ORIGINAL ARTICLE

Development and Characterization of Dual Cross-linked Microbeads for Colon Specific Targeting: Release Kinetic Modeling and Gamma Scintigraphy Studies

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Abstract

Objective: The aim of present study is to develop and characterize ionic gelation-based dual Ca_2^+ and SO_4^{2-} cross-linked gel microbeads using blend mixture of biodegradable chitosan and sodium alginate for colon targeting. **Methods:** Those diclofenac sodium (DS) loaded microbead formulations were prepared by utilizing ionic gelation based polyelectrolyte complexes technique that was effectively dual cross-linked with Ca_2^+ and SO_4^{2-} ions containing solutions. **Results:** The result for surface morphology characterization through scanning electron microscopy revealed for semispherical and wrinkled shaped rough surfaces of those optimized formulations. The mean particle size of 761.52–895.22 µm, entrapment efficiency 63.45–78.38% of, and percentage yield of 73.28–78.69% are resulted, respectively. The swelling degree of those optimized formulations is found with slighter values into acidic gastric medium (pH = 1.2), whereas increased values into alkaline intestinal mediums (pH = 6.8 and 7.4), respectively, during 4-h time intervals. The experimental vitro release studies revealed for negligible amount of DS that was released into simulated gastric fluid medium during 2 h, while slower drug released into simulated intestinal fluid medium during 3 h and augmented released into stem cell factor medium during 18 h studies, respectively. Furthermore, the drug release kinetics modeling studies performed for those optimized formulations revealed for non-fickian mechanism type that was best fitted with Higuchi and Korsemeyer peppas mathematical models. These confirmed that drug releases was guided by dual mechanisms of diffusion and erosion simultaneously. **Conclusion:** The developed experimental formulations may be used for targeting of DS into colon and possible management of inflammatory bowel disease, respectively.

Key words: Biodegradable, chitosan, diclofenac sodium, dual cross-linked beads, gamma scientigraphy, *in vitro* release studies, kinetic modeling, sodium alginate

INTRODUCTION

Ionic gelation-based polyelectrolyte complexes are gel formation process that is due to reversible type electrostatic interactions between carboxylic groups of poly anionic sodium alginate and amino groups of poly cationic chitosan moieties. It forms "egg-box-junction" like three dimensional interpenetrating molecular structures known as hydrogel beads in an aqueous medium.[1] These micron sized gel beads are prepared in laboratory by blending together opposite charged polymers for possible interactions and then cross-linking with solutions containing divalents Ca^{2+} and SO_4^{-2} ions for further rigidity and compactness of gel mass for targeting successfully into vicinity of colonic region. Xu *et al.* (2007) investigated for dual cross-linked gel beads that provides for achieving tailored mechanical properties and desired drug releasing properties into the targeted area.[2] These gel formulations are found unaffected by the acidic pH variations of the upper

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gastrointestinal (GI) tract during its transit and finally arrives successfully up to colon area.

As per reports, those cross-linked system have been proven for scores of advantages over other single cross-linked beads or conventional dosage forms.[3,4] Kesavan *et al.* (2018) reported that these beads are utilized for bypassing drug absorption and acidic assaults of drug in upper GI tract, minimum risk of dose dumping, increased drug absorption at desired site, enhanced bioavailability and biocompatibility, potential targeting to colon, etc.[5] In the present study, the colon targeting has been achieved by selection of prescribed combined dual approaches of microbial and pH-dependent systems. The repeating cationic amino groups (-NH2) on the C-2 position of chitosan have significant affinity towards anionic carboxylic acid (COO-) groups of sodium alginate to form gel complexes.[6-8]

The diclofenac sodium (DS) is phenylacetic acid derivative compound, well recognized as non-steroidal anti-inflammatory agent (NSAID) with antipyretic and analgesic actions.[9-11] In general, it is available in market into conventional dosage forms used in the treatment of rheumatoid arthritis, ankylosing spondylitis, and pain resulting from minor surgery, trauma, and dysmenorrhea.[12] Saravanan *et al.* (2008) reported that on oral administration of marketed DS containing conventional dosage form is consisted of shorter half-life, severe side effects, and suffer from major drug loss into the upper GI tract.[13] Jeganathan *et al.* (2016) investigated for DS containing polyelectrolyte based multilayer film coated tablet and reported that it is a potential drug that can be utilized for colon targeting for the management of inflammatory bowel disease (IBD), since it is well adsorbed into the colon region.[14] Glieter *et al.* (1985) also reported that DS is well absorbed into the colonic region during oral administration.[15]

Therefore, the present study is envisaged to prepare a stable DS-loaded matrix-based dual cross-linked blended gel microbeads while utilizing ionic gelation and polyelectrolyte complex techniques. During studies, those factors affecting of process and formulation variables on physicochemical properties, mechanical properties, *in vitro* drug release behavior, and *in vivo* targeting potentials were also investigated for possible management of IBD, respectively.

MATERIALS AND METHODS

The gift sample of diclofenac sodium was received from Sun Pharmaceutical Industries Ltd., Mumbai (India). Chitosan (MW ~ 3.0×10^5) polymer was received from Central Institute of Fisheries Technology, Cochin (India). While those sodium alginate polymer (viscosity of 2% solution at 25°C ~ 250 cps), calcium chloride and sodium sulfate were procured from Hi-Media Laboratories, Mumbai (India). All other reagents and chemicals utilized were of analytical grade that was used without further purification.

Preparation of chitosan-alginate blended microbeads

In the present study, the DS-loaded blend mixture of chitosan-alginate microbeads was prepared in laboratory using ionotropic gellation technique.[2] In this method, the blend mixture solution containing 2% w/v of chitosan-sodium alginate was utilized with mass ratios of 3:0.5 and 5:1, respectively. First of all, sodium alginate solution was prepared using deionized water at ambient temperature and then fixed quantity of DS (20% w/w of dry polymer weight) was suspended into it properly. That drug polymer solution was completely homogenized for 30-min time intervals. Then, the aqueous chitosan solution was prepared separately containing acetic acid (1.0%, w/v) at same laboratory condition. Thereafter, the prepared chitosan solution was added into sodium alginate solution with different mass proportions. This blended mixture was again homogenized for next 35 min to get homogenous polyelectrolyte solutions. That polyelectrolyte solutions was ultrasonicated for another 15 min for complete debubbling of gases and then pH 5 was adjusted using NaOH (0.1 mol/L), respectively. Thereafter, the blend solution was extruded through 0.45 mm inner diameter needle using hypodermic glass syringe. That solution was dropped into 3 and 6% w/v calcium chloride solution at the rate of 1 mL/min while kept falling distance of 6 cm over the container. The formation of mechanically weaker but smooth and spherical single cross-linked microbeads containing Ca_2^+ ions takes place. Those resulted that single cross-linked micro beads was allowed to remain and cured into the same solution for hardening purpose for another 60 min. These beads were decanted out from container and then gently washed with de-ionized water for 2 to 3 times. Now that calcium beads were again dipped and cured into SO_4^{-2} ions containing 4% w/v sodium sulfate solution for another 60 min for dual cross-linking purpose. This process resulted into formation of mechanically strong dual cross-linked composite micro beads containing Ca_2^+ and SO_4^{2-} ions into it. That bead was decanated and washed properly with deionized water for 2–3 times. This obtained bead was firstly air dried for 48 h at room condition and then oven dried at 50°C for 10–14 h time period. All formulation batches were prepared in triplicate. The optimization, core compositions, and independent variables that were used for preparation are depicted in Table 1, respectively.

Evaluations of prepared microbead formulation

Scanning electron microscopy (SEM) study

The SEM method was utilized for surface morphological evaluations of those dual cross-linked micro beads.[16] Those beads containing DS were perused for gold spray coated under vacuum by mounting on a brass stub while used double-sided adhesive tape. Samples were made electrically conductive while kept into an ion sputter with a thin layer of gold (~300Å) for 150 s and at 20 kV and then examined with

Table 1: Formulation variables and composition of diclofenac sodium containing microbeads using 2^3 factorial design method

Formulation code	Pectin: Chitosan (%, w/w)	Calcium chloride: Sodium sulphate (%, w/v)	Curing time (min)
D_SB_1	3:0.5 (−)	3:4 (−)	60 (−)
D_SB_2	5:1 (+)	3:4 (−)	60 (−)
D_SB_3	3:0.5 (−)	6:4 (+)	60 (−)
D_SB_4	5:1 (+)	6:4 (+)	60 (−)
D_SB_5	3:0.5 (−)	3:4 (−)	90 (+)
D_SB_6	5:1 (+)	3:4 (−)	90 (+)
D_SB_7	3:0.5 (−)	6:4 (+)	90 (+)
D_SB_8	5:1 (+)	6:4 (+)	90 (+)

Values represent those +: Higher level, −: Lower level, w/w: Weight by weight, w/v: Weight by volume

Table 2: Effect of variable polymer ratio and cross linking agents on particle size, percent yield, and entrapment efficiency and drug content of different batches of bead formulations containing diclofenac sodium drug

Batches	Particle size (μm)	Percentage yield	DEE (% w/w)	Drug content (% w/w)	Degree of swelling at pH=1.2	Degree of swelling at pH=6.8	Degree of swelling at pH=7.4
D_SB_1	878.15±0.23	74.33±0.21	64.98±0.41	85.16±0.51	0.08±0.05	0.24±0.04	0.25±0.07
D_SB_2	895.22±0.21	77.65±0.30	68.33±0.11	87.38±0.18	0.10±0.02	0.28±0.02	0.27±0.01
D_SB_3	869.65±0.16	76.28±0.28	63.45±0.19	83.73±0.62	0.11±0.03	0.25±0.01	0.26±0.02
D_SB_4	876.62±0.32	78.17±0.62	69.18±0.26	87.28±0.52	0.13±0.01	0.30±0.05	0.32±0.02
D_SB_5	767.83±0.45	75.93±0.57	72.65±0.73	81.48±0.31	0.09±0.02	0.25±0.03	0.27±0.06
D_SB_6	778.58±0.29	79.43±0.22	78.38±0.63	89.93±0.29	0.06±0.08	0.34±0.01	0.36±0.04
D_SB_7	761.52±0.40	73.28±0.18	72.62±0.28	86.73±0.13	0.09±0.07	0.26±0.05	0.27±0.09
D_SB_8	770.82±0.23	78.69±0.23	77.12±0.38	88.17±0.28	0.07±0.03	0.32±0.06	0.35±0.03

Each value represents the mean±SD (n=3). SD: Standard deviation, DEE: Drug encapsulation efficiency

proper accuracy and precision using electron microscope (JSM-5800, JEOL, Japan).

Particle size determination

Around 100 dried beads among batch formulations were selected for determination of mean particle size. Those beads diameter was calculated while utilized optical microscope (Olympus, Germany) instrument. Before measurement, the standard guideline was adopted for required calibration of that eyepiece micrometer with reference to standard stage micrometer. Each randomly selected bead among batch was used to determine the Feret's diameters while placed on the clean and sterilized glass slide. All readings were average of three trials ± standard deviation (SD) under proper 4 × magnifications with accuracy as shown in Table 2.

Drug content, percent yield, and drug entrapment efficiency estimations

Such estimations were carried out with accurately weighed 100 mg bead formulation using digital balance. These were then added into of phosphate buffer saline (100 mL) of pH = 7.4 with occasionally shaking at 37 ± 0.5°C for 24-h time intervals, respectively. Then, these samples were treated for ultrasonication for another 30 min till got completely dissolved into the medium. After that obtained polymeric dispersion was processed for centrifugation at 3000 rpm for 40 min for the purpose to eliminate those contained polymeric fragments. The obtained DS containing dispersion was filtered using Whatman filter paper number # 41 properly. Then aliquot was prepared accurately using dispersion and then assayed for quantitative drug content estimations using Shimadzu UV 1800 spectrophotometer against blank as shown in Table 2. All those determinations were performed in triplicate and results were averaged with exactness. Another study for estimation of entrapment efficiency and percent yields was performed using the following formula:

$$\text{DEE}\ (\%) = \frac{\text{Actual content}}{\text{Theoretical content}} \times 100$$

$$\text{Percent yield} = \frac{\text{Total weight of beads}}{\text{Total weight of drug and polymer}} \times 100$$

Degree of Swelling

Accurately weighed dried beads (50 mg) among batch were used to perform the equilibrium swelling degree studies. That swelling degree for bead formulation was performed

separately using three different simulated mediums containing simulated gastric fluid (SGF) at pH = 1.2, simulated intestinal fluid (SIF) at pH 6.8 and stem cell factor (SCF) at pH 7.4, respectively. Those beads were added in swelling solutions (500 mL) contained into USP dissolution apparatus II (basket type). These beads were then allowed to swell for up to constant equilibrium weight under 50 rpm speed at 37 ± 2°C for 4-h period. That swelled beads were removed out from respective medium and blotted for moisture removal using filter paper. Thereafter, that bead was separately weighed using electronic balance. All readings were carried out in triplicate and mentioned with mean values [Table 2]. The swelling ratio in percentage was estimated with respect to time using below provided formula:

$$\text{S.D. } (\%) = \frac{W_e - W_i}{W_i}$$

Whereas, W_e is equilibrium weight of swelled beads at given time, and W_i is absolutely weight of initial dried beads.

Differential scanning calorimetry (DSC)

In this experiment, differential scanning calorimeter (Mettler-Toledo India Private Limited, Mumbai) was involved to record thermograms for drug, polymers, and formulation. Separately dried sample was weighed out for about 2–2.5 mg and thereafter, that was hermetically sealed inside dust free aluminum pan of 40-µL capacity properly. Then, it was get started heating for temperature range between 20 and 380°C and scan speed of 10°C/min, respectively. Inside chamber, the temperature was controlled while used continuous nitrogen gas supply at flow rate of 70 mL/min. Those parameters for enthalpy scale and temperature were calibrated using the indium as standard reference.

X-ray diffraction

In this study, the wide angled X-ray diffractometer (Ultima-III, Reganku SmartLab, Japan) was utilized for obtaining XRD pattern of drug, polymers, and bead formulations. The diffractometer was used up with Ni-filtered Cu-K alpha as radiation sources (1.54056 Å). That instrument was set up with tube voltage of 30 kV, current of 30 mA, scan speed 5° min^{1}, over the diffraction angle (2θ) range of 20°–80°, and the count range of 3000 cps, respectively, during the experiment.[17]

In vitro drug release studies

In vitro release studies were carried out using USP Type-II dissolution apparatus (Electrolab TDT-06P, Mumbai, India) for those batch formulations. Among each batch, the weighed sample of 100 mg was taken and added into 900 ml of acidic SGF (pH = 1.2) medium. That medium was uniformly maintained at temperature of 37 ± 0.5°C, stirring speed of 50 rpm, and time interval of 2 h, respectively. The same testing was then continued with SIF (pH = 6.8) for another 3 h and then in SCF (pH = 7.4) for up to 12–24 h as similar condition maintained throughout the experiment. Then, after each fixed time intervals, 5-mL aliquots was withdrawn for quantitative estimation purpose. Whereas, at same time, an equivalent amount of fresh medium was added into the medium to maintain the sink condition properly. The withdrawn sample aliquots were got filtered, suitably diluted, and the analyzed for absorbance against blank using UV-visible spectrophotometer (UV 1800, Shimadzu, Japan). Those experiments were performed in triplicate; mean values and SD were evaluated properly.[18]

In vitro drug release studies in presence of 2, 4, and 6% w/v rat cecal content

For the reason to simulate microbial environment of human colon, the *in vitro* release studies were also performed using rat cecal content into the dissolution medium.[19] Initially, the experiment was carried out at pH = 1.2 for 2 h while using 0.1 N HCl containing dissolution medium. Then, the same medium was added up of 62.5 mL of 0.2 M trisodium phosphate to maintain pH at 6.8 and 7.4 and added 2, 4, and 6% w/v cecal content, respectively, and then continued studies for up to 24-h period. At the same time, the formulations were performed for control study in SCF at pH = 7.4 for 24 h (without rat cecal content). For quantitative estimations, 5-mL samples were withdrawn at regular intervals while maintained the sink condition and then accurately analyzed using UV-visible spectrophotometer.

Kinetic modeling and mechanism of drug releases

Mathematical models were used to obtain the possible kinetics and drug transport mechanism for matrix-based bead formulations. For this purpose, those *in vitro* release studies data were used and fitted to explore the same as shown in Table 3. Those model fittings were based on classical power law expressions such as zero order, first order, Korsmeyer-peppas, Higuchi's model, and Hixson-Crowell models as pinpointing of drug release mechanism. The accuracy and precision of obtained values were compared with that of calculation of squared correlation coefficient (R^2) values.[20] The curve plotting was carried out while utilizing Microsoft Excels software efficiently.

In vivo gamma scintigraphic imaging study

The *in vivo* gamma scintigraphic study and preparation methods for uptake of sodium pertechnetate (^{99}mTc) with optimized D_SB_6 formulation were carried out as prescribed.[21] The gamma scintigraphic study was duly approved by IAEC before carry out the experiment. Those acclimatized six healthy male rabbits weighing between 1.5 and 2 kg were divided into two groups of three animals each (one control group) and then used to examine the *in vivo* transit behavior of sample formulation. The first group of animals was orally administered with gelatin capsules containing formulation using feeding tube with sufficient drinking water and then

Table 3: Results of curve fitting of *in vitro* release data from diclofenac sodium containing chitosan-sodium alginate-based bead formulations

Formulation code	Zero order	First order	Higuchi	Hixson-Crowell
D_SB_1	0.956	0.892	0.987	0.982
D_SB_2	0.969	0.897	0.985	0.986
D_SB_3	0.946	0.914	0.978	0.991
D_SB_4	0.975	0.925	0.993	0.972
D_SB_5	0.979	0.938	0.990	0.969
D_SB_6	0.981	0.935	0.983	0.992
D_SB_7	0.984	0.888	0.984	0.973
D_SB_8	0.986	0.925	0.986	0.958

all legs were humanitarianly tied up over a piece of plywood. Then the subject was monitored and scanned after every 2-h intervals in front of the gamma camera for locating GI transit position of administered radiotracer containing formulation. During experimentation, the gamma camera was arranged with a field view of 40 cm and was fitted with a medium energy parallel-hole collimator efficiently. The 140 keV gamma rays emitted by 99m Tc were imaged for study. After definite time interval, those specific GI tract site (anterior) was imaged by E-cam Single Head Gamma Camera (Siemens, Germany). All those gamma images were recorded using an online computer system and stored on magnetic disk. Those were used as qualitative analysis to determine the distribution activities in the GI tract.

Statistical analysis

All those experimental data have been represented as mean ± SD for statistical analysis. Mean values of percent yield, particle size, and entrapment efficiency were compared using the Student's *t*-test. Those differences were considered statistically significant at level of $P < 0.05$, respectively.

RESULTS AND DISCUSSION

Preparation and optimization of microbeads systems

The present work was carried out to prepare diclofenac sodium containing matrix-based dual cross-linked chitosan-alginate microbeads system utilizing polyelectrolyte complex-based ionotropic gelation technique. The preparation of batch formulations was based on formulation and process variables for optimization purpose while used manual 2^3 (two levels and three factors) factorial design method. This had resulted into overall eight batch formulations. The blend mixtures were consisted with fixed quantity of drug and variable concentration of polymer mixtures at the ratios of 3:0.5 and 5:1 was used into the method. In this context, the curing time was set at 60 and 90 min throughout the experimentation, which was found sufficient for formation of complete cross-linked network beads system. That dispersion of drug with poly electrolytes mixture was drop-wise added into the curing $CaCl_2$ solutions at fixed flow rate and height. As soon as those drops came in contact with Ca^{2+} ions containing cross-linking solution, it get immediately changed into spherical cross-linked networks of gel beads. The spherical beads were formed because of reason that electrostatic ionic cross-linking reactions and hydrogen bonding. This reactions were taken place between positively charges of divalent Ca^{2+} ions and negatively charges of anionic carboxylic acid groups ($-COO^-$) into the given cross-linking solutions. Then, during next dual cross-linking process, those negatively charged sulfate ions (SO_4^{2-}) got electrostatically cross-linked with that of positively charged cationic amino groups ($-NH^{2+}$) into the given cross-linking solution. Those freshly prepared beads were spherical and smooth surfaced, but became semi-spherical and rough shaped after drying process with reduced micrometer size range. The prepared batch formulations were consisted with modified physicochemical properties such as good mechanical strength, proper swelling at specific pH media, appreciable drug encapsulations, compact and smaller particle size, higher stability, and controlled drug releases.

SEM

The SEM study was performed for surface analysis of micron sized dried gel beads formulations [Figure 1a-f]. That image at 100× magnification clears for rough, wrinkled, compact sized, and semi-spherical shaped surfaces of those dual cross-linked beads. At magnification of 1Kx, it was evident with minor cracks on surface of beads that may be because of shrinking during drying process and partly collapsing of polymeric gel during dual cross-linking steps. These findings are found in conformity with Srinatha *et al.* (2008).[22]

Particle size

Table 2 had shown for average particle size distributions between 761.52 ± 0.40 and 895.22 ± 0.21 µm for those drug containing dried beads while utilized calibrated optical microscope for that investigation. Those results clear that average particles sizes were decreased significantly ($P < 0.05$) with increments of mass ratio of sodium alginate polymer in comparison to mass ratio of chitosan polymer. These decreased sizes may be due to that sodium alginate forms strong and compact cross-linked networks mass in comparison to chitosan polymers. The outcomes are found in harmony with other study of Bera *et al.* (2015).[23]

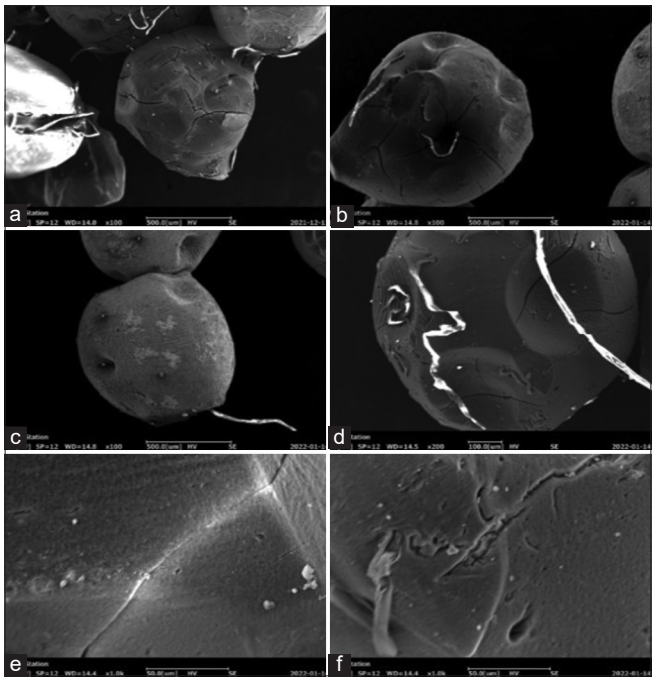

Figure 1: Scanning electron microscopy images of optimized diclofenac sodium loaded double cross-linked D_SB_6 microbeads batch formulation containing 5:1 ratios of chitosan-sodium alginate polymers (a) surface view, magnified at ×100 (b) surface view, magnified at ×100, (c) surface view, magnified at ×1000 (d) surface view, magnified at ×100 (e) surface view, magnified at ×1000, and (f) surface view, magnified at ×1000

Percentage yield, entrapment efficiency, and drug content

Quantitative estimation of those parameters of percentage yield, entrapment efficiency, and drug content was performed using UV spectroscopic method for batch formulations. Those values of percentage yield and entrapment efficiency are found increasing significantly ($p < 0.05$) with increment of mass ratio of sodium alginate in comparison to chitosan polymer into the core compositions of beads. Whereas, the drug content was also found increasing significantly ($p < 0.05$) from 81.48 ± 0.31% to 89.93 ± 0.29% with those increased concentration of alginate polymers and varying concentration of curing agents into the beads. These outcomes may be due to that sodium alginate forms more strong cross-linked networks and compactness in comparison to chitosan polymers and possible responsible for significant increments of that drug content, percent yield, and entrapment values.[24]

Swelling degree of gel beads

Those swelling degree experiments were performed in various simulated GI fluids at pH = 1.2 (SGF), 6.8 (SIF), and 7.4 (SCF), respectively, for batch formulations as presented in Table 2. The swelling degree values at pH = 1.2 were found to be very lesser for most of batch formulations. That slightly value was observed due to the mass proportions of chitosan polymer only as it is soluble at acidic pH medium. This advocates for overall acid resistant properties into acidic medium due to intense ionic cross-linking and inter-penetrating polymeric networks properties of those prepared beads. While on other hand, the same batch formulations had revealed for appreciable swelling degree into those alkaline GI fluids having pH = 6.8 and 7.4, respectively. This was resulted due to increments of composition of sodium alginate ratio only as it is soluble in alkaline medium at pH = 6.8 or 7.4, respectively. At the same time, that curing agents also affected the swelling degree of those batch formulations. It had shown that as we go on increasing the concentration of cross-linkers then vice versa, it would slightly decreased the swelling degree on other hand. Other factors governing equally for swelling degree of those pH-sensitive bead systems were based on theories of ionization and ion-exchange mechanisms. These are due to those cationic charges of chitosan and anionic charges of sodium alginates associated with functional groups of polymers. Simsek-Ege *et al.* (2003) reported that swelling degree took place due to Gibbs-Donnan effect or Donnan equilibrium.[25] Across the gel phase boundaries or diffusion barriers, the concentration gradient of ionic charges gets developed due to that the swelling of beads took place. In the same context, those protonation and deprotonation processes are also the governing factors for beads swelling at given pH medium.[2,26,27] These findings were found concordant with other researchers Anal *et al.* (2005).[28] Furthermore, those DSC and XRD reports help to confirm for effective cross-linkages took place between poly ions and functional group into the gel bead system.

DSC study

DSC thermogram was obtained for pure diclofenac sodium that exhibited sharp melting endothermic peak (Tm) at 286.00°C, while onset and endset peaks at 284.78 and 297.10°C, respectively [Figure 2a-c]. The thermal curves observed for those sodium alginate and chitosan powders were found with broader glass transition peaks (Tg) at 121.45 and 247.54°C, respectively. Thermogram of dried optimized formulations was found with small endothermic peak (Tm) at 299.82°C for entrapped drug that might be due to the peaks for evaporation of adsorbed moisture or volatile compounds. These shifted thermal curves of formulation to slight higher melting point may be due to reason that of drug entrapment or complexation with interpenetrating polymeric networks of alginate and chitosan polymers. That also says for conversion of drug state from highly crystalline state to semicrystalline (amorphism) on other hand into the formulation. This shifting curve behavior of those drugs and polymer into formulation might only be possible due to electrostatic polyelectrolyte complexation and cross-linking reactions had been completely taken place. These results are found in conformity with earlier reports of Aquino *et al.* (2012).[29]

XRD study

Further characterization of P-XRD was performed for drug, polymer(s), and drug containing optimized formulation. This was done for the purpose to investigate for any transformation of crystalline state of drugs contained into polymeric complex matrix systems [Figure 3a-d]. The report had shown sharp characteristic peaks for pure diclofenac sodium drug that confirms for its crystalline nature. Those chitosan and sodium alginate were found with weak signal intensities that may be due to its basic amorphism properties. Whereas, that sharp diffraction pattern of pure drug was found, diminished in formulation may be due to amorphism properties. Hence, these crystallographic data suggested for drug dispersion and entrapment taken place at molecular level. Here, the state of drug had been changed from its crystalline to semicrystalline state and entrapment of drugs into polymeric matrices took place because of ionic polyelectrolytic complexation process. These outcomes are found in agreement with reports of Bhattacharya *et al.* (2012).[30]

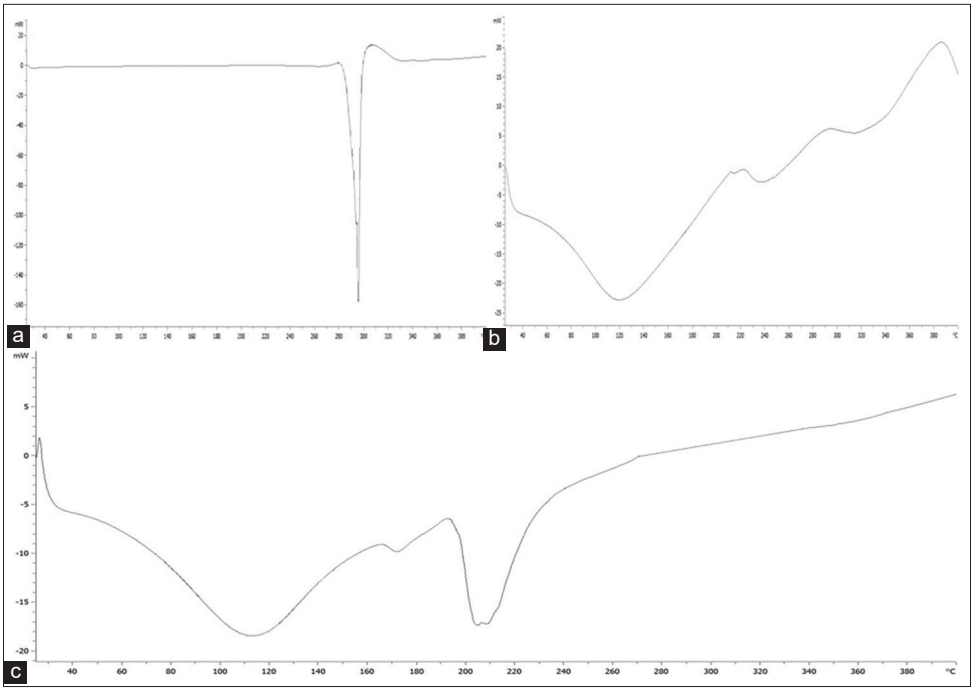

Figure 2: Differential scanning calorimetry thermogram of (a) pure diclofenac sodium (DS), (b) powder mixture of sodium alginate and chitosan polymers, and (c) chitosan-sodium alginate-based DS-loaded optimized microbead formulation

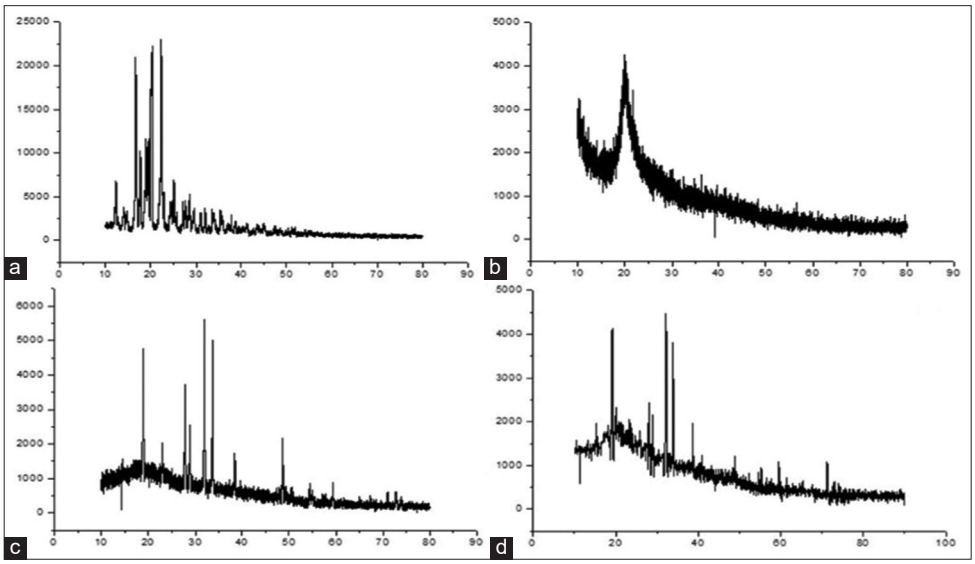

Figure 3: X-ray diffractogram of (a) diclofenac sodium drug, (b) sodium alginate polymer, (c) chitosan polymer, and (d) optimized dried D_SB_6 microbeads formulation

In vitro drug release profile

Drug release studies were carried out to investigate enteric resistance properties and targeting potential of laboratory developed drug-loaded batch formulations. The batch formulations were found resistant to adverse acidic environment of the upper GI tracts as released only up to 4.6% of surface adsorbed drug into the SGF medium (pH = 1.2) as presented in Figure 4a. Whereas, slight raised drug released into alkaline SIF medium (pH 6.8) and augmented released into SCF medium (pH = 7.4), respectively. The drug release profiles from loaded bead system into the simulated GI fluids were found completely depended on mass proportions of polymers and cross-linking agents that were used as independent formulation variables. The batch formulation containing raised proportions of alginate polymers had shown lesser drug releases in comparison to chitosan containing batches in acidic media. While augmented drug releases into SCF medium that may be due to complete polymeric relaxations and microbial degradation of polyelectrolytic complexed networks. Drug release reports had confirmed for direct influences of those variable concentrations of cross-linking agents and curing time for those batch formulations. The drug releases were found decreased from those formulations into both acidic and alkaline GI fluids at pH = 1.2 and 6.8 that were consisted with increased cross-linking agent ratios, respectively. This was due to reason of that poly ions, namely, Ca^{2+} and SO_4^{2-} which had provided intense mechanical strengths and rigidness to bead system and thereby decreases drug release profile. Those micron sized pores and channels got contracted and saturated due to presence of higher cross-linkers concentration. Concurrently, another factors also equally affected drug releases profiles into various GI fluids were based on protonation, deprotonation, chelation, and ion-exchange mechanism phenomenon, since those ions gets charged or ionized into their respective acidic or alkaline media.[31] The triggered mechanism for drug release into acidic and alkaline medium was based on ionizations of those alginates and chitosan at specific medium. The optimized mass ratio of used cross-linkers was found potential to protect the drug release into physiological environment of SGF (pH = 1.2) for 2 h and SIF (pH = 6.8) for 3 h additional and thus, mimicking lag time of 5 h, respectively. The D_SB_6 and D_SB_8 formulations that had shown optimum particle size, % yield, % entrapment efficiency, and degree of swelling at pH = 6.8 and pH 7.4 and better *in vitro* drug release profiles was selected as optimized formulation for further studies.

In vitro drug release profile in presence of rat cecal medium (2, 4, and 6%)

The specific biodegradability of alginate-chitosan containing beads system was evaluated in the presence of 2, 4, and 6% w/v rat cecal content (RCCM) for optimized formulation. Results confirmed for augmented drug releases in the presence of RCCM when compared with release without RCCM (control study) as shown in Figure 4b and c, respectively. In the presence of RCCM, 97.08% of drug was released within 24 h in contrast to 75.19% drug release in the control medium. This demonstrated that polymeric matrix systems can be degraded by bacterial enzyme activity.

Estimation of drug release mechanism

The drug release mechanism for those obtained *in vitro* data was treated for best fit into various mathematical kinetic models, namely, zero-order, first-order, Higuchi, and Hixson-Crowell, respectively [Table 3]. Those regression coefficient (R^2) values were utilized for prediction aptitude and accuracy of these models and those curve fittings. During curve fittings

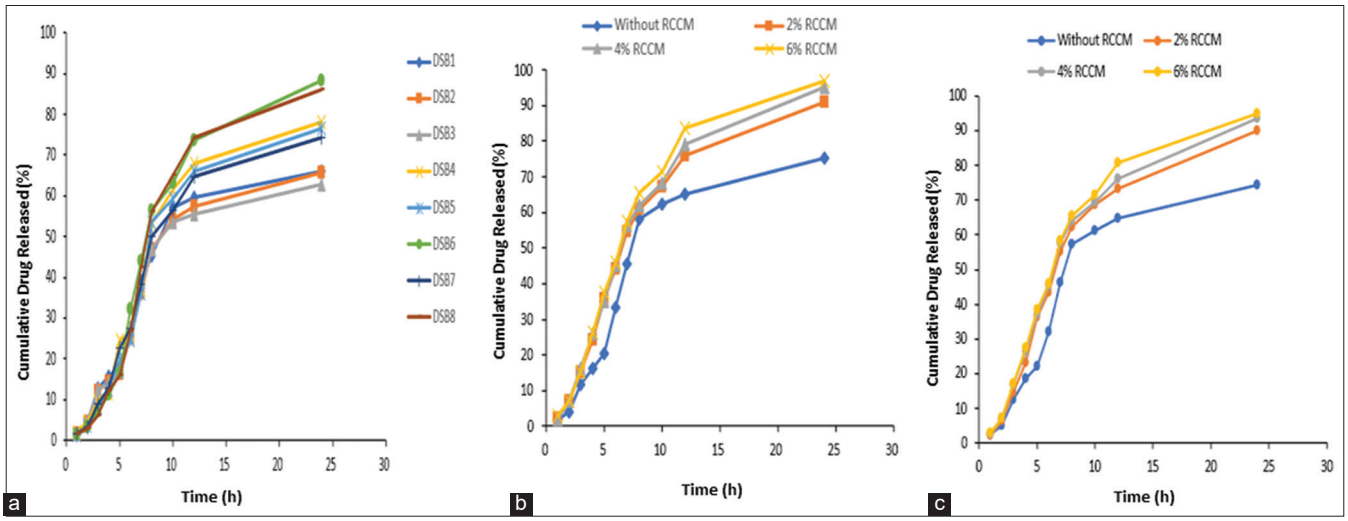

Figure 4: Cumulative percent drug release of diclofenac sodium-loaded microbead formulations at pH = 1.2, 6.8 and 7.4 medium: (a) Various batch formulations with variable polymer ratios and curing agents for up to 24-h time intervals, (b) optimized D_SB_6 formulations containing 2, 4, and 6% rat cecal content medium (RCCM) for up to 24-h time intervals, (c) optimized D_SB_8 formulations containing 2, 4 and 6% RCCM for up to 24-h period; each value represents the mean ± standard deviation; (n = 3)

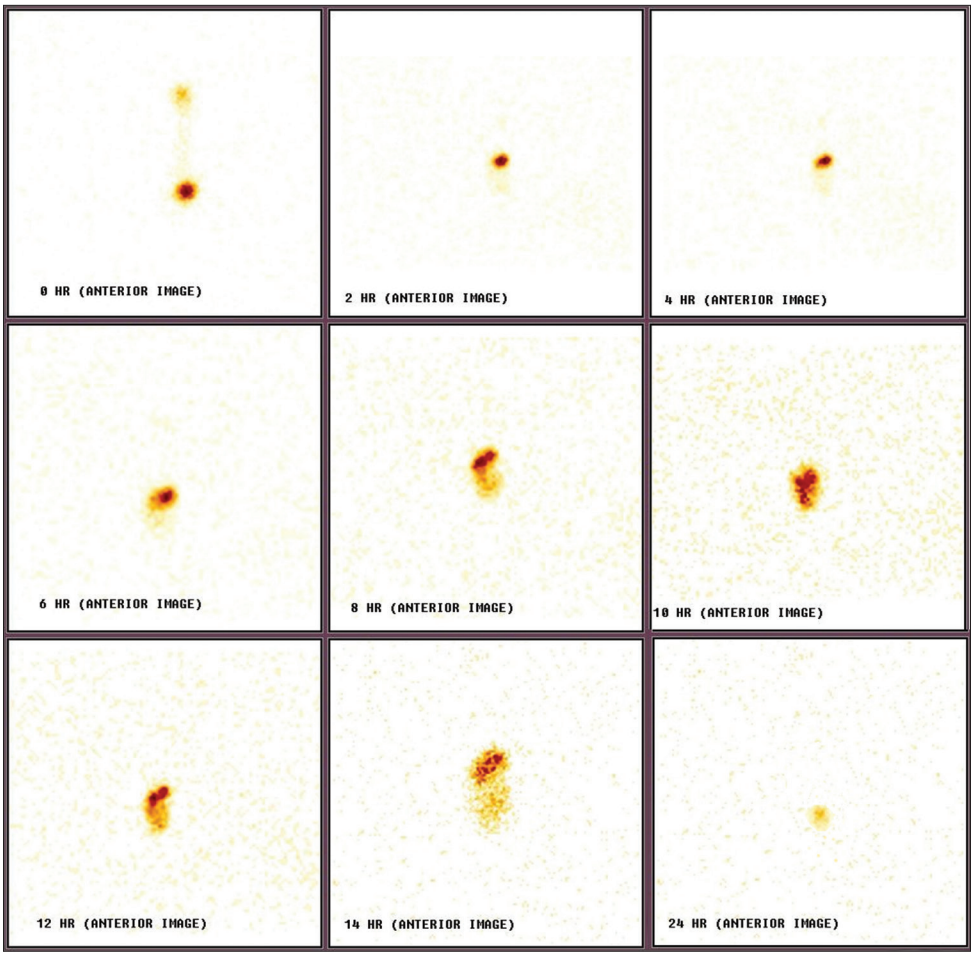

Figure 5: Gamma scintigraph showing gastrointestinal transit and *in vivo* release of loaded radioactive tracer (^{99m}TC-DTPA) from optimized microbead formulation at different time intervals of 0, 2, 4, 6, 10, 12, 14, and 24 h (n = 3)

process, the R^2 value of Higuchi matrix model was observed to be the highest and closer to unity (R^2 = 0.978 to 0.993) and diffusion exponent (n values) in range of 0.71–0.76, respectively. That was found for best-fit and the plot revealed for linearity on other hand. The drug release data gets fit linearly to Higuchi's square root kinetic equation for every sample formulations. Therefore, this confirmed that drug release was guided with anomalous non-Fickian transport mechanisms referring to combination of swelling controlled and diffusion controlled type. These findings were found agreement with the reports of Maiti *et al.* (2011).[32]

Gamma scintigraphic imaging of multiparticulate system

To finally confirm the colon targeting potential of those optimized formulation, the gamma scintigraphic imaging studies was performed using animal subject groups. During studies, those animals were orally administered with radio labeled formulations and then the anterior abdomen images of were recorded at various time intervals of 0, 2, 4, 6, 8, 10, 12, 14, and 24 h, respectively, as shown in Figure 5. The scintigrams revealed for average gastric emptying time of 2–3 h, small intestinal transit time of 3–5 h, and colonic arrival time of 6–7 h for all experimental animals. The release of radioactive tracers (^{99m}Tc-DTPA complex) confirms for exact transit time and location of formulation throughout GI tracts. It was observed that lesser amount of radioactive tracers was released after 3 h only, when formulations reached into small intestine at average 5.0 ± 0.5 h. While formulation systems were begun to disintegrate on arrival up to colon regions after 6.0 ± 0.30 h only. Thereafter that formulation got entirely disintegrated in the colon in between 12 h and 24 h time intervals that confirmed for uniform distribution of the released tracer across the entire colonic region. On other hand that also confirmed for complete degradation of core chitosan-sodium alginate consisted into polymeric matrix systems which might be due to reasons of combined pH dependent ionization and microbial degradation activities. These results are found similar with the studies of Sharma *et al.* (2016).[33]

CONCLUSION

The present matrix-based dual cross-linked microbead systems containing optimized ratios of chitosan-sodium alginate polysaccharides and cross-linkers were found successful for

potential targeting to colonic region. Those *in vitro* releases and *in vivo* gamma scintigraphic analysis further confirmed for targeting potential and controlled release profile of our laboratory prepared polyelectrolyte systems. The obtained *in vivo* results were found to be in support with those *in vitro* release profile data. Hence, it can be concluded that those ionic gelation-based dual cross-linked microbead system may be suitable for targeting of diclofenac sodium to colon specific region for efficient management of IBDs.

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