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Preparation of Sodium Alginate Microspheres Containing Hydrophilic β-lactam Antibiotics

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Alginate microspheres were prepared by the emulsification process as a drug delivery system of ampicillin sodium (AMP-Na). The preparation parameters such as the concentration of calcium chloride, the stirring time and the amount of AMP-Na were investigated. The alginate microspheres containing hydroxypropylmethylcellulose (HPMC) were found to be generally spherical, discrete and had smoother surfaces when compared to without HPMC. However, there was no significant difference in the release profile of AMP-Na from alginate microspheres prepared with or without HPMC. The concentration of calcium chloride solution and the stirring time in the preparation of alginate microspheres influenced the aggregation of alginate microspheres. The amount of AMP-Na in alginate microspheres influenced the surface morphology and the practical drug content in microspheres.

Keywords : Microspheres, Sodium alginate, Controlled release, Ampicillin sodium

INTRODUCTION

During the past 10 years or so, the increasing interest has been shown in studies on drug delivery system via polymeric carriers. Microspheres (Renbaum *et al.*, 1988, Edman *et al.*, 1986), liposomes (Gregoriadis, 1984), and polymeric micelles (Kataoka *et al.*, 1993) were studied for targeted and local drug delivery systems. Microparticles or microspheres were a new drug formulation for the purpose of transporting drugs to specific cells or organs in order to attain a more distinct or higher local concentration of drugs in the target site. Direct application of drugs to the organs or tissues is one of the most efficient ways of drug delivery compared to the indirect methods which introduce drugs into the blood stream. To this end, these carriers have to be biocompatible, non-immunogenic, nontoxic and should not induce any lasting inflammatory processes. These carriers have to be also biodegradable not to create a storage disease and stable during the systemic circulation long enough to carry drugs to the suitable target site.

Alginate is a product from marine algae and is a linear polyuronate, copolymer of D-mannuronic acid (M) and L-guluronic acid (G) (Percival *et al.*, 1967). These two monosaccharides are found as random sequences in the linear polymer. The intact polysaccharide may contain sequences that are almost entirely composed of D-mannuronosyl residues, other sequences that are almost entirely L- guluronyl residues and others that contain a random mixture of the two monomers. The extent and composition of these sequences, together with the relative molecular mass (Mr), determine the properties of the alginate, i.e. (Sutherland, 1991). The alginate gel formed in the presence of calcium ion is widely used in foodstuffs and pharmaceutical applications as suspending agent, lubricant, disintegrating agent and binder.

Ampicillin sodium (AMP-Na) was selected as a model drug of water soluble β-lactam antibiotics. AMP-Na is a broad spectrum of antibacterial agent with high efficacy against gram-positive and gram-negative bateria (Lambert *et al.*, 1992). In this study, alginate microspheres were prepared as a drug carrier of water soluble β-lactam antibiotics. The release characteristics of drug from alginate microspheres, varying drug contents, calcium concentration and stirring time were investigated.

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MATERIALS AND METHODS

Materials

Ampicillin sodium salt (AMP-Na) was provided by Chong Kun Dang Co. (Seoul, Korea). Alginic acid sodium salt (low viscosity) was purchased from Sigma Chemical Co. (St. Louis, MO). Calcium chloride and span 80 were obtained from Kanto Chemical Co. and Kakuri Pure Chemical Co. (Osaka, Japan), respectively. Hydroxypropylmethylcellulose (HPMC) was supplied from Hong Sung Chemical Co. (Seoul, Korea). Acetone and isooctane were provided by J. T. Baker Inc. (Philipsburg, NJ). Dialysis membrane bags (molecular weight cutoff : 12,000-14,000) were purchased from Spectrum Medical Industries Inc. (Los Angeles, CA). All chemicals were analytical grade and used without further purification.

Preparation of alginate microspheres

Alginate microspheres containing AMP-Na were prepared by the following method. 50 ml of isooctane and 2.0 ml of surfactant (span 80) were mixed by a mechanical stirrer for 2 min in a baffled beaker. An aqueous solution of 8 ml alginate or alginate/HPMC with or without AMP-Na was injected into the above isooctane phase via a syringe equipped with a 21 gauge needle. The fine w/o emulsion formed after the injection was stirred for 10 more min and solidified by the addition of calcium chloride solution. 10 ml of acetone was poured into the above solution to dehydrate and harden the alginate microspheres. The resulting alginate microspheres were filtered, washed with acetone and dried for 1 day before use.

Characterization of alginate microspheres

The surface morphology of alginate microspheres was examined by the scanning electron microscope (SEM, Hitachi model S-4200, Hitachi Ltd., Tokyo, Japan). The alginate microspheres were coated with gold (Eiko IB 3 ion water) before the examination. The zeta potential and size distribution of the alginate microspheres were determinated by the zeta potential analyzer (Zetamaster, ZEM5002, Malvern Instruments). 10 mg of alginate microspheres were weighed and shaken for 4 hrs in 10 ml of pH 7.4 phosphate buffered saline solution (PBS 7.4). Sample was withdrawn at a given time interval, the zeta potential of microsphere was analyzed by zeta potential analyzer.

The content of AMP-Na in alginate microspheres expressed as percentage of drug weight per total microspheres weight was calculated by the following method. 10 mg of alginate microspheres were accurately weighed and shaken for 24 hrs in 10 ml PBS 7.4 to extract AMP-Na. The above solution was filtered through the cellulose nitrate membrane (pore size : 0.45 μm). The concentration of AMP-Na was measured in triplicate by HPLC for each batch of alginate microspheres.

In vitro release of AMP-Na from alginate microspheres

Alginate microspheres of 100 mg was introduced into a dialysis membrane bag containing 5 ml of PBS 7.4 to prevent the interference of alginate in analysis process and sealed tightly by the closure. This bag was immersed in 95 ml of PBS 7.4 as release media and shaken 100 strokes per min at 37°C in waterbath. 1 ml aliquot was withdrawn at a given time interval with replacement of an equal volume of fresh PBS. The concentration of AMP-Na in the release medium was analyzed by HPLC.

HPLC analysis

The HPLC system consisted of an SP8880 autosampler, an SP88810 precision isocratic pump, and a Spectra 100 variable wavelength detector set at 223 nm and absorbance attenuation of 0.01 aufs (all from Spectra-Physics, San Jose, CA). Data acquirement and peak integration were accomplished with an SP4290 integrator (Spectra-Physics, San Jose, CA). A Waters μ-Bondapak C_{18}, 3.9 mm I.D. × 300 mm long, 10 μm particle size, stainless steel column with a Guard-Pak™ precolumn (Waters, Milford, MA) was used. The mobile phase was methanol : 0.02% ammonium phosphate monobasic (20 : 80, v/v%), at a flow rate of 1.50 ml/min. The autosampler was set at auto mode, 20 μl injection volume, and 20 min run time. Under these conditions, the retention time for AMP was about 9.70 min, and its standard curve was linear within the range of 3-100 μg/ml with r^2>0.99.

RESULTS AND DISCUSSION

Characterization of the alginate microspheres

Generally, alginate gels are made at low polymer concentration in the presence of calcium ions. The mechanism of gellation is well-known by the egg-box theory (Sutherland, 1991). In this study, the gellation of alginate was performed by the addition of calcium chloride solution into an alginate/isooctane emulsion. The droplets instantaneously formed a gelled sphere through the interaction of alginate with calcium ions. During the preparation of alginate microspheres, the baffled beaker was used to prevent the aggregation of microspheres. The gelled microspheres were washed with acetone to dehydrate and prevent aggregation each other (Wan *et al.*, 1992). Without the dehydrating agent, alginate microspheres had a high tendency to aggregate each other. The clumps of microspheres were most probably caused by the cohesion of the initially wet and soft microspheres.

The alginate microspheres formed after the addition of calcium chloride solution were stirred at various

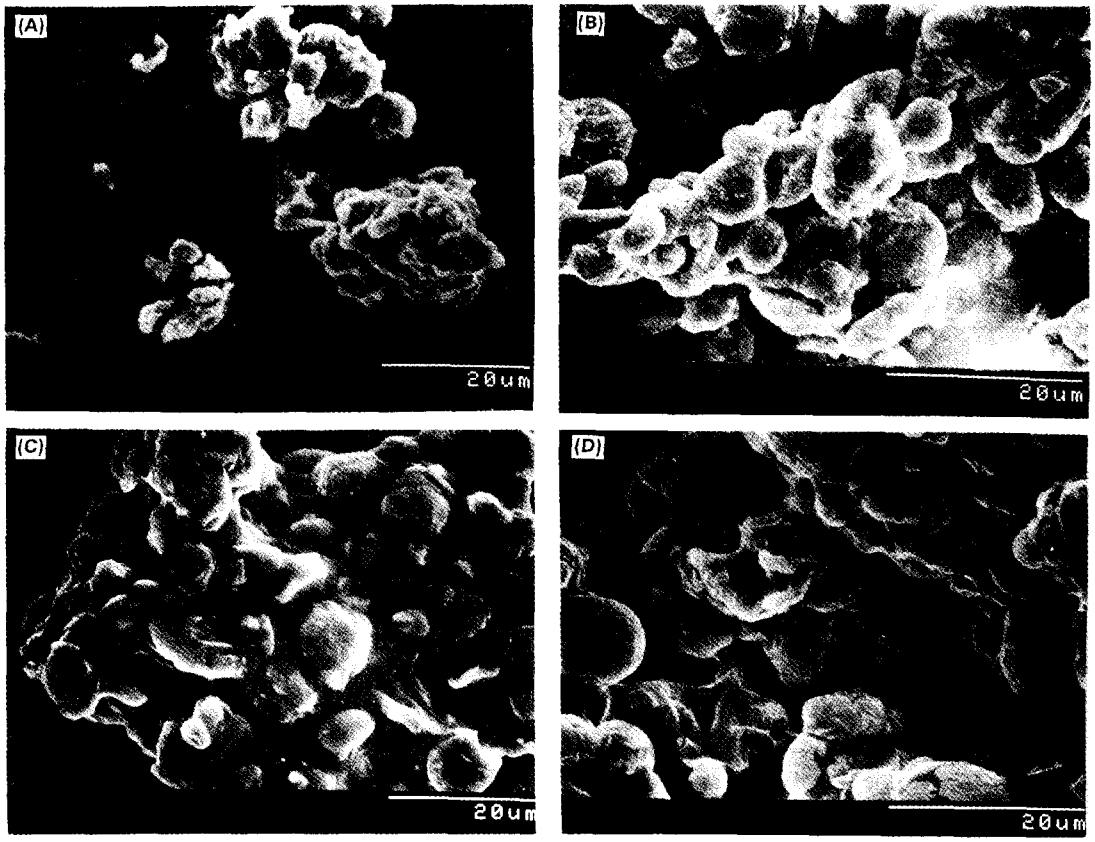

Fig. 1. The surface morphology of alginate microspheres as a function of stirring time (A; 5 min, B; 10 min, C; 20 min, D; 30 min) (magnificaton ×1000)

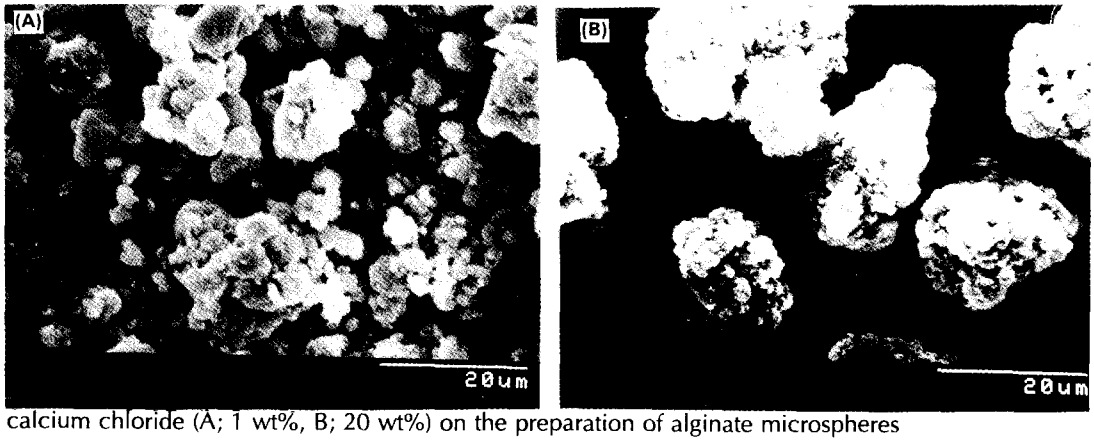

Fig. 2. The calcium chloride (A; 1 wt%, B; 20 wt%) on the preparation of alginate microspheres

length of time (5, 10, 20 and 30 min). The stirring time prominently influenced the aggregation of microspheres, as stirring time increased due to cohesive force of microspheres more aggregated (Fig. 1).

The concentration of calcium chloride was closely related to the hardness and aggregation of microspheres. When the alginate microspheres were prepared at the low concentration (< 1wt%) of calcium chloride, the formed alginate microspheres had relatively weak mechanical properties, although the aggregation of microspheres was reduced (Fig. 2). The effect of the calcium chloride concentration on the zeta potential of the microspheres is also examined in Table I. There were no significant differences in the zeta potential of the microspheres as a function of calcium chloride. This may be due to the saturation of surface charge at low concentration of calcium chloride. The high concentration of calcium chloride solution may affect the density of microspheres rather than the surface charge.

Table I. Zeta potential of alginate microspheres prepared with various concentration of calcium chloride solution (mV)

d	Zeta potential of MS-C2[a]	Zeta potential of MS-C8[b]	Zeta potential of MS-C20[c]
1 min	-22.7	-21.2	-24.5
30 min	-29.7	-24.5	-26.3
2 hr	-35.4	-37.4	-32.0
3 hr	-36.4	-33.3	-33.4
4 hr	-36.8	-35.6	-33.4

[a]alginate microspheres prepared with 2% calcium chloride
[b]alginate microspheres prepared with 8% calcium chloride
[c]alginate microspheres prepared with 20% calcium chloride
[d]swelling time of alginate microspheres in PBS 7.4

It was known that the incorporated HPMC enhances the formation of alginate microspheres and prevents aggregation (Wan *et al.*, 1992). As shown in Fig. 3, alginate microspheres containing HPMC were generally spherical, discrete, small and had smoother surfaces when compared without HPMC. The zeta potential of alginate and alginate/HPMC microspheres was examined (Fig. 4). The alginate/HPMC microspheres had higher zeta potential when compared to alginate alone. As the swelling proceeded, the zeta potential of alginate microspheres became higher because of the depletion of calcium ions from the alginate binding site.

The mean diameter, drug content and efficiency of alginate microspheres are shown in Table II, respectively. As shown this Table, the size of alginate/HPMC microspheres was markedly smaller than microspheres prepared from alginate alone. The drug content ranged from 1.8 to 2.3 wt% of polymer and the efficiency of drug loading was about 20%. The low efficiency of drug loading could be attributed to the high aqueous solubility of AMP-Na resulting in the drug loss from the alginate microspheres during the preparation process. The efficiency of drug loading decreased by 20, 17.6 and 15.6%, as a function of the amount of drug applied, while the drug content increased (Table III).

The effect of AMP-Na loading on alginate microspheres is shown in Fig. 5. The morphology of alginate microspheres was affected by drug loading. When the practical amount of drug in the microspheres was over 5% of the total weight of polymer, drug crystals appeared on the surface of the microspheres.

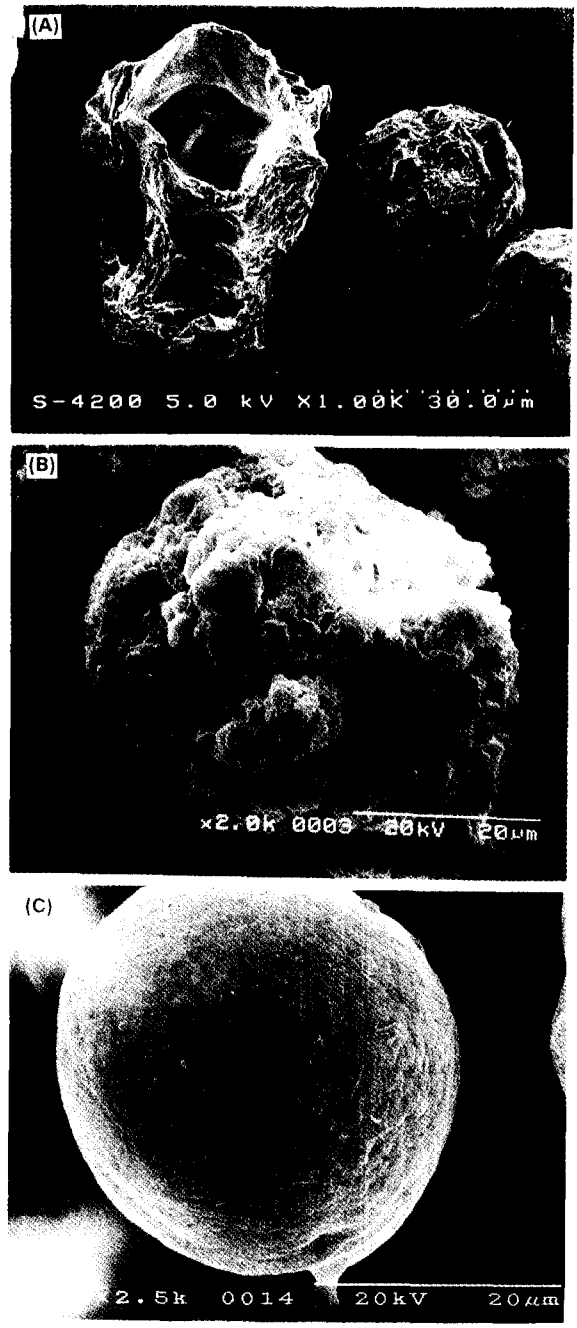

Fig. 3. The morphology of alginate microspheres prepared from 2% alginate solution (A), 5% alginate solution (B) and 4.5% alginate and 0.5% HPMC solution (C)

In vitro release of AMP-Na from alginate microspheres

The release profile of AMP-Na from alginate microspheres were investigated as a function of HPMC (Fig. 6). There was no significant difference of the drug release rates between alginate alone and HPMC incroporated alginate microspheres, although HPMC had great influence on the morphology of microspheres, the release rates of AMP-Na from alginate microspheres were unchanged. But the polymer concentration increased, the release rates of drug from microspheres retarded.

The effect of the concentration of calcium chloride solution on the release profiles of drug from alginate microspheres is shown in Fig. 7. Although the mechanical strength of alginate microspheres was af-

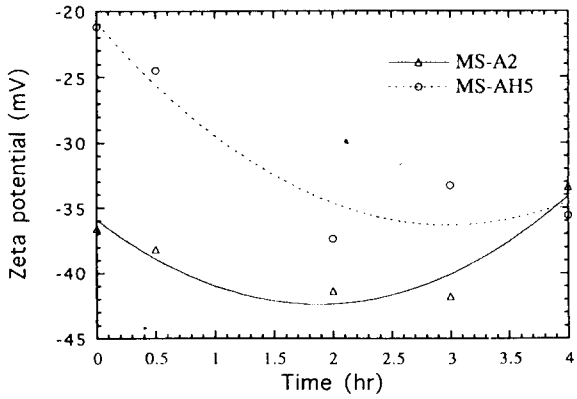

Fig. 4. Zeta potential of alginate microspheres prepared with or without 0.5% HPMC (MS-A2; Zeta potential of microspheres prepared from 2% alginate solution, MS-AH5; Zeta potential of microspheres prepared from 4.5% alginate and 0.5% HPMC solution)

Table II. Mean diameter and drug content of alginate microspheres with or without HPMC

Microspheres	Mean Diameter (μm)	Drug content (%)	Efficiency of Drug loading (%)
MS-A2[a]	12.4	1.8	18
MS-A5[b]	14.0	2.3	23
MS-AH5[c]	7.0	2.1	21

[a]Alginate microspheres prepared from 2% alginate solution
[b]Alginate microspheres prepared from 5% alginate solution
[c]Alginate microspheres prepared from 4.5% alginate and 0.5% HPMC solution

Table III. Drug content of alginate microspheres with various feed ratio of AMP-Na

Microspheres	Drug Content (%)	Efficiency of Drug Loading (%)
MS-D10[a]	2.1	20
MS-D25[b]	4.4	17.6
MS-D50[c]	7.8	15.6

[a]Total polymer weight : drug weight=9 : 1
[b]Total polymer weight : drug weight=4 : 1
[c]Total polymer weight : drug weight=1 : 1

fected by calcium chloride concentration, the release rate of drug was not significantly different.

The release profiles of AMP-Na from alginate microspheres as a function of AMP-Na loading are given in Fig. 8. When the loading of AMP-Na in alginate microspheres increased by 2.1, 4.4 and 7.8%, there was no significant effect on the release rate except for initial burst. These results indicated that the release profile of water soluble drugs was not affected by the concentration of the polymer, the binding agent and the drug loading.

CONCLUSION

Alginate microspheres were prepared by the emulsification process and the optimum condition was investigated by varying preparation parameters. Acetone was used as a hardening and dehydrating agent. The alginate microspheres containing HPMC were found to be generally spherical and had smoother surfaces. The preparation parameters had no significant effect on the release of AMP-Na from the alginate microspheres, although they had some effects

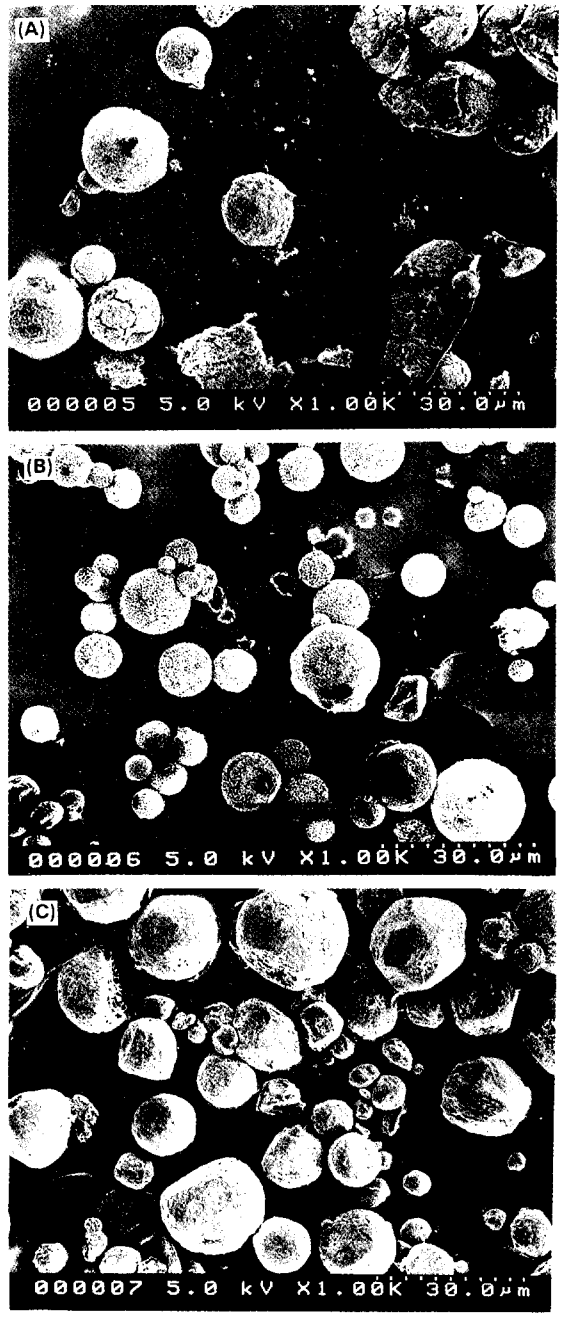

Fig. 5. The effect of feed ratio of AMP-Na (MS-D10; total polymer weight : drug weight=9 : 1, MS-D25; total polymer weight : drug weight=4 : 1, MS-D50; total polymer weight : drug weight=1 : 1) on the preparation of alginate/HPMC microspheres

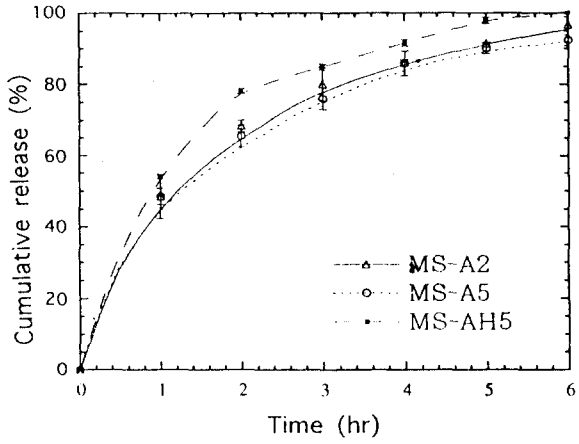

Fig. 6. The release profiles of AMP-Na from alginate microspheres with or without HPMC (MS-A2; microspheres prepared from 2% alginate solution, MS-A5; microspheres prepared 5% alginate solution, MS-AH5; microspheres prepared from 4.5% alginate and 0.5% HPMC solution)

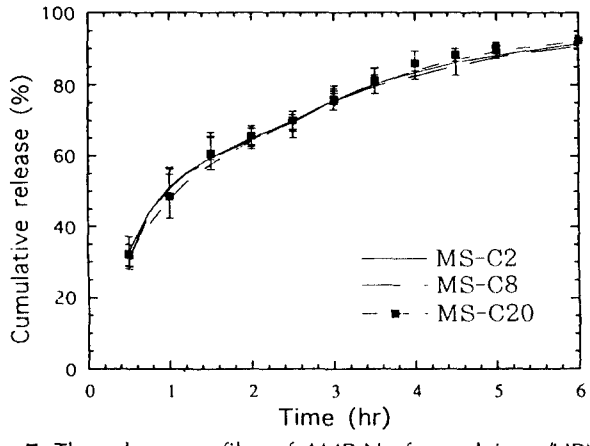

Fig. 7. The release profiles of AMP-Na from alginate/HPMC microspheres as a function of concentration of calcium chloride (MS-C2; microspheres prepared from 2% calcium chloride solution, MS-C8; microspheres prepared from 8% calcium chloride solution, MS-C20; microspheres prepared from 20% calcium chloride solution)

on the physical properties and morphology of the microspheres.

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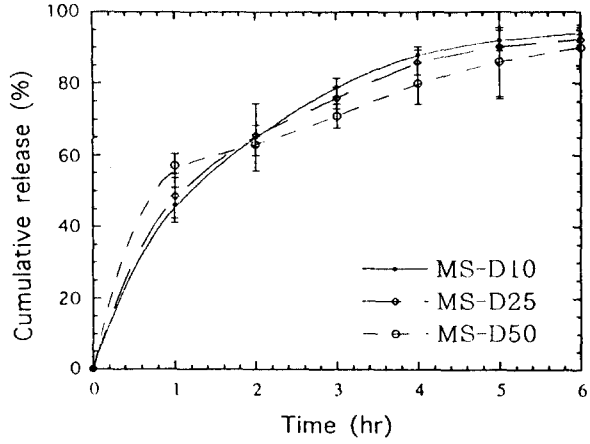

Fig. 8. The release profiles of AMP-Na from alginate/HPMC microspheres as a function of AMP-Na content (MS-D10; total polymer weight : drug weight=9 : 1, MS-D25; total polymer weight : drug weight=4 : 1, MS-D50; total polymer weight : drug weight=1 : 1)

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