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Preparation and *in vitro* Release of Melatonin-loaded Multivalent Cationic Alginate Beads

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The sustained release dosage form which delivers melatonin (MT) in a circadian fashion over 8 h is of clinical value for those who have disordered circadian rhythms because of its short half-life. The purpose of this study was to evaluate the gelling properties and release characteristics of alginate beads varying multivalent cationic species (Al^{+++}, Ba^{++}, Ca^{++}, Mg^{++}, Fe^{+++}, Zn^{++}). The surface morphologies of Ca- and Ba-alginate beads were also studied using scanning electron microscope (SEM). MT, an indole amide pineal hormone was used as a model drug. The Ca^{++}, Ba^{++}, Zn^{++}, Al^{+++} and Fe^{+++} ions except Mg^{++} induced gelling of sodium alginate. The strength of multivalent cationic alginate beads was as follows: $Al^{+++} << Fe^{+++} < Zn^{++} < Ca^{++} \cong Ba^{++}$. In case of Al^{+++}, the induced hydrogel beads were very fragile and less spherical. Fe-alginate beads were also fragile but stronger compared to Al-alginate beads. Ba-alginate beads had a similar gelling strength but was less spherical when compared to Ca-alginate beads. Zn-alginate beads were weaker than Ca- and Ba-alginate beads. Very crude and rough crystals of Ba- and Ca-alginate beads at higher magnifications were observed. However, the type and shape of rough crystals of Ba- and Ca-alginate beads were quite different. No significant differences in release profiles from MT-loaded multivalent cationic alginate beads were observed in the gastric fluid. Most drugs were continuously released upto 80% for 5 h, mainly governed by the passive diffusion without swelling and disintegrating the alginate beads. In the intestinal fluid, there was a significant difference in the release profiles of MT-loaded multivalent cationic alginate beads. The release rate of Ca-alginate beads was faster when compared to other multivalent cationic alginate beads and was completed for 3 h. Ba-alginate beads had a very long lag time (7 h) and then rapidly released thereafter. MT was continuously released from Fe- and Zn-alginate beads with initial burstout release. It is assumed that the different release profiles of multivalent cationic alginate beads resulted from forces of swelling and disintegration of alginate beads in addition to passive diffusion, depending on types of multivalent ions, gelling strength and drug solubility. It was estimated that 0.2 M $CaCl_2$ concentration was optimal in terms of trapping efficiency of MT and gelling strength of Ca-alginate beads. In the gastric fluid, Ca-alginate beads gelled at 0.2 M $CaCl_2$ concentration had higher bead strength, resulting in the most retarded release when compared to other concentrations. In the intestinal fluid, the decreased release of Ca-alginate beads prepared at 0.2 M $CaCl_2$ concentration was also observed. However, release profiles of Ca-alginate beads were quite similar regardless of $CaCl_2$ concentration. Either too low or high $CaCl_2$ concentrations may not be useful for gelling and curing of alginate beads. Optimal $CaCl_2$ concentrations must be decided in terms of trapping efficiency and release profiles of drug followed by curing time and gelling strength of alginate beads.

Key words : Melatonin, Multivalent cationic alginate beads, SEM, Release profiles, Trapping efficiency

INTRODUCTION

Sodium aiginate, a polysaccharide salt, has been used as food additives, antacid adjuvant, cell immobilizer, viscosifier and many pharmaceutical applications such as tablet binder, disintegrant, gastric emptying time delaying substance, and gelling agent (Koji *et al.*, 1981; Hwang *et al.*, 1993; Davies, 1994). The application of sodium alginate beads for the controlled delivery of various drugs has been well established (Bodmeier and Paeratakul, 1986; Kim and

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Lee, 1992; Smith, 1994; Sugawara *et al.*, 1994).

Sodium alginate is easily gelled when contacted with calcium and multivalent cations, resulting in alginate xero-gels. The gelling strength and release fashion of alginate beads may be closely dependent on the type and concentration of gel-inducing ions and alginate grades, curing time, release testing medium, and drug selected (Yotsuyanagi *et al.*, 1987; Kim and Lee 1992; Ostberg *et al.*, 1993, 1994; Smith, 1994; Wee and Gombotz, 1994; Yuk *et al.*, 1995). For example, it has been known that alginate gel beads are stable in low acidic pH but swelled and disintegrated in intestinal pH due to the affinity of multivalent ions to phosphate and sodium ion.

In this study, melatonin (MT), a pineal neurohormone was selected as a modeldrug. Although some dosage forms to deliver MT were studied (Benes *et al.*, 1993; Lee *et al.*, 1994; Lee *et al.*, 1995), little information is available for the controlled delivery of MT using alginate beads. Dosage forms must deliver MT immediately and in a controlled fashion over 8-10 h because of its short half-life so that endogenous circadian rhythms of MT are mimicked (Lee *et al.*, 1995).

The purpose of present work was to prepare MT-loaded multivalent cationic alginate beads varying multivalent ionic species (Al^{+++}, Ba^{++}, Ca^{++}, Mg^{++}, Fe^{+++}, Zn^{++}) and to evaluate gelling properties and release characteristics of MT in simulated gastric and intestinal fluids. The surface morphologies of alginate beads were also studied using scanning electron microscope (SEM). Effects of calcium concentration on the trapping efficiency and release profiles of Ca-alginate beads were well recognized.

MATERIALS AND METHODS

Materials

Melatonin (MT) was purchased from Regis Chemical Company (USA). Sodium alginate was purchased from Junsei Chemical Co. (Tokyo, Japan). Methanol was purchased from EM Industries, Inc. (New Jersey, USA). All other chemicals were of reagent grade and used without further purification.

HPLC Analysis of MT

MT concentration was determined using a reverse phase HPLC system consisting of a pump (Shimadzu, LC-9A), an UV-Vis spectrophotometeric detector at the wavelength of 229 nm (Shimadzu, SPD-6AV), a system controller (Shimadzu, SCL-6B), a sample injector, a reverse phase column (Shimadzu, CLC-ODS, 5 μm particle diameter 100 Å pore diameter) and an integrator (Shimadzu, CR4-A). Mobile phase consisted of 53% (v/v) methanol in 0.01 M sodium acetate buffer (pH 4.7). The flow rate of mobile phase was 0.8 ml/min. Methylparaben (MP) as an internal standard in the aqueous solution was prepared (33.3 μg/ml). A standard calibration curve was constructed by plotting the peak area ratio of MT and MP versus known MT concentrations from 0.42 to 2.5 μg/ml.

Preparation of multivalent cationic alginate beads

Sodium alginate (2 g) was completely dissolved in the distilled water (100 ml). MT (0.3 g) was added into above alginate solution and homogeneously mixed for 2 h. The resulting mixtures were dropped into 0.2 M multivalent cationic solution ($AlCl_3$, $BaCl_2$, $CaCl_2$, $MgCl_2$, $FeCl_3$, $ZnCl_2$) using a 10 ml pipette and then cured for 30 min for the gelation of alginate beads. In the meanwhile, the gelling properties were visually investigated. The prepared alginate beads were promptly rinsed with distilled water and then dried in an oven at 50°C. The strength of multivalent cationic alginate beads was evaluated using hardness tester (Apex, England).

Scanning electron microscope (SEM)

The surface morphology of alginate beads was investigated using a SEM. The samples were dried at room temperature and then coated with gold using a Jeol JFC-1100 sputter coater (Jeol, Japan). Micrographs were taken with a Jeol SEM at an accelerating voltage of 20 kV.

Determination of MT contents

About 70 mg of alginate beads were exactly weighted and completely dissolved in 500 ml of the phosphate buffer solution (pH 7.4). Thereafter, 2 ml of turbid solution was filtered through a millipore membrane filter (pore 0.45 μm). The concentration of MT was determined using HPLC as mentioned previously.

In vitro release studies

The *in vitro* dissolution test of MT from alginate beads formulated was performed in triplicate using the dissolution apparatus type I (Fine scientific DST 600A, Seoul, Korea) at the stirring speed of 100 rpm at 37±0.5°C. In the 500 ml of enzyme-free simulated gastric fluids (pH 1.4±0.1, NaCl-HCl buffer solution) for 5 h and 500 ml of enzyme-free simulated intestinal fluids (pH 7.4±0.1, phosphate buffer solution) for 12 h, respectively. The simulated gastric and intestinal fluids were prepared according to Lee and Lee (1995). The dissolution samples (1 ml) were collected at a given interval with replacement of the equal volume of dissolution media, and were filtered through a millipore membrane filter. The concentration of the drug released from alginate beads as a function of time was determined using a reverse

phase HPLC as mentioned previously.

RESULTS AND DISCUSSION

Preparation of multivalent cationic alginate beads

In the preparation of multivalent cationic alginate beads, the Ca^{++}, Ba^{++}, Zn^{++}, Al^{+++} and Fe^{+++} ions except Mg^{++} induced gelling of sodium alginate. The strength of multivalent cationic alginate beads was as follows: $Al^{+++} << Fe^{+++} \leq Zn^{++} < Ca^{++} \cong Ba^{++}$. In case of Al^{+++}, the induced hydrogel beads were very fragile and less spherical. As a result, Al-alginate beads were excluded in the release studies. Fe-alginate beads were also fragile but stronger compared to Al-alginate beads. Ba-alginate beads had a similar gelling strength but was less spherical when compared to Ca-alginate beads. The strength of Zn-alginate beads were weaker than that of Ca- and Ba-alginate beads.

SEM of alginate beads

The surface morphology of multivalent Ba- and Ca-alginate beads using SEM is shown in Fig. 1. The surface of morphology of Ba- (Fig. 1A) and Ca-alginate beads (Fig. 1B) were quite different at two magnifications. The Ba-alginate beads seemed to be less spherical than Ca-alginate beads. Very crude and rough crystals of Ba- and Ca-alginate beads at higher magnifications were observed. However, the type and shape of rough crystals of Ba- and Ca-alginate beads were quite different. Polymeric reinforcement and coating of these alginate beads may result in the enhanced surface morphology (Lee and Min, 1995).

In vitro Release studies

It has been known that alginate beads are stable in gastric fluid but swelled and disintegrated in intestinal fluid. Therefore, passive diffusion of drugs from alginate beads may be a predominant factor in gastric fluid for the release of drugs. However, the release of drug in the intestinal fluid is quite different from that in the gastric fluid. Generally, the swelling and disintegration of Ca-alginate beads are highly dependent on the type and concentration of gel-inducing ions and alginate grades, curing time, release testing medium, formulation excipients, and the drug selected (Yotsuyanagi *et al.*, 1987; Kim and Lee 1992; Ostberg *et al.*, 1993, 1994; Smith, 1994; Wee and Gombotz, 1994; Yuk *et al.*, 1995; Hwang *et al.*, 1995). Most of all, the composition of dissolution medium was an important factor for release profiles due to the affinity of multivalent ions to phosphate

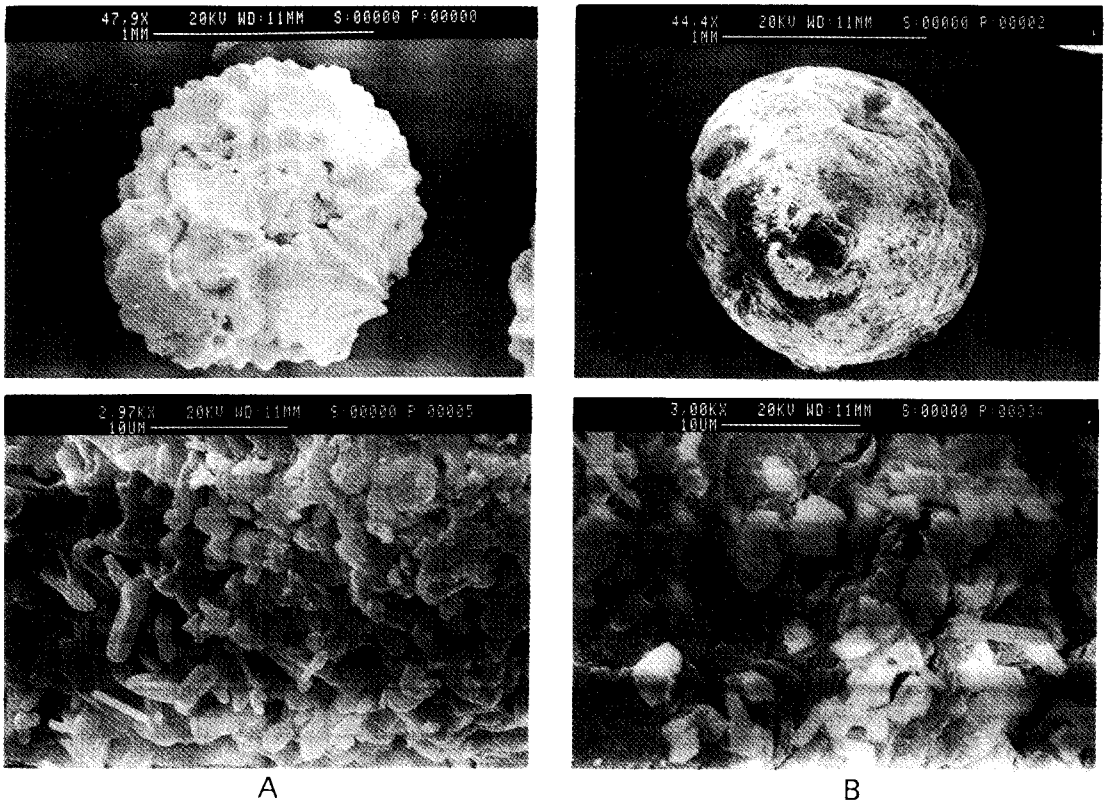

Fig. 1. Scanning electron microscope (SEM) of Ba-alginate (A), Ca-alginate beads (B) at two different magnifications of 50 times (upper) and 3000 times (lower)

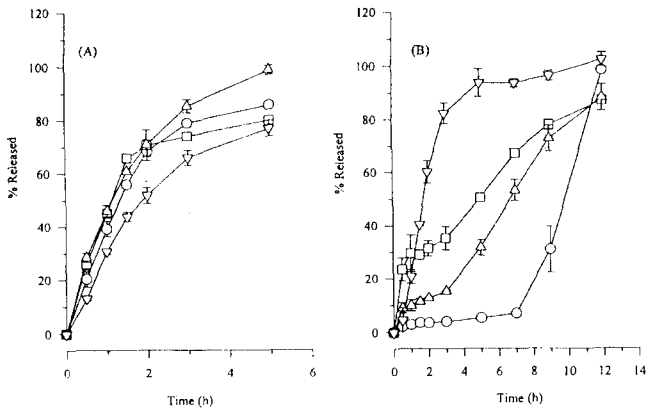

Fig. 2. The release profiles of melatonin-loaded multivalent cationic alginate beads in the simulated gastric (A) and the intestinal fluid (B). Ba^{++} (○), Fe^{+++} (□), Zn^{++} (△) and Ca^{++} (▽)

and sodium ion, and solubility of drug entrapped into alginate beads (Ostberg *et al.*, 1993; Lee and Min, 1995; Yuk *et al.*, 1995).

The *in vitro* release profiles of MT-loaded multivalent cationic alginate beads in the simulated gastric and the intestinal fluid are given in Fig. 2. As expected, in the gastric fluid, no significant difference in the release profiles of MT-loaded multivalent cationic alginate beads was observed. Most drugs were continuously released upto 80% for 5 h, mainly governed by the passive diffusion without swelling and disintegrating the alginate beads. Unlike the practically insoluble ibuprofen, MT was released in the gastric fluid because of solubility of the drug (Lee and Min, 1995).

In the intestinal fluid, there was a significant difference in the release profiles of MT-loaded multivalent cationic alginate beads. The release rate of Ca-alginate beads was faster when compared to other multivalent cationic alginate beads and was completed for 3 h. The dissolution media of Ca-alginate beads were turbid. Ba-alginate beads had a very long lag time (7 h) and then rapidly released thereafter. MT was continuously released from Fe- and Zn-alginate beads with initial burstout release. Trivalent cationic Fe-alginate beads did not induce the gelling of alginate completely, resulting in fragility of beads and initial burstout release. As mentioned previously, pH and dissolution media were very important for dissolution of alginate beads. The high affinity of Ca-alginate beads with phosphate and Sodium ion in the dissolution medium were well reported (Ostberg *et al*, 1994; Yuk *et al.*, 1995). It assumed that the different release profiles of multivalent cationic alginate beads resulted from forces of swelling and disintegration of alginate beads in addition to passive diffusion, depending on types of multivalent ions, gelling strength and drug solubility.

Ca-alginate beads has been widely studied for the

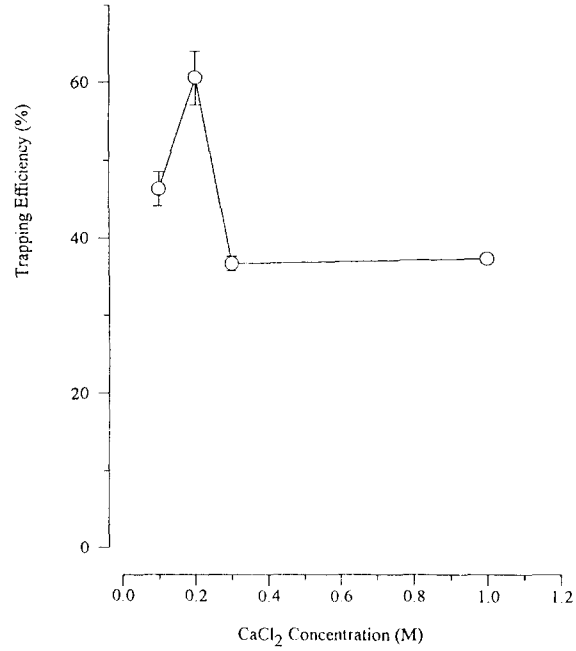

Fig. 3. Effect of $CaCl_2$ concentration on the trapping efficiency of melatonin into Ca-alginate beads

controlled delivery of various drugs because of its non-toxicity and gelling propertes (Bodmeier and Paeratakul, 1986; Kim and Lee, 1992; Smith, 1994; Sugawara *et al.*, 1994). In this study, the effects of $CaCl_2$ concentration on the trapping efficiency and release profiles of Ca-alginate beads were investigated to optimize preparation formulation. Effect of $CaCl_2$ concentration on the trapping efficiency of MT into Ca-alginate beads is shown in Fig. 3. As $CaCl_2$ concentration was increased to 1 M, the trapping efficiency was increased and then decreased at the 30 min curing time. It was estimated that 0.2 M $CaCl_2$ concentration was optimal in terms of trapping efficiency of MT and gelling strength of alginate beads. Effect of $CaCl_2$ concentration on the release rate of MT from Ca-alginate beads in the simulated gastric and the intestinal fluid is also given in Fig. 4. In the gastric fluid, the release rate of Ca-alginate beads was most retarded when 0.2 M $CaCl_2$ was used for gelling. It may be assumed that Ca-alginate beads gelled at 0.2 M $CaCl_2$ concentration had higher strength when compared to other concentrations because in the gastric fluid, the release of drug from alginate beads is mainly governed by passive diffusion without swelling and disintegration. In the intestinal fluid, the decreased release of Ca-alginate beads formed in 0.2 M $CaCl_2$ concentration was also observed. However, release profiles of Ca-alginate beads were quite similar regardless of $CaCl_2$ concentration. The higher release rate of Ca-alginate beads formed in 1.0 M $CaCl_2$ concentration resulted from improper gelling of core matrices of alginate

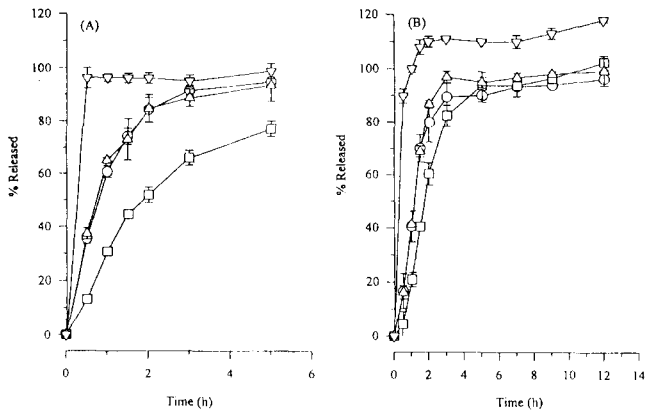

Fig. 4. Effect of $CaCl_2$ concentration on the release rate of melatonin from Ca-alginate beads in the simulated gastric (A) and the intestinal fluid (B). 0.1 M (○), 0.2 M (□), 0.3 M (△) and 1.0 M (▽)

beads. Either too low or high $CaCl_2$ concentrations may not be useful for gelling and curing of alginate beads as shown in the release profiles. Optimal $CaCl_2$ concentration must be decided in terms of the trapping efficiency and release profiles of drug followed by curing time and gelling strength of alginate beads as shown in Fig. 3 and Fig. 4.

In conclusion, the surface morphology and gelling properties of alginate beads were highly dependent on the type of multivalent cations. The release profiles of MT-loaded multivalent cationic alginate beads was similar in the gastric fluid but highly different in the intestinal fluid. It was estimated that 0.2 M $CaCl_2$ concentration was optimal judging from the trapping efficiency of MT and the controlling release rate of Ca-alginate beads.

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