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# Characterization of Calcium Alginate Beads Containing Structurally Similar Drugs

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**ABSTRACT** The aim of this study was to investigate the characteristics of alginate beads prepared by ionotropic gelation in which structurally similar drugs were incorporated. For this purpose theophylline and theobromine were selected as model drugs. The influence of incorporated drugs on bead characteristics such as size, shape, and morphology, as well as encapsulation efficiency, was examined. It was found that theobromine as well as theophylline content in beads significantly decreased with increasing hardening time due to drug diffusion into the hardening media. In theobromine beads the drug content was extremely improved by dropping the alginate and drug solution into an acidic calcium chloride solution, while theophylline content was to some extent improved by the hardening of beads in a calcium chloride solution saturated with the drug. The most evident difference between theophylline and theobromine beads was in their shape and morphology. Theobromine beads were round, while theophylline ones had an irregular shape with an extremely wrinkled surface. The distinction in shape was highly dependent on drug content. Additionally, it was demonstrated that beads' shape was dependent on preparation conditions as well. On the basis of x-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) analyses and scanning electron microscope (SEM) photographs it was found that the most of the drug in bead was present in an amorphous state. Therefore, it is suggested that some drug–alginate interactions could be present in beads and might be responsible for the different shape of theophylline and theobromine beads.

Thus it can be concluded that the preparation of beads by ionotropic gelation cannot be generalized even though structurally similar drugs are incorporated.

**KEYWORDS** Alginate bead, Ionotropic gelation, Bead characteristics, Theophylline, Theobromine

## INTRODUCTION

Alginate is a linear, naturally occurring polysaccharide extracted from brown sea algae. It contains D-mannuronic (M) and L-guluronic (G) acids which are arranged in homopolymeric MM or GG blocks separated by blocks with an alternating sequence, MG blocks (Bruneton, 1999). While alginic acid

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is insoluble in water, alginic acid salts with monovalent cations and magnesium do dissolve in water. In the presence of various divalent (generally $Ca^{2+}$ but also $Ba^{2+}$ and $Zn^{2+}$) or trivalent ions ($Al^{3+}$), an elastic gel is formed due to ionic interaction between the ions and the carboxyl groups of mainly guluronic blocks (Bajpai & Sharma, 2004; Al-Musa et al., 1999; Aslani & Kennedy, 1996; Gonzáles-Rodrígues et al., 2002) Therefore, the alginate monomer composition (proportion of poly G-blocks and their length in alginate) has an important influence on gelling properties and consequently on drug release properties of different dosage forms (Østberg & Graffner, 1994a). The ability of alginate to form a gel in the presence of multivalent ions has been used to prepare multiparticulate systems incorporating numerous drugs, proteins, cells, or enzymes (Tønnesen & Karlsen, 2002) The beads are most commonly produced by the so-called ionotropic gelation method, where the dispersion of alginate and material to be encapsulated is added dropwise into a multivalent ion solution. The contact of droplets with multivalent ions results in the instantaneous formation of gel spheres containing uniformly dispersed material to be encapsulated throughout the cross-linked alginate matrix.

Alginate is one among numerous polysaccharides (pectin, chitosan, inulin, dextran, guar gum, etc.) that are selectively degraded in the colon by colonic microbial enzymes; therefore, it can be used for preparation of colon specific drug delivery systems. However, it is well known that by incorporation of drugs into calcium alginate beads it is difficult to provide delayed release of hydrophilic, low molecular weight drugs (Østberg et al., 1994b). To avoid premature drug release, a polysaccharide degradable specifically in colon and time and/or pH controlled mechanism should be combined in the formulation. This could be achieved by coating of calcium alginate beads with suitable polymer and in this step the bead characteristics, especially bead shape and morphology, are of critical importance. Therefore, the influence of drug incorporated as well as process parameters on bead properties has to be understood in detail. It has been reported that several formulation parameters (type and concentration of alginate, combination of alginate with different polymers, and drug polymer weight ratio) and processing parameters (hardening time, calcium concentration, drying conditions, and type of multivalent ions) affect drug content and drug release as well as bead size and morphology (Bajpai & Sharma, 2004; Al-Musa et al., 1999; Gonzáles-Rodrígues et al., 2002; El-Kamel et al., 2003; Fundueanu et al., 1999; Rousseau et al., 2004; Acartürk & Takka, 1999; Østberg et al., 1993). However, to our knowledge the influence of structure similarity of drugs on bead properties prepared by ionotropic gelation has not been studied in detail. Therefore, the aim of this study was to produce alginate beads with two structurally similar model drugs incorporated, i.e., the xanthine derivatives theobromine and theophylline, which are distinguished only in the position of one methyl group. With regard to this, comparable drug–alginate interactions and thus similar bead properties were expected. The influence of these two drugs on bead characteristics such as drug loading and bead form, size and morphology was evaluated. Furthermore, the physical state of drugs in beads was examined using XRPD and DSC.

## MATERIALS AND METHODS

### Materials

Sodium alginate (Protanal LF 120M, 35–45% G–content, viscosity of 1% solution at 20°C 86 mPas) was a gift of Selectchemie AG, (Selectchemie, Zürich, Switzerland). Theophylline and theobromine were purchased from Sigma-Aldrich, (Taufkirchen, Germany).

All the other substances and solvents used for the preparation of the phosphate buffer and calcium chloride solution were of analytical grade.

### Preparation of Alginate Beads

Alginate beads containing theophylline or theobromine as a model drug were prepared by ionotropic gelation as described below. First, 3.5 g of the drug was dissolved in 100 g of a 0.24 M solution of sodium hydroxide and then 3.5 g of sodium alginate was added to the solution. After stirring on a magnetic stirrer for 4 h, the homogeneous and bubble-free solution was filled into a glass syringe equipped with plastic tube with a 1.2 mm inner diameter. The solution was then added dropwise into three different media: 0.27 M calcium chloride solution (medium 1), 0.27 M calcium chloride solution saturated with the drug (medium 2), and to 0.27 M calcium chloride solution acidified to pH 1 with 1 M HCl (medium 3) as presented in

**TABLE 1** The Preparation Conditions of Beads

| Sample | Drug | Hardening solution | Hardening time (min) |
|---|---|---|---|
| $TB_x$ / $TF_x$ / $A_x$ | theobromine/ | Medium 1: 0.27M $CaCl_2$ (pH ≈ 6) | |
| $TB_{Sx}$ / $TF_{Sx}$ | theophylline/ | Medium 2: 0.27M $CaCl_2$ saturated with theobromine/ theophylline | 1, 5, 10, 15, 20, 30 |
| $TB_{Ax}$ / $TF_{Ax}$ / $A_{Ax}$ | no drug | Medium 3: 0.27M $CaCl_2$ (pH = 1) | |

x = hardening time in minutes.

Table 1. The amounts of the drugs needed to obtain saturated solutions can be seen in Table 3. The dispersion was dropped into the hardening media for 10 sec (16–17drops/50 mL of medium) with a constant rate of 3.75 mL/min, using an infusion pump Braun-Mekungen, Germany. This procedure was repeated four times and the obtained beads represented one batch. Due to the cross-linking reaction, calcium alginate beads were formed and allowed to harden in the time interval from 1 to 30 min; after that they were isolated and washed with 500 mL of distilled water. Additionally, empty beads without any drug incorporated were prepared using the same experimental conditions (Table 1). The beads were spread on aluminium foil and dried at room temperature overnight. They were additionally dried at reduced pressure at room temperature overnight. At each experimental condition three batches of approximately 70 beads were prepared.

## Size and Shape Characterization of Beads

The bead size and shape was estimated using a Stereomicroscope Olympus SZX12, (Tokyo, Japan) equipped with a digital camera (3CCD Color Video Camera, Power HAD, Sony, Tokyo, Japan). The beads were relatively spherical, therefore only their frontal side parameters were evaluated. Ten beads of each batch (i.e., 30 beads prepared using the same experimental conditions) were examined. The mean diameter, the shape factor, and the aspect ratio of the bead frontal side was determined using an image analysis system AnalySIS (SIS, Germany, 1999). The aspect ratio was defined as the ratio between the maximal and minimal bead diameter, while the shape factor, which provides the information about the roundness of the particles, was calculated by the following equation (AnalySIS® Software Programme, 1999, SIS GmbH, Münster, Germany):

$$shape\ \ factor = \frac{4\pi \times area}{perimeter^2}$$

The closer the values of aspect ratio and shape factor were to 1, the rounder the bead was.

The morphological properties of the bead surface and the internal structure characteristics of the beads were evaluated using a scanning electron microscope (SEM) Supra™ 35 VP, Zeiss, Germany.

## Determination of Drug Content

The drug content in the beads was determined by dispersing five accurately weighed beads (approximately 10 mg) into 100 mL phosphate buffer (pH 7.4) and stirred for 24 h. The dispersion was filtered and the drug concentration was determined spectrophotometrically (EZ301, PerkinElmer, Shelton, CT, USA) at the absorption maximum (270 nm and 272.5 nm for theophylline and theobromine, respectively).

Two replicates per batch were performed for drug content determination.

Furthermore, the amount of the drug that diffused into the calcium chloride solution during the hardening of beads was determined. Immediately after isolation of beads aliquots of hardening solution were filtered, diluted, and assayed spectrophotometrically at the absorption maximum.

## Solubility

The solubility of theophylline and theobromine in media 1 and 2 and 0.27 M calcium chloride solution with pH 9 at 25°C was determined. The solubility of drugs in the calcium chloride solution with pH 9 was determined because the pH of medium 1 was elevated to approximately 9 after the bead isolation due to the diffusion of hydroxyl ions out of the beads during hardening.

The amount of the drug that exceeded solubility was placed into 4 mL of the corresponding media and stirred on a magnetic stirrer for 24 h. The temperature was maintained using a thermostat Ultra 2000, Elektromehanika (Labo, Slovenia). At 4, 6, and 24 h 0.5mL of aliquots were withdrawn and filtered. The concentration of the drug in samples was determined spectrophotometrically at the absorption maximum. The equilibrium solubility was calculated. Each determination of solubility was made in triplicate.

## Determination of the Precipitation Rate

The precipitation rate of theobromine was determined as described below. One milliletre of 35 w/w% of theobromine solution in 0.24 M NaOH was added to 10 mL of medium 1 or 2. Appropriate volume of 1 M HCl was added to the media to obtain pH values of 9 or 1 after addition of theobromine solution. The solution was stirred on the magnetic stirrer. At an appropriate time 0.5 mL samples were withdrawn, filtered, and immediately diluted to prevent further precipitation. The concentration of the drug was determined spectrophotometrically. Each determination of the precipitation rate was made in duplicate.

## Thermal Analysis

Differential scanning calorimetry (DSC) was performed using a Pyris 1 (PerkinElmer, Norwalk, CT, USA). Approximately 10 mg of the sample was weighed into an aluminum pan which was crimped non-hermetically, and heated at a scanning rate of 10°C/min from 0°C to 300°C under nitrogen purge.

## X-ray Powder Diffractometry

X-ray powder diffraction (XRPD) patterns were collected on a Siemens D-5000 diffractometer using $CuK_{\alpha}$ radiation. The samples were scanned at a range between 2 and 40° 2θ at step 0.04° 2θ with an integration time of 1 s.

## Statistical Analysis

A statistical analysis was performed using the SPSS software program (Version 12.0.1, SPSS Inc., Chicago, 2004). The one-way ANOVA followed by the LSD (least significant difference) test for post hoc comparisons was applied for evaluating the influences of the hardening time, pH, and saturation of the calcium chloride solution on the drug content, bead size, and shape. The Games-Howell test was used instead of the LSD test when homoscedasticity of variances was not proven. The significance level was set at 0.05 for all the tests.

# RESULTS AND DISCUSSION

The aim of this study was to investigate the characteristics of alginate beads prepared by ionotropic gelation in which structurally similar drugs were incorporated. For this purpose theobromine and theophylline were selected as model drugs since the only structural difference between them is in the position of one methyl group.

## Bead Size

The beads were prepared by dropping the solution of the drug and alginate into a calcium chloride solution, where beads were formed due to the crosslinking of alginate by calcium ions. The beads were allowed to harden in a calcium chloride solution from 1 to 30 min. All the wet beads were spherical and transparent with a smooth surface regardless of the drug encapsulated. Their diameters amounted to about 4.5 mm. A trend towards a slight decrease in the size of the wet beads with prolongation of hardening time was observed. The differences in bead shape as well as size became evident during drying. The water content in wet beads was high, and the significant shrinkage of the beads appeared as a consequence of water removal during drying (Table 2). It was found that the hardening time significantly affected theophylline as well as theobromine bead size ($P < 0.001$). The shorter the hardening time, the lower the extent of bead shrinkage and thus bigger beads were obtained. Increasing the hardening time from 1 to 30 min resulted in a decrease of the mean values of the bead diameter from 2.75 ± 0.14 to 1.81 ± 0.07 and from 2.23 ± 0.04 to 1.88 ± 0.07 mm for theophylline and theobromine beads, respectively. Similar results were achieved by Martinsen et al. for calcium alginate beads of different alginate types (Martinsen et al., 1989) as well as Sriamornsak for the calcium pectinate beads containing

bovine serum albumin (Sriamornsak, 1998). The decrease in bead size with prolongation of hardening could be attributed to the higher strength of the alginate gel since more extensive alginate crosslinking with a longer hardening time occurred as has been reported (Aslani & Kennedy, 1996). From the cross-sections of wet beads immediately after isolation it was evident that after 1 min of hardening, only the surface was crosslinked while the interior of the beads was still liquid. With prolongation of hardening time, the amount of liquid interior decreased and after 10 min of hardening the whole bead was crosslinked.

**TABLE 2** Size and Shape Parameters of Beads

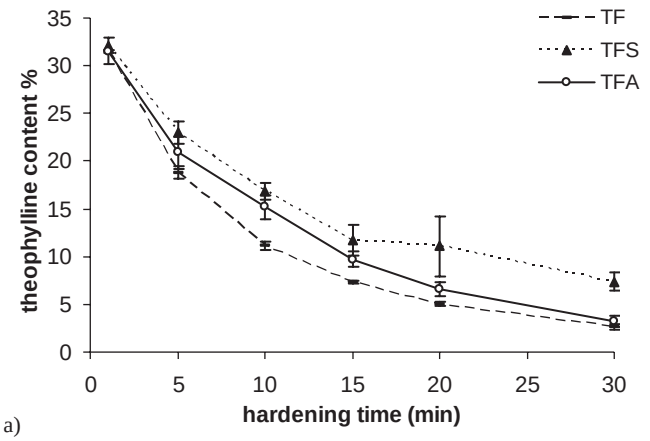
| | Dry beads | | |
|---|---|---|---|
| Sample | Mean diameter (mm) ± S.D. | Shape factor ± S.D. | Aspect ratio ± S.D. |
| $TB_1$ | 2.23 ± 0.04 | 1.08 ± 0.04 | 0.88 ± 0.03 |
| $TB_5$ | 2.04 ± 0.07 | 1.07 ± 0.03 | 0.85 ± 0.03 |
| $TB_{10}$ | 1.97 ± 0.05 | 1.06 ± 0.03 | 0.87 ± 0.03 |
| $TB_{15}$ | 1.93 ± 0.05 | 1.06 ± 0.03 | 0.86 ± 0.03 |
| $TB_{20}$ | 1.89 ± 0.05 | 1.07 ± 0.03 | 0.84 ± 0.04 |
| $TB_{30}$ | 1.88 ± 0.07 | 1.08 ± 0.04 | 0.83 ± 0.04 |
| $TB_{S5}$ | 2.07 ± 0.05 | 1.06 ± 0.02 | 0.85 ± 0.04 |
| $TB_{S10}$ | 1.98 ± 0.05 | 1.07 ± 0.03 | 0.84 ± 0.05 |
| $TB_{S15}$ | 1.91 ± 0.05 | 1.07 ± 0.03 | 0.83 ± 0.07 |
| $TB_{S20}$ | 1.88 ± 0.06 | 1.07 ± 0.03 | 0.84 ± 0.04 |
| $TB_{S30}$ | 1.84 ± 0.04 | 1.06 ± 0.04 | 0.83 ± 0.08 |
| $TB_{A5}$ | 1.88 ± 0.08 | 1.07 ± 0.03 | 0.86 ± 0.05 |
| $TB_{A10}$ | 1.93 ± 0.10 | 1.11 ± 0.08 | 0.82 ± 0.08 |
| $TB_{A15}$ | 1.85 ± 0.10 | 1.10 ± 0.06 | 0.84 ± 0.08 |
| $TB_{A20}$ | 1.91 ± 0.05 | 1.10 ± 0.04 | 0.83 ± 0.05 |
| $TB_{A30}$ | 1.81 ± 0.08 | 1.10 ± 0.05 | 0.89 ± 0.07 |
| | Dry beads | | |
| Sample | Mean diameter (mm) ± S.D. | Shape factor ± S.D. | Aspect ratio ± S.D. |
| $TF_1$ | 2.75 ± 0.14 | 1.20 ± 0.11 | 0.73 ± 0.06 |
| $TF_5$ | 2.42 ± 0.17 | 1.20 ± 0.09 | 0.62 ± 0.14 |
| $TF_{10}$ | 2.17 ± 0.14 | 1.17 ± 0.11 | 0.72 ± 0.12 |
| $TF_{15}$ | 2.05 ± 0.11 | 1.13 ± 0.06 | 0.77 ± 0.06 |
| $TF_{20}$ | 1.94 ± 0.08 | 1.10 ± 0.04 | 0.83 ± 0.03 |
| $TF_{30}$ | 1.81 ± 0.07 | 1.08 ± 0.03 | 0.80 ± 0.10 |
| $TF_{S5}$ | 2.54 ± 0.17 | 1.21 ± 0.12 | 0.67 ± 0.06 |
| $TF_{S10}$ | 2.14 ± 0.12 | 1.15 ± 0.10 | 0.73 ± 0.06 |
| $TF_{S15}$ | 1.89 ± 0.12 | 1.12 ± 0.06 | 0.81 ± 0.04 |
| $TF_{S20}$ | 1.87 ± 0.06 | 1.11 ± 0.05 | 0.83 ± 0.05 |
| $TF_{S30}$ | 1.89 ± 0.06 | 1.17 ± 0.40 | 0.81 ± 0.10 |
| $TF_{A5}$ | 2.54 ± 0.20 | 1.16 ± 0.07 | 0.59 ± 0.13 |
| $TF_{A10}$ | 2.06 ± 0.18 | 1.16 ± 0.08 | 0.78 ± 0.10 |
| $TF_{A15}$ | 1.89 ± 0.11 | 1.11 ± 0.06 | 0.82 ± 0.08 |
| $TF_{A20}$ | 1.92 ± 0.07 | 1.11 ± 0.06 | 0.72 ± 0.13 |
| $TF_{A30}$ | 1.88 ± 0.13 | 1.13 ± 0.08 | 0.71 ± 0.19 |

**TABLE 2** Continued

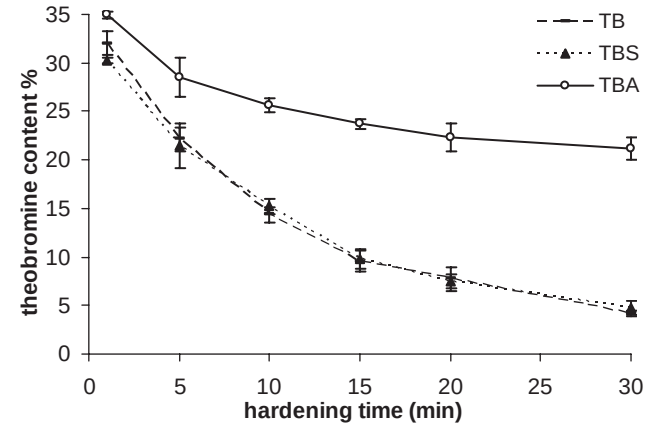
| | Wet beads | | Dry beads | |
|---|---|---|---|---|
| Sample | Mean diameter (mm) ± S.D. | Mean diameter (mm) ± S.D. | Shape factor ± S.D. | Aspect ratio ± S.D. |
| $A_1$ | 4.47 ± 0.08 | 1.86 ± 0.08 | 1.05 ± 0.02 | 0.90 ± 0.04 |
| $A_5$ | 4.39 ± 0.02 | 1.75 ± 0.05 | 1.05 ± 0.03 | 0.94 ± 0.05 |
| $A_{10}$ | 4.36 ± 0.06 | 1.75 ± 0.04 | 1.06 ± 0.02 | 0.90 ± 0.08 |
| $A_{15}$ | 4.21 ± 0.03 | 1.79 ± 0.04 | 1.05 ± 0.02 | 0.94 ± 0.03 |
| $A_{30}$ | 3.99 ± 0.07 | 1.78 ± 0.03 | 1.06 ± 0.02 | 0.89 ± 0.06 |
| $A_{A1}$ | 4.03 ± 0.09 | 1.61 ± 0.06 | 1.09 ± 0.04 | 0.84 ± 0.12 |
| $A_{A5}$ | 3.96 ± 0.09 | 1.60 ± 0.05 | 1.08 ± 0.04 | 0.88 ± 0.06 |
| $A_{A10}$ | 3.79 ± 0.06 | 1.69 ± 0.06 | 1.10 ± 0.06 | 0.75 ± 0.11 |
| $A_{A15}$ | 3.76 ± 0.09 | 1.71 ± 0.06 | 1.09 ± 0.04 | 0.81 ± 0.07 |
| $A_{A30}$ | 3.61 ± 0.08 | 1.70 ± 0.09 | 1.10 ± 0.08 | 0.84 ± 0.10 |

## Drug Content

The decrease in bead size with prolongation of hardening time could be ascribed to a lower drug content as a significant decrease in theophylline as well as in theobromine content with prolongation of hardening time was observed (Fig. 1). Similar findings were reported by some other authors (Acartürk & Takka, 1999; Garcia & Ghaly, 1996). The dramatic decline in the drug content was a result of drug diffusion through the crosslinked alginate gel into the calcium chloride solution which was confirmed by determining the drug amount in the hardening media. The sum of the amount of the drug that diffused into the hardening media and that determined in the beads was close to the amount of drug in the dispersion that was dropped into the hardening medium (data not shown). It was established that with prolongation of hardening time, the extent of the drug which diffused out of the beads increased. Furthermore, the increased amount of calcium ions in the crosslinked alginate matrix contributed to a decrease in the relative drug content. However, the influence of calcium ions on the drug content is probably of minor importance.




a)




b)

**FIGURE 1** **The Effect of Hardening Time on the Drug Content in (a) Theophylline and (b) Theobromine Beads Hardened in Medium 1 (TF or TB, Respectively), Medium 2 ($TF_S$ or $TB_S$, Respectively), or Medium 3 ($TF_A$ or $TB_A$, Respectively).**

**TABLE 3** **Solubility of Theophylline and Theobromine in 0.27 M Calcium Chloride Solution with Different pH at 25°C**

| | Solubility (mg/ml) | | |
|---|---|---|---|
| | 0.27M $CaCl_2$ pH = 1 | 0.27M $CaCl_2$ pH = 6 | 0.27M $CaCl_2$ pH = 9 |
| Theophylline | 6.22 ± 0.22 | 5.52 ± 0.10 | 5.61 ± 0.16 |
| Theobromine | 0.49 ± 0.01 | 0.46 ± 0.02 | 0.46 ± 0.02 |

A small difference between the drug content of theobromine and theophylline in beads was observed (Fig. 1). Theobromine is very slightly soluble in a calcium chloride solution (Table 3). Moreover, it is 12 times less soluble than theophylline; therefore, a higher encapsulation efficiency in theobromine beads was expected.

## *Saturation of the Hardening Medium with the Drug*

It was reported in the literature that the loss of the drug during hardening of the beads could be minimized by dropping alginate and drug dispersion into a calcium chloride solution saturated with the drug (Rousseau et al., 2004; Østberg et al., 1993). In contrast to those reports, saturation of the calcium chloride solution with the drug resulted in a noticeable increase but only in the case of theophylline content. Theobromine content did not increase significantly ($P > 0.05$). After a few minutes of hardening of beads, a white precipitate in the hardening medium was observed. Since this precipitation did not occur when preparing empty beads, the precipitation of substances other than drugs including the calcium hydroxide could be eliminated. The precipitation of calcium hydroxide was not even expected as the concentration of hydroxyl ions in the hardening media with 0.27 M concentration of calcium ions was too low to initiate the precipitation of calcium hydroxide. On this basis, we suppose that the observed precipitation in hardening medium could be ascribed to theophylline and theobromine. This means that the drugs diffused out of the beads and precipitated. Theobromine and theophylline concentrations (35 mg/mL) in alkaline alginate solution were substantially higher than in the hardening medium 2 (approximately 0.5 and 5.5 mg/mL for theobromine and theophylline, respectively, Table 3), thus significant concentration gradient existed. The diffusion was possible in spite of the fact that drug concentration in the hardening medium was equal to solubility as the dissolved drug was removed by precipitation. Consequently, a further diffusion of the dissolved drug into the hardening medium was enabled and as a result the increase in the drug content was lower than expected. The greater influence of the saturation of calcium chloride with the drug for theophylline was probably due to higher theophylline solubility and lower concentration gradient between the beads and the hardening medium.

## *Acidification of the Hardening Medium*

On the other hand, an extreme improvement in the theobromine content in the beads, prepared by dropping the initial dispersion into a calcium chloride solution acidified to pH 1 (medium 3), was observed. The theophylline content in $TF_A$ beads hardened in

medium 3 was also significantly higher than in TF beads hardened in medium 1 ($P < 0.05$); however, the increase was not as marked as in theobromine beads. We suppose that theobromine precipitated inside the beads due to acidification of the interior of beads as they became white already after a few seconds of hardening. The whitening of the beads could also occur due to the formation and precipitation of the insoluble alginic acid. Only slight whitening of the beads was observed when the empty beads were hardened in medium 3; thus, it could be concluded that some alginate did precipitate in the form of alginic acid. However, the whitening in the $TB_A$ beads was far more intensive suggesting the precipitation of the drug (Fig. 4b and 4d). This was not expected as theobromine is even slightly more soluble in an acidic than in an alkaline calcium chloride solution (Table 3). However, it was found that the rate of theobromine precipitation was much higher in an acidic medium as at pH 9 (Fig. 2). As a result of theobromine precipitation, its diffusion out of beads was much slower as reflected in better drug entrapment compared to TB beads hardened in medium 1. The difference in the drug content between beads TB and $TB_A$ increased with increasing hardening time as at the beginning of hardening when most of the theobromine was still dissolved and was able to diffuse into a calcium chloride solution. On the other hand, theophylline precipitated more slowly and almost independently of the pH of the calcium chloride solution (data not shown); therefore, only a slight improvement in encapsulation efficiency was observed.

Additionally, the formation and precipitation of alginic acid inside the beads might have some influence on drug content due to a modification of internal structure of the beads and thus hindered drug diffusion into the hardening medium. Acidification of hardening medium could also contribute to higher drug content by reducing electrostatic repulsion between the drugs and alginate. Regarding ionization constants [8.77 (pKa), 13.5 and 11.5 (pKb) for theophylline, and 10.05 (pKa) and 13.89 (pKb) for theobromine] (O'Neil et al., 2001), both drugs are negatively charged in alkaline. Since alginate is also ionized at these pH values, it could be possible that due to repulsion between negatively charged groups the drugs diffused faster into the medium 1 comparing to acidic hardening medium.

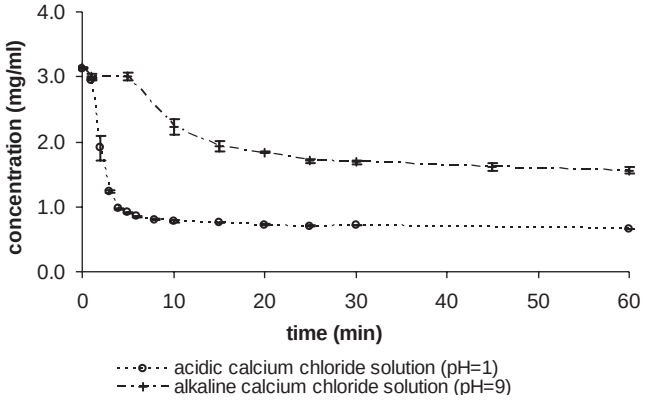



**FIGURE 2** **The Precipitation Rate of Theobromine in Acidic (pH 1) or Alkaline (pH 9) 0.27 M Calcium Chloride Solution.**

## Bead Shape and Morphology

The greatest differences between alginate beads loaded with drugs were definitely evident in bead shape and morphology (Fig. 3). As mentioned above, all the beads were spherical and transparent with a smooth surface immediately after isolation. However, during drying especially, the theophylline beads changed markedly. The modifications in shape and morphology were more perceptible in beads with a higher drug content–i.e., in beads with a shorter hardening time. The longer the hardening time, the smoother and the more spherical were the beads obtained. For instance, beads $TF_1$ were completely white and strongly wrinkled while beads $TF_{30}$ were yellow and their surface was smooth (Figs. 3a and c, respectively). Furthermore, incomplete crosslinking of theophylline beads at a shorter hardening time was reflected in the concavity of the beads. The irregularity of the shape of theophylline beads is additionally confirmed by the rather low values of the shape factor and the higher values of the aspect ratio (Table 2). With increasing hardening time, the values of these two parameters approached 1 which means that the shape of the beads extremely improved.

On the other hand, theobromine beads were not subjected to such alterations in shape and morphology during drying (Figs. 3d, e, and f). Their surface was rough but not wrinkled. All the beads remained round and spherical as can be seen also from the mean values of the aspect ratio and shape factor (Table 2). The beads which were hardened for a shorter period of time (1–6 min) were slightly flattened owing to only partial crosslinking which was evident from the liquid

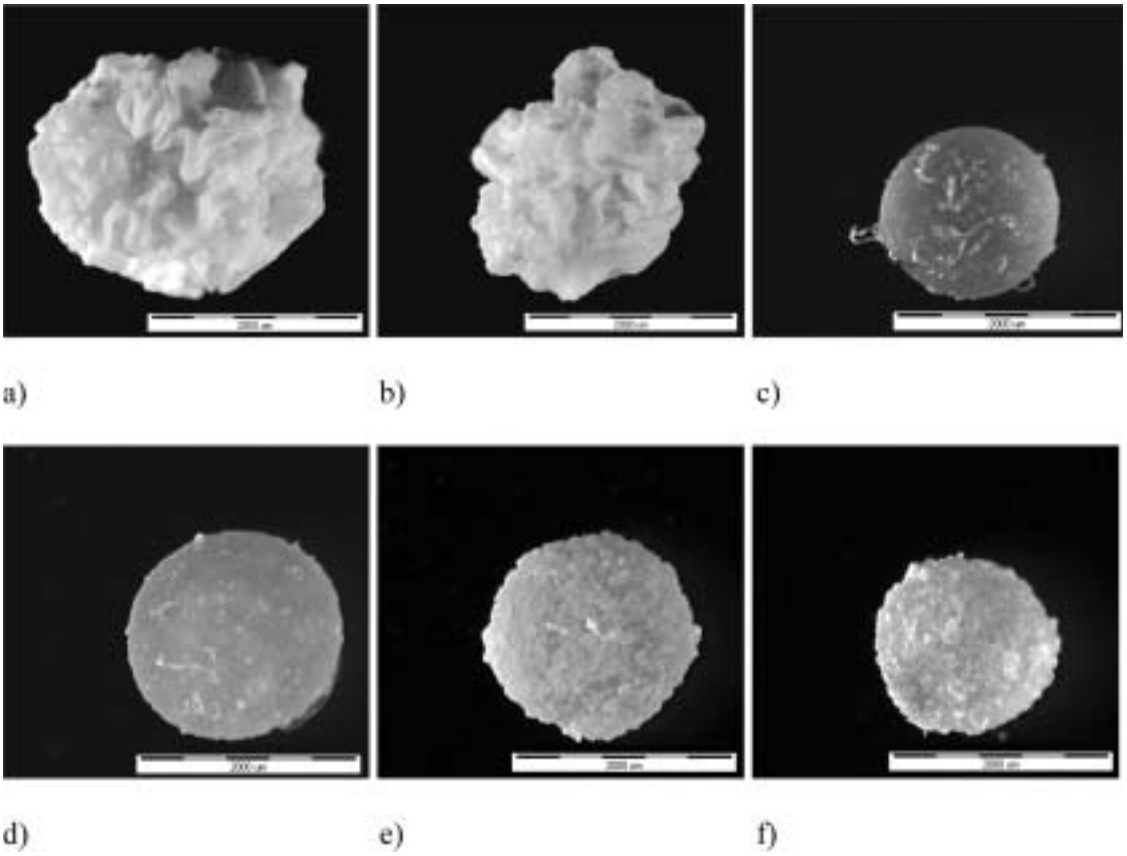



**FIGURE 3** **Stereomicrographs of TF (a, b, c) and TB (d, e, f) Beads Hardened in 0.27 M $CaCl_2$ Solution for 1 Min (a, d), 5 Min (b, e), and 30 Min (c, f). The Bar Corresponds to 2000 μm.**

interior of the bead cross-section immediately after isolation.

Furthermore, it was found that acidification of the calcium chloride solution greatly affected bead appearance. The influence of the pH of the hardening media on beads properties was obvious even on empty beads. The wet empty beads were spherical and smooth regardless of hardening media used. However, the beads hardened in the acidic medium were markedly smaller and softer comparing to the beads prepared using the medium 1 (Table 2). After drying, the beads hardened in medium 3 had a rougher surface and were more flattened on the side on which they dried (Fig. 4c) as the beads hardened in medium 1 (Fig. 4d). The smaller bead diameter is related to nonionization of carboxylic alginate groups leading to a reduction in electrostatic repulsion between the alginate chains (Velings & Mestdagh, 1995). Some amount of the alginate could even precipitate in the form of insoluble alginic acid since a slight whitening of the beads was observed during hardening in medium 3. Moreover, the affinity of calcium ions to form calcium alginate was probably lower than in medium 1 due to nonionization of carboxylic groups resulting in lower degree of alginate crosslinking. All of these probably contributed to different shapes of drug loaded beads as well. In contrast to theophylline beads $TF_A$ (Fig. 4a), which were flattened and curved, theobromine beads $TB_A$ were spherical; they were flattened only on the side on which they dried (Fig. 4b). Preparation of beads by dropping the alginate and drug solution into a calcium chloride solution saturated with the drug did not influence bead shape.

Such a difference between theophylline and theobromine bead shape was not expected. Theophylline and theobromine are both crystalline drugs; however, they differ in the size and shape of their particles. Acicula-like theophylline crystals that measure around 200 μm are approximately 10 times larger than cubic-shaped theobromine crystals. In order to avoid the influence of the different size and shape of theophylline and theobromine particles on bead shape, the drugs were dissolved in an alginate solution. It is true that wet beads did not differ; however, after drying,

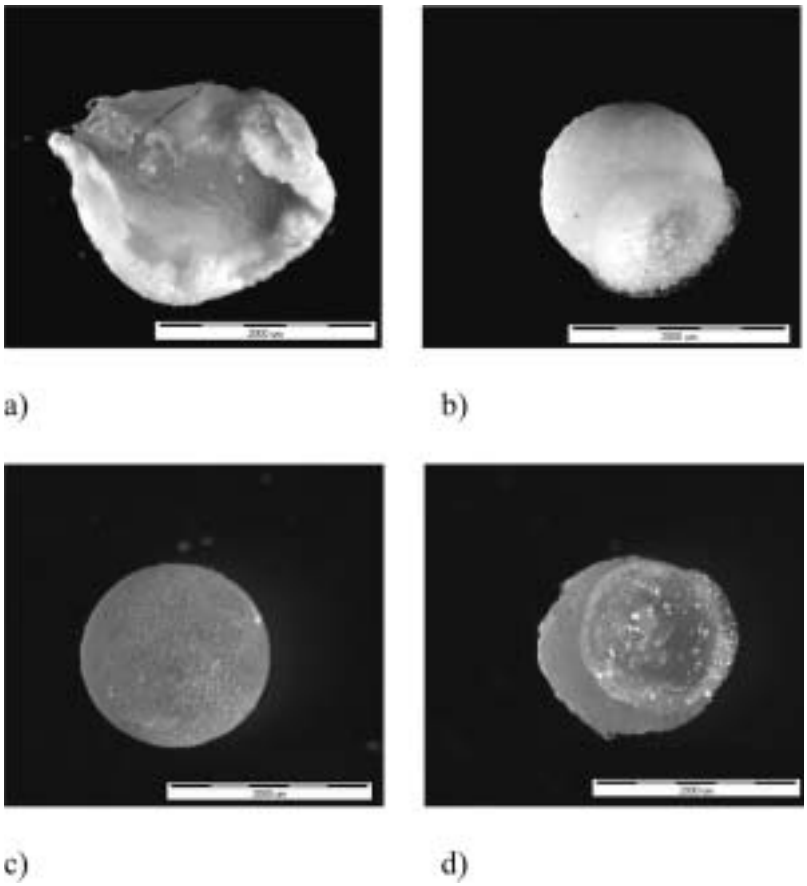



**FIGURE 4** Stereomicrographs of (a) Theophylline Beads $TF_A$, (b) Theobromine Beads $TB_A$, (c) Empty Beads $A_A$ Hardened in an Acidic Calcium Chloride Solution for 5 Min, and (d) Empty Beads Hardened in 0.27 M $CaCl_2$ Solution for 5 Min. The Bar Corresponds to 2000 mm.

beads of completely dissimilar form were obtained. In the SEM photographs of theophylline beads, acicular particles of approximately 20 μm were visible on the bead surface (Fig. 5a). On the other hand, on the surface of theobromine beads no crystalline forms could be seen (Fig. 5b). Although the interior of the beads was dense and smooth without any visible crystals (particles), the differences in the internal structure of theophylline and theobromine beads could not be excluded. However, the influence of different crystals is probably of minor importance because a very low degree of crystalline drug appeared in the beads as explained later.

In order to estimate the importance of the presence/absence of crystals, XRPD and DSC analyses were performed.

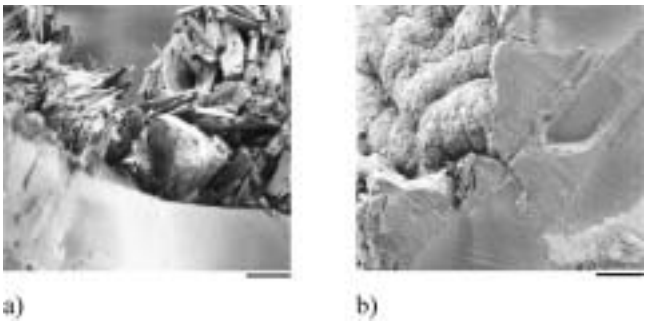



**FIGURE 5** SEM Photographs of Parts of the Cross-section of (a) TF and (b) TB Beads Hardened for 1 Min in 0.27 M $CaCl_2$ Solution. The Bar Corresponds to 20 μm.

## XRPD Analysis

The x-ray powder diffraction (XRPD) analysis of theobromine beads showed that some amount of drug was present in a crystalline state in all beads (Fig. 6). However, the intensity of diffraction peaks corresponding to theobromine in XRPD patterns of the beads hardened in acidic calcium chloride was significantly higher than diffraction peaks of beads hardened in medium 1.

On the other hand, the XRPD patterns of theophylline beads hardened in medium 1 were not comparable to XRPD patterns of theophylline beads prepared

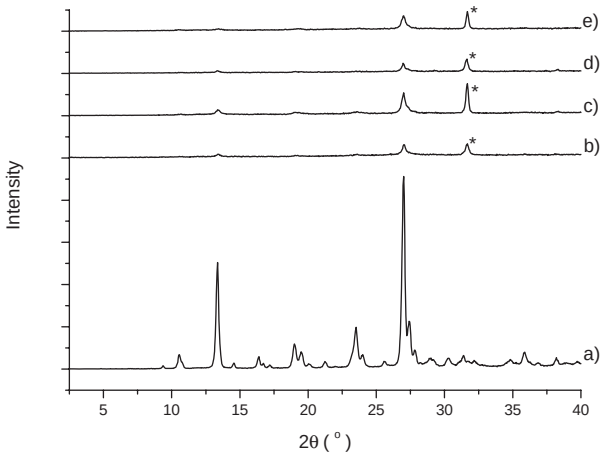



**FIGURE 6** **XRPD Powder Patterns of (a) Pure Crystalline Theobromine and Beads, (b) $TB_1$, (c) $TB_{A1}$, (d) $TB_5$, and (e) $TB_{A5}$. Diffraction Peaks Marked with an Asterisk Belong to Sodium Chloride.**

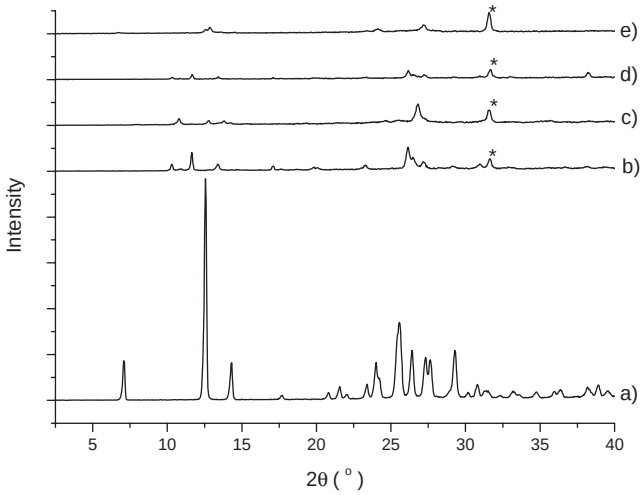



**FIGURE 7** **XRPD Powder Patterns of (a) Pure Crystalline Theophylline and Beads, (b) $TF_1$, (c) $TF_{A1}$, (d) $TF_5$, and (e) $TF_{A5}$. Diffraction Peaks Marked with Asterisks Belong to Sodium Chloride.**

in acidic hardening medium and did not match with the diffraction pattern of pure crystalline theophylline (Fig. 7). It is suggested that the beads may contain some complex form of theophylline with $Ca^{2+}$ ions since the identity of theophylline in the beads was proven by the HPLC method (data not shown). This suggestion would require further investigation. Additionally, the XRPD pattern of theophylline beads hardened in medium 3 for 1 min also showed the presence of pure crystalline theophylline which was not present in the beads after 5 min of hardening.

Nevertheless, both drugs were mostly in an amorphous state as can be seen from the low intensity of the diffraction pattern of theobromine and theophylline in the beads. Furthermore, it can be seen that the intensity of diffraction peaks decreased with the prolongation of hardening time. Taking into account decreasing drug content with increasing hardening time, it can be concluded that the degree of drug crystallinity is probably related to the drug content in the beads.

## DSC Analysis

Differential scanning calorimetry (DSC) thermograms of theophylline, alginate, and theophylline beads are shown in Fig. 8. Since theobromine sublimes prior to melting and its melting point is above the temperature of alginate decomposition, only an XRPD analysis of theobromine and its beads was made.

Crystalline theophylline melts at 271°C which is characterized by a sharp endothermic peak. The thermogram of sodium alginate shows a broad exotherm at approximately 230°C which represents its decomposition. In the case of calcium alginate beads, a shift of the alginate decomposition peak to higher temperatures was observed, as has already been reported by Gonzales-Rodriguez et al. (2002), depending on the hardening time of beads. Furthermore, no theophylline melting point could be seen in the beads even though some crystallinity of presumably the $Ca^{2+}$ complex or some form of crystalohydrate of theophylline in beads was confirmed by XRPD. This was probably due to the overlapping of the alginate decomposition exotherm and theophylline endotherm or too low amount of crystalline theophylline. The exception were beads $TF_{A5}$ where a very small endothermic peak at 259°C was observed that may represent a theophylline melting peak. This peak is shifted toward lower temperatures because of theophylline interactions with the other components. The results of DSC analyses are therefore in accordance with the XRPD results that most of the drug was in amorphous state, and that crystalline theophylline represented only a diminutive quantity of the whole drug incorporated in beads.

Nevertheless, theobromine and theophylline beads differed enormously in shape. The selected drugs differentiated in some physico-chemical characteristics like solubility and rate of precipitation as well as the size and shape of drug particles. The influence of the first two characteristics was especially evident in drug entrapment efficiency while the last one–the size and shape of the drug particles–could be accountable for the different shape formation of beads during drying. However, as

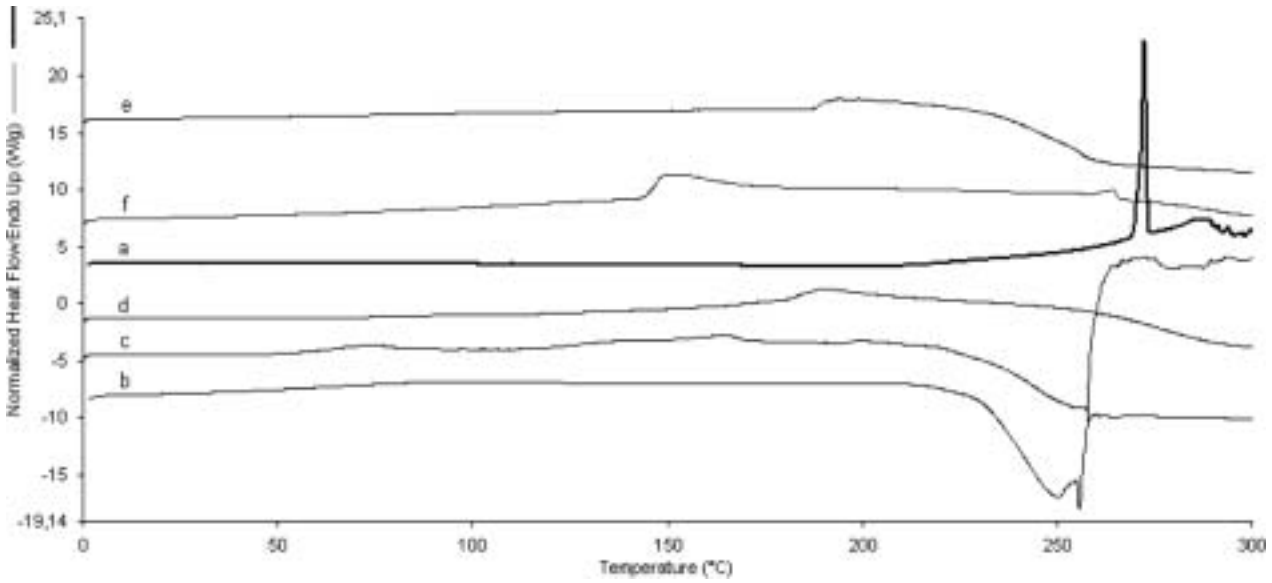



**FIGURE 8** **Thermograms of (a) Pure Crystalline Theophylline, (b) Alginate and Beads, (c) $TF_1$, (d) $TF_5$, (e) $TF_{A1}$, and (f) $TF_{A5}$.**

ascertained by XRPD and DSC analyses, the degree of drug crystallinity was very low. Because of the fact that beads of completely different shape were formed when incorporating two structurally similar drugs, it is suggested that some drug–alginate interactions could be present in beads and might be responsible for the observed shape differentiation. These interactions might appear already during preparation and drying of the beads and might have influence on different processes like gelation kinetics and, consequently, on shape and morphology of each type of beads.

Overall, all these findings verify that the preparation of beads by the ionotropic gelation method cannot be generalized, even though structurally similar drugs are incorporated. Therefore, the bead preparation procedure has to be examined for each drug separately in order that the optimal preparation conditions, which provide beads with the required characteristics, are defined.

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