Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 299 (2005) 134–145
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Cross-linking of dried paracetamol alginate granules Part 1. The effect of the cross-linking process variables

D. Mukhopadhyay [a], M. Reid [b], D. Saville [a], I.G. Tucker [a,*]

[a] *School of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand*
[b] *Department of Chemistry, University of Otago, Dunedin, New Zealand*

Received 23 September 2004; received in revised form 5 May 2005; accepted 18 May 2005

Abstract

This paper reports on the cross-linking of dried (<5% moisture) paracetamol alginate granules with calcium chloride solutions. The effect of calcium concentration, temperature of the treatment solution, stirring speed and time used during cross-linking of granules on water uptake by the granules during cross-linking and physical properties of the cross-linked and dried granules were studied. A full factorial study of these factors each at two levels was used ($CaCl_2 \cdot 2H_2O$: 20, 100 mg/ml; temperature: 25, 45° C; stirrer speed: 25, 240 rpm; time: 1.5 and 5.5 min) to treat dried stock granules (size: 0.8–1.0 mm) containing the model drug paracetamol and sodium alginate powder (1:1) which were prepared using conventional aqueous granulation under low shear. In addition to SEM and photomicrography, the physical properties studied were water uptake during cross-linking, yield, aggregation behaviour, moisture content, drug content, early stage drug release [over 10 s (R10) and the next 50 s (R50)] and calcium and sodium content of the unwashed cross-linked granules. Dry granules were successfully cross-linked. The treatment factors significantly affected most of the response variables. The variables most affected were water uptake (78–254%), drug entrapment (58–86%), early release (R10: 1.2–6.4% and R50: 3.0–12.2%), granule aggregation (0–70%), calcium (6.02–12.4%) and sodium content (1.2–6.44%). SEM photographs suggest that low calcium treated granules were less porous in nature compared to high calcium treated granules. Low shear drug alginate granules can be cross-linked in dried state. The properties of the cross-linked granules can be modified by altering the treatment process.

Keywords: Factorial study; Treatment conditions; Reduced swollen state early stage drug release

1. Introduction

Matrix type drug delivery systems have been used to control drug release for different purposes, e.g. sustained action (Baker, 1987) and taste masking (Albertini et al., 2004). Such systems are simple to prepare and have been in use for many years (Baker, 1987).

Drug alginate matrices are currently prepared by spraying or dropping an alginate drug aqueous dispersion into a bath of calcium chloride solution for cross-linking (Aoki et al., 1993; Aslani and Kennedy,

* Corresponding author. Tel.: +64 3 479 7296; fax: +64 3 479 7034.
E-mail address: ian.tucker@stonebow.otago.ac.nz (I.G. Tucker).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.05.013

1996; Kikuchi et al., 1997, 1999; Acarturk and Takka, 1999; Pillay and Fassihi, 1999). Water is lost during this cross-linking and it has been found that it takes 70 h to reach equilibrium water content (Yotsuyanagi et al., 1987). Circular dichroism suggest a molecular rearrangement in the highly hydrated matrix depending upon the cation used and the time used for cross-linking (Morris et al., 1978; Thom et al., 1982). However, it has been reported that the calcium ion content increases, replacing the sodium ions in the sodium alginate matrices and attains equilibrium within 6 min of starting the cross-linking process (Kim and Lee, 1992). Preparing granules in this fashion is cumbersome and involves large quantities of water. Also the process is slow due to long times needed to spray or deliver the drug alginate dispersion into the calcium solution. The pellets prepared by this method are usually porous (Duez et al., 2000; Hills et al., 2000; Richey, 2001). A reason could be that they lose water rapidly during drying and shrink leading to formation of cracks or fissures. Pores of size 50 μm have been reported (Hills et al., 2000). Air drying or vacuum drying at low temperatures has been used to overcome this problem (Hills et al., 2000) but this further slows the production process. Also, pellets cross-linked under a highly swollen state are less dense than polymer networks prepared under reduced swollen state (Richey, 2001). Pellets of unsuitable size can be produced if the droplet size is not controlled during pellet cross-linking in the calcium chloride bath. It is very difficult to recover drug from the pellets of unwanted size, once formed (cross-linked). Furthermore the pellets may entrap large amounts of calcium chloride (commonly used for cross-linking) which can make the granules hygroscopic. Given these problems there is need for an alternative method for cross-linking alginate to produce drug alginate matrices.

It appears that if it were possible to cross-link drug alginate granules under reduced swollen state (dried granules) then it would solve some of the problems of the current method. However, cross-linking of films (thickness about 50–60 μm) became slower when calcium solutions containing in excess of 0.5 M calcium were used (Julian et al., 1988). Apart from cross-linking of films (Julian et al., 1988; Remunan-Lopez and Bodmeier, 1997; Richey, 2001) there has been no work dealing with cross-linking of granules or pellets containing drug. So it remains to be seen if it is possible to cross-link dry granules containing drug and alginate mixtures.

This paper describes research on the cross-linking of paracetamol alginate matrices in the dry state (about 5% moisture content). A factorial design at two levels was undertaken to assess important process variables which influence the properties of the granules. The independent variables studied were calcium ion concentration, temperature, duration of treatment and level of agitation. The response variables were water uptake during cross-linking, drug entrapment, early stage drug release, extent of cross-linking (by analysis of sodium and calcium) and aggregation behaviour of granules.

2. Materials and methods

2.1. Materials

Paracetamol was obtained from BDH Poole UK (calcium: not detected; sodium: not detected), and Keltone HVCR [alginate, 400 cps (approx.); milled fine PS; medium G; calcium: 0.2%; sodium: 12.1% (based on dry weight)] from ISP Alginates USA. All other materials used were of analytical grade.

2.2. Preparation of pretreatment granules

A uniform dry mix (1:1) of paracetamol and Keltone HVCR milled (both passed through 800 micron mesh) was granulated in a planetary mixer (a low shear mixer) using water (0.725 ml/g of powder mass) and dried at 55–60 °C for 12–15 h, de-dusted and a size fraction of 0.8–1.0 mm selected. The pretreatment granules [moisture: 4.2%; drug (as is basis): 49.7%] were stored in a dessicator over silica gel until cross-linking.

2.3. Factorial design of the cross-linking treatment process

A complete factorial design for four factors at two levels (2^4) was carried out in duplicate ($n = 2$) ($2^4 \times 2 = 32$ batches). The details of the factorial study are shown in Table 1.

2.4. Cross-linking treatment of granules

The cross-linking of granules was carried out in a cylindrical thermostated stainless steel vessel of 100 ml

Table 1
Factorial study design

Factors	Low level	High level
(I) Factorial design		
Calcium chloride dihydrate (Ca concentration) (mg/ml)	20	100
Temperature of treatment solution (temperature) (°C)	25	45
Stirring time (time) (s)	30	300
Stirrer speed (speed) (rpm)	25	240

Trial no.	Ca concentration	Temperature	Time	Speed
(II) Treatment conditions used in the factorial study				
1	20	25	30	25
2	100	25	30	25
3	20	45	30	25
4	100	45	30	25
5	20	25	300	25
6	100	25	300	25
7	20	45	300	25
8	100	45	300	25
9	20	25	30	240
10	100	25	30	240
11	20	45	30	240
12	100	45	30	240
13	20	25	300	240
14	100	25	300	240
15	20	45	300	240
16	100	45	300	240

capacity with a paddle type stirrer. The stirrer blade was 10 mm above the bottom of the vessel. Batches of granules ($W_3 = 6$ g, moisture content about 5%) were treated as per conditions given in the factorial design (Table 1). The ratio of untreated granules to treatment solution was kept constant (about 1:10, w/v). Granules were immersed in the calcium chloride solution for 60 s in the case of 30 s stirring time treatment, with 15 s non-stirred periods before and after stirring and prior to filtration. Similarly, granules were immersed in calcium chloride solution for 330 s in the case of the 300 s treatment. At the end of the treatment process, the wet granules were filtered through a G1 sintered glass funnel under suction for 60 s. The weight of the wet granules before (W_1) and after drying (W_2) at 55–60 °C for 15 h were determined.

2.5. Water uptake, yield and moisture content of granules

Water uptake by the granules as a percentage was determined gravimetrically as $100(W_1 - W_2)/W_2$. The yield as a percentage was determined as $100W_2/W_3$.

Moisture content was determined gravimetrically (Yotsuyanagi et al., 1987) by exposing aliquots (250 mg; $n = 3$) of dried cross-linked granules to 110 °C in an oven for 10 h. Moisture content was calculated as $100(W_4 - W_5)/W_4$, where W_4, W_5 are the initial and final weight of the granules taken for moisture content determination.

2.6. Drug entrapment in cross-linked granules

Drug entrapment (DE) was estimated by spectrophotometric assay ($\lambda = 257$ nm) after extraction of drug from granules ($n = 3$) in phosphate buffer under stirring followed by suitable dilution in 0.1 M sodium hydroxide prior to analysis. The quantity of drug obtained was expressed as

$$\mathrm{DE} = 100 \times \left(\frac{\text{total mass of drug present in } W_2 \text{ g of granules}}{\text{total mass of drug present in } W_3 \text{ g of granules}} \right)$$

2.7. Early stage drug release from cross-linked granules

Drug release from the granules into water (80 ml, 25 °C) was estimated for the first 10 s and subsequent 50 s using an early stage drug release apparatus (Mukhopadhyay and Tucker, 2003). Granules containing about 35 mg of paracetamol were used at a stirrer speed of 240 rpm with stirrer depth 5 mm above the wire mesh of the sample holder. Analysis was carried out spectrophotometrically ($\lambda = 257$ nm) immediately after dilution with 0.1 M sodium hydroxide ($n = 3$).

2.8. Estimation of calcium and sodium content in cross-linked granules

About 250 mg of the sample granules (cross-linked granules, sodium alginate or drug) were dissolved in 10 ml of concentrated nitric acid by boiling. The samples were diluted in 1% nitric acid solution containing 1000 ppm lanthanum (matrix modifier) and

caesium (ionization suppressant) for calcium and sodium, respectively. The calcium and sodium content were determined in the linear region (0–30 ppm, $\lambda = 422.7$ nm for calcium and 0–1 ppm, $\lambda = 588.8$ nm for sodium) by a standard addition method using atomic absorption spectroscopy (Perkin-Elmer A Analyst 100 USA). The sodium and calcium content refers to the metal content of unwashed granules in the case of treated granules unless indicated otherwise.

2.9. Aggregation behavior

About 450 mg of cross-linked granules was sorted manually to separate the aggregated granules. The mass of aggregates was expressed as a percentage of the total mass of granules taken. The aggregated granules were then pressed with a spatula on a flat glass surface to determine if individual granules could be separated from the aggregated mass without crushing the individual granules.

2.10. Microscopic, and photographic characterization of cross-linked drug alginate granules

Cross-linked granules were sputter coated (BioRad SEM coating, UK) with a thin gold–palladium layer and investigated with a Cambridge Stereoscan S360 scanning electron microscope (SEM, Cambridge, UK) which was operated with an acceleration voltage of 10 kV.

The microphotography of the selected cross-linked granules in water was carried out at selected times using a camera (Nikon Cool pix 990, Digital imaging system, Tokyo, Japan) mounted on a compatible microscope (Nikon Optiphot, Tokyo, Japan).

Particle size of 100 granules from stock untreated granules and Trials 1, 2, 15 and 16 (Table 1) were determined by measuring the average length and breadth using optical microscopy.

2.11. Data Analysis

A balanced ANOVA was performed unless mentioned otherwise on the results using Minitab 12.1 Pennsylvania USA. *P*-values <0.05 were considered significant.

3. Results

3.1. Water uptake during cross-linking, yield and moisture content of granules after cross-linking

3.1.1. Water uptake

During the cross-linking process water uptake by the granules ranged from 78 ± 1.52 to $254 \pm 6.56\%$. The water uptake during treatment was reproducible depended on calcium chloride concentration used for treatment of granules. The other factors, treatment solution temperature, stirring time, and stirrer speed did not affect the water uptake significantly (Fig. 1).

3.1.2. Yield

In general high calcium treated batches showed higher yield compared to the corresponding batches prepared with low calcium. The average granule yield ranged from 80% (Trial 15) to 92% (Trial 1) for low calcium granules and from 89% (Trial 16) to 100% (Trial 2) for high calcium treated granules (Table 1). The yield reduced when the granules were exposed to high temperature, high stirring speed and longer stirring time during treatment compared to low levels of these factors.

In order to study the granule erosion process the granule particle sizes of the above trials were studied. The average particle size ($L \times B$) of untreated granules, Trials 1, 2, 15 and 16 were $1627 \pm 197\ \mu m \times 981 \pm 112\ \mu m$, $1250 \pm 199\ \mu m \times 932 \pm 118\ \mu m$, $1328 \pm 222\ \mu m \times 955 \pm 104\ \mu m$, $1052 \pm 106\ \mu m \times 829 \pm 78\ \mu m$ and $1341 \pm 189\ \mu m \times 935 \pm 116\ \mu m$, respectively. The low calcium short time treated granules were larger than the corresponding low calcium granules treated for longer times under high stirrer speed and high temperature of treatment solution temperature. Such a difference was not observed between the high calcium treated granules (i.e. Trials 2 and 16). In general the reduction was more in length than breadth.

3.1.3. Moisture content

The average moisture contents ranged from 3.9 to 4.6% for low calcium trials and 4.4–5.7% for high calcium trials.

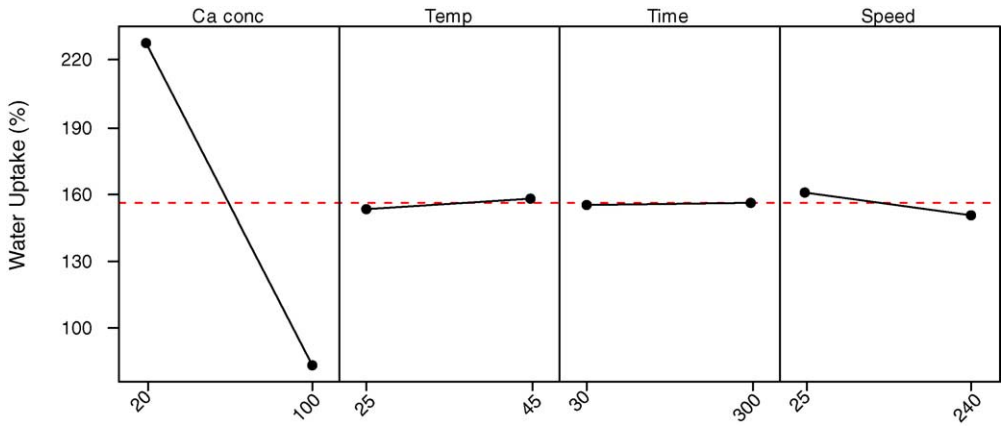

Fig. 1. Effect of treatment conditions on the uptake of water by the granules during treatment process. Data are treatment means.

3.2. Drug entrapment

Granules undergoing cross-linking lost about 11–26% drug depending upon the treatment conditions used. Low calcium concentration, higher temperature of the treatment solution, higher agitation rate and longer time lead to low drug entrapment (Fig. 2). The average drug entrapment ranged from 58% (Trial 15) to 79% (Trial 1) for low calcium treated granules and from 64% (Trial 16) to 86% (Trial 2) for high calcium treated granules. The effect of calcium concentration on drug entrapment was lower

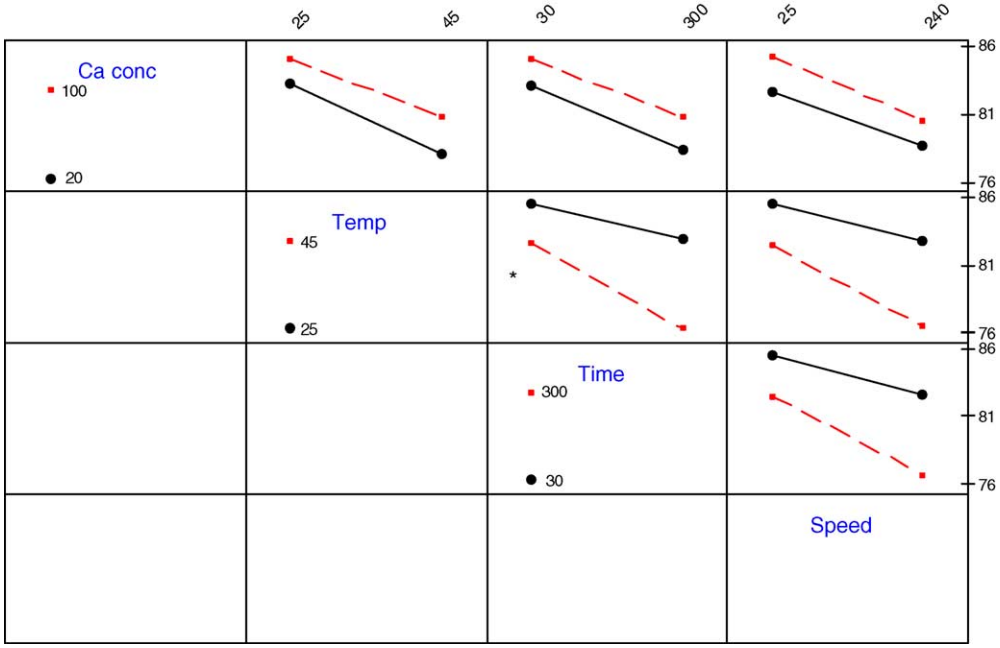

Fig. 2. Interaction plots showing the effect of treatment conditions on drug entrapment (%) of drug-alginate granules during treatment process. For example 'asterisk' marks cell 2, 3 which shows the temperature × time interaction and indicates that stirring time had a greater effect at 45 °C (greater slope) than at 25 °C.

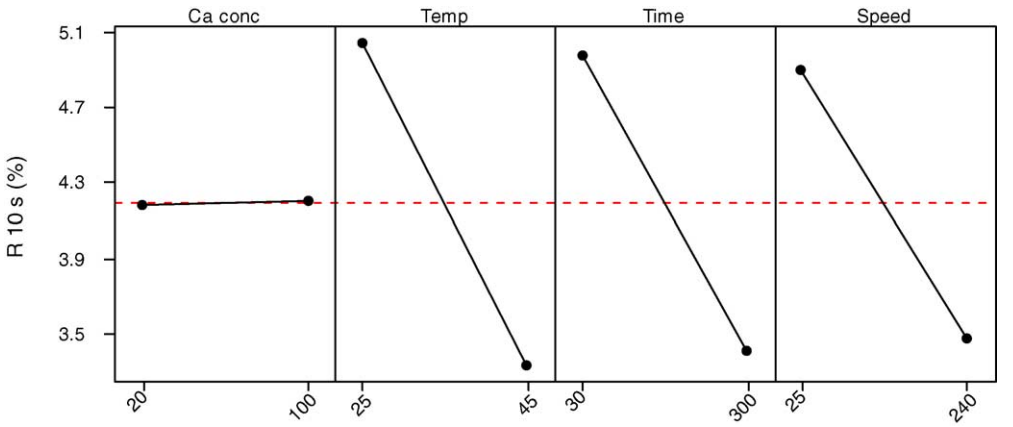

Fig. 3. Effect of treatment conditions on the early stage (10 s) drug release (R10 s) from cross-linked drug-alginate granules using early stage drug release apparatus.

than the other three factors, e.g. treatment solution temperature, agitation rate and time (Fig. 2).

Significant interactions were observed between treatment solution temperature and stirring time (Fig. 2, cell 2, 3) and between treatment solution temperature and stirring speed (Fig. 2, cell 2, 4). The other significant interaction was stirring time × stirrer speed (Fig. 2, cell 3, 4). Some three factor interactions were observed but they were of no practical importance. Four factor interactions were not significant.

3.3. Early stage drug release

Lowest drug release was observed for granules prepared under high treatment solution temperature, high stirrer speed and high stirring time compared to the granules prepared under low levels of these factors (Fig. 3). During treatment the former granules lost higher amounts of drug compared to the latter granules (Fig. 2) and this resulted in lower early stage drug release from the former granules.

The effect of calcium concentration on R10 release is more complex. Although on average, calcium content had no effect on 10 s, release (Fig. 3) there were significant interactions (Fig. 4, cell 1, 3); hence, granules treated with low calcium for short times showed higher 10 s release than those treated with high calcium for short time. This effect was reversed at longer treatment times. Drug release over the subsequent 50 s was significantly affected by all treatment conditions (Fig. 5) with high calcium treatment leading to higher release in the 50 s period. These granules under scanning electron microscope appeared to be more porous (Fig. 6).

3.4. Microphotography

When untreated granules were exposed to low calcium treatment solution (20 mg/ml) the edges of the granules did not break or fall apart at short times (20 s) under a microscope. Usually during swallowing sample residence time in our mouth is about 30 s (Taylor, 1996). Rupture of granules within this period can potentially cause unpalatability if it contains an unpalatable drug. Additionally high calcium short time treated granules and granules treated with low calcium for long times did not break down at short times in water (e.g. 20 s). Only granules treated with low calcium for short times (Trial 1 and 9) broke down after coming into contact with water.

3.5. Calcium and sodium contents of the cross-linked granules

The calcium and sodium levels of the treated granules were significantly inversely correlated ($r = -0.932$; $p = 0.0001$; Fig. 7). The granules treated with high calcium treatment solution showed higher calcium levels and lower sodium levels compared to the corresponding granules treated with low calcium

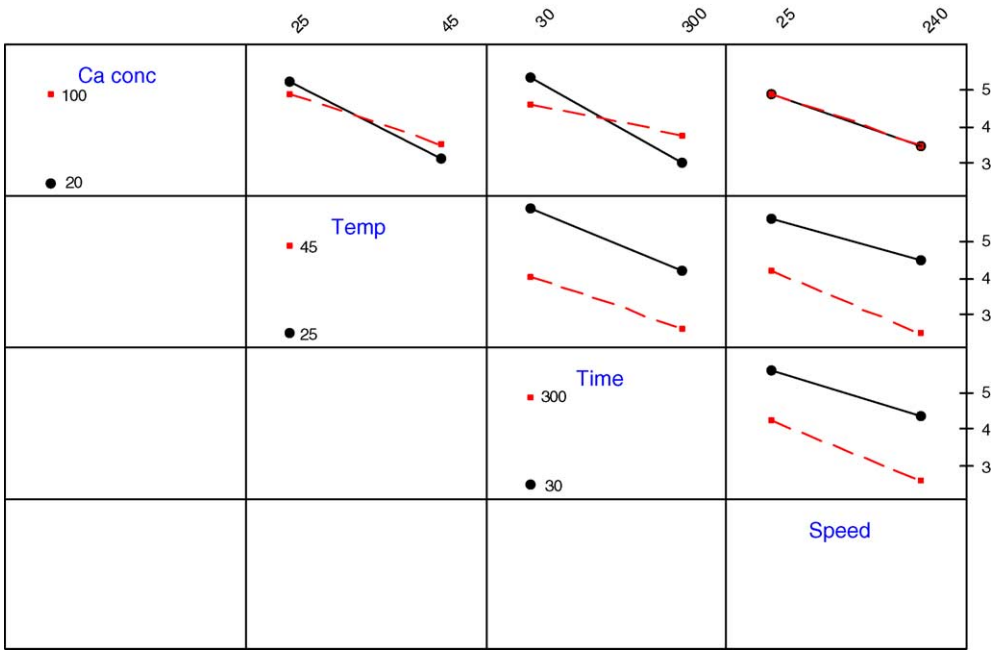

Fig. 4. Interaction plot showing the effect of granule treatment conditions on 10 s drug release (right axis).

treatment solutions. In addition, the calcium content depended on the other treatment conditions used during treatment but agitation rate, time and solution temperature had a smaller effect than calcium concentration. Although the calcium concentration of the treatment solution affected the sodium levels of the matrices the treatment time had a larger effect on the sodium levels than calcium concentration.

3.6. Aggregation level of the cross-linked granules

Granules treated with low calcium treatment solutions for short times showed about 70% aggregation (Trial 1) as opposed to 3% observed for long time low calcium treated granules (Trial 15). Aggregation was not observed for short time high calcium treated granules (Trial 2). In general the aggregated granules could

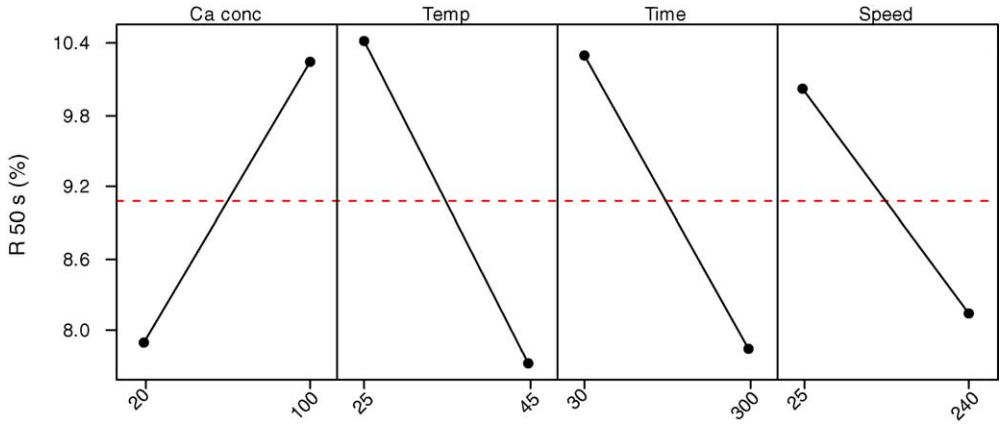

Fig. 5. Effect of treatment conditions on the early stage (50 s) drug release (R50 s) from cross-linked drug-alginate granules using early stage drug release apparatus.

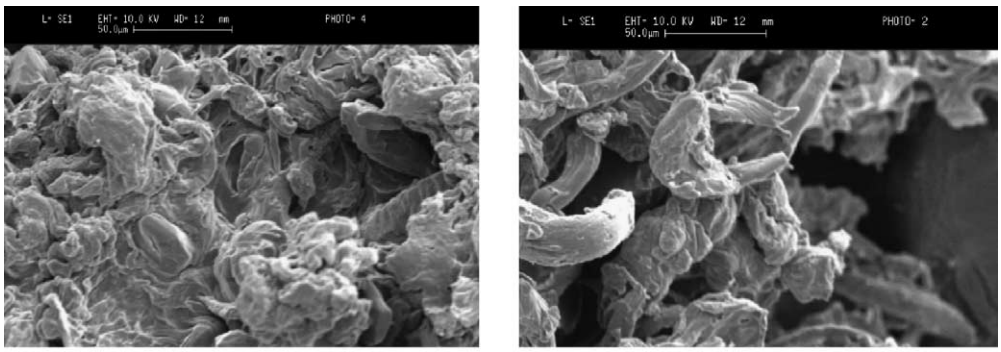

Fig. 6. Scanning electron micrographs of the granules treated with low calcium (a) and high calcium (b) stirred for 300 s (Trials 13 and 14).

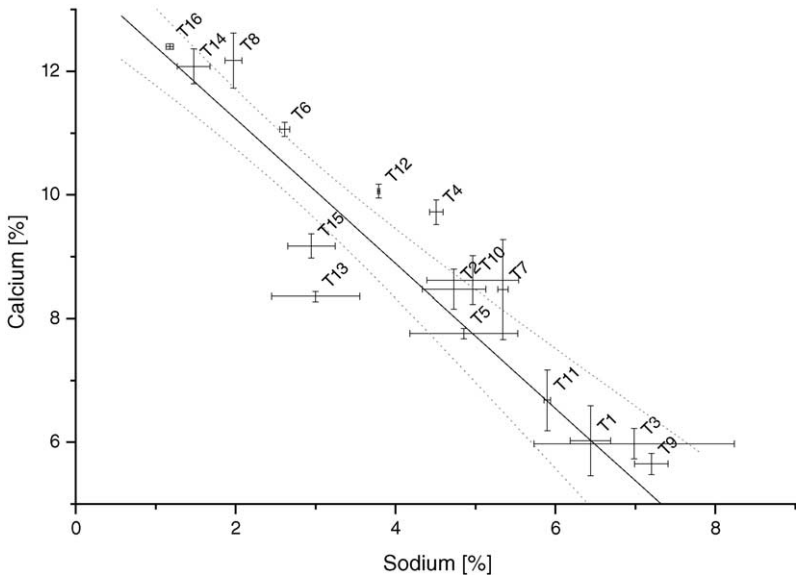

Fig. 7. Scatter plot showing a linear correlation (with 95% confidence interval) between calcium and sodium contents of the unwashed granules from the factorial design (Table 1). Trials 1, 2, 3, . . . are abbreviated as T1, T2, T3, . . ., respectively, and the calcium and sodium contents as shown in the plot have been expressed as a percentage after making corrections for moisture and drug present in the matrices. The sodium content of the Keltone HVCR used in the study on a dried basis was 11.4%.

be separated easily so it is unlikely to pose any practical separation problems during preparation.

4. Discussion

4.1. Introduction

Preparation and characterization of drug alginate matrix systems cross-linked under hydrated state have been widely reported in literature. However, no studies have been done thus far to cross-link drug alginate matrices in a dehydrated or reduced swollen state. In an attempt to overcome manufacturing related problems associated with cross-linking of drug alginate aqueous dispersions we have studied the cross-linking process in a reduced swollen state. The process consists of preparation of drug alginate granules by a conventional granulation process followed by dipping the granules in a calcium chloride solution bath prior to drying of the

cross-linked granules. In this study the preparation process of the dried drug alginate granules was kept constant (i.e. same stock granules used). The cross-linking process was varied to study the effect of cross-linking conditions on the stock granules which in turn affected the response variables. The important cross-linking conditions like calcium concentration (Yotsuyanagi et al., 1987; Oestberg et al., 1993; Oestberg and Graffner, 1994; Pillay et al., 1998) and agitation rate during cross-linking (Al-Zahrani, 1999) have been studied by workers in the case of cross-linking of drug alginate matrices in a hydrated state but had not been studied in case of cross-linking of granules under reduced swollen state. In addition to the above factors the other treatment factors studied were calcium solution temperature and the agitation time.

4.2. The cross-linking process

Gel matrices (granules) in a reduced swollen state or dried state absorb (Osada and Kajiwara, 2001) water (in our case calcium chloride) rapidly and swell during the initial stages of the treatment process. However, the concentration of calcium has two contrary effects. Low calcium concentration leads to greater swelling of granules facilitating calcium ion penetration (Julian et al., 1988) but the low concentration slows replacement by calcium of the sodium ions from the carboxylic acid residues present in the polymer network. On the other hand, high calcium reduces polymer swelling and ion penetration but supplies higher levels of calcium ions to the polymer undergoing cross-linking.

As the calcium solution penetrates the granule and cross-links the water-soluble polymeric part of the granule, a cage like water insoluble but permeable (Julian et al., 1988; Bhagat et al., 1991; Remunan-Lopez and Bodmeier, 1997; Kibbe, 2000) polymeric macro-structure is formed in which the drug particles are embedded. This macro-structure is made up of dense (Richey, 2001) cross-linked calcium alginate networks formed by the ion exchange between sodium and calcium ions depending on the calcium treatment solution used. Similar macrostructure formations have been reported in the case of polymer matrices containing a high proportion of dispersed drug (Baker, 1987; Aslani and Kennedy, 1996).

During treatment, when the granules first come into contact with the calcium chloride solution, an insoluble gel is precipitated immediately at the hydrating polymer and treatment solution interface where the calcium ions preferentially bond to the polycarboxylic acid residues (Haug and Larsen, 1962). As the calcium chloride solution penetrates inside the polymeric network via the hydrated and insoluble calcium alginate gel layer an additional amount of sodium alginate hydrates and is cross-linked in a similar fashion. This leads to a drop in the concentration of the calcium ion inside the polymer matrix which favors further entry of calcium ions (Osada and Kajiwara, 2001). The formation of the outer calcium alginate gel layer is believed to maintain the integrity of the granule during treatment by reducing the alginate dissolution from the granules.

The influx of calcium chloride is slowed as the cross-linked polymer probably resists the influx of the calcium ions through the dense polymer network (Julian et al., 1988; Remunan-Lopez and Bodmeier, 1997). Secondly, the elastic deformation forces, working inside the swelling matrix counter balance the pressure generated due to further entry of calcium solution. This too slows the calcium chloride influx. Unlike calcium ions, the sodium ions migrate out of the matrix as they are exchanged with the calcium ions. This process goes on till the granules are recovered from the treatment solution and it is postulated that the cross-linking and polymer rearrangement continues during the drying step. Further study is needed to confirm this.

4.3. The calcium and sodium ion content of the matrices and extent of cross-linking

The hydration of the polymer matrix and the availability of the calcium ions to replace the sodium ions largely control the extent of cross-linking and the changes in the macro-structure formation during the treatment process. The cross-linking process is slow and the swelling is higher in case of low calcium treated granules compared to high calcium treated granules. The sodium and calcium ion composition in the unwashed matrices were inversely correlated (Fig. 7) and depended on the calcium concentration of the treatment solution and treatment time but to a lesser extent on the other two factors. Additionally the unwashed matrices showed slightly higher total calcium and sodium contents than the washed granules.

This is most probably due to incomplete diffusion of the excess ions during the short treatment times used in the study (max. 330 s). However, the ratio of the sodium to calcium was unchanged after washing. Others have indicated the presence of extra calcium ions in alginate matrices (Murata et al., 1993). The possibility of short ion exchange times of about 5–6 min have also been indicated in the case of hydrated matrices by some researchers (Kim and Lee, 1992).

Overall these results suggest that the metal ion composition of the dried unwashed granules is a good indicator for the cross-linked state of the polymer matrix. Though the changes in the ion composition of the granule ceases after the granules are recovered from the treatment solution it is possible that cross-linking followed by rearrangement goes on during the drying step after treatment.

In the study with cross-linking of films in the dehydrated state the researchers reported that as the calcium ion concentration is increased the cross-linking process becomes slower and takes more than 6 min for the completion of this process (Julian et al., 1988). However, this did not affect cross-linking of drug containing granules (size: 0.8–1.0 mm) and is possibly due to the following reasons. Firstly the amount of drug present in the polymer matrix is high (1:1) so the polymer cross-section between drug particles is quite thin. As a result the calcium ions have to permeate through thin films of polymeric material to become cross-linked. Secondly the ion permeation is further assisted by the porosity of the granules. Lastly it could be due to the formation of interconnecting networks (as the drug content is high) which facilitates the calcium ion penetration and sodium ion replacement (Burns et al., 1990; Kaewvichit and Tucker, 1994).

4.4. Treatment conditions and drug entrapment

The granules being porous in nature lose drug during the treatment process. The drug loss depends on the treatment conditions as expected being higher at higher temperature at longer times and at higher stirring speeds. Low calcium levels resulted in higher swelling of the granules during the treatment process caused higher erosion of granule mass (drug and polymer) especially during the early stages of granule treatment. As a result the yield, drug entrapment and granule size were low in the case of low calcium treated granules compared to the corresponding high calcium treated granules.

In the case of cross-linking of hydrated alginate drug dispersions the matrices lose water and drug during cross-linking (Oestberg and Graffner, 1994; Aslani and Kennedy, 1996). During the granule treatment this type of drug loss is less as the water uptake by the granules during treatment is much less compared to the method reported in literature.

The drug loss during treatment weakens the matrix and is responsible for lowering the yield due to erosion of the porous polymer mass left after loss of drug particles.

When drug saturated treatment solutions were used for treatment of granules there was a significant increase in drug entrapment (about 10%) and yield but some drug loss still occurred as free drug particles (data not shown). However, it remains to be seen whether the particulate drug loss occurring during the treatment process from the granule periphery is related to the degree of granule swelling.

These results suggest that the matrices in the reduced swollen state lose drug as particles and also in solution during treatment using calcium solutions devoid of drug.

4.5. Treatment conditions and early stage drug release from treated granules

Granules treated with low calcium swell and erode more when compared to high calcium treated granules. In addition low calcium treated granules appear less porous than the corresponding high calcium treated granules (Fig. 6) suggesting there is some rearrangement during cross-linking. Early stage drug release from the low calcium treated granules in general was lower than the high calcium treated granules R50 (Fig. 4). Similar behaviours were also observed in the case of R10 excepting for the granules treated with low calcium for short times (Trials 1 and 9). Microphotography of these exceptional granules indicated that the surface of these granules disintegrated within 20 s after coming into contact with water due to improper cross-linking. These data suggest that probably the early release from these granules would have been less if the granules had not disintegrated in the presence of the drug-releasing medium (water).

The low drug release from the low calcium treated granules may be due to the low porosity of the granules. Another possibility could be that these smaller, less cross-linked dry granules (having larger surface area) after coming in contact with the releasing medium swell at a faster rate compared to the bigger and more cross-linked high calcium treated granules. Movement of water (moving inside the granule) opposite in direction to the diffusion of drug (moving out of the granule) could cause a reduction in the early stage drug release.

4.6. Treatment conditions and granule aggregation

The aggregation of the granules was greater for short time low calcium treated granules having higher sodium content than the long time low calcium treated batches having lower sodium content. As the sodium alginate on the granule surface is cross-linked, aggregation behaviour is reduced which is reflected by the inverse relation between the calcium ion composition and the granule aggregation behaviour ($r = -0.894$, $P = 0.003$).

Similarly higher surface cross-linking has been reported for the cross-linking of sodium alginate films (Julian et al., 1988).

5. Conclusions

Cross-linked granules can be prepared by cross-linking dried drug alginate granules after short exposure (5–6 min) to calcium chloride solutions. The cross-linking of the matrices is rapid in the case of treatment with high concentrations of calcium chloride solutions compared to the low calcium treatment solutions. Yield, drug entrapment, early release and extent of cross-linking depend on the granule treatment conditions used. The preparation process is simple and scalable industrially and uses limited water compared with current methods of cross-linking alginates.

Acknowledgements

The authors would like to thank Ms. Liz. Girvan and Mr. Mark Gould all from The University of Otago, for help with the scanning electron microscopy.

References

Acarturk, F., Takka, S., 1999. Calcium alginate microparticles for oral administration. II. Effect of formulation factors on drug release and drug entrapment efficiency. J. Microencapsulation 16, 291–301.

Albertini, B., Cavallari, C., Passerini, N., Voinovich, D., Gonzalez-Rodriguez, M.L., Magarotto, L., Rodriguez, L., 2004. Characterization and taste-masking evaluation of acetaminophen granules: comparison between different preparation methods in a high-shear mixer. Eur. J. Pharm. Sci. 21, 295–303.

Al-Zahrani, S.M., 1999. Investigation of factors influencing drug release using calcium alginate polymer. Bioprocess Eng. 21, 57–60.

Aoki, S., Ando, H., Tatsuishi, K., Uesugi, K., Ozawa, H., 1993. Determination of the mechanical impact force in the in vitro dissolution test and evaluation of the correlation between in vivo and in vitro release. Int. J. Pharm. 95, 67–75.

Aslani, P., Kennedy, R.A., 1996. Effect of gelation conditions and dissolution media on the release of paracetamol from alginate gel beads. J. Microencapsulation 13, 601–614.

Baker, R., 1987. Controlled Release of Biologically Active Agents. Wiley, New York, USA, pp. 39–83.

Bhagat, H.R., Mendes, R.W., Mathiowitz, E., Bhargava, H.N., 1991. A novel, self-correcting membrane coating technique. Pharm. Res. 8, 576–583.

Burns, R., Peterson, K., Sanders, L., 1990. A one year controlled release implant for the luteinizing hormone releasing hormone superagonist RS-49947. I. Implant characterization and analysis of in vitro results. J. Controlled Release 14, 221–232.

Duez, J.M., Mestdagh, M., Demeure, R., Goudemant, J.F., Hills, B.P., Godward, J., 2000. NMR studies of calcium-induced alginate gelation. Part I. MRI tests of gelation models. Magn. Reson. Chem. 38, 324–330.

Haug, A., Larsen, B., 1962. Determination of the uronic acid composition of alginates. Acta Chem. Scand. 16, 1908–1918.

Hills, B.P., Godward, J., Debatty, M., Barras, L., Saturio, C.P., Ouwerx, C., 2000. NMR studies of calcium induced alginate gelation. Part II. The internal bead structure. Magn. Reson. Chem. 38, 719–728.

Julian, T.N., Radebaugh, G.W., Wisniewski, S.J., 1988. Permeability characteristics of calcium alginate films. J. Controlled Release 7, 165–169.

Kaewvichit, S., Tucker, I.G., 1994. The release of macromolecules from fatty acid matrices: complete factorial study of factors affecting release. J. Pharm. Pharmacol. 46, 708–713.

Kibbe, H.A., 2000. Hand book of Pharmaceutical Excipients, 3rd ed. American Pharmaceutical Association, Washington DC, NW, USA, pp. 465–467.

Kikuchi, A., Kawabuchi, M., Sugihara, M., Sakurai, Y., Okano, T., 1997. Pulsed dextran release from calcium-alginate gel beads. J. Controlled 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. Controlled Release 58, 21–28.

Kim, C.K., Lee, E.J., 1992. The controlled release of blue dextran from alginate beads. Int. J. Pharm. 79, 11–19.

Morris, E.R., Rees, D.A., Thom, D., Boyd, J., 1978. Chiroptical and stoichiometric evidence of a specific, primary dimerization process in alginate gelation. Carbohydr. Res. 66, 145–154.

Mukhopadhyay, D., Tucker, I.G., 2003. Design and evaluation of an early stage drug release apparatus. Int. J. Pharm. 265, 47–54.

Murata, Y., Nakada, K., Miyamoto, E., Kawashima, S., Seo, S.H., 1993. Influence of erosion of calcium-induced alginate gel matrix on the release of Brilliant blue. J. Controlled Release 23, 21–26.

Oestberg, T., Vesterhus, L., Graffner, C., 1993. Calcium alginate matrixes for oral multiple unit administration. II. Effect of process and formulation factors on matrix properties. Int. J. Pharm. 97, 183–193.

Oestberg, T., Graffner, C., 1994. Calcium alginate matrixes for oral multiple unit administration. III. Influence of calcium concentration, amount of drug added and alginate characteristics on drug release. Int. J. Pharm. 111, 271–282.

Osada, Y., Kajiwara, K., 2001. Gels Handbook Volume 1: The Fundamentals. Academic Press, San Diego, CA, USA, pp. 65–85.

Pillay, V., Dangor, C.M., Govender, T., Moopanar, K.R., Hurbans, N., 1998. Ionotropic gelation: encapsulation of indomethacin in calcium alginate gel disks. J. Microencapsulation 15, 215–226.

Pillay, V., Fassihi, R., 1999. In vitro release modulation from crosslinked pellets for site-specific drug delivery to the gastrointestinal tract. II. Physicochemical characterization of calcium-alginate, calcium-pectinate and calcium-alginate-pectinate pellets. J. Controlled Release 59, 243–256.

Remunan-Lopez, C., Bodmeier, R., 1997. Mechanical, water uptake and permeability properties of crosslinked chitosan glutamate and alginate films. J. Controlled Release 44, 215–225.

Richey, T., 2001. Alginate networks for controlled release of proteins. In: Proceedings of the International Symposium on Controlled Release of Bioactive Material, vol. 2, San Diego, CA, USA, pp. 978–979.

Taylor, A.J., 1996. Volatile flavor release from foods during eating. Crit. Rev. Food Sci. Nutr. 36, 765–784.

Thom, D., Grant, G.T., Morris, E.R., Rees, D.A., 1982. Characterization of cation binding and gelation of polyuronates by circular dichroism. Carbohydr. Res. 100, 29–42.

Yotsuyanagi, T., Ohkubo, T., Ohhashi, T., Ikeda, K., 1987. Calcium-induced gelation of alginic acid and pH-sensitive reswelling of dried gels. Chem. Pharm. Bull. 35, 1555–1563.