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Oral controlled release of melatonin using polymer-reinforced and coated alginate beads

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Abstract

Melatonin (MT) is an indole amide pineal hormone. The sustained release dosage form which delivers MT in a circadian fashion over 8 h is of clinical value, because of its short half-life, for those who have disordered circadian rhythms. The purpose of this study was to prepare polymer reinforced and coated alginate beads and to evaluate in vitro release characteristics in simulated gastric and intestinal fluids, varying drug loadings, and the amount of polymer and plasticizer. The Eudragit® RS100 as a polymer and aluminium tristearate (AT) as a plasticizer were used, respectively. Plain (simple) alginate beads were prepared by dropping the mixture of MT and sodium alginate into 0.2 M $CaCl_2$ solution. The polymer reinforced alginate beads were prepared by dropping the mixture of drug, sodium alginate and polymer with plasticizer into 0.2 M $CaCl_2$ solution. The coated alginate beads were also prepared by adding plain alginate beads into polymer solution using the solvent evaporation method. Acetone was used as a solvent of polymer. The dissolution test was carried out using the basket method at a stirring speed of 100 rpm at 37°C in simulated gastric (pH 1.4) and intestinal fluid (pH 7.4). The concentration of MT was determined by reverse phase HPLC. In the study of scanning electron microscope (SEM), the surface crystal and roughness were reduced by polymeric reinforcing and coating alginate beads. However, higher coatings of alginate beads resulted in cracks and holes on the surface of coated alginate beads. The longer curing time into 0.2 M $CaCl_2$ solution, the lower trapping efficiency of MT was observed due to release of MT during gelling process. The release rate of MT in gastric and intestinal fluid when drug loading increased was not changed. The polymeric reinforcement of alginate beads on the release rate of MT was not significant in gastric fluid, but pronounced in intestinal fluid with initial burst out release for 1 h. The release rate of the drug from coated alginate beads was retarded, both in gastric and intestinal fluid when compared to plain alginate beads. As the polymer contents increased, the release rate was significantly decreased in the intestinal fluid due to hindrance of swelling and disintegration of coated alginate beads unlike gastric fluid. The release rate of drug from coated alginate beads was more efficiently sustained in the gastric and intestinal fluid when 0.1 g of AT as a plasticizer was used for coating. However, the higher amount of AT was not useful for retarding the release rate. From the current studies, the sustained release formulation of MT using alginate beads may provide as an alternative for oral delivery of MT.

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1. Introduction

Sodium alginate, a polysaccharide salt, has been used as food additives, an antacid adjuvant, cell immobilizer and viscosifier (Koji et al., 1981; Bodmeier and Paeratakul, 1989; Hwang et al., 1993; Sugawara et al., 1994). In pharmaceutical aspects, the alginate has been used as tablet binder, disintegrant, gastric emptying time delaying substance, and gelling agent because of its protection effect on gastrointestinal mucus and nontoxicity.

Sodium alginate is gelled when contacted with calcium and multivalent cations. Alginate xerogels are stable in low pH solution but swelled in weak basic solution. Recently, these properties of alginate beads have been investigated as a drug carrier and vehicle for the controlled delivery of low molecules or macro molecules (Kim and Lee, 1992; Tomida et al., 1993; Sugawara et al., 1994; Lee and Min, 1995). Polymer reinforced and coated alginate beads were previously investigated for the controlled delivery of drugs (Lin and Ayres, 1992; Lee and Min, 1995). Murata et al. (1993) also investigated the release characteristics of drug from chitosan-reinforced alginate beads. It has been reported that the physicochemical properties of alginate beads are influenced by concentration and viscosity of alginate grades, the alginate proportion of guluronic acid and mannuronic acid, cationic concentration, gelling time, size of beads and formulation compositions (Kim and Lee, 1992; Ostberg et al., 1993; Sugawara et al., 1994).

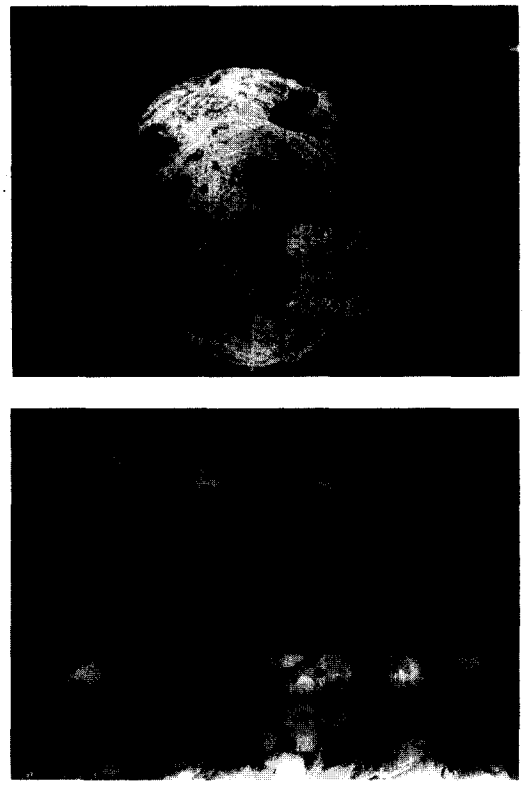
In this study, melatonin (MT) was selected as a model drug using alginate beads. MT (*N*-acetyl-5-methoxytryptamine) is a neurohormone secreted by the pineal gland in circadian rhythm. Although some dosage forms to deliver MT were studied (Benes et al., 1993, Lee et al., 1994; Lee et al., 1995), little information is available for the controlled delivery of MT using polymer reinforced and coated alginate beads. Dosage forms which mimic the physiologically produced endogenous MT plasma concentration versus time profile will be valuable to fully evaluate the clinical potential of MT in the treatment of disorder sleep syndrome, jet-lag, seasonal affective disease, shift work syndrome, and other circadian disorders. 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 by simultaneously administering immediate and sustained release dosage form (Lee et al., 1995). The dosage form design of MT may be helpful in the future because no commercial dosage forms of MT that mimic endogenous MT circadian rhythm are currently available.

The purpose of present work was to prepare plain (simple), polymer-reinforced and coated alginate beads and to evaluate release characteristics of MT in simulated gastric and intestinal fluids, varying drug loadings, and amount of polymer and plasticizer.

2. Materials and methods

2.1. Materials

Melatonin (MT) was purchased from Regis Chemical Company (USA). Sodium alginate was purchased from Junsei Chemical Co. (Tokyo, Japan). Eudragit® RS100 was kindly supplied courtesy of Duckwoo Trading, Ltd. (Seoul, Korea). Aluminium tristearate (AT) and polyethylene glycol 400 (PEG 400) from Katayama Chemical Co. (Osaka, Japan) and liquid paraffin from Junsei Chemical Co. were purchased respectively. *n*-Hexane and acetone were purchased from Duksan Pharmaceutical Co. (Seoul, Korea). Methanol was purchased from EM Industries, (New Jersey, USA). All other chemicals were of reagent grade and used without further purification.



A-I

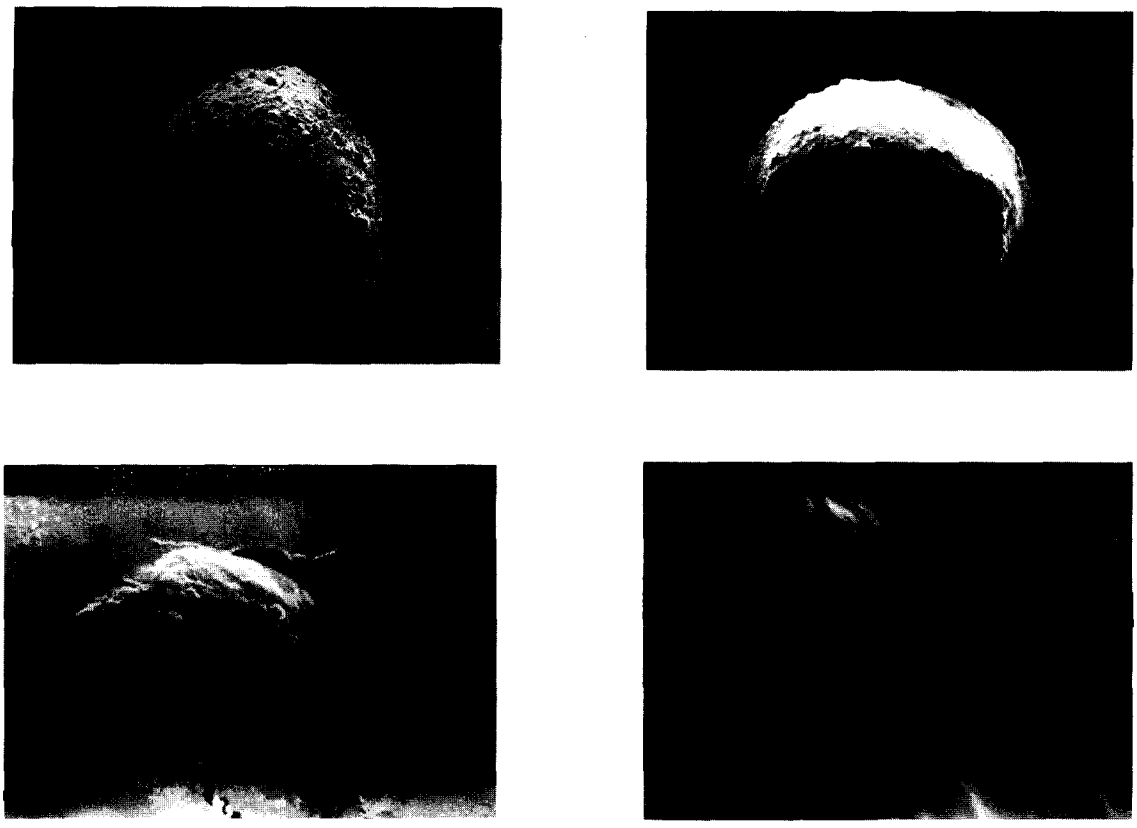
Fig. 1. Scanning electron micrographs (SEM) of plain (A), polymer-reinforced (B) and coated alginate beads (C) at two magnifications, × 50 times (upper) and × 3000 times (lower). A-I: no polymer; B-II: polymer 1.0 g; B-III: polymer 4.0 g, C-IV, polymer 2.0 g; C-V: polymer 4.0 g.

2.2. HPLC analysis of MT

MT concentration was determined using a reverse phase HPLC system consisting of pump (Shimadzu, LC-9A), UV-Vis spectrophotometeric detector at the wavelength of 229 nm (Shimadzu, SPD-6AV), system controller (Shimadzu, SCL-6B), sample injector, reverse phase column (Shimadzu, CLC-ODS, 5 μm particle diameter 100 Å pore diameter) and 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. Mobile phase was filtered using 0.45 μm nylon membrane filter (Gelman Sciences, Michigan, USA) and degassed under vacuum and sonication. MT and internal standard solution (100 μl and 20 μl, respectively) were mixed and then 20 μl of mixed solution was injected in duplicate for HPLC analysis. Methylparaben as an internal standard in aqueous solution was prepared (33.3 μg/ml). Standard calibration curve was constructed by plotting the peak area ratio of MT and internal standard versus known MT concentrations from 0.42 to 2.5 μg/ml.

2.3. Preparation of plain alginate beads

Sodium alginate (2 g) was completely dissolved in distilled water (100 ml). MT (0.3 or 0.5 g) was added into the above alginate solution and mixed for 2 h. Then, the mixtures were dropped into each 0.2 M $CaCl_2$ solution using a pipette and then cured for further gellation of alginate beads.



B-II B-III

Fig. 1 continued.

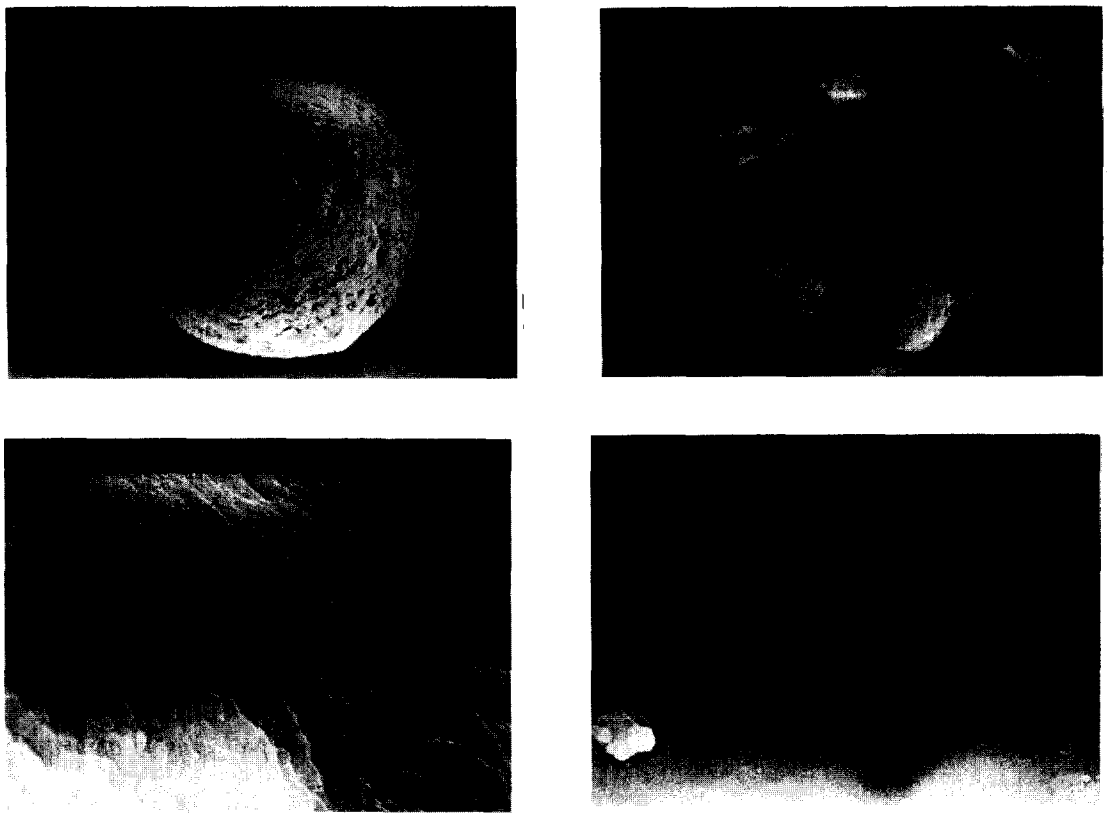
The prepared alginate beads were promptly rinsed with distilled water, and then dried in an oven at 50°C over 18 h. The dried alginate beads were then weighed.

2.4. Preparation of polymer reinforced alginate beads

For the preparation of Eudragit® RS100 reinforced alginate beads, Eudragit® RS100 (1 g, 4 g) suspended in PEG400 (10 g) and AT (0.1 g) as a plasticizer was stirred for 4 h. Sodium alginate solution (2 g/90 ml) containing MT (0.3 g) was mixed for 2 h. The polymer solution and MT-alginate dispersion were mixed together over 24 h. The final mixtures were gently dropped into 0.2 M $CaCl_2$ solution as reported previously (Lee and Min, 1995).

2.5. Preparation of coated alginate beads

The plain alginate beads were further coated using Eudragit® RS100 for the controlled oral delivery of MT with a minor modification as reported previously (Lee and Min, 1995). The plain alginate beads with Eudragit® RS100 polymer solution were coated as follows. Eudragit® RS100 as a polymer was added to 20 ml of acetone as a solvent in a six-baffled glass vessels and completely dissolved with the stirring speed of 400 rpm in a water-bath at 20°C. AT as a plasticizer was added to the above solution with additional stirring for 10 min. AT was used to prevent electrification and flocculation of alginate beads. The alginate beads were added to the above coating solution followed by pouring the



C-IV C-V

Fig. 1 continued.

liquid-paraffin (20 ml) after 15 min. The solution temperature was gradually increased to 40°C by 5°C intervals. The stirring speed was also increased to 500 rpm by 50 rpm intervals every 30 min. Thereafter, the stirring speed was increased to 900 rpm for reducing the aggregation of beads. The coated alginate beads were finally formed as a result of evaporation of acetone. The coated beads were filtered through filter paper (diameter 110 mm), washed with *n*-hexane (50 ml) to eliminate residual liquid paraffin and then dried in an oven at 50°C for 30 min. The coated beads were further dried at room temperature. The amount of polymer (1, 2, 4 g) and AT (0.1, 0.25, 0.5 g) in coating solution was varied during coating procedure of plain alginate beads.

2.6. Scanning electron microscope (SEM) of alginate beads

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 scanning electron microscope at an accelerating voltage of 20 kV.

2.7. Trapping efficiency of drug in alginate beads

About 70 mg of beads were completely dissolved in 500 ml of phosphate buffer solutions (pH 7.4), and stirred for 12–15 h. Then, 2 ml of solution was filtered through a millipore membrane filter (pore size 0.45 μm, diameter 13 mm). The concentration of MT was determined using reverse phase HPLC as mentioned previously.

2.8. In vitro release studies

The in vitro dissolution test of MT from alginate beads formulated was carried out in triplicate using the dissolution apparatus type I (Fine scientific DST600A, Seoul, South 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 equal volume of dissolution media, and were filtered through a millipore membrane filter. The concentration of drug released from alginate beads as a function of time was determined using a reverse phase HPLC as mentioned previously.

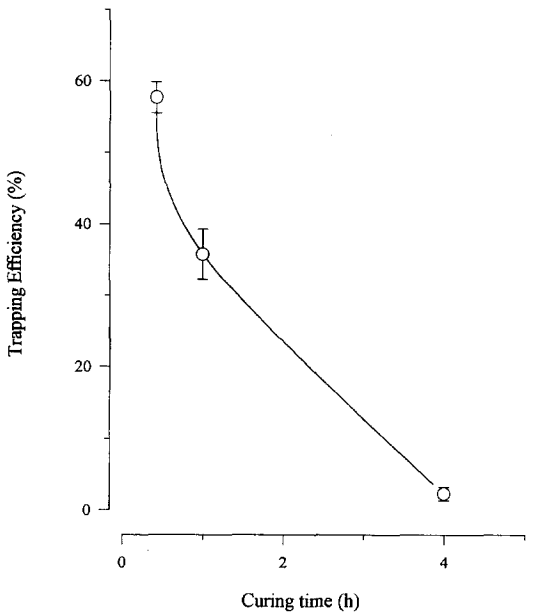


Fig. 2. The trapping efficiency (%) of MT as a function of curing time.

3. Results and discussion

3.1. SEM of alginate beads

The surface morphology of plain, polymer-reinforced and coated alginate beads using SEM is shown in Fig. 1. The surface of plain alginate beads showed very crude and roughness as viewed at two different magnifications (Fig. 1A-I). The surface cracks and rough crystals of plain alginate beads were reduced when polymer-reinforced (Fig. 1B-II, III). As polymer contents increased in the matrices of alginate beads, the surface was getting smoother when compared to plain alginate beads (Fig. 1B-III). The surface crystals and roughness of plain alginate beads were more efficiently reduced by coating (Fig. 1C-IV). However, higher coatings of alginate beads resulted in cracks and holes on the surface of coated alginate beads (Fig. 1C-V). The surface morphology and roughness of alginate beads were further evaluated in release studies.

3.2. Trapping efficiency of drug in alginate beads

The trapping efficiency of MT into alginate beads was studied as a function of curing time. Solubility of MT is low and slightly soluble in water at 25°C (1.5 mg/ml). Excessively long curing time might result in low trapping efficiency of soluble drugs in alginate beads. As expected, the longer curing time into 0.2 M $CaCl_2$ solution, the lower trapping efficiency of MT was observed (Fig. 2). It was observed that curing time for 30 min gave good trapping efficiency (60%) and proper gelling of sodium alginate beads.

3.3. In vitro release studies

It has been known that alginate gel beads are stable in low acidic pH but swell and disintegrate in intestinal pH (Yotsuyanagi et al., 1987; Kim and Lee, 1992). The swelling and disintegration of calcium alginate beads are dependent on compositions of dissolution medium, e.g. sodium and phosphate, and solubility of drug entrapped into alginate beads. Generally, a poor-water-soluble drug was not released from Ca-alginate beads in

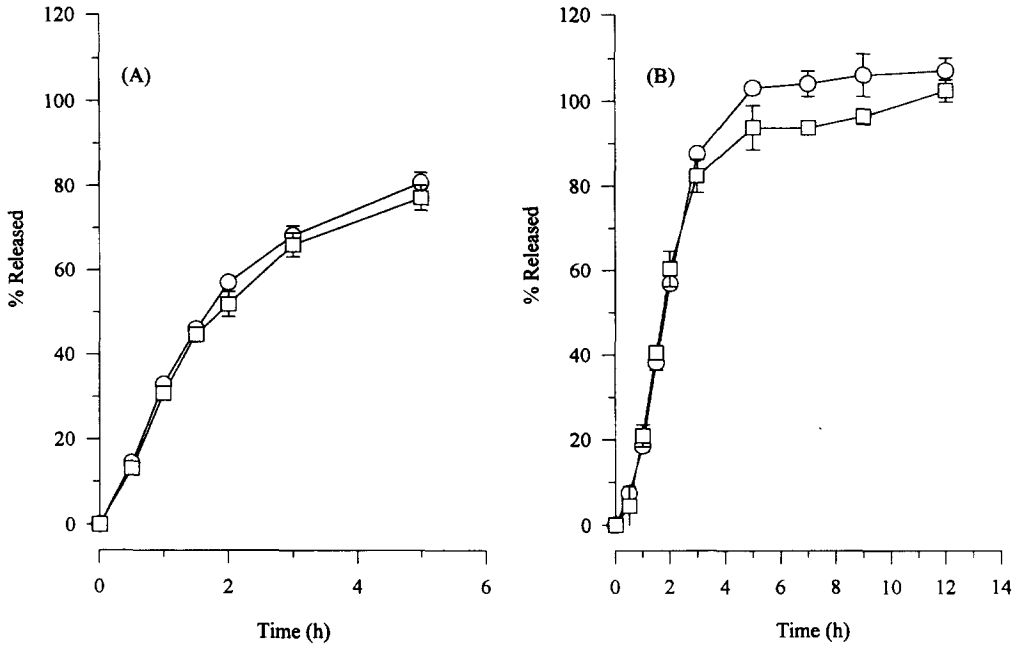


Fig. 3. Effect of loading dose on the release rate of MT from plain alginate beads in the simulated gastric (A) and intestinal fluid (B). (○) 0.5 g; (□) 0.3 g.

the simulated gastric fluids because of stability of Ca-alginate beads in acidic environment and non swelling property of alginate beads. MT was released from alginate beads, unlike the ibuprofen as studies previously although alginate beads are not swelled and disintegrated in gastric fluids (Lee and Min, 1995). The amount of drug released in the intestinal fluid was higher due to possible passive diffusion and disintegration when compared to gastric fluid. The swelling and disintegration of alginate beads in intestinal fluid were due to the affinity of calcium to phosphate and sodium/calcium exchange (Ostberg et al., 1994). The effect of drug loading on the release of MT from plain alginate beads in simulated gastric and intestinal fluid is given in Fig. 3. The release rate of MT in gastric and intestinal fluid was not changed when drug loadings increased.

It is necessary to retard the rate of swelling and disintegration of alginate beads for the sustained delivery of drug, otherwise, most of drug would be released in the intestinal fluid within a few hours. A commercially available Eudragit® RS100 copolymer synthesized from acrylic and methacrylic acid esters with low content of quaternary ammonium group was used for the reinforcement and coating of Ca-alginate gel matrices (Lehmann, 1989). Effect of polymer contents with constant amount of AT (0.1 g) and PEG (10 g) on the release rate of MT from polymer-reinforced alginate beads in the simulated gastric and intestinal fluid is given in Fig. 4. It was observed that release rate of drug from alginate beads can be changed by polymeric reinforcement of alginate gel matrices as a result of protecting effect from swelling and disintegration. Murata et al. (1993) previously used chitosan to reinforce alginate gel matrices and investigate effect of chitosan on gel matrix erosion. Likewise, Eudragit® RS100 could be sited within the alginate gel matrices, resulting in change of MT-release (Lee and Min, 1995).

The release rate of MT from polymer-reinforced alginate beads was not retarded in the simulated gastric fluid when compared to plain alginate beads unlike practically soluble ibuprofen (Lee and Min, 1995). It may assume that poly-

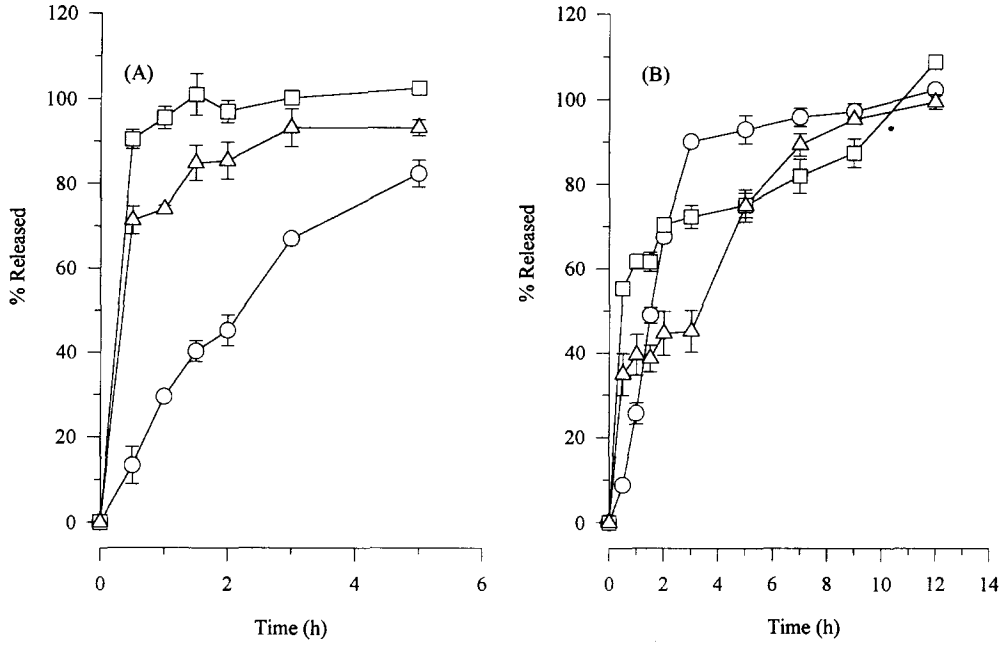


Fig. 4. Effect of polymer contents with constant amount of AT (0.1 g) and PEG (10 g) on the release rate of MT from plain (○) and polymer-reinforced alginate beads with 1 g (□) and 4 g (△) of polymer in the simulated gastric (A) and intestinal fluid (B).

meric incorporation into alginate gel matrices may provide more passive diffusion because alginate bead are stable in the gastric fluid. However, higher content of polymer in alginate beads resulted in hindering release rate of drug due to enhanced morphology of alginate beads (see Fig. 1B-III).

Polymeric reinforcing effect was more pronounced in the simulated intestinal fluid. Initial drug-release rate from polymer-reinforced alginate beads was more rapid for 1 h when compared to plain Ca-alginate beads. However, release rate of drug significantly retarded thereafter because swelling and disintegration of polymer-reinforced alginate beads were reduced as polymer contents increased.

Ca-alginate beads were further coated with polymer to efficiently control the release of MT from alginate beads. Although the release rate of drug can be changed by polymeric reinforcement of alginate beads, coated alginate beads may be more useful not only to retard drug release and erosion but also to overcome the possible defects of outer surface of Ca-alginate beads with cracks and pores. The porosity and any structural defects of Ca-alginate beads give a fast release and low efficiency of incorporating drug (Kim and Lee, 1992).

Effect of polymer contents with constant amount of AT (0.1 g) on the release rate of MT from plain and coated alginate beads in the simulated gastric and intestinal fluid is given in Fig. 5. In gastric medium, MT was linearly released from coated beads for 5 h by passive diffusion. The release rate of drug from coated alginate beads was decreased when compared to plain alginate beads. However, no significant difference of release rate was not observed as polymer contents. In intestinal fluid, effect of coating on the release rate of drug was more pronounced. As polymer content increased, the release rate decreased due to hindrance of swelling and disintegration of coated alginate beads. However, no significant difference of drug release was observed when 2 and 4 g of polymer was used. Although higher coatings provided higher thickness of film, excess coating resulted in cracks and pores of outer surface as shown in Fig. 1C-V. In case of coated alginate beads with 1 g of polymer, the release rate was higher when compared to other coatings due to weak protection of swelling and erosion from polymeric coated membrane.

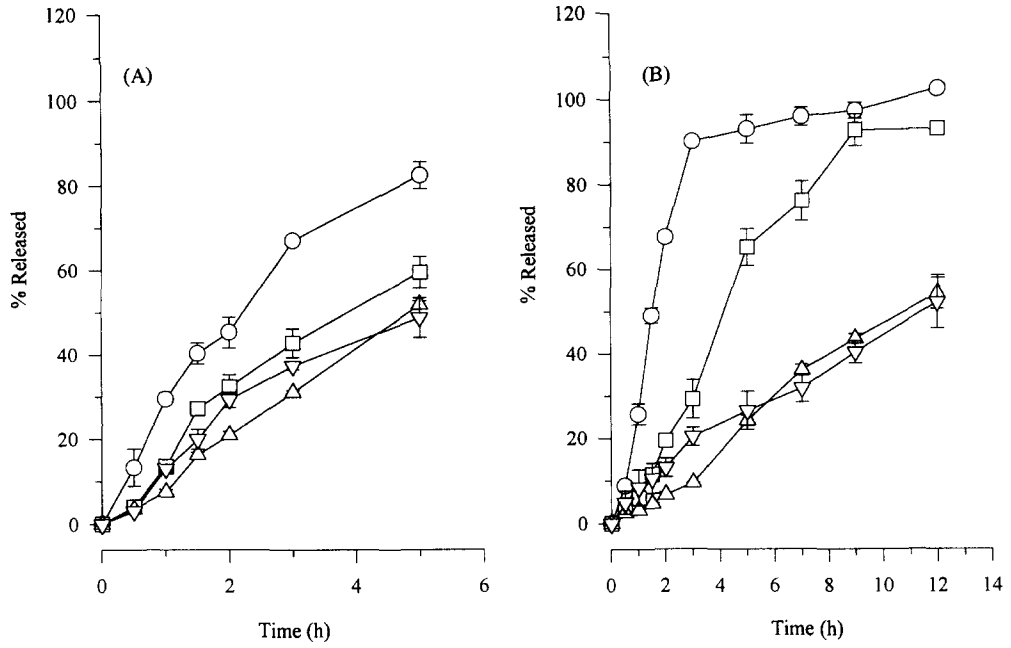


Fig. 5. Effect of polymer contents with constant amount of AT (0.1 g) on the release rate of MT from plain (○) and coated alginate beads with 1 g (□), 2 g (△) and 4 g (▽) of polymer in the simulated gastric (A) and intestinal fluid (B).

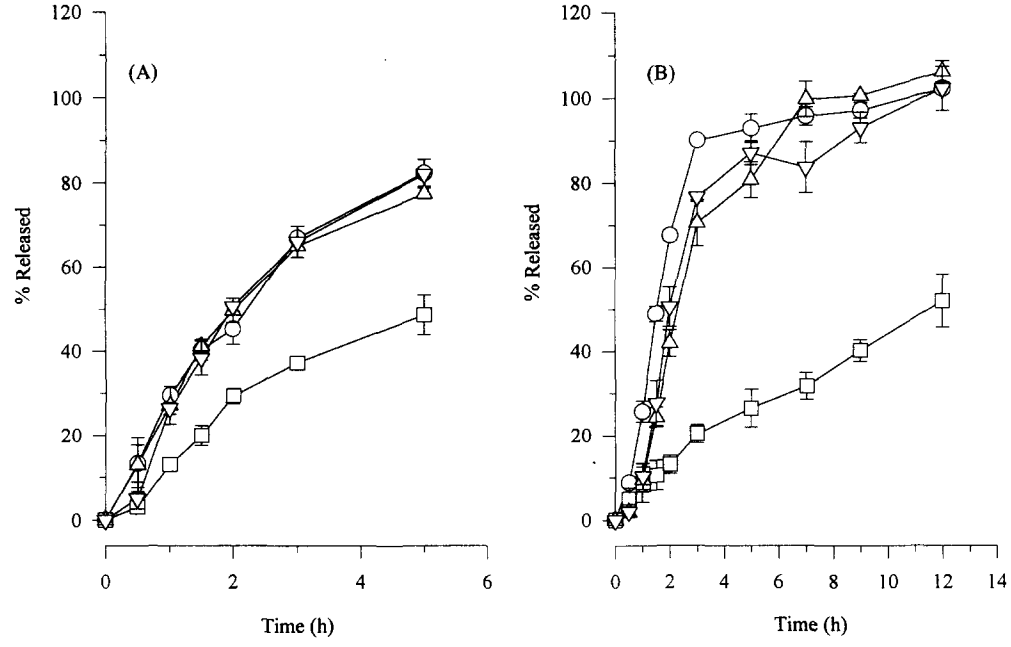


Fig. 6. Effect of AT contents on the release rate of MT from plain (○) and coated alginate beads with 4 g of polymer solution containing 0.1 g (□), 0.25 g (△) and 0.5 g (▽) of AT in the simulated gastric (A) and intestinal fluid (B).

Effect of AT contents on the release rate of MT from plain and alginate beads coated with 4 g of polymer solution containing 0.1, 0.25 and 0.5 g of AT in the simulated gastric and intestinal fluid is shown in Fig. 6. AT was used to improve plasticity of Eudragit® RS100. The release rate of drug was more efficiently sustained in the gastric and intestinal fluid when 0.1 g of AT as a plasticizer was used for coating. However, the higher amount of AT was not useful for retarding the release rate. It suggested that optimal amount of AT might be present for plasticization of polymethacrylate polymer.

In conclusion, swelling and disintegration of alginate beads were dependent on the components of dissolution medium and drug selected. The release profiles could be changed by polymeric reinforcement and coating of Ca-alginate beads. Most of all, polymeric coating of Ca-alginate beads would be proper and efficient for oral MT delivery system because the release rate of MT was easily controlled without initial burstout release. The detailed in vivo pharmacokinetics of alginate beads will be validated in the future. From the current studies, the sustained release formulation of MT using alginate beads may provide as an alternative for oral delivery of MT.

Acknowledgements

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