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Development of Cross-Linked Alginate Beads by Ionotropic Gelation Technique for Controlled Release of Diclofenac Sodium

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ABSTRACT

The aim of the study was to formulate colon targeted diclofenac sodium alginate beads. The beads were formulated by ionotropic gelation using sodium alginate as polymer, calcium carbonate as an internal cross linking agent and calcium chloride as an external cross linking agent. The optimized beads contained Neusilin and coated with Eudragit L100. The beads were analyzed in terms of, encapsulation efficiency (EE%), particle size and morphology. Swelling properties were studied in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4). *In vitro* drug release was studied in SGF, pH 1.2, SIF, pH 6.8 and SIF, pH 7.4. The results show that the particle size of the beads ranged from 1.98 ± 0.40 to 2.80 ± 0.30 mm. The optimized batch containing Neusilin® had the highest EE% of 72 % significantly higher than other batches ($p < 0.05$). The degree of swelling of the beads was zero in SGF and about 90 % in SIF at 100 min. The results of the *in vitro* release showed that the beads had 0 % release in SGF, pH, 1.2 at 2 h, about 3 % in SIF, pH 6.8 at 4 h and 9 % drug release in SIF, pH 7.4 at 9 h. Therefore, sodium alginate beads could be used for colon delivery of diclofenac sodium.

Keywords: Colon targeting, Alginate beads, Diclofenac sodium, NSAIDs

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INTRODUCTION

Diclofenac sodium is one of the routinely prescribed anti-inflammatory agents available for the management of pain and inflammation. It is marketed as injections, oral normal release and sustained release tablets and topical formulations. The drug is sparingly soluble in water and almost completely absorbed after oral administration, but is subjected to approximately 50 % hepatic first pass metabolism. However, diclofenac has a short half life of 1 – 2 hours and like other non steroidal anti-inflammatory drugs (NSAIDs), it causes gastrointestinal irritation [1,2]. Owing to the high gastric irritation effect and short biological half life of this NSAIDs, a sustained action dosage form is generally preferred, so as to reduce the frequency of administration, reduce the incidence of gastric irritation, enhance patient compliance, and improve the bioavailability profile of these drugs.

Among all the factors that make for an ideal therapy, the most difficult to achieve is maintaining serum concentrations of drugs at therapeutic levels for a long period of time. This is usually done by repeated administration and thus, poses a serious problem of patient compliance [3]. Sustained release dosage forms are those preparations with controlled rate of absorption of drug into the body, which is achieved mainly by controlling the dissolution rate of the formulations [3].

Beads are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability, stability and target drug to specific sites. Beads can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing the dosing frequency and improving patient compliance [4,5].

Ionic gelation technique involves ionic cross-linking between polyelectrolyte biopolymers such as alginates, carboxymethylcellulose, chitosan, etc. and counter ions to produce cross-linked matrix which would provide the desired controlled release rate [6,7]. Sodium alginate was chosen as the biopolymer because alginates are non-toxic, biodegradable, naturally occurring anionic polysaccharides having high biological safety, and can absorb 200-300 times their own weight of water [8]. One of the most important and useful property of alginates is their ability to form gels in the presence of metal ions such as calcium. Sodium alginate is water-soluble and can be crosslinked with divalent or polyvalent cations to form insoluble meshwork. It can be easily gelled by the addition of calcium ions to form an insoluble calcium alginate network. This occurs due to cationic exchange between Na^+ and Ca^{2+} ions and the technique is called ionotropic gelation [6,9]. The alginate matrix obtained possess many advantages including non-toxicity, protection of mucous membrane from irritation upon oral administration, controlled release of

drug due to its ability to undergo swelling when it comes in contact with water, and ability to incorporate acid sensitive drugs into the matrix [6,9]. The objective of the work is to formulate sustained release diclofenac sodium by producing diclofenac sodium-loaded alginate beads for possible delivery to the colon.

MATERIALS AND METHODS

Materials

The following materials were used as procured from their local suppliers without further purification: Calcium chloride (Merck, Germany), calcium carbonate (May and Baker, England), ethanol, hydrochloric acid (Sigma-Aldrich, Germany), sodium alginate (Qingdao Jiashidi seaweed Ltd, China), diclofenac Sodium (Triveni interchem pvt. Ltd, India), Eudragit L100 (Lianyungana Wantai pharmaceutical material co., ltd, China), sodium hydroxide (BDH, Poole, England), monobasic potassium phosphate (BDH, Poole, England), Neusilin® (Fuji Chemical Industry Co., Ltd in Japan).

Preparation of Diclofenac sodium-alginate beads

The diclofenac sodium-loaded alginate beads were formulated by ionotropic gelation. A 3 % w/v of sodium alginate was prepared in 9 ml of deionized water and an appropriate quantity of diclofenac sodium and calcium carbonate (internal cross linking agent), Neusilin ® as presented in Table 1 were weighed accurately in an analytical balance (Adventurer, Ohaus, China) and dispersed in a 100 ml beaker containing the sodium alginate solution. The mixture was stirred on a magnetic stirrer at 500 rpm (SR1 UM 52188, Remi Equip., India) for 15 min in order to obtain a homogenous mix. The volume of this suspension was then made up to 10 ml with deionized water. The viscous solution formed was then drawn into a 10 ml syringe. It was thoroughly shaken and was introduced drop wise into a beaker containing 100 ml of 4 % $CaCl_2$ (external cross linking agent).

Table 1: Composition of diclofenac sodium alginate beads

Batch	Diclofenac sodium (mg)	Sodium alginate (%)	Calcium chloride (%)	Calcium carbonate (mg)	Neusilin® (mg)	Eudragit L100 (%)
A1	360	3	4	120	--	2.0
A2	307	3	4	120	--	2.0
A3	255	3	4	120	--	2.0
A4	360	3	4	120	300	2.0
A5	360	3	4	--	--	-

Batches A1, A2, A3 and A4 were coated with Eudragit L100, batch A5 was uncoated, batch A4 contains an additional polymer-Neusilin®.

Five minutes were allowed as the curing time, thereafter, the beads formed were rinsed with water and filtered using filter paper (Whatman no 1). The beads were then spread on a plate and were dried in a tray dryer (Memmer, U25, Western Germany) at 50 ^{o}C. The beads were then collected into an air tight container. About 10 % of Eudragit L100 was prepared in 20 ml of ethanol. The beads were coated with Eudragit L100 by spraying. These processes were repeated for the remaining batches of beads using their respective formula.

Determination of drug content and EE%

Beers calibration curve was obtained for diclofenac sodium in alcoholic buffer (5 ml ethanol: 2.5 ml SIF 7.4) at a predetermined wavelength of 283 nm wavelength. A 500 mg quantity of the beads was crushed in a mortar containing 10 ml of the medium. The solution was filtered (Whatman no 1), diluted appropriately and assayed spectrophotometrically at a wavelength of 283 nm. The drug concentration in each batch of the microsphere was calculated from the Beer's plot.

The percentage encapsulation efficiency was calculated using equation 1.

$$\% \text{ Encapsulation Efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \text{ x } 100 \qquad (1)$$

Analysis of the particle morphology and size

The particle morphology was determined using about 10 beads per batch randomly selected and placed on a microscope slide. It was imaged under a microscope attached with a digital camera (Kodak Easyshare C143). The sizes of the beads were calculated by measuring the bead diameter in spherical samples or the length and the width in non spherical samples (n = 100).

Swelling studies

The swelling behavior of diclofenac sodium-loaded alginate beads was observed by dispersing an accurately weighed quantity of beads in 20 ml of simulated intestinal fluid (SIF) (pH 7.4) and simulated gastric fluid (SGF) (pH 1.2). The spheres were separated from the swelling media after 20 min by filtering the solution through a filter paper (Whatman no 1). The beads were placed on a dry filter paper to remove excess media and then weighed [10]. The spheres were returned to the media and the process was repeated for 1 h. The percent weight change was calculated using Eq. 2.

$$\text{Weight change } (\%) = \frac{(w_s - w_i)}{w_i} \text{ x } 100 \qquad (2)$$

In this equation, w_s is the weight of swollen spheres and w_i is the initial weight of the spheres.

***In vitro* drug release studies**

The USP paddle method was adopted in the study. The dissolution medium consisted of 900 ml of freshly prepared simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8 and pH 7.4). The *in vitro* release was studied for 2 h in SGF, pH 1.2, after which the beads were transferred into SIF, pH 6.8 for 2 h and finally to SIF, pH 7.4 for 5 h. The medium was maintained at 37 ± 1 ^{o}C. Three batches of beads were selected (A3, A4 and A5) based on the results of encapsulation efficiencies and the swelling studies. An amount of beads containing 100 mg of diclofenac sodium was employed in the study. The beads were placed inside a tightly secured basket and the paddle was rotated at 100 rpm. At various intervals, 5 ml sample was withdrawn from the dissolution medium, filtered with a non adsorbent filter paper (Whatman no 1) and analysed for drug content using UV-spectrophotometer (Jenway 6305 spectrophotometer, Barloworld Scientific Ltd., Essex CMB 31BWL, UK) at a predetermined wavelength of 275 (SGF), 282 (SIF, pH 6.8) and 283 nm (SIF, pH 7.4). Sink condition was maintained by replacing the withdrawn sample with a fresh medium.

***In vitro* release kinetics**

The dissolution data were analysed to determine the *in vitro* release kinetic and mechanisms for the release in SIF (pH, 7.4) using three kinetic models including the first order equation, Higuchi square root equation and Ritger-Peppas empirical model.

$$\ln Q_t = \ln Q_0 - K_1 t \quad (3)$$

$$Q_t = K_2 t^{1/2} \quad (4)$$

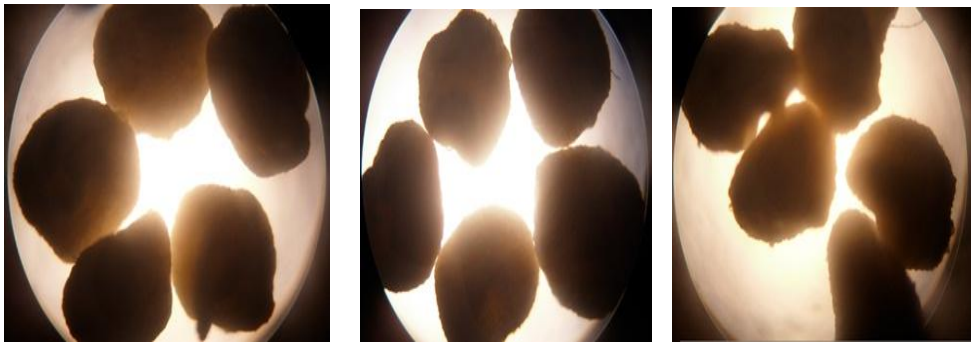
$$M_t/M_\infty = K_3 t^n \quad (5)$$

where Q_t is the amount of drug released or dissolved at time t, Q_0 is amount of drug released or dissolved at time t = 0, k_1 is first-order release rate constant, k_2 is Higuchi rate constant, M_t/M_∞ is fraction of drug released at time t, n is diffusion exponent and is indicator of the mechanism of transport of drug through the polymer, k_3 is Ritger-Peppas kinetic constant [11,12].

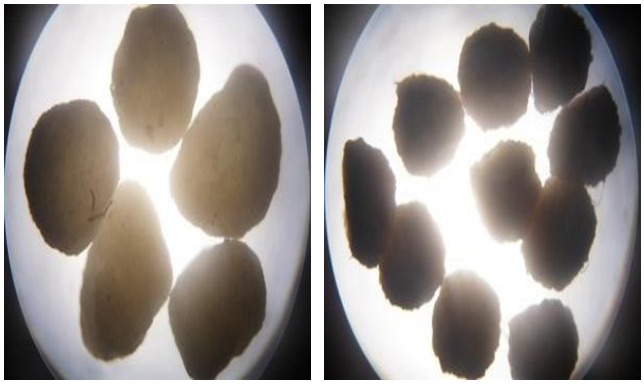
RESULTS AND DISCUSSION

Particle morphology and size

The results of the morphology of the diclofenac sodium bead are shown in Figure 1 and the results show that most of the beads were spherical. However, some were oblong in shape as shown in Figure 1. The particle size of the beads shown in Table two show that the particle size of the beads ranged from 1.98 ± 0.40 to 2.80 ± 0.30 mm. The results showed that the beads were of narrow size range as depicted in Table 2. Batch A5 which was uncoated had smaller particle size than the Eudragit L 100 coated beads (batches A1, A2, A3 and A4) as shown in Table 2.



(a) (b) (c)



(d) (e)

Figure 1: Photomicrographs of diclofenac sodium alginate beads. Batches A1, A2, A3 and A4 were coated with Eudragit L100, batch A5 was uncoated, and batch A4 contains an additional polymer-Neusilin.

Table 2: Some properties of diclofenac sodium alginate beads

Batch	TDL (mg ± SD)	ADL (mg ± SD)	Particle size (mm ± SD)	EE (%)
A1	360	178.13	2.80 ± 0.30	49.5
A2	307	150.12	2.33 ± 0.44	48.9
A3	255	122.15	2.38 ± 0.25	47.9
A4	360	262.22	2.55 ± 0.70	72.8
A5	360	211.89	1.98 ± 0.40	58.9

Batches A1, A2, A3 and A4 were coated with Eudragit L100, batch A5 was uncoated, and batch A4 contains an additional polymer-Neusilin. TDL: Theoretical drug loading, ADL: Actual drug loading, EE; Encapsulation efficiency, SD: Standard deviation**.**

Encapsulation efficiency (EE%)

The results of EE% of diclofenac sodium alginate beads are shown in Table 2 and the results show that the beads generally exhibited high encapsulation efficiency values. However, the optimized batch containing Neusilin® had the highest EE% of 72 % and this value was significantly higher than the EE of all the other batches without this stabilizer Neusilin® ($p <$

0.05). The ability of the bead to accommodate drugs could be expressed by the EE and it could be a function of formulation technique and excipients used in the formulation amongst others.

Swelling properties of beads

The results of the swelling properties of diclofenac sodium alginate beads studied in both the SGF, pH 1.2 and SIF, pH 7.4 are shown in Figure 2 and 3 respectively. The results show that the degree of swelling of diclofenac sodium beads in SGF was zero in all the batches from 40 to 100 min as shown in Figure 2. However, when the beads was transferred into SIF, pH 7.4, the degree of swelling increased with time as shown in Figure 3. For a substantial amount of drug to be released, the beads should absorb some solvent and swell significantly. The results therefore, show that the beads are expected to release the drug in SIF, pH 7.4 and not in SGF, pH 1.2.

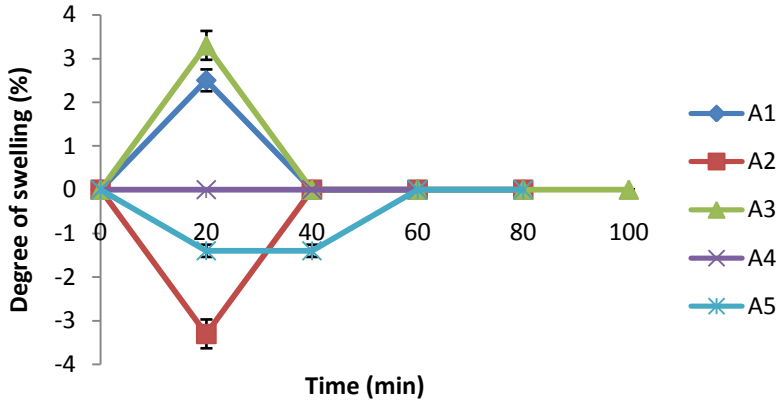


Figure 2: Swelling behaviour of beads in simulated gastric fluid (SGF, pH 1.2). Batches A1, A2, A3 and A4 were coated with Eudragit L100, batch A5 was uncoated, and batch A4 contains an additional polymer-Neusilin.

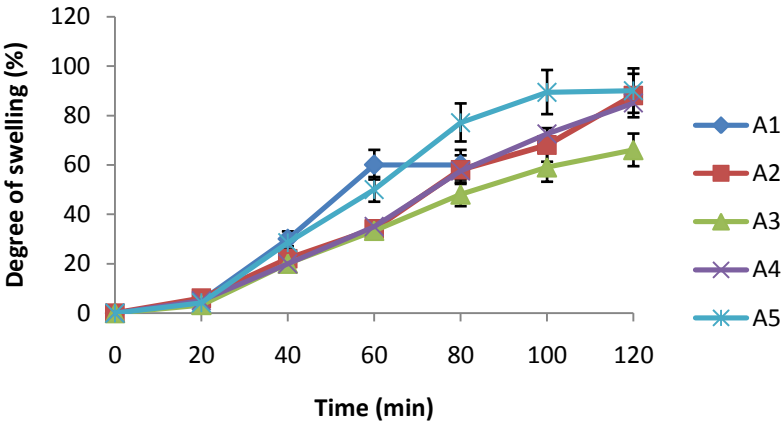


Figure 3: Swelling behaviour of beads in simulated intestinal fluids (SIF, pH 7.4). Batches A1, A2, A3 and A4 were coated with Eudragit L100, batch A5 was uncoated, and batch A4 contains an additional polymer-Neusilin®.

***In vitro* drug release**

The results of the *in vitro* drug release studied in SGF, (pH 1.2), SIF, pH 6.8 and 7.4 respectively are shown in Figure 4. The results showed that the drug was not released in SGF, pH, 1.2 during the first two hours of the study. However, when the diclofenac sodium beads were transferred into SIF, pH 6.8 about 0.2 to 3 % of diclofenac sodium was released from batch A5 i.e. the uncoated batch within the two hours of the study i.e. from 2 – 4 h of the study. In SIF, pH 7.4 there was significantly higher ($p < 0.05$) release of the diclofenac sodium from the Eudragit L100 coated alginate beads (A3 and A4) and the uncoated beads (A5). Also the uncoated beads (A5) exhibited higher release of the drug than the Eudragit L100 coated alginate beads (A3 and A4). The optimized alginate beads (A4) containing an extra polymer- Neusilin® had more sustained release of drug than the other batches as shown in Figure 4. These results show revealed that the formulated diclofenac sodium alginate beads could be used for colon delivery.

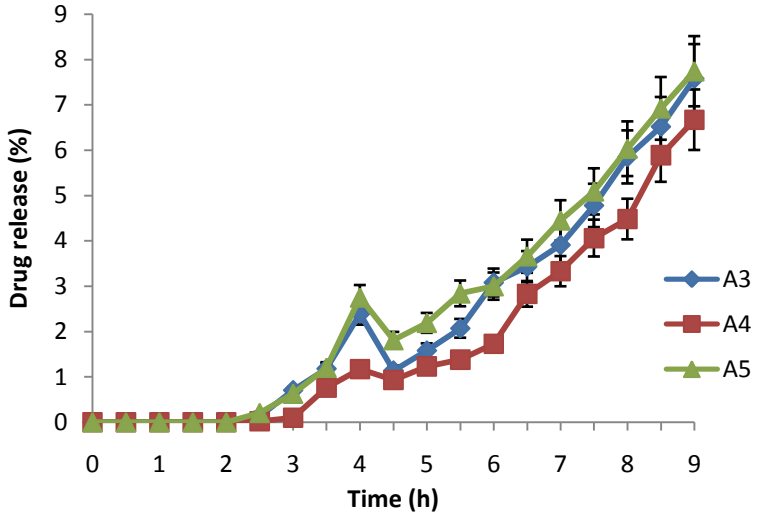


Figure 4: Percentage of drug released in SGF (0-2 h), SIF, pH 6.8 (2 – 4 h) and pH 7.4 (4 – 9 h) against time. Batches A3 and A4 were coated with Eudragit L100, batch A5 was uncoated, and batch A4 contains an additional polymer-Neusilin.

Kinetics and mechanisms of the *in vitro* drug release

The results of the *in vitro* release of diclofenac sodium from the alginate beads in SIF, pH 7.4 are shown in Figure 5 (a –d). The results show that the first order plots of amount of drug remaining versus time (Figure 5a) were linear for all the batches ($r^2 = 0.9$). Therefore, the drug release followed first order kinetic models. The Higuchi plot of amount of drug release against square root of time (Figure 5b) were also linear showing that diffusion is implicated in the release of drug from the beads. The plot of the integral form of Higuchi (Figure 5c) were also linear, but

had n values (slope) that were greater than 0.5 showing that diffusion was not the only predominant mechanism of drug release from the beads [13,14]. The Ritger-Peppas models (Figure 5d) were also linear for all the batches, however, the n values were 0.812, 0.867 and 0.610 for batches A3, A4 and A5 respectively. Therefore drug release was by non-Fickian diffusion release, 0.43 < n < 0.85 i.e. anomalous release from swellable spherical matrix [15]. Therefore, the mechanism of drug release was by erosion and diffusion [11].

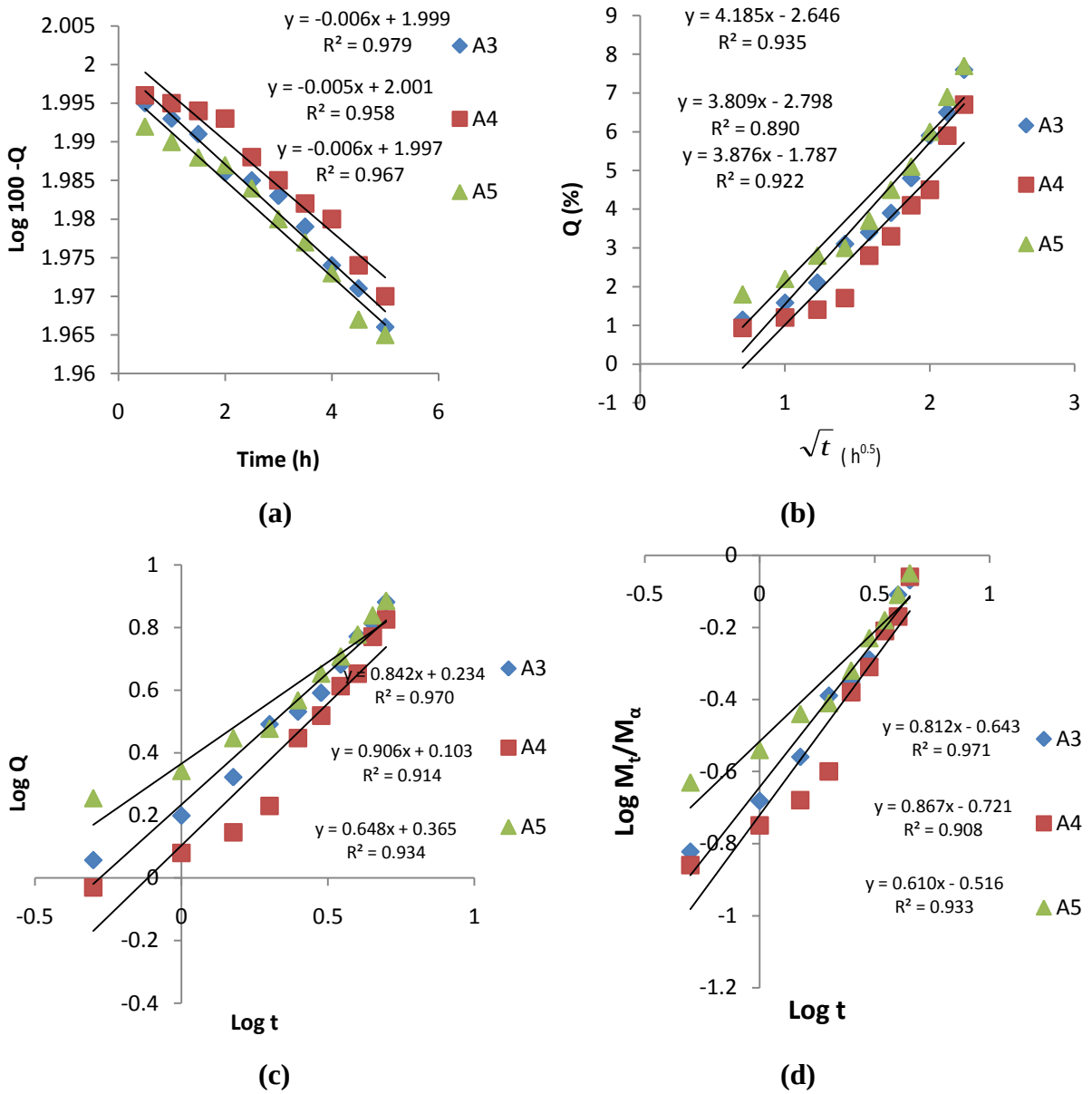


Figure 5: *In vitro* release kinetic of diclofenac sodium alginate beads in SIF (pH, 7.4): (a): First order plot of amount of drug remaining versus time, (b): Higuchi plot of percentage amount of drug released versus square root of time, (c): Higuchi plot of log of amount of drug released versus log time and (d): Ritger-Peppas plot of log fraction of drug released versus log time.

CONCLUSION

The diclofenac sodium alginate beads coated with Eudragit L100 exhibited good sustained release of the drug and could be used to deliver this drug to the colon in order to avoid gastric irritation often encountered with the use of this drug. The optimized formulation containing Neusillin® exhibited higher sustained release properties than the other formulations without it. Also the Eudragit coated alginate beads had more sustained release properties than the uncoated beads. The formulations had good encapsulation efficiency and high degree of swelling in simulated intestinal fluid SIF, pH 7.4.

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