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Effect of formulation and processing variables on the characteristics of microspheres for water-soluble drugs prepared by w/o/o double emulsion solvent diffusion method

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Abstract

Water-soluble drugs were encapsulated within anionic acrylic resin (Eudragit® S100) microspheres by water in oil in oil (w/o/o) double emulsion solvent diffusion method. Dichloromethane and corn oil were chosen as primary and secondary oil phases, respectively. The presence of internal water phase was essential in forming stable emulsion droplets and it accelerated the hardening of microspheres. Tween 80 was used as a surfactant for stabilizing internal water phase and Span 80 was used for stabilizing corn oil phase. The optimum concentration of Tween 80 was 0.25% (v/v) and that of Span 80 was above 0.02% (v/v). The temperature of continuous phase affected stability of emulsion and the morphology of microspheres. As the volume of continuous phase increased, the size of microspheres decreased. The loading efficiency was $>80\%$ except for acetaminophen, due to its lower solubility in water and higher solubility in corn oil. The release profile of the drug was pH dependent. In acidic medium, the release rate was much slower, however, the drug was released quickly at pH 7.4. Tacrine showed unexpected release profiles, probably due to ionic interaction with polymer matrix and the shell structure and the highest release rate was obtained at pH 2.0. The prepared microspheres had a sponge-like inner structure with or without central hollow core and the surface was dense with no apparent pores.

Keywords: w/o/o double emulsion solvent diffusion method; Acrylic microspheres; Water-soluble drug; Acrylic resin; Release profile

1. Introduction

Microencapsulation has been used as one of the methods to deliver a drug in a controlled fashion. Most of the microencapsulation techniques have been used for lipophilic drugs, since hydrophilic drugs usually showed low loading efficiency. To

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entrap the water-soluble drugs, several preparation methods were developed such as phase separation (Ruiz et al., 1989), spray-drying (Takada et al., 1994) and solvent evaporation (Ogawa et al., 1988; Wada et al., 1990). The problems of phase separation technique, are aggregation and residual solvent in resulting microspheres. In spray-drying method, the active substance can be exposed to the heat. Conventional oil in water (o/w) solvent diffusion and evaporation method may not be used for water-soluble drugs, due to low loading efficiency, resulting from partitioning of drug into continuous phase. To reduce the partitioning of a drug into the continuous phase, various method such as chemical modification, modification of the continuous phase or dispersed phase of the emulsion were used. As an example of the latter case, water in oil in water (w/o/w) (Ogawa et al., 1988), water in oil (w/o) (Iwata and McGinity, 1992), oil in oil (o/o) solvent evaporation method or other multiple emulsion solvent evaporation methods have been used (Iwata and McGinity, 1993; O'Donnell and McGinity, 1998). When microspheres are prepared by w/o/w emulsion diffusion method, stability of the internal water phase influences the loading efficiency of the water-soluble drugs and the morphology of the internal structure of microspheres (Florence and Whitehill, 1981; Nihant et al., 1994; Schugens et al., 1994).

We have shown in the previous study (Lee et al., 1999) that floating acrylic microspheres could be prepared, using a solvent diffusion evaporation method, based on the use of solvent mixtures having different water miscibility, ethanol, isopropanol and dichloromethane. The prepared microspheres can float on gastric fluid and moves to intestinal region slowly to release the drug due to enteric nature of acrylic polymer. One disadvantage of the method was low loading efficiency for water-soluble drugs. In order to improve loading efficiency for water-soluble drugs and to modulate release profiles, the present study was conducted. We also investigated the effect of various processing parameters, such as ratios of ethanol (EtOH) to dichloromethane (DCM), amount of internal aqueous phase, surfactant concentration and amount of polymer on the size, yield, loading efficiency, morphology and release profile of a drug. The results were compared with those of the microspheres produced by o/w solvent diffusion method.

2. Materials and methods

2.1. Materials

Eudragit® S100 (methacrylic acid copolymer type B) was a gift from Röhm Pharma. (Darmstadt, Germany). Propranolol HCl and theophylline were purchased from Sigma (St Louis, MO). Acetaminophen was obtained from Dong-Hwa Pharmaceuticals (Seoul, Korea). Tacrine HCl was a gift from Jeil Pharmaceutical (Seoul, Korea). All other chemicals were reagent grade and were used as received without further purification.

2.2. Preparation of microspheres

Microspheres were prepared using the procedure illustrated in Fig. 1. Eudragit® S100 and a drug were dissolved in 5 ml of DCM/EtOH mixture, followed by addition of 1 ml of aqueous phase containing 0.25% (v/v) Tween 80 (polyoxyethylene sorbitan monooleate).The drug could also be dissolved or dispersed in internal water phase and then emulsified in the polymer phase. The initial w/o emulsion was prepared by stirring the mixture for 20 s. The w/o emulsion was slowly added into 500 ml of corn oil, the second oil phase, containing 0.02% (v/v) Span 80 (sorbitan monooleate) as a surfactant with stirring at 250 rpm at 25°C. It was stirred for 1h and the hardened microspheres were collected by filtration. Collected microspheres were washed with *n*-hexane three times and soaked in fresh hexane with gentle shaking for 24 h. The microspheres were separated and then dried in an oven overnight at 50°C.

2.3. Particle size analysis

Prepared microspheres (0.5 g) were dispersed in 0.02% (v/v) aqueous Tween solution with stirring and under ultrasonification. Average particle size

and particle size distribution of acrylic microspheres were determined by a laser light scattering method (Mastersizer S, Malvern Instrument UK).

2.4. Yield of microsphere formation

The prepared microspheres were weighed after drying in an oven. The weight of collected microspheres was divided by the total weight of all the non volatile components used for the preparation of the microspheres.

2.5. Loading efficiency

Microspheres prepared with 100 mg of a model drug, were dissolved in EtOH. Diluted sample solution was analyzed by high pressure liquid chromatography (HPLC) system with an UV detector (Shimadzu Scientific Instruments, MD). Loading efficiency was calculated, by comparing the amount of the drug initially used to prepare microspheres, to that encapsulated in microspheres.

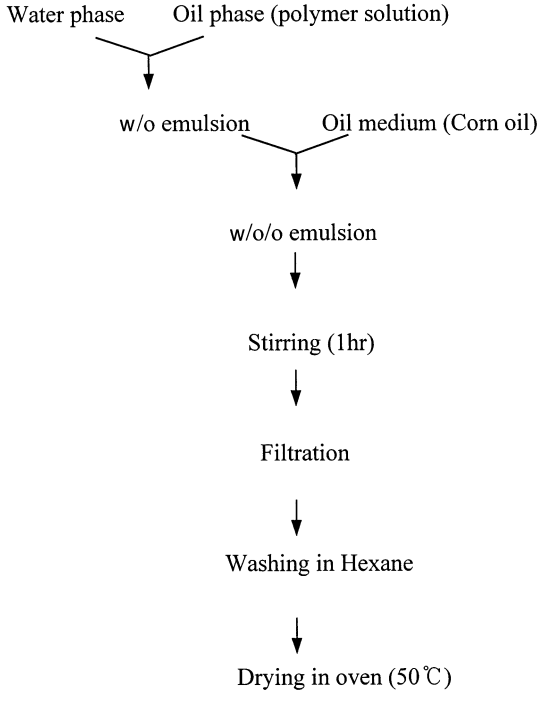

Fig. 1. Procedure of w/o/o double emulsion solvent diffusion method.

2.6. Drug release test

A drug release test was carried out using a dissolution tester (DST 600A, Fine Science Institute, Korea). Microspheres containing the active principles were placed in 500 ml of dissolution medium and the microspheres were stirred at 100 rpm at 37°C. The pH values of dissolution medium tested were set at 2.0 (HCl-Soln), 6.8 and 7.4 (70 mM phosphate buffer saline solution). An aliquot of the release medium was withdrawn at predetermined time intervals and equivalent amount of fresh medium was added to the release medium. Collected samples were analyzed by HPLC, to determine the amount of the active principle released from the microspheres.

2.7. Dissolution of microspheres without the drug

Dried microspheres were placed in 500 ml of dissolution medium at pH 2.0, 6.8 and 7.4. They were agitated by a paddle rotating at 100 rpm at 37°C. They were filtered at a predetermined time point and the dry weight was measured. The percentage of dissolved fraction of the microspheres was determined at each time interval.

2.8. Morphology

The morphology and surface characteristics of the microsphere were examined by a scanning electron microscopy (JMS 840-A, JEOL, Japan). The sample was mounted onto an aluminum stub and sputter-coated for 90 s with gold particles in an argon atmosphere.

2.9. Assay

The model drugs were analyzed by HPLC system (Shimadzu Scientific Instruments, MD) consisting of a UV detector (SPD-10A), a pump (LC-10AD) and an automatic injector (SIL-10A). A reversed phase column (Alltima C8, Alltech, IL) was used as a stationary phase. The mobile phases used were methanol/water/phosphoric acid (70/29.9/0.1) for acetaminophen, acetonitrile/water/phosphoric acid/methanol (24/56/0.08/20) for propranolol HCl, acetonitrile/0.1M KH_2PO_4 (13/

87) for theophylline and methanol/acetonitrile/water/triethylamine (25/20/55/1) for tacrine HCl.

3. Result and discussion

3.1. Preparation of microspheres

Microspheres were prepared by w/o/o double-emulsion solvent diffusion method. Corn oil was selected as a continuous phase, since water-soluble drugs and Eudragit® were hardly soluble in corn oil. After the microspheres were formed, corn oil could be washed with *n*-hexane which acted to further solidify the prepared microspheres. Water phase was emulsified in EtOH/DCM co-solvent solution containing acrylic polymer (Eudragit® S100) to form primary w/o emulsion. EtOH was chosen, since it diffuses out into corn oil phase slowly, due to its low solubility in corn oil phase and acrylic polymer is soluble in it. To form stable emulsion droplets, a certain amount of time was required before the organic solvents diffused into corn oil phase and the acrylic polymer solidified (Lee et al., 1999). DCM was chosen as a primary oil phase, since it was miscible with corn oil and diffused out as soon as the w/o emulsion was poured into the corn oil phase. When the microspheres were prepared using only 5 ml of EtOH and 1 ml of water without DCM, small and transparent emulsion droplets were produced. They were not solidified, even after 2 h and then aggregated together. It seems that DCM played a role in increasing the partitioning of EtOH to external oil phase and reduced the solidification time as Sprokel and Prapaitrakal (1990) reported. To select the optimum preparation condition, the effects of various experimental parameters, such as surfactant concentration, stirring speed, solvent ratios and the amount of internal water phase, on the morphology and size of microspheres were investigated. When EtOH/DCM mixture without water was used as a dispersed phase, it did not form emulsion droplets and the solution remained in a transparent state. The internal water phase seemed to accelerate the hardening of microspheres. When w/o emulsion was dispersed in corn oil medium initially, transparent liquid droplets were formed and gradually turned into white. It was supposed, that DCM diffused out preferentially over EtOH from the droplets, resulting in the solidification of acrylic polymer. Water in the dispersed phase reduced the solubility of acrylic polymer and helped solidification of the shell. The shape of the primary emulsion, traced to that of the final particles, since the solidification process underwent relatively fast after the emulsification of primary emulsion in corn oil phase (Quintanar-Guerrero et al., 1996).

3.2. Effect of solvent composition

The effects of various ratios of solvents, used in the primary emulsion on the formation of microspheres were investigated. The varying volume ratios of EtOH/DCM were tested at 2.5 ml/2.5 ml, 3 ml/2 ml and 3.5 ml/1.5 ml, respectively, with a fixed amount of water volume at 1 ml. The volume of internal water, played a key role in the formation of microspheres. It affected the solidification time, as well as the size of microspheres. As the amount of water increased, the size of microspheres increased. Regardless of EtOH/DCM ratio, when the volume of water phase was equal to or more than 1.5 ml, larger microspheres with irregular shapes were observed and microspheres were not formed, when < 1 ml of aqueous phase was used. When the volume of DCM was greater than EtOH, large microspheres were formed in a non spherical shape. As the proportion of EtOH was greater, the size of microspheres decreased. After DCM diffuse out into corn oil phase preferentially, EtOH and water mainly constitute emulsion droplets. Consequently, the relative volume ratio of water and EtOH is important in determining droplet size, as it will be smaller when the proportion of ethanol is larger. The droplets with smaller proportion of water, would form smaller microspheres, since the polymer would solidify around the water droplets serving as an internal core region.

Sizes of microspheres were found to increase with the increase in the concentration of polymer or drug (Tables 1 and 2).

Table 1
Effect of various parameters on average particle size of microspheres[a]

	Processing parameter	Mean particle size (mm)
Stirring speed	200 rpm	267 ± 55
	300 rpm	201 ± 73
Amount of polymer		
	0.25 g	122 ± 20
	0.4 g	173 ± 16
	0.6 g	329 ± 15
Surfactant (Tween 80) concentration		
	0.25%	174 ± 21
	0.5%	227 ± 23
	1%	214 ± 58
	2%	225 ± 21
	5%	235 ± 32
Drug (tacrine HCl)		
	100 mg	216 ± 44
	200 mg	257 ± 7
Drug (acetaminophen)		
	100 mg	205 ± 6
	200 mg	220 ± 19
Solvent ratio (EtOH:DCM:W)		
	3:2:1	192 ± 10
	3:2:1.5	215 ± 10
	3.5:1.5:1	196 ± 4
	3.5:1.5:1.5	239 ± 56
Temperature of medium		
	20°C	223 ± 18
	30°C	238 ± 38
Volume of medium		
	150 ml	339 ± 25
	250 ml	238 ± 38
	1000 ml	203 ± 8
Surfactant (Span) concentration		
	0%	91 ± 30
	0.02%	222 ± 22
	0.05%	238 ± 32
	0.1%	266 ± 8
	0.5%	244 ± 39

[a] Stirring speed was 250 rpm, the amount of acrylic polymer was 0.5 g, the concentration of Tween 80 was 0.25%, the medium temperature was 25°C, the volume of medium was 500 ml, if it is not otherwise specified.

3.3. Effect of surfactant concentration

Two surfactants were used in this study. Tween 80, having a high HLB value was added to internal water phase and Span 80 with a low HLB value was added to corn oil phase. Tween 80 was examined at the range of 0–5.0% (v/v). When an aqueous phase without Tween 80 was used as an internal phase, microspheres were aggregated. Above 5.0% (v/v), the droplets were broken and corn oil medium became opaque and then the solidified broken pieces aggregated around the propeller. The concentration of Tween 80 was fixed at 0.25% in the subsequent experiments. Span 80 also influenced the formation of microspheres. When its concentration is over 0.02%, microspheres were prepared with high yield and spherical shape. In the literature, it was reported that increasing the concentration of emulsifying agent, resulted in reduction in the size of microspheres (Jalil and Nixon, 1990). Without Span 80 in the corn oil phase, larger microspheres were aggregated and only some of smaller microspheres were left unaggregated. The concentration of Span 80 above 0.02% (v/v) did not significantly affect the size of microspheres, as shown in Table 1.

3.4. Effect of stirring speed

The rotating speed of the stirrer usually controls the size of microspheres, however, it does not change the shape of microspheres significantly. In the present study, stirring speed had more impact on the shape than on the size of prepared microspheres. Short rod or cometary structures were formed at the rotation speed of higher than 300 rpm. As shown in Table 1, optimum rotation speed range was 200–300 rpm and the size of the microspheres was relatively uniform in this stirring range. The stirring speed was set at 250 rpm.

3.5. Effect of temperature

The temperature of continuous phase also affected the morphology of microspheres. At 20°C, w/o emulsion droplets were solidified in 4–5 min

Table 2
Solubility of drug in the solvents used as dispersed phase and continuous phase

Drug	Solubility (mg/ml)				Loading efficiency (%)
	EtOH	DCM	Water	Corn oil	
Tacrine HCl	>50	0.92	>50	0.014	87 ± 4
Acetaminophen	>50	0.21	>50	0.059	83 ± 3
Propranolol HCl	48.6	44.0	>50	4.97	85 ± 3
Theophylline	3.90	1.86	8.80	0.025	60 ± 4

after pouring into corn oil. When the temperature was lower than 20°C, the introduced w/o emulsion, was aggregated around the propeller shaft and microspheres were not formed. At lower temperature, the diffusion and evaporation rate of solvents became slow and the transient emulsion droplets could not maintain a spherical shape until the solidification. Above 30°C, liquid droplets were formed and solidified in ≈1.5 min, but they started to aggregate afterwards. At 40°C aggregation of microspheres occurred to a great extent and formed a large mass, generating very small microspheres to some extent. It can be explained, that it is easier for the microspheres to collide with each other and they may coalesce together at the time of solidification, since the viscosity of oil medium is lower at higher temperature.

3.6. Volume of continuous phase

As the volume of medium increased, the size of microspheres decreased (Table 1). When the volume of continuous phase was reduced, microspheres had more opportunities to collide with each other and fuse together to form larger microspheres. When the w/o emulsion was first introduced into oil medium, the size of emulsion droplets was similar in any case. But after the solidification, the size of resulting microspheres was different, since the size of emulsion droplets can be changed while they remain in a transparent state. After the droplets were turned into white and completely solidified, collisions may result in flocculated microspheres, however, they can be dispersed again in *n*-hexane at washing process.

3.7. Loading efficiency

Loading efficiency of the drug depended on solubility of the drug in the solvents and continuous phase and physicochemical properties of the drug and polymer. Water-soluble drugs, acetaminophen, propranolol HCl, theophylline and tacrine HCl were used as model drugs. In cases of acetaminophen, tacrine and propranolol, loading efficiencies were over 80%. It was increased by 6–20 times when compared to o/w solvent diffusion method as shown in Fig. 2. The increase in the loading efficiency, resulted from poor solubility of the drug in continuous phase and high solubility in internal water. On the contrary, the loading efficiency of theophylline was lower (≈ 60%) than the others, due to low solubility in water. Acetaminophen gradually leaked out from

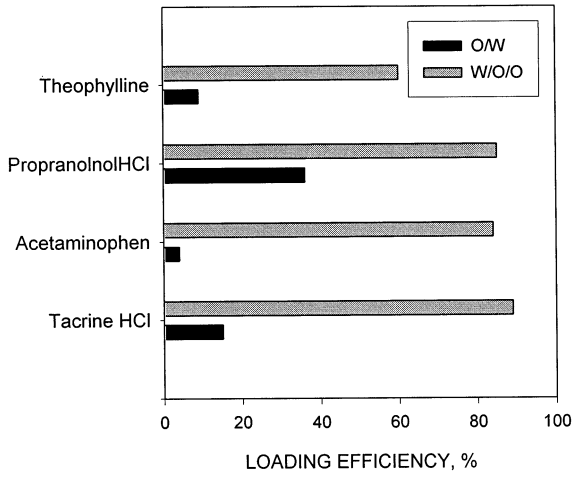

Fig. 2. Comparison of loading efficiencies of water-soluble drugs prepared by w/o/o solvent diffusion method and o/w solvent diffusion method.

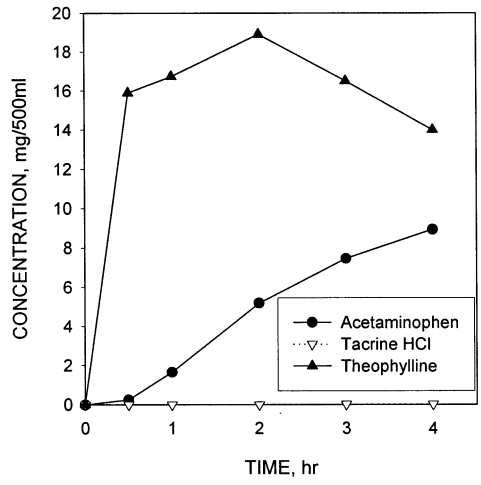

Fig. 3. Concentrations of various drugs in corn oil phase during microencapsulation process.

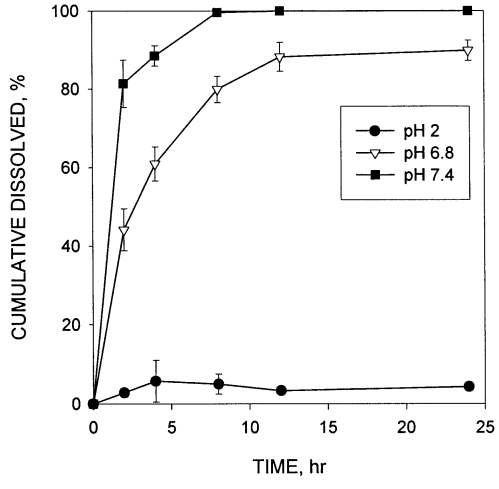

Fig. 4. Dissolution of acrylic microspheres without a drug at pH 2.0, 6.8 and 7.4. Each point represents an average value of three experiments. The error bar shows S.D.

the microspheres, as the time in the dispersion medium was increased, even after the solidification was complete (Fig. 3). It might diffuse out of microsphere together with EtOH, due to high solubility in ethanol. When the microsphere was prepared with higher EtOH/DCM ratio, loading efficiency decreased, due to high solubility of the drug in EtOH, delayed solidification of microspheres and larger surface area of small microspheres. On the contrary, tacrine HCl showed higher loading efficiency than acetaminophen, which may due to the ionic interaction between amino group of tacrine and carboxyl group of acrylic polymer.

3.8. Drug release

In our previous study, drug release from microspheres, was attributed to dissolution of polymeric matrix and/or drug diffusion through hydrated matrix or through the pores. At pH 7.4, the release profile of acetaminophen and theophylline were similar to the dissolution rate of acrylic polymer itself (Fig. 4) and the most of microspheres were dissolved within 4 h. As shown in Fig. 5, at pH 2.0, microspheres were not dissolved or swollen and remained intact, however, 30–40% of the drugs were released. At pH 6.8, microspheres were swollen and slowly dissolved and the drugs were released more rapidly than at pH 2.0. On the other hand, 100% of tacrine was released after 24 h at pH 2.0, while $<40\%$ was released at pH 6.8 and 7.4. The release profile of tacrine, was quite different from that obtained with microspheres prepared by o/w solvent diffusion method, where the highest release rate was obtained at pH 7.4. The release rate of tacrine from the microspheres prepared by o/w solvent diffusion method was faster at pH 2.0 than pH 6.8, due to the ionic interaction between the drug and acrylic polymer at neutral pH. It is unclear why unexpectedly high release rate was obtained at pH 2.0 and low release rate at pH 7.4 in this study, however, it might have something to do with different shell structure and higher degree of ionic interaction between the drug and the polymer. More studies need to be done to explain the reason for the difference.

3.9. Morphology

Spherical microspheres had a sponge-like inner structure with or without a hollow core, but the surface of microspheres was dense with no apparent pores as shown in Fig. 6. These results are in contrast with the hollow structure obtained in the previous study using o/w solvent diffusion method

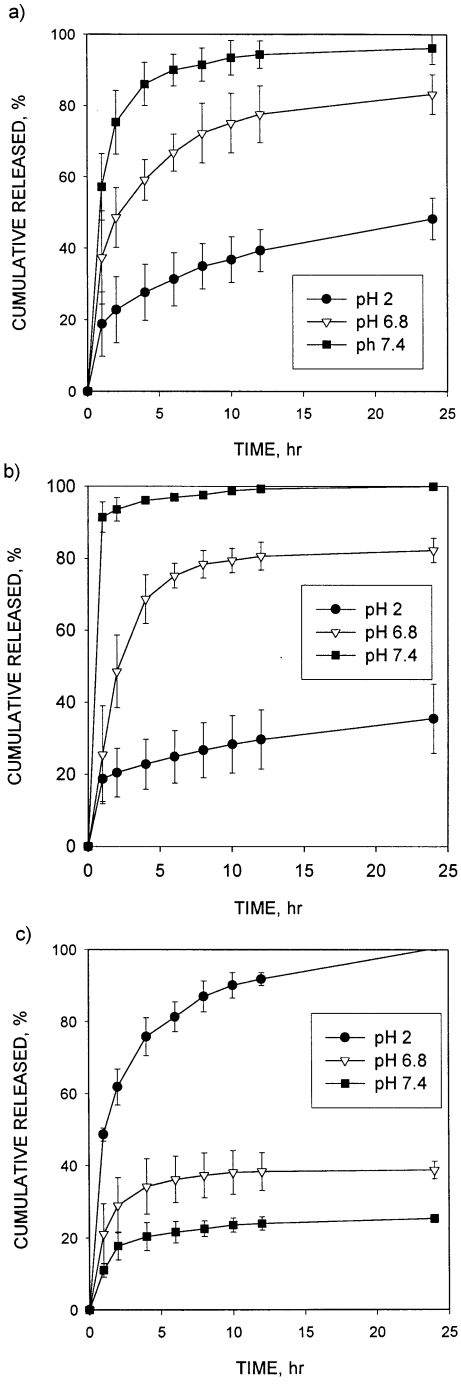

Fig. 5. The release profiles of the drugs from acrylic microspheres at pH 2.0, 6.8 and 7.4; acetaminophen; (a) theophylline; (b), tacrine HCl; (c) each point represents average of three experiments. The error bar shows S.D..

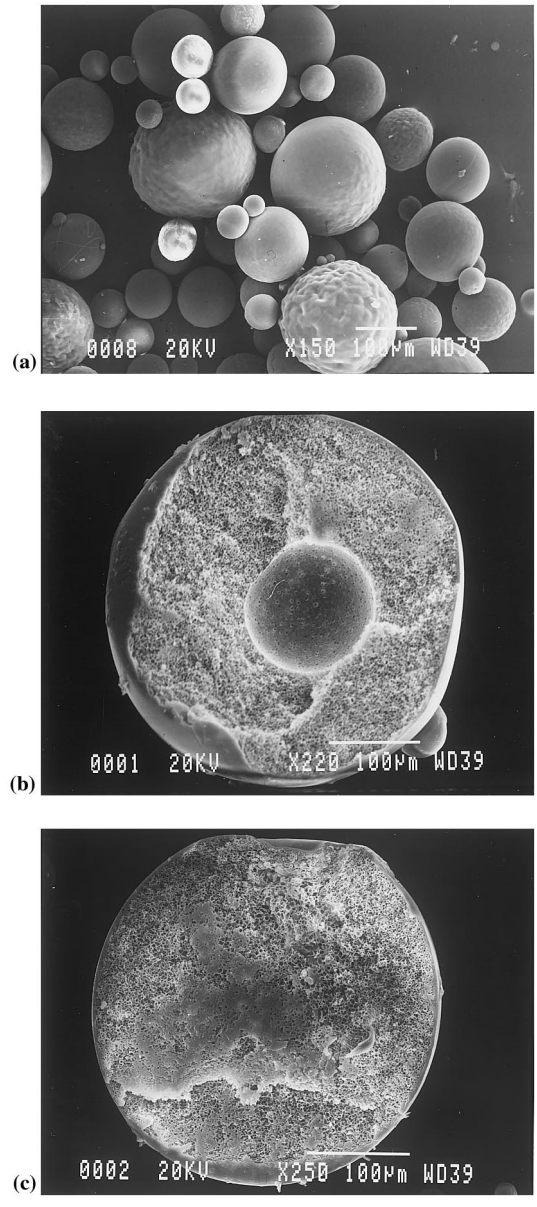

Fig. 6. Scanning electron micrographs: (a) acrylic microspheres; (b) internal view; internal view of microspheres with a hollow core; (c) view of the shell having a porous structure.

(Lee et al., 1999). Both water and ethanol, which remained in microspheres for relatively longer period, are expected to play a role in generating internal pore structure. The polymer was dissolved in the primary w/o emulsion and if the w/o emulsion was stable until the solidification was complete, the inside of microspheres would be

filled with w/o emulsion droplets and the polymer would solidify around the emulsion droplets. Once the remained solvents, ethanol and water, diffuse out, microspheres filled with porous sponge-like structure would result. When the stability of w/o emulsion is poor, larger pores can be seen due to coalescence of emulsion droplets, prior to the solidification of acrylic polymer.

Acknowledgements

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