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FORMULATION OF ORAL SUSTAINED RELEASE ACECLOFENAC SODIUM MICROBEADS

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ABSTACT: The objective of the present study was microencapsulate the Aceclofenac sodium (NSAIDs) by ionotropic gelation technique by using sodium alginate as hydrophilic carrier in various proportions and examines the influences of various process parameters like drug: polymer ratio, concentration of calcium chloride, stirring speed and cross-linking time on physicochemical properties of drug loaded microbeads. This system was able to prolong the drug release, minimizing the drug related adverse effects and improve bioavailability in different GI-tract conditions. Formulated drug loaded microbeads were investigated for physicochemical properties and drug release potential. All investigated properties showed satisfactory results. While increasing in the concentration of sodium alginate, calcium chloride and cross-linking time increased sphericity, size distribution, flow properties, mean particle size, swelling ratio and drug entrapment efficiency. No significant effect of drug polymer interactions were observed in FT-IR studies. The drug entrapment efficiency obtained in the range of 63.24-98.90% Particle size of drug loaded formulations were measured by an optical microscope. The mean particle size of drug-loaded microbeads were found to be in the range 596.45±1.04 to 880.10±0.13. Increase in the stirring rate and cross-linking time tremendous decrease in mean particle size. The shape and surface characteristics were determined by scanning electron microscopy (SEM) using gold sputter technique. The physical state of the drug in the formulation was determined by differential scanning calorometry(DSC). *In-vitro* drug release profile of aceclofenac sodium from microbeads was examined in simulated gastric fluid pH1.2 for initial 2h, mixed phosphate buffer pH6.8 upto 6h and simulated intestinal pH 7.2 at end of 24h studies. The release of drug from the microbeads was pH dependent, showed negligible drug release in pH1.2. Under neutral conditions the beads will swell and the drug release depend on the swelling and erosion process resulting optimum level of drug released in a sustained manner and exhibited zero-order kinetics followed by super case-II transport.

Keywords: Sustained drug delivery, Sodium alginate, Ionotropic gelation, aceclofenac sodium.

INTRODUCTION

Oral drug delivery is the most desirable and preferred method of administering therapeutic agents for their systemic effects. In addition, the oral medication is generally considered as the first avenue investigated in the discovery and development of new drug entities and pharmaceutical formulations, mainly because of patient acceptance, convenience, and cost effective manufacturing process. For many drug substances, conventional immediate release formulations provide clinically effective therapy while maintaining the required balance of pharmacokinetic and pharmacodynamic profiles with acceptable level of safety to the patient[1]

In recent years a wide variety of newer oral drug delivery systems like sustained/controlled release dosage forms are designed and evaluated in order to overcome the limitations of conventional therapy. These products are able to maintain steady drug plasma levels for extended periods of time as a result the variations of the drug levels in the blood are prevented and minimized drug related side effects [2]. Aceclofenac sodium is non-steroidal anti-inflammatory drug used extensively in the treatment of

rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Aceclofenac sodium is newer derivative of diclofenac and having less GIT complication. It is rapidly and completely absorbed after oral administration, peak plasma concentrations are reached 1 to 3 hours after oral dose. The plasma elimination half-life of the drug is approximately 4h, and dosing frequency 2-3 times daily with dose range 100-200mg.[3] To reduce the frequency of administrations and improve patient compliances, Aceclofenac sodium is suitable candidate for making sustain release dosage form. Microencapsulation is well accepted technique employed to sustain the drug release and reduce or/ eliminate gastrointestinal irritation, dose intake and ultimately improve the compliance in the pharmacotherapy of arthritis, inflammation and pain [4,5]

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(1-4) mannuronic acid (M) and α (14)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), homopolymeric M blocks (MM), and heteropolymeric 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 solution of sodium alginate is added to 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 beads have the advantages of being nontoxic orally , high biocompatibility, inability to reswell in acidic environment, whereas they easily reswell in an alkaline environment. So acid sensitive drugs incorporated into the beads would be protected from gastric juice.[6]

The aim of the present study, which was to develop sustained release oral product namely microbeads of Aceclofenac sodium using sodium alginate as the hydrophilic carrier and calcium chloride as cross-linking agent. Further, examines influences of various process parameters on physicochemical properties and drug release potential. In the proposed method ionotropic gelation we drop the mixture of drug and polymer dispersion into aqueous calcium chloride solution, gelation occurs instantaneously resulting to the formation of spherical micro-scale sized beads, with narrow particle size, high yield, low porosity and optimum sustained release in various physiological gastrointestinal conditions.[7,8]

MATERIALS AND METHODS

Aceclofenac sodium was a gift sample from Microlabs, Bangalore. Sodium alginate gift sample from F.M.C.International biopolymers,willingtown,Ireland .Calcium chloride(Fused) was purchased from S.B. Fine chemicals Ltd, Mumbai. India. All other reagents and solvents used were of analytical grade satisfying pharmacopoeial specifications.

PREPARATION OF ACECLOFENAC SODIUM – LOADED ALGINATE MICROBEADS

The microbeads were prepared by ionotropic external gelation technique. Sodium alginate was dissolved in deionized water at a concentration of 1-3%w/v. using gentle heat and magnetic stirring. On complete solution, an accurately weighed quantity of aceclofenac sodium was added and dispersed uniformly. The dispersion were sonicated for 30 min to remove any air bubbles that may have been formed during the stirring process. The bubble free sodium alginate-drug dispersion (50ml) were added drop wise via a 18-guage hypodermic needle fitted with a 10ml glass- syringe into 50ml of calcium chloride solution (1-5%w/v) and stirred at 200rpm for 30min. The droplets from the dispersion instantaneously gelled into discrete matrices upon contact with the solution of gelling agent. The formed drug loaded microbeads were further stirred in the solution of gelling agent for an additional 0.5-3.h. After specified stirring time and stirring speed the gelled beads were separated by filtration, washed with 3x50ml volumes of deionized water, finally dried at 80^0c for 2h in a hot air oven.[9]

Twenty batches of drug loaded microbeads were prepared 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, keeping the others constant and optimized to get small, discrete, uniform, smooth surfaced, and spherical microbeads. The detailed composition of the various formulations mentioned in Table1

CHARECTERIZATION AND EVALUATION OF MICROBEADS:

GRANULOMETRIC STUDY

The particle size has significant effect on the release profile of microbeads. Size and size distribution was 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 1.4mm, 1.2mm, 1.0mm, 0.85mm and 0.71mm (sieve

no 12, 14, 16, 18 and 22, respectively). Particles that passed through one sieve but were retained on the other 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 [10].

Mean particle size =Σ(mean particle size of the fraction x weight fraction) /Σ (weight fraction)

MEASUREMENT OF MICROMERITIC PROPERTIES OF MICROBEADS

The flow properties were investigated by measuring the angle of repose of drug loaded microbeads using fixed-base cone method. Microbeads were allowed to fall freely through a funnel fixed at 1cm above the horizontal flat surface until the apex of the conical pile just touches to 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].

$\theta = \tan^{-1}(h/r)$

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

The bulk and tapped densities were measured in a 10ml graduated cylinder as a measure of packability of the microbeads. The sample contained in the measuring cylinder was tapped mechanically by means of 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:

Corr's index (%)=

$$= \left[\left(\frac{\text{Tapped density-Bulk density}}{\text{Tapped density}}\right)\right] \times 100$$

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

Hausner's ratio= Tapped density / Bulk density

PARTICLE SIZE ANALYSIS

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

SCANNING ELECTRON MICROSCOPY ANALYSIS (SEM)

The shape and surface characteristics were determined by scanning electron microscopy (model-JSM, 35CF, jeol, Japan) using gold sputter technique. The particles were vacuum dried, coated to 200 A^o thickness with gold palladium using prior to microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15kv were the operating parameters. Photographs were taken with in a range of 50-500 magnifications.[13]

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Differential scanning calorimetry (DSC) was performed using DSC-60 (Shimadzu, Tokyo, Japan) calorimeter to study the thermal behaviors of drug alone and mixture of drug and polymer. The instrument 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 (30ml/min) at a scanning rate of $5\ ^0$ C/min from 24±1 to 250°C. Empty aluminum pan was used as reference. The heat flow as a function of temperature was measured for the drug and drug -polymer mixture.[14]

FOURIER TRANSFORM- INFRARED SPECTROSCOPIC ANALYSIS (FT-IR)

Drug polymer interactions were studied by FT-IR spectroscopy. One to 2mg of Aceclofenac sodium, placebo microbeads, and drug loaded microbeads samples were weighed and mixed properly with potassium bromide to a uniform mixture. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR- spectrum of the pellet from 450-4000cm^{-1} was recorded taking air as the reference and compared to study any interference.

X-RAY POWDER DIFFRACTOMETRY[X-RD] STUDY

The X-ray diffraction patterns of pure drug and the optimized drug loaded formulations were recorded using Philips X-ray diffract meter (model;PW 1710)with copper target to investigate the effect of microencapsulation on crystallinity of drug. Powder X-RD patterns were recorded using a radiation at 30kv and 25mA, scanning speed 2^0 /min^{-1} , over the 4^0 to 40^0 diffraction angle(2θ)range.[15]

`MEASUREMENT OF BIOADHESION:-

In-vitro bioadhesion was determined for drug loaded microbeads by following a previously reported method (Ranga Rao and Buri, 1989). Microbeads were placed on albino rats small intestine (2cm) and kept for 20min in a humidity temperature control cabinet (Metrex international, India) at 75% relative humidity and temperature of 25±2^{o}C to allow hydration of the microbeads. This is followed by thorough washing of the mucosal lumen with isotonic phosphate buffer pH 7.2. The washing was then dried at 70^{o}C in a hot air oven.[16] Percent bioadhesion was determined by the following formula;

% Bioadhesion=

[Weight of adhered microbeads] x100.
Weight of applied microbeads

DETERMINATION OF ENTRAPMENT EFFICIENCY

Aceclofenac sodium content in the microbeads was estimated by a UV-spectrophotometric method. Accurately weighed 50mg of microbeads were suspended in 100ml of phosphate buffer pH 7.2±0.1. The resulting solution was kept for 24hrs. Next day it was stirred for 15min. The solution was filtered, after suitable dilution, Aceclofenac sodium content in the filtrate was analyzed at 275nm using Shimadzu 1201 UV-Visible spectrophotometer. The obtained absorbance was plotted on the standard curve to get the exact concentration of the entrapped drug. Calculating this concentration with dilution factor we get the percentage of actual drug encapsulated in microbeads.[17] The drug entrapment efficiency was determined using following relationship;

% Drug Entrapment Efficiency =

[Actual drug content] x 100
Theoretical drug content

LOOSE SURFACE CRYSTAL STUDY (LSC)

This study was conducted to estimate the amount of drug present on the surface of the microbeads which showed immediate release in dissolution media. 100mg of microbeads were suspended in 100ml of phosphate buffer (pH 7.2), simulating the dissolution media. The samples were shaken vigorously for 15min in a mechanical shaker. The amount of drug leached out from the surface was analyzed spectrophotometrically at 275nm.[17] Percentage of drug released with respect to entrapped drug in the sample was recorded.

SWELLING PROPERTIES;

The swelling properties of the drug loaded microbeads were determined in various pH range (i.e.pH 1.2, 4.8, and 6.8 buffer solutions) Thirty dried beads were placed in a small beaker to which 100ml of buffer solutions was added and then allowed to swell at 37^{0}C. After 2h interval, the equilibrium swollen beads were observed and measured by Optical microscopy (Olympus model SZX-12). The magnitude of swelling was presented by the ratio of the mean diameter of swelling beads to the mean diameter of the dried beads before the test.[18] Swelling ratio was determined from the following relation.

Swelling ratio=

[(Mean diameter at time t-initial diameter)]X100
initial diameter of beads

***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-tract. The media of pH 1.2 was represent the gastric condition; pH 6.8 was a compromise condition between pH of the gastric and small intestine and pH 7.2, which is simulated intestinal fluid. The dissolution process was carried out by using USP XIII rotating basket apparatus (Microlabs, Mumbai,India). The drug loaded microbeads (equivalent to 200mg of aceclofenac sodium) filled in empty capsule shells were put into the basket rotated at a constant speed at 75rpm and maintained temperature 37^{0}C.The 900ml of the dissolution medium, pH1.2 containing 0.01% SLS and the test was done for 2h. At the end of 2h continued the test with changing the dissolution media with pH6.8 buffer solution up to 6h and pH 7.2 phosphate buffer up to the end of 24h.At scheduled time intervals, the sample (5ml) was withdrawn and replaced with same volume of fresh medium. The withdraw sample were filtered through a 0.45µm membrane filter and after appropriate dilution, then estimated for aceclofenac sodium concentration 275nm spectrphotometrically (Shimadzu 1201,Japan). Finally, corresponding drug content in the samples were calculated from the calibration curve of aceclofenac sodium to determine the drug release pattern.

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 equation. Coefficient of correlation (r) values were calculated for

the linear curves obtained by regression analysis of the above plots.

STABILITY STUDIES OF MICROBEADS

After determining the drug content, the optimized drug-loaded microbeads were charged for the accelerated stability studies according ICH guidelines. To assess long-term stability, accurately weighed drug loaded microbeads equivalent to 200mg of Aceclofenac sodium were filled into a hard gelatin capsules manually and sealed in a aluminum packaging coated inside with polyethylene. The studies were performed at 40 $\pm 2^0$Cand 75±5% relative humidity (RH) in the desiccators with saturated salt solution for up to 6 months. A visual inspection, drug content, in-vitro drug release was conducted every 15 days for the entire period of stability study.

RESULTS AND DISCUSSION:

Side effects, mainly at the gastric level are well known, following oral administration of an NSAID. 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 NSAID release in this gastric region. In this paper we evaluate the potential utility of the natural material such as sodium alginate to inhibiting the release of aceclofenac sodium in the gastric environment.[19] Since among the microparticulate systems, microbeads have a special interest as carriers for NSAID, mainly to extend the duration period of the dosage form, we aimed to investigate possible applicability of sodium alginate in various proportions as drug release modifier for the preparation of microbeads of aceclofenac sodium as a sustained release manner. We prepared microbeads containing aceclofenac sodium by ionotropic gelation method and examined the effects of various processing and formulation factors like concentration of sodium alginate, concentration of calcium chloride, curing time, stirring speed and nature of beads, these may be effects the physical characteristics and drug release potential.

The microbeads were prepared in an environment free from organic solvents by dropping a mixture of aceclofenac sodium and sodium alginate polymer dispersion in calcium chloride solution, which acted as a counter ion. The droplets instantaneously formed gelled spherical beads due to cross- linking of calcium ions with the sodium ions of alginate which remain ionized in the solution. Chemical reaction between sodium alginate and calcium chloride to form calcium alginate gel was utilized for the microencapsulation of aceclofenac sodium core material..

Aceclofenac sodium loaded microbeads formulated with 0.5 percent of sodium alginate which were cured for 2h at 2000rpm in 0.5 percent calcium chloride solution were not spherical and had a flattened base at the points of contact with the drying vessel. However, increase in the concentration of sodium alginate tended to make the particles more spherical. This indicates that at low alginate concentration the particles were composed of loose net-works structure which collapsed during drying. On the other hand higher sodium alginate concentration formed dense matrix structure which prevented collapse of microbeads. But forming high viscous polymer dispersion did not pass easily in the needle during the manufacturing process moreover found a small tail at one end of beads which significantly affects the flow properties and particle size distribution. It was found that optimum concentration of sodium alginate could influence the microbeads size, average diameter, recovery, encapsulation efficiency, size distribution swelling behavior and the release characteristics.

The total percentage yield of drug-loaded microbeads obtained were in the range between 72.40 to 88.30%w/w (Table1). It was observed that increasing the polymer ratio in the formulation significantly lower the product yield, due to the formation of high viscous polymer dispersion which may be lost during manufacturing process. Further observation, when the drug polymer ratio was constant, an increase in the concentration of calcium chloride and curing time slightly increased the percent of yield. There was no significant change on the product yield with increasing the speed of agitation. Actual drug concentration in the microbeads was evaluated and were found to be in the range 56.20±0.50 to 72.80±0.55mg/100mg.The polymer concentration increases consequently the actual drug loading high due to increase in hydrophobicity, leading to better precipitation of polymer at the boundary phase of the droplets.

The effect of various process and formulation parameters on the drug entrapment efficiency of microbeads were investigated, while keeping the concentration of calcium chloride, stirring speed and cross-linking time fixed at 4%w/v, 2000rpm and 2h respectively. By increasing the drug-polymer ratio concentration from 1:5 to 1:15 w/w, the drug entrapment efficiencies were found to in the range 63.24±0.66 to 98.90±0.86 (Table1) It was observed that the drug entrapment efficiencies increased progressively with increasing the concentration of sodium alginate resulting in the formation of larger beads entrapping the greater amount of the drug This may be attributed to the greater availability of active calcium binding sites in the polymeric chains and, consequently, the greater degree of cross-linking as the amount of sodium alginate increased.. Alginate concentration increases may also reduced loss of drug in the curing medium due to the formation of dense matrix structure.

Keeping the concentration of drug polymer ratio, stirring speed and cross-linking time fixed at, 1:10, 2000rpm and 2h 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±0.23 (Table1).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, resulting in more dug entrapment in the microbeads. On other hand further increase in the concentration of calcium chloride above (5%w/v) did not enhance the drug loading. This could be due to possible saturation of calcium binding sites in the guluronic acid chain, preventing further Ca^{2+}ions entrapment and, hence, cross-linking was not altered with higher concentration of calcium chloride solution.

The cross-linking time also effects the drug entrapment efficiencies of formulated drug loaded microbeads. We evaluated the drug entrapment efficiencies while keeping the drug: polymer ratio, concentration of calcium chloride, and stirring speed constant at 1:10w/w, 4%w/v, and 2000rpm respectively. Increasing cross-linking time from 0.5 to 2.5h the drug entrapment efficiencies were found to be found in the range 85.40±0.55 to 96.77±0.30 (Table1) Increasing the cross-linking time resulted decrease in the drug entrapment efficiencies, 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 2h, with no further decrease after 4 and 5h of curing time. This could be due to the formation of tight junction between calcium ions and the active sites on the guluronic acid chain.. Consequently, the drug was entrapped in highly bound calcium alginate matrix resulting in no further diffusion of drug in the curing medium.. Increasing the stirring speed 500 to 2500rpm the drug entrapment efficiencies were found to be in the range84.25±0.90 to 90.56±0.35(Table1). There was no significant change on encapsulation efficiency of drug with increased in the speed of agitation.

Table 1: Drug loading capacity and entrapment efficiency.

Batch code	D : P Ratio (%w/w)	Calcium chloride (%w/v)	Stirring rate (rpm)	Stirring Time (h)	Yield (%)	Drug loading capacity (mg/100mg)	Drug entrapment efficiency (%)
F1	1:5	4	2000	2	88.30	56.20±0.50	63.24±0.66
F2	1:7.5	4	2000	2	80.60	60.80±0.75	75.43±0.42
F3	1:10	4	2000	2	76.40	68.70±0.60	89.95±0.25
F4	1:12.5	4	2000	2	74.80	70.20±0.74	93.85±0.50
F5	1:15	4	2000	2	73.40	72.60±1.20	98.90±0.86
F6	1:10	4	500	2	74.10	64.80±0.85	87.44±0.90
F7	1:10	4	1000	2	75.30	66.10±0.66	87.96±0.98
F8	1:10	4	1500	2	75.80	67.30±1.30	88.35±0.93
F9	1:10	4	2000	2	76.40	68.70±0.60	89.95±0.25
F10	1:10	4	2500	2	76.30	69.15±0.15	90.56±0.35
F11	1:10	1	2000	2	72.40	60.30±0.67	83.30±0.75
F12	1:10	2	2000	2	74.60	64.20±1.10	86.05±0.96
F13	1:10	3	2000	2	75.10	66.80±0.97	88.94±0.84
F14	1:10	4	2000	2	76.40	68.70±0.60	89.95±0.25
F15	1:10	5	2000	2	77.60	72.30±0.35	93.30±0.23
F16	1:10	4	2000	0.5	75.65	72.80±0.55	96.23±0.30
F17	1:10	4	2000	1.0	76.15	70.15±0.58	92.15±0.48
F18	1:10	4	2000	1.5	76.30	69.50±0.95	91.08±0.87
F19	1:10	4	2000	2.0	76.40	68.70±0.60	89.95±0.25
F20	1:10	4	2000	2.5	77.70	66.35±1.45	85.40±0.55

D: P ratio:-Drug: Polymer ratio [Aceclofenac sodium : Sodium alginate] Each formulation containing 200mg of Aceclofenac sodium.. Data are expressed as mean ±SD, n=3

The effect of various process and formulation parameters on the micromeritic properties of drug loaded microbeads were evaluated, the size distribution of the microbeads in different sieves were observed , showed 32.46% to89.50% of microbeads retained ≠22 sieve, which proves the uniformity in size. It was observed that by an increase in the concentration of sodium alginate and calcium chloride solution tends to form the particles more spherical and obtaining the uniform size spheres. On other hand increase in the cross-linking time and stirring speed are also favorable to the formation of more spherical beads and the distribution of particle size slightly shifts to the lower pore size.

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 represent in terms of angle of repose ($<40^0$)[20,] Carr's index, and Hausner's ratio(Table2). Here, too, the sodium alginate concentration has a significant positive effect on the angle of repose. Particle size increased with increase in the concentration of sodium alginate and resulted in a decrease 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. Bulk and tapped density of beads showed good acceptable range indicates that 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 content. Corr's index and Hausner's ratio of all the formulations were estimated and found to be in the range 12.10 to 20.14 and 1.14±0.78 to 1.29±0.12 respectively (Table2), and explains the formulated microbeads had excellent compressibility and good flow properties. The improvement of flow properties suggest that the microbeads can easily handled during processing.

The SEM photomicrographs of the dried drug-loaded microbeads and their surface morphology are shown (Figure 2) Morphology of the various formulations of drug loaded microbeads. were discrete and spherical in shape with a rough outer surface and visible large wrinkles, have a sandy appearance because of the surface-associated crystals of drug. The drug crystals observed on the surface were probably formed as a result of their migration along with water to the surface during drying.

The mean particle sizes of drug loaded microbeads were performed by Optical microscopy. The mean particle size of the various formulations (F1-F20) of microbeads were obtained in the range between 596.45±1.04 to 880±1.23 (Table 2).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 concentration 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, penetrates into interior of droplets, water is squeezed out of the interior of droplets resulting in contraction of beads.(Table2) The size of the spherical matrix could easily be controlled by varying the stirring speed of the system. The mean particle size of microbeads were tremendous decreased with increasing the rotational speed (Table2). At a stirring speed of 500rpm, 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 vessel hence resulting in a wider diameter of the final beads. Consequently at higher stirring speed, a vigorous, uniform, increased mechanical shear might have been influenced in 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 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 variable shear force experienced by particulate system. All the observed data suggest that the stirring speed 2000rpm and stirring time 2h were found to be optimal for the drug loaded microbeads.

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 increasing in the concentration of sodium alginate and calcium chloride solution the LSC decreased significantly owing to high entrapment of drug in the dense matrix structure.

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.[20] Under neutral conditions the beads will swell and the drug release depend on the swelling and erosion process(Figure1). Being a polyelectrolyte, alginate can exhibit swelling properties that are sensitive to the pH, ionic strength and ionic composition of the medium. Optical microscopy was

used to investigate the hydration and swelling of microbeads at pH1.2, 4.8 and 6.8 up to 4hrs.(Figure 3a, 3b, and 3c) The equilibrium swelling studies showed, with increase in the polymer concentration, swelling of beads were significantly increased. The low swelling in acidic media pH1.2 was probably due to proton-calcium ion exchange forming insoluble alginic acid regions and followed by solvent penetration into the gel net work. The swelling of beads were ultimately increases in pH 4.8 and pH6.8 at the end of 4h. 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 in higher pH which displaces the Ca^{2+} ions within the beads.(Figure3a) Increasing the concentration of calcium chloride produces the beads with higher levels of Ca^{2+} ions that could reduce the swelling of the beads in acidic medium. However, the amount of calcium in swollen gel films after 4h in the medium was about 10-30%, which has apparently to prevent total breakdown of the gel structures. The swelling behavior of beads in pH4.8 and 6.8 were observed as a result the swelling ratio slightly increases due to ionic exchange between the phosphate ions in the buffer and higher level of Ca^{2+} ions within the beads.(Figure3b) When we compared the swelling ratio with prolonged cross-linking time maintaining same drug-polymer ratio and concentration of calcium chloride in the system showed appreciably maximum swelling with increased pH level. This results 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 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 time may be correlated to incomplete cross-linking of sodium alginate.(Figure3c) We further observed the swelling ratio of microbeads prepared by various stirring speed could not much effect on 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 at increased pH level of the medium initially, further they were broken after 2h. The overall results suggest that the dried beads swell slightly in the stomach. When they are subsequently transferred to upper intestine, the particles are begin to swell and they behave as matrices for sustained release of incorporated drug but they are subject to erosion in the lower intestine.21 Optimized aceclofenac sodium microbeads were found to possess good bioadhesion (78.50%), which resulted in prolonged retention in small intestine.

Differential scanning calorimetry thermograms of pure drug and drug loaded sodium alginate microbeads was observed, calcium chloride shows two endotherm peaks in the temperature range 180-200^{0}C; while sodium alginate decomposes at about 240^0 C with broad exotherm. Pure drug of aceclofenac sodium showed a sharp endotherm at 154.50^{0}C corresponding 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. It may indicate that the drug was uniformly dispersed at the molecular level of polymers. The compatibility of aceclofenac sodium with polymer was investigated by IR-spectrscopy study. The IR spectra of the drug and polymer combination were compared with the spectra of the pure drug and individual polymer spectra. In which no considerable changes in the IR peaks of aceclofenac in the physical mixture thereby indicating the absence of any interaction. The X-ray powder diffraction patterns of pure drug and formulation are compared and found that the intensity of the peaks for the pure drug is sharper than that of the drug in polymer matrix. The loss of sharpness is due to decreased crystallinity of the drug in the formulation.

Aceclofenac sodium release from formulated microbeads have been performed in different media, either in simulated gastric fluid (SGF) pH1.2 for initial 2h, mixed phosphate buffer pH6.8 for the period upto 6h and simulated intestinal (SIF) pH 7.2 at end of 24h studies.(Table3). The aceclofenac sodium being slightly soluble in water and showed very poor solubility in the buffer media as result of which we had to use 0.01%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, all the formulations showed negligible drug release in acidic pH 1.2 (<5%w/w) may be due to the stability of alginate at lower pHs and conversion of Ca-alginate to the insoluble alginic acid to formed tightening of the gel mesh work. In 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 behaviour of drug-loaded Ca-alginate beads at higher pH could be explained by the ionotropy that occurs between Ca^{2+} ion of alginate and Na^+ ions present in phosphate buffer and consequently, capturing of the Ca^{2+} by phosphate ions.[21] The ion exchange with phosphate buffer which resulted in swelling and erosion of the beads and formation of the solute Ca-phosphate all have influence on increase in the drug release rate at higher pH levels When we changed the pH from 1.2 to 6.8 mixed phosphate buffer, the drug release rate was slightly increased and found to be in the range 37.80±0.32 to 64.80±0.12 (Table3), that might be the lower number of Na^+ ions present in that buffer and consequently slower rate of ion exchange and swelling of the polymer at this pH. On further changing the pH from 6.8 to 7.2 (SIF) till 24h, the maximum drug released at constant rate and found to be in the range 78.60±0.67 to 97.20±0.36 (Table3). Based on these results we reported, that the swelling is the main parameter controlling the

release rate of aceclofenac sodium from alginate matrices is modulated by a swelling-dissolution-erosion process

Effect of drug-polymer ratio

The effect of drug-polymer ratio on aceclofenac sodium release from different batches of microbeads is shown in Figure4(a). As the drug-polymer ratio increased, the release rate of aceclofenac sodium from the microbeads decreased. The slower in the release rate can be explained by the increase in the extent for 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 for 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.

Effect of Calcium chloride Concentration

The effect of cross-linking agent on aceclofenac sodium release from different batches of microbeads is shown in Figure 4 (c). The results indicate that rate and extent of drug release decreased significantly with increase of concentration of calcium chloride, because sodium alginate as a linear copolymer consisting of β (1→4) mannuronic acid and α (1→4) L-guluronic acid residues; a tight junction is formed between the residues of alginate with calcium ions. However, in case of higher calcium chloride concentration due to increased surface roughness and porosity (Figure2) and also poor entry of dissolution medium into the polymer matrix may be delayed drug release.

Effect of Stirring Time

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

Effect of Stirring Speed

Effect of variation in the stirring speed on drug release profile were also been studied for selecting the best optimized formulation and observed aceclofenac sodium release from different batches 0f microbeads is shown in Figure 4(b)when the stirring rate was increased, the drug release was found to be faster. This may be due to the reduction in the size of microbeads, which provided a large surface area for increasing in the drug release.

The *in-vitro* dissolution data were analyzed by different kinetic models in order to find out the n- value, which describes the drug release mechanism (Table; 3). The values of correlation (r) were caluculated and were found to be more linear for first-order release as compared to zero order. Cumulative % drug release was analyzed using PRISM software. The kinetic data was best fitted to Korsmeyer and Peppa's model and good regression co-efficient was observed. The values of diffusion co-efficient ranged between n=0.8806 and 1.4503 indicating the drug release from the microbeads followed by Zero-order and super case-II transport mechanism controlled by swelling and relaxation of polymer chains.

For the developed optimum formulations were subjected to stability studies at 40^0C/ 75% RH up to 6 months. The dissolution profiles, 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 and chemically stable for more than 6 months.

CONCLUSION

In conclusion, ionotropic gelation technique can be successfully used for preparation of aceclofenac sodium microbeads using sodium alginate as drug release modifier. Various formulation variables such as polymer concentration, calcium chloride concentration, stirring speed, and cross-linking time were used, which are influenced to the drug entrapment efficiency, size distribution, mean particle size, surface morphology, swelling behavior and *in- vitro* drug release. The drug release from the microbeads was affected by the pH of the dissolution medium results more sustained effect in alkaline medium. The alginate drug loaded microbeads swelled at pH 1.2 predominantly very slow but underwent increases at pH 6.8 FT-IR and DSC studies did not reveal any significant drug interactions. Aceclofenac sodium release from microbeads formulated by fixing the drug: polymer ratio1:10, concentration of calcium chloride 4%w/v, stirring speed 2000rpm, and cross-linking time 2h showed a satisfactory sustained release profile. Therefore, one can assume that the aceclofenac sodium microbeads are promising pharmaceutical dosage forms by providing sustained release drug delivery systems and avoiding the dose related side effects in the entire physiological region. The entire process is feasible in an industrial scale and demands pilot study.

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Table 2: Micromeritic Properties of Drug-Loaded Microbeads

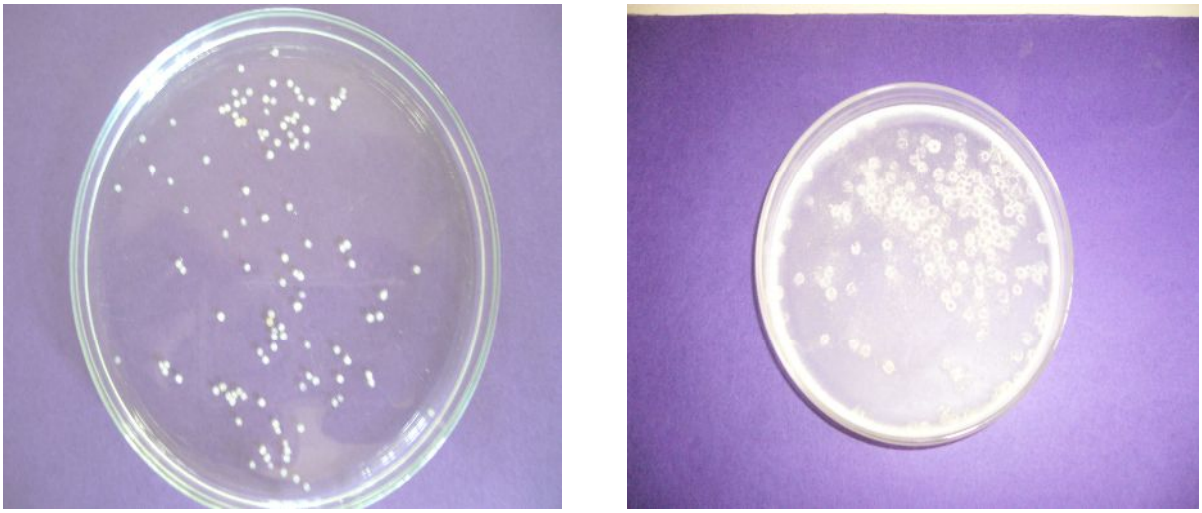
Formulation code	Mean Particle size [µm]	Angle of Repose [θ]	Bulk Density [g/ml]	Tapped Density [g/ml]	Carr's Index (ci) %	Hausner's ratio
F1	596.45±1.04	32.20±1.96	0.475±0.07	0.593±0.03	19.89	1.24±0.20
F2	624.86±0.98	28.16±0.62	0.566±0.92	0.675±0.06	16.14	1.19±0.30
F3	703.55±0.75	22.65±0.55	0.665±0.75	0.782±0.05	14.96	1.17±0.58
F4	844.75±1.10	20.55±1.07	0.695±0.05	0.807±0.87	13.87	1.16±0.15
F5	880.10±1.23	19.85±0.54	0.745±0.08	0.855±0.16	12.86	1.14±0.78
F6	784.60±1.08	23.65±1.65	0.585±0.85	0.727±0.15	19.55	1.24±0.08
F7	764.55±1.06	25.15±0.93	0.595±0.96	0.735±0.80	19.05	1.23±0.20
F8	743.20±1.44	26.78±0.75	0.622±1.10	0.755±0.36	17.64	1.21±0.40
F9	703.55±0.75	28.65±0.55	0.665±0.73	0.782±0.05	14.96	1.17±0.58
F10	716.80±0.96	20.15±0.05	0.685±0.09	0.790±0.18	13.30	1.15±0.32
F11	746.60±0.73	30.65±0.85	0.515±0.16	0.665±0.22	22.55	1.29±0.12
F12	734.10±0.54	27.75±0.96	0.565±0.25	0.697±0.45	18.95	1.23±0.45
F13	724.40±0.34	26.25±0.55	0.635±0.35	0.753±0.96	15.70	1.18±0.68
F14	703.55±0.75	22.65±0.55	0.665±0.75	0.782±0.05	14.96	1.17±0.58
F15	688.56±1.25	19.10±1.23	0.712±0.15	0.810±0.46	12.10	1.13±0.77
F16	804.35±1.43	25.75±0.64	0.555±o.77	0.695±0.55	20.14	1.25±0.84
F17	764.45±1.05	24.66±0.77	0.588±0.93	0.724±0.15	18.80	1.24±0.56
F18	724.64±1.54	23.15±0.87	0.625±0.66	0.758±0.35	17.62	1.20±0.34
F19	703.55±0.75	22.65±0.55	0.665±0.75	0.782±0.05	14.96	1.17±0.58
F20	708.10±0.86	18.85±1.15	0.695±0.82	0.805±0.77	13.65	1.15±0.55

* Data are expressed as mean ±SD of at least triplicate

Table 3. In-vitro Drug Release and Various Kinetic Data of Drug-Loaded Microbeads

Batch Code	Cumulative % drug Release			Various Kinetic models				
	pH 1.2 #	pH 6.8 $	pH 7.2 ≠	Zero-Order [r]	First-Order [r]	Higuchi-Matrix[r]	Korsmeyer-peppas [r]	'n'-Values
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	2.60±0.07	37.80±0.37	83.60±0.77	0.9943	0.9026	0.9689	0.9806	1.3520
F7	2.83±0.04	42.50±0.78	84.80±0.73	0.9964	0.9591	0.9772	0.9968	1.2206
F8	2.90±0.03	44.10±0.67	85.90±0.44	0.9918	0.9818	0.9884	0.9848	1.4503
F9	3.06±0.05	54.60±0.46	86.40±0.36	0.9890	0.9885	0.9933	0.9747	1.0745
F10	4.68±0.06	56.80±0.18	90.40±0.33	0.9815	0.9807	0.9876	0.9856	1.3229
F11	4.04±0.03	58.20±0.16	95.40±0.90	0.9756	0.9143	0.9675	0.9855	1.0791
F12	3.42±0.02	56.40±0.66	90.20±0.15	0.9866	0.9843	0.9910	0.9834	1.2962
F13	3.24±0.08	55.80±0.74	88.80±0.19	0.9955	0.9784	0.9870	0.9904	1.2304
F14	3.06±0.07	54.60±0.65	86.40±0.36	0.9978	0.9865	0.9943	0.9967	1.0745
F15	2.44±0.04	47.80±0.45	81.50±0.55	0.9993	0.9877	0.9930	0.9987	1.4023
F16	3.96±0.06	58.70±0.43	97.20±0.36	0.9786	0.9712	0.9890	0.9915	0.9406
F17	3.10±0.07	56.10±0.55	94.30±0.56	0.9852	0.9087	0.9648	0.9833	1.3720
F18	3.20±0.05	55.60±0.36	90.50±0.84	0.9844	0.9765	0.9908	0.9867	1.2882
F19	3.06±0.04	54.60±0.48	86.40±0.36	0.9976	0.9863	0.9917	0.9877	1.0745
F20	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,i.e. #- values expressed at the end of 2h. $-values at the end of 6h. ≠values expressed at the end of 24h. n=Diffusion exponent related to mechanism of drug release, according to equation Mt/M_=Ktn , r-Correlation Coefficient



a **b**

Figure 1: Photographs swelling behavior of aceclofenac sodium microbeads (a) pH1.2 and (b) pH 6.8

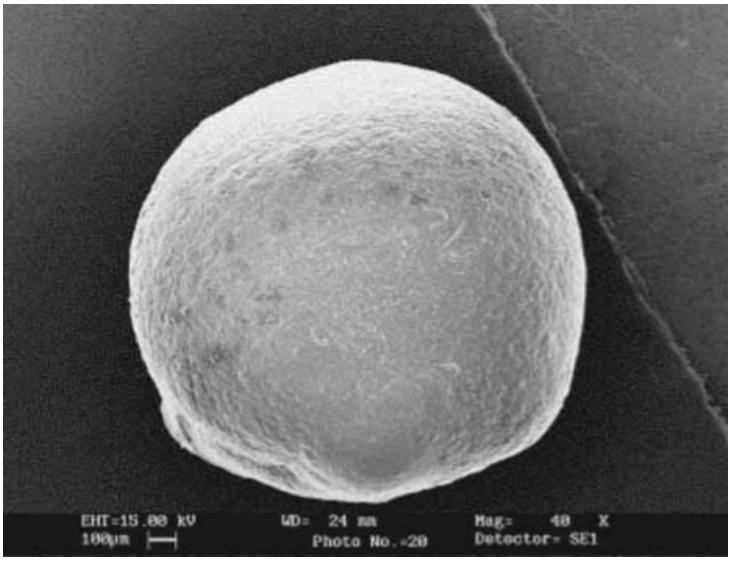
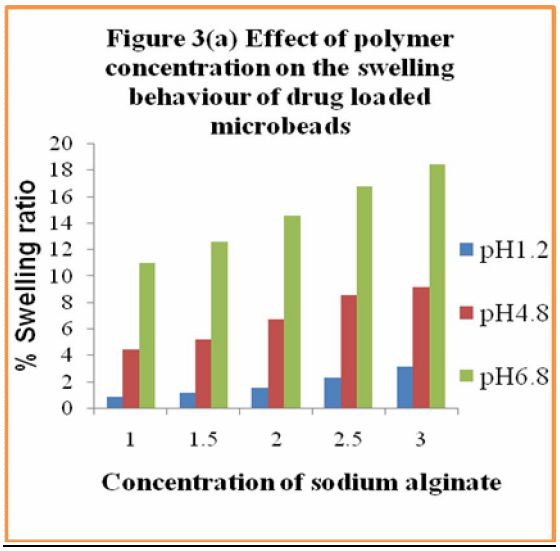
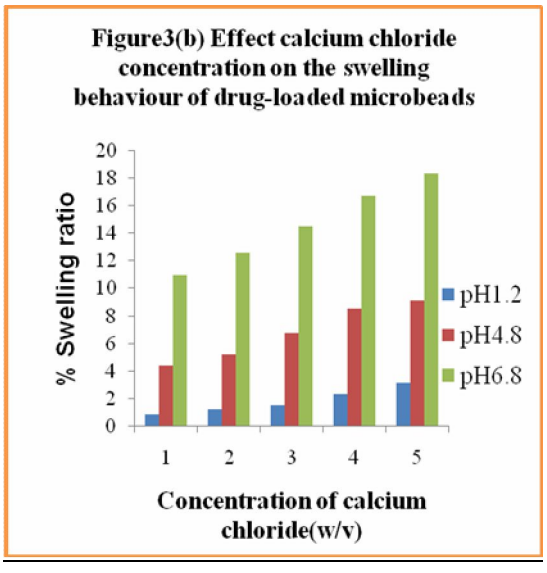


Figure 2: Scanning Electron Micrographs of Aceclofenac sodium Microbeads





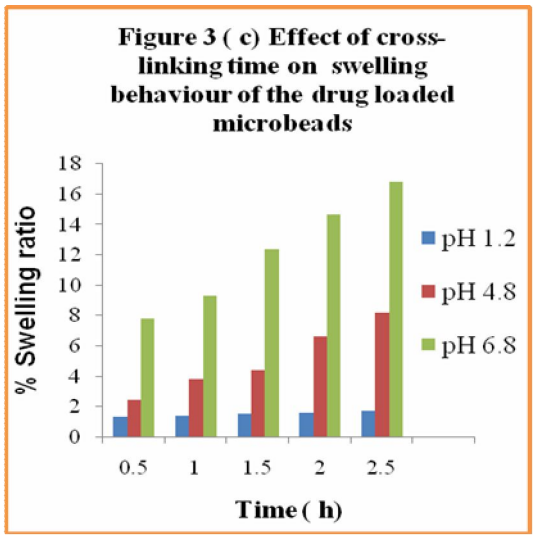


Figure 3 (c) Effect of cross-linking time on swelling behaviour of the drug loaded microbeads
% Swelling ratio
18
16
14
12
10
8
6
4
2
0
0.5
1
1.5
2
2.5
Time (h)
pH 1.2
pH 4.8
pH 6.8

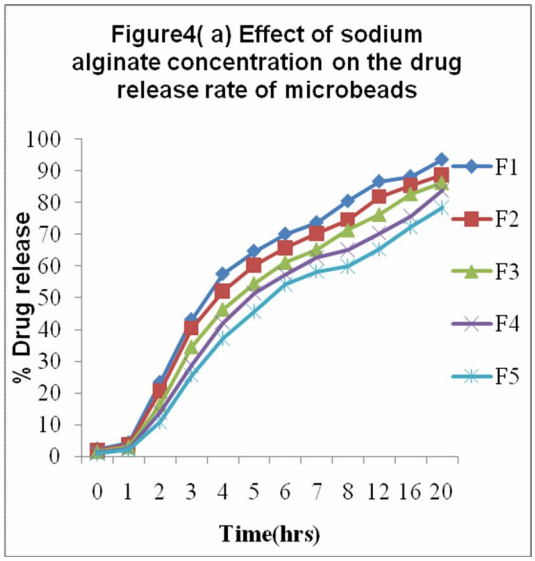


Figure4(a) Effect of sodium alginate concentration on the drug release rate of microbeads
% Drug release
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 12 16 20
Time(hrs)
F1
F2
F3
F4
F5

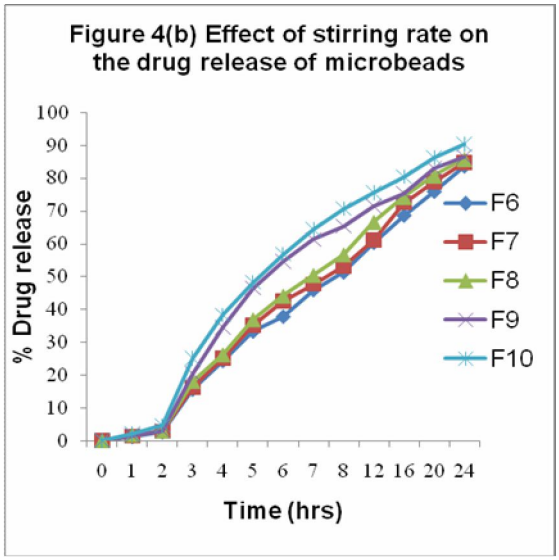


Figure 4(b) Effect of stirring rate on the drug release of microbeads
% Drug release
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 12 16 20 24
Time (hrs)
F6
F7
F8
F9
F10

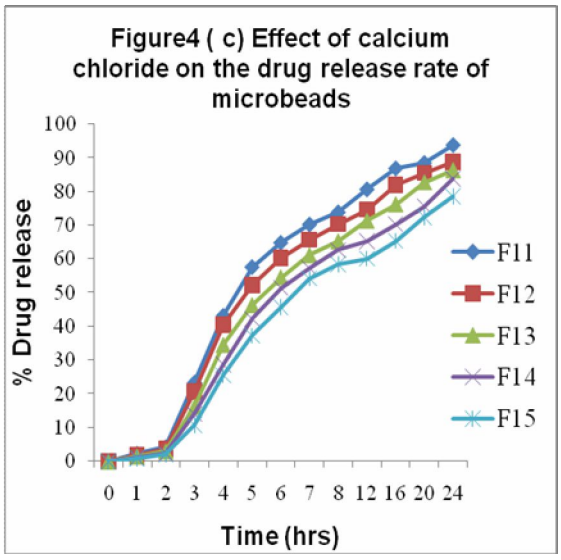


Figure4 (c) Effect of calcium chloride on the drug release rate of microbeads
% Drug release
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 12 16 20 24
Time (hrs)
F11
F12
F13
F14
F15

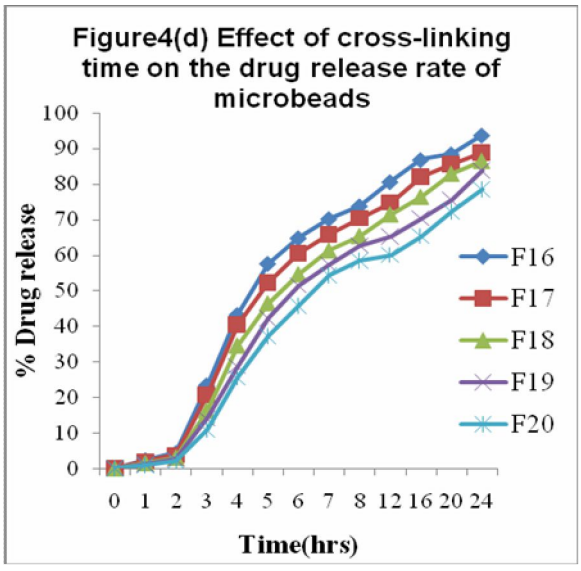


Figure4(d) Effect of cross-linking time on the drug release rate of microbeads
% Drug release
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 12 16 20 24
Time(hrs)
F16
F17
F18
F19
F20

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