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RESEARCH PAPER

Propranolol Hydrochloride Binding in Calcium Alginate Beads

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ABSTRACT

The interaction of propranolol hydrochloride with alginate molecular chains in calcium alginate beads was investigated. The drug was either incorporated into formed calcium alginate gel beads or incorporated simultaneously with the gelation of alginate beads by Ca^{2+}. Beads produced by the former method had a higher drug content and lower Ca^{2+} level compared to those prepared by the latter method. The extent of drug binding to the alginate molecules increased with decreasing Ca^{2+} levels in the beads, indicating that propranolol and Ca^{2+} shared common binding sites in the alginate chains. The appearance of the beads and the morphology of the alginate polymer in the beads were affected by the amounts of both propranolol and Ca^{2+} in the beads. Differential scanning calorimetry (DSC) analyses showed that the formation of the calcium alginate gel structure was impeded in the presence of propranolol molecules.

INTRODUCTION

Alginic acid is a polysaccharide found in brown seaweeds. It is a linear copolymer of D-mannuronate (M) and L-guluronate (G) residues arranged in blocks of one type of residue (G blocks or M blocks) or of both types of residues in an alternating fashion (MG blocks)(1-2).

Gels can be obtained by reacting alginic acid with polyvalent ions such as Ca^{2+}. These gels have been investigated for use as carriers for drug delivery (3-5). In particular, calcium alginate beads, prepared by adding droplets of a sodium alginate sodium into a calcium chloride solution, have potential as an oral drug delivery system. The effects of process and formulation factors on the characteristics of the beads have been studied (6-7).

Alginic acid has ion-exchange properties because of the presence of carboxylate groups in both the M and G residues. It is therefore unlikely that when an incorporated drug is cationic, there is interaction between the alginate and the drug. Such interaction has been reported for propranolol (8) (pK_a 9.45) and imipramine (4) (pK_a 9.5). Drug interaction may comprise the formation of

calcium alginate gel as the drug molecule, and the Ca^{2+} ions may have common binding sites in the alginate molecular chain. An alteration in gel structure will, in turn, affect the drug release kinetics from the gel. Whether the cationic drug is incorporated before or after gel formation is likely also to influence the extent to which drug molecules disrupt the structure of the alginate gel. In this study, propranolol, chosen as a model drug, was incorporated into calcium alginate beads by one of two methods. In the first method, the drug was loaded onto formed calcium alginate beads, and in the second, drug loading was carried out simultaneously with the gelation of alginate beads by Ca^{2+} ions. The characteristics of the drug-loaded beads were then examined with respect to the method of drug loading.

MATERIALS

Sodium alginate (*laminaria hyperborea*) (BDH Ltd., U.K.), calcium chloride dihydrate (analytical grade, E. Merck, Germany), and propranolol HCl (USP XXII grade, Beacons Chemicals Pte. Ltd., Singapore) were used as supplied. All other reagents were of analytical grade.

METHODS

For bead formation, 50 ml of a 2% aqueous solution of sodium alginate was introduced dropwise from a blunt size-14 needle (Bopper and Sons Inc.) into 100 ml of an aqueous calcium chloride solution being stirred at 300 rpm. The concentration of $CaCl_2$ in the solution ranged from 0.25% w/v to 7.5% w/v. One hour after the first drop of alginate was added to the counterion solution, the calcium alginate beads were harvested by filtration, washed with distilled water, and dried at 60°C for 10 hr in an oven.

Drug loading was carried out by two methods designated as the sequential and the simultaneous methods. In the sequential method, calcium alginate beads were prepared as described in the previous paragraph. The wet beads were then immersed and stirred for 2 hr in a solution containing 5% w/v propranolol HCl. In the simultaneous method, the gelation of beads by calcium ions occurred simultaneously with the drug loading into the beads. The sodium alginate solution was introduced dropwise into $CaCl_2$ solutions (concentration ranging from 0.5% to 7.5%), which also contained 5% w/v propranolol HCl. After 2 hr of interaction, the beads were removed from the counterion solutions. The drug-loaded beads were washed and dried in a manner similar to that described for the blank beads.

For the determination of calcium content, duplicate 20-mg samples of beads were dissolved in 100 ml of 0.05 M disodium ethylenediamine tetracetic acid (EDTA) solution. The solution, after suitable dilution, was assayed for calcium content using atomic absorption spectrophotometry (Varian AA-1275).

For the determination of propranolol content, duplicate 20-mg samples of beads were immersed in 100 ml of 0.1 M sodium chloride solution for 24 hr. The filtered solution was measured for propranolol content using UV spectrophotometry (Perkin-Elmer Lamda 4A) at 288 nm.

The surface morphology of the beads was observed under a scanning electron microscope (SEM) (Jeol JSM 5200) operating at 25°C with a beam voltage of 15 kV. Polymer morphology in the beads was studied using differential scanning calorimetry (Perkin-Elmer DSC 4). Five-milligram samples of beads were heated from 30°C to 250°C at a rate of 20°C/min.

RESULTS AND DISCUSSION

The calcium alginate beads could not be formed successfully when the counterion solutions contained 0.25% of $CaCl_2$. The large and soft beads collapsed upon drying to form translucent thin disks. When the $CaCl_2$ concentration in the counterion solution was increased to 0.5% w/v or higher, round, translucent, firm beads were obtained. Upon drying, the beads maintained their sphericity but had a flattened base at the point of contact with the drying vessel. The appearance and size of the beads appeared to be independent of the $CaCl_2$ concentrations used in the counterion solution. Viewed under the SEM, all the beads had smooth surfaces.

Increasing the $CaCl_2$ concentration in the counterion solution produced beads with higher levels of Ca^{2+} ions (Table 1). Fully cured calcium alginate beads have been reported to contain between 1.89×10^{-3} and 2.44×10^{-3} mol/g polymer of associated Ca^{2+} (9). The calcium alginate beads in this study should thus contain Ca^{2+} ions associated with alginate molecular chains as well as unassociated Ca^{2+} ions. The amount of associated Ca^{2+} ions in these beads may, however, differ from the value reported (9) because of variations in the proportion of G blocks in different sources of alginates (10). Of the G, M, and MG blocks in an alginate sample, Ca^{2+} ions preferentially interact with the G chains because of the favorable orientation of carboxylate groups in these

Table 1

Ca^{2+} (10^{-3} mol/g of Alginate) and Propranolol HCl (% w/w) Content in Calcium Alginate Beads Prepared with Solutions Containing Different Amounts of Calcium Chloride

$CaCl_2$ Solution (% w/w)	Blank Beads Ca^{2+}	Simultaneous Method		Sequential Method	
		Ca^{2+}	Propranolol HCl	Ca^{2+}	Propranolol HCl
0.25	3.33 ± 0.27	-	-	-	-
0.50	3.70 ± 0.30	2.47 ± 0.03	59.78 ± 0.92	2.01 ± 0.03	67.07 ± 0.20
1.00	4.52 ± 0.13	3.48 ± 0.07	50.26 ± 0.35	1.80 ± 0.03	63.16 ± 1.38
2.50	5.46 ± 0.17	5.50 ± 0.02	38.34 ± 0.21	3.21 ± 0.03	57.09 ± 0.74
5.00	8.09 ± 0.08	5.28 ± 0.14	22.74 ± 0.47	4.27 ± 0.06	57.55 ± 2.11
7.50	8.42 ± 0.15	7.21 ± 0.08	23.18 ± 0.07	3.94 ± 0.04	45.63 ± 0.74

chains (11). The specific interchain linkages of the G chains by Ca^{2+} ions result in the formation of a gel network structure in the alginate (12).

DSC thermograms of the dried calcium alginate beads show two peaks, the Ca^{2+}magnitudes of which increased with enhanced Ca^{2+} content in the beads (Fig. 1). Peak 1, which appeared at lower temperatures and was broader than peak 2, may be attributed to the presence of water molecules trapped within the calcium alginate gel structure. Peak 2 indicates the transition of the dried gel network, the structural integrity of which is reflected in the magnitude of peak 2.

The integrity of the gel structure depends on the proximity of alignment of the G molecular chains by Ca^{2+} ions. When sufficient Ca^{2+} ions were available in the counterion solutions, a large number of G molecular chains in the alginate beads were interlinked to form a tight network structure (12). Water molecules en-

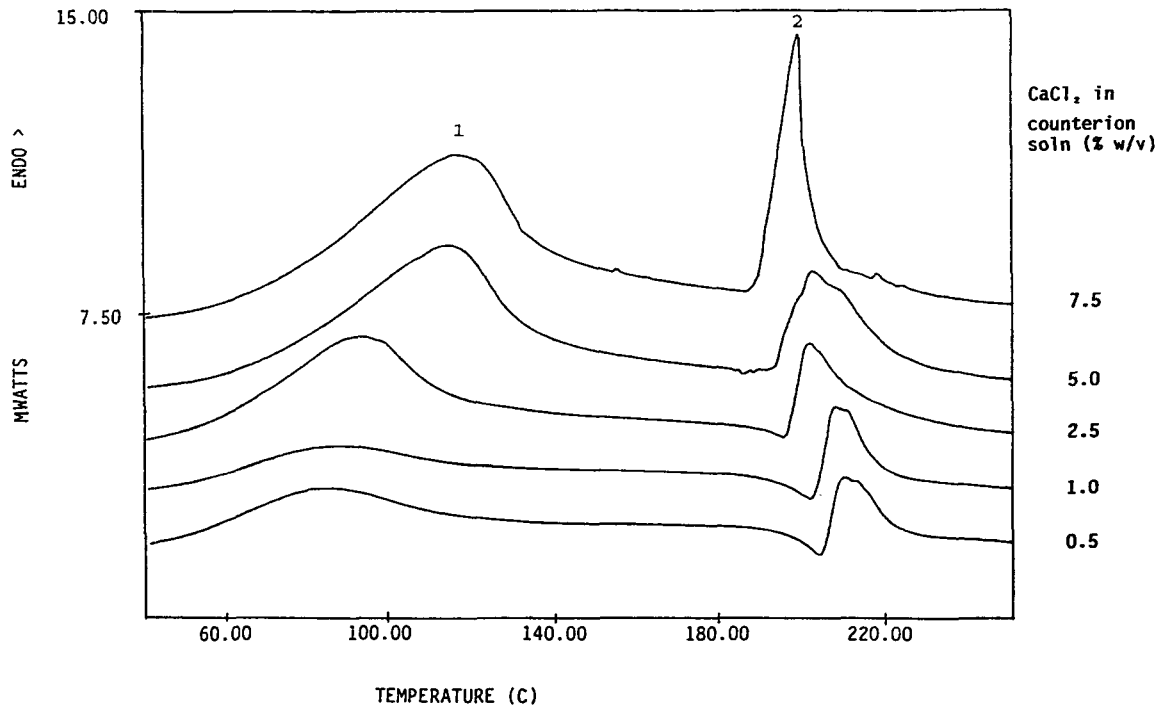

Figure 1. DSC thermograms of dried calcium alginate beads prepared with solutions containing different amounts of $CaCl_2$.

trapped within the network contributed to a high water content in the beads even after drying. At low levels of Ca^{2+} ions in the counterion solution, the number of linkages formed between G molecular chains was less, resulting in the beads having a loose network structure. The soft gel constituted an inefficient barrier to the loss of water from the beads during drying. Such beads, after drying, may not exhibit peak 1.

Although propranolol is cationic, it is not polyvalent, and the formation of alginate beads were unsuccessful when only the drug was used as the counterion. Introduction of a sodium alginate solution dropwise into a 5% propranolol solution produced opaque beads, which when stirred, collapsed immediately to give a stringy precipitate. DSC analyses of the precipitate indicated that propranolol was unable to form a gel with the alginate. Ion-pairing of propranolol molecules with carboxylate groups in the alginate chains caused the salting out of the chains.

The incorporation of propranolol into calcium alginate beads reduced the calcium content in the beads, the reduction being greater when the drug was loaded sequential to the gelation of the beads by Ca^{2+} ions (Table 1). During the process of drug loading by the sequential method, unassociated Ca^{2+} ions in the calcium alginate beads diffused into the drug solution while drug molecules move in the counter-direction along their respective concentration gradients. The propranolol molecules may further displace associated Ca^{2+} ions from common binding sites in the alginate chains because of a more favorable concentration gradient. The degree of displacement would be inversely proportional to the Ca^{2+} content in the wet beads, and therefore to the concentration of $CaCl_2$ in the counterion solution used to prepare the beads.

On the other hand, when drug loading was carried out simultaneously with the gelation of alginate by Ca^{2+}, the counterion solution contained both the drug molecules and $CaCl_2$. The drug molecules and Ca^{2+} ions diffused in the same direction, from the counterion solution into the alginate beads, and competed simultaneously for common binding sites along the alginate chains. The final amount of associated Ca^{2+} ions (and of drug molecules) in the beads was a function of the concentration ratio of $CaCl_2$:propranolol in the counterion solution. This method thus provides for a more efficient binding of Ca^{2+} ions to the alginate chains in the presence of propranolol molecules. In addition, the presence of $CaCl_2$ in the solution also contributed to a higher amount of unbound Ca^{2+} ions in these beads compared to beads prepared by the sequential method.

By the same reasoning, the efficiency of loading propranolol into the calcium alginate beads by the simultaneous method was lower than by the sequential method (Table 1). For both types of drug-loaded beads, the drug content decreased with increasing Ca^{2+} content in the beads, further confirming that Ca^{2+} ions and propranolol molecules share common binding sites in the alginate molecules.

Fig. 2 shows the cumulative amount of propranolol released into distilled water from calcium alginate beads prepared by the sequential method. Calcium alginate beads are not known to swell or dissolve significantly in distilled water, therefore the released drug molecules would be unassociated molecules. Regardless of the drug content in the beads, there was no difference in the total amount of drug released with time, indicating that the beads contained similar amounts of unassociated drug molecules. The different initial drug contents may then be attributed to the varying extent of drug binding in the beads. It is observed that a higher extent of drug binding was achieved in the presence of lower levels of Ca^{2+} in the beads.

Unlike the drug release profiles from beads prepared by the sequential method, the amount of drug released with time from beads prepared by the simultaneous method decreased with the initial drug content in the beads (Fig. 3). For beads containing less than 38% w/w propranolol, more than 90% of the initial drug con-

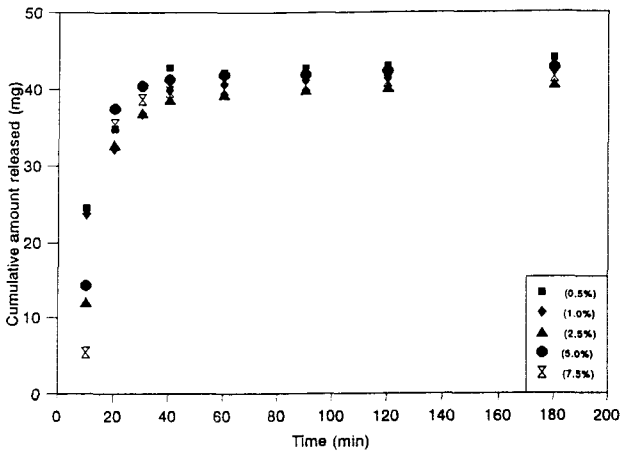

Figure 2. The cumulative amounts of propranolol released into distilled water from beads prepared by the sequential method. The legend shows the concentration of $CaCl_2$ used to prepare the beads.

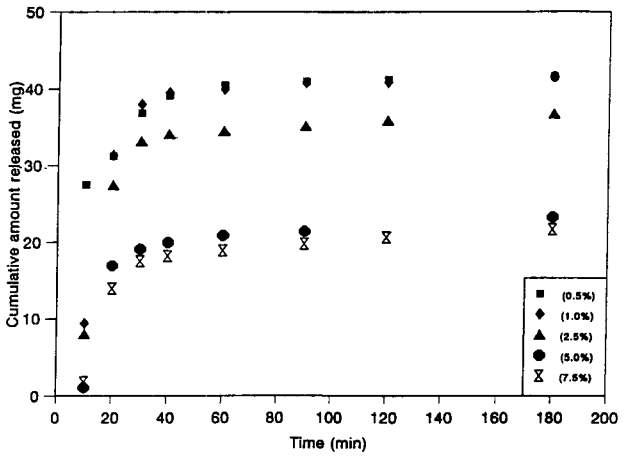

Figure 3. The cumulative amounts of propranolol released into distilled water from beads prepared by the simultaneous method. The legend shows the concentration of $CaCl_2$ used to prepare the beads.

tent was released within 3 hr, i.e., most of the drug molecules in these beads were not bound to the alginate chains. These beads were prepared using counterion solutions containing $CaCl_2$:propranolol ratios of greater than 0.5. It may be implied that, at high salt:drug ratios, the drug molecules were less efficient in coupling with Ca^{2+} ions for the common binding sites in the alginate chains. Furthermore, since the beads released different amounts of drug with time, it may be inferred that the amount of unbound drug in the beads also decreased with increasing salt:drug ratios in the counterion solution.

The simultaneous method of preparation yielded round and opaque beads when the counterion solution contained low $CaCl_2$ concentrations of between 0.5% and 1.0%. The opacity suggests that more propranolol molecules than Ca^{2+} ions interacted with the alginate molecules. Upon drying, the degree of opacity lessened, a consequence of drug loss during the process of filtering, washing, and drying. Using counterion solutions containing higher $CaCl_2$ concentrations of 2.5% -7.5% resulted in translucent and firm beads that dried to give white, opaque, and friable beads. The translucent wet beads are indicative of a relatively greater extent of gel formation (Ca^{2+}-induced) compared to precipitate formation (drug-induced) in the beads. The opacity of the dried beads cannot be attributed to a high drug content (Table 1), but may be due to phase separation of the precipitate from the gel structure during the drying process. The flaking off of the precipitate at the surface of the beads made the beads friable.

Beads prepared by the sequential method were firm, but had various degrees of opacity after drug loading. The degree of opacity, a measure of the drug loading efficiency, decreased in beads prepared using increasing concentrations of $CaCl_2$ in the counterion solution. As a result, beads prepared with a solution containing 7.5% $CaCl_2$ were translucent even after drug loading. The ability of propranolol molecules to displace Ca^{2+} ions from the beads therefore depended on the Ca^{2+} content in the wet beads. All the beads prepared by the sequential method, except for those formed using solutions containing 0.5% $CaCl_2$, were free-flowing, whitish spheres upon drying. In the exceptional case, the beads collapsed upon drying to yield thin disks. Unlike beads prepared by the simultaneous method, none of the beads prepared by the sequential method was friable.

Fig. 4 shows the SEM micrographs of drug-loaded calcium alginate beads prepared by the simultaneous method. Beads formed using 0.5% $CaCl_2$ solutions showed some surface corrugation, a consequence of their low Ca^{2+} content (Table 1) and therefore, poor gel strength. Beads formed using increasing amounts of $CaCl_2$ showed diminishing surface porosity. However, surface sloughing was observed on beads obtained with solutions containing more than 2.5% $CaCl_2$. The surface sloughing correlated to the friability of the beads observed visually.

SEM micrographs of drug-loaded beads prepared by the sequential method (Fig. 5) show a similar dependence of bead porosity on the Ca^{2+} content in the beads. Beads containing less than 3.5×10^{-3} mol/g polymer of Ca^{2+} had corrugated surfaces, and the extent of corrugation decreased when Ca^{2+} content in the beads was increased. Beads with Ca^{2+} content greater than 3.5×10^{-3} mol/g polymer had a compact surface. Surface sloughing was not apparent in any of the beads prepared by the sequential method.

Neither peak 1 nor 2 was present in the DSC thermograms of calcium alginate beads loaded with propranolol sequential to the gelation process (Fig. 6). The loading of propranolol had therefore interfered with the structural integrity of the gel structure, probably by the displacement of associated Ca^{2+} by drug molecules. On the other hand, the DSC thermograms of beads prepared by the simultaneous method showed peaks 1 and 2 when the Ca^{2+} content in the beads was 5.5×10^{-3} mol/g alginate or greater (Fig. 7). Comparison of these figures with Fig. 1 indicates that the drug-loaded beads exhib-

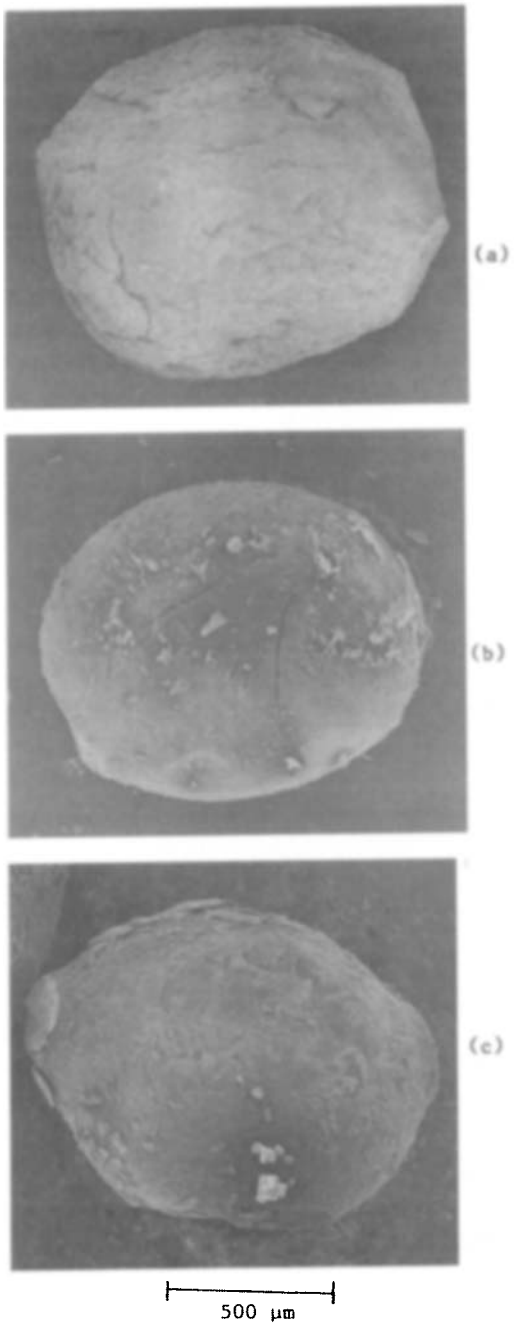

Figure 4. SEM micrographs of propranolol-loaded calcium alginate beads prepared by the simultaneous method using 0.5% (a), 2.5% (b), and 7.5% (c) w/v $CaCl_2$ solutions.

ited peaks 1 and 2 at higher Ca^{2+} content compared to the blank beads, further affirming that the propranolol molecules hindered the formation of a calcium alginate gel in the beads.

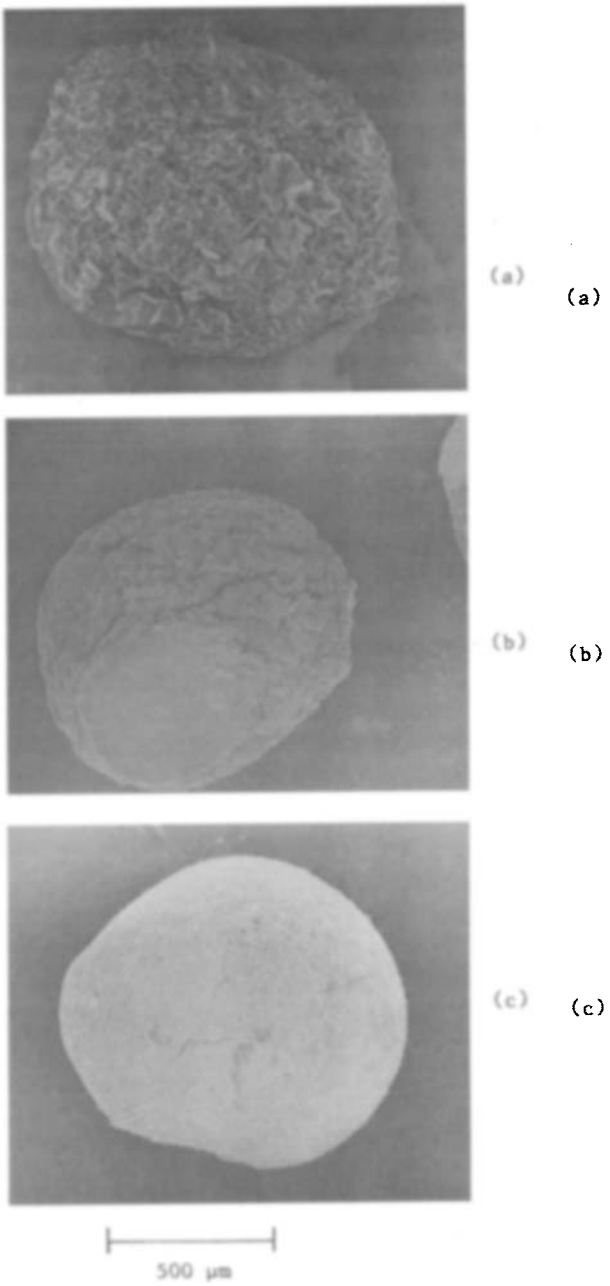

Figure 5. SEM micrographs of propranolol-loaded calcium alginate beads prepared by the sequential method using 0.5% (a), 2.5% (b), and 7.5% (c) w/v $CaCl_2$ solutions.

It is apparent from these results that the efficiency of loading and extent of binding of a cationic drug such as propranolol in calcium alginate beads are influenced by the method of drug incorporation and the concentration of Ca^{2+}, both bound and unbound, in the beads. Concurrently, the formation of alginate gel structure is af-

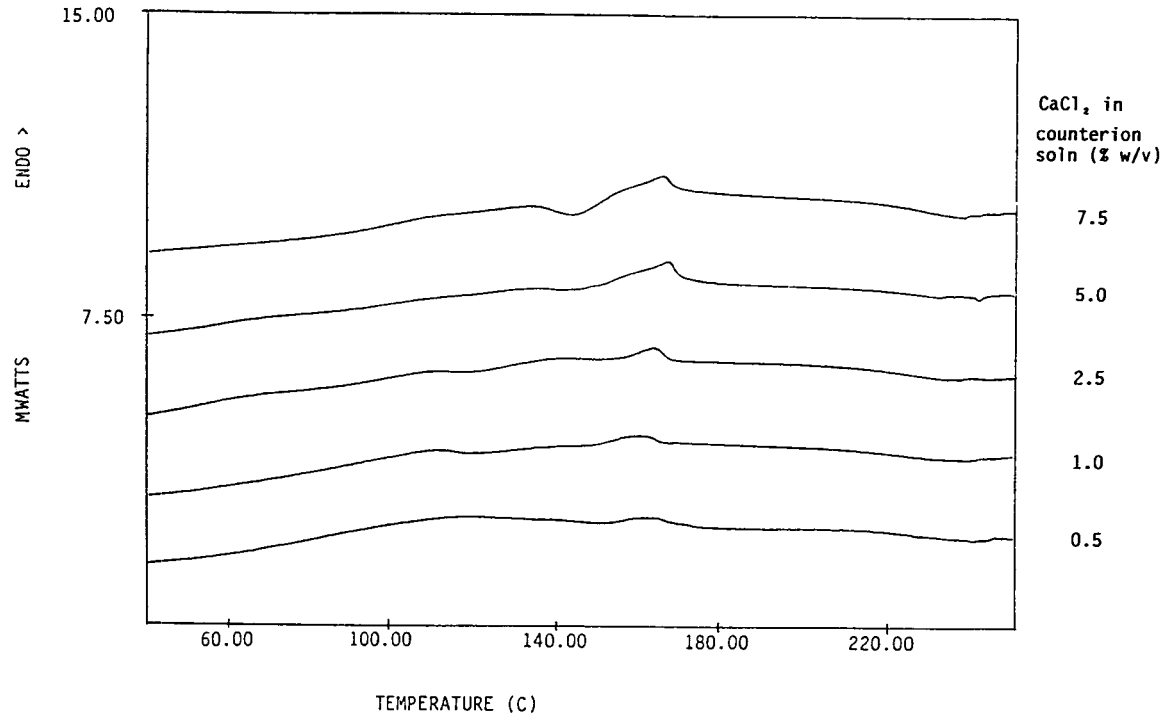

Figure 6. DSC thermograms of propranolol-loaded calcium alginate beads prepared by the sequential method using different strengths of $CaCl_2$ solutions.

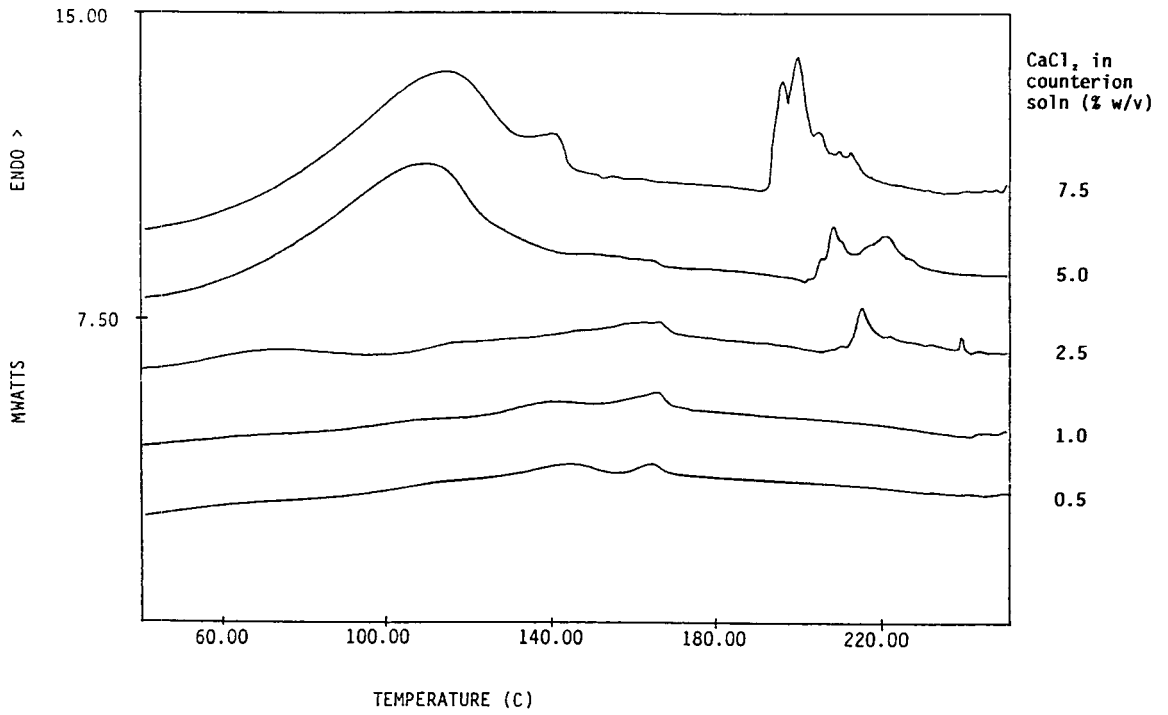

Figure 7. DSC thermograms of propranolol-loaded calcium alginate beads prepared by the simultaneous method using different strengths of $CaCl_2$ solutions.

fected. These factors should be of interest when calcium alginate is chosen as a carrier in controlled-release systems of cationic drugs.

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