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SODIUM ALGINATE-ZEOLITE COMPOSITE GEL BEADS FOR CONTROLLED RELEASE OF 5-FLUOROURACIL

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ABSTRACT

In this paper, controlled release of 5- Fluorouracil (5-FU) through sodium alginate (NaAlg) and zeolite embedded NaAlg composite gel beads has been investigated. Beads were prepared by precipitating the viscous solution of zeolite dispersed NaAlg in alcohol followed by ionic cross linking with calcium chloride. The formed gel beads were characterized by Fourier transform infrared (FT-IR), X-ray diffraction pattern (X-RD), thermal analysis by differential scanning calorimetry (DSC) and surface morphology was studied by scanning electron microscopy (SEM). In vitro drug release profiles of the composite gel beads at pH 7.4 in aqueous phosphate buffer medium confirmed controlled release nature of the composite beads. The drug loading, encapsulation efficiency and drug release patterns are found to be dependent on the concentrations of calcium chloride and zeolite. The embedded zeolites maintained their structural stability in the composite gel beads. The obtained results suggested the versatility of these hybrid materials as promising drug carriers.

INTRODUCTION:

The development and application of polymeric biomaterials as drug carriers have been played a significant role in improving the treatment of various diseases and the quality of health care. Use of natural polymers in the growth of pharmaceutical systems have long been the theme, which received immense attention during last few years because of their abundance, water solubility, swelling and low price (Seeli et al., 2016). Among all types of natural polysaccharides, alginates are special materials which are widely used to make variety of foods, heel tap care and as drug carrier devices (Seo et al., 2013). Alginate exist in a salt form of alginic acid, naturally occurring polysaccharide derived from brown marine algae, is made of mucoadhesive block polymers of mannuronic acid (M), guluronic acid (G) and mannuronic-guluronic (MG) blocks. It shows excellent biodegradability and/or biocompatibility and also nontoxic in nature besides a good bacteriostatic (Seo et al., 2013). Alginates showed pH responsive nature with high swelling in aqueous fluids and are relatively cheap (Li et al., 2011). It experiences ionotropic gelation in water in the presence of di- and tri-valent cations like calcium, barium, zinc, aluminum, etc due to intermolecular ionic linking between carboxylic moieties of alginate

chains and cations of metals and fit into electronegative cavities of the sodium alginate like eggs in "Egg-Box" to form cross linked alginate gels (Nayak et al., 2011). Sodium alginate (NaAlg) is widely used in lipid nanoparticles (Li et al., 2011), tissue engineering (Wang et al., 2003), protein adsorption (Wang et al., 2014), controlled release of liquid pesticide (Kulkarni et al., 2000) and adenovirus delivery (Park et al., 2012).

Now-a-days, numerous alginate gel beads have been used and it has been the best vehicle for delivery of drug in sustained release manner (Al-Kassas et al., 2007). However, in physiological environment, these beads have major drawbacks such as poor mechanical stability, low drug encapsulation due to drug leaching through the pores and rapid degradation and burst release of in intestinal fluids (Nayak et al., 2011). Consequentially, many researchers have evinced keen interest in alginates for improving the long time release of drug in controlled manner (Nayak et al., 2013; Nayak et al., 2013; Sinha et al., 2015; Sinha et al., 2015).

Recently, polymer-inorganic composites have gained significance as they are ideal drug delivery vehicles due to their improved thermal and mechanical stability (Ninan et al., 2013). Zeolites are porous solid inorganic crystalline materials composing of silicon, aluminum and oxygen elements in a three dimensional (3-D) manner. The porous structure allows the accommodation of small molecules such as drug and/or biological molecules inside the pores, and releases via an ion exchange process (Malekian et al., 2011). There are many reports the use of zeolites for biomedical applications including the improvement and identification of low occurrence of peptides/proteins (Zhang et al., 2004), in the field of magnetic resonance imaging (MRI) and drug delivery (Prado et al., 1999; Horcajada et al., 2006), etc. The main benefit of using zeolite materials in drug carriers is that the drug molecules can diffuse out of the channels by slow degrees so that the rate of release of drug will be under control (Spanakis et al., 2014). Recently, Tugba et al., developed zeolite 4A nanocomposite drug delivery carrier for cancer applications. They studied the biological assays measuring the association of the 5-FU to the zeolite magnetic complex and investigated their functionality in a cell based assay system and inhibit the proliferation of human gastric carcinoma (AGS) cells and induce greater apoptosis to AGS cell lines (Sagir et al., 2002).

5-Fluorouracil (5-FU) belongs to the family of pyrimidine analogue, anticancer agent, broadly used in the healing of a broad range of solid tumors (Boyer et al., 2004) and the main objective of three definite enzymes concerned in the metabolism of pyrimidines, such as thymidylate synthase (TS), thymidine phosphorylase (TP) and di-hydropyrimidine dehydrogenase (DHDH). 5-FU and its derivative compounds showed their anti-cancer activity by changing the thymidine bio-synthesis by stopping the enzymatic conversion of thymine (ribosylation and phosphorylation) to the nucleotide, thus stopping the regular job of Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) and, as a result, cell proliferation (Longley et al., 2003). However, 5-FU shows some abnormal activities due to its low tumor affinity, narrow therapeutic window and its effectiveness is acutely reduced due to drug resistance (Alvarez et al., 2012). Moreover, it has very short-life (10-20 min) period in the tissue (Winer et al., 1998) and its bioavailability is also low (less than 20% of an injected dose undergoes enzymatic activation (Tanaka et al., 2000). Its unpredictable behavior (Bono et al., 2001) is due to its enzymatic degradation, which strictly related to the individual patient characteristics (Grem et al., 1990).

The main objective the of the current experimental work in developing composite NaAlg-zeolite gel beads is to improve the drug loading, increasing the bioavailability of the drug and also extending the drug residence time. i.e. release period.

MATERIALS AND METHODS

Materials

Sodium alginate (NaAlg) was purchased from Sigma Aldrich, USA. Zeolite 3A was obtained from M/S Zeolite and Allied products, Mumbai. Anhydrous calcium chloride, methanol, sodium hydroxide pellets and 5-Fluorouracil (5-FU) were purchased from Himedia, Mumbai, India. Hydrochloric acid and potassium dihydrogen orthophosphate were procured from Merck, Mumbai, India. Double distilled water produced itself in the laboratory and used throughout the study.

Preparation of sodium alginate – zeolite composite gel beads

Sodium alginate-zeolite composite gel beads were prepared by inotropic gelation method. A 4 wt. % solution of NaAlg in distilled water was prepared under constant magnetic stirring. Different concentrations (2.5, 5 and 7.5 wt. %) of zeolite dispersion in water were prepared and sonicated for 30 min. Then, zeolite dispersion was added to polymer solution by syringe addition. The mixture was transferred to a syringe and extruded drop wise in to a beaker containing required amount (1 to 3 wt. %) of cross linking agent, $CaCl_2$ in methanol under constant stirring condition. NaAlg-zeoltie composite gel beads (CGB) were formed immediately, however the stirring was continued for 30 min. Finally, the beads were filtered, washed two times with distilled water to remove unreacted $CaCl_2$. Total 6 formulations were prepared by varying the concentrations of cross linking agent and zeolite and the details are given in Table 1. In the first set of experiments, three formulations were developed by varying the amount of cross linking agent 1, 2 and 3 wt. % with respect to polymer at fixed wt.% of zeolite and these are designated, respectively as CGB-1, CGB-2 and CGB-3. In next set of studies, formulations were prepared by varying zeolite concentration (0, 5 and 7.5 wt. % with respect to NaAlg wt.%) by keeping cross linking agent concentration constant and are represented as GB-4, CGB-5 and CGB-6 respectively. The protocol used in making the beads was outlined schematically in scheme 1.

Characterization of composite gel beads

FT-IR spectra of plain NaAlg gel beads, zeolite, pure 5-FU and 5-FU drug-loaded composite gel beads were taken on FT-IR spectrophotometer (Perkin Elmer, model two, UK) by grinding the samples separately with KBr and making the pellet under a hydraulic pressure of 600 kg/cm^2. X-ray diffraction (X-RD) patterns of plain NaAlg gel beads, 5-FU loaded composite gel beads, pure 5-FU and zeolite were recorded on X-ray diffractometer (RIGAKU SMART LAB, Mini flex 600, Japan) operated at 30 kV 100 mA with Cu Kα as a radiation source. Thermal analysis was performed for plain NaAlg gel beads, zeolite 3A, pure 5-FU and 5-FU drug-loaded composite gel beads on SHIMADZU DSC-60 (Tokyo, Japan) machine by heating the samples at the rate of 20 ^{o} C/min up to 100 ^{o}C and cooled back to 30 ^{o}C and then the thermo grams were recorded from 30^{o}C- 500 ^{o}C at a heating rate of 10 ^{o}C/min. Scanning electron microscope (SEM) photographs were taken on placebo composite gel beads prepared by cross linking with 2 wt.% of $CaCl_2$. Beads were sputtered with gold to make them conductive and placed on copper stub. Image was taken on HITACHI S-3700 (Germany) operated at an acceleration voltage of 15 kV. Thickness of the gold layer obtained with sputtering was $\leq$1 µm. For observation of the internal structure of the beads, they were cut into half with a steel blade and cross sectional image was taken in the same way.

Swelling studies

The swelling behavior of the composite gel beads was measured in both acidic (pH 1.2) and basic (pH 7.4) buffer media. Composite gel beads were allowed to swell completely in the buffer for 24 hrs at 37 °C temperature. After that the swollen beads were taken out, the solution adhered to the bead surface was blotted gently with tissue paper and weight of the beads was taken. In order to gain reproducible and accurate results, the swelling studies were performed in triplicate for each formulation. The percentage equilibrium swelling (% ES) was calculated using the following equation 1.

$$\% \text{ ES} = \left(\frac{\text{Weight of swollen bead } (W_1) - \text{Weight of dry bead } (W_2)}{\text{Weight of dry bead } (W_2)}\right) x 100 \quad (1)$$

Drug loading and encapsulation efficiency

Known quantity of composite gel beads were equilibrated with known concentration of 5-FU drug solution for 48 hrs with occasional shaking. In the drug solution, the beads swell and absorbs drug. After 48 hrs, the drug loaded beads were separated by filtration and dried at ambient conditions.

It is important to estimate the amount of drug present in the drug carrier so as to analyze its drug release behavior. The drug 5-FU from the drug loaded composite gel beads was extracted by stirring vigorously the known amount of dried samples in known concentration of buffer solution for 2 days. Finally, the solution was filtered and the amount of drug present in the solution was analyzed by measuring the absorbance at lambda maximum values of the drug viz., 270 nm. The results of percent drug loading (% DL) and percent encapsulation efficiency (% EE) were calculated by using the following equations 2 and 3, respectively.

$$\% \text{ DL} = \left(\frac{\text{Weight of drug in gel beads}}{\text{Weight of gel beads}}\right) x 100 \quad (2)$$

$$\% \text{ EE} = \left(\frac{\text{Actual loading}}{\text{Theoritical loading}}\right) x 100 \quad (3)$$

RESULTS AND DISCUSSION

Preparation of NaAlg -zeolite composite gel beads

NaAlg is a water soluble anionic natural polysaccharide and its gelling ability with divalent positively charged ions (calcium) is well recognized for the formulation of beads. When aqueous suspension of NaAlg-zeolite was dropped into cross linking methanol solution containing calcium chloride, spherical and transparent gel beads were formed. Within the composite gel bead matrix, zeolite xmight have dispersed in the cross links formed between Ca^{+2} ions with carboxylic groups of neighboring NaAlg polymer chains, leading to the formation of egg box like structure.

Fourier transform infrared (FT-IR) results

Fig. 1 shows the FT-IR results of (a) plain NaAlg, (b) zeolite 3A (c) 5-FU and (d) 5-FU drug-loaded composite gel beads. In the spectrum of NaAlg, peak at high frequency region of 3444 cm^{-1} corresponds to O-H vibrations and sharp peak at 1610 cm^{-1} can be assigned to carbonyl group of carboxylate (Olukman et al., 2012). In FT-IR spectrum of zeolite 3A, the bands at low frequency region of 456, 547 and 970 cm^{-1} correspond to the stretching vibrations of Si–O or Al–O bending vibrations, double ring and MeO_4 asymmetric stretching vibrations (Me = Si or Al), respectively. The peak at 3000–3600 cm^{-1} represents inter and intra molecular hydrogen bonding. The broad absorption band in the high frequency region of 3374 cm^{-1} can be attributed to last silanol groups on the outer surface of the zeolite crystals (Orha et al., 2011; Flanigen et al., 1971). This broad band overlapped with wide and strong peaks of

hydroxyl stretching between 3100 and 3500 cm^{-1}. Absorption band at 1673 cm^{-1} corresponds to O-H bending vibration (Orha et al., 2011). In the spectrum of 5-FU drug, characteristic peak observed at 1736 cm^{-1} is due to vibration of C=O group and peak at 1642 cm^{-1} corresponds to nitrile stretching, peak at 1244 cm^{-1} may be due to C-N vibration (in plane) and carbonyl vibration was observed at 1186 cm^{-1} observed. In spectrum of drug loaded NaAlg-zeolite composite gel bead wide broad band at 3500 cm^{-1} may be due to the overlapping of hydroxyl group of alginate with –NH band of 5-FU. The peak observed at 1326 cm^{-1} in pure 5-FU is due to stretching vibration of C-F (Zhang et al., 2012) was observed with less intensity in the drug loaded NaAlg-zeolite beads. In addition to this, most of the zeolite peaks were also observed in drug loaded composite gel beads. This confirms the encapsulation of drug 5-FU in compsoite gel beads along with zeolite.

X-ray diffraction (X-RD) results

X-RD was employed to understand the crystallinity of 5-FU drug in the composite gel beads. Fig. 2 shows the X-ray diffractograms of (a) plain NaAlg gel bead, (b) zeolite 3A (c) 5-FU and (d) 5-FU drug-loaded composite gel beads. In the case of plain NaAlg gel bead (Fig. 2a), two major diffraction peaks at 2θ of 13.5º and 22º due to the reflection of its (110) plane from the polyguluronate unit and (200) plane from the polymannuronate, respectively were observed (Seeli et al., 2016). The diffraction pattern for zeolite 3A (Fig. 2b) showed the crystalline alumino silicate characteristic peaks at 2θ of 30.92, 32.66, 34.16, 35.5, 35.82, 36.6, 38.18, 40.28, 41.66, 42.98, 43.64, 44.26, 47.44, 47.96, 49.82, 52.26, 54.36, 56.56, 57.68 and 58.74 indicated the micro porous structure of zeolite (Jahangirian et al., 2013). 5-FU has specific peaks at 2θ of 17º, 29º and 31º due to the crystalline nature of the drug (Naidu et al., 2011) presented in Fig. 2c. 5-FU loaded composite beads exhibits less intensive characteristic peaks of 5-FU displayed in Fig. 2 d. This may due to the molecular level distribution of 5-FU within polymeric matrices and further, the large of size of the polymer chains compared to drug could shield the drug molecules (Datt et al., 2013). However, zeolite peaks are observed in the composite gel beads suggested that zeolite maintained its crystalline nature even after embedded into NaAlg matrix.

Differential scanning calorimetric (DSC) results

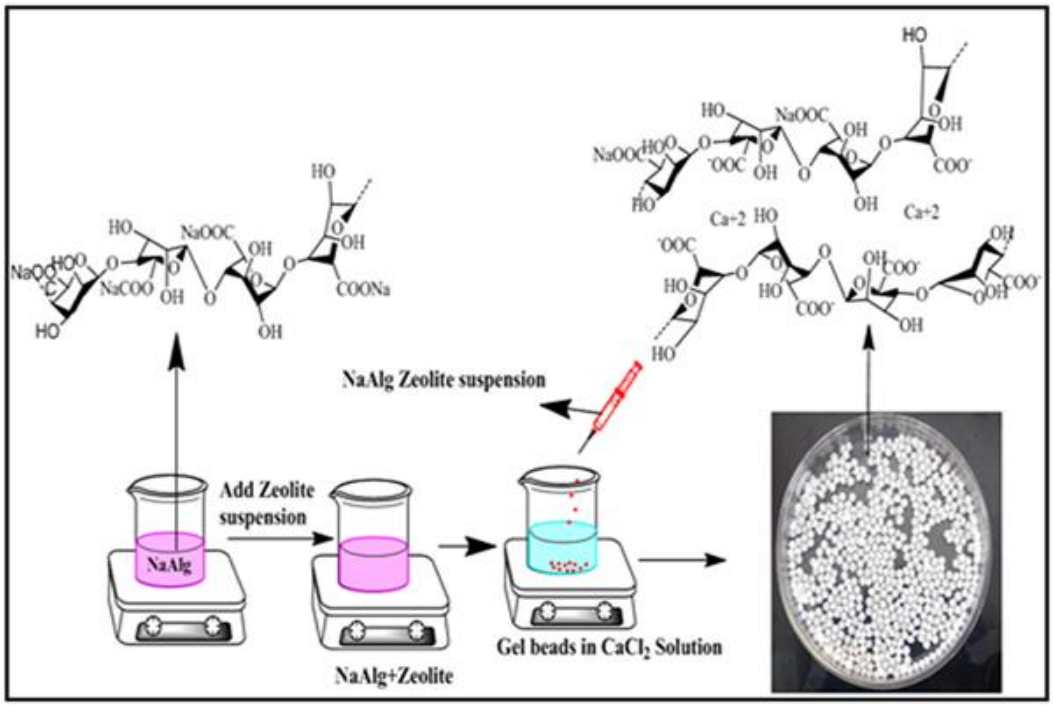
DSC profiles of (a) plain NaAlg gel beads, (b) zeolite 3A, (c) pure 5-FU and (d) 5-FU drug- loaded composite gel beads are displayed in Fig. 3. 5-FU drug shows a characteristic melting endothermic transition at 287 ºC due to polymorphism and melting. In the case of plain and drug loaded composite gel beads broad endothermic peak was observed around 100 ºC, which could be due to evaporation of moisture from the gel beads. However, in case of drug loaded beads, characteristic endothermic peak of 5-FU was not observed suggesting that the 5-FU was well distributed at molecular level (Ding et al., 2007).

Scanning electron microscopy (SEM) results

SEM study was performed to find out a topographical characterization of prepared composite gel beads. It revealed that the composite gel beads are closely spherical in appearance with a size ranging from 1 to 2 mm as shown in Fig. 4. The beads are hard without any aggregation and slightly rough external surface, which is due to the inward shrinkage of the blank matrix during drying. The cross sectional image showed that zeolites are uniformly dispersed throughout the composite gel bead matrix.

Swelling study results

The results of swelling studies were presented in Table 1.



Scheme 1. Schematic representation of the preparation of sodium alginate – zeolite composite gel beads.

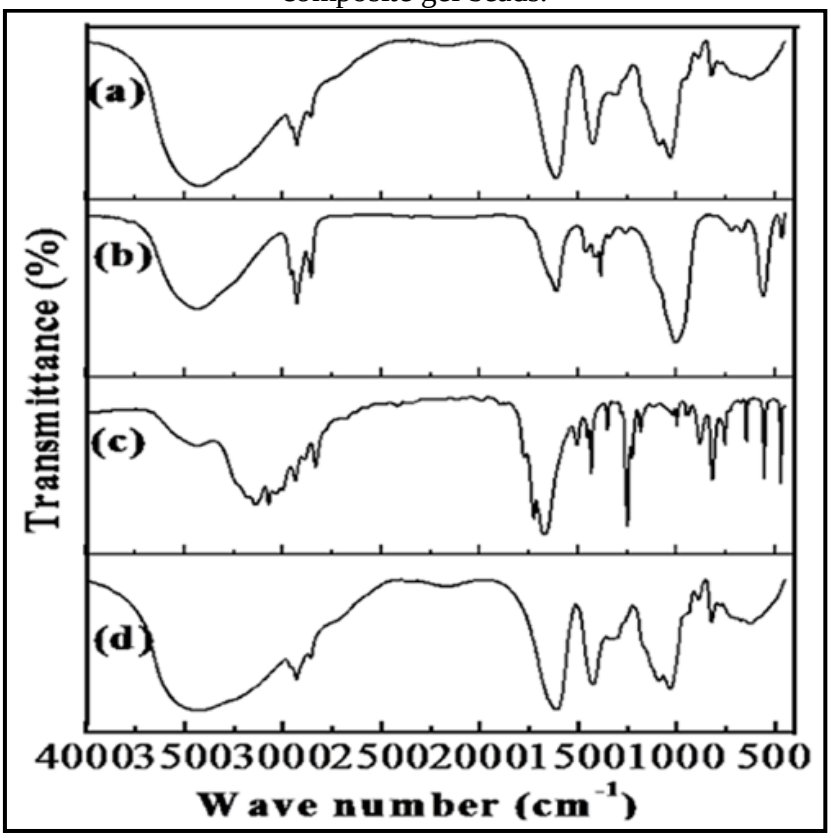


Figure 1.FT-IR Spectra (a) plain NaAlg gel beads, (b) zeolite-3A, (c) 5-FU and (d) 5-FU drug -loaded NaAlg composite gel beads.

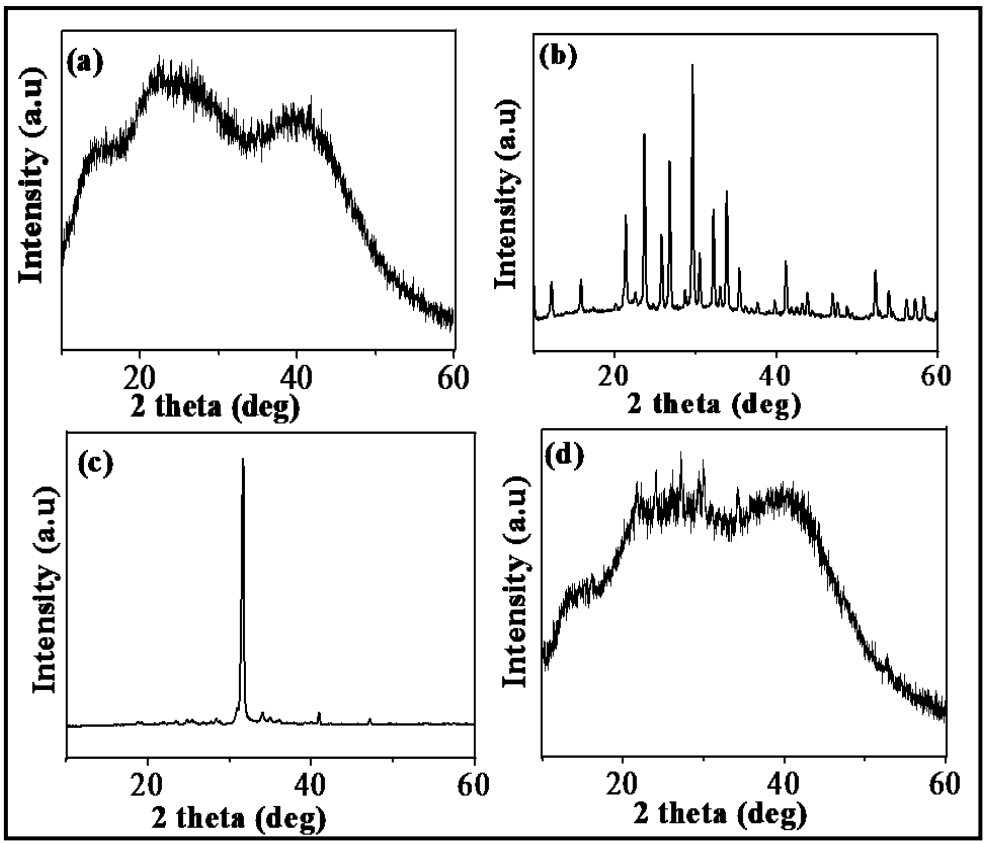


Figure 2. X-RD patterns of (a) plain NaAlg gel beads, (b) zeolite-3A, (c) 5-FU and (d) 5-FU drug-loaded NaAlg composite gel beads.

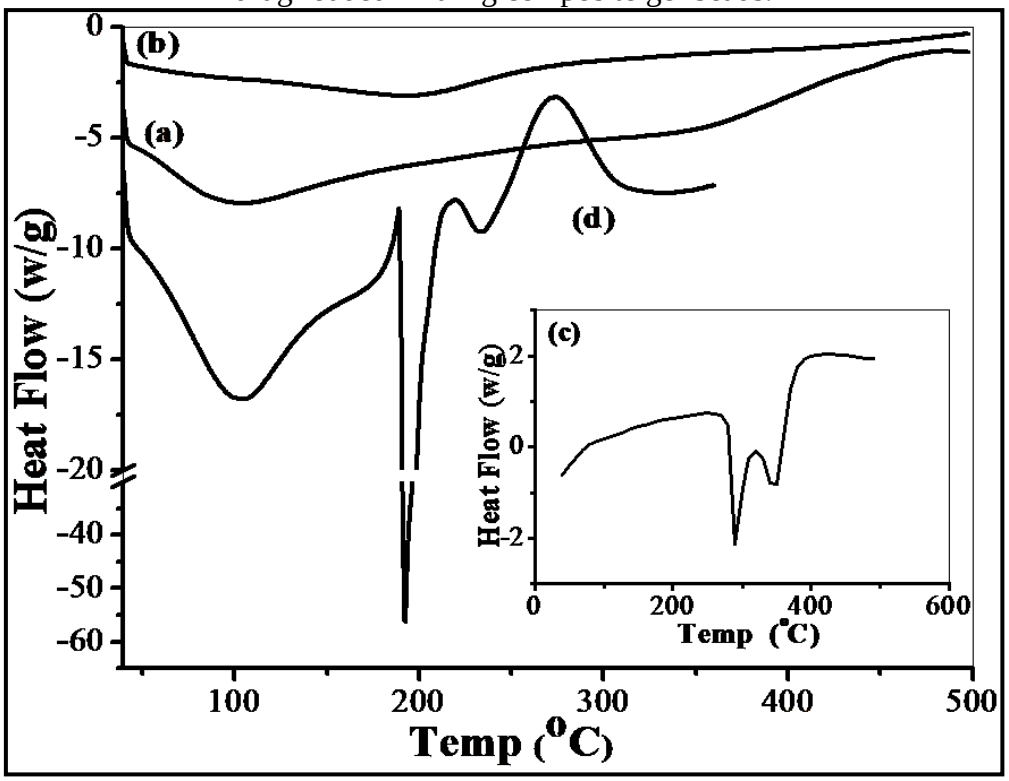


Figure 3. DSC tracings of (a) plain NaAlg gel beads, (b) zeolite 3A, (c) 5-FU and (d) 5-FU drug- loaded NaAlg composite gel beads.

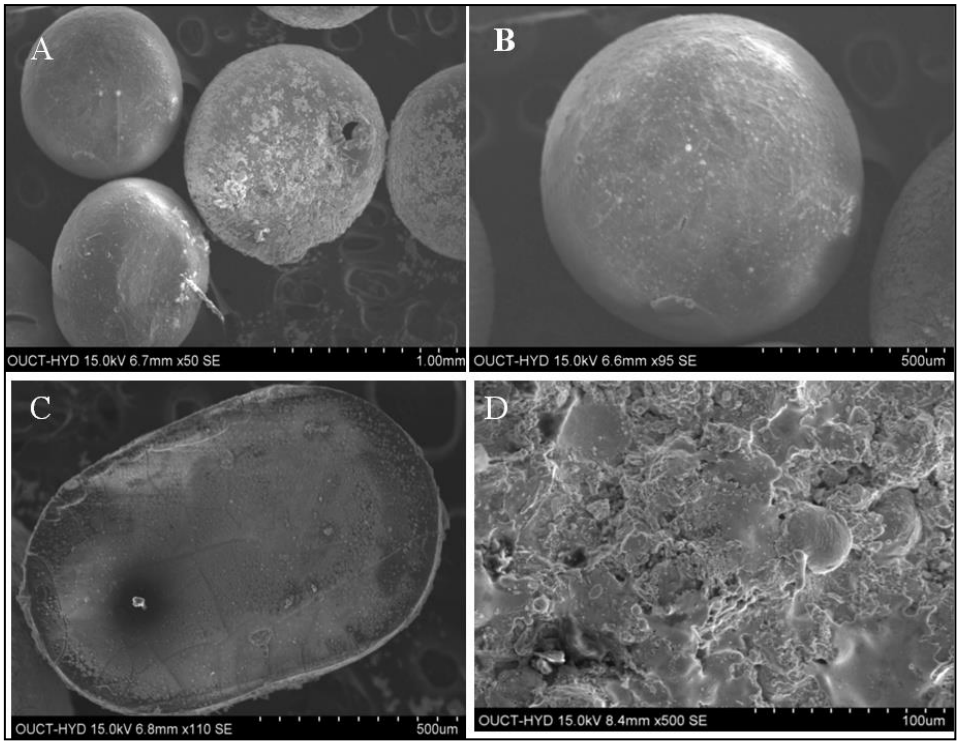


Figure 4. (A) and (B) SEM micrographs of composite gel beads, (C) cross section of composite gel bead and (D) surface morphology of porous NaAlg composite gel bead.

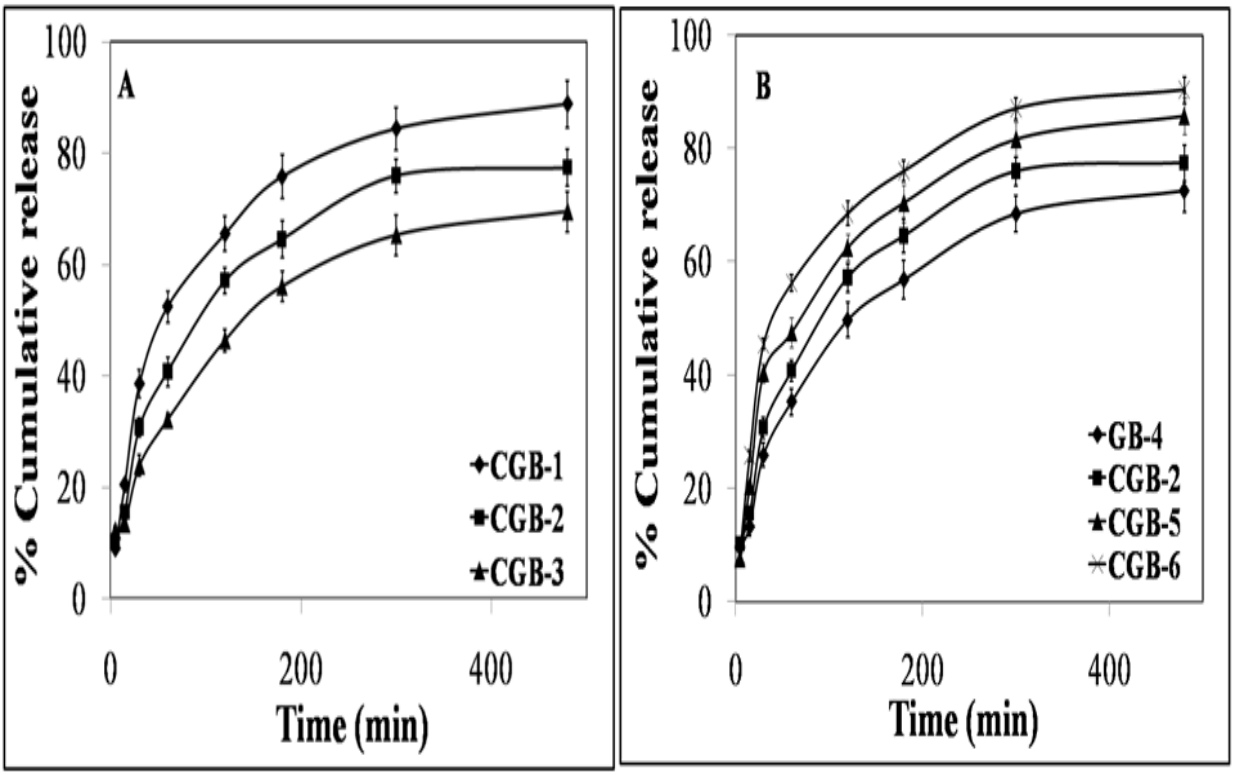


Figure 5. Percent cumulative release of 5-Fluorouracil from NaAlg composite gel beads at pH 7.4. (A) cross linking variation and (B) zeolite variation. (The data presented is average three independent measurements and errors bars are drawn from standard deviation).

Table 1. Release parameters calculated by cumulative release data

Gel bead Code	Concentration of $CaCl_2$ (w/v)%	Zeolite (wt.%)	Percent encapsulation efficiency± Standard deviation	Percent equilibrium swelling ± Standard deviation		n	k
				At pH 1.2	At pH 7.4		
CGB-1	1	2.5	40.67±1.3	75.80±1.06	180.56±1.12	0.407	7.379
CGB-2	2	2.5	35.55±2.5	70.47±1.15	165.85±1.06	0.403	6.576
CGB-3	3	2.5	30.45±1.5	52.85±1.44	128.54±1.32	0.382	6.426
GB-4	2	0	25.68±1.9	44.67±1.38	106.66±1.84	0.409	5.688
CGB-5	2	5	48.26±2.1	68.09±1.02	148.45±1.32	0.409	6.902
CGB-6	2	7.5	77.75±.05	80.50±1.03	210.25±1.19	0.393	8.260

Swelling capacity of composite gel beads is low in acidic medium compared to alkaline medium. The matrix polymer chains contain carboxylic group which accept or release protons in response to the changes in external pH of the media. In acidic medium (pH 1.2), the occurrence of high concentration of H^+ ions induces the exchange of ions between H^+ and Ca^{2+}, transforming the alginate salt into alginic acid on the surface of the composite gel beads. The low solubility of alginic acid restricts the penetration of the fluid inside the beads, as a result, the beads swell less (Agarwal et al., 2015). But, at higher pH 7.4, the carboxylic groups will be deprotonated and converting into carboxylate $–COO^–$ groups. Owing to the negatively charged ionic groups present on the chains of polymer structure causes the electrostatic repulsion, as a result the distance between the polymer chains increases.

The void space of network increases, so that the network becomes more permeable to large molecules and much water can penetrate into the network, leading to the higher degree of swelling. Moreover, the ionic exchange process between Na^+ and Ca^{2+} takes place. Na^+ ions diffused into alginate beads substituting calcium ions out of the network and leading to the swelling of beads. When the ionic exchange involved the Ca^{2+} ions in the egg-box network causes the loss of the rigidity of the structure and disruption of the system.

Encapsulation efficiency results

The results of percent encapsulation of 5-FU drug in composite NaAlg gel beads and plain NaAlg gel bead were given in Table 1. These values showed a dependence on the concentration of cross linker ($CaCl_2$) and also on the amount of zeolite in the composite gel matrix. The percent encapsulation efficiency values decreased from 40.67±1.3 to 30.45 ± 1.5 % with increase in amount of $CaCl_2$ from 1 to 3 wt.%. As the concentration of calcium salt i.e. cross linking agent increased, more cross linking networks will be formed between calcium ions with carboxylate groups of NaAlg and forms hard gel beads. Due to the rigid nature, only few void spaces are available between the networks and hence, only less amount of drug could load into the composite gel beads. The percent encapsulation efficiency of composite gel beads increased from 25.68 ±1.9 to 77.75 ±.05 % with increase in the wt. % of zeolite from 0 to 7.5 %. This is obvious, as the more and more zeolite is available in the composite gel beads, more drug molecules can accommodate because of increased porosity of the composite gel matrix. In NaAlg composite gel beads, hydrogen bonding may be possible between the electronegative atoms of 5-FU and zeolites with hydroxyl groups of NaAlg, which also helps in increasing the encapsulation efficiency of 5-FU in the composite gel beads.

***In vitro* drug delivery results**

The release results of 5-FU from composite gel beads in 7.4 pH aqueous buffer medium at 37 ^{o}C were presented in Fig. 5. The drug release pattern of composite gel beads were affected by the amount of cross linking agent as well as zeolite used. All formulations showed release profile as an initial small burst release followed by slower release.

Effect of amount of cross linking agent on drug release

The percentage cumulative drug release data against time for different amounts of $CaCl_2$ at fixed amount of zeolite are displayed in Fig. 5 (A). The cumulative release of 5-FU from CGB-1 beads prepared using 1 wt. % $CaCl_2$ solution was faster compared with other formulations. At 1wt. % $CaCl_2$, the gel beads formed were inhomogeneous, where higher amount alginate was present at surface of the bead compared to the core causes the more drug on the surface of the gel beads. Consequently, a faster release of the 5-FU was observed at the beginning of the dissolution studies. As the amount of cross linking agent increased, polymeric matrix becomes more rigid due to more number of cross links formed between polymer chains reduces the swelling of the beads in release medium thereby decreasing the release of the drug from the beads.

Effect of zeolite content on drug release

The plots of % cumulative release against time from beads with varying amount of zeolite (0, 2.5, 5 and 7.5 wt. %) at fixed concentration of cross linking agent are displayed in Fig. 5 (B). As the amount of zeolite increases, the cumulative drug release from the beads gets increased. This is obvious, because as the zeolite content increases more drug was encapsulated in to the composite gel beads thereby making the drug release at faster rates.

Drug release kinetics

The release kinetics of all the formulations of 5-FU loaded composite gel beads were estimated from percentage cumulative release (M_t/M_∞) values with time studied at 37 ^{o}C in aqueous buffer solution using the equation. 4.

$$M_t/M_\infty = kt^n \quad (4)$$

Here, M_t/M_∞ denotes release of drug at time t, n is a diffusion parameter characterizing the release phenomenon and k is constant corresponds to drug-polymer system. The type of release mechanism is governed by the n value in equation, where $n \leq 0.5$ follows Fickian diffusion pattern; while $0.5 < n < 1$ follows non-Fickian trend (Jahangirian et al., 2013). The n and k values were calculated for the various formulations are included in Table 1. The n values were between 0.382–0.409 suggest that the release mechanism follows Fickian diffusion behavior. The k values gradually decreases from 7.3 for formulation CGB-1 to 6.4 for formulation CGB-3 due to reduced interaction of drug within the gel bead matrix due to the formation of tight rigid polymer networks. Moreover, k values gradually increase from 5.6 for formulation GB-4 to 8.2 for formulation CGB-6 due to more interaction between drug and polymer gel matrix (Babu et al ., 2006).

CONCLUSIONS

In this paper, we have developed stable new NaAlg-zeolite composite gel beads by using $CaCl_2$ as cross linker through simple ionotropic gelation method for encapsulation of 5-FU drug. The developed composite gel beads were characterized by FT-IR, X-RD and DSC confirms the encapsulation and good distribution of drug in the composite beads. SEM micrograph revealed the rough surface, closely spherical shape and size of the beads ranges from 1-2 mm. The swelling ratio of beads depicted an opposite relationship of cross linker concentration and zeolite with water uptake capacity of gel beads. The drug release from these composite beads were extended over 8 hrs suggests the controlled release nature of these beads.

CONFLICT OF INTEREST

The authors declare that we have no conflict of interest.

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