arshady, 1990, Jeffery *et al.*, 1991). The oil-in-water (o/w) emulsion method was used for the drugs which are practically insoluble in water or soluble in organic solvents. However, in the delivery system for water soluble drugs, the efficiency of drug loading was very low when the microspheres were made by the o/w emulsion method. Therefore the w/o/w multiple emulsion system is usually used for water-soluble drugs (Wang *et al.*, 1991, Ogawa *et al.*, 1988). The purpose of this study was to develop a biodegradable microsphere dosage form containing ampicillin sodium (AMP-Na) at a high entrapment ratio and to investigate the parameters which affect the preparation of microspheres. AMP-Na loaded microspheres can be used for the delivery system at the local inflammatory regions for a few weeks.

MATERIALS AND METHODS

Materials

Poly (*l*-lactic acid) (PLLA) (MW100,000) was pur-

chased from Polysciences Inc. (Warrington, PA). Ampicillin sodium (AMP-Na) was kindly donated by Chong Kun Dang Pharm. Co. (Seoul, Korea). Dichloromethane (CH_2Cl_2, AR®) was supplied by Mallinckrodt Inc. (Paris, Kentucky) and polyvinyl alcohol (PVA) (MW 13,000-23,000, 87-89% hydrolyzed) was purchased from Aldrich Chemical Co. (Dorset, USA). Sorbitan monooleate (Span80) was purchased from Yakuri Pure Chemicals Co. Ltd. (Osaka, Japan).

Preparation of microspheres

A solution of AMP-Na (100 mg, 200 mg) in distilled water (200 μl, 400 μl, respectively) was emulsified in a CH_2Cl_2 solution of PLLA (0.7 g/5 ml) by the ultrasonicator (Branson Model 3210, Bransonic® ultrasonics Corp., Danbury, USA) for 30 min. The above w/o emulsion was then added into 30 ml or 100 ml aqueous solution of 0.5w/v% or 1w/v% PVA with the homogenizer (Yamato Model LK 41, IKA Werke, Germany) at 2000-8000 rpm. After 5 min, the stirring rate was reduced to ca. 500 rpm and the resulting (w/o/w) emulsion was heated to 35°C to evaporate dichloromethane. After 2 hours, the hardened microspheres were filtered with a 0.45 μm membrane, washed with distilled water and vacuum-dried for a few days.

Characterization of microspheres

A scanning electron microscope (SEM, Hitachi Model S-510, Hitachi Ltd., Tokyo, Japan) was used to examine the surface characteristics and the cross-sectional view of microspheres. The internal construction of microspheres was observed by the paraffin-embedding method and cross-sectioning.

To determine the size distribution of microspheres, the dried microspheres were poured into the sieves which were piled up in order of 53 μm (270), 106 μm (140), 212 μm (70) and 355 μm (45) from the bottom. The microspheres in each sieve were weighed and the size distribution was calculated from that.

To determine the content of AMP-Na in microspheres, they were dissolved in 10 ml of chloroform in a screw-capped vial. Ten milliliters of 5N NaOH solution was added into the above solution and shaken for 30 min for extraction of AMP-Na into the aqueous layer. After centrifugation at 4000 rpm for 10 min, an aliquot of the aqueous layer was taken and the UV absorbance (Hewlett-Packard 8451A Diode Array Spectrophotometer, USA) was read at 280 nm (CA 74,6412k, 1971).

The recovery and the content of drug in the microspheres were computed by the following equations (1) and (2), respectively.

$$\text{Recovery of drug}(\%) = \frac{\text{total amount of drug in microspheres}}{\text{total amount of drug fed in system}} \quad (1)$$

$$\text{Content of drug}(\%) = \frac{\text{amount of drug in microspheres}}{\text{amount of microspheres sampled}} \quad (2)$$

The recovery of microspheres was calculated by the equation (3).

$$\text{Recovery of microspheres}(\%) = \frac{\text{amount of microspheres obtained}}{\text{amount of polymer and drug added initially}} \quad (3)$$

In vitro release experiment

Three hundred milligrams of microspheres were filled in a wire-netted basket and immersed in a 30 ml of PBS (pH 7.2) solution as a release medium. The release medium was shaken at 90 cpm and the temperature of the release medium was kept at 37°C. The release medium was exchanged with fresh PBS solution at each sampling time and the amount of drugs released from microspheres was detected by the UV spectrophotometer.

RESULTS AND DISCUSSION

The effect of parameters on the preparation of PLLA microspheres

The effect of components in the inner water phase : As shown in Table I, AMP-Na loaded PLLA microspheres were prepared in various preparation conditions. The concentration of AMP-Na in the inner water phase was fixed at 50% (100 mg in 200 μl water or 200 mg in 400 μl water). The effect of volume in the inner water phase was not significant on the size and shape of microspheres as shown in Fig. 1. However the recovery of drug was enhanced when the volume of the inner water phase was doubled from 100 mg/200 μl (MS 1) to 200 mg/400 μl (MS 2). All the microspheres were spherical and had many pits on the surface which could have been often observed when the solvent evaporation method had been used.

The effect of components in the organic phase : To study the effect of the concentration of polymer in the organic phase, microspheres were prepared with the three different concentrations, 6w/v% (PLLA 0.3 g in dichloromethane 5 ml, MS 8), 10w/v% (PLLA 0.5 g in dichloromethane 5 ml, MS 9), and 14w/v% (PLLA 0.7 g in dichloromethane 5 ml, MS 10). The concentration of polymer in the organic phase induced significant effects on both the morphology of microspheres and the recovery of drug (Fig. 2 and Table I).

The microspheres, MS 10, had round shapes and smooth surfaces with pits on the surface. The microspheres, MS 9, had rough and porous surface although they are round-shaped. However MS 8 mi-

Table I. The preparation conditions, the recovery of microspheres and the recovery of drug for AMP-Na loaded PLLA microspheres

MS No.	Inner water phase[a] AMP-Na sol'n (μl)	Organic phase[b] PLLA (w/v%)	Span80 (w/v%)	Outer phase PVA (w/v%)	water (ml)	Stirring rate (rpm)	Recovery of microspheres (%)	Recovery of drug (%)
1	200	14	3	1	30	5000	92.1	2.5
2	400	14	3	1	30	5000	82.2	9.1
3	200	14	0.4	0.5	30	8000	35.7	—
4	200	14	1.2	0.5	30	8000	70.2	2.6
5	200	14	3	0.5	30	8000	71.3	6.3
6	200	14	3	0.5	100	8000	75.0	10.5
7	200	14	3	0.5	100	5000	77.3	54.6
8	400	6	5	0.5	100	2000	79.9	2.2
9	400	10	5	0.5	100	2000	86.4	24.1
10	400	14	5	0.5	100	2000	93.5	71.5
11	400	14	5	0.5	100	8000	86.0	9.7
12	400	14	5	0.5	30	8000	84.7	6.5

[a]50% AMP-Na in aqueous solution
[b]5 ml of dichloromethane was used as an organic phase

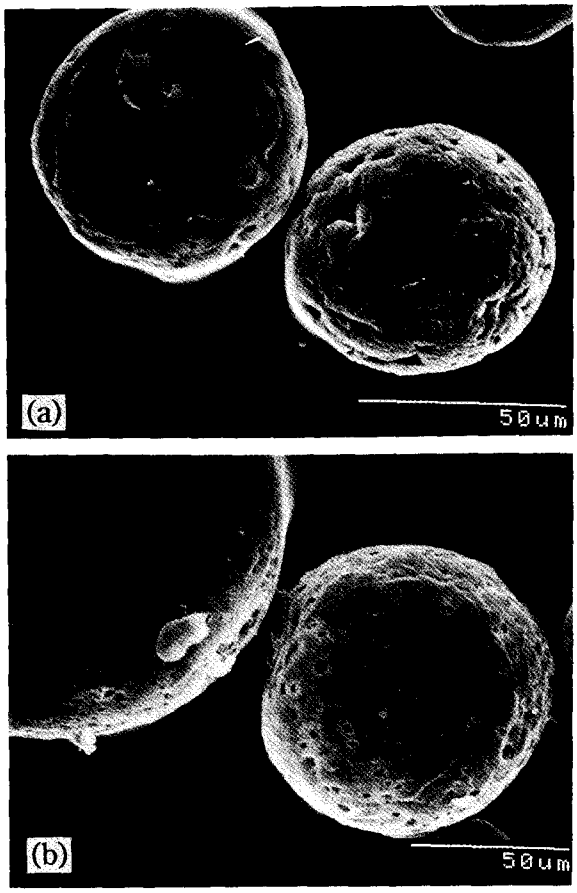

Fig. 1. Scanning electron micrographs of AMP-PLLA MS prepared with a) 200 ml of inner water volume (MS 1) and b) 400 ml of inner water volume (MS 2)

crospheres were very small and irregular compared with MS 9 or MS 10. The microspheres, MS 8, had the lowest recovery of microspheres and recovery of drug among them. The recovery of microsphere was high for MS 9 but the recovery of drug was only 1/3 of MS 10 which had the highest drug loading in our experiment.

The size distributions of MS 10 (14w/v%) and MS 9 (10w/v%) were measured and compared in Fig. 3. Microspheres of MS 10 were distributed mainly in the range of 200-350 μm, on the other hand microspheres of MS 9 were distributed mainly in the range of 100-200 μm. The size of microspheres of MS 8 reduced significantly compared to MS 9 or MS 10, and it was impossible to measure their size distribution. Therefore the increase in the polymer concentration at a fixed volume (5ml) of dichloromethane resulted in the increase in the particle size. The recovery of microsphere and the recovery of drug were enhanced with increase in the concentration of polymer. This may be due to the enhanced viscosity of the organic phase and stability of the outer o/w emulsion.

The other component of the organic phase is the surfactant (Span80) which affects the stability of the inner w/o emulsion. Microspheres prepared with three different concentrations of surfactant (0.4w/v% (MS 3), 1.2w/v% (MS 4) and 3w/v% (MS 5)) were compared. Both the recovery of microsphere and the recovery of drug were significantly affected by the concentration of the surfactant. When the concentration of the surfactant, Span80, was low (0.4w/v% for 5 ml dichloromethane, MS 3), the microspheres were irregularly shaped and large holes appeared on the surface (Fig. 4). This may be due to the instability of the inner w/o emulsion and this may also affect the recovery of microspheres and drug. The recovery of microspheres for MS 3 was lowest among the three microspheres (MS 3, MS 4 and MS 5) and the recovery of drug was undetectable for MS 3.

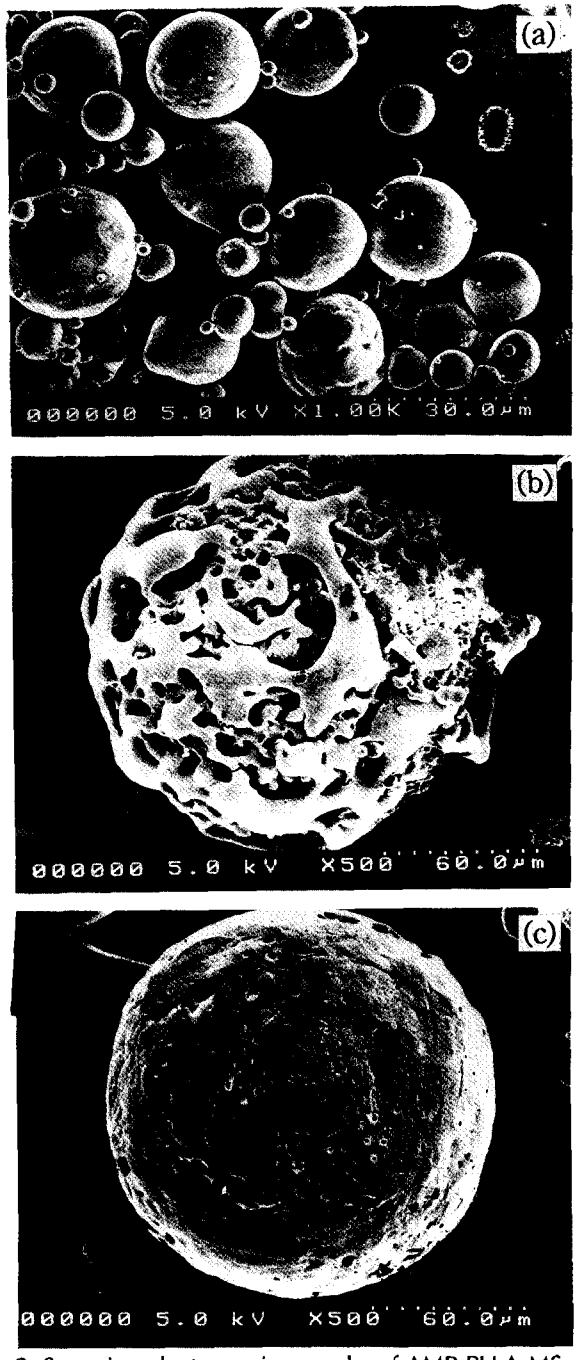

Fig. 2. Scanning electron micrographs of AMP-PLLA MS prepared with a) 6w/v% (MS 8), b) 10w/v% (MS 9) and c) 14w/v% (MS 10) of PLLA in 5ml dichloromethane solution

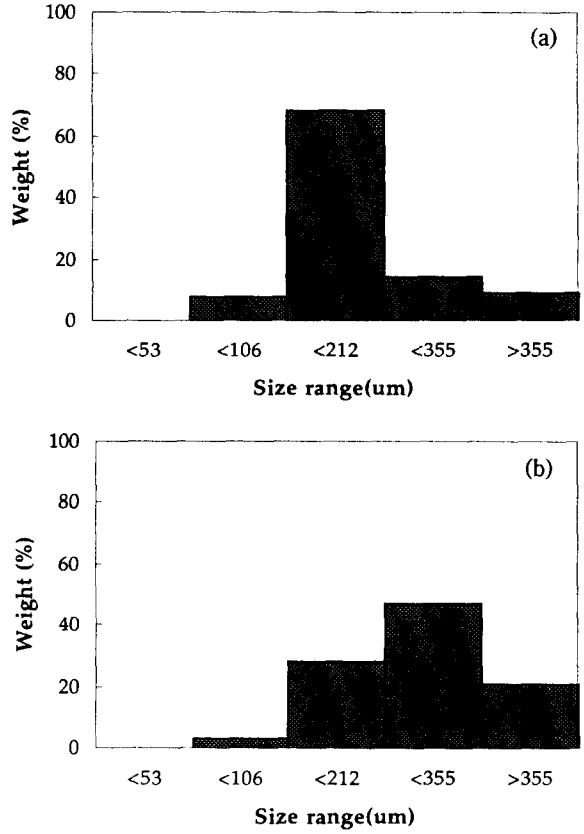

Fig. 3. Size distributions of AMP-PLLA MS prepared with a) 10w/v% (MS 9) and b) 14w/v% (MS 10) of PLLA in 5ml dichloromethane solution

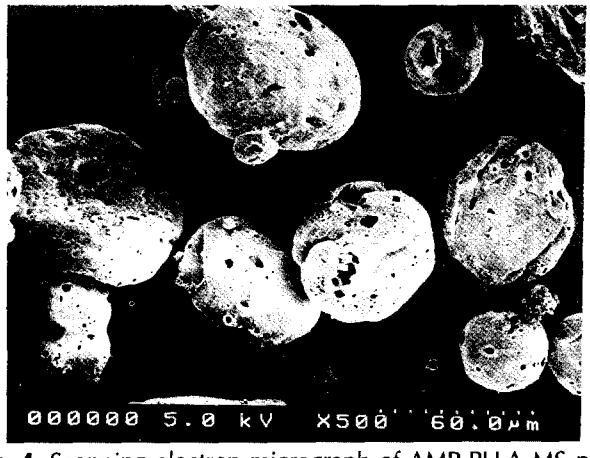

Fig. 4. Scanning electron micrograph of AMP-PLLA MS prepared with 0.4w/v% surfactant (MS 3) in 5 ml organic phase

The recovery of microspheres and the recovery of drug were both enhanced with the increase in the concentration of the surfactant.

The effect of components in the outer water phase : Various kinds of stabilizers for the outer water phase were study by Jeffery *et al.* (1991). The stabilizers used for the preparation of polylactide microspheres were polyvinyl alcohol (PVA), sodium dodecyl sulfate (SDS), cetyltrimethyl ammonium bromide (CTAB), methyl cellulose (MC) and gelatin. Most of the stabilizers showed good results in the preparation of polylactide microspheres. Among them PVA was the most frequently studied stabilizer in the preparation of PLLA microspheres (Benita, 1984, Alex and Bodmeier, 1990).

In this study, the concentration of PVA solution was

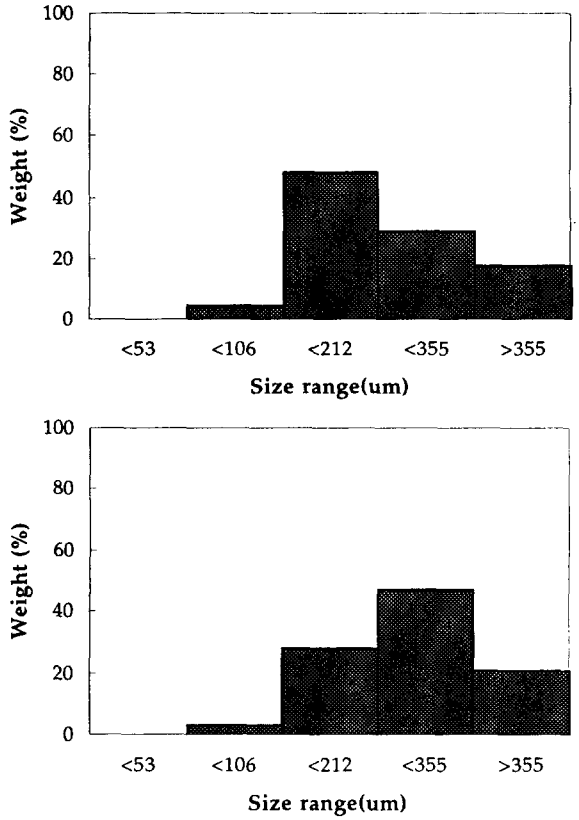

Fig. 5. Size distributions of AMP-PLLA MS prepared with a) 5000 rpm (MS 7) and b) 2000 rpm (MS 10) of stirring rate

fixed at 0.5w/v% and two different volumes of the outer water were examined. The MS 5 and MS 12 were prepared with 30 ml of PVA solution, and MS 6 and MS 11 were prepared with 100 ml of PVA solution. MS 6 and MS 11 showed better results in both the recovery of microspheres and the recovery of drug compared with MS 5 and MS 12. This may also be closely related to the stability and the frequency of collisions between the w/o emulsion droplets.

The effect of the stirring rate : The effect of the stirring rate in the preparation of microsphere was examined at 2000 rpm, 5000 rpm and 8000 rpm. The stirring rate was considered as a main factor of preparation parameters due to the stability of w/o/w emulsion. The stability of w/o/w emulsion was significantly affected by the mechanical stress during the stirring process.

The higher stirring rate reduced both the recovery of microsphere and the recovery of drug. However the sizes of microspheres prepared at a higher stirring rate (8000 rpm) , MS 6 and MS 11, were very small compared with MS 7 and MS 10. The microspheres, MS 6 and MS 11, were laid under the sieve of 53 μm size, although the microspheres, MS 7 and MS 10, were distributed mainly in the range of 100-200 μm and 200-350 μm, respectively (Fig. 5).

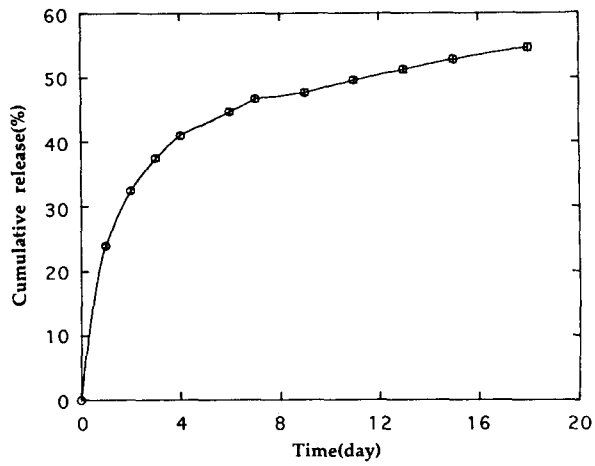

Fig. 6. *In vitror* elease of AMP-PLLA microsphere (MS 10) in 37°C PBS solution (pH 7.2) at 90 cpm shaking

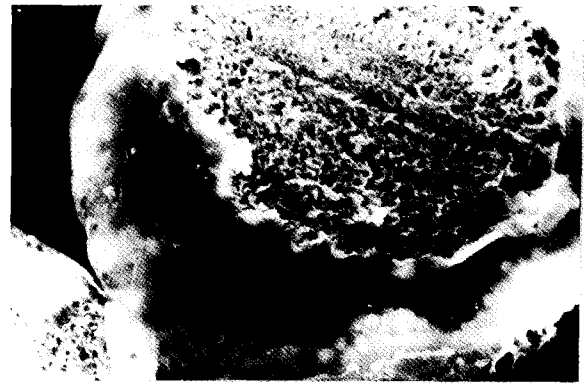

Fig. 7. Scanning electron micrograph of the cross-sectional view of AMP-PLLA microsphere (MS 10)

In vitro release study

For the study of *in vitro* release, the microspheres, MS 10, which showed the highest loading of drug (15. 89w/w%), were selected. The microspheres prepared in six different batches were collected and used for release study. The release experiment was performed for 18days.

The result of *in vitro* release test was shown in Fig. 6. The early burst effect (40% release) was detected during the first 4 days and the drug was released sustainedly at an average rate of 1.68 μg/ml/day per 1 mg of microspheres.

The release rate of AMP-Na from PLLA microspheres was relatively faster than other microspheres (Wang *et al.*, 1991) due to both the solubility of AMP-Na in PBS and the porous inner structure of microspheres. As shown in the cross-sectional view in Fig. 7, the microspheres are composed of the hard outer skin and the porous inner layer which may be affected by the good stability of the inner water phase.

CONCLUSION

The preparation parameters of PLLA microspheres by the w/o/w emulsion-solvent evaporation method were investigated in this study.

As a parameter on a component of the inner water phase, the volume of the AMP-Na solution was examined. The increase in the volume of the inner water phase enhanced the recovery of drug in microspheres. As parameters on the components of the organic phase the concentration of the polymer and the surfactant (Span80) were also investigated. Both of the parameters affected significantly the formation of microspheres, size distribution and drug loading. With the increase in concentration of polymer and surfactant both the recovery of microspheres and the recovery of drug were enhanced although the size of microspheres was reduced. As a parameter on the component of the outer water phase, the volume of PVA solution was examined. The increase in the volume of PVA aqueous solution enhanced the recovery of microspheres and the recovery of drug. Among the fabrication parameters, the effect of the stirring rate was examined. The higher stirring rate reduced both the recovery of drug and the size of microspheres.

An *in vitro* release of 15.89w/w% AMP-Na loaded PLLA microsphere showed a sustained release of AMP-Na for 2 weeks after an early 4 days burst effect.

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