

Original Article**Calcium alginate cross-linked polymeric microbeads for oral sustained drug delivery in arthritis****K. M. Manjanna^{1,*}, T. M. Pramod Kumar², B. Shivakumar³**¹ Department of Pharmaceutics, T. V. M. College of Pharmacy, Bellary Karnataka, India;² Department of Pharmaceutics, J.S.S. College of Pharmacy, JSS University, Mysore, Karnataka, India;³ Department of Pharmaceutical Chemistry, S. C. S. College of Pharmacy, Harapanahalli, Karnataka, India.

ABSTRACT: After the successful optimization and development of a drug entity, design of dosage form then plays an important role. Hence, research continuously keeps on searching for ways to deliver drugs over an extended period of time. With aceclofenac, a novel NSAID used in the treatment of rheumatoid arthritis, frequency of administration may cause certain GI-adverse effects. The objective of the present research work was to develop a microparticulate oral sustained release dosage form, to reduce dosing frequency, to eliminate the dose related adverse effects and to ultimately improve compliance in the pharmacotherapy of arthritis. The microbeads were prepared by an ionotropic external gelation technique, by using sodium alginate as the hydrophilic carrier and calcium chloride as the cross-linking agent. The shape and surface characteristics were determined by scanning electron microscopy (SEM). Particle size distribution was determined by an optical microscope. The physical state of the drug in the formulation was determined by differential scanning calorimetry (DSC). While increasing the concentration of sodium alginate dispersion increased flow properties, mean particle size, swelling ratio and drug entrapment efficiency. The mean particle sizes of drug-loaded microbeads were found to be in the range 596.45 ± 1.04 to $880.10 \pm 0.13 \mu\text{m}$. The drug entrapment efficiency was obtained in the range of 63.24-98.90% (w/v). The release of drug from the microbeads at pH 1.2 is negligible. Under neutral conditions, the beads will swell and the drug release depends on swelling and the erosion process resulting in an optimum level of drug released in a sustained manner which exhibits zero-order kinetics.

Keywords: Oral sustained release, sodium alginate, aceclofenac sodium, ionotropic external gelation, zero-order kinetics

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1. Introduction

Arthritis is a term that includes a group of disorders that affect joints and muscles. Arthritis symptoms include joint pain, inflammation and limited movement of joints. When a joint is inflamed it may be swollen, tender, warm to the touch or red. Surrounding each joint is a protective capsule holding a lubricating fluid to aid in motion. Cartilage, a slippery smooth substance, covers most joints to assure an even, fluid motion of the joint. With joint arthritis, the cartilage may be damaged, narrowed and lost by a degenerative process or by inflammation making movement painful (1). Most of the conventional NSAIDs are used in the treatment of arthritis but have short biological half-lives and hence require repeated administration 3 to 4 times a day. This leads to patient non-compliance and also fluctuation in blood level drug concentration.

The design of effective drug delivery systems has recently become an integral part of the development of new medicines. Hence, research continuously keeps on searching for ways to deliver drugs over an extended period of time, with a well-controlled release profile. The oral administration of pharmaceutical dosage forms is the more usual, convenient and comfortable route for active drug delivery to the body. An oral modified release drug-delivery system should be able to achieve optimum therapeutic drug concentration in the blood with minimum fluctuation, to predict and reproduce release rates for extended duration, to enhance pharmacotherapy of short half life drugs, to reduce frequent dosing, to minimize/or eliminate dose related adverse effects, and to improve therapy, safety, efficacy and better patient compliance (2). The use of hydrogel systems for controlling the release of drugs has been increasingly well known to respond to surrounding conditions such as pH, ionic strength, temperature, and frequent changes of environment in the GI-tract, which has a variation of pH from the stomach to intestine. Hydrogels from natural polymers, especially polysaccharides, have been widely used because of their advantageous properties over synthetic polymers such as non-toxicity, biocompatibility, biodegradability, ability

to modify the properties of aqueous environments, and capacity to thicken, emulsify, stabilize, encapsulate, swell, and form gels and films (3). Alginate is one of the natural polysaccharides that has been widely used in numerous biomedical applications. Sodium alginate is a salt of alginic acid, a natural polysaccharide found in all species of brown algae and certain species of bacteria. It is a linear polymer of β -(1 \rightarrow 4) mannuronic acid (M) and α -(1 \rightarrow 4) L-guluronic acid (G) residues in varying proportions and arrangements. It has been shown that the G and M units are joined together in blocks, and as such, the following 3 types of blocks may be found: homo-polymeric G blocks (GG), homo-polymeric M blocks (MM), and hetero-polymeric sequentially alternating blocks (MG). The reactivity with calcium and the subsequent gel formation capacity is a direct function of the average chain length of the G-blocks. Hence, alginates containing the highest GG fractions possess the strongest ability to form gels. This initially arises from the ability of the divalent calcium cation to fit into the guluronate structures like eggs in an "egg box junction". Consequently, this binds the alginate chains together by forming junction zones, sequentially leading to gelling of the solution mixture and bead formation. When aqueous solutions of sodium alginate are added drop wise to an aqueous solution of calcium chloride, it forms a spherical gel with regular shape and size, also known as an "alginate bead". Alginate microbeads have the advantages of being nontoxic orally, have high biocompatibility, and inability to reswell in an acidic environment, whereas they easily reswell in an alkaline environment. Therefore, acid sensitive drugs incorporated into the beads would be protected from gastric juice (4).

Aceclofenac sodium is non-steroidal anti-inflammatory drug used extensively in the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It is rapidly and completely absorbed after oral administration, and peak plasma concentrations are reached 1 to 3 h after oral dose. The plasma elimination half-life of the drug is approximately 4 h and dosing frequency is 2-3 times daily with a dose range of 100-200 mg (5). An adverse gastrointestinal reaction has been observed and due to its short biological half-life aceclofenac requires multiple dosing. This leads to fluctuation in the drug blood levels and dose related adverse effects. Multiple dosing also fails to release the drug at the desired rate and in the desired amount which often results in poor patient compliance and inefficient therapy (6).

Microencapsulation is a well accepted technique for development of homogeneous, monolithic particles in a range of about 0.1-1,000 μ m and is employed to sustain drug release. Among the microparticulate systems, microbeads have special interest as carriers for NSAIDs, mainly to reduce and/or eliminate gastrointestinal irritation, dose intake and ultimately

to improve compliance in the pharmacotherapy of arthritis, inflammation and pain. In the proposed method of ionotropic gelation we drop the mixture of drug and polymer dispersion into aqueous calcium chloride solution and gelation occurs instantaneously resulting in the formation of spherical micro-scale sized beads, with narrow particle size. Calcium induced alginate beads have been developed in recent years as a unique vehicle for modified drug delivery systems. Their preparation is quite easy and is usually based on the gelling properties of the polysaccharide in the presence of several divalent ions (7).

The aim of the present study was to develop sustained release oral microbeads of aceclofenac sodium using sodium alginate as the hydrophilic carrier and calcium chloride as the cross-linking agent and to examine the effects of various process parameters on the physicochemical properties and drug release potential of the product.

2. Materials and Methods

2.1. Materials

Aceclofenac sodium was obtained as a gift sample from Microlabs, Bangalore, Karnataka, India. Sodium alginate was a gift sample from F. M. C. International Biopolymers, Willingtown, Ireland, through Signet Chemical Corporation Pvt. Ltd., Mumbai, India. Calcium chloride (fused) was purchased from S. B. Fine Chemicals Ltd., Mumbai, India. All other reagents and solvents used were of analytical grade satisfying pharmacopoeias specifications.

2.2. Preformulation studies

2.2.1. Saturation solubility study

The saturation solubility of aceclofenac sodium was determined with various concentrations of surfactants *i.e.*, 0.5, 1.0, 1.5, and 2% (w/v) of sodium lauryl sulfate (SLS) in double distilled water, 0.1 N HCl, pH 4.5 acetate buffer, pH 6.8, and pH 7.4 phosphate buffers at 37°C. An excess quantity of aceclofenac sodium was added to 100 mL of dissolution medium in a conical flask and agitated continuously at room temperature for 8 h on a shaker. The solutions were kept aside for 6 h until equilibrium was achieved. The solutions were then filtered through No. 41 Whatman filter paper, and the filtrate suitably diluted and analyzed spectrophotometrically at 275 nm (8). The results of the solubility study are summarized in Table 1.

2.2.2. Solubility of aceclofenac sodium in calcium chloride solution

The solubility of drug in a calcium chloride (1%, w/v)

Table 1. Solubility of the aceclofenac sodium in different dissolution media

Dissolution media	Concentration of SLS (% w/v)	Solubility (mg/mL)
Double-Distilled water	--	0.067 ± 0.12
	0.5	0.126 ± 0.56
	1.0	0.455 ± 0.23
	1.5	0.643 ± 0.55
	2.0	0.924 ± 0.68
0.1 N HCl	--	0.016 ± 0.87
	0.5	0.098 ± 0.77
	1.0	0.208 ± 0.87
	1.5	0.389 ± 0.65
	2.0	0.487 ± 0.08
Acetate buffer, pH 4.5	--	0.996 ± 0.76
Phosphate buffer, pH 6.8	--	3.963 ± 1.06
Phosphate buffer, pH 7.4	--	5.567 ± 0.98
Calcium chloride solution (1%, w/v)	--	0.096 ± 1.54

was determined by adding an excess of drug into the medium containing vials and shaking at constant temperature 37°C in a water bath for 12 h. The samples were filtered and diluted with distilled water, and then assayed spectrophotometrically at 275 nm (9). The results are summarized in Table 1.

2.3. Drug polymer compatibility studies

2.3.1. Thin layer chromatography (TLC)

Silica gel 60 g plates activated by heating at 105°C for 1 h were used. The mixture of methanol/acetonitrile/buffer solution (pH 6.8) in a ratio of 45:45:10 was used as a solvent system. The drug extracted from different microbeads was spotted on the plates. The R_f values were determined for comparison of pure drug and extracted drug by examining spots under UV-light.

2.3.2. Fourier transform-infrared (FT-IR) spectroscopic analysis

Drug polymer interactions were studied using FT-IR spectroscopy. One to 2 mg of aceclofenac sodium alone, and a mixture of drug and polymer were weighed and mixed properly with potassium bromide uniformly. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR-spectrum of the pellet from 450-4,000 cm^{-1} was recorded taking air as the reference and compared to study interference.

2.3.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was performed using a DSC-60 (Shimadzu, Kyoto, Japan) calorimeter to study the thermal behavior of drug alone and mixtures of drug and polymer. The instrument was comprised of calorimeter (DSC-60), flow controller

(FCL-60), thermal analyzer (TA-60) and operating software (TA-60). The samples were heated in sealed aluminum pans under nitrogen flow (30 mL/min) at a scanning rate of 5°C/min from 24 ± 1 to 250°C. An empty aluminum pan was used as reference. The heat flow as a function of temperature was measured for the drug and drug-polymer mixture (10).

2.4. Preparation of aceclofenac sodium-loaded microbeads

The microbeads were prepared by the ionotropic external gelation technique. Sodium alginate was dissolved in deionized water at a concentration of 1-3% (w/v) by gentle heat at 40°C on a magnetic stirrer. An accurately weighed 200 mg of aceclofenac sodium was added and dispersed uniformly. The dispersion was sonicated for 30 min to remove any air bubbles that may have been formed during the stirring process. The bubble free sodium alginate-drug dispersions (50 mL) were added drop wise *via* an 18-gauge hypodermic needle fitted with a 10 mL glass-syringe into 50 mL of calcium chloride solution (1-5%, w/v) and stirred at 200 rpm for 30 min. The droplets from the dispersion instantaneously gelled into discrete matrices upon contact with the solution of gelling agent. The drug-loaded microbeads were further stirred in the solution of gelling agent for an additional 2,000 rpm up to 1-3 h. After the specified stirring time and stirring speed gelled beads were separated by filtration, washed with 3×50 mL volumes of deionized water and finally dried at 80°C for 2 h in a hot air oven (10).

Fifteen batches of drug-loaded microbeads were prepared by the ionotropic gelation method to investigate the effect of certain formulation and process variables, such as drug to polymer ratio, concentration of cross-linking agent, cross-linking time and stirring time on the mean particle size, yield, distribution pattern, drug entrapment efficiency and *in vitro* drug release. To study the effect of these variables, each time one variable was varied, the other variables were kept constant and optimized to get small, discrete, uniform, and smooth surface spherical microbeads. The detailed composition of the various formulations is stated in Table 2.

2.5. Characterization and evaluation of microbeads

2.5.1. Granulometric study

Particle size has a significant effect on the release profile of microbeads. Size and size distribution, determined by sieve analysis, was carried out on mechanical sieve shaker. The drug-loaded microbeads were separated into different size fractions by sieving for 5 min using standard sieves having nominal mesh apertures of 1.4 mm, 1.2 mm, 1.0 mm, 0.85 mm, and 0.71 mm (sieve

Table 2. Composition and physical characteristics of various formulations

Batch code	D/P ratio (%, w/w) ^a	Calcium chloride (%, w/v)	Stirring time (h)	Yield (%)	Drug loading capacity (mg/100 mg)	Drug entrapment efficiency (%)
F1	1:5	4	2	88.30	56.20 ± 0.50	63.24 ± 0.66
F2	1:7.5	4	2	80.60	60.80 ± 0.75	75.43 ± 0.42
F4	1:12.5	4	2	74.80	70.20 ± 0.74	93.85 ± 0.50
F5	1:15	4	2	73.40	72.60 ± 1.20	98.90 ± 0.86
F6	1:10	1	2	72.40	60.30 ± 0.67	83.30 ± 0.75
F7	1:10	2	2	74.60	64.20 ± 1.10	86.05 ± 0.96
F8	1:10	3	2	75.10	66.80 ± 0.97	88.94 ± 0.84
F9	1:10	4	2	76.40	68.70 ± 0.60	89.95 ± 0.25
F10	1:10	5	2	77.60	72.30 ± 0.35	93.30 ± 0.23
F11	1:10	4	0.5	75.65	72.80 ± 0.55	96.23 ± 0.30
F12	1:10	4	1.0	76.15	70.15 ± 0.58	92.15 ± 0.48
F13	1:10	4	1.5	76.30	69.50 ± 0.95	91.08 ± 0.87
F14	1:10	4	2.0	76.40	68.70 ± 0.60	89.95 ± 0.25
F15	1:10	4	2.5	77.70	66.35 ± 1.45	85.40 ± 0.55

^a Drug/polymer ratio (aceclofenac/sodium alginate). Each formulation contains 200 mg of aceclofenac sodium. Data are expressed as mean ± S.D. (*n* = 3).

No. 12, 14, 16, 18, and 22, respectively). Particles that passed through one sieve but were retained on the next sieve were collected and weighed and the distribution was analyzed based on the weight fraction on each sieve. The particle size distribution and mean particle size of microbeads were calculated using the following formula (11):

$$\text{Mean particle size} = \frac{\sum(\text{particle size of the fraction} \times \text{weight fraction})}{\sum(\text{weight fraction})}$$

2.5.2. Measurement of micromeritic properties

The flow properties were investigated by measuring the angle of repose of drug-loaded microbeads using the fixed-base cone method. Microbeads were allowed to fall freely through a funnel fixed 1 cm above the horizontal flat surface until the apex of the conical pile just touches the tip of the funnel. The height and diameter of the cone was measured and angle of repose was calculated by using the following formula (11). Each experiment was carried out in triplicate (*n* = 3).

$$\text{Angle of repose } (\theta) = \tan^{-1}(h/r)$$

(*h* = cone height, *r* = radius of circular base formed by the microbeads on the ground.)

The bulk and tapped densities were measured in a 10 mL graduated cylinder as a measure of packability of the microbeads. The sample contained in the measuring cylinder was tapped mechanically by means of a constant velocity rotating cam. The initial bulk volume and final tapped volume were noted from which their respective densities were calculated (11).

Compressibility index or Carr's index value of microbeads was computed according to the following equation:

$$\text{Carr's index } (\%) = \frac{[(\text{Tapped density} - \text{Bulk density}) / \text{Tapped density}] \times 100}{}$$

Hausner's ratio of microbeads was determined by comparing the tapped density to the bulk density by using the equation:

$$\text{Hausner's ratio} = \text{Tapped density} / \text{Bulk density}$$

2.5.3. Mechanical strength study

To precisely measure mechanical strength of the alginate gel beads, large beads were prepared with sodium alginate polymer dispersion dropped through a 1 mL pipette into calcium chloride solution. The fully formed beads were collected, washed with distilled water and subsequently dried at 80°C for 2 h. Compression testing was performed with an Instron (4460). Ten beads of identical size were selected, crosshead speed and probe diameter were set at 1 mm/min and 3.5 cm, respectively (12).

2.5.4. Water uptake determination

Weighed drug-loaded microbeads were placed in a small basket, soaked in pH 6.8 phosphate buffer or distilled water and shaken occasionally at room temperature. After a predetermined time to remove excess water, beads were immediately weighed on an analytical balance. The water uptake can be calculated from the following equation (13):

$$\text{Water uptake } (\%) = [(W_t - W_0) / W_0] \times 100$$

where *W_t* and *W₀* are the wet and initial mass of beads, respectively.

2.5.5. Determination of calcium content in the beads

Alginate drug-loaded microbeads (250 mg) were dissolved in 10 mL by boiling in concentrated nitric acid. Samples were diluted with 1% (w/v) of nitric acid solution and calcium content was determined spectrophotometrically (14).

2.5.6. Disintegration test of drug-loaded microbeads

Disintegration studies were carried out in 50 mL of buffer media pH 1.2 and pH 7.2 in 100 mL conical flasks. A maximum of 5 pellets were used in each trial and stirred on a magnetic stirrer maintained at 37°C, at 25 rpm. Each batch of microbeads was run in triplicate and the time taken for all 5 pellets to disintegrate leaving behind polymer in the soluble form and drug in the insoluble form was noted as the disintegration time.

2.5.7. Particle size analysis

The particle sizes of both placebo and drug-loaded formulations were measured using an optical microscope fitted with an ocular and stage micrometer to calculate particle size distribution. The Olympus model (SZX-12) having a resolution of 30 μ m was used for this purpose. The instrument was calibrated at 1 unit of eyepiece micrometer equal to 1/30 mm (33.33 μ m). In all measurements at least 100 particles in five different fields were examined (15). Each experiment was carried out in triplicate.

2.5.8. Scanning electron microscopy (SEM) analysis

The shape and surface characteristics were determined by scanning electron microscopy (model-JSM, 35CF, Jeol, Japan) using a gold sputter technique. The particles were vacuum dried and coated to 200 Å thicknesses with gold palladium prior to microscopy. A working distance of 20 nm, a tilt of zero-degrees and an accelerating voltage of 15 kV were the operating parameters. Photographs were taken within a range of 50-500 magnification.

2.5.9. Determination of entrapment efficiency

Aceclofenac sodium content in the microbeads was estimated using a UV-spectrophotometric method. Accurately weighed 50 mg of microbeads were suspended in 100 mL of phosphate buffer pH 7.2 \pm 0.1. The resulting solution was kept for 24 h. The next day it was stirred for 15 min. The solution was filtered, after suitable dilution. Aceclofenac sodium content in the filtrate was analyzed at 275 nm using a Shimadzu 1201 UV-Visible spectrophotometer. The absorbance obtained was plotted on a standard curve to get the exact concentration of the entrapped drug. Calculating this concentration with the dilution factor the percentage of actual drug encapsulated in microbeads

was obtained (16). The drug entrapment efficiency was determined using the following relationship:

$$\% \text{ Drug entrapment efficiency} = \left[\frac{\text{Actual drug content}}{\text{Theoretical drug content}} \right] \times 100$$

2.5.10. Loose surface crystal (LSC) study

This study was conducted to estimate the amount of drug present on the surface of the microbeads which showed immediate release in dissolution media. A hundred mg of microbeads were suspended in 100 mL of phosphate buffer (pH 7.2), simulating the dissolution media. The samples were shaken vigorously for 15 min in a mechanical shaker. The amount of drug leached out from the surface was analyzed spectrophotometrically at 275 nm (16). The percentage of drug released with respect to entrapped drug in the sample was recorded.

2.5.11. Swelling properties

Swelling properties of drug-loaded microbeads were determined in various pH ranges (*i.e.*, 1.2, 4.8, and 6.8 buffer solutions), thirty dried beads were placed in a small beaker to which 100 mL of buffer solutions was added and then allowed to swell at 37°C. After a 2 h interval, the equilibrium swollen beads were observed and measured by optical microscopy (Olympus model SZX-12). The magnitude of swelling was presented as the ratio of the mean diameter of swelling beads to the mean diameter of the dried beads before the test (17). Swelling ratio was determined from the following relation:

$$\text{Swelling ratio} = \left[\frac{\text{Mean diameter at time } t\text{-initial diameter}}{\text{Initial diameter of beads}} \right] \times 100\%$$

2.6. In vitro drug release studies

The release profiles of aceclofenac sodium from microbeads were examined in three different buffer solutions to mimic the various physiological GI-tracts. The media of pH 1.2 represented the gastric condition; pH 6.8 was a compromise condition between the gastric pH and the small intestine and pH 7.2, which is phosphate buffer solution. The dissolution process was carried out using a USP XIII rotating basket apparatus (Microlabs, Mumbai, India). The drug-loaded microbeads (equivalent to 200 mg of aceclofenac sodium) filled in empty capsule shells were put into the basket, rotated at a constant speed of 75 rpm and a temperature of 37°C. The 900 mL of dissolution medium, pH 1.2, containing 2% (w/v) sodium lauryl sulfate (SLS) and the test was conducted for 2 h. At the end of 2 h the test was continued changing the dissolution media with pH 6.8 buffer solution up to 6 h and pH 7.2 phosphate buffer up to the end of 12 h. At

scheduled time intervals, a 5 mL sample was withdrawn and replaced with the same volume of fresh medium. The samples were filtered through a 0.45 μm membrane filter and after appropriate dilution, aceclofenac sodium concentration was estimated spectrophotometrically at 275 nm (Shimadzu 1201, Japan). Finally, the corresponding drug content in the samples was calculated from the calibration curve of aceclofenac sodium to determine the drug release pattern (18).

2.7. Kinetics of drug release

In order to understand the mechanism and kinetics of drug release, the drug release data of the *in vitro* dissolution study was analyzed with various kinetic equations like zero-order (% release v/s time), first-order (Log% retained v/s time) and korsmeyer and peppas equations. Correlation coefficients (*r*) values were calculated for the linear curves obtained by regression analysis of the above plots.

2.8. Stability studies of microbeads

After determining the drug content, the optimized drug-loaded microbeads were charged for the accelerated stability studies according to ICH guidelines. To assess long-term stability, accurately weighed drug-loaded microbeads equivalent to 200 mg of aceclofenac sodium were filled into hard gelatin capsules manually and sealed in aluminum packaging coated inside with polyethylene. The studies were performed at $25 \pm 2^\circ\text{C}$, $40 \pm 2^\circ\text{C}$ with $60 \pm 5\%$, and $75 \pm 5\%$ relative humidity (RH) in desiccators with saturated salt solution for up to 6 months (19). A visual inspection for drug content was conducted every month for the entire period of the stability study.

3. Results and Discussion

Side effects, mainly at the gastric level are well known, following oral administration of NSAIDs. Therefore the efforts of many researchers have been concerned to solve these problems, through a variety of techniques of protection of the gastric mucosa or alternatively to prevent the NSAIDs release in this gastric region. In this paper we evaluate the potential utility of natural material such as sodium alginate to inhibit the release of aceclofenac sodium in the gastric environment. Among microparticulate systems, microbeads have special interest as carriers for NSAIDs, mainly to extend the duration of the dosage form.

Aceclofenac sodium loaded microbeads formulated with 0.5% sodium alginate which were cured for 2 h at 2,000 rpm in 0.5% calcium chloride solution were not spherical and had a flattened base at the points of contact with the drying vessel. However, an increase in the concentration of sodium alginate tended to make

the particles more spherical. This indicates that at low alginate concentrations the particles were composed of a loose networks structure which collapsed during drying. On the other hand, higher sodium alginate concentrations formed a dense matrix structure which prevented collapse of microbeads. However, forming a highly viscous polymer dispersion did not pass easily in the needle during the manufacturing process. Moreover, we found a small tail at one end of the beads which significantly affected the flow properties and particle size distribution. It was found that optimum concentration of sodium alginate (1:10), calcium chloride (4%), and cross-linking time (2 h), could influence the microbead size, average diameter, recovery, encapsulation efficiency, and size distribution swelling behavior.

3.1. Preformulation studies (saturation solubility studies)

The available data on the solubility profile of aceclofenac sodium indicated that the drug is freely soluble in acetone and practically insoluble in water. The results of the solubility study and the influence of sink conditions are summarized in Table 1. The results showed, that there was a significant increase in solubility with increasing pH. The addition of different concentrations of SLS in 0.1 N HCl significantly increased solubility up to 0.487 mg/mL. A dissolution study of dosage forms necessitates modifications in the dissolution medium to increase the solubility of practically insoluble drugs. Aceclofenac sodium is a weak acid; the solubility of aceclofenac sodium in HCl was much less compared to distilled water. However, the addition of surfactant is a reasonable approach for solubilizing such drugs, because various surfactants are present in the GI-fluid. Saturation solubility of aceclofenac sodium in different media increased with an increase in buffer pH as well as with an increase in surfactant concentration. The significant increase is attributed to the micellar solubilization by SLS. Aceclofenac sodium showed sufficient solubility in 0.1 N HCl with 2% (w/v) of SLS which was adequate to maintain sink condition and was selected as the dissolution medium for *in vitro* drug release studies.

The solubility of aceclofenac sodium in calcium chloride was found to be 0.96 ± 1.54 mg/mL. The solubility of aceclofenac sodium was more in 1% (w/v) calcium chloride solution than in double-distilled water, which induces a certain amount of drug release, when there is prolonged exposure of the beads in curing medium during the manufacturing process.

3.2. Drug/polymer compatibility studies

3.2.1. Thin layer chromatography (TLC)

In results from TLC studies the R_f value of aceclofenac

sodium pure drug was 22, and linearity was between 700-2,400 ng/zone. The R_f value of extracted drug from microbeads was 21.40, and linearity was in the range between 2,350 ng/zone. In images of spots observed on the TLC plates, there is no overlap of spots; this indicates that the drug did not contain any extraneous matter. Moreover, extracted drug R_f values obtained from microbeads are almost equal to that of the standard drug. This indicates there is no deformation during the process.

3.2.2. Fourier transform-infrared (FT-IR) spectroscopic analysis

IR-spectra of pure aceclofenac sodium, sodium alginate and the physical mixture of drug and polymer are shown in Figure 1. The characteristic absorption peaks were obtained at 2966.36, 2915.5, 1716.5, 1589.3, 1479.6, and 665.4 cm^{-1} for pure aceclofenac sodium, 2814.98, 1603.34, 1519.3, 1359.60, and 674.05 cm^{-1} for sodium alginate, 2820.28, 1591.72, 1384.91, 1102.03, and 666.4 cm^{-1} for physical mixture of drug and polymer (Figure 1). The compatibility of aceclofenac sodium with polymer was investigated by an IR-spectroscopy study. The IR spectra of the drug and polymer combination were compared with the spectra of the pure drug and individual polymer spectra. There were no considerable changes in the IR peaks of aceclofenac in the physical mixture, thereby indicating the absence of any interaction.

3.2.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry thermo grams of pure drug and drug-loaded sodium alginate microbeads was observed, calcium chloride shows two endotherm peaks in the temperature range 180-200°C; while sodium alginate decomposes at about 240°C with a broad exotherm. Pure drug aceclofenac sodium showed a sharp endotherm at 154.50°C corresponding to its melting point. There was no appreciable change in the melting endotherm of the physical mixture as compared to pure drug. The peak of the drug did not appear in the thermogram of any type of the prepared microbeads containing the drug. This may indicate that the drug was uniformly dispersed at the molecular level of polymers.

3.3. Characterization and evaluation of microbeads

The total percentage yields of drug-loaded microbeads obtained were in the range between 72.40 to 88.30% (w/w). It was observed that increasing the polymer ratio in the formulation significantly lowered the product yield due to the formation of a highly viscous polymer dispersion which may be lost during the manufacturing process. A further observation, when the drug/polymer ratio was constant, an increase in the concentration of

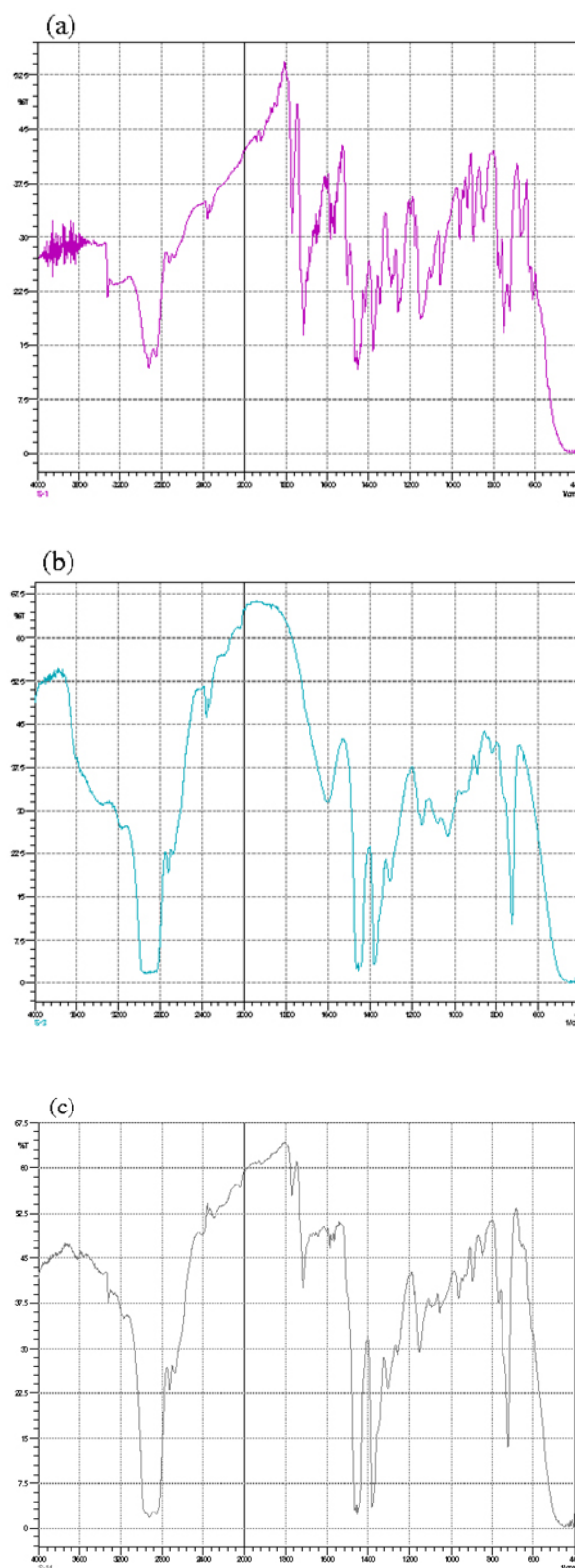


Figure 1. IR-spectra. (a) Aceclofenac sodium pure drug. (b) Sodium alginate. (c) Physical mixture.

calcium chloride and curing time slightly increased the percent yield. Actual drug concentration in the microbeads was evaluated and found to be in the range of 56.20 ± 0.50 to 72.80 ± 0.55 mg/100 mg. The polymer concentration consequently makes the actual drug loading higher due to the increase

in hydrophobicity, leading to better precipitation of polymer at the boundary phase of the droplets.

3.3.1. Granulometric study

The effects of various processes and formulation parameters on the micromeritic properties of drug-loaded microbeads were evaluated, and the size distribution of the microbeads in different sieves was observed and showed 32.46% to 89.50% of microbeads retained \neq 22 sieve. The size distribution of microbeads was observed and that an increase in the concentration of sodium alginate and calcium chloride solutions tends to form more spherical particles and more uniform size spheres. On the other hand, an increase in cross-linking time and stirring speed are also favorable for the formation of more spherical beads and distribution of particle size slightly shifts to the lower pore size.

3.3.2. Measurement of micromeritic properties

The rheological parameters like angle of repose, bulk density and tapped density of all microbeads confirms better flow and packaging properties. All the formulations showed excellent flowability represented in terms of angle of repose ($< 40^\circ$) (20). Here, the sodium alginate concentration also has a significant positive effect on the angle of repose (Table 3). Particle size increased with an increase in the concentration of sodium alginate and resulted in a decreased angle. However, higher calcium chloride concentration, cross-linking time and high stirring speed influenced the formation of smaller beads because of shrinkage and showed an increased angle of repose (Table 3). Bulk and tapped density of beads showed an acceptably good range which indicates that the beads have good packability. The density of the beads increases as the concentration of the polymer increases suggesting that

the beads formed at high polymer concentration are more compact and less porous than those prepared at low polymer contents (Table 3). Carr's index and Hausner's ratio explain that the formulated microbeads had excellent compressibility and good flow properties. The improvement of flow properties suggest that the microbeads can be easily handled during processing. Mechanical testing was performed in order to study the effect of concentration of calcium chloride and cross-linking time. The results show increasing the concentration of calcium chloride and cross-linking time significantly increases the mechanical strength due to formation of a dense matrix between sodium and Ca^{2+} divalent ions.

3.3.3. Water uptake determination

The % water uptake of the aceclofenac sodium loaded microbeads in distilled water was significantly lower than that in pH 6.8 phosphate buffer. The equilibrium time of water uptake in distilled water of the beads was between 0.5-2 h. In the case of phosphate buffer pH 6.8 the equilibrium time of water uptake reaches 1 h because calcium ions cross-linked with alginate were rapidly exchanged with sodium ions in phosphate buffer with a prolonged cross-linking time. Moreover, calcium alginate gels could be solubilized by the addition of phosphate ion, which acted as a complexing agent for calcium ions at a pH above 5.5 (20). The sodium alginate concentration increased water uptake of the beads ultimately increases the swelling behavior of the beads at higher pH levels which would significantly delay drug release.

3.3.4. Determination of calcium content in the beads

Calcium content in the drug-loaded microbeads was performed and obtained in the range 0.346 ± 0.116

Table 3. Micromeritic properties of drug-loaded microbeads

Batch code	Mean particle size (μm)	Angle of repose (θ)	Bulk density (g/mL)	Tapped density (g/mL)	Carr's index (CI) (%)	Hausner's ratio
F1	596.45 \pm 1.04	32.20 \pm 1.96	0.475 \pm 0.07	0.593 \pm 0.03	19.89	1.24 \pm 0.20
F2	624.86 \pm 0.98	28.16 \pm 0.62	0.566 \pm 0.92	0.675 \pm 0.06	16.14	1.19 \pm 0.30
F3	703.55 \pm 0.75	22.65 \pm 0.55	0.665 \pm 0.75	0.782 \pm 0.05	14.96	1.17 \pm 0.58
F4	844.75 \pm 1.10	20.55 \pm 1.07	0.695 \pm 0.05	0.807 \pm 0.87	13.87	1.16 \pm 0.15
F5	880.10 \pm 1.23	19.85 \pm 0.54	0.745 \pm 0.08	0.855 \pm 0.16	12.86	1.14 \pm 0.78
F6	746.60 \pm 0.73	30.65 \pm 0.85	0.515 \pm 0.16	0.665 \pm 0.22	22.55	1.29 \pm 0.12
F7	734.10 \pm 0.54	27.75 \pm 0.96	0.565 \pm 0.25	0.697 \pm 0.45	18.95	1.23 \pm 0.45
F8	724.40 \pm 0.34	26.25 \pm 0.55	0.635 \pm 0.35	0.753 \pm 0.96	15.70	1.18 \pm 0.68
F9	703.55 \pm 0.75	22.65 \pm 0.55	0.665 \pm 0.75	0.782 \pm 0.05	14.96	1.17 \pm 0.58
F10	688.56 \pm 1.25	19.10 \pm 1.23	0.712 \pm 0.15	0.810 \pm 0.46	12.10	1.13 \pm 0.77
F11	804.35 \pm 1.43	25.75 \pm 0.64	0.555 \pm 0.77	0.695 \pm 0.55	20.14	1.25 \pm 0.84
F12	764.45 \pm 1.05	24.66 \pm 0.77	0.588 \pm 0.93	0.724 \pm 0.15	18.80	1.24 \pm 0.56
F13	724.64 \pm 1.54	23.15 \pm 0.87	0.625 \pm 0.66	0.758 \pm 0.35	17.62	1.20 \pm 0.34
F14	703.55 \pm 0.75	22.65 \pm 0.55	0.665 \pm 0.75	0.782 \pm 0.05	14.96	1.17 \pm 0.58
F15	708.10 \pm 0.86	18.85 \pm 1.15	0.695 \pm 0.82	0.805 \pm 0.77	13.65	1.15 \pm 0.55

Data are expressed as mean \pm S.D. of at least triplicate.

to $0.676 \pm 0.232\%$ (w/w) in 10 mg beads. Results are known that calcium chloride concentration affects the amount of Ca^{2+} ions in alginate beads. Formulations prepared with 5% (w/v), have higher Ca^{2+} contents than those of the formulations prepared with low concentrations of calcium chloride. On the other hand, sodium alginate concentration and cross-linking time increases the calcium content in the beads.

3.3.5. Disintegration test of drug-loaded microbeads

In all formulations the disintegration time was found in the range between 116 to 185 min. The disintegration times of the drug-loaded microbeads increased with increased sodium alginate concentrations because it leads to increased viscosity of the polymeric matrix and increased cross-linking in turn to form stronger beads which take longer times for disintegration.

3.3.6. Particle size analysis

The mean particle size of drug-loaded microbeads were performed using optical microscopy, and mean particle size of the various formulations (F1-F15) of microbeads were obtained in the range between 596.45 ± 1.04 to 880 ± 1.23 (μm) (Table 3). It was found that the particle size distribution of each formulation was within a narrow size but the mean particle size was different among the formulations. The results indicated that the proportional increase in the mean particle size of microbeads increased with the amount of sodium alginate in the formulations. This could be attributed to an increase in relative viscosity at higher concentrations of sodium alginate and formation of large droplets during addition of polymer solution to the gelling agent. On the other hand, the mean particle size of microbeads was found to decrease with an increase in the concentration of calcium chloride. It has been stated that when a drop of alginate solution comes in contact

with calcium ions, gelation occurs instantaneously. As Ca^{2+} ions penetrate into the interior of droplets, water is squeezed out of the droplets resulting in contraction of beads (Table 3). The size of the spherical matrix could be easily controlled by varying the stirring speed of the system. The mean particle size of microbeads was tremendously decreased with an increase of rotational speed. At a stirring speed of 500 rpm, the mean particle diameter and the size distribution of the beads increased significantly. This low stirring speed might have decreased the uniformity of the mixing force throughout the emulsion mixture, and the particles were found to settle at the bottom of the vessel resulting in a wider diameter for the final beads. Consequently, at a higher stirring speed, a vigorous, uniform, increased mechanical shear might have influenced the formation of lesser diameter beads. A higher mixing rate did not further reduce the mean diameter, because high turbulence caused frothing and adhesion to the container wall. The effect of cross-linking time at a particular stirring speed was also observed, and it was recorded that cross-linking time influenced the shape as well as the size distribution of microbeads, possibly because of the variable shear force experienced by the particulate system.

3.3.7. Scanning electron microscopy analysis (SEM)

The SEM photomicrographs of the dried drug-loaded microbeads and their surface morphology are shown in Figure 2. Morphology of the various formulations of drug-loaded microbeads was discrete and spherical in shape with a rough outer surface and visible large wrinkles with a sandy appearance because of the surface-associated crystals of drug.

3.3.8. Determination of entrapment efficiency

The effect of various process and formulation

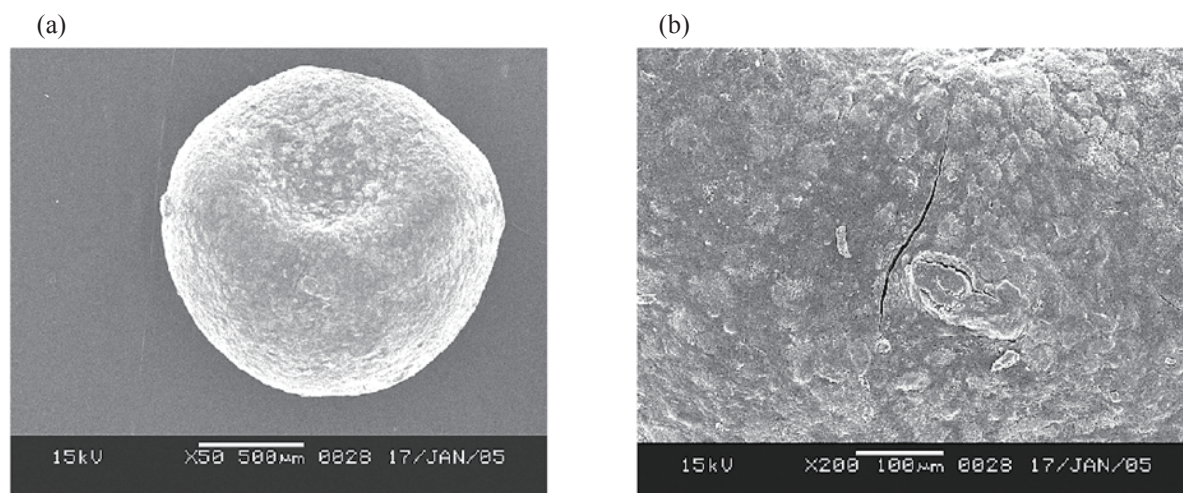


Figure 2. Scanning electron micrographs of aceclofenac sodium microbeads at 15 kV. (a) Overall. (b) Surface.

parameters on the drug entrapment efficiency of microbeads were investigated, keeping the concentration of calcium chloride, stirring speed, and cross-linking time fixed at 4% (w/v), 2,000 rpm, and 2 h, respectively. By increasing the drug/polymer ratio concentration from 1:5 to 1:15 (w/w), the drug entrapment efficiencies were found to be in the range of 63.24 ± 0.66 to $98.90 \pm 0.86\%$ (w/w). It was observed that the drug entrapment efficiencies increased progressively with increased concentration of sodium alginate resulting in the formation of larger beads entrapping a greater amount of drug. This may be attributed to the greater availability of active calcium binding sites in the polymeric chains and, consequently, a greater degree of cross-linking as the amount of sodium alginate increased. Alginate concentration increases may also reduce loss of drug in the curing medium due to the formation of a dense matrix structure.

When the concentration of drug/polymer ratio, stirring speed, and cross-linking time were fixed at 1:10, 2,000 rpm, and 2 h, respectively, increasing calcium chloride concentration from 1-5% (w/v) the drug entrapment efficiencies were found to be in the range 83.30 ± 0.75 to $93.30 \pm 0.2\%$ (w/w). From the results, it is obvious that increasing calcium chloride concentration produced beads with higher levels of Ca^{2+} ions. Consequently, the cross-linking of the polymer and compactness of the formed insoluble dense matrices also increased, which resulted in more drug entrapment in the microbeads (Table 2). On the other hand, further increase in the concentration of calcium chloride above 5% (w/v) did not enhance drug loading. This could be due to possible saturation of calcium binding sites in the guluronic acid chain, which prevented further Ca^{2+} ion entrapment and, hence, cross-linking was not altered with higher concentrations of calcium chloride solution.

Drug entrapment efficiencies were evaluated while keeping the drug/polymer ratio, concentration of calcium chloride, and stirring speed constant at 1:10 (w/w), 4% (w/v), and 2,000 rpm, respectively. Increasing cross-linking time from 0.5 to 2.5 h, the drug entrapment efficiencies were found to be in the range 85.40 ± 0.55 to $96.77 \pm 0.30\%$ (w/w). The cross-linking time also effects the drug entrapment efficiencies of formulated drug-loaded microbeads. Increasing the cross-linking time resulted in a decrease in the drug entrapment efficiencies (Table 2), since the solubility of aceclofenac sodium was slightly higher in calcium chloride than in distilled water. Prolonged exposure in the curing medium caused greater loss of drug through weakly cross-linked alginate beads. However, constant drug loading was achieved at 2 h, with no further decrease after 4 and 5 h of curing time. This could be due to the formation of a tight junction between calcium ions and the active sites on the guluronic acid chain. Consequently, the drug was entrapped in the

highly bound calcium alginate matrix which resulted in no further diffusion of drug in the curing medium.

3.3.9. Loose surface crystal study (LSC)

The loose surface crystal (LSC) study was an important parameter giving an indication of the amount of drug on the surface of the microbeads without proper entrapment. With increased concentration of sodium alginate and calcium chloride solutions, the LSC decreased significantly due to high entrapment of drug in the dense matrix structure.

3.3.10. Swelling properties

The "Swelling-Dissolution-Erosion" process is highly complex. In systems based on sodium alginate cross-linked with calcium chloride, the osmotic pressure gradient that exists between the alginate gel and the environment comprises an important factor in the swelling process. The swelling ratio of the beads was dependent on the pH of the solution. Under acidic conditions, swelling of calcium alginate beads occurs scarcely. Under neutral conditions, the beads will swell and the drug release depends on the swelling and erosion process. Being a polyelectrolyte, alginate can exhibit swelling properties that are sensitive to pH, ionic strength, and ionic composition of the medium (21). Optical microscopy was used to investigate the hydration and swelling of microbeads at pH 1.2, 4.8, and 6.8 up to 4 h (Figures 3a-3c). The equilibrium swelling studies showed that, with an increase in the polymer concentration, swelling of beads was significantly increased. The low swelling in acidic media at pH 1.2 was probably due to proton-calcium ion exchange forming insoluble alginic acid regions followed by solvent penetration into the gel network. The swelling of beads was ultimately increased at pH 4.8 and pH 6.8 at the end of 4 h. This was due to increased solubility of the polymer in basic pH leading to relaxation of the cross-linked polymeric network. It has been reported that the swelling can be enhanced by the presence of phosphate ions at higher pH which displaces the Ca^{2+} ions within the beads (Figure 3a). Increasing the concentration of calcium chloride produces beads with higher levels of Ca^{2+} ions that could reduce the swelling of beads in acidic medium. However, the amount of calcium in swollen gel films after 4 h in the medium was about 10-30%, which apparently prevented total breakdown of the gel structures. The swelling behavior of beads at pH 4.8 and 6.8 was observed as a result of slight increases of swelling ratio due to ionic exchange between the phosphate ions in the buffer and a higher level of Ca^{2+} ions within the beads (Figure 3b). When we compared the swelling ratio with a prolonged cross-linking time while maintaining the same drug/polymer ratio

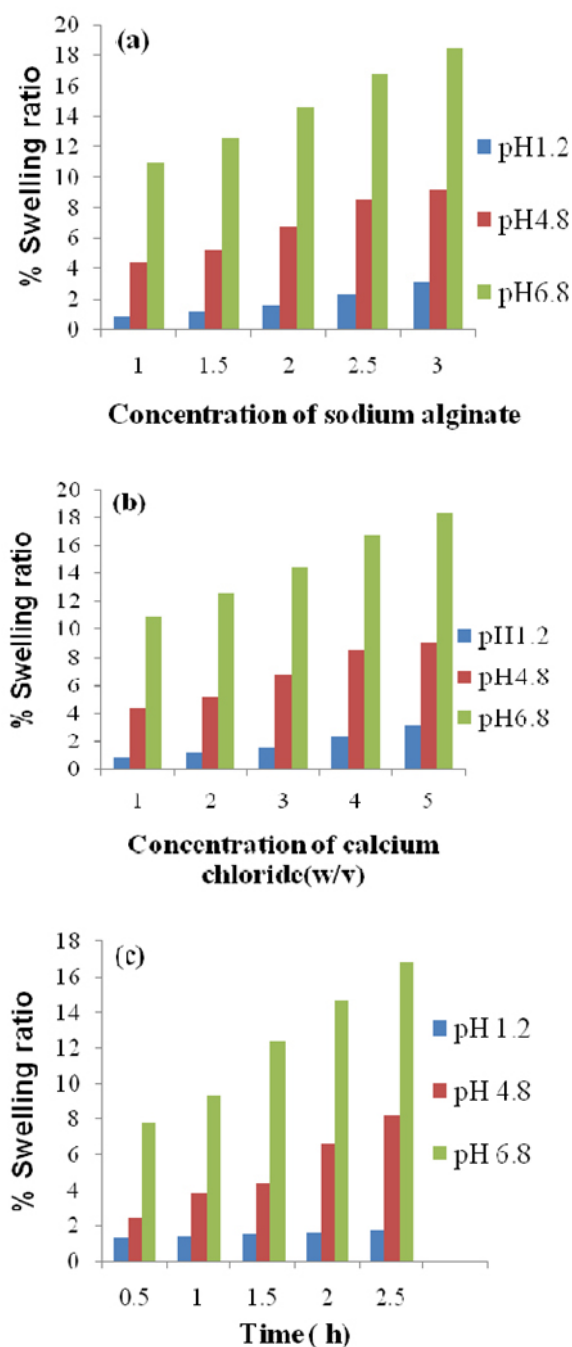


Figure 3. Effect of various parameters on the swelling behavior of drug-loaded microbeads. (a) Polymer concentration. (b) Calcium chloride concentration. (c) Cross-linking time.

and concentration of calcium chloride in the system, appreciable maximum swelling was shown with increased pH levels. These results may be due to the maximum extent of cross-linking that yielded compact beads, which might have rehydrated to a greater extent. The sequestering action of phosphate ions in higher pH media on Ca^{2+} ions may have contributed to the swelling of cross-linked beads. The lower rehydration of beads that were prepared at shorter cross-linking times may be correlated with incomplete cross-linking of sodium alginate (Figure 3c). We further observed that

the swelling ratio of microbeads prepared at various stirring speeds could not affect much of the swelling equilibrium of the beads. When we compared the overall results of the swelling ratio of all formulations, the slowest swelling ratio was obtained at pH 1.2, whereas the highest was at increased pH levels of the medium, initially. Further they were broken after 2 h. The overall results suggest that the dried beads swell slightly in the stomach. When they are subsequently transferred to the upper intestine, the particles begin to swell and they behave as matrices for sustained release of incorporated drug but they are subjected to erosion in the lower intestine.

3.4. *In vitro* drug release studies

Acetoclofenac sodium release from formulated microbeads have been performed in different media, either in simulated gastric fluid (SGF) at pH 1.2 for an initial 2 h, and mixed phosphate buffer at pH 6.8 for a period up to 6 h and pH 7.2 at the end of 12 h studies. When we changed the pH from 1.2 to 6.8 by mixing with the phosphate buffer, the drug release rate was slightly increased and found to be in the range of 37.80 ± 0.32 to 64.80 ± 0.12 (%). On further changing the pH from 6.8 to 7.2 (SIF) till 12 h, the maximum drug released at a constant rate was found to be in the range of 78.60 ± 0.67 to 97.20 ± 0.36 (%) (Table 4).

The acetoclofenac sodium was slightly soluble in water and showed very poor solubility in the acidic buffer media as a result of which we had to use 2.0% (w/v) SLS in the media to aid the dissolution of the drug. It is generally seen that when microbeads formulated with hydrophilic polymer are immersed in water, they swell and form a gel diffusion layer that hinders the outward transport of the drug, hence producing a sustained release effect. However, the drug release from alginate beads was pH dependent, and all of the formulations showed negligible drug release in acidic pH 1.2 ($< 5\%$, w/w) which may be due to the stability of alginate at lower pHs and conversion of Ca-alginate from the insoluble alginic acid to a formed tightening of the gel mesh work. On the other hand, the polymer is eroded at alkaline pH and the contents are released in a sustained manner by both diffusion and slow erosion of polymer matrix. However, the swelling behavior of drug-loaded Ca-alginate beads at higher pH could be explained by the ionotropic effect that occurs between the Ca^{2+} ion of alginate and Na^+ ions present in phosphate buffer and consequently, capturing of the Ca^{2+} by phosphate ions (22). The ion exchange with phosphate buffer which resulted in swelling and erosion of the beads and formation of the solute Ca-phosphate all have an influence on the increase of drug release rate at higher pH. This might be due to the lower number of Na^+ ions present in that buffer and consequently a slower rate of

Table 4. *In vitro* drug release and various kinetic data of drug-loaded microbeads

Batch code	Cumulative % drug release			Various Kinetic models				
	pH 1.2 ^a	pH 6.8 ^b	pH 7.2 ^c	Zero-order (r) ^d	First-order (r) ^d	Higuchi-matrix (r) ^d	Korsmeyer-peppas (r) ^d	'n'- values ^e
F1	4.32 ± 0.05	64.80 ± 0.12	93.70 ± 0.19	0.9980	0.9515	0.9737	0.9984	0.8806
F2	3.80 ± 0.06	60.40 ± 0.44	88.80 ± 0.43	0.9960	0.9076	0.9694	0.9932	0.9160
F3	3.06 ± 0.12	54.60 ± 0.32	86.40 ± 0.36	0.9981	0.9098	0.9674	0.9955	1.0745
F4	2.60 ± 0.23	57.40 ± 0.56	83.90 ± 0.88	0.9942	0.9854	0.9951	0.9937	1.0682
F5	2.06 ± 0.44	45.80 ± 0.32	78.60 ± 0.67	0.9988	0.9887	0.9940	0.9969	1.1304
F6	4.04 ± 0.03	58.20 ± 0.16	95.40 ± 0.90	0.9756	0.9143	0.9675	0.9855	1.0791
F7	3.42 ± 0.02	56.40 ± 0.66	90.20 ± 0.15	0.9866	0.9843	0.9910	0.9834	1.2962
F8	3.24 ± 0.08	55.80 ± 0.74	88.80 ± 0.19	0.9955	0.9784	0.9870	0.9904	1.2304
F9	3.06 ± 0.07	54.60 ± 0.65	86.40 ± 0.36	0.9978	0.9865	0.9943	0.9967	1.0745
F10	2.44 ± 0.04	47.80 ± 0.45	81.50 ± 0.55	0.9993	0.9877	0.9930	0.9987	1.4023
F11	3.96 ± 0.06	58.70 ± 0.43	97.20 ± 0.36	0.9786	0.9712	0.9890	0.9915	0.9406
F12	3.10 ± 0.07	56.10 ± 0.55	94.30 ± 0.56	0.9852	0.9087	0.9648	0.9833	1.3720
F13	3.20 ± 0.05	55.60 ± 0.36	90.50 ± 0.84	0.9844	0.9765	0.9908	0.9867	1.2882
F14	3.06 ± 0.04	54.60 ± 0.48	86.40 ± 0.36	0.9976	0.9863	0.9917	0.9877	1.0745
F15	2.43 ± 0.15	48.30 ± 0.90	82.30 ± 0.64	0.9991	0.9923	0.9912	0.9886	0.9684

All the results shows S.D. ($n = 3$). ^a Values expressed at the end of 2 h; ^b Values at the end of 6 h; ^c Values expressed at the end of 12 h; ^d Correlation coefficient; ^e Diffusion exponent related to mechanism of drug release, according to equation of $Mt/M = Kt^n$.

ion exchange and swelling of the polymer at this pH. The present results revealed that swelling is the main parameter controlling the release rate of aceclofenac sodium from alginate matrices, which is modulated by a "swelling-dissolution-erosion" process.

3.4.1. Effect of drug/polymer ratio on drug release

The effect of drug/polymer ratio on aceclofenac sodium release from different batches of microbeads is shown in Figure 4a. As the drug/polymer ratio increased, the release rate of aceclofenac sodium from the microbeads decreased. The slower release rate can be explained by the increase in the extent of swelling and the gel layer thickness that acted as a barrier for the penetration medium, thereby retarding the diffusion of drug from the swollen alginate beads. However, the steady state release was achieved after an initial lag time and it was directly proportional to the concentration of sodium alginate. The first phase might be due to the negligible dissociation of alginate beads in phosphate buffer mainly based on drug diffusion through the small pores and cracks. The second phase exhibited a burst-like release pattern, which was accompanied by alginate disintegration. The sodium alginate concentration in the formulation greatly influenced the steady state release of drug from the microbeads.

3.4.2. Effect of calcium chloride concentration on drug release

The effect of cross-linking agent on aceclofenac sodium release from different batches of microbeads is shown in Figure 4c. The results indicate that rate and extent of drug release decreased significantly with increase

of calcium chloride concentration. This is because sodium alginate is a linear copolymer consisting of β -(1 \rightarrow 4) mannuronic acid and α -(1 \rightarrow 4) L-guluronic acid residues and a tight junction is formed between the residues of alginate with calcium ions. However, higher calcium chloride concentrations, increased surface roughness and porosity (Figure 2) and also poor entry of dissolution medium into the polymer matrix may delay drug release.

3.4.3. Effect of stirring time on drug release

Variation in cross-linking time was also studied for selecting the most optimized formulation. The effect of stirring time on aceclofenac sodium release from different batches of microbeads is shown in Figure 4d. An increase in the cross-linking time from 0.5-2.5 h significantly decreased drug release due to penetration of calcium into the interior of the beads. Faster drug release was observed with 0.5-1 h, which can be attributed to the poor binding of drug within the polymer matrix and also incomplete gelling of sodium alginate. Increasing the cross-linking time to more than 2 h, however, caused no significant change in the amount of drug release.

3.5. Kinetics of drug release

The *in vitro* dissolution data were analyzed using different kinetic models in order to find out the n -value, which describes the drug release mechanism (Table 4). Correlation values (r) were calculated and were found to be more linear for first-order drug release compared to zero-order. Cumulative % drug release was analyzed using PRISM software. Kinetic data was a reasonable best fit to Korsmeyer and Peppas's model and a good

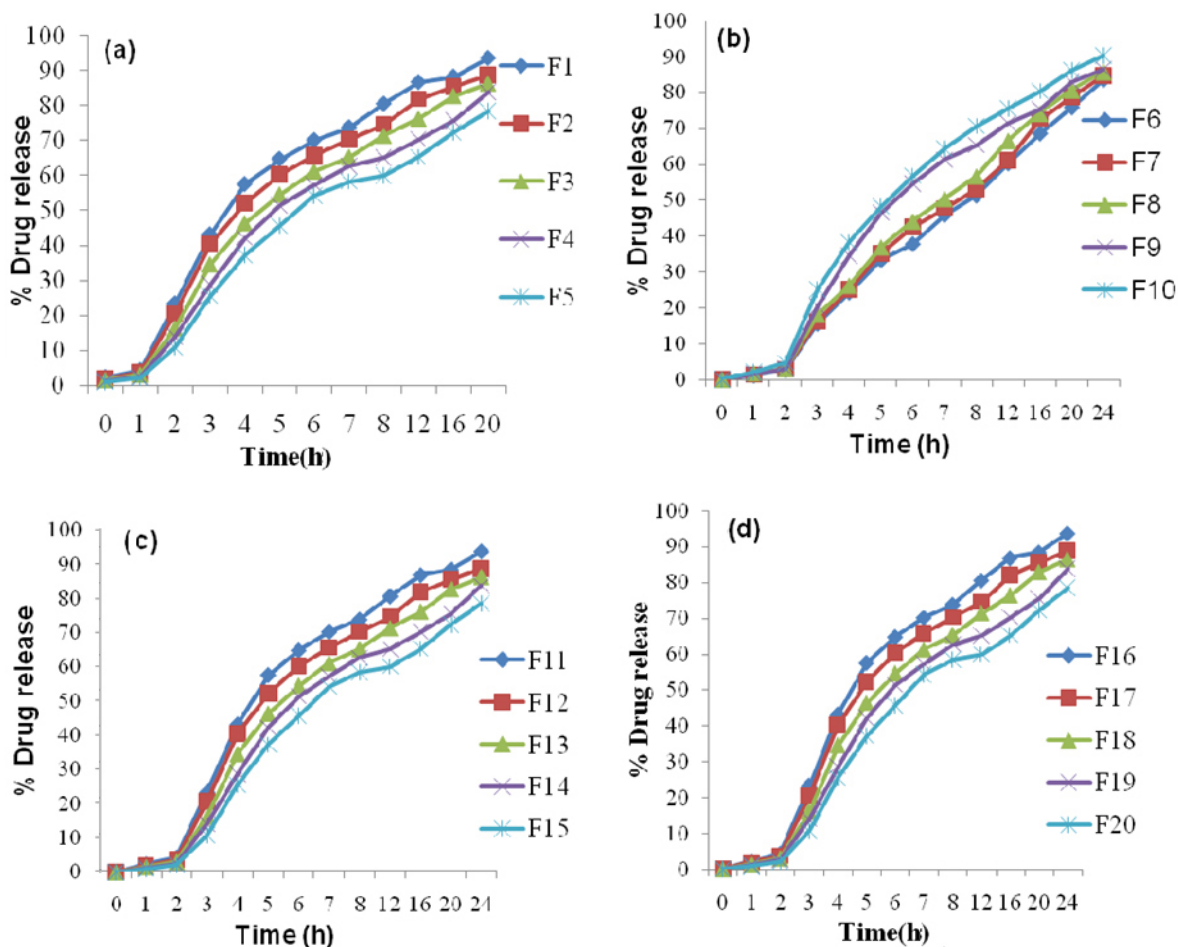


Figure 4. Effect of various parameters on the drug release rate of microbeads. (a) Sodium alginate concentration. (b) Stirring rate. (c) Calcium chloride concentration. (d) Cross-linking time.

Table 5. Stability data for aceclofenac sodium drug-loaded microbeads

Serial No.	Sampling interval (months)	Drug-content of microbeads			Physical characteristics		
		5°C %, w/w	25°C/60%RH %, w/w	40°C/75%RH %, w/w	5°C	25°C/60%RH	40°C/75%RH
0	0	100	100	100	*	*	*
1	1	100	100	99.56	*	*	*
2	2	100	99.89	98.67	*	*	*
3	3	100	98.76	97.45	*	*	*
4	4	100	97.90	96.16	*	*	*
5	5	100	97.32	95.55	*	*	*
6	6	100	96.87	94.64	*	*	*

* No change.

regression coefficient was observed. The values of the diffusion coefficient ranged between $n = 0.8806$ and 1.4503 indicating that the drug released from microbeads followed Zero-order kinetics controlled by swelling and relaxation of polymer chains.

3.6. Stability studies

The developed optimum formulations were subjected to stability studies at 25°C/60%RH, and 40°C/75%RH for up to 6 months. The dissolution profiles, and capsule

potency results for all of the stability conditions were within 90% to 110% of the label claim. Overall, results from the stability studies indicated that capsules were physically stable but the drug content at 40°C/75%RH was slightly reduced to 94.64% (w/w) after 6 months (Table 5). Good stability was observed at the lower temperature for more than 6 months.

4. Conclusion

It can be concluded from the above investigation

that the proper selection of optimized formulation conditions is very important to achieve high encapsulation efficiency and to control the release of aceclofenac sodium from the alginate microbeads. The alginate drug-loaded microbeads swelled at pH 1.2 predominantly very slowly but underwent increases at pH 6.8. The drug release from the microbeads was affected by the pH of the dissolution medium and results in a more sustained effect in alkaline medium. The ionotropic gelation technique is very easy to prepare, is free from any organic solvents and has a low manufacturing cost that can be successfully used for preparation of aceclofenac sodium microbeads using sodium alginate as drug release modifier. Therefore, one can assume that the aceclofenac sodium microbeads are a promising pharmaceutical dosage form that provides a sustained release drug delivery system. This system reduces the dosing frequency, eliminates the dose related adverse effects in the entire physiological region and ultimately improves compliance in the pharmacotherapy of arthritis, inflammation and pain. The entire process is feasible on an industrial scale and demands a pilot study.

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