AMERICAN JOURNAL OF PHARMTECH RESEARCH

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Xyloglucan Calcium Alginate (Ca) Coated Microbeads of Aceclofenac Sodium For Oral Controlled Drug Delivery

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ABSTRACT

The aceclofenac sodium loaded calcium alginate (CA) based microbeads prepared by ionotropic external gelation technique with calcium chloride as cross-linking agent. Calcium alginate microbeads represent a useful tool for oral sustained/ controlled drug delivery but show several problems, mainly related to the stability, and rapid drug release at higher pH that, in most cases, is too fast due to increase porosity. To overcome such inconveniences, which was to develop CA microbeads coated with xyloglucan (XG) as drug release modifier to improve stability and prolong the drug release. The mean particle sizes of drug-loaded microbeads were found to be in the range 476.45±12 to 765.10 ± 0.22. The drug entrapment efficiency was obtained in the range of 62.24±0.66 to 102.75 ± 0.87.The shape and surface characteristics were determined by scanning electron microscopy (SEM). No significant drug-polymer interactions, physical changes and crystallinity of the drug in the formulations were determined by FT-IR spectroscopy, differential scanning calorimetry (DSC) and X-ray powder diffraction [XPRD]. *In-vitro* drug release profiles of microbeads were pH dependent and were analyzed by different kinetic models. The mechanism of drug release from microbeads depends on swelling and erosion process resulting CA microbeads was diffusion controlled followed by First order kinetics and whereas CA microbeads coated with XG approaching to near Zero- order kinetics.

Keywords: Sodium alginate, Xyloglucan aceclofenac sodium, Oral bioavailability, Natural hydrogels, pH dependent, Zero-order kinetics

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Received 13 November 2013, Accepted 26 November 2013

Please cite this article in press as: Manjanna KM. *et al.*, Xyloglucan Calcium Alginate (Ca) Coated Microbeads of Aceclofenac Sodium For Oral Controlled Drug Delivery American Journal ofPharmTech Research 2014.

INTRODUCTION

Aceclofenac sodium is non-steroidal anti-inflammatory drug used extensively in the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis but observed certain side effects like abdominal pain, gastritis, constipation, etc. 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.[1] An adverse gastrointestinal reaction has been observed and due to its short biological half-life 1.8-3.5h requires multiple dosing. It leads to fluctuation in the drug blood levels and dose related adverse effects, multiple dosing also fail to release the drug at the desired rate and in the desired amount which often results in poor patient compliance and inefficient therapy [2]

An oral sustained / controlled release drug-delivery system should be able to achieve optimum therapeutic drug concentration in the blood with minimum fluctuation, improving therapy, safety, efficacy and patient compliance.[3] Natural polysaccharides hydrogels have been widely used as drug release modifiers in several controlled drug delivery systems of their advantageous properties over synthetic polymers such as biocompatibility, biodegradability ability to modify the properties of aqueous environment, capacity to thicken, emulsify, stabilize, encapsulate, swell and to form gels, films.[4] It is well known that polysaccharides like sodium alginate, pectin; locust bean gum, guar gum, xyloglucan, gellan gum etc are used as binders, disintegrant, suspending agents, stabilizing agents and play a fundamental role in determining the mechanism and rate of drug release from the dosage form.[5]

Sodium alginate (SA) 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 (1-4) guluronic acid (G) residue in varying proportions and arrangements. Gelation of SA occurs when uronic acids (-l-guluronic and -d-mannuronic acids) are cross-linked with divalent cation, such as calcium ions. [6] Gelation occurs when the extended chain sequences of these acids adopt a regular twofold conformation and dimerize by chelating calcium, forming the so called egg-box' structure.[7] Each calcium ion takes part in nine coordination bonds with each oxygen atom, resulting in a three-dimensional network of calcium alginate (CA). This phenomenon has been applied to the preparation of CA beads for use as a drug delivery system, by dropping the drug-containing SA dispersion into a calcium chloride bath. [8] The CA microbeads have the advantages of being nontoxic orally, high biocompatibility, and inability to reswell in acidic

environment, whereas they easily reswell in an alkaline environment. CA beads could protect an acid-sensitive drug from gastric juice, and the drug was consequently released from the beads in the intestine. [9]

Xyloglucan is a natural polysaccharide hydrogel derived from tamarind seed and it is composed of a (1→4)-β-D-glucan backbone which has (1→6)-α-D-xylose branches that are partially substituted by (1→2)-β-D-galactoxylose. Xyloglucan used in various novel drug delivery systems as a drug release retardant to maintain the plasma drug concentration for extended period of time. [10]

Microencapsulation is well accepted technique for development of homogeneous, monolithic particles in the range of about 0.1-1000µm and employed to sustain the drug release. [11] Calcium induced alginate beads have been developed in recent years as a unique vehicle for drug delivery systems. Their preparation is quite easy and is usually based on the gelling properties of the polysaccharide in the presence of divalent ions; nevertheless, microbeads prepared only with CA show several problems, mainly related to the mechanical stability and rapid drug release at higher pH that, in most cases, is too fast [12]. To overcome such inconveniences, alginate microbeads coated with natural polysaccharide polymers, and/or appropriately interpenetrating polymer network (semi-IPNs and IPNs) structures formed with SA and other macromolecules were developed.

The aim of the present study, which was to develop oral products namely microbeads of aceclofenac sodium using sodium alginate as the hydrophilic carrier coated with xyloglucan polymer and calcium chloride as cross-linking agent. Further, examines influences of various process parameters on physicochemical properties and drug release potential.

MATERIAL AND METHODS

Materials:

Aceclofenac sodium was obtained as a gift sample from Microlabs Bengaluru. Karnataka. India. SA was gift sample from F.M.C. International biopolymers, willingtown, Ireland, through Signet Chemical Corporation Pvt. Ltd, Mumbai, India. Xyloglucan (tamarind powder) procured from local market and 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 specification.

PREFORMULATION STUDIES

Saturation solubility study:

The saturation solubility of aceclofenac sodium was determined with various concentration of surfactants i.e. 0.5, 1.0, 1.5, and 2%w/v of sodium lauryl sulfate (SLS) in double distilled water, 0.1N HCl, pH 4.5 acetate buffer, pH 6.8 and pH7.2 phosphate buffers at 37^0 C. Excess quantity of aceclofenac sodium was added to 100ml of dissolution medium in a conical flask and agitated continuously at room temperature at 8h on a shaker. The solutions were kept aside for 6h until equilibrium was achieved. The solutions were then filtered through No-41 Whatman filer paper, and the filtrate suitably diluted and analyzed spectrophotometrically at 275nm. [13].The results of the solubility study are summarized in table 1

FT-IR Spectroscopic Analysis;

Drug polymer interactions were studied by FT-IR spectroscopy. One to 2mg of aceclofenac sodium alone, 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 450-4000cm^{-1} was recorded taking air as the reference and compared to study any interference

Table 1. Saturation solubility of 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
0.1N 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

All data are expressed as mean ±SD, n=3

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 (80ml/min) at a scanning rate of 10 ^{0} C/min from 25±1 to 450°C. Empty aluminum pan was used as reference.[14] The heat flow as a function of temperature was measured for the drug and drug -polymer mixture.

X-Ray Powder Diffractometry (XRD)

X-ray diffraction patterns of pure drug and the drug loaded formulations were recorded using Philips X-ray diffractometry (Model; PW 1710) with copper target to investigate the effect of microencapsulation on crystallinity of drug. Powder XRD patterns were recorded using radiation at 30kv & 25mA, scanning speed 20/min^{-1}, over the 4^0 to 40^0 diffraction angle (2θ) range.

Preparation of sodium alginate microbeads

The microbeads were prepared by ionotropic external gelation technique. SA 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 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 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 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.[15]

Preparation of alginate xyloglucan microbeads:

50ml of deionized water, XG was added and stirred with the electric stirrer to form mucilage. Then SA (2%w/v) was added to form uniform dispersion. Weighed quantity of aceclofenac sodium (200mg) was added and homogenized for 5 min. The resulting dispersion was dropped through syringe with needle into 100ml of 4%w/v aqueous calcium chloride solution and stirred at 200rpm for 2h. After stirring the formed beads were separated by filtration, washed with distilled water, dried at 80^0C for 2h in an oven.

Characterizations and Evaluation of Microbeads:

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. [16] 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 vacuum dried particles were coated to 200 A^o thicknesses 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 within a range of 50-500 magnifications.

Drug entrapment efficiency (DEE)

Aceclofenac sodium content in the microbeads was estimated by a UV-spectrophotometrically. Accurately weighed 100 mg of drug loaded microbeads were suspended in 100ml of phosphate buffer pH 7.4 ± 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 /Theoretical drug content] x 100

Swelling properties;

The swelling properties of the drug loaded microbeads were determined in various pH range (i.e. 1.2, 4.8, and 6.8 buffer solutions) Thirty dried microbeads were placed in a small beaker to which 100ml of buffer solutions was added and then allowed to swell at 37^0C. 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) / initial diameter of beads)] X100

***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 was representing the gastric condition and pH 7.4 which is simulated intestinal fluid. The dissolution process was carried out by using USP XIII rotating basket apparatus (Micro labs, 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 37^0C. The 900ml buffer solution of pH1.2 containing 2% SLS used as dissolution medium for initial 2h. At the end of 2h continued the test with changing the dissolution media with pH 7.4 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 at 275nm using Shimadzu 1201 UV-Visible spectrophotometer.[19]Finally, corresponding drug content in the samples was calculated from the calibration curve of aceclofenac sodium to determine the drug release pattern.

RESULTS AND DISCUSSION

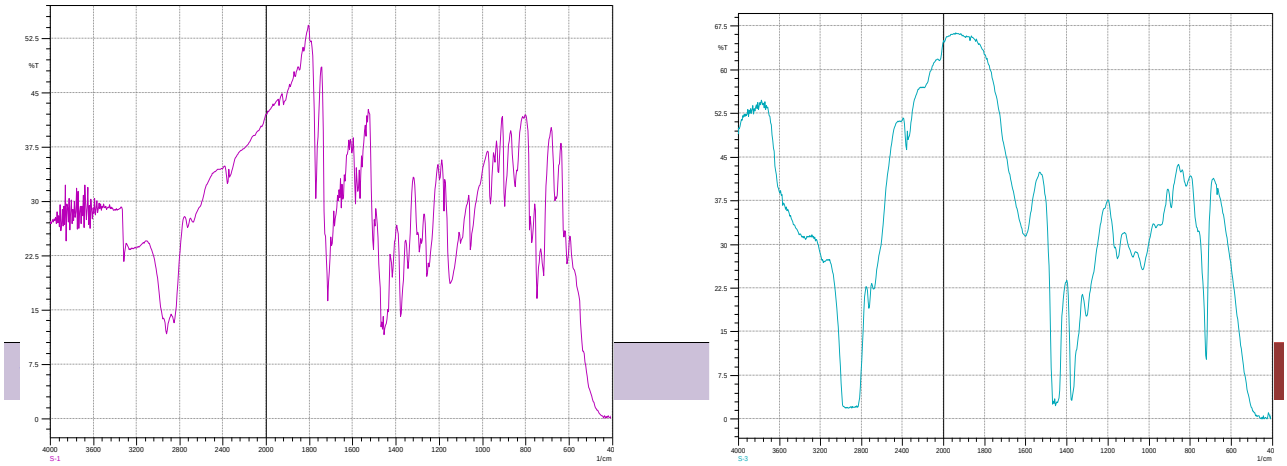
Saturation Solubility Studies;

The available data on 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 0.1N HCl significantly increased up to 0.487mg/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 very less compared with 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 0.1N 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±1.54mg/ml The solubility of aceclofenac

sodium was more in 1%w/v of calcium chloride solution than in double-distilled water, which induces certain amount of drug release, when prolonged exposés of the beads in curing medium during the manufacturing process.

FT-IR spectroscopic analysis;

The molecular interactions of aceclofenac sodium, SA and XG in the microbeads were investigated using FTIR spectroscopy. The characteristic absorption peaks of pure aceclofenac sodium were obtained 3276.5, 2915.5, 1716.5, 1589.3, 1279.6 and 749.4cm^{-1} corresponding to NH- stretching, C=O stretching of –COO and –COOH group respectively.(Figure1a) The characteristic absorption peaks SA powder showed peaks around 3077.15, 2914.98, 1615.34, 1359.60 and 754.05 cm^{-1} reflective of O–H, C-H, COO− (asymmetric), COO− (symmetric), and C–O–C stretching respectively.(Figure1b) The cross-linking process of SA with calcium caused an obvious shift to higher wave numbers and a decrease in the intensity of COO− stretching peaks. Additionally, a change to lower wave numbers and a decrease in the intensity of the C–O–C stretching peak of SA was observed. This indicated the presence of an ionic bond between the calcium ion and the carboxyl groups of SA and partial covalent bonding between the calcium and oxygen atoms of the ether groups.[20] The physical mixture SA powder and aceclofenac sodium characteristic peaks were obtained at 2820.28, 1591.72, 1384.91, 1102.03 and 766.4cm^{-1} caused a shift in the O–H, COO− (asymmetric), and COO− (symmetric) stretching peaks to lower wave numbers,(Figure 1c) suggesting that a molecular interaction between SA and aceclofenac was formed due to hydrogen bonding and electrostatic force. Further, characteristic IR peaks of XG shows around 3078.18, 2836.84, 1628.19, 1503.56 and 718.81 cm^{-1} and physical mixture of aceclofenac sodium and XG 30556.97, 2940.01, 1708.99, 1509.35 and 761.24 cm^{-1}.(Figure 1d,e). The IR spectra of the drug and polymer combination were compared with the spectra of the pure drug and individual polymer spectra. The peaks showed at 2916.5, 1589.3 1279.6, and 749.4cm^{-1} as major peaks for drug are presents in all physical mixture of drug and polymer that confirms the drug was molecularly dispersed in the polymer without any interaction.



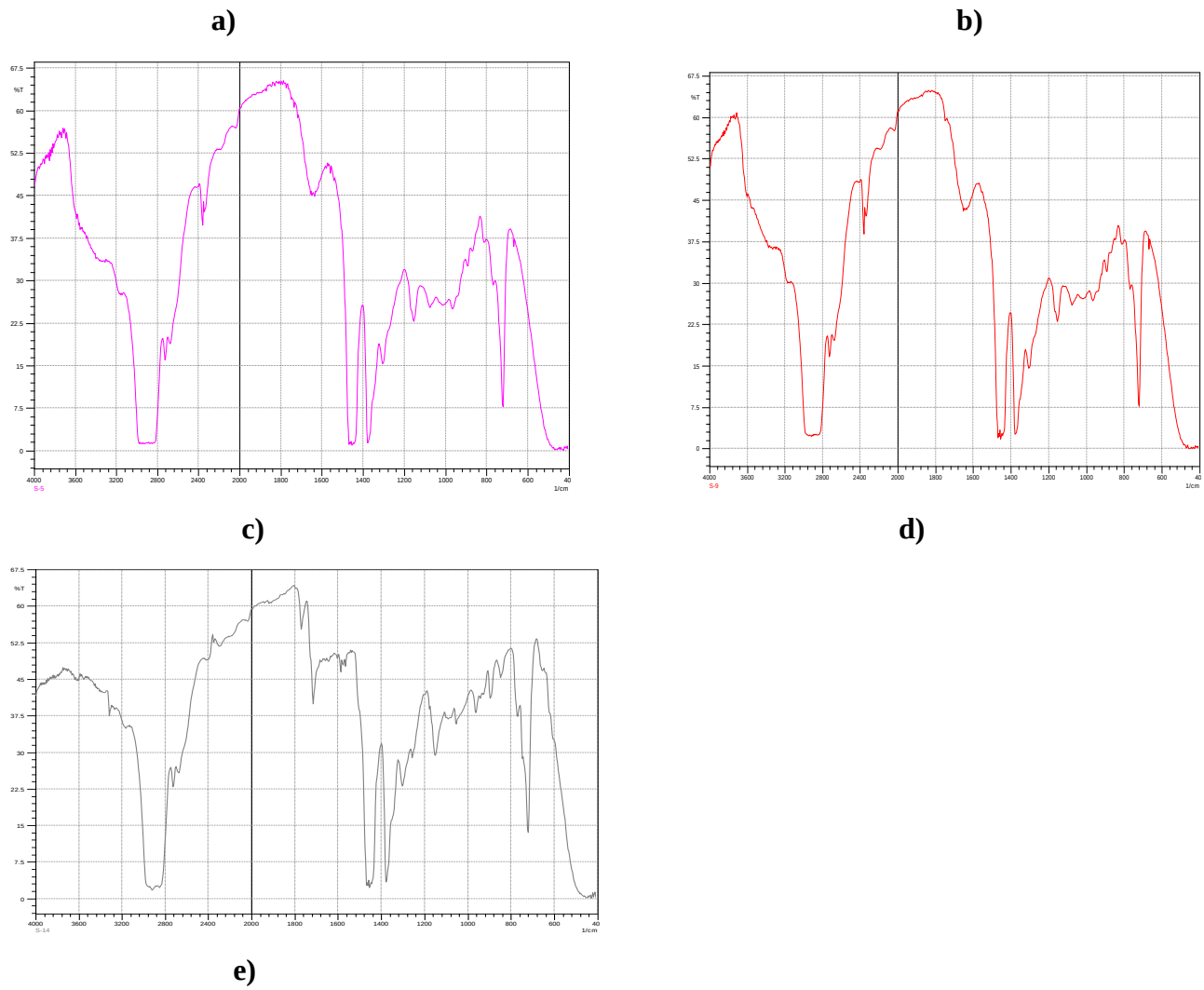
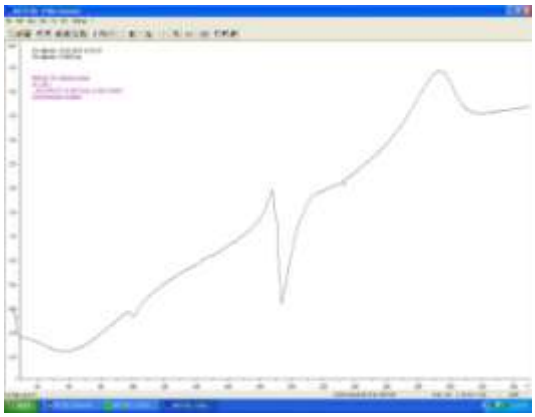


Figure 1: FT-IR Spectra a) Pure aceclofenac sodium b) Sodium alginate c) Xyloglucan d) Physical mixture of aceclofenac sodium and sodium alginate e) Physical mixture of aceclofenac sodium and Xyloglucan

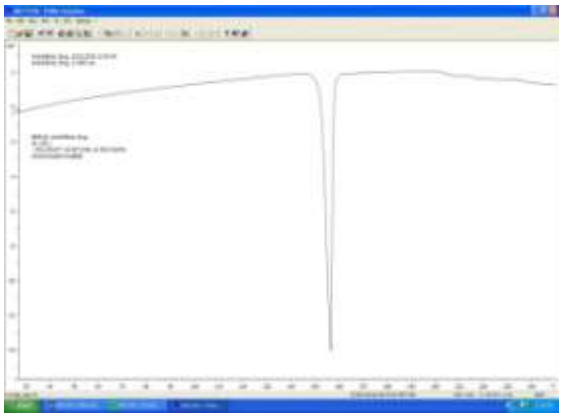
Differential scanning calorimetry (DSC)

DSC is a well-established method often used as a qualitative technique to characterize physical and chemical changes in either enthalpy or heat capacity of a crystalline drug in the polymer matrix during the manufacturing process. The thermal behavior of the pure aceclofenac sodium, drug loaded SA, and SA-XG microbeads were characterized using DSC, as shown in Figure 2. The thermogram of pure aceclofenac showed a sharp endothermic peak at 158.50^{0}C followed by corresponding meting point. (Figure 2a] However, the drug-loaded SA microbeads showed a sharp endothermic peak at 193.5^{o}C. However, the DSC thermogram of drug loaded SA-XG microbeads were observed at 189.50C and 195.60C respectively. It can clearly suggested the

drug loaded microbeads containing SA and SA-XG showed an increase in the exothermic peak temperature (Figure 2b,c,d) and improves the thermal stability of the alginate beads. The extra obvious peak of the drug (158.5^{0}C) was not observed in any type of the prepared microbeads containing the drug. There was no appreciable change in the melting endothermic peak of the physical mixture as compared to pure drug. It may indicate that there were no changes in thermal behavior of drug and also the drug was molecularly dispersed in hydrogel matrix.



a)



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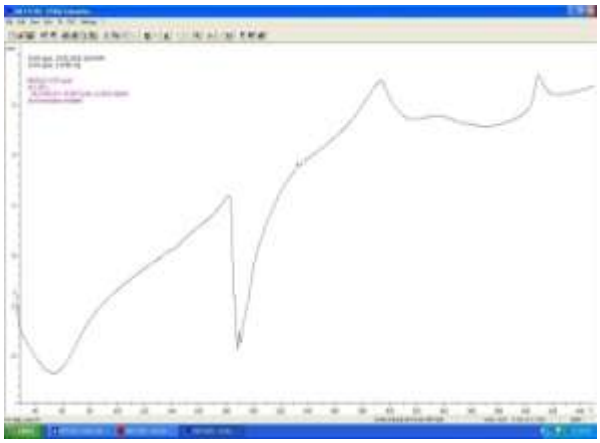


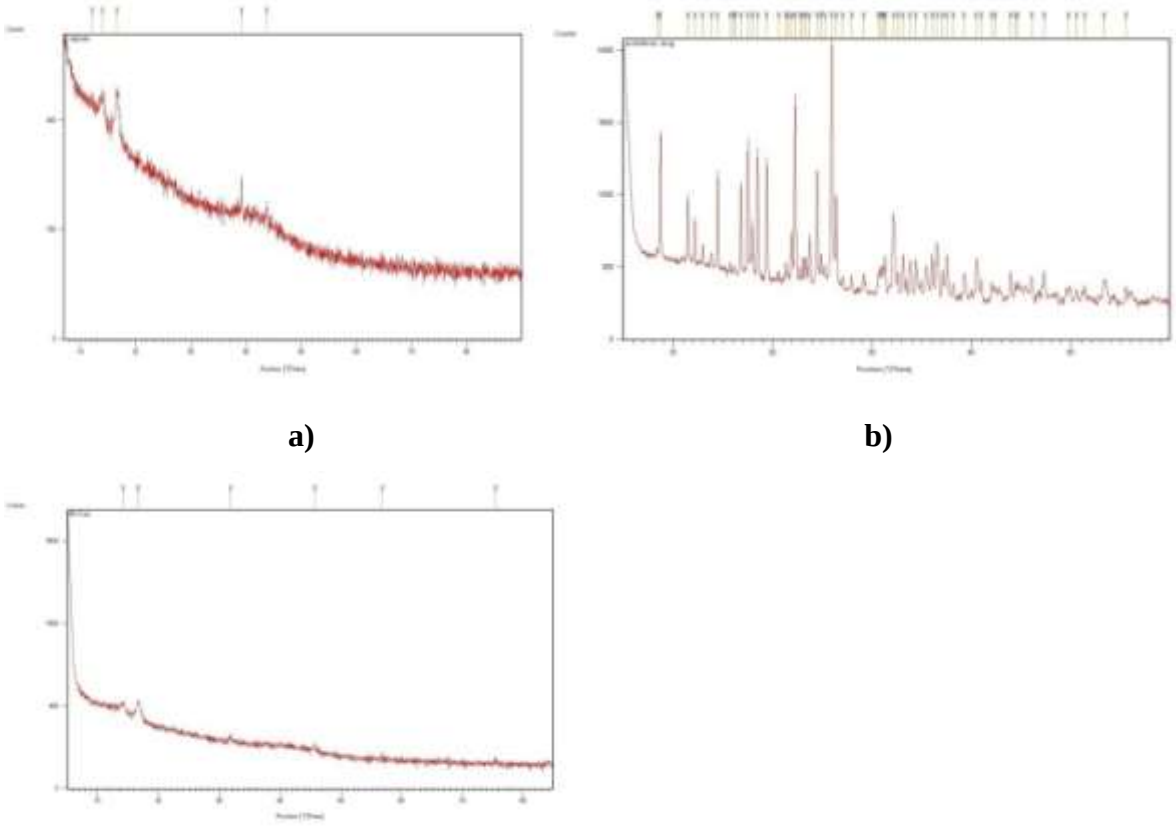
c)

Figure 2: DSC Thermo-grams a) Pure aceclofenac sodium b) CA drug loaded microbeads (F3) c) CA-XG drug loaded microbeads (F12)

X-Ray Powder Diffractometry (X-RD)

The X-ray powder diffraction patterns of pure drug are compared with drug-loaded microbeads. The XRD scan of plain aceclofenac showed sharp intense peaks of crystallinity (Figure 3a); whereas the XRD pattern of the drug-loaded microbeads exhibited halo pattern with less intense and more denser peaks compared to plain aceclofenac indicating the decrease in crystallinity or partial amorphization of the drug in the microbeads (Figure 3b).. The intensity of the aceclofenac peaks at 14.47, 24.87, 26.26 and 29.95 and 36.55^{0}C (2 θ), CA microbeads (F3) at 16.29, 39.23,

43.76^{0}C (2 θ), CA-XG microbeads (F12) shows at 14.06, 16.65 45.65^{0}C (2 θ) was calculated using D8 TOOLS software. The calculated percentage relative crystallinity value for pure aceclofenac was 92.40% and that of formulations containing SA (F3) and XG (F12) were 89.25 and 83.09 respectively.(Figure 3b,c,d) This is possibly due to the decrease in the degree of crystallinity of the drug following dispersal in the polymer matrix.



a) **b)**

c)

Figure 3: X-Ray diffraction patterns of a) Pure aceclofenac sodium b) CA drug loaded microbeads (F3) c) CA-XG drug loaded microbeads (F12)

Particle Size Analysis:-

The mean particle sizes of drug loaded microbeads were performed by Optical microscopy, and mean particle sizes of the SA formulations (F1-F5) 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 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 SA and formation of large droplets during addition of polymer solution to the gelling agent. On the other hand which increases in the concentration

of XG coating polymer the mean particle size was found to decreases due to the formation of dense matrix with SA and covers irregular pores and wrinkles present on the surface of SA microbeads and influences the sphericity, smooth surface of the microbeads. Further, the concentration of calcium chloride increases would significantly decreases the mean particle size of microbeads. (Table 2) 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.[21] The size of the spherical matrix could easily be controlled by varying the stirring speed and cross-linking time of the system.(Results are not mentioned)

Table 2. Effect of drug-polymer ratio, Concentration of calcium chloride, XG on physical characteristics of drug loaded microbeads

Formulation Code	Variables	Actual drug content (mg)	Drug entrapment efficiency (%)	Mean Particle size [µm]
F1	Drug: Sodium alginate (1:05, 1:7.5,	56.92 ± 0.39	62.24±0.66	476.45±1.12
F2	1:10, 1:12.5, and 1:15w/w)	60.82 ± 0.30	75.43±0.42	624.86±0.98
F3		68.99 ± 0.20	89.95±0.25	703.55±0.75
F4		70.47 ± 0.80	93.85±0.50	844.75±1.10
F5		72.60 ± 0.62	98.90±0.86	880.10±1.23
F6	Calcium chloride (1%, 2%, 3%,	60.45 ± 0.68	83.30±0.75	746.60±0.73
F7	4%, and 5%w/v)	64.53 ± 0.45	86.05±0.96	734.10±0.54
F8		66.76 ± 0.57	88.94±0.84	724.40±0.34
F9		68.99 ± 0.20	89.95±0.25	703.55±0.75
F10		72.33 ± 0.40	93.30±0.23	688.56±1.25
F11	Xyloglucan (0.5, 1.0, 1.5 %w/v)	64.30 ± 0.38	92.95±1.19	708.37±1.21
F12		69.80 ± 0.96	94.56±1.34	748.92±0.45
F13		76.30 ± 0.54	102.75±0.87	765.75±0.22

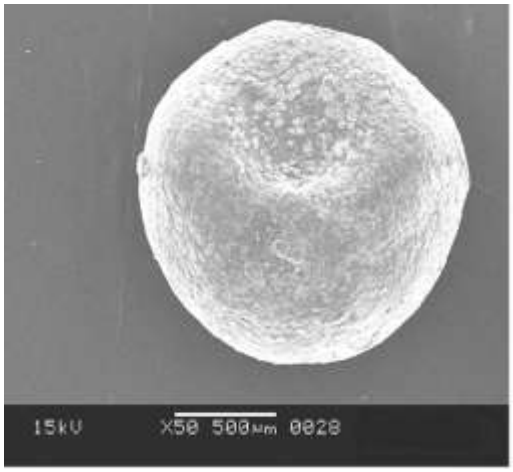
All the formulations containing 200 mg of aceclofenac sodium. All data are expressed as mean ±SD, n=3

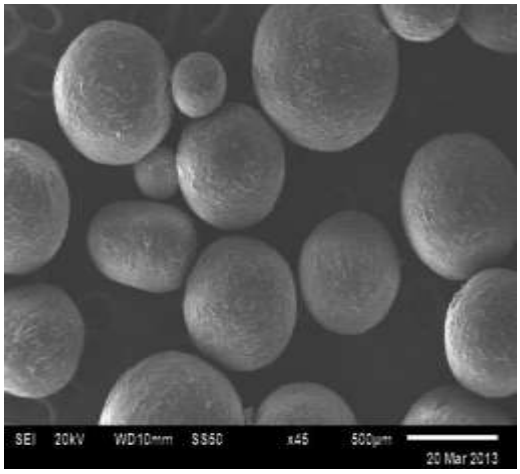
The mean particle sizes of drug-loaded microbeads were found to be in the range 476.45±12 to 765.10 ± 0.22. The drug entrapment efficiency was obtained in the range of 52.24±0.66 to 102.75 ± 0.87

Scanning electron microscopy analysis (SEM)

The SEM photomicrographs are shown in Figure 4(a, 4b) ; concentration of SA influences the surface morphology of CA beads at higher concentration SA formed discrete and spherical shape with a rough outer surface and visible large wrinkles have a sandy appearance because of the surface-associated crystals of drug. Addition of XG alters morphological regularity due to

formation of smooth surface; uniform sphericity and formation of thick coat on outer surface of the CA beads followed by complete entrapment of drug into interior polymer network.(Figure 4c, 4d)





a) **B)**

Figure 4: SEM Photographs magnification a) CA drug loaded microbeads (b) CA-XG drug-loaded microbeads before drying at 20kv c) CA-XG drug loaded microbeads sfter drying at 50kv

Drug entrapment efficiency (DEE)

Actual drug concentration in the microbeads was evaluated and found to be in the range 56.20±0.50 to 76.30±0.54mg/100mg. (Table 2). The polymer SA 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. On other hand, actual drug content in CA-XG was determined and observed in range 60.45±0.68 to 76.30±0.54/100mg. (Table 2). Increasing the concentration of XG coating polymers influences higher drug content in

the microbeads may be formation of thick surface and decreases loss of drug in the curing medium.

As showed in Table 2; By increasing the drug-polymer ratio concentration from 1:5, 1:7.5, 1:10, 1:12.5 and 1:15 w/w, and fixed other variables constant the drug entrapment efficiencies were found to in the range 63.24±0.66, to 98.90±0.86.(F1-F5) 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. 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±0.2. (F6-F10) 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 drug 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 [22]. Increasing in the concentrations of XG in CA beads the drug entrapment efficiency progressively increases and were found 92.95±1.19, 94.56±1.34, 102.75± 0.87 respectively. It may be the formation of dense matrix and also insoluble aceclofenac uniformly encapsulate within the hydrophilic helix of SA-XG interpenetrating polymer net-work.

Swelling properties

The swelling ratio of the CA, CA- XG 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 the pH, ionic strength and ionic composition of the medium[23]. Optical microscopy was used to investigate the hydration and swelling of drug loaded microbeads at pH1.2, 4.8 and 6.8 up to 4hrs. The equilibrium swelling studies showed, with increase in the SA concentration, swelling of beads was 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 [24]. The swelling of beads was ultimately increased in pH 4.8 and pH6.8 at the end of 4hrs. (Figure 5a). 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. 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. 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 [25](Figure 5b). The water uptake behavior of CA-XG bipolymeric beads in the media of varying pH 1.2, 4.8 and 6.8 was also studied. The beads swelled to less in simulating gastric fluid (SGF) of pH 1.2 in 4 hrs. On transferring the beads into higher pH the swelling was significantly enhanced due to degradation of galactan and mannan units combined through glycosidic linkages of the polysaccharide with phosphate ions of the buffer solution.[26] (Figure 5c). 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 diffuse formation of thick diffusion layer and they behave as matrices for sustained release of incorporated drug but they are subjected to erosion in the lower intestine.

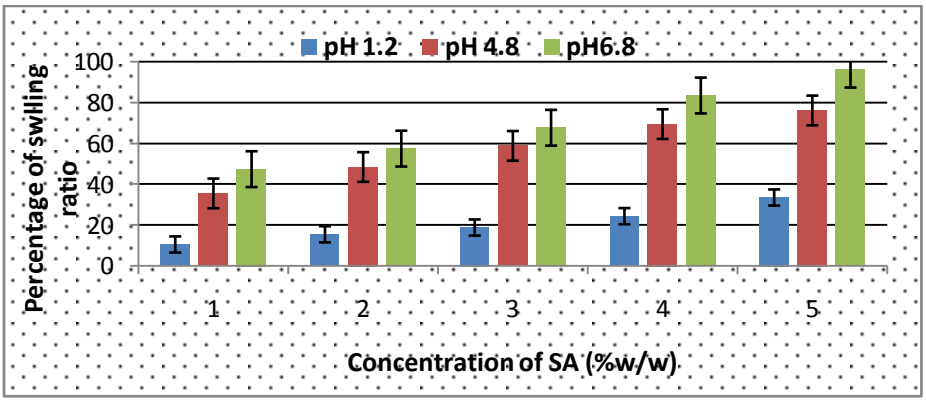


Figure 5(a); Effect of SA concentration on swelling behavior of drug loaded CA microbeads

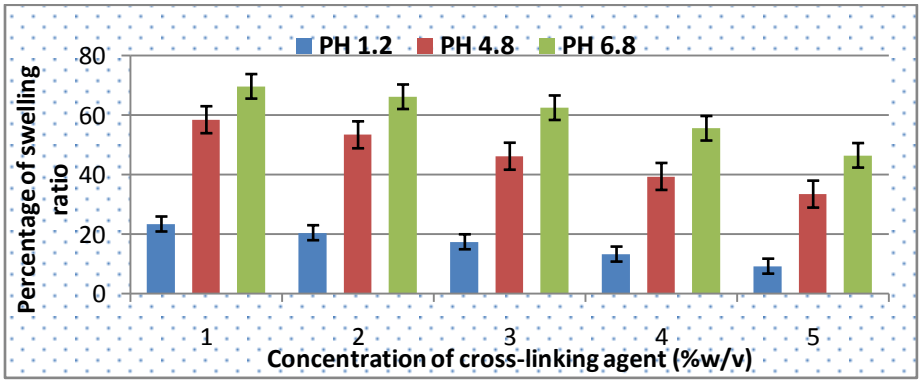


Figure 5(b) Effect of calcium chloride concentration on swelling behavior of drug loaded CA microbeads

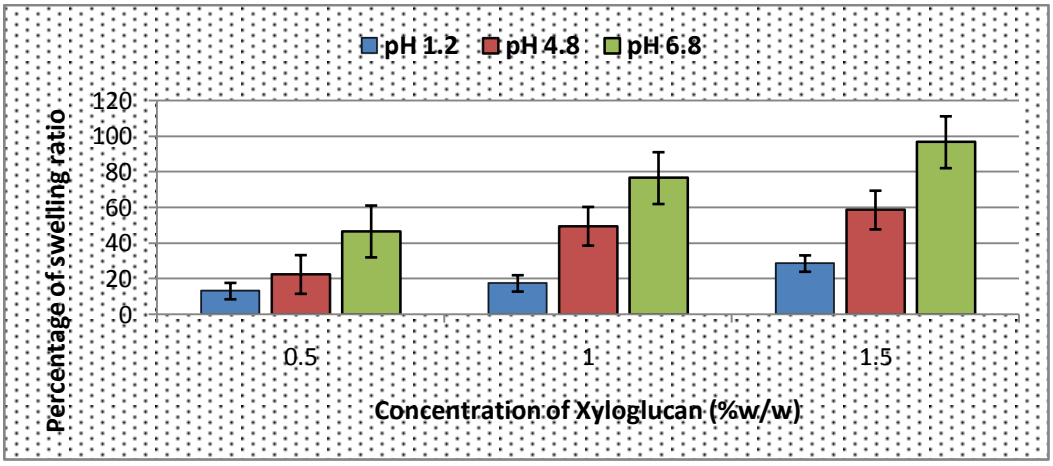


Figure 5(c) Effect of XG concentration on swelling behavior of drug loaded CA microbeads

***In-vitro* drug release studies**

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 up to 6h and simulated intestinal (SIF) pH 7.2 at end of 12 h studies. 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 2%w/v SLS in the media to aid the dissolution of the drug. The drug release from CA 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 polymers 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. [27]

The effect of drug-polymer ratio on aceclofenac sodium release from different batches of microbeads is shown in Figure 6a. Formulations F1 to F5 were showed the percentage of drug release in pH6.8 buffer media at the end of 6h was 80.74, 76.38, 69.28, 68.01, and 63.23. Moreover, at the end of 12hr in pH7.2 phosphate buffer solution the percentage of drug released in the range 98.54, 94.61, 90.63, 88.04, and 85.06 respectively. 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.[28]

The effect of cross-linking agent on aceclofenac sodium release from different batches of microbeads is shown in Figure 6b. The percentage of drug release from the formulations F6 to F10 was observed in pH6.8 buffer solution at the end 6hr 76.15, 72.37, 64.66, 60.80, 57.72 and at the end of 12hr in pH7.2 phosphate buffer media was 94.20, 91.84, 88.44, 84.26, and 81.25 respectively. 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 and also poor entry of dissolution medium into the polymer matrix may be delayed drug release.[29]

The effect of XG concentration on release of aceclofenac is shown in Figure 6c; the percentage of drug release from F11 to 13 in pH 6.8 buffer solutions was observed in the range 78.25, 70.57 65.36 and at the end of 12hr in pH7.2 phosphate buffer media was 92.24, 82.36, 75.65 respectively. The results explain that the initial burst release of CA was reduced by increasing the coating polymer concentration of XG resulting better entrapment efficiency, as discussed earlier and formation of diffusional bridges due to swelling of hydrophilic linkage in pH 6.8 buffer. On other hand, the optimum release of drug observed in pH 7.2 phosphate buffers due to relaxation of polymer net-work at higher pH level followed by erosion process shows near to zero-order kinetics.

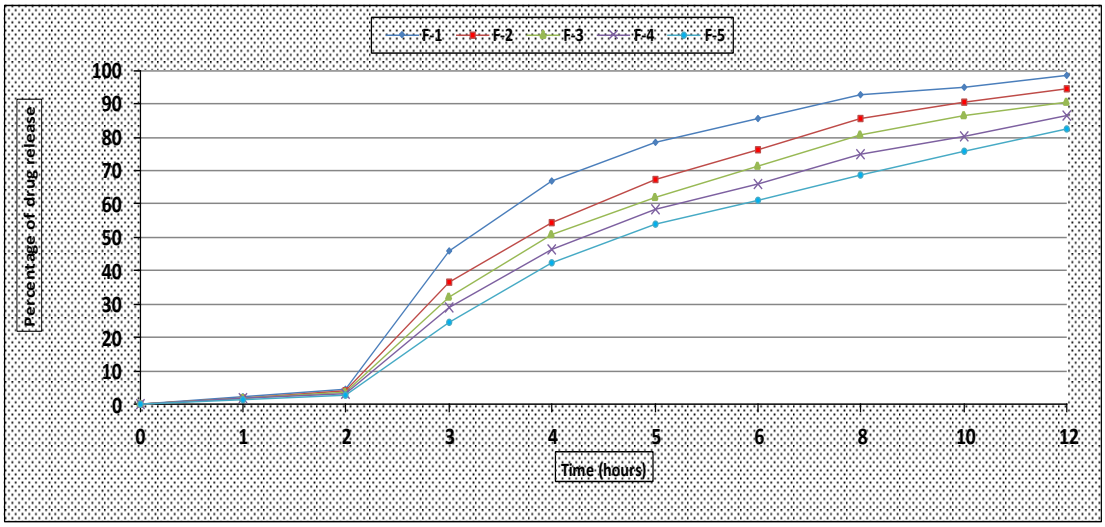


Figure 6(a) Effect of drug to polymer ratio on *in-vitro* drug release of CA microbeads.

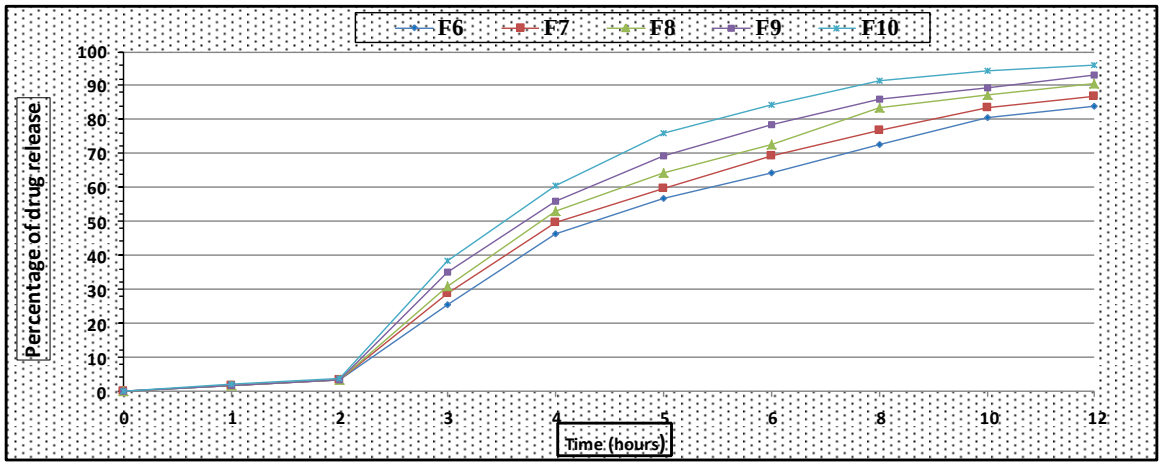


Figure 6(b) Effect of crosslinking agent on *in-vitro* drug release profile of CA microbeads

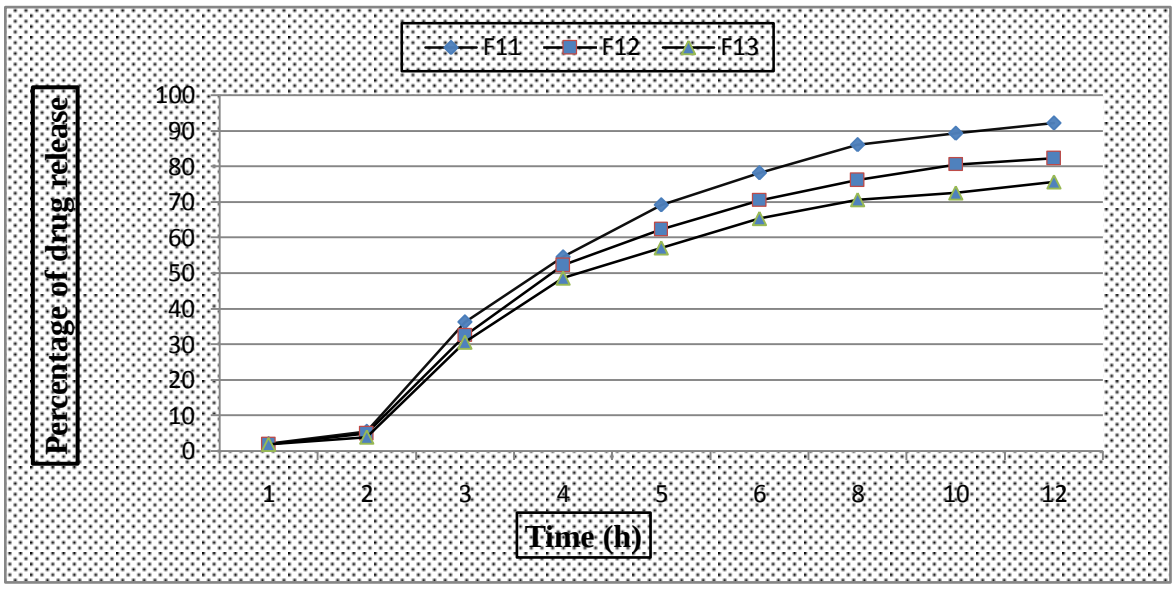


Figure 6(c) Effect of Xyloglucan(XG) on *in-vitro* drug release profile of CA microbeads

Kinetics of 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 calculated and were found to be more linear for first-order release as compared to zero order. Cumulative % drug release was analyzed using PCP DissoV2.08 software. The kinetic data was best fitted to Korsmeyer and Peppa's model and good regression co-efficient was observed[30]. The values of diffusion co-efficient ranged between n=0.6448 and 1.6988 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.

Table 3: Study of various kinetic models on *In-vitro* drug release of CA and CA-XG microbeads of aceclofenac sodium

Formulation code	Various Kinetic Models on drug release					
	Zero-Order	First-Order	Huguchi Matrix	Korsmeyer-Peppas	Korsmeyer-Peppas n-values	k-values
F1	0.9117	0.9814	0.9124	0.9162	0.6448	3.0689
F2	0.9209	0.9774	0.9113	0.9201	0.6756	2.7783
F3	0.9392	0.9821	0.9108	0.9240	0.7315	2.3240
F4	0.9474	0.9829	0.9103	0.9250	0.7711	2.0693
F5	0.9521	0.9830	0.9097	0.9257	0.7764	1.9066
F6	0.9399	0.9803	0.9082	0.9291	0.8535	2.0130
F7	0.9372	0.9811	0.9088	0.9262	0.8449	2.1643
F8	0.9278	0.9801	0.9076	0.9217	0.8523	2.2625
F9	0.9129	0.9775	0.9602	0.9148	0.8482	2.4075
F10	0.9007	0.9768	0.9020	0.9115	0.8361	2.6019
F11	0.9826	0.9224	0.9142	0.9162	1.6704	2.8220
F12	0.9846	0.9300	0.9168	0.9153	1.6788	2.6173
F13	0.9860	0.9410	0.9156	0.9186	1.6988	2.3596

All the results shows S.D. n=3, n=Diffusion exponent (slope) related to mechanism of drug release, according to equation $Mt/M=K^{tn}$, r- regression coefficient

CONCLUSION:

In conclusion, ionotropic gelation technique can be successfully used for preparation of aceclofenac sodium microbeads using SA, XG as drug release modifiers. Various formulation variables such as polymers concentration, calcium chloride concentration 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. All the 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. XG natural polymer was significantly affects mechanical properties, decreases porosity, controlled drug release due to increases swelling properties in higher pH of CA aceclofenac sodium microbeads. Therefore, one can assume that the Xyloglucan (XG) is promising natural biopolymers used in 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.

ACKNOWLEDGEMENTS:

Authors thank Micro labs pharmaceuticals Ltd, Bangalore for gift sample of aceclofenac sodium, F.M.C. international biopolymers, willing town, Ireland through Signet Chemical Corporation Pvt. Ltd, Mumbai, India for gift sample of sodium alginate.

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