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PharmTech

International Journal of PharmTech Research
CODEN(USA): IJPRIF ISSN : 0974-4304
Vol.1, No.2, pp 317-327, April-June 2009

Diclofenac Sodium Microbeads for Oral Sustained Drug Delivery

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ABSTRACT: In the present study, spherical microbeads are able to prolong the release of Diclofenac sodium were prepared by ionotropic gelation method, using sodium alginate as the hydrophilic carrier in combination with HPMC, chitosan and pectin polymers as drug release modifiers in various proportions to overcome the drug related adverse effects, improve drug bioavailability in different GI tract conditions. Formulated microbeads were investigated for physicochemical properties and drug release potential. All investigated properties showed satisfactory results. While increase in concentration of sodium alginate and other polymer dispersion increased sphericity, size distribution, flow properties and mean diameter of microbeads No significant drug-polymer interactions were observed in FT-IR studies. The drug entrapment efficiency obtained in the range of 70.4% to 95.2%. Increase in concentration of calcium chloride significantly affects the mean diameter but no appreciable change in morphology and release behavior. The shape and surface characteristics were determined by scanning electron microscopy (SEM) using gold sputter technique. Particle size of both placebo and drug loaded formulations were measured by SEM and particle size distribution was determined by an optical microscope. The microbeads had a smoother surface and were found to be discreet and spherical in shape. The physical state of the drug in the formulation was determined by differential scanning colorometry(DSC). *In-vitro* drug release profile of Diclofenac sodium from microbeads was examined in simulated gastric fluid (SGF pH1.2) and simulated intestinal fluid (SIF pH 7.2). Microbeads coated with chitosan and HPMC aqueous polymer dispersion shows optimum level of sustained release and exhibited zero-order kinetics followed by super case-II transport.

Keywords:-Ionotropic gelation, microbeads, Diclofenac sodium, Drug release modifiers.

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]. The non-steroidal anti-inflammatory drug Diclofenac sodium is a good candidate for the development of oral sustained release formulations. It is used for the treatment of rheumatoid arthritis, osteoarthritis with dose range 100-200mg 2-3 times, as conventional tablets/capsules.[3] An adverse gastrointestinal reaction has been observed and due to its short biological half-life 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.[4] 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.[5, 6].

The aim of the present study, which was to develop sustained release oral product namely microbeads of Diclofenac sodium using sodium alginate as the hydrophilic carrier in combination with HPMC, Chitosan and pectin polymers as drug release modifiers in various proportions to overcome the drug related adverse effects, improve drug bioavailability. 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

Diclofenac sodium was a gift sample from Sun Pharmaceuticals Ltd, Mumbai, India. Sodium alginate gift sample from F.M.C.International biopolymers,willingtown,Ireland Pectin (classic AF707)gift sample from Herbsteith & Fox K.G.Neuenburg.. Chitosan gift sample from Central institute of fisheries technology, Cochin, India. Hydroxypropylmethylcellulose was a gift sample from colorcon U.K.Calcium chloride was purchased from S.B. Fine chemicals Ltd, Mumbai. India. All other reagents and solvents used were of analytical grade.

PREPARATION OF MICROBEADS:

Microbeads of Diclofenac sodium were prepared by ionotropic gelation technique. In this present work four sets of microbeads were prepared by using sodium alginate alone and combination with coating polymers like HPMC, chitosan, pectin and calcium chloride used as counter ion. The detailed composition of the various formulations mentioned in table 1

PREPARATION OF SODIUM ALGINATE MICROBEADS;

In the first set three batches of drug-loaded microbeads were prepared (F1, F2, and F3). A solution of sodium alginate was prepared in 100ml of deionized water. In 50ml of sodium alginate solution, weighed quantity of Diclofenac sodium was dispersed uniformly. Bubble free dispersion was dropped through a syringe with needle into 100ml aqueous calcium chloride solution and stirred 500rpm. After stirring for 30minutes, the gelled beads were separated by filtration, washed with distilled water and finely dried at 70^0C for 6h in an oven.[9, 10.]

PROCESS VARIABLES AND PROCESS OPTIMIZATION

The following process variables were investigated, i.e. concentration of sodium alginate, concentration calcium chloride, curing time, stirring speed and drying condition. The different batches thus produced were analyzed for size, shape, ease of preparation, drug content and drug release. On the basis of the result obtained the process parameters were optimized as follows-

Sodium alginate concentration-3%w/v

Calcium chloride concentration-5%w/v

Curing time-2h

Stirring time-500rpm

Drying condition-06hrs at 70^0C

A different batch of microbeads was then prepared by using the optimized process variables and then only changes the concentration of different coating polymers. The final formulations were subjected to several characterization studies.

PREPARATION OF ALGINATE -HPMC MICROBEADS:

In the second set two batches of drug loaded microbeads were prepared using sodium alginate and HPMC as a coating polymer. To 50ml of deionized water, HPMC was added and stirred with the electric stirrer to form mucilage. Then sodium alginate was added to form uniform dispersion. Weighed quantity of Diclofenac sodium was added and homogenized for 5 min. The resulting dispersion was dropped through syringe with needle into 100ml of 5%w/v aqueous calcium chloride solution and stirred at 500rpm. After stirring for 30min the formed beads were separated by filtration, washed with distilled water, dried at 70^0C for 6h in an oven.[11]

PREPARATION OF ALGINATE –CHITOSAN MICROBEADS:

In third set, two batches of microbeads were prepared using sodium alginate and chitosan as a coating polymer. To 50ml aqueous sodium alginate solution, weighed quantity of Diclofenac sodium was dispersed uniformly. Bubble free dispersion was dropped through a syringe with needle into 100ml of chitosan solution containing 5%w/v of calcium chloride (chitosan dissolved in 10ml of 5% w/v glacial acetic acid). Stirred

at 500rpm. After stirring for 30min, the coated beads were separated by filtration, washed with distilled water, dried at 70^0C for 6h in an oven.[12]

PREPARATION OF ALGINATE-PECTIN MICROBEADS:

In fourth set, two batches of microbeads were prepared using sodium alginate and pectin as a coating polymer. To 50ml of deionized water, pectin was added and stirred with electric stirrer to form mucilage. Then sodium alginate was added to form homogenous dispersion. Weighed quantity of Diclofenac sodium was added and homogenized for 5min. The resulting dispersion was dropped through syringe with needle into 100ml of 5%w/v aqueous calcium chloride solution and stirred at 500rpm. After stirring for 30min the formed beads were separated by filtration, washed with distilled water, dried at 70^0C for 6h in an oven.[13]

CHARECTERIZATION AND EVALUATION OF MICROBEADS:

GRANULOMETRIC STUDY:

The particle size has significant effect on the release profile of microbeads. Size distribution determined by sieve analysis was carried out on mechanical sieve shaker, using different meshes (#12, #16,#22, #30) of American society of testing materials (ASTM). 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 [14].

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 angle of repose was determined by $\theta=\tan^{-1}(h/r)$: 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. Each experiment was carried out in triplicate [14]

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. Each experiment was carried out in triplicate.[15]

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.[16]

DIFFERENTIAL SCANNING COLORIMETRY (DSC)

The DSC analysis of pure drug, blank microbeads, and drug loaded microbeads was carried out using Perkin Elmer DSC-7 model, to evaluate any possible drug polymer interaction. Samples containing 3mg of the drug /placebo/formulation were placed in aluminium pans and heated from 30^oC to 225^oC at a heating rate of 10^oC/ minute under inert atmosphere flushed with nitrogen at the rate of 20ml/min [17.]

FOURIER TRANSFORM- INFRARED SPECTROSCOPIC ANALYSIS (FT-IR)

Drug polymer interactions were studied by FT-IR spectroscopy. One to 2mg of Diclofenac 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.

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. Percent bioadhesion was determined by the following formula; [18]

% Bioadhesion=

Weight of adhered microbeads x100.
weight of applied microbeads

DETERMINATION OF ENTRAPMENT EFFICIENCY:

Diclofenac 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, Diclofenac sodium content in the filtrate was analyzed at 276nm 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.The drug entrapment efficiency was determined using following relationship; [10]

% 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 276nm. Percentage of drug released with respect to entrapped drug in the sample was recorded.[10]

SWELLING PROPERTIES

The swelling properties of the drug loaded microbeads were determined in simulated gastric fluid (SGF-pH1.2). Thirty dried beads were placed in a small beaker to which 200ml of acidic buffer solution was added and then stirred with a magnetic stirrer at a speed 50rpm. After 1h 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.[19]

***IN-VITRO* DRUG RELEASE STUDIES**

The release profiles of Diclofenac sodium from microbeads were examined in simulated gastric fluid (SGF-pH 1.2) and simulated intestinal fluid (SIF-pH 7.2) by using USP XIII rotating basket apparatus (Microlabs, Mumbai,India). The drug loaded microbeads (equivalent to 100mg of diclofenac sodium) filled in empty capsule shells were put into the basket rotated at a constant speed of 75rpm and placed in 900ml of the dissolution medium, thermostated at 37^{o} C. At scheduled time intervals agitation was stopped, the samples were withdrawn and replaced with fresh medium. The samples were diluted, filtered and the drug content was determined spectrphotometrically at 276nm.

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

To assess long-term stability, accurately weighed drug loaded microbeads equivalent to 100mg of Diclofenac 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^{0}C/75% 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 condected 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 materials, such as sodium alginate, HPMC, chitosan and

pectin in inhibiting the release of Diclofenac sodium in the gastric environment. [20] 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 microbeads coated with HPMC, Chitosan and Pectin as drug release modifiers as a sustained release system. We prepared microbeads containing Diclofenac sodium by ionotropic gelation method and examined the effects of various factors (concentration of sodium alginate, concentration of calcium chloride, concentration of coating polymer ratio, curing time, stirring speed and nature of beads).

The microbeads were prepared in an environment free from organic solvents by dropping a mixture of colloidal copolymer dispersion, the dispersed drug Diclofenac sodium, formed mucilage of sodium alginate in calcium chloride solution, which acted as a counter ion. The droplets instantaneously formed gelled spherical beads due to cross- linking of calcium ion with the sodium ion which remain ionized in the solution. Chemical reaction between sodium alginate and calcium chloride to form calcium alginate was utilized for the microencapsulation of Diclofenac sodium core material. Preliminary work on the preparation of microbeads revealed that stirring speed and curing time greatly affected the size of microbeads. Smaller particles can be prepared by adjusting stirring rate 500rpm and curing time for 2h and also depending upon the height of the syringe from the level of counter ion solution, compressed force on the plunger of the syringe. The gelled particles were cured to get sufficiently hardened, filtered and dried. The colloidal polymer particles fused into the polymer matrix during drying with the drug being dispersed in the latex. The microbeads thus formed using three different coating polymers showed significant results on the evaluation.

Diclofenac sodium loaded sodium alginate microbeads which were cured for 2h at 500rpm in 1 percent calcium chloride solution were not spherical and had a flattened base at the points of contact with the drying vessel. Variation in the concentration of calcium chloride (1-7%) did not improve the sphericity appriciability. 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. It was found that sodium alginate concentration influence the microbeads size, average diameter, recovery, encapsulation efficiency, wall thickness, size distribution and the release characteristics. The total yields of microbeads obtained were between 80-95%. Yields were slightly lower 70-75% when the concentration of the polymer solution for preparation was 2%w/v. The yields were slightly high 90-95% from the microbeads which were prepared with coating polymer. The size distribution of the microbeads in different sieves were observed , that showed 42.46% to 79.50% of microbeads retained on ≠20 sieve, which proves the uniformity of microbeads. It was observed that by an increase in the concentration of sodium alginate and calcium chloride solution it tends to make the particles more spherical and obtaining the uniform size spheres. On other hand with an increase in the concentration of coating polymers, the distribution of the particle size slightly shift to the higher pore size, due to increase in the physical behaviors of the microbeads.

The physical parameters like shape, surface morphology and also particle size were analyzed by scanning electron microscopy (SEM).Scanning electron micrographs of drug loaded microbeads prepared by sodium alginate (F2) and with coating polymers (F5, F7,F9) are shown in figures 1a, 1b, 1c, 1d, respectively. The surface of the sodium alginate microbeads were discrete and spherical in shape with a rough outer surface and have a sandy appearance because of the surface-associated crystals of drug. Moreover, higher concentration of drug uniformly dispersed at the molecular level in the alginate matrices (Figure;1a). The surface morphology of drug-loaded microbeads prepared with sodium alginate and HPMC was spherical in shape and has large bridges observed on the outer surface (Figure;1b). In case of drug loaded sodium alginate microbeads coated with chitosan appeared to be more spherical, the outer surface was smooth due to adhesion of the chitosan membrane with alginate core, visible large wrinkles observed by partial collapsing of the polymer network during dehydration. (Figure;1c). However, in case of drug loaded sodium alginate microbeads coated with pectin formed more robust beads that were more resistant in acidic pH but faster disintegration at pH 7.2. The mean particle sizes of drug loaded microbeads were performed by Optical microscopy. The mean particle size of the various formulations (F1-F9) of microbeads were obtained in the range between 778±0.007 and 982±0.009µm (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 the proportional increase in the mean particle size of microbeads with increasing 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 and curing time. 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. In fixed concentration of sodium alginate and calcium chloride and increases in the coating polymer concentration results increase in diameter of microbeads.(F4, F5, F6, F7,F8 and F9).

The size of the spherical matrix could easily be controlled by varying the stirring speed of the system and the concentration of sodium alginate added to the aqueous medium. At a stirring speed of 50rpm, the mean particle diameter and the size distribution of the beads increased significantly. The tendency of the droplets to coalesce and aggregate at the slower stirring speed, appeared to be correspondingly high, and resulting in larger mean bead diameters. This low stirring speed might have decrease the uniformity of the mixing force throughout the emulsion mixture, hence resulting in a wider size distribution of the final beads. At a stirring speed of 500rpm the particle size is changed to a lesser extent. At this higher stirring speed, a vigorous, uniform, increased mechanical shear might have resulted. This suggests that the size of the droplets formed during microencapsulation might, therefore, be closely related to the size of the final beads, which increased significantly by decreasing the stirring speed.

The rheological parameters like angle of repose and bulk density of all the microbeads confirms better flow and packing properties. All the formulations showed excellent flowability, as represented in terms of angle of repose ($<40^0$)[20]reported (Table; 1). The batches prepared with coating polymer shows good flowability due to formation of smooth layer on the surface of the microbeads. Bulk and tapped density of the microbeads were also determined and shows good acceptable range and found to have higher packability. The improvements of micromeritic properties suggest that prepared micobeads can be easily handled.

The drug entrapment efficiency increased progressively with increasing sodium alginate concentration (Table; 2). resulted in the formation of larger microbeads entrapping greater amounts 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. Since the solubility of Diclofenac sodium was slightly higher in calcium chloride than in distilled water, prolonged exposure caused greater loss of drug in the curing medium. Increase in alginate concentration reduced loss of drug in the curing medium due to the formation of dense matrix structure. On other hand drug entrapment efficiency was significantly increase with an increase in the concentration of coating polymers (F4, F5, F6, F7, F8, and F9).because adhering property of coating polymers was further reduced the loss of drug in the curing medium.

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 increase in concentration of sodium alginate and coating polymers, percentage of 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. Under acidic conditions swelling of calcium alginate beads occurs scarcely.[21] Optical microscopy was used to investigate the hydration and swelling of microbeads at pH1.2 up to 2hrs (Table; 2) .Being a polyelectrolyte, alginate can exhibit swelling properties that are sensitive to the pH, ionic strength and ionic composition of the medium. The swelling ratio of the beads was dependent on the pH of the solution. The low swelling of sodium alginate microbeads (F1, F2, F3) in acidic media was probably due to proton-calcium ion exchange forming insoluble alginic acid regions and followed by solvent penetration into the gel net work. Increasing the concentration of calcium chloride produces the beads with higher levels of Ca^{2+} ions 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 sodium alginate microbeads coated with different polymers (F4, F5, F6, F7, F8 and F9) was observed, by increasing the concentration of coating polymer ratio which enables decreasing of the swelling properties of microbeads in acidic medium, whereas chitosan coated microbeads(F6, F7) showed an unexpected swelling increment. Swelling of dry microbeads is mainly attributed to the hydration of the hydrophilic groups of alginate and chitosan, causeing protonization of the amino groups of chitosan which causes improvement in the solubility of the chitosan membrane, which results in slightly increased swelling behavior at pH 1.2. When we compared 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 45-60min. These results suggest that the dried beads swell slightly in the stomach. When they are subsequently transferred to upper intestine, the

particles are begain to swell and they behave as matrices for sustained release of incorporated drug but they are subject to erosion in the lower intestine. Optimized Diclofenac sodium microbeads were found to possess good bioadhesion (78.50%), which resulted in prolonged retention in small intestine.

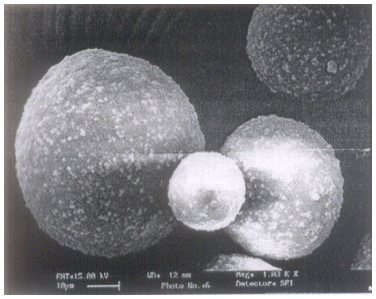
Differential scanning colorimetry thermograms of pure drug and drug loaded sodium alginate microbeads prepared with different coating polymers was observed, calcium chloride shows two endotherm peaks in the temperature range 180-200^{0}C; while sodium alginate decomposes at about 240^0 C with broad exotherm. 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 Diclofenac sodium with various polymers 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 spectras. In which no shifting of peaks was significantly found, indicating the stability of the drug during encapsulation process.

Diclofenac sodium release from microbeads was studied in acidic buffer (SGF pH1.2) for initial 2h and phosphate buffer (SIF pH7.2) for a period of 10 h. The release of Diclofenac sodium from microbeads causes more sustained nature with an increase in sodium alginate concentration (Table; 2). Alginic acid is composed of D-mannuronic acid and L-guluronic acid residues at varying proportion of GG-, MM-, and MG-blocks. Cross-linking takes place only between the corboxylate residue of GG-blocks and Ca^{+2} ions via egg-box model to give a tight gel network structure. The toughness of the network structure and subsequent disintegration of dissolution of alginate particles taking place through ion exchange between bound Ca^{+2} and Na^{+} ions present in dissolution medium, which might have hindered the penetration of dissolution medium into alginate particles, leading to extended release of drug in different physiological pH range. However, at acidic pH the alginate beads shrink due to tightening of the gel network, resulting decreased drug release. The polymer is eroded at alkaline pH and contents are released in a sustained manner by both diffusion and slow erosion of polymer matrix. Based on these suggestions *in-vitro* release kinetic data proves that the drug release was very slow in acidic medium (pH1.2) and subsequently increases at higher pH levels. The maximum drug release of about 93%- 98%w/w (F1-F3) was observed (Figure 2), due to the alginate beads fast disintegration occurs in simulated intestinal fluid (SIF pH7.2) and has high porosity which results in rapid drug release. Whereas more sustained drug release in sodium alginate microbeads with different coating polymers were observed 81-87%w/w (F4, F5), 86-88%w/w (F6, F7), and 89-92% w/w (F8, F9) (Figure;3) 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 (Table--). The values of diffusion co-efficient ranged between n=0.8806 and 1.1506 indicating the drug release from the microbeads followed the anomalous transport and super case-II transport mechanism controlled by swelling and relaxation of polymer chains.

For the developed formulations containing sodium alginate and combination with different coating polymers were subjected to stability studies at 40^{0}C/ 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 Diclofenac sodium microbeads using sodium alginate and with other coating polymers like chitosan, HPMC, pectin as drug release modifiers. Various formulation variables such as polymer concentration, calcium chloride concentration, stirring speed, and coating polymer 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. The alginate drug loaded microbeads swelled at pH 1.2 predominantly very slow but underwent erosion at pH 7.2. FT-IR and DSC studies did not reveal any significant drug interactions.Diclofenac sodium release from microbeads formulated with sodium alginate with 1%HPMC and 1%chitosan coating polymers showed a satisfactory sustained release profile. Therefore, one can assume that the Diclofenac 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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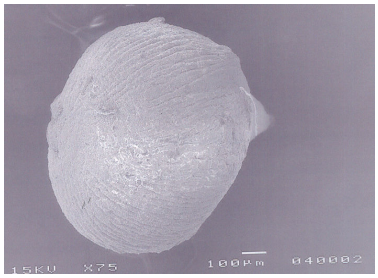

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d

Figure1: Scanning Electron Micrographs of Diclofenac sodium microbeads 1(a), 1(b), 1(c) and 1(d) for formulations F2, F5, F7, and F9 at 15kv

TABLE 1; COMPOSITION AND PHYSICAL CHARACTERISTICS OF MICROBEADS

Formulation code	Sodium Alginate %(w/v)	Calcium Chloride % (w/v)	HPMC %(w/v)	Chitosan % (w/v)	Pectin % (w/v)	Angle of Repose θ	Bulk Density (g/cc)	Tapped Density (g/cc)
F1	2	3	-	-	-	32.25±2.09	0.485±0.040	0.693±0.031
F2	3	5	-	-	-	30.15±1.87	0.695±0.032	0.755±0.067
F3	4	7	-	-	-	28.20±1.95	0.785±0.091	0.862±0.056
F4	3	5	0.5	-	-	26.20±0.78	0.735±0.076	0.806±0.0202
F5	3	5	1.0	-	-	24.20±0.96	0.764±0.043	0.810±0.0105
F6	3	5	-	0.5	-	25.40±0.86	0.725±0.052	0.835±0.039
F7	3	5	-	1.0	-	22.30±0.98	0.745±0.056	0.844±0.042
F8	3	5	-	-	1.0	27.15±1.87	0.775±0.085	0.852±0.084
F9	3	5	-	-	2.0	26.80±2.10	0.765±0.076	0.866±0.0992

Each formulation containing 100mg of Diclofenac sodium.Results show are the±SD, n=3 for Angle of repose, Bulk density and Tapped density.

TABLE 2: PHYSICAL CHARECTERASTICS AND *IN-VITRO* DRUG RELEASE

Formulation code	Drug Entrapment Efficiency (%)	Mean Diameter (µm)	Swelling Ratio pH 1.2 in 2h (µm)	Cum Percent Drug Release pH1.2 # [%]	Cum percent Drug Release pH7.2 * [%]
F1	70.40 ±1.65	822.15±1.34	862.30±0.042	6.20±0.12	98.40±0.68
F2	77.28 ±1.32	794.25±1.65	823.40±0.048	4.80±0.23	95.60±0.98
F3	85.32±1.20	778.50±0.76	803.20±0.056	4.20±0.32	93.80±0.94
F4	90.84±0.86	860.20±0.86	910.40±0.024	2.80±o.13	87.40±1.12
F5	94.70±0.65	890.50±.0.54	931.30±0.043	3.60±0.26	81.40±1.65
F6	91.92±0.89	970.60±0.75	1029.10±0.096	5.10±0.34	88.40±1.43
F7	95.28±1.10	982.15±.0.68	1038.15±0.087	6.80±0.45	86.10±0.97
F8	86.30±1.96	908.60±0.56	958.70±0.020	4.50±0.36	90.60±0.76
F9	88.20±0.97	920.25±0.93	962.65±0.055	4.10±0.30	88.40±0.87

Results show are the mean ±SD, n=3 for Drug entrapment efficiency, Mean diameter, Swelling ratio. #-values expressed as mean of triplicate at the end of 2h, *-values are expressed as mean of triplicate at the end of 10h.

TABLE 3: MODEL FITTING DATA FOR *IN-VITRO* RELEASES KINETIC PARAMETERS OF MICROBEADS.

Formulation code	Zero Order [r]	First Order [r]	Higuchi Matrix [r]	Korsmeyer – peppas [r]	'n'-Values
F1	0.9980	0.9515	0.9737	0.9984	0.8806
F2	0.9960	0.9076	0.9694	0.9932	0.9160
F3	0.9981	0.9098	0.9674	0.9955	0.9271
F4	0.9942	0.9854	0.9951	0.9937	1.0682
F5	0.9988	0.9887	0.9940	0,9969	1.1304
F6	0.9943	0.9026	0.9689	0.9806	1.3520
F7	0.9964	0.9591	0.9772	0.9968	1.2206
F8	0.9918	0.9818	0.9884	0.9848	1.4503
F9	0.9890	0.9885	0.9933	0.9747	1.1506

n=Diffusion exponent related to mechanism of drug release, according to equation $Mt/M\infty=Kt^n$, r-Correlation coefficient

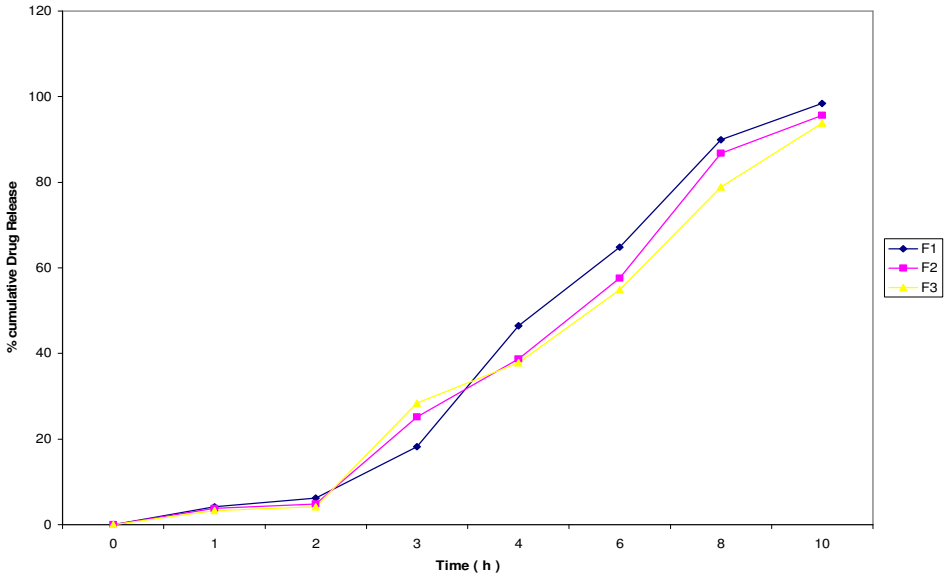

Figure 2: In-vitro Drug Release Profile of Microbeads Containing Sodium Alginate [F1, F2, and F3] Results indicate ±SD (n=3)

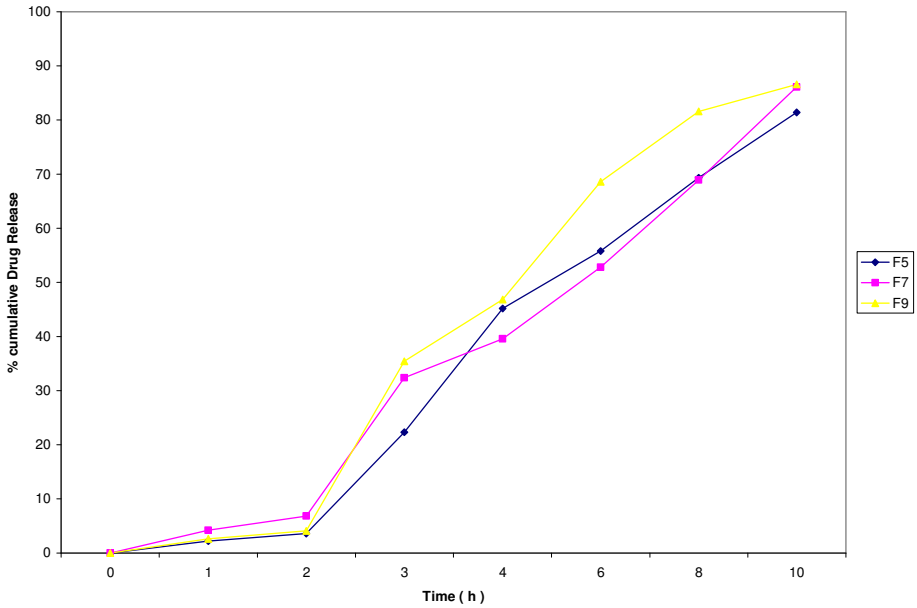

Figure 3: In-vitro Drug Release Profile of Microbeads Containing Sodium Alginate with HPMC [F5], Chitosan [F7], and Pectin [F9]. Results indicate ±SD (n=3)

ACKNOWLEDGEMENTS

Authors thank sun pharmaceuticals Ltd, Mumbai for gift sample of Diclofenac sodium, Colorcon , U.K., Central Institute of Fisheries Technology for gift sample of chitosan and Herbsteith & Fox K.G. Newenburg for gift sample of Pectin (Classic AF 707)

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