The Effect of Drug Concentration and Curing Time on Processing and Properties of Calcium Alginate Beads Containing Metronidazole by Response Surface Methodology

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ABSTRACT

The purpose of present research work was to prepare calcium alginate beads containing water-soluble drug metronidazole using 3^2 factorial design, with drug concentration and curing time as variables. Curing time was kept as low as possible to improve entrapment with increasing drug concentration. Mostly the drugs which had been encapsulated were water insoluble to facilitate drug encapsulation; a characteristic drug release as whole process is aqueous based. Entrapment efficiency was in the range of 81% to 96% wt/wt, which decreased with decrease in polymer concentration and increase in curing time. The beads were spherical with size range between 1.4 and 1.9 mm. Scanning electron microscope (SEM) photomicrographs revealed increase in the leaching of drug crystals with increased curing time and high drug concentrations. In acidic environment, the swelling ratio was 200% in 30 minutes, but in basic medium, it increased to a maximum of 1400% within 120 minutes. In acidic medium, the swelling and drug release properties were influenced by drug solubility, whereas in phosphate buffer these properties were governed by the gelling of polymer and exhibited curvilinear and quadratic functions of both the variables, respectively.

KEYWORDS: calcium alginate, ionotropic gelling, water-soluble drug, curing time, metronidazole.

INTRODUCTION

Natural hydrophilic polymers, owing to their characteristic biocompatibility and biodegradability properties, are widely exploited in the pharmaceutical industry for the development of novel drug delivery systems. Among these polymers, alginate is one that has been widely used in numerous biomedical applications, processed in various dosage forms (eg, tablets, capsules, beads, rafts, liquid suspensions), and used in sutures and dressing materials with characteristic features such as mucoadhesion, bioadhesion, and modifying drug release profile. Chemically, alginates are linear, anionic block copolymer heteropolysaccharides consisting of monomers of (-d-mannuronic acid) (M) and its C-5 epimer, (-l-guluronic acid) (G), residues joined together by 1,4-glycosidic linkages. The simple, mild, aqueous-based gel formation is achieved by the ionotropic gelling, on the addition of bivalent alkaline earth metals Ca^{2+}, Sr^{2+}, and Ba^{2+} or trivalent Fe^{3+} and Al^{3+}, due to an ionic interaction and intramolecular bonding between the carboxylic acid groups located on the polymer backbone and these cations.[1-8]

Calcium-induced alginate gel beads have been developed in recent years as a unique vehicle for drug delivery system. These beads have been used in formulations as single or multiple units, with or without the addition of other hydrogels or polymers, intrapenetrating networks (IPNs), nanospheres, polycations, and many more dosage forms for achieving temporal and spatial drug release.[7-10] Various categories of drugs have been encapsulated such as gastro-irritant nonsteroidal antiinflammatory drugs, enzymes, peptides/proteins, and acid labile. All these drugs show different patterns of drug entrapment and release profiles depending upon their physico-chemical characteristics and method of preparation. In these applications, the uses of low molecular weight and water-soluble drugs have not been exploited much. It was presumed that the incorporation of the water-soluble drug in beads was not significant because of the surrounding aqueous environment, which causes low encapsulation and fast drug release governed by the pore size and inherent solubility of drug in a matrix system. Thus, during ionotropic gelation, curing time can be a crucial factor in governing the entrapment efficiency.[1-7,11-14]

Metronidazole (MZ) was selected as a model drug for incorporation in calcium alginate beads. With drug concentration and curing time as variables, 3^2 factorial design was applied. MZ, a low molecular weight, water-soluble drug is widely used as an anti-amoebic and antiprotozoal as well as to prevent recurrence of peptic ulcer disease caused by *H pylori*. Because of its solubility, the calcium alginate bead

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containing additives such as oil and chitosan has been developed with low drug entrapment and floating characteristics.[13] The scope of the present work was to study the effect of the curing time, which was kept as short as possible to limit the effect of drug solubility, while simultaneously increasing the drug amount. The concentration of the polymer was kept constant. The beads were evaluated with respect to micromeritic properties, moisture content, practical yield, drug content and encapsulation efficiency, surface morphology by scanning electron microscopy (SEM), crystallinity by differential scanning calorimetry (DSC), and swelling and in-vitro drug release in both acidic and alkaline medium.

Materials and Methods

Materials

Sodium alginate (Protanal LF-200 M) was a generous gift from Signet Chemical Co (Mumbai, India) having G/M ratio 40% to 45% : 55% to 60% and viscosity of 1% wt/wt aqueous solution as 200 to 400 megapascal seconds (MPAS). MZ was gifted from Aarti Drugs Ltd (Ahmedabad, India). Other chemical and solvents were of analytical grade.

Preparation of Metronidazole-loaded Calcium Alginate Beads

Sodium alginate solution of 2% wt/vol was prepared in deionized water; MZ was dispersed in 10 mL of this solution. Then, 8 mL of MZ-alginate suspension was added dropwise into 5% wt/vol $CaCl_2$ solution at ambient temperature by constant stirring with magnetic stirrer. The falling distance was kept constant at 6 cm. The beads formed were allowed to stand in 5% wt/vol $CaCl_2$ solution to be cured. Beads were separated and washed with deionized water and dried in air for 24 hours.

Factorial Design Experiments

To study the effect of variables, batches were prepared using 3^2 factorial design. The independent variables selected were drug concentration (X1) and curing time (X2). Coded and actual value of variables for each batch and the experimental design are given in Table 1.

Evaluation and Characterization of Beads

Determination of the Drug Content

One hundred milligrams of beads were kept in 100 mL of phosphate buffer (Indian Pharmacopeia [IP]) pH 7.4 in a reflux assembly for 12 hours, and then the filtrate was assayed by spectrophotometry at 320 nm. The encapsulation efficiency was calculated according to the following relationship:

Table 1. 3^2 Factorial Design: Drug Content and Encapsulation Efficiency of MZ-loaded Ca-Alginate Beads*

Batch	Drug Amount (mg) (X_1)/code	Curing Time (minutes) (X_2)/code	% Encapsulation Efficiency ± SD	% Drug Content (wt/wt) ± SD
YM-1	200 (–1)	5 (–1)	96 ± 3.2	36 ± 2.4
YM-2	200(–1)	15 (0)	95 ± 3.4	35 ± 1.2
YM-3	200(–1)	25 (+1)	90 ± 1.6	34 ± 1.3
YM-4	400(0)	5 (–1)	89 ± 2.8	54 ± 3.2
YM-5	400(0)	15 (0)	87 ± 3.5	53 ± 2.6
YM-6	400(0)	25 (+1)	83 ± 5.6	50 ± 2.5
YM-7	600(+1)	5 (–1)	96 ± 6.4	67 ± 2.6
YM-8	600(+1)	15 (0)	95 ± 2.6	64 ± 2.8
YM-9	600(+1)	25 (+1)	81 ± 7.2	57 ± 3.1

*SD indicates standard deviation.

$$\text{Encapsulation efficiency} = \frac{\text{\% Drug content} \times \text{Amount of dried beads produced}}{\text{Amount of drug added} - \text{Amount of drug remaining in apparatus}} \quad (1)$$

Determination of Moisture Content

Moisture content of the batches was determined by Karl Fisher method using Karl Fisher titrator (Veego Matic D, Veego Instruments Corp, Mumbai, India). It involved titration of the moisture present in methanolic solution with Karl Fisher reagent and the end point was detected visually.

Surface Topography

Photomicrographs of the beads were observed at 50× and 500× magnification using SEM Cambridge Stereoscan 120 (Cambridge, UK) operated with an acceleration voltage of 10 kV. The beads were mounted on the standard specimen mounting stubs and were coated with a thin layer (20 nm) of gold by sputter coater unit (VG Microtech, Uckfield, East Sussex, UK).

Differential Scanning Calorimetric Analysis

Thermograms of Metronidazole, unloaded calcium alginate beads, and MZ-loaded calcium alginate beads were obtained using a Mettler-Toledo DSC 821^e instrument (Mettler Toledo, Greifensee, Switzerland) equipped with an intracooler. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The powder samples were hermetically sealed in perforated aluminum pans and heated at constant rate of 10°C/min over a temperature range of 25°C to 300°C. The system was purged with nitrogen gas at the rate of 100 mL/min to maintain inert atmosphere.

Powder X-ray Diffraction

X-ray powder diffraction patterns of MZ, sodium alginate, unloaded calcium alginate beads, and MZ-loaded calcium alginate beads of different batches were recorded by using a Philips PW 1729 X-ray diffractometer (Philips, Amsterdam, The Netherlands). Samples were irradiated with monochromatized Cu Kα radiation (1.542 Å) and analyzed between 2°C and 60°C. The voltage and current used were 30 kV and 30 mA, respectively. The range and the chart speed were 2×10^4 cps and 10 mm/2θ, respectively.

Fourier Transform Infrared Analysis

Fourier Transform Infrared Analysis (FTIR) measurements of MZ, sodium alginate, and MZ- loaded calcium alginate beads were obtained on JASCO V5300 FT-IR (Tokyo, Japan). The pellets were prepared on KBr-press (Spectra Lab, Pune, India) under hydraulic pressure of 150 kg/cm^2. The spectra were scanned over the wave number range of 3600 to 400 cm^{-1} at the ambient temperature.

Particle Size Determination

The mean diameter was determined using a stereomicroscope (Carl Zeiss, Oberkochen, Germany) attached with a digital camera (Watec, Wat-202, Tokyo, Japan). The captured images were analyzed using Biovis Image Plus software (Expert Tech Vision, Mumbai, India). Around 100 particles were analyzed. Average diameter and different surface factors such as circulatory factor, elongation, roundness, and perimeter ratio were determined.

Swelling Studies

Beads of each batch were studied for swelling characteristics. Drug-loaded beads were placed in wire basket of United States Pharmacopeia (USP) dissolution apparatus II. The basket containing bead was put in a beaker containing 100 mL of 0.1 N HCl (pH 1.2) and phosphate buffer IP pH 7.4 maintained at 37°C. The beads were periodically removed at predetermined intervals and weighed. Then the swelling ratio was calculated as per the following formula:

$$\text{swelling ratio} = \frac{\text{weight of wet beads}}{\text{weight of dried beads}} \qquad (2)$$

Drug Release Studies

The dissolution of MZ-loaded Ca-alginate beads was studied using USP 26 Type II dissolution test apparatus Electrolab TDT-06P (Mumbai, India) containing 900 mL of 0.1 N HCl (pH 1.2) and phosphate buffer IP pH 7.4 maintained at 37°C ± 0.5°C and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. Analysis of data was done using PCP Disso v2.08 software, (Poona College of Pharmacy, Pune, India).

Results and Discussion

The metronidazole containing alginate beads were evaluated for the micromeritic properties. The moisture content of beads was found in the range of 4% to 5% wt/wt. The drug content was in proportion to that of loaded drug ranging from 34% ± 1.3% to 67% ± 2.6% wt/wt. Encapsulation efficiency was in range of 81% ± 2.8% to 96% ± 3.2%, which was higher as compared with a previous study done by Muruta et al.[13] Their developed beads containing oil had MZ encapsulation efficiency of 72% to 77% and other beads containing chitosan had it up to 65%. Drug content increased with increasing drug amount in the batches, whereas it decreased with the increasing curing time and showed much difference as the polymer concentration decreased. This finding could be because of metronidazole's inherent solubility in media influenced by the extent of cross-linking time. The same observations seem to hold true for the encapsulation efficiency also. Results of percentage drug content and encapsulation efficiency are given in Table 2.

DSC thermograms of MZ, empty Ca-alginate beads, and MZ-loaded Ca-alginate beads are depicted in Figure 1. Metronidazole shows sharp melting endotherms at 160.79°C. Empty alginate beads show melting endotherm at 183.83°C, and MZ-loaded Ca-alginate beads show melting endotherm at 155.18°C and 194.05°C. This finding indicates molecular dispersion of drugs within the beads (data of only single batch shown).

FTIR spectra of MZ, empty Ca-alginate beads, and MZ-loaded Ca-alginate beads of batch YM-2 are shown Figure 2. The figure reveals drug characteristics such as OH stretch (3219 cm^{-1}), C=CH, C-H stretch (3101 cm$^{-1)}$, NO_2, N-O stretch (1535 cm^{-1}), C-OH, C-O stretch (1074 cm^{-1}), C-NO_2, and C-N stretch (825 cm^{-1}). The characteristics of the peak

Table 2. Regression Analysis Data of Drug Release From Ca-Alginate Beads in Phosphate Buffer Ph 7.4*

Coefficient	$t_{10\%}$	$t_{50\%}$	$t_{80\%}$	$t_{90\%}$
β_0	15.1	173	38	470
β_1	–7.28	–27.9	–25	–20.5
β_2	3.11	1.2	–5.6	–18.1
β_{11}	16	97.1	16.2	176
β_{22}	–8.88	–100	–225	–271
β_{12}	2.32	31.10	71.1	76.7
R^2	0.893	0.8	0.651	0.6
Significance	0	.001	.015	.032
F	20.13	9.68	4.49	3.61

*t indicates time with percent release.

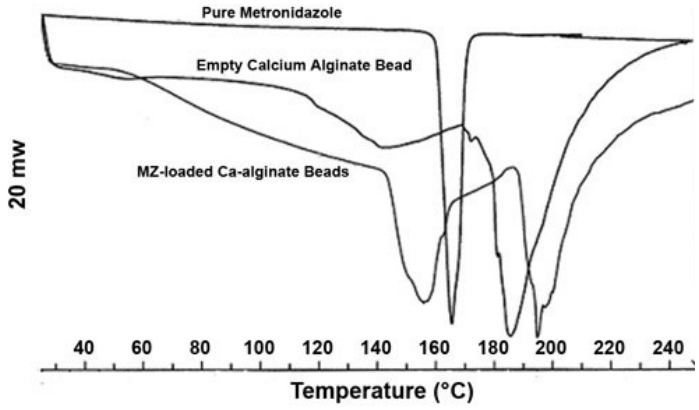

Figure 1. DSC thermograms of Pure Metronidazole; Empty Ca-alginate beads; MZ-loaded Ca-alginate beads (YM-2).

of MZ were not altered after encapsulation, indicating no chemical interaction between drug and polymer (data of only single batch shown).

SEM photomicrographs of dried MZ-loaded Ca-alginate beads are shown in Figure 3. The bead surface of batch YM-2 containing lowest drug amount prepared in 15 minutes of curing time was comparatively smooth and tightly packed, with surface deposition of irregular clusters of MZ crystals, which could have leached out onto the surface through the viscous gel during shrinking. The increased amount of the drug deposition on bead surface of batch YM-8 indicates

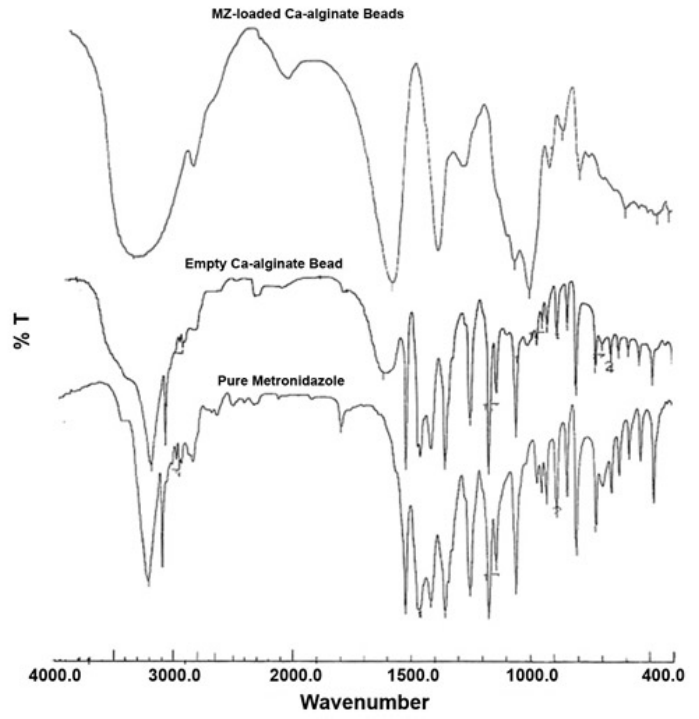

Figure 2. Fourier transform infrared spectra of Pure metronidazole (MZ); MZ-loaded Ca-alginate beads; Empty Ca-alginate beads (YM-2).

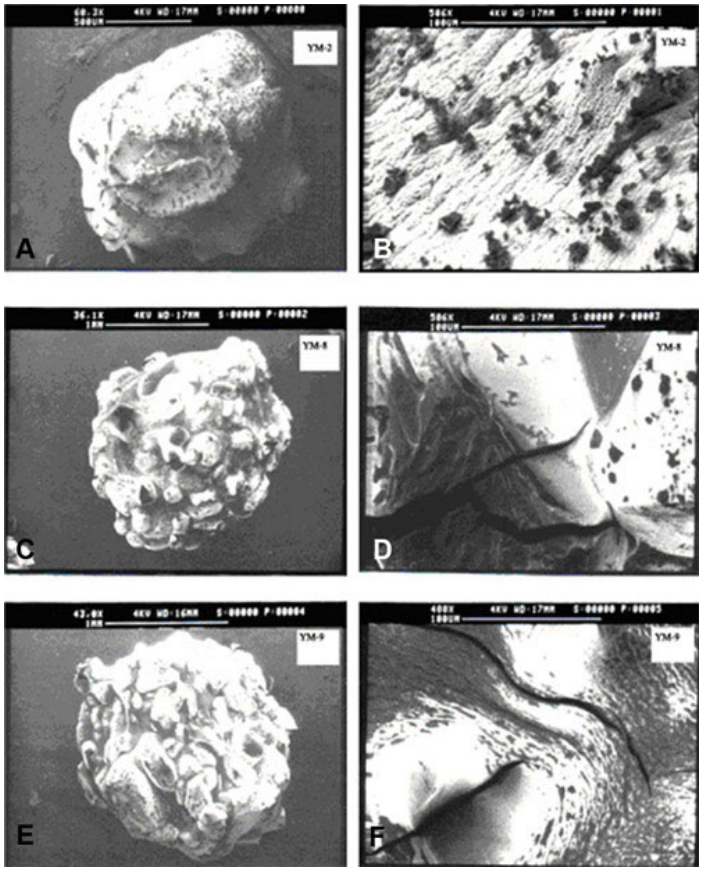

Figure 3. Scanning electron microscopy of metronidazole-loaded Ca-alginate beads (A) original magnification ×60.3; (B) ×506; (C) ×36.1; (D) ×506; (E) ×43; and (F) ×488.

increased leaching lead by large crystals, which facilitated their path outwards thereby causing the roughness of the surface due to high drug concentration. Plate-shaped crystals with sharp corners were visible because of the thin polymer coat in the case of batch YM-8. The beads containing same drug:polymer ratio, but obtained after 25-minute curing time as for batch YM-9, showed more surface irregularity and deposition of the drug in an organized manner. This increased leaching could be owing to the lower amount of polymer as well as the increased curing time. The prominent

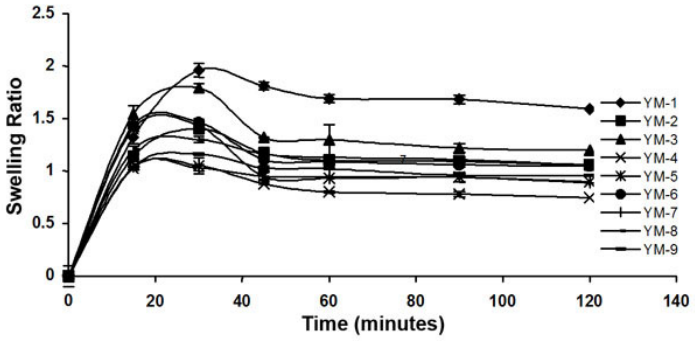

Figure 4. Swelling ratio in 0.1 N HCl (pH 1.2) vs time relationship of metronidazole-loaded Ca-alginate beads.

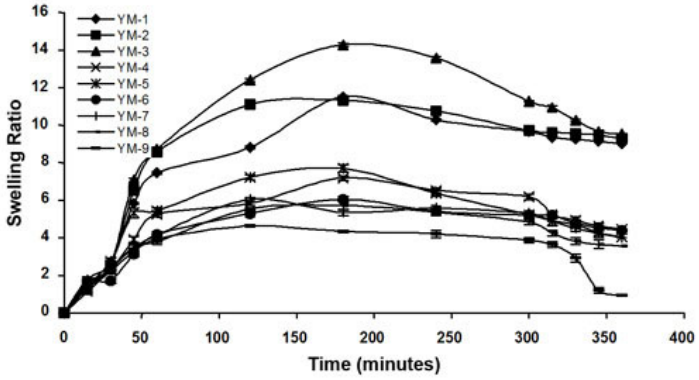

Figure 5. Swelling ratio in phosphate buffer IP pH 7.4 vs time relationship of metronidazole-loaded Ca-alginate beads. IP indicates Indian Pharmacopeia.

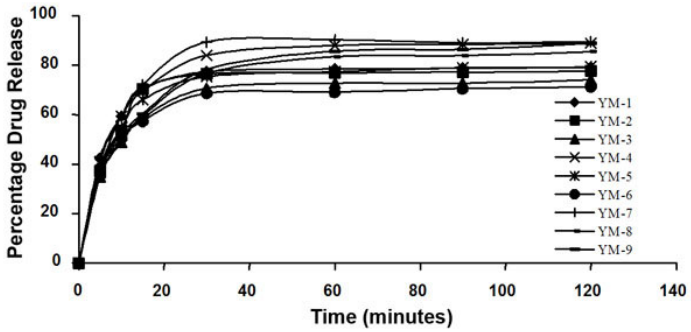

Figure 7. Percentage release profile of metronidazole (MZ) in pH 1.2 acid buffer from MZ-loaded Ca-alginate beads.

surface cracks in case of batch YM-8 and YM-9 may be attributed to weak inter-particulate bonding due to low polymer content.

The data analysis of parameters obtained from various batches for particle size distribution, swelling, and drug release was subjected to multiple regression analysis using statistical software UNISTAT (Statistical Version 3, Megalon, Novato, CA). The equation fitted was:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (3)$$

where, Y represents measured response; X, levels of factors, and β, coefficient computed from the responses of the formulations.

The beads obtained were evaluated for diameter, circularity, and roundness. The beads were spherical with circulatory factor in range of 1.2 to 1.3 and roundness in range of 0.4 to 0.7, but no significant difference was observed in these parameters. The mean particle size of all formulations was between 1.398 ± 0.057 mm and 1.884 ± 0.075 mm. The regression equation for particle size was

$$Y = 1.66 + 0.162 X_1 + 0.05 X_2 \ (r^2 = 0.9730) \quad (4)$$

The multiple regression data obtained for particle size showed higher β1 values and lower β2 values, indicating the dominating effect of polymer:drug ratio on the particle size because of the availability of sufficient amount of polymer to hold the drug.

Sodium alginate exhibits pH-dependent swelling with lowest swelling ratio in water and pH 1.2 and highest in pH 2.5 and 4.5. They also show disintegration in pH 7 to 7.5 within 45 to 50 minutes.[9] The time required for maximum swelling was processed for statistical regression data for both the mediums. The regression equation of swelling of beads in acidic pH at 30 minutes was

$$Y = 1.397 - 0.276 X_1 \ (r^2 = 0.5761) \quad (5)$$

These results can be attributed to the decrease in polymer ratio with increase in drug amount. In the present study, the

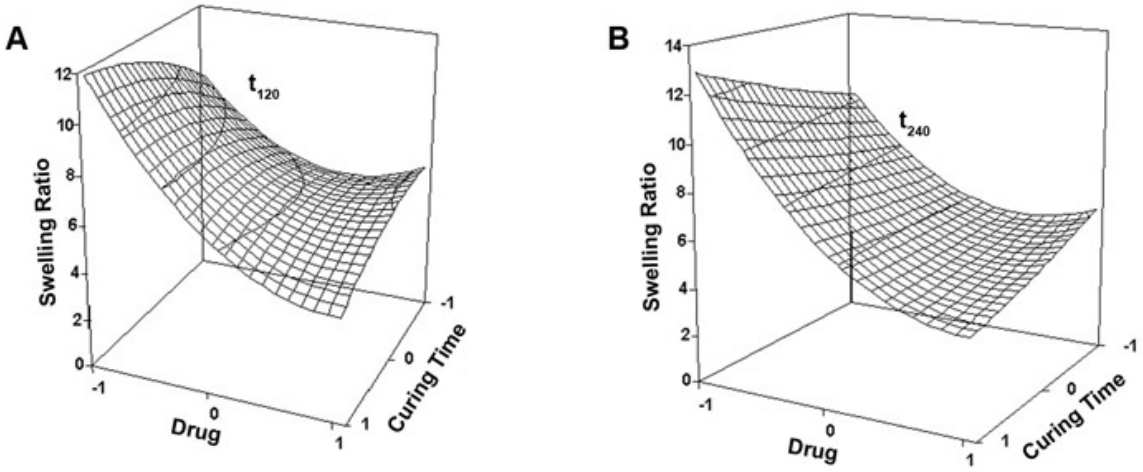

Figure 6. Response surface graph for the effect of variables on swelling ratio at different time intervals: (A) t_{120} minutes; (B) t_{240} minutes.

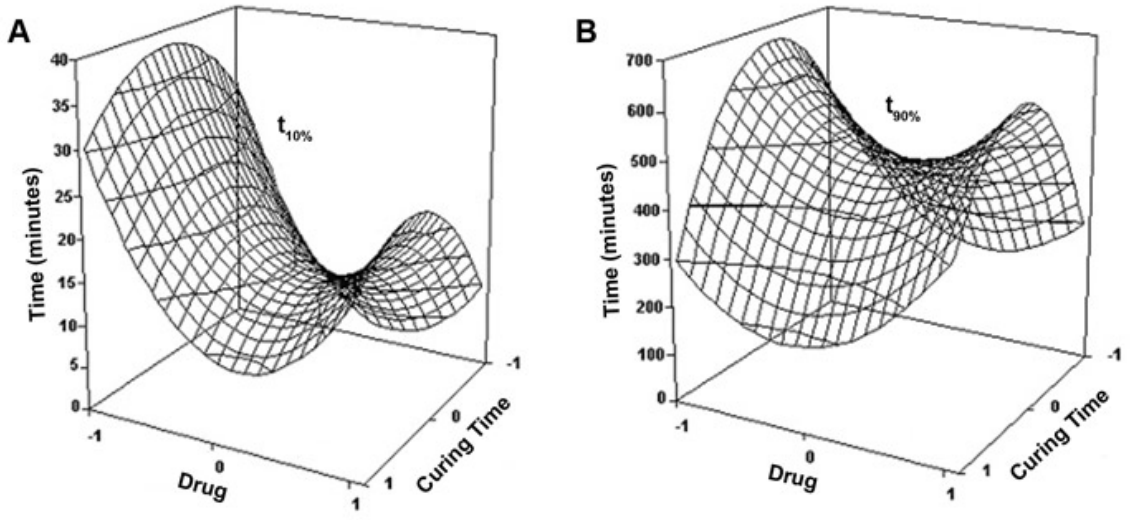

Figure 8. Response surface graph for the effect of variables on drug release: (A) $t_{10\%}$; (B) $t_{90\%}$.

maximum swelling up to 200% wt/wt was observed within 25 to 30 minutes in pH 1.2 followed by sudden reduction in weight in the next 15 to 20 minutes as shown in Figure 4. This effect might be owing to the acid solubility of drug that might have influenced swelling behavior of the beads; otherwise the acid gels of sodium alginate are resistance to erosion. Figure 5 shows the swelling ratio of Ca-alginate beads in phosphate buffer IP pH 7.4. The beads undergo maximum swelling up to 1400% wt/wt after 120 minutes. The polymer:drug ratio had significant effect on the swelling ratio and exhibited a curvilinear relationship as shown in Figure 6A and B. The regression equation for swelling at 180 minutes is as follows:

$$Y = 7.08 - 0.324X_1 + 0.102X_2 \ (r^2 = 0.9540) \quad (6)$$

Maximum swelling was observed in the beads containing high polymer with prolonged curing time. Beads of batch YM-3 showed maximum swelling with rapid hydration as compared with batches YM-1 and YM-2. Among these, YM-2 beads prepared at 15-minute curing time maintained plateau throughout study period of 6 hours. This result may be because of the maximum extent of cross-linking that yielded compact beads, which might have rehydrated to a greater extent. The sequestering action of phosphate on Ca^{2+} may have contributed to the swelling of cross-linked beads. The lower rehydration of beads that were prepared at shorter curing time may be correlated to the incomplete cross-linking of the sodium alginate.[9,14,15]

When MZ-loaded Ca-alginate beads were evaluated for drug release in 0.1 N HCl, pH 1.2 (Figure 7), 70% to 90% release of drug from the beads occurred within 20 to 30 minutes, which was in accordance with Murata et al,[13] who found complete MZ release from floating sodium alginate beads within 30 minutes. The regression equation of drug release pattern in pH 1.2 at 30 minutes was as follows:

$$Y = 77.37 + 3.315X_1 - 5.526X_2 \ (r^2 = 0.9540) \quad (7)$$

Multiple regression data revealed retarding effect of curing time on drug release, which might be caused by slower penetration of medium in highly cross-linked beads, but the overall drug release was governed by solubility of drug in dissolution medium and not by swelling properties of beads.

Complete dissolution of drug from the beads occurred within 6 hours in phosphate buffer IP pH 7.4. The drug release was significantly affected by both variables as predicted from response surface graphs at various $t_{X\%}$, which was a quadratic function of these variables given in Figure 8A and B and Table 2. The $t_{50\%}$ increased initially with increase in curing time and decreased thereafter. Rapid rate drug release from beads that were prepared by 5-minute and 25-minute curing time, respectively, may be owing to softness of beads and surface deposition of leached drug. The slow release from beads prepared at medium curing time confirms the drug release by diffusion from a strong and thick hydrated gel. The drug release was more extended from beads containing higher concentration of polymer, which may be associated with swollen gel with greater structural integrity and greater entanglement holding excess of medium.

CONCLUSION

Modulation of cross-linking time has a positive effect on the encapsulation efficiency of water-soluble drug. The leaching out of water-soluble drug from calcium alginate beads is not only the function of curing time but polymer concentration and fineness of crystals. Drug release in acidic medium

is governed by drug solubility and not by gel properties of calcium alginate, whereas drug release in basic medium is controlled by swollen gel.

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