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Alginate microparticles prepared by spray–coagulation method: Preparation, drug loading and release characterization

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Abstract

A spray–coagulation method was developed for the preparation of large scale of porous alginate microparticles. The effect of three variables on porosity was evaluated: (1) alginate solution concentration (2) the concentration of $CaCl_2$ in the coagulation medium and (3) the ratio of guluronic acid to manuronic acid of the alginate. Methylene blue (MB), a highly water-soluble compound and a practically water-insoluble compound, 4-phenylazoaniline (PAA) were used as the model drugs to study drug loading and release characteristics from alginate microparticles. The release of the model compounds from the microparticles was found to depend upon the release medium. Incomplete in vitro release of both model drugs in deionized (DI) water was observed. The release of MB in simulated gastrointestinal fluid (0.1N HCl) was fast and complete, while the release of PAA was slow in 0.1N HCl and fast in phosphate buffer solution (pH 6.8). Interactions between the model drugs and alginate microparticles were identified from scanning electron microscopy (SEM), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR) analysis. The results indicated that (1) porous alginate microparticles can be produced by the spray–coagulation method; (2) drugs can be loaded by the adsorption method; (3) and the obtained microparticles may be used for delaying the release of drugs of low water solubility in acidic conditions.

Keywords: Alginate; Microparticles; Spray–coagulation method; Methylene blue; 4-Phenylazoaniline

1. Introduction

Alginates are linear unbranched block polymers consisting of two monomeric units, guluronic acid (G) and manuronic acid (M) in various configurations. Multivalent cations such as calcium form gels with alginates by reacting with carboxylic acid groups and bringing guluronate chains together stoichiometrically in an egg-box-like conformation leaving mannuronate intact (Vauthier and Couvreur, 2000). The instantaneous gelation of alginate caused by calcium ions results in the formation of particles with different diameters and porosity based on the preparation method

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used (Fundueanu et al., 1999). Alginate microparticles are being widely studied as carriers for immobilization of cells, enzymes and proteins as well as for controlled release of drugs (Chan et al., 2002a; Tonnesen and Karlsen, 2002) due to their unique characteristics, such as natural origin, biocompatibility and relatively lower costs.

Even though sodium alginate has been widely used as a binder and a disintegrating agent in tablets, as a suspending and thickening agent in water-miscible gels, lotions and creams and as a stabilizer for emulsion, the other potential applications of alginate lie in the development of controlled delivery systems. The application of alginate in oral dosage forms with systemic effect is mainly based on alginate microparticles from which the release of incorporated drug is controlled by diffusion mechanism (Tonnesen and Karlsen, 2002). Alginate microparticles were shown to have excellent bioadhesive properties, especially in presence of chitosan, which showed strong affinity for gastric mucosa. Alginate/chitosan tablets have been prepared for a mucoadhesive delivery system (Miyazaki et al., 1995). Alginate microparticles have been widely investigated for the delivery of macromolecules such as DNA and proteins (Gometz and Wee, 1998). Biomolecules can be loaded into alginate microparticles under mild conditions to retain their three-dimensional structure. The loading capacity is controlled by the porosity of alginate microparticles. Porous alginate microparticles will allow a higher loading content and a faster release rate than regular non-porous microparticles (Mandal et al., 2001).

Alginate microparticles are primarily prepared by either the emulsification method (Poncelet, 2001; You et al., 2001; Chan et al., 2002b) or the dripping method (Fundueanu et al., 1999; Shu and Zhu, 2002). Residual organic solvent is a shortcoming of the emulsification method, while the dripping method is unsuitable for scale-up and subsequent applications. Recently Mofidi et al. (2000) reported a large-scale preparation method for alginate microparticles. However, the residual organic solvent remained as a concern in this method. An air-atomization technique was investigated by Kwok et al. (1991) to produce alginate–polylysine microcapsules of Bacillus Calmette Guerin (BCG).

The aims of the present studies were to produce alginate microparticles with high porosity using spray–coagulation method and to study the release behavior of two model drugs loaded by the adsorption method. Additionally, the effect of alginate composition on porosity and the influence of media on model drug release behavior were also investigated.

2. Materials and methods

2.1. Materials

Sodium alginate: Protanal LF 10/60 was obtained from FMC BioPolymer, Norway (Batch No. S12727) and Manugel were provided by ISP Ltd. All other reagents were purchased from Sigma (St. Louis, MO, USA) and used as received.

2.2. Preparation of alginate microparticles by spray–coagulation method

Sodium alginate solution (5%, w/v) was sprayed into calcium chloride solution (1 M) through a nozzle (3/8 in.) at air pressures (40–60 psi). The microparticles were stirred for an hour, then filtered and washed three times with deionized (DI) water. The cross-linked microparticles were dispersed in 10 mL of water and frozen using liquid nitrogen. The frozen microparticles were lyopholized at 100–150 mTorr by using a freeze-dryer (Flexi-dry^{TM}, model FD-3-85A-μP, FTS System Inc., USA) for 48 h. Dried microparticles in the size range of 0.110–0.423 mm were collected by sieving. The microparticles were also prepared with lower concentrations (0.05 or 0.1 M) of calcium chloride to study the influence of this variable on porosity.

2.3. Determination of specific surface area of alginate microparticles

One hundred milligrams of microparticles were weighed accurately and oven-dried for 2 h before measurement. The surface area of the weighed microparticles was determined by a single point Brunauer–Emmett–Teller (BET) method from the adsorption of nitrogen gas at liquid nitrogen temperature using a Flowsorb II 2300 (Micromeritics, USA). The specific surface area of the microparticles was determined by dividing the surface area by the weight of microparticles.

2.4. Drug loading

Methylene blue (MB, methylthionine chloride trihydrate, Sigma Chemical Co.) was selected as a water-soluble model drug. Blank alginate microparticles (572 mg) were suspended in 30 mL of MB solution (2.5 and 25 mM). The suspension was stirred for 24 h at room temperature and then lyophilized. MB-loaded microparticles were then dried and stored in a desiccator for further evaluation.

4-Phenylazoaniline (PAA, Aldrich Chemical Co., USA) was selected as a water-insoluble model drug. PAA (320 mg) was dissolved in 400 mL of ethanol. To this PAA solution, 4.18 g of blank alginate microparticles were added and the suspension was stirred for 24 h. Ethanol was evaporated in a rotary evaporator (Ratavapor® R-3000, Buchi, Switzerland) at 65 °C under vacuum. PAA-loaded microparticles were then stored in a desiccator for further evaluation.

2.5. Scanning electron microscopy (SEM) studies

The surface properties of blank and drug-loaded microparticles were studied by using SEM (Hitachi-260, Japan). The samples were mounted on metal stubs and sprayed with gold to a thickness of 200–5000 Å using a gold splutter. The electron micrographs were scanned at 150–2000 magnification.

2.6. In vitro release of MB or PAA from alginate microparticles

Release studies of MB or PAA from alginate microparticles were conducted in two media, deionized water (for PAA 1% SLS solution) and simulated GI fluid using paddle method (USP Type II Apparatus). The effect of salt concentration on the release was evaluated by using sodium chloride at concentrations of 0.45, 0.70 and 0.90%.

Alginate microparticles (60 mg) loaded with MB or PAA were added into the release medium. Release studies were conducted at 37 °C. Aliquots of 2 mL samples were collected at predetermined time points (0, 15, 30, 45, 60, 90, 120, 150 and 180 min), filtered and 2 mL of fresh release medium was replenished to maintain a constant volume. The concentrations of MB and PAA in the samples were quantified by spectroscopic method (UV-1601, Shimadzu, Japan) using an external standard curve by monitoring at 663 and 381 nm, respectively.

2.7. Differential scanning calorimetry (DSC) studies

Differential scanning calorimetry analysis of MB, PAA, blank alginate microparticles, alginate microparticles loaded with MB and alginate microparticles loaded with PAA were scanned in the range of 30–300 °C using a Shimadzu DSC-50 (Shimadzu, Japan) at the rate of 10 °C/min. Thermograms of blank alginate microparticles, PAA, alginate microparticles of PAA, MB and alginate microparticles of MB were recorded. The endothermic peaks were measured using Shimadzu DSC-50 software.

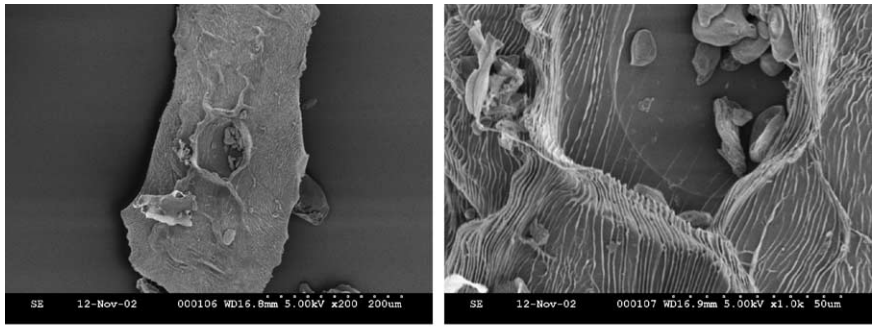
2.8. FT-IR spectra

Fourier transform infrared spectroscopy (FT-IR) spectra of MB, PAA, blank alginate microparticles, alginate microparticles loaded with MB and alginate microparticles loaded with PAA were determined by the KBr pellet method using a NICOLET Impact 400 FT-IR spectrophotometer (Nicolet Instrument Technologies Inc., USA).

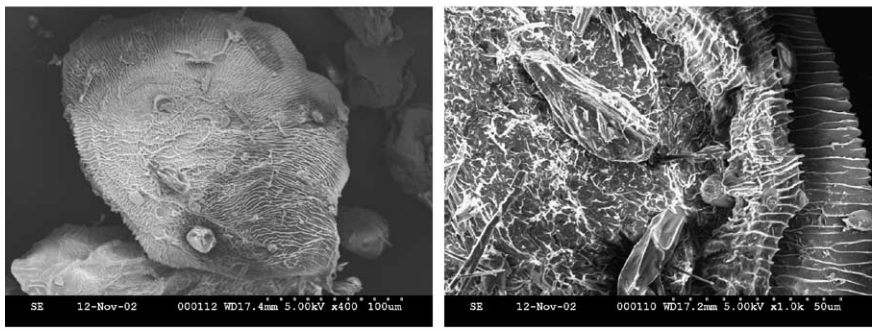
3. Results and discussions

3.1. Preparation and characterization of alginate microparticles

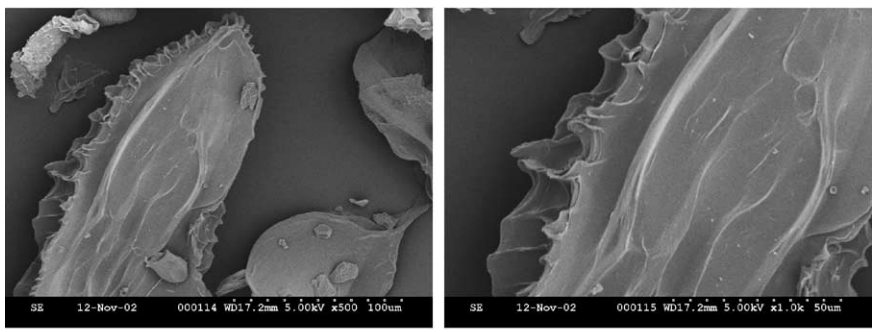
Currently available methods for the preparation of alginate microparticles are not amenable to scale-up, so, a spray–coagulation method was investigated for this purpose. When sodium alginate solution (5%, w/v) was sprayed into calcium chloride solution (1 M) through a nozzle (3/8 in.) at air pressures (40–60 psi), microparticles were formed in $CaCl_2$ solution. After freeze-drying, the resultant alginate microparticles were found to be porous and irregularly shaped as shown in SEM photograph (Fig. 1a). The yield of porous microparticles was $68.9 \pm 5.9\%$ ($n = 5$) of alginate polymer used and the process was scaled up to 25 g of scale (alginate) without difficulty. A larger scale can be achieved by altering the method slightly such as employing a continuous flow system and extending



(a): Blank Alginate Microparticles (Left: 200×, right: 1000×)



(b): Alginate Microparticles of MB (Left: 400×, right: 1000×)



(c): Alginate Microparticles of PAA (Left: 500×, right: 1000×).

Fig. 1. SEM photographs of blank alginate microparticles (a), alginate microparticles of MB (b) and alginate microparticles of PAA (c).

the time of spraying. The specific surface area of the microparticles with non-porous structure alginate is calculated as 0.0276 m^2/g based on the average size, volume and density of alginate polymer (Martin et al., 1993). The specific surface area of alginate microparticles prepared by spray–coagulation method was determined to be 32 m^2/g, about 1100 times higher than non-porous alginate particles of the same size. Because of the higher specific surface area, porous alginate microparticles produced by spray–coagulation method are expected to hold higher amounts of drug. Since alginates consist of guluronic acid and manuronic acid, the effect of guluronic acid content on specific surface area of alginate microparticles was investigated. As shown in Table 1, the specific surface areas of alginate microparticles prepared from high to low guluronic acid content alginates are comparable and the differences are not statistically significant ($p > 0.5$). In general, alginates with high guluronic acid content results in strong and brittle microparticles with good heat

Table 1
The effect of guluronic acid on surface area of alginate microparticles

Type of alginate used	Specific surface area (m^2/g) $\bar{X} \pm$ S.D. ($n = 3$)	
	Particle size 0.149–0.212 mm	Particle size 0.110–0.149 mm
Protanal LF 10/60 (high guluronic acid polymer)	6.65 ± 1.30	7.02 ± 2.20
Protanal LF 10/60D (low guluronic acid polymer)	7.10 ± 1.50	5.90 ± 1.70

All microparticles were prepared by spraying 2.5% alginate into 0.05 M $CaCl_2$.

stability but prone to water syneresis on freeze-thaw, whereas alginates with high manuronic acid content result in weaker and more elastic gels with good freeze-thaw behavior (Chan et al., 2002a). Therefore, alginate with high guluronic acid content was used in further studies.

The influence of calcium chloride concentration on the porosity of alginate microparticles was determined in the range of 0.05–1.0 M (Table 2). The microparticles prepared with 0.05 M calcium chloride have significantly lower specific surface area ($7.02 \pm 2.20\ m^2/g$, $n = 3$) than that with 0.5 and 1 M calcium chloride ($p < 0.01$) indicating less porous microparticles were obtained due to a lower degree of cross-linking of alginate at a low concentration of calcium. Alginate microparticles of all size ranges prepared under similar conditions have comparable surface area ($p > 0.5$). Alginate microparticles prepared with 0.5 M $CaCl_2$ exhibited higher surface area than those prepared with lower concentration of $CaCl_2$. The specific surface area of microparticles obtained with 1 M $CaCl_2$ is slightly higher than those with 0.5 M $CaCl_2$, the difference was not statistically significant ($p > 0.5$). This lack of further increase in specific area of microparticles with an increase in $CaCl_2$ concentration ($p > 0.5$) suggests the depletion of available carboxylic groups for cross-linking.

Table 2
Effect of concentration of $CaCl_2$ on the specific surface area

Concentration of $CaCl_2$ (M)	Specific surface area (m^2/g) $\bar{X} \pm$ S.D. ($n = 3$)	
	Particle size 0.149–0.212 mm	Particle size 0.110–0.149 mm
0.05	6.65 ± 1.30	7.02 ± 2.20
0.05[a]	2.24 ± 0.70	5.23 ± 1.10
0.5	14.81 ± 1.91	16.90 ± 1.92
1.0	18.90 ± 2.30	16.33 ± 3.40

The concentration of alginate was 2.5%.
[a] The concentration of alginate was 5.0%.

The influence of alginate concentration on the specific surface area of alginate microparticles was also studied by spraying aqueous solution of alginate at 2.5% (w/v) and 5% (w/v) into 1 M $CaCl_2$ and the results are shown in Table 3. At 1 M $CaCl_2$ concentration, the specific surface area of alginate microparticles prepared with 5% alginates with a particle size between 0.318 and 0.423 mm was significantly higher than that with 2.5% alginate ($p < 0.01$). Microparticles of other size ranges also showed comparable porosities ($p > 0.5$) from both 2.5 and 5% alginate solutions. The microparticles prepared by spraying 5% alginate solution into 0.05 M $CaCl_2$ solution had lower surface area, indicating lower cross-linking degree of the carboxylic acid groups in these microparticles (Table 2).

Model drugs, MB and PAA were loaded into alginate microparticles of particle size between 0.110 and 0.423 mm by the adsorption method. A polar solvent which solubilizes model compounds but not alginate microparticles was chosen. Consequently, MB was dissolved in water and PAA in ethanol to load them into alginate microparticles. The scanning electron microscopic examination of the loaded microparticles of MB and PAA were compared with blank alginate microparticles. As shown in Fig. 1, blank alginate microparticles have pores (Fig. 1a) that entrapped MB in MB-loaded alginate microparticles (Fig. 1b). MB and PAA crystals were found on the surface of loaded alginate microparticles (Fig. 1b and c). However, the PAA-loaded microparticles did not show pores and the microparticles were found to be dense and smooth when compared to blank or MB-loaded microparticles. The difference in the morphology of microparticles can be traced to the solvent used in the loading process. PAA was dissolved in ethanol and then loaded into alginate microparticles whereas MB was dissolved in water. The pores of alginate microparticles seem to be sealed after PAA was entrapped resulting in smooth surface morphology.

Table 3
Effect of concentration of alginate on the specific surface area

Concentration of alginate (%)	Specific surface area (m^2/g) $\bar{X} \pm$ S.D. ($n = 3$)			
	A (0.318–0.423 mm)	*B* (0.212–0.318 mm)	*C* (0.149–0.212 mm)	*D* (0.110–0.212 mm)
2.5	24.04 ± 1.30	20.85 ± 3.30	18.90 ± 2.30	16.33 ± 3.40
5.0	31.97 ± 4.20	20.13 ± 1.87	16.28 ± 1.28	13.86 ± 1.50

The concentration of $CaCl_2$ was 1 M.

3.2. Release of MB from alginate microparticles

The release profiles of MB from alginate microparticles in water and simulated gastrointestinal fluid are shown in Fig. 2. The release of MB from alginate microparticles in water was incomplete (65%) whereas in 0.1N HCl, near complete MB release occurred within 5 min. Complete release (99%) of MB was observed from all the NaCl media (0.45, 0.70 and 0.9%), however the release of MB was faster in 0.9% NaCl than in 0.45% NaCl, which was faster than water (Fig. 3). The order of MB release rate in different release media was 0.1N HCl > NaCl solution > water. The release profiles of MB fitted well with the Higuchi equation: $Q = 0.0695 + 0.171t^{0.5}$, $r^2 = 0.987$, indicating that MB release was controlled by diffusion (Vauthier and Couvreur, 2000).

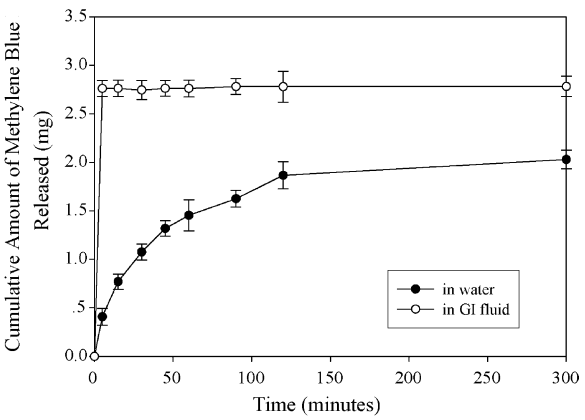


Fig. 2. Release profiles of MB from alginate microparticles in water and simulated gastrointestinal fluid.

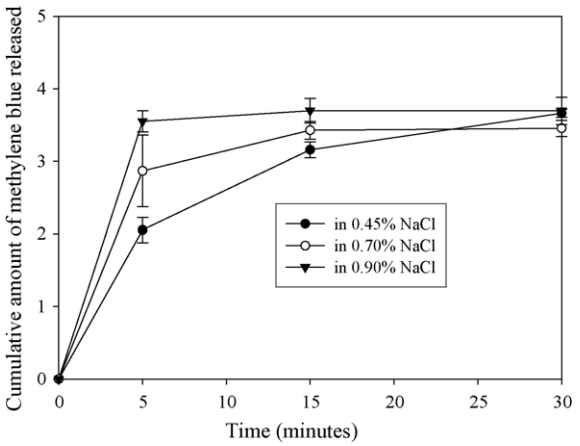


Fig. 3. Effect of release media on the release of MB from alginate microparticles.

3.3. Release of PAA from alginate microparticles

The release profile of PAA from alginate microparticles is shown in Fig. 4. The release of PAA from alginate microparticles in water was incomplete and the release followed the Higuchi equation: $Q = 0.148 + 0.115t^{0.5}$, $r^2 = 0.962$, suggesting that PAA release was also diffusion controlled, similar to MB release (Vauthier and Couvreur, 2000).

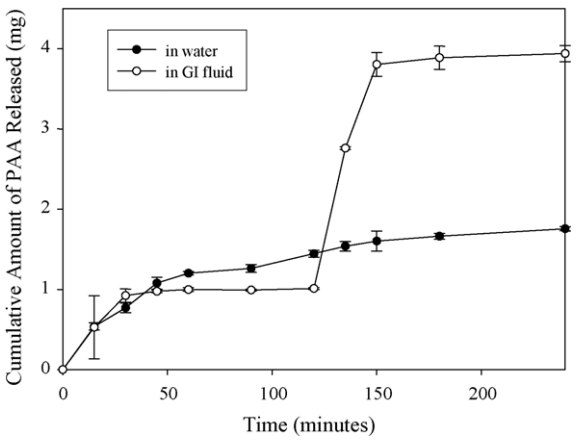


Fig. 4. Release profiles of PAA from alginate microparticles in water and GI fluid.

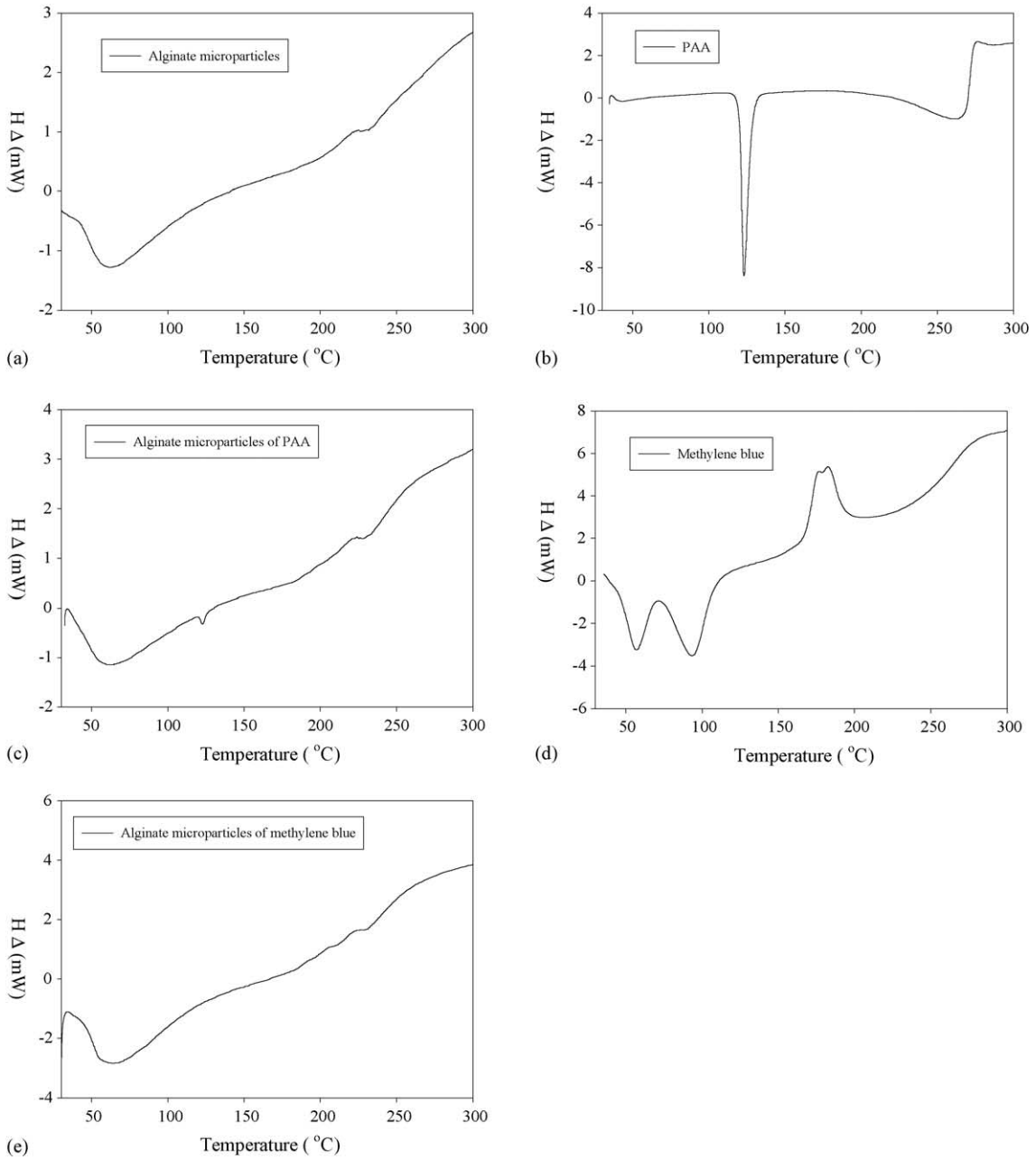


Fig. 5. DSC diagram of alginate microparticles (a), PAA (b), alginate microparticles of PAA (c), MB (d) and alginate microparticles of MB (e).

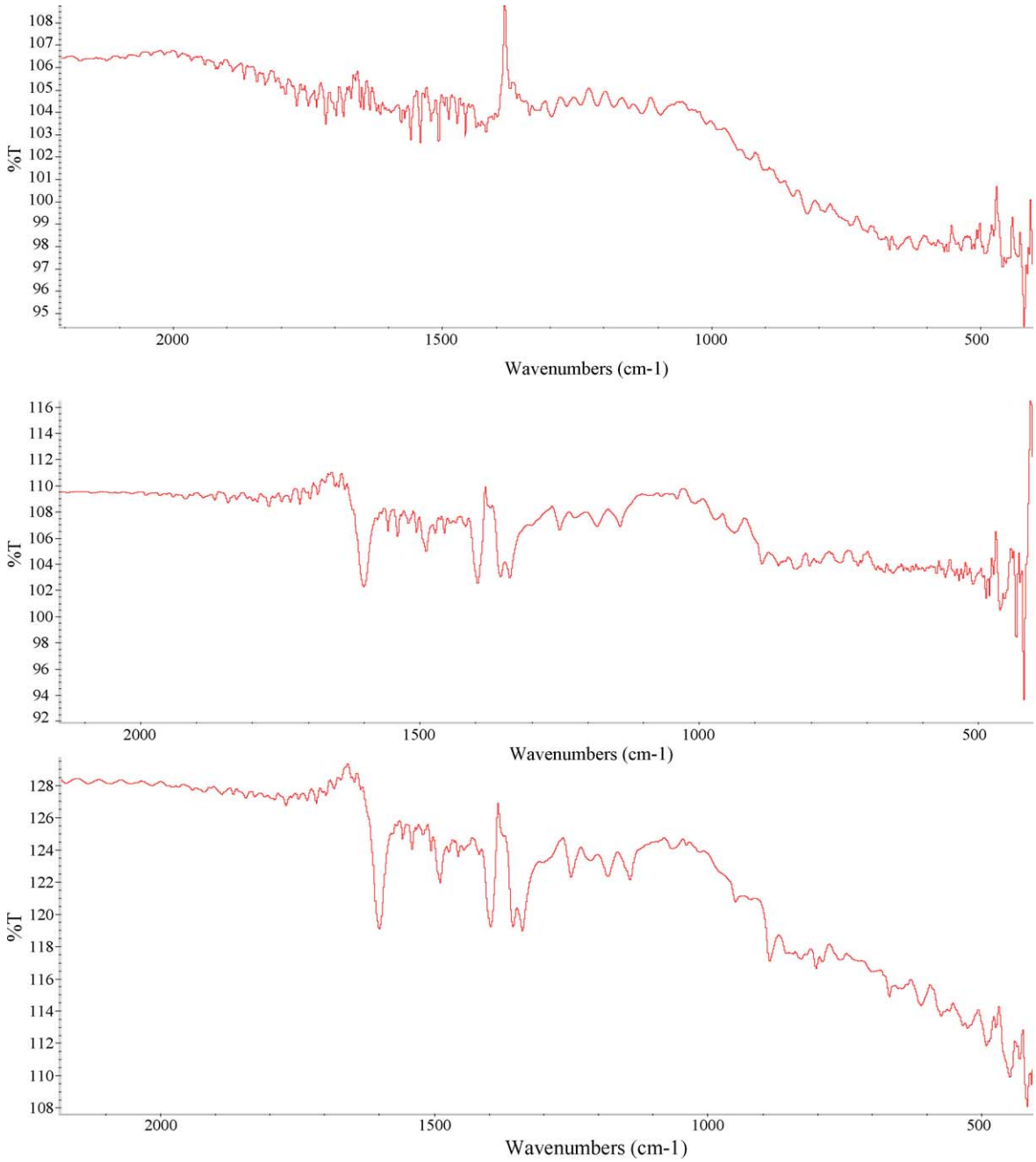


Fig. 6. FT-IR spectra of alginate microparticles, MB and alginate microparticles of MB.

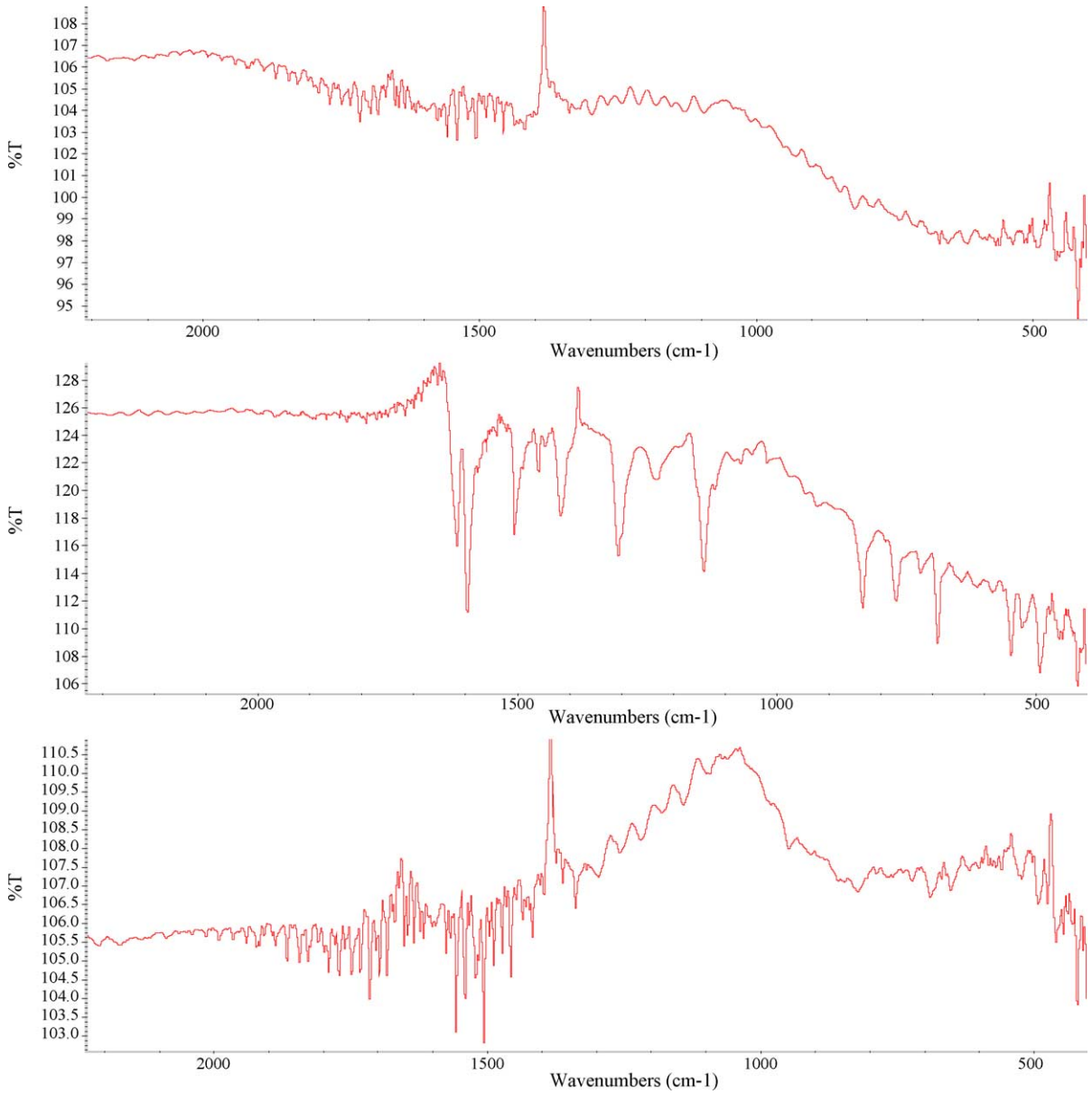


Fig. 7. FT-IR spectra of alginate microparticles, PAA and alginate microparticles of PAA.

The incomplete dissolution of MB and PAA from alginate microparticles in water may due to the ionic interaction between alginate and MB or PAA, with consequent maintenance of the microparticles structure during the release study. When exposed to NaCl solution, ionic exchange between Na^+ and Ca^{2+} occurred resulting in the disintegration of alginate microparticles and complete release of the model compounds. An increase in the concentration of NaCl can increase the release rate of model drug from alginate microparticles. When 0.9% NaCl solution was used as the release media, complete release occurred within 15 min along with complete disintegration of the alginate microparticles.

The release of PAA from microparticles of alginate microparticles in simulated gastrointestinal fluid occurred in two phases (Fig. 4). The initial phase lasted for 45 min where only 25% of loaded PAA was released. In this phase, the release of PAA followed the Higuchi equation: $Q = -0.0071 + 0.153t^{0.5}$, $r^2 = 0.978$, suggesting that the release of PAA was controlled by diffusion (Vauthier and Couvreur, 2000). After 2 h, when the pH was changed to 6.80, PAA release rate increased. This may be due to ionic exchange between Na^+ and Ca^{2+} causing the dissolution of microparticles, following exposure to Na^+ containing medium.

The release profiles of PAA from alginate microparticles in simulated GI fluid were different from the release profiles of MB from alginate microparticles in simulated GI fluid. Water-soluble MB was completely released within 5 min, whereas with practically water-insoluble PAA, only 30% of loaded PAA was released in 0.1N HCl during the first 2 h of the release studies. In 0.1N HCl, MB release was near complete, whereas PAA release was slow. To identify the reason for the slow release of PAA from alginate microparticles, dissolution studies were conducted comparing pure PAA with a physical mixture of PAA and blank alginate microparticles. PAA by itself or as a physical mixture in 0.1N HCl released more than 80% in 2 h. This data suggested that poor dissolution is not the cause for this slow release of PAA. Slow release of dextran from alginate–Ca^{2+} beads was observed in 0.1N HCl by Kikuchi et al. (1997, 1999), where the slowest release occurred from the beads loaded with the highest molecular weight. No such delay was observed with smaller molecular weight dextran molecules (M.W. 9400). Also, addition of sodium chloride to the release media accelerated the release of dextran from these alginate beads. In the current study, delayed release of PAA could be attributed to the ionic interaction between PAA and alginate in the initial release period. As the ion exchange between sodium and calcium occurred in the later stage of release (after 2 h in simulated GI fluids), the alginate microparticles began to disintegrate. Therefore, the release of PAA from alginate microparticles was accelerated.

3.4. DSC analysis

DSC thermograms of blank alginate microparticles (a), PAA (b), alginate microparticles of PAA (c), MB (d) and alginate microparticles of MB (e) are shown in Fig. 5. Melting peaks at 61.3 °C (a), 123.5 °C (b), 62.9 and 122.0 °C (c), 57.3, 93.2, 175.0 °C (exothermic) (d) and 62.9 °C (e) were observed. The endothermic peak of alginate microparticles at 61.3 °C was shifted to 62.9 °C after loading of MB and PAA. The endothermic peak of PAA at 123.5 °C was shifted to 122.0 °C after loading to microparticles, while all peaks of MB disappeared. The lowering of PAA and disappearance of MB melting peaks suggested that alginate microparticles interacted with both MB and PAA.

3.5. FT-IR spectrometry

FT-IR spectra of alginate microparticles, MB, alginate microparticles of MB, PAA, alginate microparticles of PAA are shown in Figs. 6 and 7. Distinct absorption peaks of MB corresponding to the aromatic amine (1600 cm^{-1}) and tertiary amine (1400 cm^{-1}) were observed with pure sample. After loading into alginate microparticles, both above-mentioned peaks disappeared (Fig. 6). These results indicated an interaction between MB and free carboxylic groups of alginate. Similar results were observed in PAA and alginate microparticles of PAA, i.e., the peaks corresponding to primary amine (1600 cm^{-1}) and azo (1306 cm^{-1}) disappeared (Fig. 7). As shown by DSC and FTIR investigation, both MB and PAA interacted with free carboxylic acid groups, which explain their incomplete release in water.

4. Conclusion

Spray–coagulation method was found to be suitable for preparation of porous alginate microparticles. Cationic drugs can be loaded by ionic interaction. For poorly water-soluble drugs, release of drug can be slowed down rendering alginate microparticles as an attractive carrier for the development of enteric bypass delivery systems.

References

Chan, L., Jin, Y., Heng, P., 2002a. Cross-linking mechanisms of calcium and zinc in production of alginate microspheres. Int. J. Pharm. 242, 255–258.

Chan, L., Lee, H., Heng, P., 2002b. Production of alginate microspheres by internal gelation using an emulsification method. Int. J. Pharm. 242, 259–262.

Fundueanu, G., Nastruzzi, C., Carpov, A., Desbrieres, J., Rinaudo, M., 1999. Physio-chemical characterization of Ca–alginate microparticles produced with different methods. Biomaterials 20, 1427–1435.

Gometz, W., Wee, S., 1998. Protein release from alginate matrices. Adv. Drug Deliv. Rev. 31, 267–285.

Kikuchi, A., Kawabuchi, M., Sugihara, M., Sakurai, Y., Okano, T., 1997. Pulsed dextran release from calcium–alginate gel beads. J. Control Release 47, 21–29.

Kikuchi, A., Kawabuchi, M., Watanabe, A., Sugihara, M., Sakurai, Y., Okano, T., 1999. Effect of Ca^{2+}–alginate gel dissolution on release of dextran with different molecular weights. J. Control Release 58, 21–28.

Kwok, K.K., Groves, M.J., Burgess, D.J., 1991. Production of 5–15 mm diameter alginate–polylysine microcapsules by an air-atomization technique. Pharm. Res. 8, 341–344.

Mandal, T.K., Bostanian, L.A., Graves, R.A., Chapman, S.R., Idodo, T.U., 2001. Porous biodegradable microparticles for delivery of pentamidine. Eur. J. Pharm. Biopharm. 52, 91–96.

Martin, A., Bustamente, P., Chou, A., 1993. Physical Pharmacy, Physical Chemical Principles in the Pharmaceutical Sciences, fourth ed. Lea & Febiger, New York.

Miyazaki, S., Nakamura, A., Oda, M., Takada, M., Attwood, D., 1995. Drug release from oral mucosal adhesive tablets of chitosan and sodium alginate. Int. J. Pharm. 118, 257–263.

Mofidi, N., Aghai-Moghadam, M., Sarbolouki, M.N., 2000. Mass preparation and characterization of alginate microspheres. Process Biochem. 35, 885–888.

Poncelet, D., 2001. Production of alginate beads by emulsification/internal gelation. Ann. N.Y. Acad. Sci. 944, 74–82.

Shu, X.Z., Zhu, K.J., 2002. The release behavior of brilliant blue from calcium–alginate gel beads coated by chitosan: the preparation method effect. Eur. J. Pharm. Biopharm. 53, 193–201.

Tonnesen, H.H., Karlsen, J., 2002. Alginate in drug delivery systems. Drug Dev. Ind. Pharm. 28, 621–630.

Vauthier, C., Couvreur, P., 2000. Handbook of Pharmaceutical Controlled Release Technology. Marcel Dekker, New York.

You, J.O., Park, S.B., Park, H.Y., Haam, S., Chung, C.H., Kim, W.S., 2001. Preparation of regular sized Ca–alginate microspheres using memethylene bluerane emulsification method. J. Microencapsul. 18, 521–532.