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Release of Anticancer Drug 5-Fluorouracil from Different Ionically Crosslinked Alginate Beads

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ABSTRACT

In this research, the release of 5-Fluorouracil (5-FU) from different ionically crosslinked alginate (Alg) beads was investigated by using Fe^{3+}, Al^{3+}, Zn^{2+} and Ca^{2+} ions as crosslinking agent. The prepared beads were characterized by Fourier Transform Infrared Spectroscopy (FTIR) Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM). The drug release studies were carried out at three pH values 1.2, 6.8 and 7.4 respectively each for two hours. The effects of the preparation conditions as crosslinker type, drug/polymer (w/w) ratio, crosslinker concentration and time of exposure to crosslinker on the release of 5-FU were investigated for 6 hours at 37˚C. It was observed that 5-FU release from the beads followed the order of Fe > Zn > Al > Ca-Alg and increased with increasing drug/polymer ratio. At the end of 6 hours, the highest 5-FU release was found to be 90% (w/w) for Fe-Alg beads at the drug/polymer ratio of 1/8 (w/w), crosslinker concentration of 0.05 M, exposure time of 10 minutes respectively. The swelling measurements of the beads supported the release results. Release kinetics was described by Fickian and non-Fickian approaches.

Keywords: Anticancer Drug; pH Responsive Release; Alginate; Ionically Crosslinking; Controlled Release; 5-Fluorouracil

1. Introduction

5-Fluorouracil (5-FU) is one of the most widely used agents in cancer theraphy. Since its active form inhibits DNA synthesis by inhibiting the normal production of thymidine. It has a relatively high response in colon, rectal, breast, gastrointestinal tract pancreas, head, ovarion cancers [1-4]. The common method of administration of 5-FU is in the form of injections into vein [1,5,6] (intravenous) or as an infusion. However, such an administration causes severe gastrointestinal (vomiting, nausea, poor appetite) neural, hematological, cardiac, dermatological toxic effects. Since the drug is rapidly adsorbed through blood capillaries into systematic circulation, results in relatively low levels of the drug near the side action with subsequent loss of efficiency and increased risk of toxicity. By using oral rate controlled formulations, the incidence of side effects may be reduced since the drug has short biological half-life due to fast metabolism incomplete and non uniform oral absorption [6]. There are many studies in the literature for encapsulation of 5-FU in polymeric materials. In those studies [1,2,4, 6-13]. Generally natural polymers were preffered to synthetic polymers for the encapsulation because of their free aviability, nontoxicity and biodegrability characteristics. These polymers eventually undergo hydrolytic scission, producing by products that can be metabolized in the body. Polymers like gelation [14]. Chi-tosan [15], copoly(D,L-lactic/glycolic acid [16], poly(D-L-Lactide-coglycolide) [1] have been used in the controlled delivery of 5-FU.

There are also some studies concerning the encapsulation of 5-FU into alginate matrix [12,17,18] but they are in a limited number. Alginate is a lineer copolymer of D-Mannuric acid (M) and Gluronic acid (G) units which is found in brown seaweeds and is commercially available as sodium salt. It is a cellulose based biodegredable type polymer and is widely used in pharmaceutical applicatinos [18-29]. Gel formation of alginate matrix is generally achieved by using divalent Ca^{2+} ions [19,21,22, 24-29]. However calcium-alginate beads or microparticles when exposed to highly acidic (pH: 1.2) environment of stomach may result in insoluble alginic acid form causing reduction in their degree of crosslinking hence the beads degrades in a very short time after arriving to colon (pH: 6.8, 7.4). For this reason Arıca *et al*. [27] and Sure-

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kha *et al*. [28] studied the release of 5-FU only at pH conditions of 7.4, Chui-Yu *et al*. Reinforced alginate microparticles by chitosan during gelation [13]. Dodova *et al*. [12] prepared lectin-conjugated chitosan-Caalginate microparticles and loaded with 5-FU for the same purpose.

In the present study we have aimed to increase the strength of alginate beads so that after passing through stomach they can stand for a longer time period in intestinal medium conditions. For this reason we have tried different metal ions as Fe^{3+}, Al^{3+}, Zn^{2+}, Ca^{2+} for the crosslinking of alginate matrix, tried to find most suitable cation for the crosslinking and evaluated the physicochemical properties of the beads prepared. Attention has been paid for the effects of various factors on the release such as drug/polymer ratio, pH of the dissolution medium, crosslinking time and concentration. Although Fe^{3+} ions were previously used for the preparation of crosslinked alginate-carboxymethyl cellulose beads for protein theraupeutics [30] and carboxymethyl chitin nanoparticles for 5-FU delivery [31]. There is no study concerning the use of these ions in the delivery of 5-FU from the alginate matrix.

2. Experimental

2.1. Materials

NaAlg (medium viscosity) was purchased from Sigma Chemical Co (Louis, USA). 5-FU was provided by Sigma-Aldrich (Steinem, Germany). Na_2HPO_4 and NaH_2PO_4 were all supplied from Merck (Darmstadt, Germany) and were used as received. Iron (III) chloride, Aluminum chloride, zinc chloride and calcium chloride were provided by Merck (Darmstadt, Germany).

2.2. Preparation of the 5-FU Loaded Beads

NaAlg was dissolved in distilled water to prepare 2% (w/v) NaAlg solution. Different amounts of 5-FU were added and mixed using a magnetic stirrer. The polymer solution containing 5-FU was added drop wise into crosslinking solution using a peristaltic pump (Masterflex, L/S Digital Economy Drive, USA). The formed beads were then removed from the crosslinker solution. To remove the adhered crosslinker, beads were washed with water repeatedly then dried completely in an oven at 40˚C. Preparation conditions were displayed in **Table 1**. In order to estimate the size of beads completely dry beads from the different formulations were selected and their sizes were measured by using a micrometer screw gauge (Aldrich, Germany) and given in **Table 1**.

2.3. Equilibrium Swelling Study of the Beads

Equilibrium swelling degree of the crosslinked empty beads was determined by measuring gravimetrically the extent of their swelling in solutions at pH 1.2, 6.8 and 7.4 at 37˚C. To ensure complete equilibration, the samples were allowed to swell for 24 h. The excess surface-adhered liquid drops were removed by blotting. The swollen beads were weighed using electronic balance (Precise XB 220 A, USA). The beads were then dried in an oven at 40˚C, until there was no change in the dried mass of the samples. The percent equilibrium swelling degree was calculated as follows:

$$\text{Equilibrium swelling degree (\%)} = \frac{M_s - M_d}{M_d} \times 100 \quad (1)$$

where M_s and M_d were the mass of the swollen beads and dry beads, respectively.

Table 1. Preparation conditions of the 5-Fluorouracil loaded NaAlg beads.

Code	Drug/Polymer ratio (w/w)	Crosslinking agent	Concentration of crosslinking agent (M)	Exposure time to crosslinking agent (min)	Entrapment efficiency (%)	Bead yield (%)	Bead diameter (mm)
A1	1/8	$FeCl_3$	0.1	10	12	96	1.25
B1	1/8	$AlCl_3$	0.1	10	5	93	1.17
C1	1/8	$CaCl_2$	0.1	10	4	84	1.11
D1	1/8	$ZnCl_2$	0.1	10	7	89	1.13
A2	1/8	$FeCl_3$	0.2	10	15	75	0.62
A3	1/8	$FeCl_3$	0.05	10	10	82	1.00
A4	1/8	$FeCl_3$	0.05	5	17	70	0.55
A5	1/8	$FeCl_3$	0.05	15	7	78	1.10
A6	1/4	$FeCl_3$	0.05	10	13	77	1.06
A7	1/2	$FeCl_3$	0.05	10	14	62	1.10
A8	1/1	$FeCl_3$	0.05	10	25	50	1.20

2.4. Determination of 5-FU Content of the Beads

The known mass of beads was crushed in an agate mortar with a pestle, and then polymeric powder is taken in a flask. Water (50 mL) was added and refluxed at 25˚C for 1 h, to ensure the complete extraction of 5-FU from the beads. At the end of the 1 h, precipitated NaAlg was filtered and 5-FU was analyzed by using a UV spectrophotometer (Unico 4802 UV/VIS) at a wavelength of 266 nm using a calibration curve and water as the blank. Percentage of entrapment efficiency was then calculated as follows:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Practical 5-FU loading}}{\text{Theoretical 5-FU loading}} \times 100 \quad (2)$$

2.5. Fourier Transforms Infrared Measurements (FTIR)

FTIR spectra of the 5-FU, NaAlg and 5-FU/NaAlg beads crosslinked with Fe^{3+} were taken with a Mattson 1000 FTIR spectrometer and presented in **Figure 1**.

2.6. Differential Scanning Calorimetry (DSC)

The thermal analysis was carried out with differential scanning calorimeter (DSC, Shimadzu, Japon). Measure ments were performed over the temperature range of 0˚C - 300˚C at the heating rate of 10˚C/min. and displayed **Figure 2**.

2.7. Scanning Electron Microscopy (SEM)

SEM micrographs were taken with QUANTA 400F Field

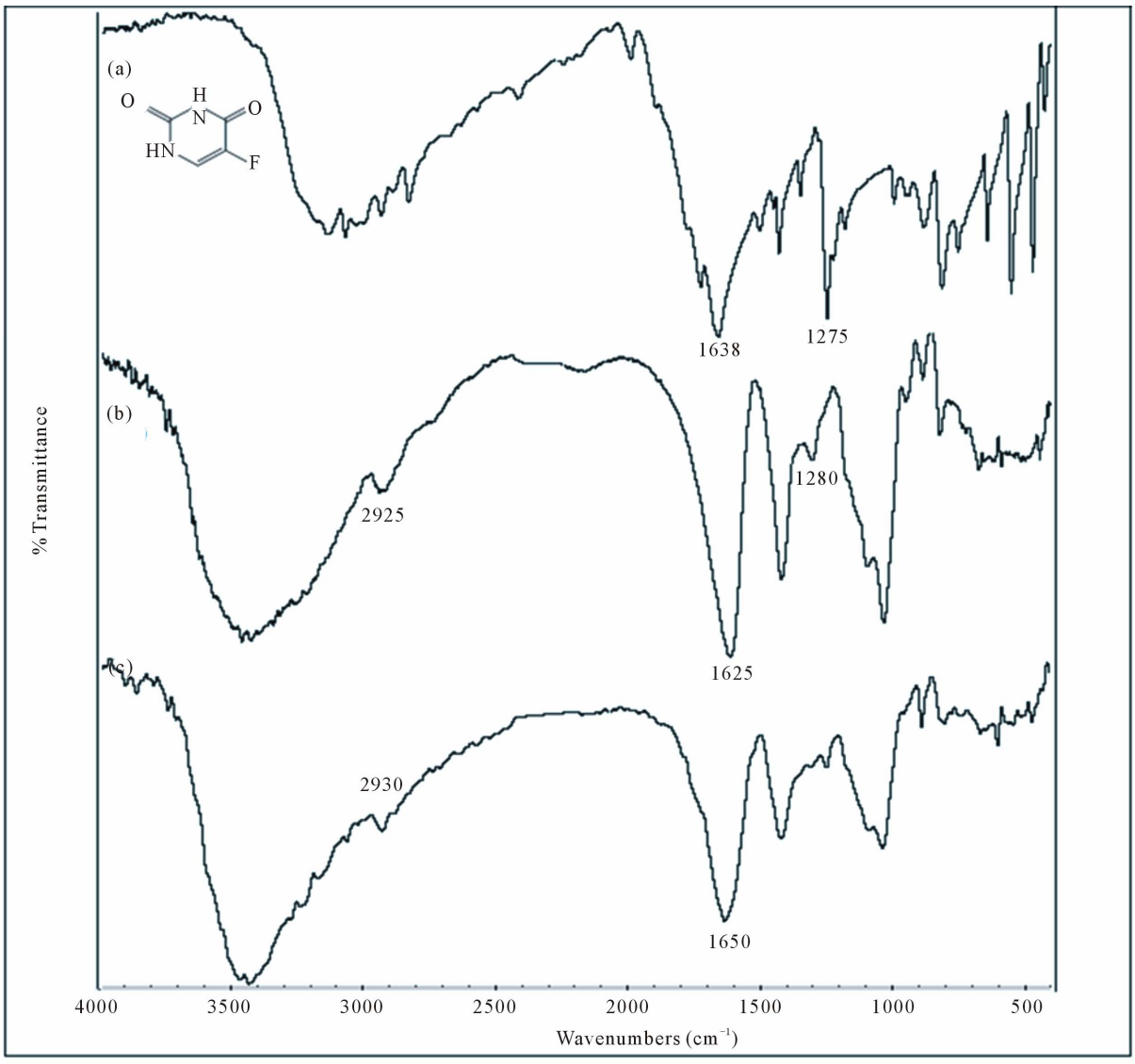


Figure 1. FTIR spectra of (a) 5-FU; (b) 5-FU loaded NaAlg (1/8 w/w) beads crosslinked with Fe^{3+}; (c)NaAlg.

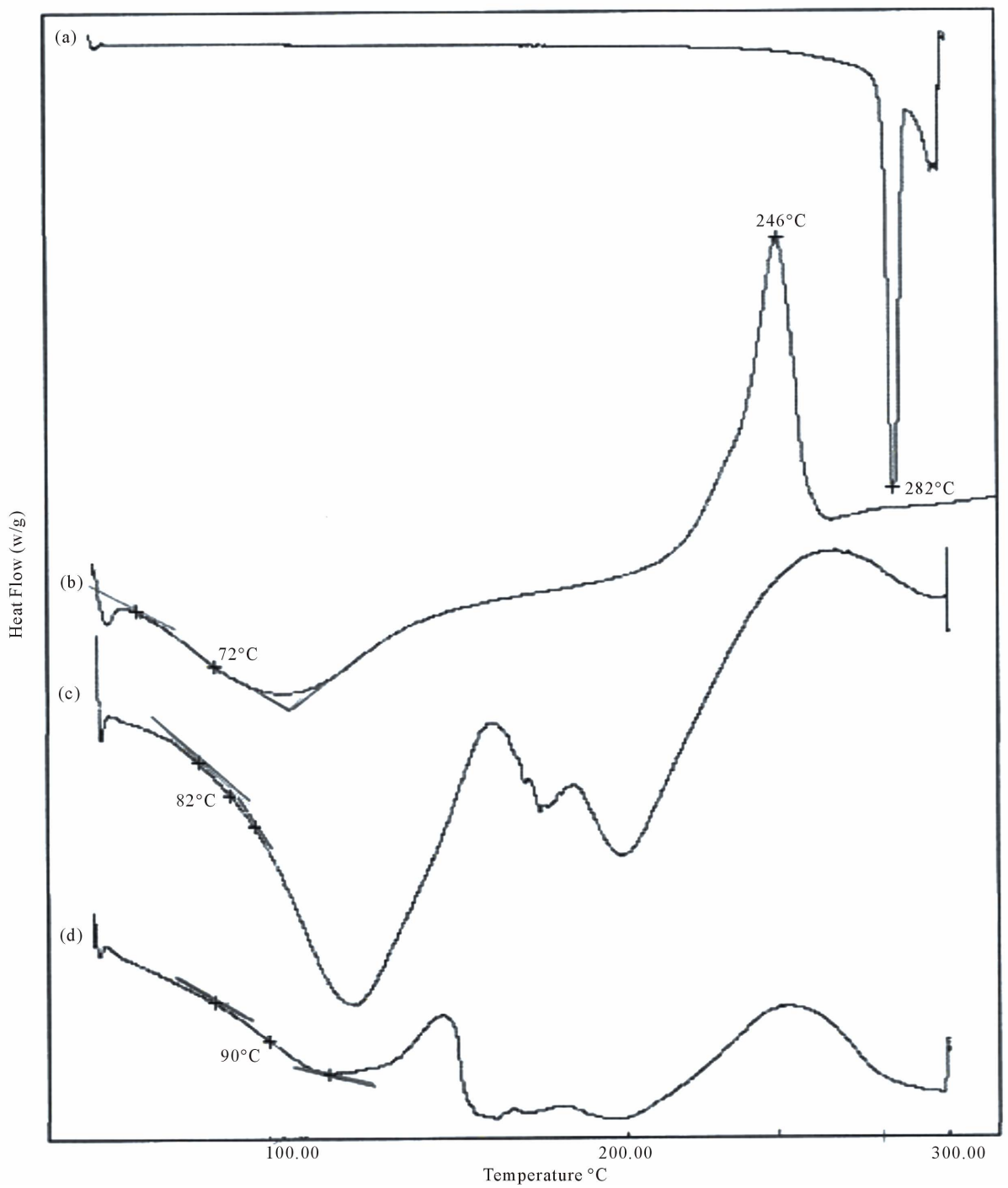


Figure 2. DSC termograms of (a) Pure 5-FU; (b) NaAlg; (c) NaAlg beads crosslinked with $FeCl_3$; (d) 5-FU loaded beads crosslinked with $FeCl_3$.

Emission SEM to examine the morphology and surface structure of the beads at the required magnification at room temperature and shown in **Figure 3**.

2.8. *In Vitro* Drug Release

In vitro drug release from the beads was studied in 250 mL, pH 1.2 HCl solution, pH 6.8 and pH 7.4 phosphate buffer solutions and incubated in a shaking water bath (Medline BS-21, Korea) at 37˚C. At 2 h intervals medium was changed to be pH: 1.2, 6.8 and 7.4, respectively, to follow the gastrointestinal tract. At specific time intervals, the 5-FU content was determined using UV spectrophotometer at 266 nm. Equal volume of fresh

HCl or phosphate buffer solution was added into the dissolution media to maintain a constant volume. From the absorbance values the cumulative released amount percentage was determined. All experiments were performed in triplicate to minimize the variational error. Standard deviations from the average values were calculated.

3. Results and Discussion

3.1. Effect of Type of Crosslinker and Crosslinker Concentration on the 5-FU Release

The release of 5-FU from the Fe-Alg, Al-Alg, Ca-Alg and Zn-Alg beads were carried out for three pH values at 37˚C and the amount of drug release within a given time was evaluated by UV spectroscopy. Effect of type and valency of the ions in the crosslinking agents on the cumulative release of 5-FU were shown in **Figure 4**. It was reflected from the figure that cumulative drug release from beads followed the order of Fe > Zn > Al > Ca-Alg and the beads of Zn-Alg, Ca-Alg, Al-Alg eroded at the pH values higher than 6.8 (**Table 2**).

The release results can be related to the mechanism of the bonding of iron, aluminum, zinc and calcium ions with NaAlg. Since calcium and zinc cations are divalent, their bonding to alginate was expected to occur in a planar two-dimensional manner as represented in the egg-box model [32] shown in **Scheme 1**. Trivalent aluminium and iron cations were expected to form a three dimensional valent bonding structure with the alginate. Possible scheme for the crosslinking of NaAlg with Fe^{3+} and Al^{3+} was given in **Scheme 2**. Reaction mechanism of sodium alginate with Zn^{2+} and Ca^{2+} ions is similar to Fe^{3+} and Al^{3+}. Dissimilarly two alginate chains were used in the crosslinking with Zn^{2+} and Ca^{2+}.

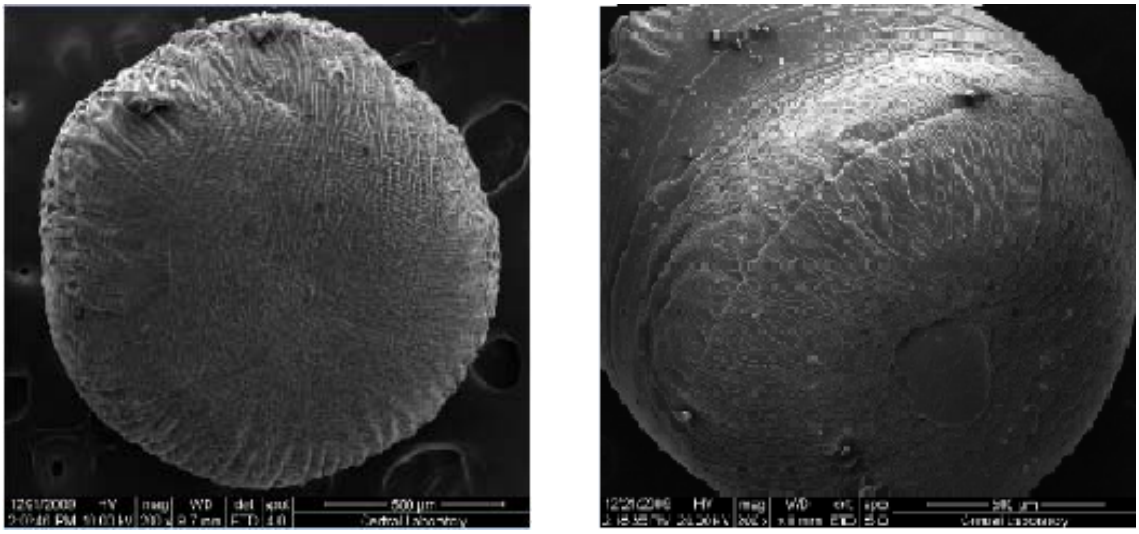


Figure 3. Microscopic pictures of (a) Empty Fe-Alg and (b) 5-FU loaded Fe-Alg beads.

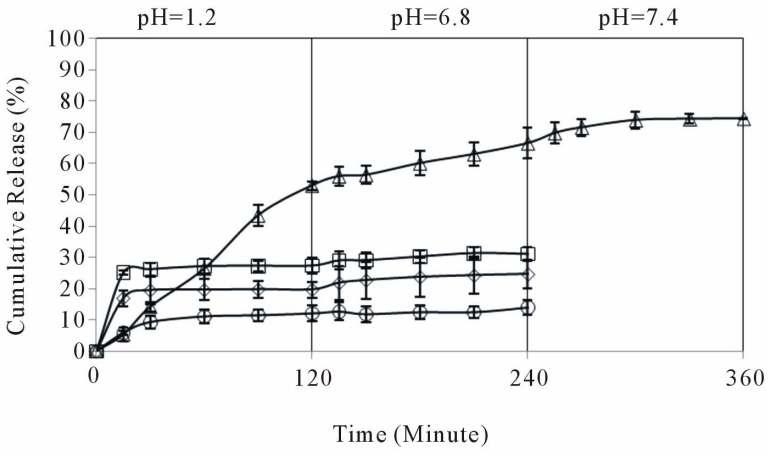


Figure 4. Effect of type and valency of the ions in the crosslinking agents on the cumulative release of 5-FU (Δ: $FeCl_3$, □: $ZnCl_2$, ◊: $AlCl_3$, ○: $CaCl_2$).

Table 2. Equilibrium swelling degree for empty beads.

Formulation Code	pH = 1.2	pH = 6.8	pH = 7.4
A	51.19 ± 1.07	108.17 ± 1.58	188.65 ± 2.46
B	217.65 ± 2.56	Beads eroded	Beads eroded
C	135.58 ± 1.44	Beads eroded	Beads eroded
D	229.23 ± 1.73	Beads eroded	Beads eroded

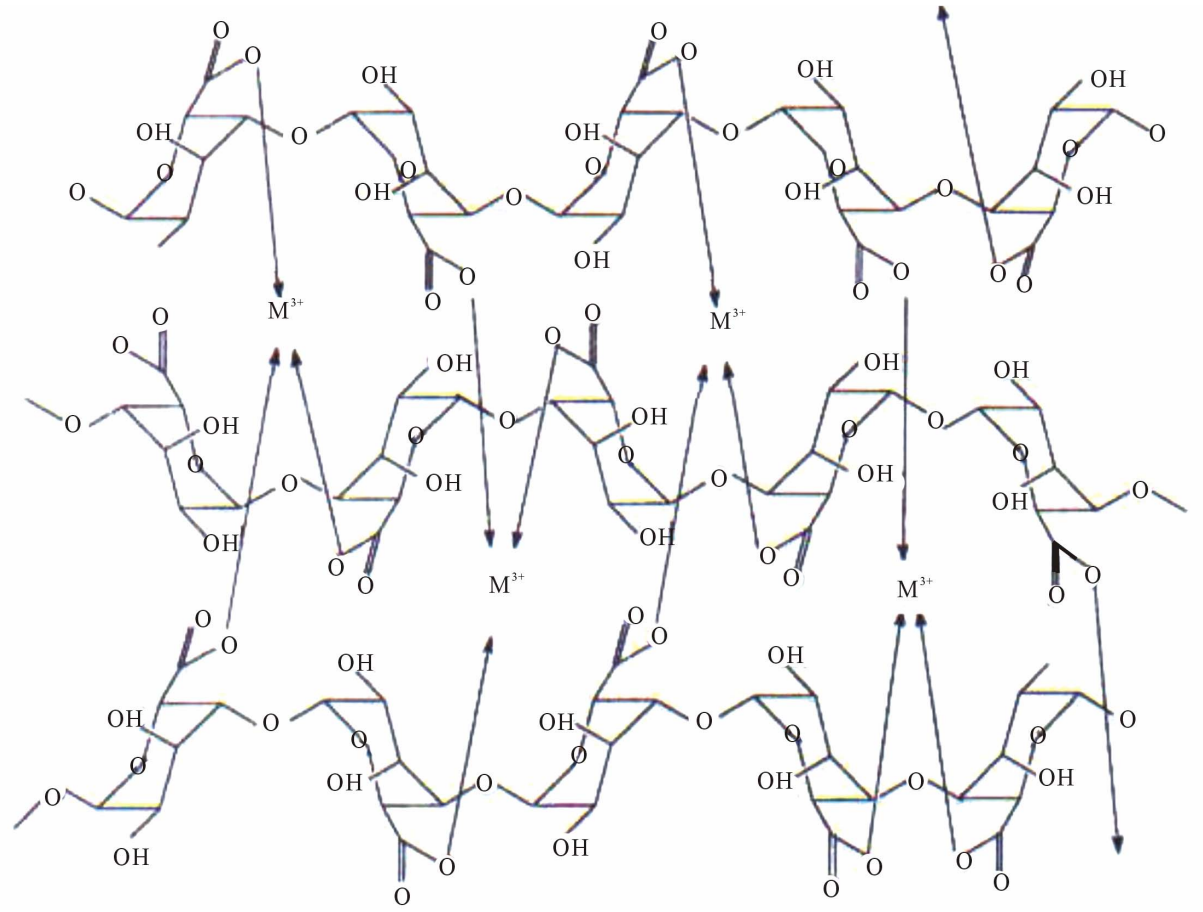
Scheme 1. Egg-box model representing M^{2+} cations reacting with alginates.

Three dimensional bonding model was expected to be the reason for the extended crosslinking through the whole body of matrix. On the other hand diffusion ability of the NaAlg crosslinked matrix can be explained by the ionic size of the crosslinker cations. The size of aluminum (0.50 Å) was smaller than the size of the iron (0.64 Å) cations. Iron ions were expected to fill larger space between the alginate chains producing a loose arrangement in the matrix leading to high release. Musa and coworkers [29] have studied the parameters involved in the preparation and release of metoclopramide hydrochloride and cisapride in calcium, barium, aluminum crosslinked matrices of alginate and reported that the crosslinker type was shown to have a pronounce influence on the drug release. In addition aluminum ion constitute complex with 5-FU causes low drug release. In the rest of the study due to the high release of 5-FU from Fe-Alg beads we have continued with Fe^{3+} as the crosslinker ion [32].

Figure 5 shows the effect of crosslinker concentration on the 5-FU release. It was seen from the figure that the cumulative release of 5-FU beads increased as the crosslinker concentration decreased from 0.2 to 0.05 M. Similar results were also observed in the literature [20, 30]. In the rest of the study crosslinker concentration was selected as 0.05 M due to the high release at this concentration.

3.2. Effect of Exposure Time to Crosslinker on the 5-FU Release

One of the ways of changing drug release from the beads



Scheme 2. Scheme for the crosslinking of NaAlg with [M^{3+}: Fe^{3+}, Al^{3+} cations].

is to change the crosslinking density of the matrix by employing various time of exposure to crosslinking agent. The effect of the exposure time to $FeCl_3$ on the release rate of 5-FU has been investigated by varying the time of exposure to $FeCl_3$ as 5 - 15 min. The results were given in **Figure 6**, which clearly indicated that increasing exposure time to $FeCl_3$ decreased the cumulative release of 5-FU. Similar results were given in the literature [4, 11,12,14]. Although the maximum 5-FU release from the Fe-Alg beads was obtained with the exposure time of 5 min., since these beads did not stand to pH value of 7.4. We have continued in the rest of the study with exposure time of 10 min.

3.3. Effect of Drug/Polymer Ratio on the 5-FU Release

Effect of 5-FU/NaAlg ratio on 5-FU release from Fe-Alg beads was shown in **Figure 7**. The figure showed that a decrease in the 5-FU/polymer ratio from 1/1 to 1/8 causes an increase in the release of 5-FU from the beads. The highest cumulative 5-FU release obtained at the end of 6 hr is 90 % for the 1/8 drug/polymer ratio. As 5-FU content of the bead decreases, a loose structure in the polymeric bead forms and this loose structure causes the liquid to easily penetrate into the bead and eases the diffusion of the 5-FU. As the drug/polymer ratio decreased from 1/1 to 1/8 particle size of the beads also decreased (**Table 1**). Release from smaller size bead is faster than those from the large size bead due to smaller diffusional path length for the drug and the larger surface area of contact of small particle with the dissolution media [33, 34].

3.4. Characterization of the Beads

FTIR spectra of 5-FU, 5-FU/NaAlg (1/8 w/w) beads crosslinked with Fe^{3+} and NaAlg were shown in **Figure 1**. A broad band between the 3000 and 3500 cm^{-1}, is attributed to—NH stretching vibrations in the spectrum of 5-FU. This band was seen approximately at 3500 cm^{-1} in the spectrum of drug loaded NaAlg, because the over-

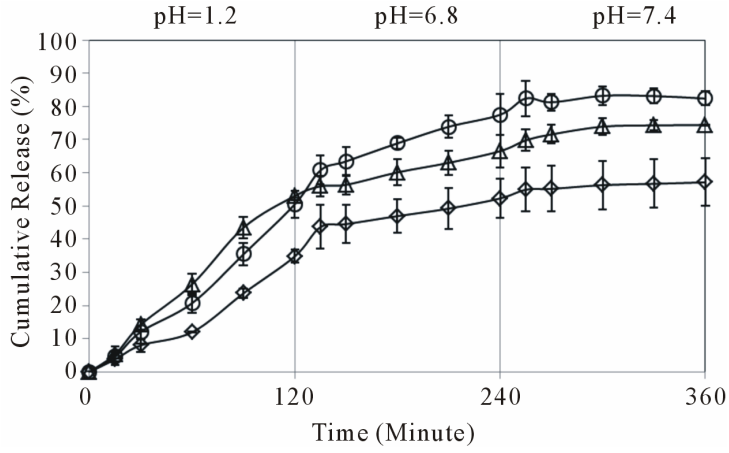


Figure 5. Effect of crosslinker concentration on the 5-FU release. Concentration of $FeCl_3$: 0.05 M, exposure time to $FeCl_3$ 10 min. drug/polymer; 1/8 (◊: 0.2 M, Δ: 0.1 M, ○: 0.05 M).

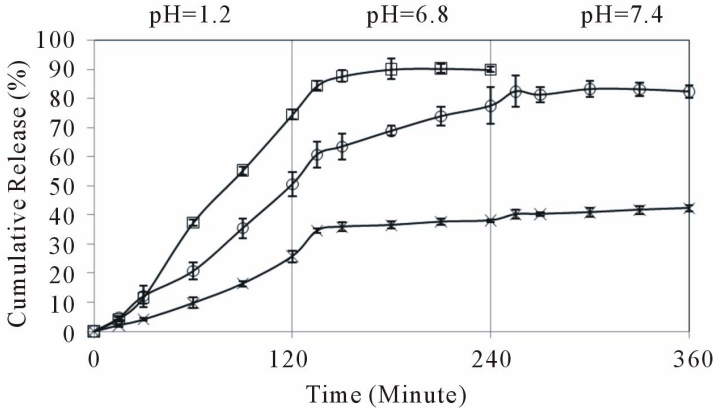


Figure 6. Effect of exposure time to crosslinker on the 5-FU release. Concentration of $FeCl_3$: 0.05 M, drug/polymer: 1/8. (□: 5 min., ○: 10 min., x: 15 min).

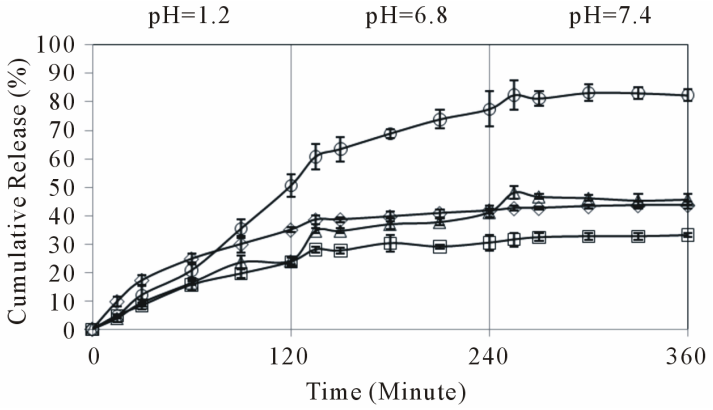


Figure 7. Effect of drug/polymer ratio on 5-FU release. Concentration of $FeCl_3$: 0.05 M, exposure time to $FeCl_3$ 10 min. (drug/polymer ratio; 1/8 (○), 1/4 (Δ), 1/2 (◊), 1/1 (□)).

overlapping of -OH band of NaAlg with -NH band of 5-fluorouracil. In the spectrum of NaAlg, drug loaded NaAlg and 5-FU appeared band of carbonyl stretching (C=O) at 1650 cm^{-1}, 1625 cm^{-1} and 1638 cm^{-1}, respecttively. C-H group stretching band of NaAlg and drug loaded NaAlg appeared were seen at 2930 cm^{-1} 2925 cm^{-1}, respectively. The peak at 1275 cm^{-1} was belong to C-F stretching band in the spectrum of 5-FU. This peak was seen at 1280 cm^{-1} in the spectrum of drug loaded NaAlg which can be taken as the evidence of encapsulation.

DSC trackings of pure 5-FU, NaAlg, NaAlg beads crosslinked with $FeCl_3$, 5-FU loaded beads crosslinked with $FeCl_3$ were displayed in **Figure 2** melting peak of 5-FU was observed at 282˚C. However no characteristic peak of 5-FU was observed in DSC curves of the 5-FU loaded beads suggesting that drug is molecularly dispersed in the polymer matrix.

Shape of dried empty NaAlg and 5-FU loaded NaAlg beads were shown in **Figure 3**. As it was reflected from the figure that, both empty and 5-FU loaded beads almost maintain spherical form at empty and loaded conditions.

The results of bead diameter, entrapment efficiency (%) and bead yield (%) were shown in **Table 1**. As can be seen from the table the beads formed have particle sizes ranging from 0.55 to 1.25 mm in diameter. The size of the beads changed with drug/polymer (w/w) ratio, crosslinker type and crosslinker concentration. Entrapment efficiency percentage increased with the increase in crosslinker concentration whereas decreased with the exposure time to crosslinker. Similar results were observed in the literature. Şanlı and coworkers [21] prepared poly(vinyl alcohol)/sodium alginate and poly(vinyl alcohol)-grafted-poly (acrylamide)/sodium alginate blend beads for the delivery of diclofenac sodium and reported that with increasing exposure time to crosslinking agent (2.5 - 5 min.) the entrapment efficiency decreased.

3.5. Analysis of Kinetic Results

Solvent sorption by a bead depends mechanistically on the diffusion of water molecules into the gel matrix and subsequent relaxation of macromolecular chains of the bead [35]. The release data of all the systems were further substantiated by fitting the fraction release data M_t/M_∞ to an empirical equation proposed by Peppas [36].

$$kt^n = \frac{M_t}{M_\infty} \quad (6)$$

where M_t is the amount of 5-FU released at time t and M_∞ is the drug released at equilibrium time; k, a constant characteristic of the drug-polymer system; and n, the diffusional exponent which suggests the nature of the release mechanism. Fickian release is defined by initial $t^{1/2}$ time dependence of the fractional release for slabs, cylinders and spheres. Analogously Case-II transport is defined by an initial linear time dependence of the fractional release for all geometries [37]. A value of n; 0.5 indicates the Fickian transport (mechanism), while n; 1 is of Case II or non-Fickian transport (swelling controlled) [38]. The intermediary values ranging between 0.5 and 1.0 are indicative of the anomalous transport. The least squares estimations of the fractional release data along with the estimated correlation coefficient values, r, are presented in **Table 3**. From these data, the n value ranged between 0.4486 - 1.1506, indicating 5-FU release from the Fe-Alg beads deviates from the Fickian transport.

The values of diffusion coefficients, D, for the transport of aqueous drug solution from the beads were calculated using the sorption and desorption results as in Equation (7).

$$D = \left(\frac{r\theta}{6M_\infty} \right)^2 \pi \quad (7)$$

where θ is the slope of the linear portion of the plot of

Table 3. The results of k, n and r calculated from Equation (6).

Formulation Code	k (min^{-n})	n	r	D (cm^2/s) × 10^{-13}	Diffusion Mechanism
A1	0.0040	0.7832	0.965	50.7	Anomalous Transport
A2	0.0039	0.8926	0.976	8.72	Anomalous Transport
A3	0.0067	0.8659	0.981	55.1	Anomalous Transport
A4	0.0025	1.1506	0.972	31.9	Case II
A5	0.0017	0.9911	0.969	15.2	Anomalous Transport
A6	0.0065	0.7624	0.978	13.3	Anomalous Transport
A7	0.0119	0.5979	0.970	0.0078	Anomalous Transport
A8	0.0363	0.4486	0.966	8.27	Anomalous Transport

M_t/M_∞ vs $t^{1/2}$, and r is the radius of the beads; M_∞ is equilibrium sorption. To calculate D from desorption experiments, θ was computed from the initial linear portion of the desorption plot, *i.e.* $\ln(1 - M_t/M_\infty)$ vs. time, t. The calculated values of D from Equation (7) for sorption and desorption runs are also presented in **Table 3**. The D values for desorption were smaller than those observed for sorption, and these ranged from 0.0078 × 10^{-13} to 55.1 × 10^{-13} cm^2/s [38].

4. Conclusion

Studies on the release of 5-FU from sodium alginate beads crosslinked with Fe(III), Al(III), Zn(II) and Ca(II) ions indicated that the crosslinking with Fe(III) lead to highest release of 5-FU from the beads. Release of 5-FU from NaAlg beads crosslinked with $FeCl_3$ increased with the decrease in the drug content. It was also observed that release of 5-FU was much higher at high pH values compared to low pH values. Optimum conditions for 5-FU release were determined as crosslinker concentration of 0.05M, exposure time to crosslinker of 10 min and drug/polymer ratio of 1/8. The highest 5-FU release at these conditions was found to be 90% (w/w).

5. Acknowledgements

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