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Rosin Microparticles as Drug Carriers: Influence of Various Solvents on the Formation of Particles and Sustained-release of Indomethacin

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Abstract The aim of this study was to formulate a sustained release system for indomethacin (IND) with rosin gum obtained from a pine tree. Rosin microparticles were prepared by a dispersion and dialysis method without the addition of surfactant. In order to investigate the influence of solvents on the formation of colloidal microparitcles, various solvents like ethanol, DMF, DMAc, and acetone were used. The rosin microparticles containing IND were characterized by X-ray diffractometry (XRD) and differential scanning calorimetry (DSC). The morphologies of rosin microparticles observed by scanning electron microscopy (SEM) were spherical. The solvents used to dissolve rosin significantly affected the drug content and drug release rate of IND. The release behaviors of IND from the rosin microparticles were dependent on the drug content and size of the particles. Rosin micorparticles with a higher drug content and of a larger particle size had a slower drug release rate. Also, the IND release rate from the rosin microparticles could be regulated by the rosin content in the microparticles. From these results, rosin microparticles have the potential of being used as a sustained release system of IND.

Keywords: rosin gum, indomethacin, sustained release, microparticle

INTRODUCTION

Rosin, a natural polymer obtained from pine trees, and its derivatives has attracted much interest in the field of pharmaceutical applications due to their characteristic properties such as biocompatibility, biodegradability and low cost [1]. They have also been used in the fields of cosmetics, chewing gum and dental varnishes [2]. In a drug delivery system, rosin has been evaluated as a novel film-forming material for sustained-release preparations [3-5]. Recently, the research group of Fulzele suggested the possibility of rosin and its derivatives to be used as coating and microencapsulating agents for sustained drug release [3]. In particular, these researchers demonstrated through subcutaneous rat model studies that rosin and its derivatives have characteristic properties such as biodegradation and biocompatibility *in vivo* [3]. These results show the usefulness of rosin in drug delivery application.

Indomethacin (IND), a non-steroid anti-inflammatory drug (NSAID) is commonly used for the relief of pain and stiffness in rheumatoid, osteoarthritic and acute gouty arthritis diseases, *etc.* [6]. However, the high incidence and severity of side effects, which are dose-related and associated with long-term administration, have limited its use [7,8]. The antiphlogistic effect of IND has been presented as the suppression of the biosynthesis of prostaglandin through a hindering activity of cyclooxygenase. Prostaglandin inhibits the excessive secretion of acid in the stomach, increases the blood flow on the region of mucosa, and hastens the secretion of mucosa for cell protection in the intestine [9]. Accordingly, the reduction of prostaglandin by an oral administration of IND produces gastrointestinal side effects such as gastrointestinal mucosal lesions and an ulcer. Many attempts have been made to prevent these side effects of IND. Major improvements have been achieved by designing sustained release systems or by using rectal and transdermal

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administration as an alternative to the oral route [10-12]. A sustained release system can provide a desirable blood concentration of the drug for a suitable period without toxicity [10].

The aim of this study was to prepare rosin microparticles containing IND for the sustained release of this drug, and to evaluate this release through *in vitro* dissolution tests. Furthermore, the influence of various organic solvents used in the preparation of the rosin microparticles was investigated for their affect on formulation, drug content, and drug release.

MATERIALS AND METHODS

Materials

Rosin gum and indomethacin (IND) were obtained from Sigma Chemical Co. (St. Louis, MO). *N,N*-dimethyl formamide (DMF), *N,N*-dimethyl acetamide (DMAc), ethanol and acetone were purchased from Duksan Co. (Korea). All other chemicals were of a regent grade and used without further purification.

Preparation of IND-loaded Rosin Microparticles

Rosin microparticles were prepared by a dispersion and dialysis method without the addition of surfactant [13, 14]. Briefly, 100 mg of rosin was dissolved in 5 mL of different organic solvents such as ethanol, acetone, *N,N*-dimethyl formamide (DMF) and *N,N*-dimethyl acetamide (DMAc), respectively. To form the microparticles, the solution was dispersed in 20 mL of distilled water and stirred for 5 min. The suspension was then dialyzed using a molecular weight cut off (MWCO) 12,000 g/mol dialysis tube (Sigma Chemical Co., St. Louis, USA) against distilled water. The distilled water was exchanged every 2 h for 24 h and the dialyzed solution was then freeze-dried. IND-loaded microparticles were prepared as follow: rosin and IND were dissolved in organic solvent, and dispersed in distilled water as described earlier [15,16]. The suspension was then dialyzed, and the dialyzed solution was freeze-dried.

To measure the drug content of the particles, the freeze-dried samples of IND-loaded microparticles were suspended in 5 mL of methanol. After vigorously stirring for 24 h, the resulting solutions were centrifuged at 5,000 rpm for 15 min and the drug concentration in the supernatant of each sample was measured with a UV spectrophotometer (Shimadzu UV-1201, Shimadzu Co. Ltd., Tokyo, Japan) at 319 nm. Drug loading efficiency and content were calculated according to the following equation:

Drug loading content (%)
= (weight of loaded drug in microparticles / weight of microparticles) × 100
Loading efficiency (%)
= (weight of loaded drug in microparticles / initial feeding weight of drug) ×100

Scanning Electron Microscope (SEM)

The surface morphology of the rosin microparticles was observed using SEM (Jeol, JSM 5400, Japan). The freeze-dried samples were mounted onto stubs and then coated with gold/palladium by an Ion Sputter (Jeol, JFC-1100, Japan). The coating was carried out at 20 mA for 4 min and then the samples were observed with the microscope operated at 20 kV.

X-ray Diffractometry (XRD)

In order to investigate the crystallinity of the polymer, X-ray diffractograms were obtained using a Rigaku D/Max-1200 (Rigaku, Japan) with Ni-filtered Cu Kα radiation (40 kV, 30 mA). The instrument was operated in the step scan mode with an increment of 0.02°(2θ).

Differential Scanning Calorimetry (DSC)

The melting points of rosin, the rosin microparticles including IND, IND itself, and the physical mixture of rosin and IND were measured by DSC (PerkinElmer instruments, Japan) at a temperature range of 50 to 200°C under nitrogen and at a rate of °C/min.

In vitro Drug Release Studies

The *in vitro* release rate measurements were carried out as follows: 40 mg of drug-loaded rosin microparticles were suspended in 1 mL of phosphate buffered saline (PBS, pH 7.4) and the solution was placed in a dialysis tube (MWCO = 12,000 g/mol). The tube was then introduced into 500 mL of PBS, and the medium was stirred at 100 rpm at 37°C. At predetermined times, 2 mL of sample solution was removed and the same volume of fresh PBS was added. The concentration of drug released into PBS was detected by a UV spectrophotometer (Shimadzu UV-1201) at 319 nm.

RESULTS AND DISCUSSION

Preparation of Rosin Microparticles Using Various Organic Solvents

One of the characteristic points of this study was to investigate the influence of various organic solvents on the formation of microparticles without the addition of surfactant. Ethanol, acetone, DMF and DMAc were selected from various organic solvents, and the studies for the influence of these solvents were performed in the condition of a rosin to IND ratio of 200:100. All presented organic solvents were observed to form a microparticle without the addition of surfactant (Fig. 1). In addition, the influences of these organic solvents on this process were detected in the drug-loading and drug release from the particles (the results of drug loading are demonstrated below). In similar studies, it has been reported that the polarity and non-polarity of organic solvents can

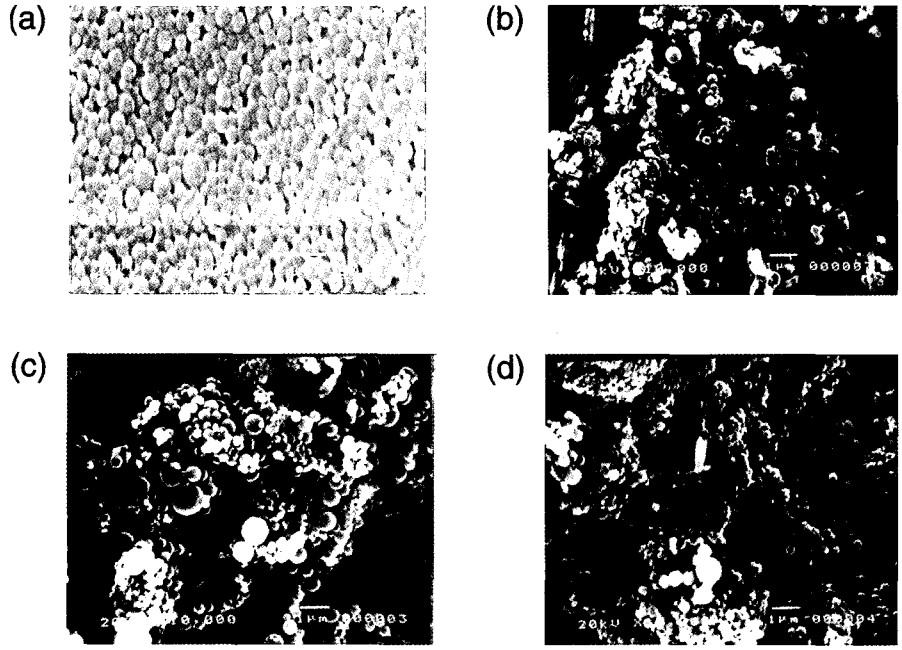

Fig. 1. Scanning electron microscopic photographs of the rosin microparticles prepared by various solvents, (a) ethanol, (b) DMAc, (c) acetone, and (d) DMF.

have an influence on the size, drug-loading and physicochemical properties of particles [13]. The selection of organic solvents is, so, very important for the preparation of particles using a solvent evaporation method and an emulsion method. These results indicate that the solvents used in the preparation can significantly affect the particle size, and the physicochemical properties of particles. In addition, drug loading can be changed by a selected solvent. This may be due to that the solvents having a different miscibility in a water media by dialysis [13,15]. Upon consideration of this influence, ethanol was chiefly used as a solvent in the following experiments because the morphological shapes of the microparticles prepared by ethanol were uniform and the drug content in the microparticles was better than those in the other solvents.

Morphological Observation by SEM

The rosin microparticles were prepared by a dispersion and dialysis method with various organic solvents. The dialysis method has been used for preparing polymeric micelles, self-assembled particles and liposomes [15]. Using SEM, the morphologies of the rosin microparticles containing IND were observed to be spherical for the all solvents tested (Fig. 1). A difference in uniformity of the microparticles, however, was observed according to the various solvents. In particular, the microparticles prepared by ethanol as a solvent were mostly uniform and smaller in size than some of those prepared by other solvents (Fig. 1). The diameter of the rosin microparticles prepared using ethanol was generally smaller than 500 nm. On the other hand, the diameter of the rosin microparticles formed using acetone was about 0.8~1 μm.

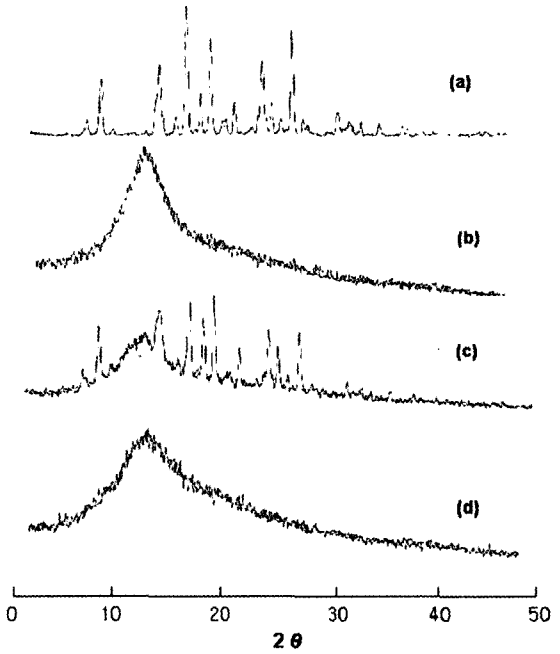

Fig. 2. Powder X-ray diffraction patterns of (a) IND, (b) rosin, (c) the physical mixture of rosin and IND, and (d) IND-loaded rosin microparticles.

These differences may be caused by various physicochemical properties between the polymer and solvents such as difference in solubility, and viscosity, or by a miscibility difference between the solvent and water [15].

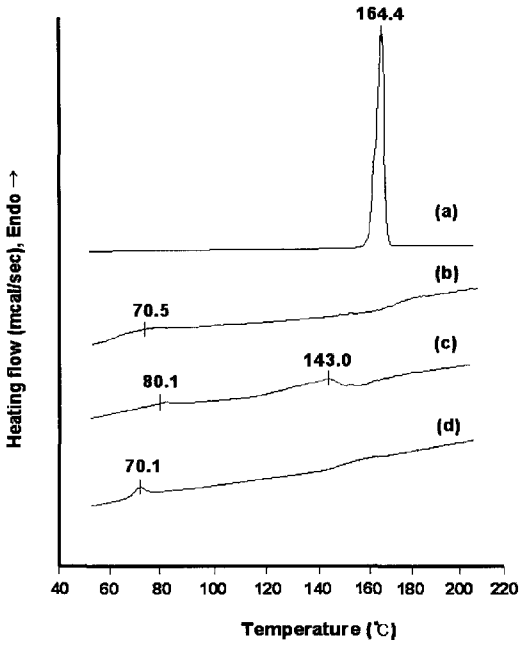

Fig. 3. DSC thermograms of (a) IND, (b) rosin, (c) the physical mixture of rosin and IND, and (d) IND-loaded rosin microparticles.

Physical State of the Drug in Rosin Microparticles

The XRD and DSC analyses were used to investigate the physicochemical characteristics of rosin and IND in the microparticles (Figs. 2 and 3). During the formulation of particles, hydrophobic drug, IND, could be incorporated in the rosin microparticles because it is completely soluble in the polymer solution [2,3].

Fig. 3 shows that the XRD test confirmed the physical state of IND and rosin. A specific broad peak associated with rosin was observed, whereas, for pure IND, a crystalline peak was shown. For the physical mixture of IND and rosin (mixture ratio, 1:1) crystalline and amorphous peaks were revealed. The crystalline peaks, however, were significantly decreased for the rosin microparticles including IND. This result indicates that IND was almost always included in rosin microparticles [13,16]. The incorporation of IND in the rosin microparticles is demonstrated by the results of the thermal analysis as shown in Fig. 4. The softening point of rosin was observed at 70.5°C and the melting point of pure IND was at 164.4°C. The endothermic peak of physical mixture of IND and rosin (mixture ratio, 1:1), which coincided with the melting point of IND and the softening point of rosin, was observed at 143.08 and 80.16°C, respectively. The reason why the melting temperature of IND was depressed may be due to the decrease of the specific crystallinity of IND by the mollification of rosin at a low temperature. On the other hand, the rosin microparticles including IND had only an endothermic peak at 70.1°C, but an IND peak was not observed. Consequently, these data suggest that IND is included in rosin microparticles and it is dispersed in the co-polymer matrix [13,17].

Table 1. Drug contents and loading efficiencies of IND-loaded rosin microparticles against the various solvents used (Values indicate mean ± S.D.)

Solvent	Drug content (wt.-%)	Drug loading efficiency (wt.-%)
Ethanol	28.1 ± 3.1	52.9 ± 5.0
Acetone	24.9 ± 0.8	55.3 ± 4.2
DMF	24.5 ± 1.2	65.4 ± 3.7
DMAc	27.6 ± 1.4	69.3 ± 5.1

*Abbreviations: IND; indomethacin, DMF; *N,N*-dimethyl formamide, DMAc; *N,N*-dimethyl acetamide, S.D.; standard deviation.

Table 2. Drug content and loading efficiencies of rosin microparticles prepared by ethanol at various concentrations of rosin (values indicate mean ± S. D.)

Initial polymer (mg) / IND (mg)	Drug content (wt.-%)	Drug loading efficiency (wt.-%)
100 / 100	40.3 ± 1.5	42.7 ± 4.0
130 /100	32.3 ± 1.6	44.6 ± 3.8
140 / 100	31.2 ± 1.5	45.0 ± 2.6
150 / 100	30.0 ± 2.9	46.0 ± 1.2
200 / 100	28.1 ± 3.1	52.9 ± 5.4
250 / 100	23.0 ± 0.9	52.7 ± 1.5
300 / 100	22.5 ± 0.5	57.8 ± 2.8
400 / 100	17.8 ± 0.7	58.0 ± 1.0

Drug Loading and Release Studies *In Vitro*

The drug loading efficiencies of the rosin microparticles prepared by various organic solvents are shown in Table 1. All rosin microparticles had a >50% of drug loading efficiency, and the drug loading content levels differed according to which solvent was used. The order of drug content per solvent used was ethanol ≥DMAc >acetone = DMF. In particular, the use of ethanol resulted in the smallest particle size and in lowest drug loading when compared to these same parameters of the other solvents. Table 2 shows the drug loading efficiencies of the microparticles prepared by ethanol at various concentrations of rosin. As the initial rosin concentration was increased, the drug loading efficiencies were gradually increased. These results could be expected such that the differences of solubility among rosin, IND and the solvent, or between water and the solvent would affect the shape, drug loading and drug contents of the microparticles [18-19].

To study the drug release behavior, the IND-loaded rosin microparticles were suspended in PBS (pH 7.4, 0.1

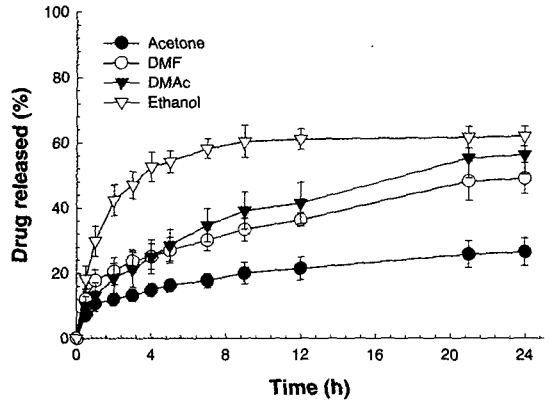

Fig. 4. IND release from rosin microparticles prepared by various solvents.

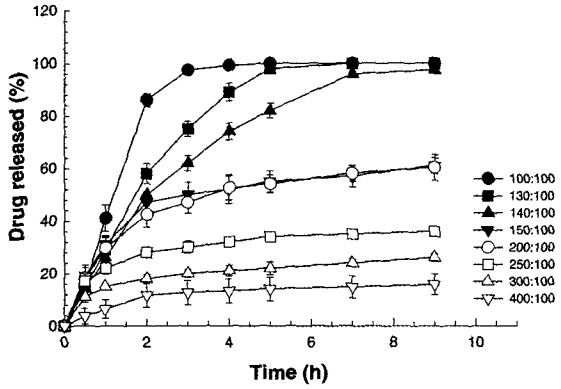

Fig. 5. IND release from rosin microparticles at different rosin concentrations.

M) and drug release was determined *in vitro*. Fig. 4 shows the *in vitro* IND release from rosin microparticles versus the various solvents used in the preparation. The rate of IND released from the microparticles prepared by ethanol was higher than the rates obtained from the other solvents, and the release rate of the microparticles prepared by acetone was the slowest of the solvents used in this study. Generally, the drug release rate from microparticles is relatively faster than that of microparticle systems due to their high surface area and small size [20, 21]. The order of drug release rate kinetics was ethanol > DMAc > DMF expect for the case of acetone. These results indicated that the drug release rate from the higher drug-loaded particles was slower than that of the lower drug-loaded particles. The reason may be extrapolated from the results reported by several other researchers [18,22]. Gref *et al.* reported that hydrophobic drugs can be crystallized inside microparticles and a phase separation occurs at higher drug contents of the drug in the microparticles [23]. Interestingly, despite the rosin microparticles prepared by acetone having the lower drug loading efficiency, the release rate from the microparticle was slower than that of DMF. It was thought that the shape and size of particles affected drug release rate. In addition, it was found that the solvent affects the formulation of microparticles and the drug content. According to the results, the drug release rate from microparticles was changed by the various solvents used in the experiment. As shown in Fig. 5, the drug release rate was regularly controlled by the rosin ratio. This result indicates that drug release behaviors can be regulated by the rosin content of a particle.

CONCLUSION

Rosin microparticles were prepared by a dispersion and dialysis method without the addition of surfactant and their physicochemical characteristics, such as particle shape, drug content and drug release rate were investigated against various solvents used for the preparation of microparticles. The particle shape and drug content of rosin microparticles were changed by the various solvents (*i.e.*, ethanol, DMF, DMAc and acetone) used in the test. The morphological shape of the rosin microparticles prepared with ethanol as a solvent was uniform and smaller than those formed with the other solvents. From the analyses of XRD and DSC, it was confirmed that IND was included in the rosin microparticles. The release behaviors of IND from the rosin microparticles were dependent on the drug content and particle size parameters. A higher drug content and larger particle size resulted in a slower the drug release. The drug release behaviors of the rosin microparticles can be regulated by the rosin content of a particle. Consequently, the rosin microparticles formed without the addition of surfactant can be used for an efficacious sustained-drug release system.

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REFERENCES

[1] Mandaogade, P. M., P. M. Satturwar, S. V. Fulzele, B. B. Gogte, and A. K. Dorle (2002) Rosin derivatives: Novel film forming materials for controlled drug delivery. *Reactive Func. Polymers* 50: 233-242.

[2] Fulzele, S. V., P. M. Satturwar, and A. K. Dorle (2002) Polymerized rosin: Novel film forming polymer for drug delivery. *Int. J. Pharm.* 249: 175-84.

[3] Fulzele, S. V., P. M. Satturwar, and A. K. Dorle (2003) Study of the biodegradation and *in vivo* biocompatibility of novel biomaterials. *Eur. J. Pharm. Sci.* 20: 53-61.

[4] Ramani, C. C., P. K. Puranik, and A. K. Dorle (1996) Study of diabietic acid as matrix forming material. *Int. J. Pharm.* 137: 11-19.

[5] Puranik, P. K. and A. K. Dorle (1991) Study of a abietic acid-glycerol derivatives as microencapsulating materials. *J. Microencapsulation* 8: 247-252.

[6] Flower, R. J. (1994) Drugs which inhibit prostaglandin biosynthesis. *Pharm. Rev.* 26: 33-67.

[7] Karasulu, E., H. Y. Karasulu, G. Ertan, L. Kirilmaz, and T. Gűneri (2003) Extended release lipophilic indomethacin microspheres: Formulation factors and mathematical equations fitted drug release rates. *Eur. J. Pharm. Sci.* 19: 9-104.

[8] Eis, M. J., B. M. Watkins, A. Philip, and R. E. Welling (1998) Non-steroidal-induced benign structures of colon: A case report and review of the literature. *Am. J. Gastroenterol.* 93: 120-121.

[9] Kam, S. H., E. S. Park, and S. C. Chi (1995) Skin Permeation of indomethacin from Gels. *J. Kor. Pharm. Sci.* 25: 129-136.

[10] Uzunkaya, G. and N. Bergisadi (2003) *In vitro* drug liberation and kinetics of sustained release indomethacin suppository. *Il Farmaco* 58: 509-512.

[11] Khanfar, M. S., N. M. Najib, and G. K. Pillai (1997) Dissolution behaviour of sustained release formulation of indomethacin with Eudragit RS. *Acta Pharm. Hung.* 67: 235-239.

[12] Joseph, I. and S. Venkatoran (1995) Indomethacin sustained release from alginate-gelatin or pectin-gelatin coacervates. *Int. J. Pharm.* 126: 161-168.

[13] Jeon, H. J., Y. I. Jeong, M. K. Jang, Y. H. Park, and J. W. Nah (2000) Effect of solvent on the preparation of surfactant-free poly(DL-lactide-co-glycolide) nano-particles and norfloxacin release characteristics. *Int. J. Pharm.* 207: 99-108.

[14] Yeo, Y., N. J. Baek, and K. N. Park (2001) Microencapsulation methods for delivery of protein drugs. *Biotechnol. Bioprocess Eng.* 6: 213-230.

[15] Jung, S. W., Y. I. Jeong, and S. H. Kim (2003) Characterization of hydrophobized pullulan with various hydrophobicities. *Int. J. Pharm.* 254: 109-121.

[16] Na, K., Y. E. Kim, and K. Y. Lee, (1999) Preparation of microparticles in drug delivery system using guar derivatives and dialysis method. *J. Microbiol. Biotechnol.* 9: 50-55.

[17] Lee, C. M., D. W. Kim, H. C. Lee, and K. Y. Lee, (2004) Pectin microspheres for oral colon delivery: Preparation using spray drying method and *in vitro* release of indomethacin. *Biotechnol. Bioprocess Eng.* 9: 191-195.

[18] La, S. B., T. Okano, and K. Kataoka (1996) Preparation and characterization of the micelle-forming polymeric drug indomethacin-incorporated poly(ethylene oxide)-poly (β-benzyl L-aspartate) block copolymer micelles. *J. Pharm. Sci.* 85: 85-90.

[19] Kim, S. Y., I. G. Shin, and Y. M. Lee (1999) Amphiphilic diblock copolymeric nanospheres composed of methoxy poly(ethylene glycol) and glycolide: Properties, cytotoxicity and drug release behavior. *Biomaterials* 20: 1033-1042.

[20] Wilhelm, M., C. L. Zhao, Y. Wang, R. Xu, M. A. Winnik, J. L. Mura, G. Riess, and M. D. Croucher (1991) Poly (styrene-ethylene oxide) block copolymer micelle formation in water: A fluorescence probe study. *Macromolecules* 24: 1033-1040.

[21] Jeong, Y. I., J. B. Cheon, S. H. Kim, J. W. Nah, Y. M. Lee, Y. K. Sung, T. Akaite, and C. S. Cho (1998) Clonazepam release from core-shell type microparticles *in vitro*. *J. Control. Release* 51: 169-178.

[22] Kown, G. S., M. Naito, M. Yokoyama, T. Okano, Y. Sakurai, and K. Kataoka (1995) Physical entrapment of adriamycin in AB block copolymer micelles. *Pharm. Res.* 12: 192-195.

[23] Gref, R., Y. Minamitake, M. T. Peracchia, V. Trubetskoy, V. Torchilin, and R. Langer (1994) Biodegradable long-circulating polymeric nanospheres. *Science* 263: 1600-1603.

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