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Synthesis and Characterization of biodegradable Poly (Vinyl caprolactam) grafted on to sodium alginate and its microgels for controlled release studies of an anticancer drug

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ABSTRACT

Biodegradable sodium alginate-g-poly (vinyl caprolactam) synthesized by graft copolymerization of *N*-vinyl caprolactam (VCL) on to sodium alginate (NaAlg) via free radical initiation mechanism using a redox initiation system. Grafting (%), efficiency (%), and conversion (%) were all found to depend on the content of potassium persulfate (KPS), VCL reaction temperature and time. The maximum % of grafting was ascertained to be 251 at the optimum conditions of 65 ^{o}C reaction temperature, 180 min of reaction time, $1.1098X10^{-3}$ mol of KPS and $7.1844X10^{-3}$ mol of VCL. Evidence of graft copolymerization was obtained by fouriertransform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC). Further, graft copolymer was used for preparation of microgels (MGs) using Ca^{+2} as a crosslinking agent. SEM results showed that the MGs are spherical in structure with smooth surface. The effects of pH and temperature on the swelling behaviour of MGs were studied and ascertained that they were sensitive to both pH and temperature. 5-FU drug was successfully loaded in to these MGs and encapsulation efficiency was found 84%. The release of 5-FU was systematically investigated as a function of temperature, pH, amount of crosslinker and % of drug loading concentration. The results indicate that the responsive MGs have the potential to be used as an effective pH and temperature controlled delivery of 5-FU for more than 12 h.

INTRODUCTION

Carbohydrate polymers and their derivatives represent a group of polymers widely used in the pharmaceutical and biomedical fields for the controlled release of drugs. Controlled drug delivery systems have attracted increasing attention during the past three decades (Kost and Langer, 2001; Bromberg and Ron, 1998; Hirayama and Uekama, 1999; Santini *et al.*, 1999).

For the standard aim of optimization of the pharmacotherapy, the release of drug molecules should be controlled in agreement with the therapeutic purpose and the pharmacological properties of the drug. There is a growing interest in developing rate or time-controlled devices because the level of drug released from a delivery system is of particular importance in the realization of therapeutic efficacy. Also, drug delivery systems can be more effective therapeutically by eliminating the potential for both under- and overdosing, maintaining the drug levels within a desired range, decreasing the administration times and increasing patient compliance. Of the controlled drug release systems, responsive matrices have been widely investigated because they closely resemble natural physiological processes, during which the amount of drug released can be modulated by the physiological conditions. The polysaccharides do hold advantages over the synthetic polymers, generally because they are nontoxic, less expensive, biodegradable, biocompatible, renewable, and freely available, compared to their synthetic counterparts. Therefore, in the years to come, there is going to be continued interest in the polysaccharides and their modifications with the aim to have better materials for drug delivery systems. Several polysaccharides such as sodium alginate, chitosan, guar gum, xanthan gum, pectin, and gellan gum have been employed either alone or in combination with their native or modified forms to control the drug release from different types of

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delivery systems, but these just had a limited degree of success (Badwan *et al.,* 1985; Halder and Mukherjee, 2005; Yassin *et al.,* 2006; Maiti *et al.,* 2007; Patil *et al.,* 2006). In order to improve their properties the graft copolymers designed primarily for medical applications have entered the arena of controlled release. A graft copolymer is a macromolecular chain with one or more species of block connected to the main chain as side chains (Athawale and Rathi, 1999; Zohuriaan-Mehr, 2005). This fascinating technique may be considered as an approach to achieve novel polysaccharide-based materials with improved properties including all the expected usefulness of these biomaterials.

Sodium alginate (NaAlg) is a renewable and biodegradable natural polymer that is used in a variety of commercial applications because of its capacity for gelatinization. Alginates are linear anionic polysaccharides of (1, 4)-linked α-L-guluronate and β-D-mannuronic acid residues and are obtained mainly from brown algas belonging to the Phaeophyceae. Alginates and their derivatives are widely used in food, cosmetic, drug delivery (Babu *et al.,* 2007; Pongjanyakul and Puttipipatkhachorn, 2007), pervaporation (Adoor *et al.,* 2008), and agriculture applications (Mishra *et al.,* 2004). Graft copolymerization is widely used chemical modification method for Sodium alginate in order to improve its properties. However, modification of NaAlg via grafting by vinyl monomers is one of the most effective methods to incorporate desirable properties in to NaAlg without sacrificing its biodegradable nature (Yadav *et al.,* 2011). Recently many researchers have reported the pH and temperature responsive sodium alginate graft copolymers and their carriers which are potentially used for drug delivery applications (Gao *et al.,* 2009; Işıklan *et al.,* 2011; Abd El-Ghaffar *et al.,* 2012; Deng *et al.,* 2010; Kulkarni *et al.,* 2010). In our previous reports we have also developed several graft copolymers for different types of carbohydrate polymers for drug delivery systems (Krishna Rao *et al.,* 2010; Prasad *et al.,* 2010; Lakshmi Narayana Reddy *et al.,* 2010; Yerriswamy*et al.,* 2011). One of the most extensively studied thermoresponsive polymer is poly (*N*-isopropyl acrylamide) (PNIPAM). Poly vinyl caprolactam (PVCL) is another example of a polymer with LCST behavior in water (Meeussen *et al.,* 2000). PVCL precipitates from aqueous solution in a range of physiological temperatures (32-34 ^{0}C). As hydrolysis of the amide group of PVCL does not produce small amide compounds, PVCL is suitable for biomedical applications. The use of PVCL instead of PNIPAM is considered advantageous because of the assumed lower toxicity of PVCL (Vihola *et al.,* 2005). At the moment, the number of studies on PVCL is still low, at least compared to those on PNIPAM. Apart from that, PVCL is also attractive because the LCST behavior is sensitive to changes of the polymer concentration, molecular weight of the polymer, and the composition of the solution (Krish *et al.,* 1999; Lau and Wu, 1999; Wu *et al.,* 2002). The combination of graft copolymer of sodium alginate with PVCL exhibits dual responsive nature (pH and temperature sensitivity). This is the main advantage of this system for drug delivery applications. 5-Fluorouracil is an antimetabolic drug, used extensively in cancer chemotherapy (Heidelberger, 1982; Waxman *et al.,* 1982; Sommadossi et al; 1982) and is an antimetabolite, which is used to prevent the subsequent scarring following trabeculectomy and to improve the prognosis for long-term retinal reattachment. 5-Fluorouracil is an acidic, water soluble (Ermis and Yuksel, 1999), hydrophilic drug and is an antineoplastic agent of extensive use in clinical chemotherapy for the treatment of solid tumours.

It has been widely used in drug administration due to its large number of secondary effects that accompany its conventional administration. In the present work, the results of a study on grafting of VCL on to NaAlg in terms of monomer concentration, temperature and time using different concentrations of potassium persulphate as a free radical initiator, under inert conditions are described. Graft copolymeric microgels (MGs) were prepared using Ca^{+2} as a crossliker. The swelling experiments were performed at different pH and temperatures. The 5-FU was introduced in to these MGs and its encapsulation efficiency also studied. Furthermore, release profiles have been analyzed using Korsemeyer-Peppas equations. The utility of graft copolymer based MGs are suitable for colon cancer drug delivery in order to improve their drug release in the intestinal tract and minimise in the gastric regions.

EXPERIMENTAL

Materials

N-Vinyl caprolactam was purchased from Aldrich chemicals co. U.S.A., Sodium alginate, potassium persulphate, acetone, and calcium chloride were purchased from s.d. fine chemicals Mumbai, India. H_3PO_4, KH_2PO_4, K_2HPO_4, HCl and NaOH were purchased from Merck, India. 5-fluorouracil (5-FU) received from Himedia chemicals, India. All the chemicals were used without further purification and the experiment work double distilled water was used throughout the experiment work.

Synthesis of Graft copolymer

The graft copolymer derived from VCL and NaAlg was prepared by free radical polymerization. Briefly, 0.25 g of NaAlg was dispersed in 25 mL of water and dissolved overnight under constant stirring in a 250 mL round bottom flask. Then, 1.7961-10.7766X10^{-3} moles of VCL were mixed with 10 mL of water, added to solution and stirred for 1 h. A given amount of redox initiator ($K_2S_2O_8$/$NaHSO_4$) equivalent to 0.1850-1.4798X10^{-3} mol) was dissolved in 10 mL of water and added to the above solution. Polymerization was carried out at 45-70 ^{o}C under continuous purging of nitrogen gas for 60-240 mins in a thermostatic water bath under constant stirring. After complete polymerization, the product was cooled by running under tap water and poured into excess acetone to induce precipitation. The graft solid polymer was washed several times with methanol: water (80:20, v/v) mixture to remove the homopolymer formed in the grafting reaction and was vacuum dried at 40 ^{o}C to a constant weight. The

percentage of grafting (% G), grafting efficiency (% GE) and % conversion (% C) were calculated by using:

$$\%\ Grafting\ (\%\ G) = \left(\frac{W_1 - W_0}{W_0}\right)100 \quad \ldots(1)$$

$$\%\ Grafting\ efficiency\ (\%\ GE) = \left(\frac{W_1 - W_0}{W_2}\right)100 \quad \ldots(2)$$

$$\%\ Conversion\ (\%\ C) = \left(\frac{W_1}{W_2}\right)100 \quad \ldots(3)$$

where, W_0, W_1 and W_2 denote the weight of NaAlg, graft copolymer and monomer, respectively. The results of these graft parameters have shown in table 1.

Preparation of microgels (MGs)

0.5 gms of graft copolymer was dissolved in 25 mL of distilled water until clear solution was formed. The solution was taken in hypodermic syringe and added drop wise into aqueous methanol solution containing $CaCl_2$ (2%) (80:20 methanol:water) for 10 sec under constant stirring.

This solution further stirred for 10 mins. The MGs formed were removed from the solution and washed with water for several times. The resulting MGs were dried at 40 ^oC until constant weight was reached. The 5-FU drug loaded MGs were prepared by adding to graft copolymer solution before crosslinking. Various formulations were prepared by varying % of drug, and crosslinker variation (0.5%, 1% and 2%).

pH and temperature sensitive nature of graft copolymer MGs

pH and temperature sensitivity of graft copolymer MG was studied through swelling experiments. First the MGs were immersed in buffer solution with various pH values (pH buffer solutions were prepared using NaH_2PO_4, Na_2HPO_4, NaCl and NaOH solution and pH values were measured using ELICO pH meter, India) at 30 ^oC for 12 h.

The swollen MGs were taken out for every 30 min and removed surface adhered buffer solution using tissue paper. The MGs were further immersed in various buffer solutions to reach equilibrium swelling. Swelling experiments were carried out in water by mass measurements at various temperatures to study temperature responsive behaviour of MGs. The percentages of swelling ratio (% SR) were calculated using the following equations.

$$\%\ SR = \left[\frac{W_s - W_d}{W_d}\right] X\ 100 \quad \ldots(4)$$

Where, W_s is the weight of swollen gel at time t, and W_d is the dry weight of the hydrogel. Mass measurements were made on a digital ADAMS microbalance (Model AF 210L, U.K) with sensitivity of ± 0.01 mg. Each value was averaged over three parallel measurements. Statistical analysis was performed using one-way ANOVA way in ORIGIN 8.0. All quantitative data are presented as means±standard deviation.

Estimation of drug loading and encapsulation efficiency

The loading efficiency of 5-FU in the MGs was determined spectrophotometrically. About ~10 mg of the drug-loaded MGs were placed in 10 mL of buffer solution and stirred vigorously for 48 h to extract drug from the beads.

The solution was filtered and assayed by UV spectrophotometer (Lab India, Mumbai, India) at fixed λ_{max} value of 270 nm. The results of % drug loading and % encapsulation efficiency were calculated, using Eqs. (5) and (6) respectively.

$$\%\ \text{Drug loading} = \left(\frac{\text{Amount of drug in MGs}}{\text{Amount of MGs}}\right) x100 \quad .(5)$$

$$\%\ \text{Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) x100 \quad .(6)$$

In vitro release studies

In vitro release studies were carried out using Tablet dissolution tester (Lab India, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37± 0.5 ^oC at constant speed of 100 rpm.

Drug releases from the beads were carried out in pH 1.2 and 7.4 phosphate buffer solutions at 37 ^oC and also 25 ^oC. At regular intervals of time, sample aliquots were withdrawn and analyzed using UV spectrophotometer (Lab India, Mumbai, India) at the fixed λ_{max} value of 270 nm.

After each sample collection, the same amount of fresh medium at the same temperature was added to the release medium to maintain the sink condition. All measurements were carried out in triplicate, and values were plotted with standard deviation errors.

Characterization of graft copolymer and micro gels

FT-IR analysis

Fourier transform infrared spectroscopy (FTIR) spectral measurements of pure NaAlg, graft copolymer and MGs were performed using Perkin Elmer (model Impact 410, Wisconsin, MI, USA) spectrophotometer.

The MGs were finely grounded with KBr to prepare the pellets under a hydraulic pressure of 600 dynes/m^2 and spectra were scanned between 4000 to 500 cm^{-1}.

Differential Scanning Calorimetry (DSC)

DSC curves of pure NaAlg, graft copolymer, pristine drug, pristine MGs and drug loaded MGs were recorded using TA instruments sequential thermal analyzer (Model-SDT Q600, U). Analysis of the samples was performed at heating rate of 10^oC/min under N_2 atmosphere at a purging rate of 100mL/min.

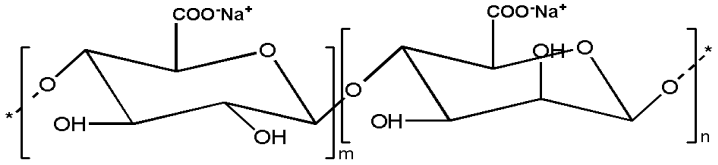
Scanning electron microscopy (SEM)

SEM images of graft copolymeric MGs were recorded using a JSM 6400 SEM (JEOL Ltd., Akishima, Tokyo, Japan) at 53x, 200x, 500xand 1.0Kx magnification. Working distance of 8.5-9.5 mm was maintained and the acceleration voltage used was 20 kV, with the secondary electron image (SEI) as a detector.

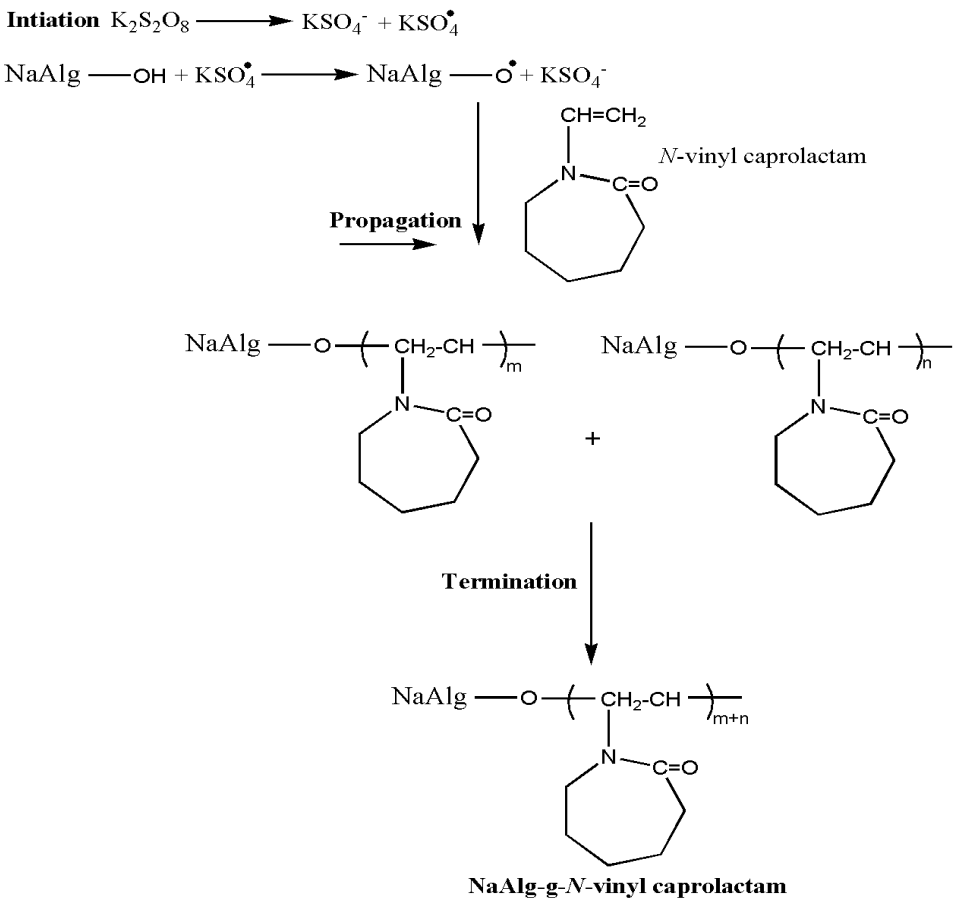
RESULTS AND DISCUSSIONS

Mechanism of graft copolymerization

Kulkarni *et al.*, 2009 developed a pH-sensitive graft copolymer of poly (acrylamide) and NaAlg by free radical polymerization using ammonium persulphate (APS) as initiator under a nitrogen atmosphere. Recently Yadav *et al.*, 2011 developed pH sensitive NaAlg-g-poly (acrylamidoglycolic acid) using potassium peroxodisulphide/silver nitrate redox system and to study their physico chemical properties. Liu *et al.*, 2005 also synthesized graft copolymerization of methyl acrylate on to NaAlg using potassium diperiodatocuprate (III) as initiator. In the present study the graft copolymerization of VCL onto NaAlg was carried out by a KPS/SB as redox initiator in homogeneous solution. This was achieved by dissolving in the monomer solution, then adding initiator at the required temperature. The probable reaction mechanism is given in **scheme 1.** KPS is used as an initiator to produce radicals on the polymer backbone. The reactive hydroxyl groups act as powerful radical producing in presence of free radical initiating agent. The free radicals formed during the first steps then react with the double bond of the vinyl monomer, resulting in a covalent bond between the monomer and NaAlg to propagate the chain. Termination takes place when two radicals combine.



NaAlg



NaAlg-g-*N*-vinyl caprolactam

Scheme. 1: Reaction mechanism of *N*-vinyl caprolactam on to Sodium alginate.

The results of grafting parameters (%G, %GE, %C, yield, %HP) obtained are summarized in table 1. The effect of temperature on grafting parameters has been studied. It has been found that as the temperature is increased from 45 ^{o}C to 65 ^{o}C, grafting ratio, conversion and efficiency increase. But beyond 65 ^{o}C, these parameters decrease. The increase in grafting ratio, and efficiency with temperature from 45 ^{o}C to 65 ^{o}C might be due to the rate of production of primary free radicals increases which generates grafting sites at greater rate, thereby, increasing the value of these parameters. However, beyond 65 ^{o}C grafting parameters decreased and homopolymer increased. The decrement in grafting parameters is attributed to the premature termination of growing grafted chain at higher temperature (decomposition of potassium peroxydiphosphate into HSO_4, H_2O, and O_2. Since O_2 acts as a scavenger for free radicals, which reacts with primary free radicals thereby lowering the free radical concentration).

Table 1: Grafting parameters of NaAlg-g-poly (VCL)

Temperature	**1.1098X10^{-3} moles KPS, 7.1844X10^{-3} NVCL, 180 min**				
(^{o}C)	**%G**	**%GE**	**%C**	**Yield**	%HP
45	107.6	26.9	51.9	41.52	73.1
50	135.6	33.9	58.9	47.12	66.1
55	156.8	39.2	64.2	51.36	60.8
60	238.8	59.7	84.7	67.76	40.3
65	251.6	62.9	87.9	70.32	37.1
70	225.2	56.3	81.3	65.04	43.7
Time (min)	**1.1098X10^{-3} KPS, 7.1844X10^{-3} NVCL, and at 65 ^{o}C**				
	%G	**%GE**	**%C**	**Yield**	**%HP**
60	178.4	44.6	69.6	55.68	55.4
120	238.8	59.7	84.7	67.76	40.3
180	251.6	62.9	87.9	70.32	37.1
240	230.4	57.6	82.6	66.08	42.4
NVCL	**1.1098X10^{-3} KPS and at 65 ^{o}C, 180 min**				
(X10^{-3} moles)	**%G**	**%GE**	**%C**	**Yield**	**%HP**
1.7961	45.6	45.6	145.6	72.8	54.4
3.5922	108.0	54.0	104.0	69.3	27.2
5.3883	180.8	60.2	93.6	70.2	39.7
7.1844	251.6	62.9	87.9	70.3	37.1
8.9805	243.6	48.7	68.7	57.2	29.6
10.7766	235.6	39.2	55.9	47.9	60.7
KPS (X10^{-3}	**7.1844X10^{-3} NVCL, and at 65 ^{o}C, 180 min**				
moles)	**%G**	**%GE**	**%C**	**Yield**	**%HP**
0.1850	147.6	36.9	61.9	49.52	63.1
0.3699	238.8	59.7	84.7	67.76	40.3
0.7399	250.4	62.6	87.6	70.08	37.4
1.1098	251.6	62.9	87.9	70.32	37.1
1.4798	239.2	59.8	84.8	67.84	40.2

Effect of time

The effect of change in reaction time on grafting parameters has been studied by varying the time period from 60 to 240 min. The results are summarized in table 1. It is clear from table that grafting ratio, conversion and efficiency increase with increase in time period from 60 to 180 min but homopolymer decreases and thereafter these parameters decrease and homopolymer increases. On increasing the time period propagation of grafting chains takes place at faster rate due to availability of more active sites, which account for higher grafting. On further increasing time interval, the mutual annihilation of growing grafted chains might occur so grafting parameters decrease.

Effect of monomer

The effect of concentration of monomer on grafting parameters has been studied by varying the concentration N-vinylcaprolactam from 1.7961 to 10.7766X10^{-3} moles. The results are shown in table 1. It has been observed that %G, and %GE increases whereas % C and homopolymer decreases continuously with increase in monomer concentration. The increase in grafting parameters might be attributed due to accumulation of monomer molecules at the close proximity to the polymeric backbone. The monomer molecules, which are at immediate vicinity of reaction sites become acceptors of alginate macro radicals resulting in chain initiation and thereafter themselves become free radical donors to neighboring molecules causing the lowering of termination.

Effect of initiator

Increasing the concentration of KPS initially leads to an increase in the %G, %GE, and %C, but further increases in concentration have a negative effect (table 1). The increase in the % grafting may be due to an increase in NaAlg macro radicals in the propagation step (Scheme 1), since an increase in KPS concentration means that more KPS free radicals can attack the saccharide unit of NaAlg. This would generate more NaAlg macro radicals and more active sites to react with VCL. By further increasing the amount of KPS, the concentration of $^{\cdot}OSO_3K$ radicals would increase, which could help to initiate the polymerization of VCL, thereby resulting in a decrease of the % grafting and grafting efficiency.

Characterization studies

Evidence of grafting by FTIR and DSC techniques

The structural changes of Sodium alginate and Sodium alginate grafted poly vinyl caprolactam were confirmed by FTIR spectroscopy and DSC studies. The figure 1 shows the spectrum of sodium alginate and its characteristic peaks. From this figure it can be observed that the absorption bands around 1610 cm^{-1}, 1416 cm^{-1}, and 1306 cm^{-1} are attributed to stretching vibrations of asymmetric and symmetric bands of carboxylate anions, respectively.

The peak appeared at 3430 cm^{-1} corresponds to stretching vibrations of hydroxyl groups. Further, the formation of NaAlg-*g*-poly (VCL) supported by the weak intensity band at 3419 cm^{-1} due to O-H stretching vibrations when compared to FTIR spectrum of neat NaAlg (3430 cm^{-1}$_{(S)}$). The weakening of band is due to the utilization of some –OH groups of NaAlg during the formation of the graft copolymer. In the spectrum of the graft copolymer, besides retaining the above mentioned bands of pure NaAlg, It shows an additional stronger absorption band at 1631 cm^{-1}, characteristic absorption of carbonyl groups of amide in ring structure (N, N'- dialkyl amide) of graft copolymer, which confirmed the formation of NaAlg-gt-poly (VCL) effectively.

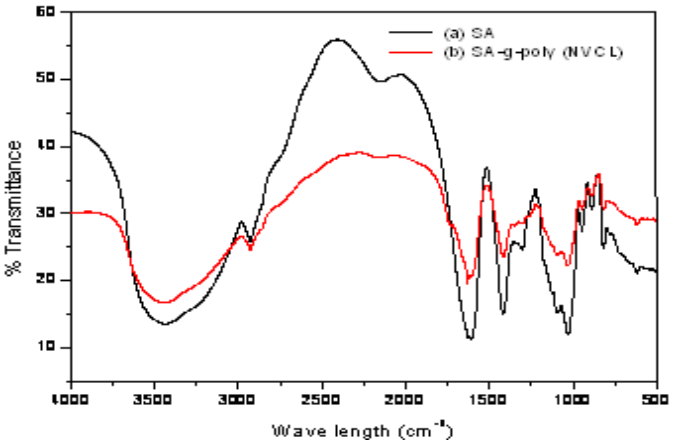


Fig. 1: FTIR spectra of (a) pure Sodium alginate (b) Sodium alginate-g- poly (N-vinyl caprolactam).

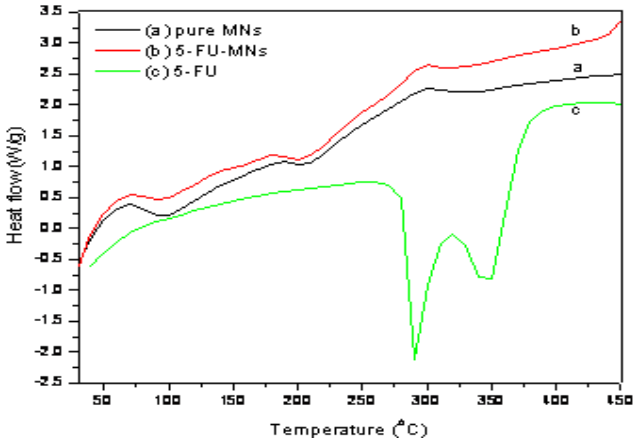


Fig. 3: DSC thermograms of (a) pristine MGS, (b) 5-FU loaded MGs and (c) pure 5-FU.

DSC thermograms of and NaAlg-gt-poly (VCL) are shown in figure 2. The DSC curve of NaAlg shows a broad endothermic peak around 100 ^{o}C and a sharp exothermic peak at 250 ^{o}C. The endothermic peak of NaAlg and NaAlg-g-poly (VCL) around 100 ^{o}C may be caused by the loss of water and moisture content of the polysaccharide, respectively. The exothermic peak at 250 ^{o}C corresponds to its thermal decomposition. The NaAlg-gt-poly (VCL) shows exothermic peak appeared at 263 ^{o}C. The increase in exothermic peak temperature indicated that the structure of NaAlg chains has been changed by the introduction of the poly (VCL). This is confirmed for the formation of graft copolymer.

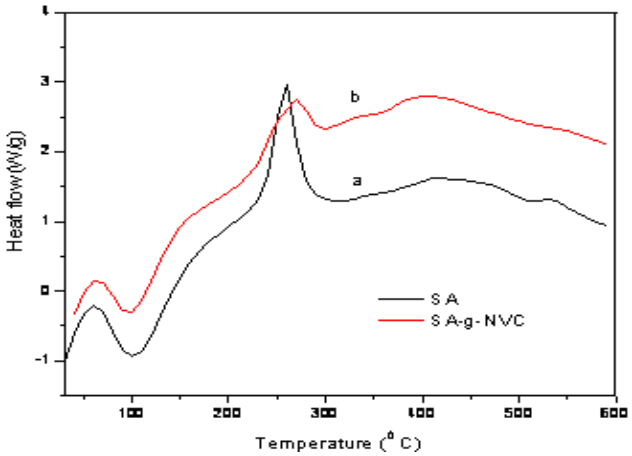


Fig. 2: DSC thermograms of (a) pure Sodium alginate (b) Sodium alginate-g-poly (N-vinyl caprolactam).

Characterization of MGs

The structure and morphology of MGs were characterized by DSC. The figure 3 shows DSC curve of pure MGs (curve a) which indicates an endothermic peak appeared at 219 ^{o}C is corresponds crosslinking of Ca^{+2} between two –COONa of graft copolymer. The pure 5-FU exhibits a sharp peak at 285 ^{o}C (curve c) is due to polymorphism and melting. However, this peak is not appeared in the case of drug loaded MGs (curve b) which conforms that the drug is molecularly dispersed in the polymeric MGs.

SEM micrographs of MGs are shown in figure 4. The average size of MGs is around 100 µm measured from the shape of the MGs. Grafting led to a substantial increase in the surface smoothness; (fig 4a & 4b) this might be due to the formation of their own domains and morphologies at the surface by grafted chains. These results further indicate that the surface morphology of MGs is significantly affected by grafting. VCL grafted chains are hydrophilic in nature and hence lead to compatibility with matrix, resulting in the formation of single phase with a smooth surface (fig 4c & 4d). It has been observed that the particle size did not vary significantly either by increasing the exposure time to Ca^{+2} or by varying the amount of the drug.

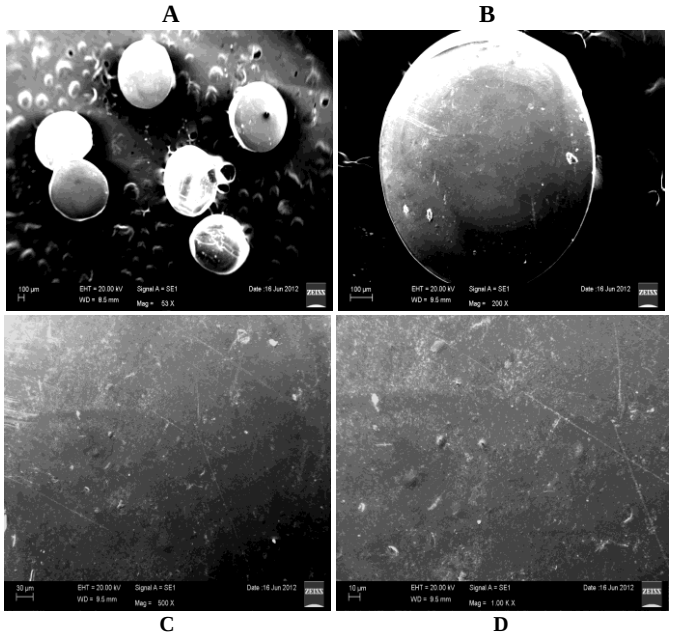


Fig. 4: Scanning electron micrographs of MGs for different magnifications

pH and temperature responsive behaviour of MGs

Figure 5a shows the swelling ratio of MGs at various pH solutions. As we can clearly seen that the swelling ratio of MGs slowly increases when pH increases upto 5.0 after that it increases

rapidly upto pH 8. Because at low pH i.e., < 5.0 carboxyl groups present in NaAlg are converted in to –COOH and hydrogen bonding developed. Beyond pH 5, the carboxyl groups are ionic nature and repelling effect increases so, the swelling also increases dramatically. The sharp increase in swelling ratio observed up to pH 8.0, and then further increases slowly with further increase in pH upto 12.0. These changes were indicates the prepared MGs exhibits pH sensitive behavior.

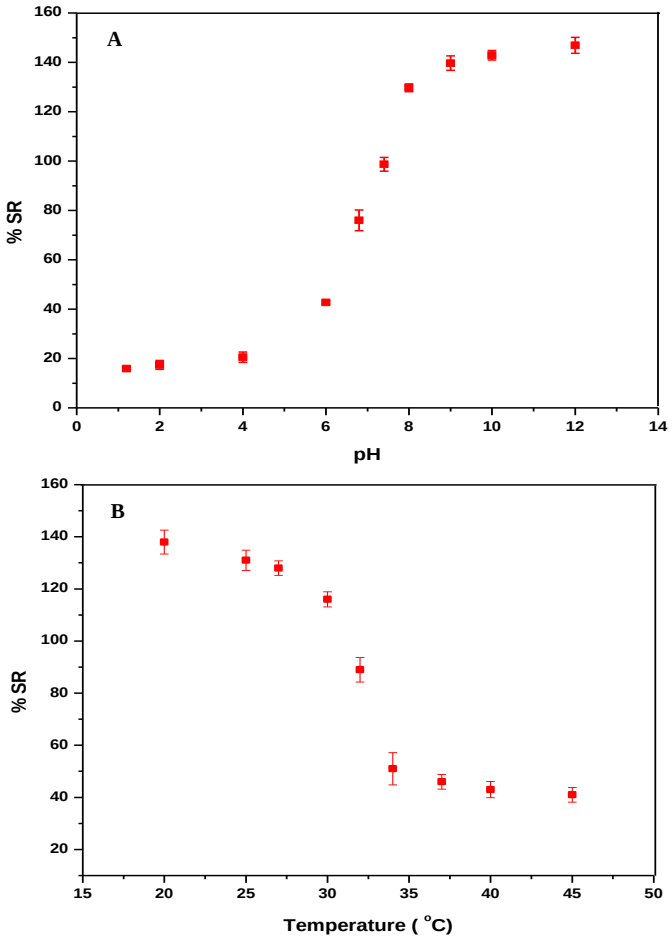


Fig. 5: Swelling studies of MGs (a) various pH conditions, and (b) different temperatures .

The effect of temperature on the equilibrium swelling ratios for MGs is shown in figure 5b. The swelling ratio of MGs is higher at low temperature (< LCST) and lower at higher temperature (> LCST). This is because below LCST VCL contains a hydrophilic group (-CONH-) and hydrophobic isopropyl group present in the linear polymer chain. So, the hydrophilic group in the polymer structure will form an intermolecular hydrogen bond with surrounding water at low temperature (below the gel transition temperature); above LCST the hydrogen bonds are broken and the water molecules are expelled from the polymer. These two results make the water molecule inside the gel change from a bound state to a free State and release from the gel. This phenomenon makes the swelling ratios of the MGs decrease rapidly at the gel transition temperature.

In-vitro release studies

Variation of drug

Figure 6 shows the release profiles of drug loaded MGs at different amount of drug loadings. Release data showed that formulations containing the higher amount of drug loading (30 wt %) displayed (M8) the fast and higher release rates than formulations containing smaller amount of 5-FU (M3 and M7 formulations). A prolonged release was observed for the formulation containing a lower amount of 5-FU. Notice that the release rate becomes quite slower at the lower amount of drug in the polymeric matrix, due to availability of more free void spaces through which a lesser number of drug molecules will transport.

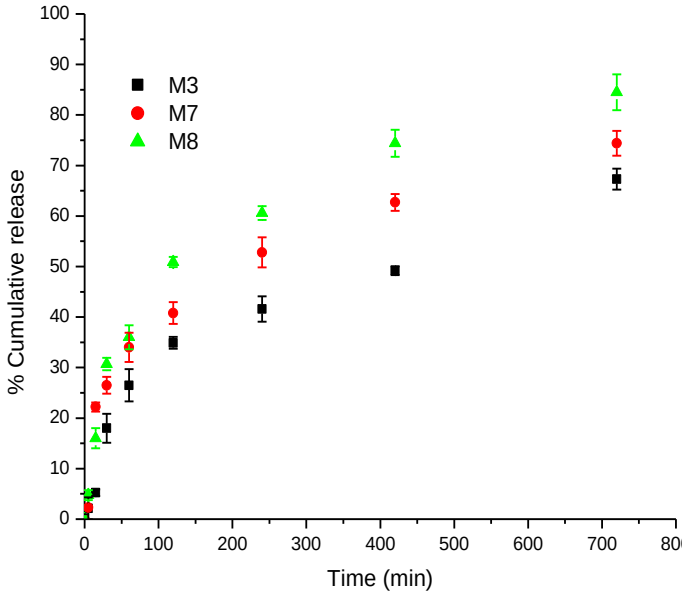


Fig. 6: % Cumulative release of 5-FU through MGs containing 2% $CaCl_2$ (pH 7.4 and at 37 ^{0}C) with different amounts of 5-FU (M3) 10%, (M7) 20 and (M8) 30%.

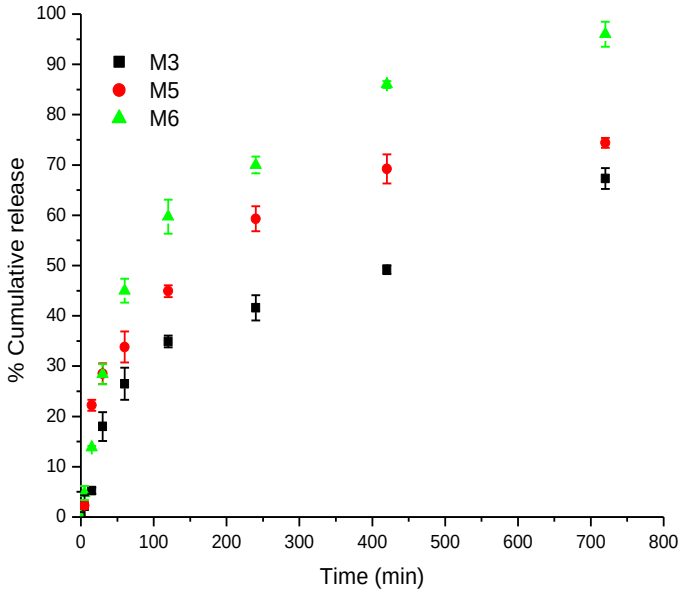


Fig. 7: % Cumulative release of 5-FU through MGs containing 10% 5-FU (pH 7.4 and at 37 ^{0}C) with different % of $CaCl_2$ (M3) 2%, (M5) 0.5% and (M6) 1%.

Crosslinker variation

The % cumulative release vs time curves for varying % of $CaCl_2$ i.e., 1, 2 and 0.5 at a fixed amount of drug (10 wt%) are displayed in figure 7**.** The % cumulative release is quite fast and large at lower amount of $CaCl_2$ (M5 formulation), where as the release is quite slower at higher amount of $CaCl_2$ (M3 formulation). The increase in the % of $CaCl_2$ leads to the formation of rigid network structure due to the contraction of microvoids. As expected, the release becomes slower at higher amount of crosslinker, but becomes faster at lower amount of Ca^{+2}.

Effect of pH and temperature

The in vitro release experiments were carried out 5-FU loaded MGs in gastric and intestinal pH conditions. Releases of 5-FU from MGs at 1.2 pH and 7.4 pH solutions with different temperatures (25 ^{o}C and 37 ^{o}C) are shown in figure 8.

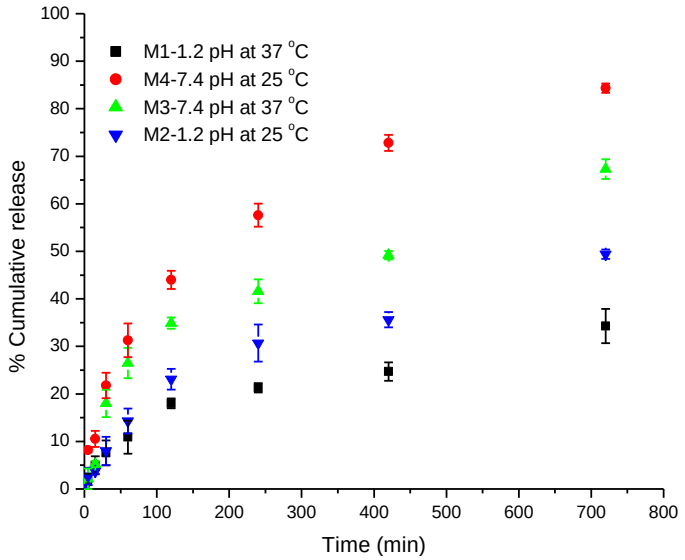


Fig. 8: % Cumulative release of 5-FU through MGs containing 2% $CaCl_2$ and 10% 5-FU (M1) 1.2 pH 37 ^{0}C, (M2) 1.2 pH 25 ^{0}C, (M3) 7.4 pH 37 ^{0}C, and (M4) 7.4 pH 25 ^{0}C.

The release of 5-FU is higher at pH 7.4 than at pH 1.2 for both temperatures (25 ^{o}C and 37 ^{o}C). This can be explained on the basis of higher degree of swelling as explained above due to ionization of carboxylic groups in the networks at pH 7.4. This indicates the pH sensitivity of MGs. At both pH conditions (pH1.2 and pH 7.4) the release of 5-FU is higher at 25 ^{o}C than at 37 ^{o}C. This is due to the temperature responsive nature of MGs (this can also explained on the basis of swelling studies).

To understand the drug release from 5-FU loaded graft copolymeric MGs, in vitro release studies data were fitting these data to the exponential equation (Ritger and Peppas, 1987).

Drug release kinetics was analyzed by the following equation type.

$$\frac{Mt}{M\alpha} = kt^{n} \qquad (7)$$

Here, $Mt/M\alpha$ represents the fractional drug release at time *t*, *k* is a constant characteristic of the drug-polymer system, and n is an exponent parameter characterizing the release mechanism. Using the least squares procedure; we have estimated the values of *n* and *k* for all the formulations, and these values are given in table 2. The log value of percent drug dissolved is plotted against log time for each formulation according to equation 7. If n=0.5, drug diffuses and releases out of the polymer matrix following the Fickian diffusion. For n>0.5, anomalous or non-Fickian type drug diffusion occurs. If n=1, a completely non-Fickian or case II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to the anomalous type of diffusive transport (Siepmann and Peppas, 2001). In the present study the values n are obtained in the range of 0.616-0.918 are attributed to the anomalous type of diffusive transport.

Table. 2: Release kinetics parameters of different formulations of MGs.

Formulation code	n	r	k
M1	0.616	0.99	0.9015
M2	0.761	0.95	1.4256
M3	0.812	0.959	0.7979
M4	0.654	0.976	0.8933
M5	0.878	0.909	1.0092
M6	0.884	0.987	1.1331
M7	0.867	0.903	1.0244
M8	0.846	0.969	1.226

CONCLUSION

New type of graft copolymer synthesized from NaAlg and NVC by free radical graft copolymerization. The maximum % grafting was ascertained to be 251 at the optimum conditions of 65 ^{o}C reaction temperatures, 180 min of reaction time, $1.1098X10^{-3}$ mol of KPS and $7.1844X10^{-3}$ mol of VCL. The evidence of grafting was obtained by FTIR and DSC techniques. The MGs were prepared using Ca^{+2} as a cross linker. Based on swelling studies the MGs exhibited excellent pH and temperature behaviour. A SEM study indicates the MGs are spherical in nature and good compatibility between graft chains and NaAlg. DSC studies showed the 5-FU dispersed at molecular level in the MGs. In-vitro release studies of 5-FU indicates that these responsive MGs can be used effectively for colon cancer drug delivery.

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