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Evaluation of Tramadol Hydrochloride Loaded Calcium Alginate Beads

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ABSTRACT



In this study, an attempt was made to develop oral sustained delivery formulations based on ionotropic gelation of sodium alginate using calcium chloride as the cross-linking agent. Tramadol hydrochloride was either incorporated into preformed calcium alginate gel beads (Method-1) or simultaneously incorporated during the gelation of sodium alginate by calcium chloride (Method-II). The beads prepared by the method I resulted in higher drug loading when compared to those prepared by the method II. The morphology of the beads was studied by means of scanning electron microscopy (SEM) and the physical state of the drug in formulations was characterized using X-ray powder diffraction analysis (X-RD) and differential scanning calorimetry (DSC). The results indicated that the drug was present in an amorphous state in the case of beads prepared by method I. The drug - polymer interactions were evaluated using FTIR and no interaction between the drug and polymer was observed. The *in-vitro* drug release was studied using USP XXIII (type II) dissolution test apparatus with distilled water as the medium. Further, the release data was analyzed to understand the mechanism of drug release and it was found to follow diffusion model. From this present study, it can be concluded that tramadol hydrochloride loaded calcium alginate beads prepared by ionotropic gelation method with calcium chloride as the cross-linking agent can be utilized for the development of a sustained release drug delivery system for tramadol hydrochloride.

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Key Words

Calcium alginate beads, XRD, SEM
Tramadol hydrochloride, FTIR, DSC

INTRODUCTION

Calcium alginate beads represent one of the most widely used carriers for the sustained/ controlled release of drug(s)¹. The success of the ionotropic gelation method is largely attributed to the mild conditions required for the process, in addition to the low cost¹. Alginic acid is an anionic polysaccharide found in brown seaweeds. It is a linear co-polymer of D-mannuronate (M) and L-guluronate (G) residues arranged in blocks of one type of residue (G or M blocks) or of both types of residues in an alternating fashion (MG blocks)². Their gelling property is derived from the cooperative binding of divalent cations localized between homopolymeric blocks of guluronate residues. Calcium ions are located into electronegative cavities like eggs in an egg box and from this similitude arises the term "egg box" model. Such a cross-linking process stiffens and roughens the polymer and reduces its swelling in solvents. These gels prepared in the form of beads have been investigated for use as carriers in drug delivery³⁻¹¹. The use of non-steroidal anti-inflammatory drugs (NSAIDs) are widespread, but in many cases they fail to provide sufficient analgesic effects. It has also been reported that side effects such as gastro-intestinal problems are very much prevalent with such type of drugs. Tramadol hydrochloride is positioned as a painkiller (moderate analgesic) that fills the niche between powerful analgesics conventionally used to treat symptoms such as cancer pain and non-steroidal anti-inflammatory drugs (weak analgesics) used to treat mild symptoms, such as headache and joint pain. At present, oral immediate release formulations containing tramadol hydrochloride are used worldwide for

treatment of chronic pain. Tramadol hydrochloride has a biological half-life in the terminal phase ($t_{1/2}/\beta$) of 5.5 ± 0.9 h¹² and the usual oral dosage regimen is 50-100mg taken two to three times a day, which necessitates dosing of immediate release formulations every 4 - 6 h. It is expected that a sustained release pharmaceutical formulation can improve patient compliance by reducing the dosing frequency and minimize the peak-to-trough fluctuations¹³⁻¹⁶.

MATERIALS AND METHODS

Materials

A gift sample of Tramadol hydrochloride was obtained from Unichem Pharmaceuticals, Mumbai, India; Sodium alginate was purchased from Fluka chemie GmhH, Buchs. All other chemicals used were of analytical grade.

Preparation of Beads

A. Preparation of Calcium Alginate Beads

An aqueous solution of sodium alginate (2 to 3% w/v) was added dropwise using a 5 ml hypodermic syringe having a 21 gauge needle under constant stirring into an aqueous calcium chloride solution (1 to 3% w/v) taken in a beaker to form spherical calcium alginate beads, and the interaction was allowed to proceed for about 1 h. The calcium alginate beads were then harvested by filtration washed with distilled water and dried at room temperature for 24 h. The dried beads were then collected and stored in an airtight container.

B. Preparation of Tramadol Hydrochloride Loaded Calcium Alginate Beads

Drug loading was carried out by two methods designated as sequential (method I) and simultaneous (method II). In method I, wet calcium alginate beads prepared by the above mentioned method were immersed and stirred for 2.5 h in a solution containing tramadol hydrochloride (1- 4%w/v). In the case of method II, the beads were formed by calcium ions with simultaneous drug loading. The sodium alginate solution (2-3%) was added dropwise using a 5 ml hypodermic syringe having a 21 gauge needle under constant stirring into the solution containing calcium chloride (1-3%) which also contained 1-3% w/v of the drug. After 1h of interaction, the beads were filtered, washed with distilled water and dried at room temperature for 24 h, and then the dried beads were collected and stored in an airtight container.

Determination of Tramadol Hydrochloride Content in the Beads

For the determination of tramadol hydrochloride content in the beads, 20mg of beads were placed in 100 ml of distilled water for 24 h. The filtrate was assayed spectrophotometrically for tramadol hydrochloride content at 270 nm using a UV spectrophotometer (Beckman DU64). The studies were performed in triplicate.

Mean Particle Size and Surface Morphology

Particle size of about 100 beads for each batch was determined using an optical microscope (Olympus, New Delhi, India) fitted with a calibrated ocular micrometer. Scanning electron micrograph was obtained using a SEM (Jeol, JSM 6360) operating between 5-24 Kv. The scanning electron micrographs of

unloaded calcium alginate beads, tramadol hydrochloride loaded beads prepared by method I and II, and of bead formulations after dissolution studies were obtained.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) measurements were performed using a Perkin Elmer 1600 series instrument. The samples of the tramadol hydrochloride, calcium alginate beads and tramadol hydrochloride loaded beads prepared by both methods were grounded with KBr and pellets were made by applying hydraulic pressure and then measured by IR spectroscopy in the range of 4000-400 cm^{-1} in order to confirm interaction between tramadol hydrochloride and calcium alginate matrix.

Differential Scanning Calorimetric Studies

Thermal analysis (Mettler TA 4000) was performed on pure tramadol hydrochloride, unloaded calcium alginate beads and tramadol hydrochloride loaded beads prepared by both methods I and II. The samples were heated at the rate of 10° C/min over a temperature range of 30 to 300° C under a constant flow of nitrogen gas.

Powder X-Ray Diffractometry

Powder X-ray diffraction (Miniflex goniometer) pattern, over a range of 10-100 2 θ , of pure Tramadol hydrochloride, drug loaded beads prepared by methods I and II was obtained to understand the physical state of the drug in the polymer matrix.

In-Vitro Drug Release Studies

The *in-vitro* drug release of the different batches of beads were carried out using USP XXIII (type II) dissolution test apparatus (Thermonik, Model no.C-DR-2) for 8 h with continuous stirring of 50 rpm at $37 \pm 0.2^\circ\text{C}$. Accurately weighed beads equivalent to 25mg of tramadol hydrochloride were placed in 350 ml of distilled water. 3ml samples were withdrawn at predetermined time intervals and replaced with the same volume of fresh medium. The withdrawn samples were then analyzed spectrophotometrically (Beckman DU64) at 270 nm for drug content.

RESULTS AND DISCUSSION

Preparation of Beads

The minimum concentrations of Sodium alginate and Calcium chloride necessary for the formation of beads with proper structure were found to be 2%w/v and 1%w/v respectively. The beads were spherical except at the point of contact with the petridish used for drying the preparation, where it was slightly flattened.

Tab 1. Formulation Design, Drug Loading and Mean Particle Size for Tramadol Hydrochloride Loaded Beads Prepared By Sequential Method (method I) (n = 3)

Formulation Code*	Polymer concentration	Cross linking agent Concentration	Drug Concentration	Drug loading (%)	Mean particle size (mm)
	SA (%w/v)	CaCl ₂ (%w/v)	TH (%w/v)	mean \pm SD	mean \pm SD
SQA1	2	1	2	52.1 \pm 0.57	1.065 \pm 0.005
SQA2	2	2	2	51.0 \pm 1.02	1.060 \pm 0.010
SQA3	2	3	2	48.6 \pm 0.36	1.057 \pm 0.007
SQA4	2.5	2	2	49.8 \pm 0.34	1.076 \pm 0.075
SQA5	3	2	2	47.6 \pm 0.07	1.005 \pm 0.005
SQA6	2	1	1	49.5 \pm 0.03	1.043 \pm 0.003
SQA7	2	1	3	43.6 \pm 0.06	1.055 \pm 0.011

*Only a few relevant bead formulations are given in the Table

SA= sodium alginate, TH= Tramadol hydrochloride

Tab 2. Formulation Design, Drug Loading and Mean Particle Size for Tramadol Hydrochloride Loaded Beads Prepared By Simultaneous Method (method II) (n = 3)

Formulation size Code*	Polymer	Cross linking agent		Drug	Drug loading	Mean particle size
	concentration	Concentration		Concentration	(%)	(mm)
	SA (%w/v)	CaCl ₂ (%w/v)	TH (%w/v)	mean ± SD	mean ± SD	
SMA1	2	1	2	38.4±0.52		1.207±0.002
SMA2	2	2	2	22.5±1.32		1.158±0.007
SMA3	2	3	2	20.2±0.52		1.106±0.003
SMA4	3	1	2	27.6±0.07		1.151±0.011
SMA5	2	1	1	26.8±0.50		1.057±0.002
SMA6	2	1	3	19.7±0.65		1.162±0.011

*Only a few relevant bead formulations are given in the Table

SA= sodium alginate, TH= Tramadol hydrochloride

Determination of Tramadol Hydrochloride Content in the Beads

The purpose of the drug loading studies was to examine the ability of the polymer as well as the manufacturing process to entrap the drug. In general, drug loading for beads prepared by sequential method (Method I) was found to be higher than that for beads prepared by simultaneous method (Method II). The highest drug loading for beads prepared by method I was 52.1% (SQA1) and 38.4% (SMA1) respectively. For method I, the concentrations of sodium alginate,

calcium chloride and drug had an effect on drug loading. With respect to the above three variables, increase in their concentrations resulted in decreased drug loading (Tab 1). Similar results were also obtained from the drug loading studies performed for beads prepared by method II (Tab 2).

Mean Particle Size and Surface Morphology

The mean particle size of Tramadol hydrochloride loaded alginate bead

formulations prepared by method I and II were in the range of 1.005-1.076 mm and 1.057-1.207 mm respectively. The method adopted for the preparation of beads had no major effect on the particle size of the drug loaded beads. It was observed that there was a slight decrease in mean particle size of beads with increment in sodium alginate and calcium chloride concentrations (Table 1&2). Morphological characterization of the drug loaded calcium alginate beads was done by scanning electron microscopy (Fig 1 to 4).

The beads prepared by methods I and II were found to be spherical in shape. The scanning electron micrographs (Fig 2 & 3) reveal the absence of any clear demarcation of core or coat in the bead formulations prepared by methods I and II, indicating the bead formulation exists as a matrix system, where the drug is dispersed in the polymeric matrix (calcium alginate). Surface morphology of the drug-loaded beads prepared by methods I and II (Fig 2 & 3) show difference in surface morphology with that of unloaded calcium alginate beads (Fig 1).

The surface of the beads prepared by both methods showed the presence of drug on their rough external surface. In the case of beads prepared by the method II, lesser amount of drug was observed on the surface when compared to beads prepared by method I.

The SEM photographs presented in the Fig 4 also show difference in surface morphology of the prepared beads before the commencement and after the completion of *in-vitro* drug release study for beads prepared by methods I and II respectively. The surface of beads after dissolution was rougher than prior to dissolution in both the cases.

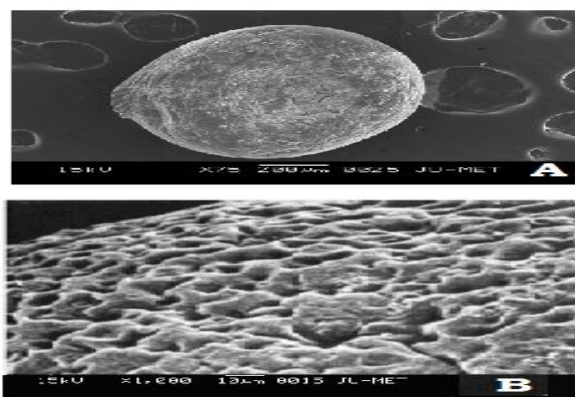


Fig 1. Scanning electron micrographs of: (a) unloaded calcium- alginate beads; (b) Surface morphology of unloaded calcium alginate bead.

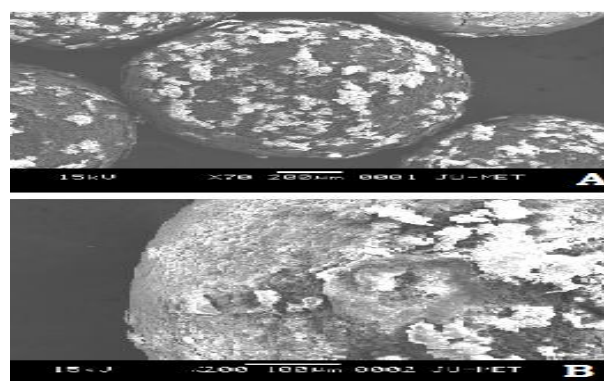


Fig 2. Scanning electron micrographs of: (a) Tramadol hydrochloride loaded calcium alginate beads prepared by sequential method (Method I); (b) surface morphology Tramadol hydrochloride loaded calcium alginate beads prepared by sequential method (Method I) (C4)

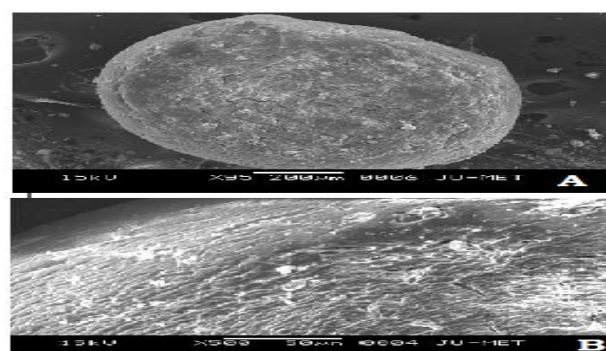


Fig 3. Scanning electron micrographs of Scanning electron micrographs of: (a) tramadol hydrochloride loaded calcium alginate beads prepared by simultaneous method (Method II) (A9); (b) surface morphology tramadol hydrochloride loaded calcium alginate beads prepared by simultaneous method (Method II) (A9)

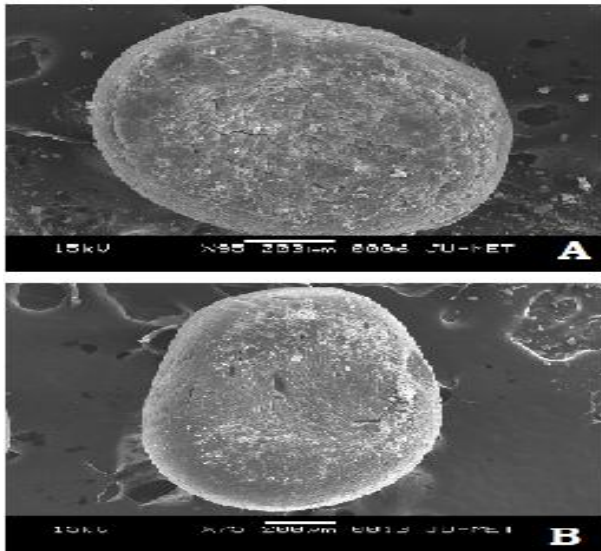


Fig 4. Scanning electron micrographs of: (a) Tramadol hydrochloride loaded beads after the completion of *in-vitro* drug release studies (SQA4); (b) Tramadol hydrochloride loaded beads after completion of *in-vitro* drug release studies (SMA6).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of the Tramadol hydrochloride, unloaded calcium alginate. The characteristic FTIR absorption peak of Tramadol hydrochloride (Fig.5a) showed the N-H stretching of the tertiary amine group¹⁷ in the range of 2300-2600 cm^{-1} . The FTIR spectrum of unloaded calcium alginate beads at 3414 cm^{-1} for OH stretching, 1617 cm^{-1} for -COOH stretching and 1024.02 cm^{-1} for C-O-C stretching¹⁷. FTIR spectra of the drug loaded beads prepared by both methods shows a peak for Tramadol hydrochloride between 2300-2600 cm^{-1} which is due to N-H tertiary amine group as for the pure drug. This indicates that Tramadol hydrochloride present in the drug-loaded beads is not involved in any interaction with the polymer matrix (calcium alginate).

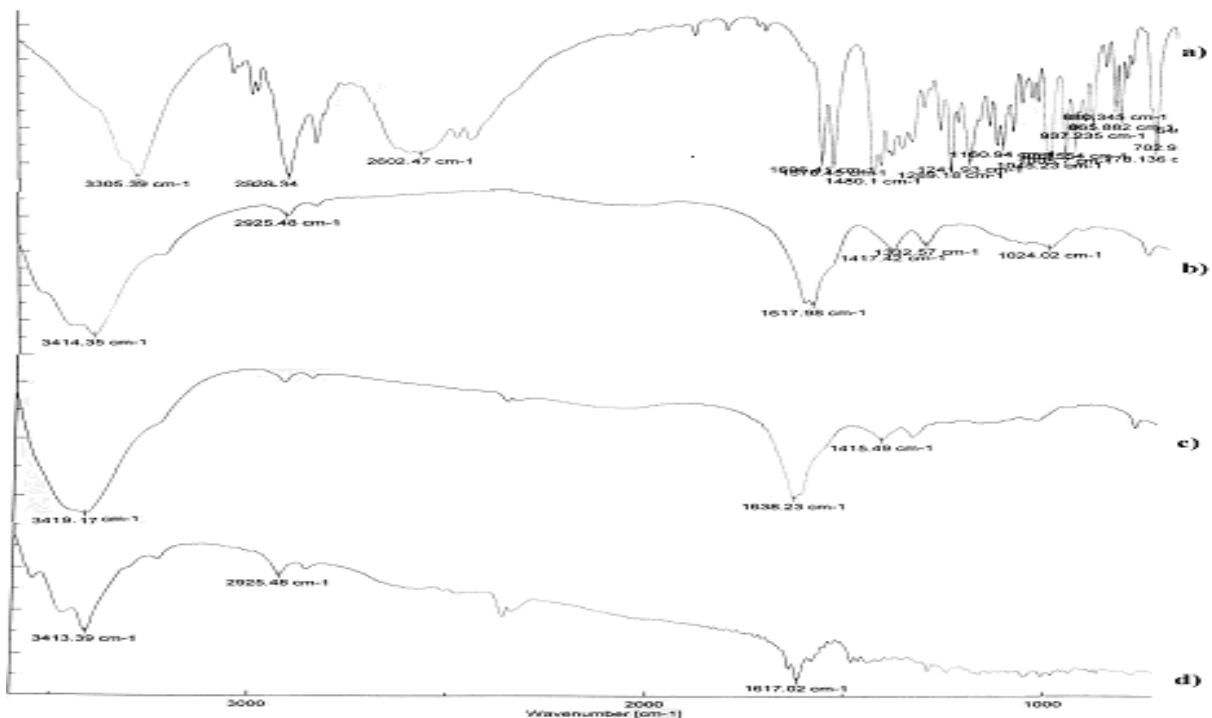


Fig 5 . FTIR spectra of : (a) Tramadol hydrochloride; (b) Unloaded calcium-alginate bead; (c) Tramadol hydrochloride loaded beads prepared by method I (SQA4); (d) Tramadol hydrochloride loaded beads prepared by method II (SMA6).

Differential Scanning Calorimetric Studies

DSC scans of the tramadol hydrochloride loaded calcium alginate beads prepared by methods I and II, unloaded polymeric matrix and tramadol hydrochloride are presented in Fig 6. The endothermic peak of tramadol hydrochloride appeared between 178 -181 °C which is its melting point (Fig 6a). This endotherm was not observed in the DSC plots of the tramadol hydrochloride-loaded beads prepared by methods I and II. This indicates that tramadol hydrochloride exists as a molecular level dispersion in the polymeric matrix¹⁸.

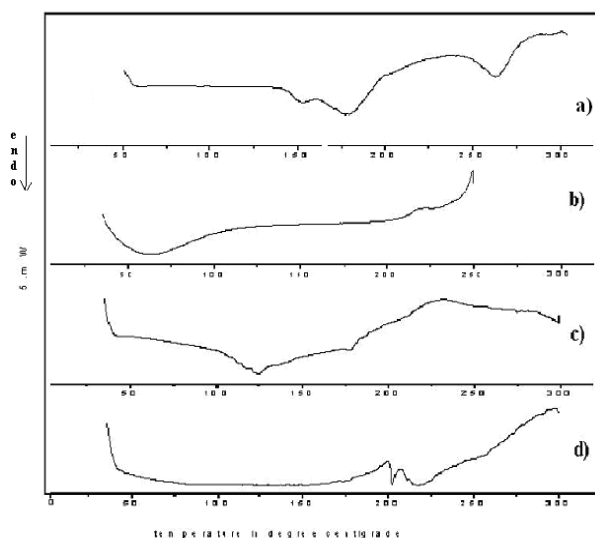


Fig 6. DSC thermograms of: (a) Tramadol hydrochloride; (b) unloaded calcium-alginate bead; (c) Tramadol hydrochloride loaded beads prepared by method I (SQA4); (d) Tramadol hydrochloride loaded beads prepared by method II (SMA6).

Powder X-Ray Diffractometry

The X-ray diffraction patterns of tramadol hydrochloride, unloaded calcium alginate beads, beads loaded with tramadol hydrochloride prepared by methods I and II are presented in

Fig 7. The X-ray diffraction pattern of tramadol hydrochloride is given in Fig 7a. In the X-ray diffraction pattern (Fig 7 c) of tramadol hydrochloride loaded beads prepared by the method I, no peak intensities were observed. From this it may be concluded that drug loaded by method I in the polymeric matrix is in an amorphous form¹⁸. In the case of Tramadol hydrochloride loaded beads prepared by the method II (Fig 7 d), only a very few peaks with lower intensities are observed when compared to the pure drug. This indicates that the major portion of tramadol hydrochloride is present in the amorphous form and is dispersed at the molecular level in the polymeric matrix system.

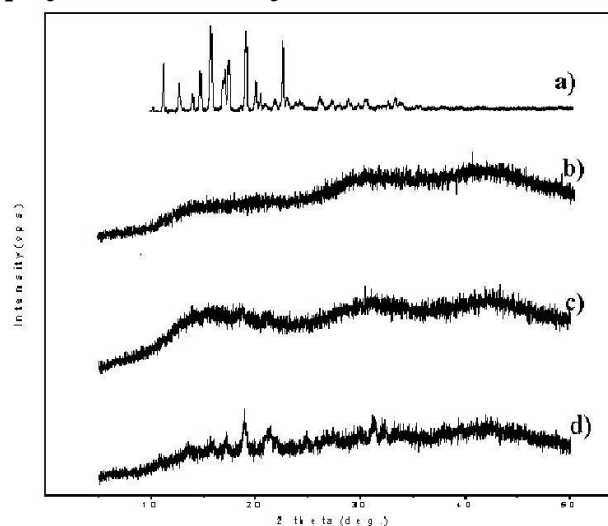


Fig 7. XRD spectra of: (a) Tramadol hydrochloride; (b) unloaded calcium-alginate bead; (c) Tramadol hydrochloride loaded beads prepared by method I (SQA4); (d) Tramadol hydrochloride loaded beads prepared by method II (SMA6).

In-Vitro Drug Release Studies

The drug release from the alginate beads depend on the penetration of the dissolution medium into the beads, with eventual swelling and burst out effect ultimately leaching the drug through the swollen matrix.

Figs 8-11 illustrate the effects of sodium alginate concentration and calcium chloride concentration on tramadol hydrochloride release from calcium alginate bead. A decrease in the rate and extent of drug release was observed with the increase in sodium alginate concentration, which may be due to an increase in the density of the polymer matrix formed and increase in the diffusional path length that the drug molecules might have to traverse. In the present study, the effect of calcium chloride (cross-linking agent) at three different concentrations, namely 1%, 2%, 3%w/v as showed in Figs 9&11.

The results confirm that the rate and extent of drug release was decreased with increase in the concentration of calcium chloride, which is due to formation of tight junction between the MM / GG residues of sodium alginate with calcium ion ¹⁹.

Further, almost similar drug release profiles were observed for the beads by both the methods.

The release of tramadol hydrochloride from the different batches prepared was characterized by an initial phase of high release (burst effect) followed by a second phase of sustained release. The initial burst effect was considerably reduced with increased sodium alginate concentration (Figs 8&10).

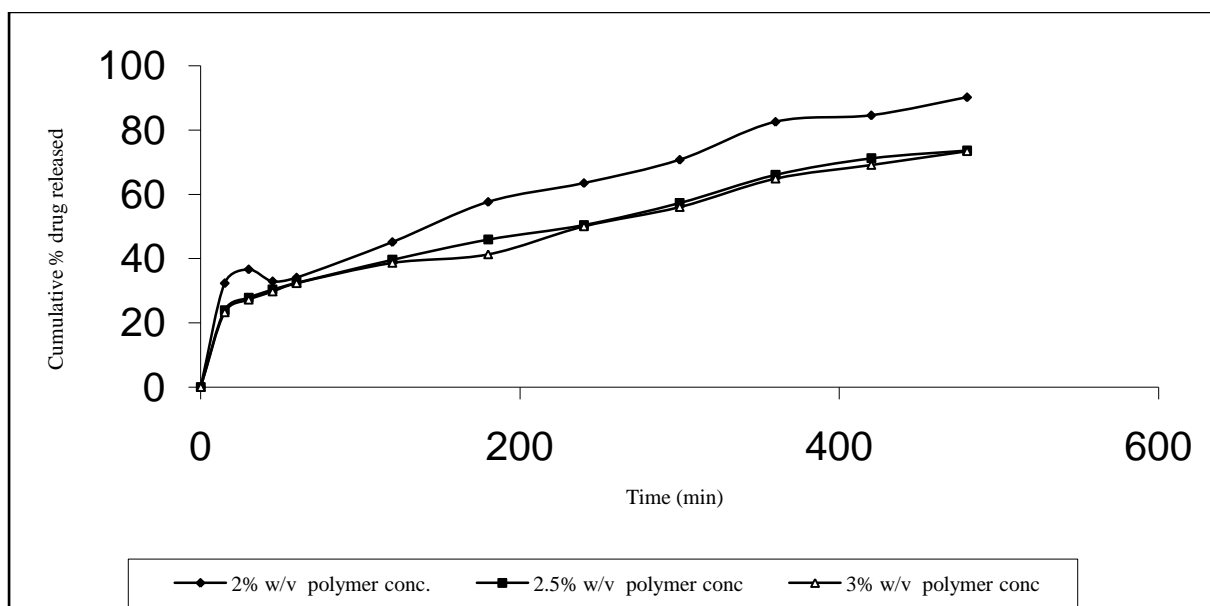


Fig 7. XRD spectra of: (a) Tramadol hydrochloride; (b) unloaded calcium-alginate bead; (c) Tramadol hydrochloride loaded beads prepared by method I (SQA4); (d) Tramadol hydrochloride loaded beads prepared by method II

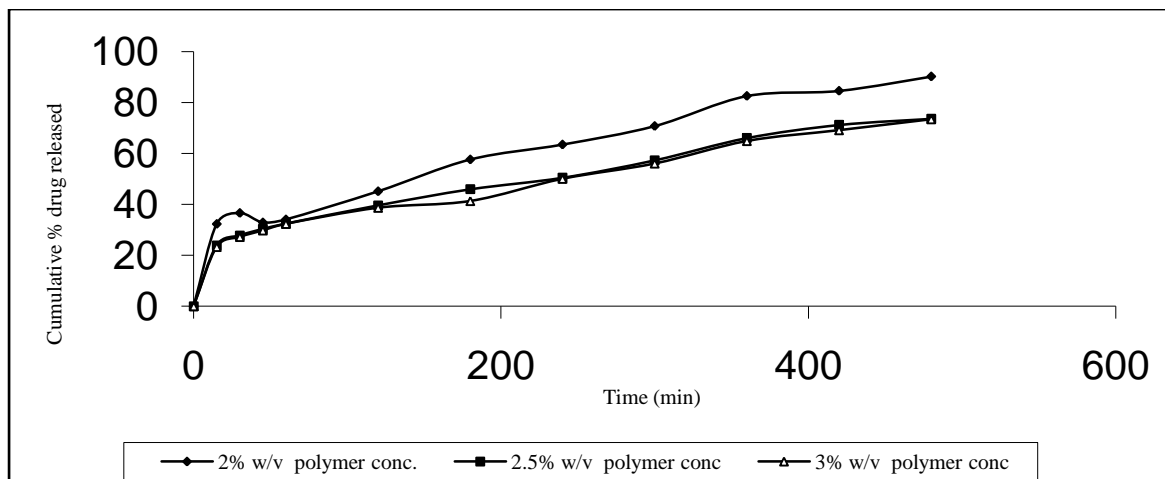


Fig 8. Effect of polymer concentration on the cumulative percentage drug released from the formulation prepared by method I

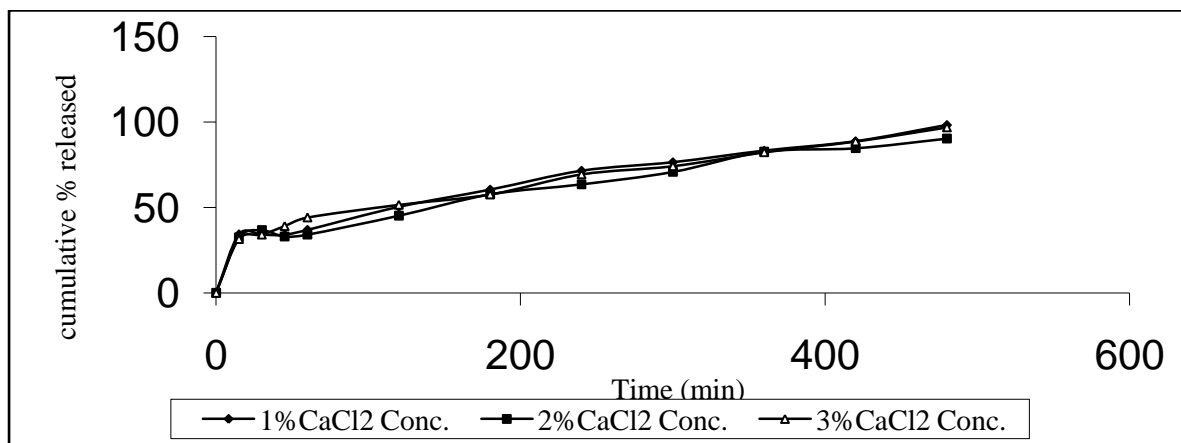


Fig 9. Effect of CaCl₂ concentration on the cumulative percentage drug released from the formulation prepared by method I

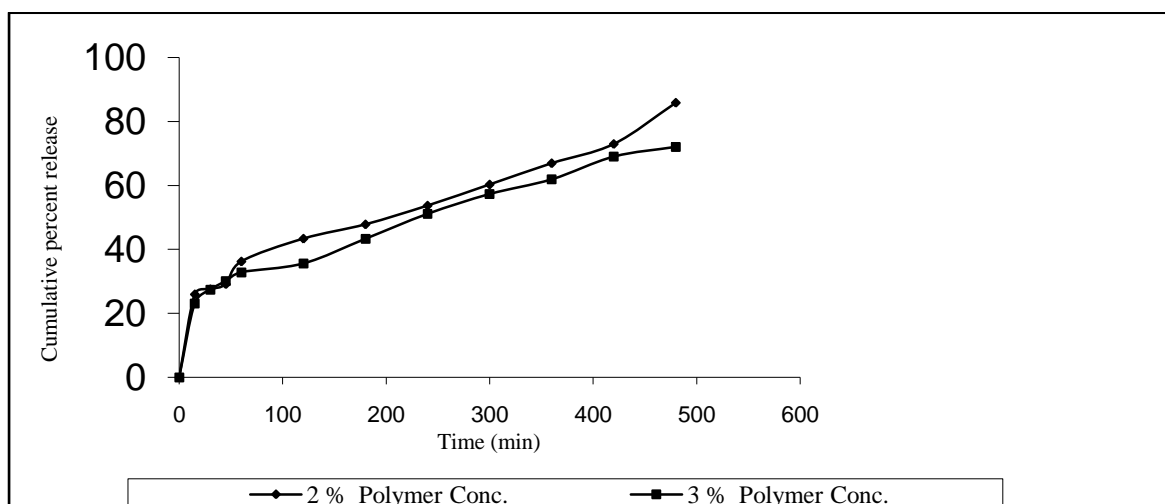


Fig 10. Effect of polymer concentration on the cumulative percentage drug released from the formulation prepared by method II

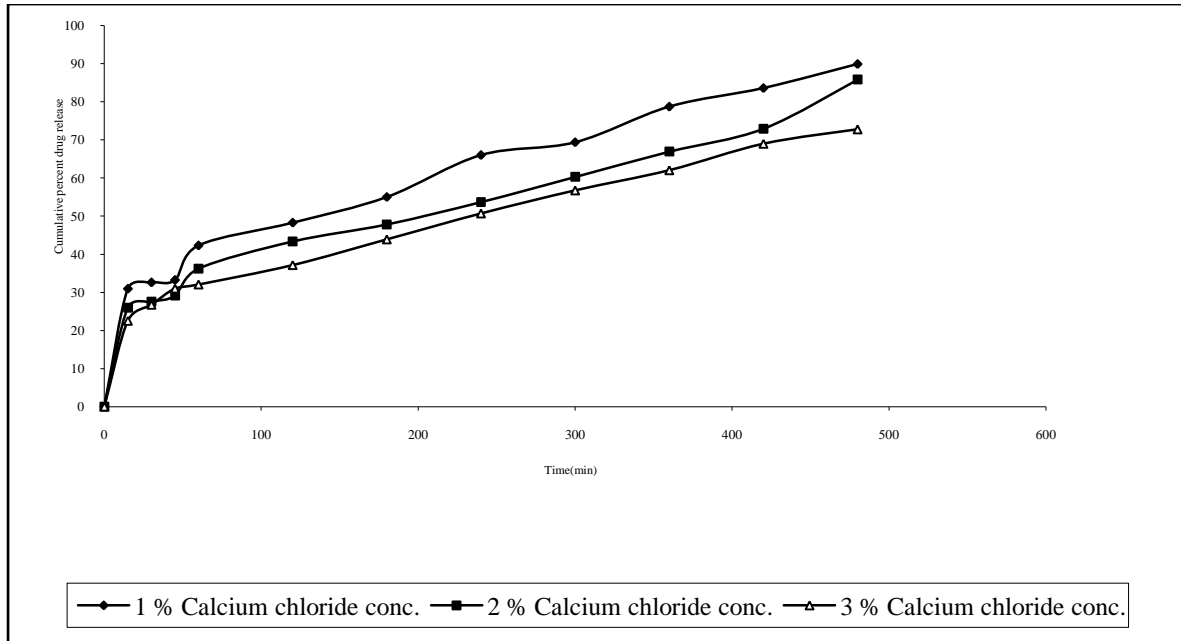


Fig 11. Effect of CaCl₂ concentration on the cumulative percentage drug released from the formulation prepared by method II

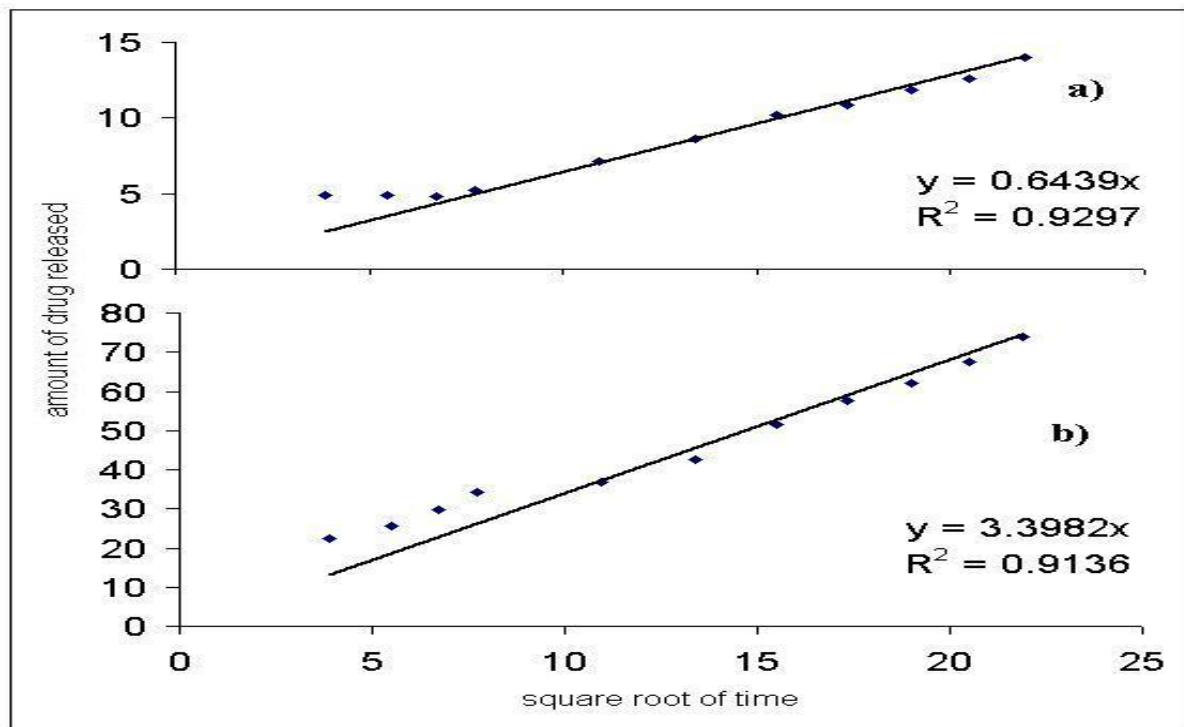


Fig 12. Higuchi diffusion kinetic model for a) the formulation (SQA1) prepared by method I and b) for the formulation (SMA5) prepared by method II.

Tab 3. Correlation coefficient of Higuchi kinetic model for Tramadol hydrochloride loaded calcium alginate beads.

Formulation code	Higuchi's kinetic model	
	K (mg/min)	r ²
SQA1	0.6439	0.9297
SQA2	0.5919	0.9034
SQA3	0.5976	0.9031
SQA4	0.474	0.9085
SQA5	0.4576	0.9059
SQA6	0.2952	0.9048
SQA7	1.1196	0.9037
SMA1	0.4498	0.9024
SMA2	0.7521	0.9014
SMA3	0.3401	0.9133
SMA4	0.2638	0.9106
SMA5	0.3024	0.9136
SMA6	0.2566	0.9016

The drug release from the tramadol hydrochloride loaded beads prepared by methods I and II followed Higuchi model²⁰ (Table 4). The Correlation Coefficients (r²) for the Higuchi model of drug release for beads prepared by method I method II are in the range of 0.9031 to 0.9297 and 0.9014 to 0.9136 respectively.

The Higuchi plot between amounts of drug released as a function of the square root of time is given in Fig 12. The amount of drug released from the beads increased linearly with the square root of time indicating that the

diffusion of drug from the beads, which is affected by the porosity and tortuosity of the matrix may be the rate-limiting step in the release of tramadol hydrochloride from calcium alginate beads.

CONCLUSION

From the present study, it can be concluded that tramadol hydrochloride loaded calcium alginate beads prepared by ionotropic gelation method with calcium chloride as cross-linking agent can be utilized for

the development of sustained release drug delivery system for tramadol hydrochloride based on information reported here in.

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