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Swelling Kinetics of a pH-sensitive polyelectrolyte complex of polyacrylamide-g-alginate and chitosan

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Abstract: Preparation and characterization of a new polyelectrolyte complex (PEC) based on polyacrylamide-g-alginate and chitosan has been carried out. The experimental conditions for efficient grafting and subsequent complex formation have been optimized. The polyelectrolyte complex has been characterized by IR and TGA techniques. The morphology of the polyelectrolyte complex was evaluated by scanning electron microscopy (SEM). The swelling study has been performed under different pH and ionic strength conditions. The results indicated ten times higher swelling under neutral pH conditions compared to highly acidic conditions. Also, the increasing ionic strength of the swelling medium enhanced swelling up to a particular level above which the polyelectrolyte complex dissociates. The kinetic analysis of the swelling data indicated 'second-order' swelling kinetics. Due to the pH and salt responsive character, the polyelectrolyte complex may find use as matrix material for controlled drug release formulations.

Keywords: Acrylamide, Sodium Alginate, Polyelectrolyte complex, Chitosan, Swelling kinetics.

1. Introduction

The physical modification of the polymer with specific physicochemical properties can be achieved by formation of polyelectrolyte complexes. Polyelectrolyte complexes (PECs) are the association complexes formed between oppositely charged particles (e.g. polymer-polymer, polymer-drug and polymer-drug-polymer). These are formed due to electrostatic interaction between oppositely charged polyions.[1-4] This avoids the use of chemical cross linking agents, thereby reducing the possible toxicity and other undesirable effects of the reagents in biomedical applications. Polyelectrolyte complexes are biocompatible and are sensitive to changes in environmental conditions. [5]

Chitosan (Cts) is a natural, non-toxic, biodegradable, and biocompatible polysaccharide, obtained by the extensive N-deacetylation of chitin. It is composed of randomly distributed units of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine. [6] Cts is slightly a crystalline polysaccharide, which is insoluble when the pH value is around or above 7. However, the free amino groups of Cts become protonated in an acidic environment, which makes the molecule soluble with a positive charge.[7] Because of its cationic character, Cts can interact with oppositely charged macromolecules, such as alginate, carrageenan, pectin, carboxy methylcellulose etc giving rise to polyelectrolyte complexes (PEC).

Sodium alginate (NaAlg) is a high-molecular mass, natural, non-toxic and biodegradable polyanionic copolymer which can be extracted from marine brown algae. It consists of 1, 4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues arranged either as consecutive blocks or in a random distribution. The relative amount of the two uronic acid monomers and their sequential arrangement along the polymer chain vary widely, depending on the origin of the alginate. Alginate is chemically very stable at pH values between 5 and 10. High acid concentrations cause decarboxylation of alginate. [8] Alginate is used as an entrapment matrix for cells and enzymes and as a pharmaceutical and food adjuvant. [9]

Preparation of PECs from oppositely charged graft copolymers increases their applications. Recillas et.al, [10] synthesised PEC from chitosan graft N-isopropylacrylamide and pectin and studied their thermo and pH responsive behaviour. Krishna Rao et.al, [11] studied the preparation and characterization of poly (acrylamidoglycolic acid) grated onto chitosan and its PEC with hydroxyapatite. The composition, stability and swelling behavior of polyelectrolyte complexes depend on pH, ionic strength, temperature, charge density of the component polymers among other environmental conditions. [12]

In the present study we have developed a PEC between Cts and acrylamide grafted NaAlg. The aim of the present work was to establish the conditions for formation of PEC between and Cts and to evaluate the pH- and ionic responsiveness of the complex. A detailed kinetic study of swelling of the PEC under different pH and ionic strength conditions has been undertaken with an intention of using the material in the design of effective drug delivery system.

2. Experimental

2.1 Materials

Cts and NaAlg were purchased from Sigma–Aldrich Chemical Co. Ltd (India). acrylamide (Am), potassium peroxodisulfate (KPS), acetic acid, potassium chloride, hydrochloric acid, potassium dihydrogen phosphate, disodium hydrogen phosphates, sodium chloride were obtained from Merck (India). All the chemicals and reagents were used as received. All experiments were carried out with distilled water.

Solution containing 0.05M KCl and 0.085M HCl was made to obtain buffer of pH-1.2; solution containing disodium hydrogen phosphate (0.013M), potassium dihydrogen phosphate (0.0014M) and sodium chloride (0.13M) was made to obtain buffer of pH-7.4.

2.2. Methods

2.2.1. Synthesis of Acrylamide grafted Sodium Alginate copolymer (NaAlg-g-Am)

Graft polymerization of Am onto NaAlg was carried out following the method published elsewhere. [13] Briefly, 0.1g of NaAlg was dissolved in deionized water under constant stirring. After complete dissolution, solution containing measured amounts of Am and KPS was added and the mixture was maintained at $60^{\circ}C$ for 2 h. The reaction mixture was then precipitated in excess of acetone. The homopolymer of acrylamide was removed by soxhlet extraction with methanol for 48 h. The grafted polymer, designated as NaAlg-g-Am was separated, dried and stored. Percentage of Am incorporated into the polymer was calculated from the following equation. [14]

$$\% \text{ conversion} = \frac{[[W]_g - W_p]}{W_m} \times 100 \qquad (1)$$

Where W_g is the weight of grafted copolymer, W_p is the weight of NaAlg and W_m is the weight of acrylamide initially taken. The preparation conditions and percentage conversion of graft copolymer samples obtained are presented in Table 1.

Table 1. Details of preparation of NaAlg-g-Am.

Polymer Fomulation code	**NaAlg (g)**	**Am (g)**	**KPS (g)**	**Conversion (%)**
NaAlg-g-Am-1	0.1	0.1	0.02	77.2
NaAlg-g-Am-2	0.1	0.1	0.03	79.6
NaAlg-g-Am-3	0.1	0.1	0.05	81.3
NaAlg-g-Am-4	0.1	0.2	0.02	86.5

NaAlg-g-Am-5	0.1	0.2	0.03	88.1
NaAlg-g-Am-6	0.1	0.2	0.05	89.8
NaAlg-g-Am-7	0.1	0.4	0.02	87.6
NaAlg-g-Am-8	0.1	0.4	0.03	90.3
NaAlg-g-Am-9	0.1	0.4	0.05	93.8
NaAlg-g-Am-10	0.1	0.5	0.02	88.7
NaAlg-g-Am-11	0.1	0.5	0.03	85.8
NaAlg-g-Am-12	0.1	0.5	0.05	78.8

2.2.2. Preparation of NaAlg-g-Am /Cts polyelectrolyte complex ((NaAlg-g-Am)-Cts).

The polyelectrolyte complex (PEC) was made with NaAlg-g-Am-6 and Cts. Aqueous solution (1% w/v) of NaAlg-g-Am-6 was made in distilled water. Cts was dissolved in 5% acetic acid solution (1% w/v). The pH of the graft copolymer and Cts solutions were adjusted to ~6.5 and ~4.0 respectively using 0.1M HCl and 10% (w/v) NaOH solution. These pHs ensured that the copolymer was fully deprotonated and that the Cts was fully protonated, which is to favour the formation of maximum number of interchain ionic bonds.[15] The NaAlg-g-Am6 solution was added drop wise to the Cts solution at a rate of 1 mL/sec. The mixture was maintained under stirring for about half an hour. The precipitate formed was washed with distilled water until no change in conductivity of filtrate solution was observed. The product designated as (NaAlg-g-Am)-Cts was dried under vacuum at 40°C .

2.2.3. IR spectroscopy

The IR spectra of Cts, NaAlg-g-Am and PEC ((NaAlg-g-Am)-Cts) samples were recorded on a IR-Prestige-21, FTIR spectrophotometer (Shimadzu, Japan). Samples were prepared as KBr pellets and scanned in the range 4000-400cm^{-1}.

2.2.4. Thermogravimetric analysis

TGA of Cts, NaAlg-g-Am and PEC ((NaAlg-g-Am)-Cts) samples were recorded on SDT Q600 V20.9 (Japan) thermogravimetric analyser. The samples were heated from zero to 800°C under nitrogen atmosphere, at a rate of 5°C/min and the weight loss were recorded.

2.2.5. Scanning Electron Microscopic (SEM) analysis:

The micrographs of Cts, NaAlg-g-Am and PEC ((NaAlg-g-Am)-Cts) samples were recorded on a JEOL-JSM5800LV scanning electron microscope. The micrographs were recorded with magnifications of 1000 under a voltage of 20 KV.

2.2.6. Swelling studies

The swelling behaviour of the PEC was investigated under different pH conditions by carrying out weight measurements in distilled water (pH 7.0) and in buffer media of pH-1.2 and 7.4. The weight measurements were made using an electronic balance with an accuracy of ±0.1mg. Pre-weighed dry complexes were immersed in excess of the buffer solution, maintained at 30°C. After specific intervals of the time, the complexes were removed from the medium, the surface adhered liquid drops were wiped with blotting paper and the increase in weight was measured. The measurements were continued till the weights of the swollen complexes attained constant values. The swelling ratio (SR) was calculated using the following expression,

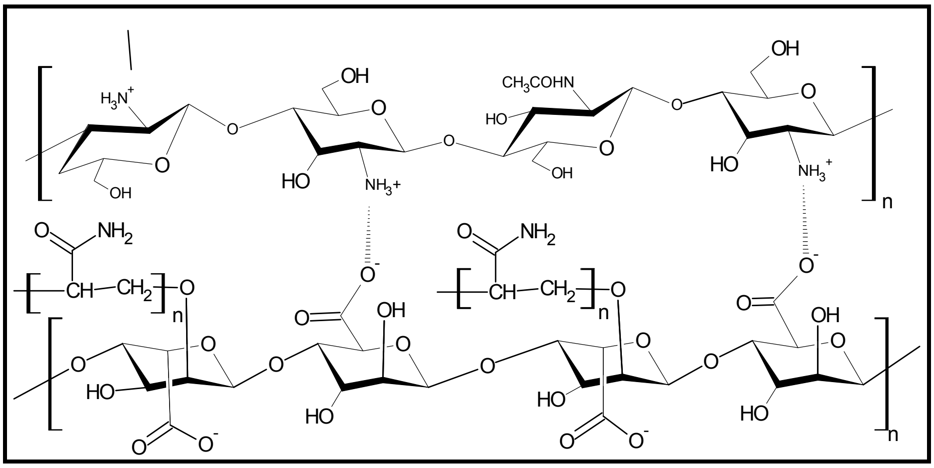
$$SR\ (g/g) = \frac{[W_t - W_o]}{W_o} \qquad (2)$$

where W_o and W_t are the weights of the PEC sample in the dry state and in the swollen state respectively.

3. 0. Results and discussion

Grafting of acrylamide on sodium alginate has been achieved by free radical polymerization technique in aqueous medium using KPS as initiator. The higher weights of the grafted product as compared to that of the starting NaAlg, has been taken as evidence of grafting. An attempt to polymerize just acrylamide under similar conditions resulted in no homopolymer formation. Hence it was concluded that under the reaction conditions employed in this investigation, the free radicals were generated exclusively on the polysaccharide backbone and polyacrylamide chains were formed as graft on NaAlg. Similar results were reported by Desmukh et al. [12, 16]

The electrostatic attraction between the cationic amino groups of Cts and the anionic carboxyl groups of the NaAlg-g-Am is the main interaction leading to the formation of the PEC.[17] The yield of the complex depends on the degree of interaction between the oppositely charged polymers, the maximum electrostatic interaction occurring in their fully ionized forms.[18] The maximum yield of complex is the signal for complete neutralization of charge between the oppositely charged polymers.[19] Experiments were performed with different ratios of polymer components and the yield of PEC precipitated in each case was determined. The results are tabulated in Table 3. The maximum yield of PEC precipitate was observed at 2:1 weight ratio of NaAlg-g-Am to Cts. The probable structure of the complex formed is shown in scheme 1.



Scheme 1. The structure of PEC from NaAlg-g-Am and Cts.

Table 2. Effect of polymer composition on the yield of the PEC at pH 5.5

Cts (%)	NaAlg-g-Am (%)	PEC (%)* (mean)
1	0.5	49.3
1	1	55.1
1	1.5	55.6
1	2	58.8
1	3	52.3
1	4	48.2

* % yield = (observed yield / theoretical yield) X 100

The degree of ionization depends on the pH, which sequentially dictates the yield of complex. Hence, the effect of pH on the yield of PEC formed was studied. The pH of aqueous solutions of NaAlg-g-Am and Cts were varied in the range, 4 to 6.0. As Cts was insoluble above pH 6.0, the experiment could not be conducted above the same. The yield of complex formation gradually increased as the pH of the solutions changed from 4.0 up to 5.5 and with a further increase in pH, a slight decrease in the yield was observed. This is may be due to the fact that with increase in pH, the number of ionized carboxylic acid groups on grafted copolymer increased where as the percentage of ionization of Cts decreased. Hence, the maximum yield was observed at the intermediate pH of 5.5, where maximum numbers of ionized groups exist on component polyelectrolytes. The data is tabulated in Table 3.

Table 3. Effect of pH on the yield of the PEC.

pH	% yield* (mean)
4.0	52.8
4.5	56.2
5.0	59.3
5.5	64.2
6.0	53.6

3.1. IR Spectroscopy

The grafting is supported by IR spectroscopy. The spectrum of NaAlg-g-Am displayed in Figure 1.(a), exhibited a peak at 3447cm^{-1} for N-H stretching vibration, a peak at 1670cm^{-1} which represented the overlapping of COO^{-} anion and primary amide C= O absorption bands, and a peak at 1317cm^{-1} due to C– N vibration. The FTIR spectrum of NaAlg-g-Am, as discussed above had characteristic peaks of both NaAlg and Am which can be considered another experimental proof of grafting.

The FTIR spectra of Cts (1.b.) exhibits a broad band at around 3443 cm^{-1} attributed to –NH and –OH stretching vibration, as well as inter- and intra-molecular hydrogen bonding. A weak band at 2926 cm^{-1} is attributed to –CH stretching. The two bands at 1640 and 1591 cm^{-1} represent bending vibrations of the free amine and acetylated amine, respectively. [20]

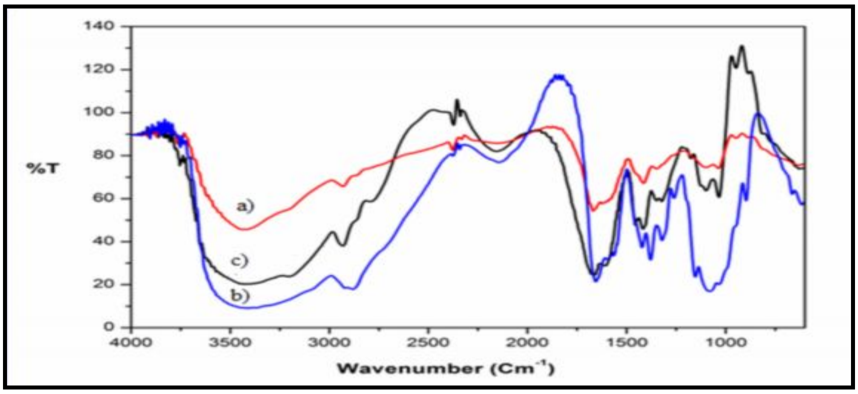


Fig.1. FTIR spectra of a) NaAlg-g-Am, b) Cts and c) ((NaAlg-g-Am)-Cts)

The spectrum of PEC (1.c) shows the characteristic peaks of both NaAlg-g-Am and Cts with minor shifts in the absorption. The doublet peaks of amide bond at 1654 and 1583 cm^{-1} observed in the spectra of Cts (1.b) has been changed nearly to a single band at 1635 cm^{-1} with increased sharpness. Moreover, the N–H bending vibration of non-acylated 2-aminoglucose primary amines (band at 1570 cm^{-1}) and asymmetric and symmetric –C–O stretching at 1596 and 1431 cm^{-1}, respectively, disappeared, indicating that the ($-NH_3^{+}$) of the Cts has reacted with the $-COO^{-}$ of the NaAlg-g-Am.

3.2 Thermogravimetric analysis

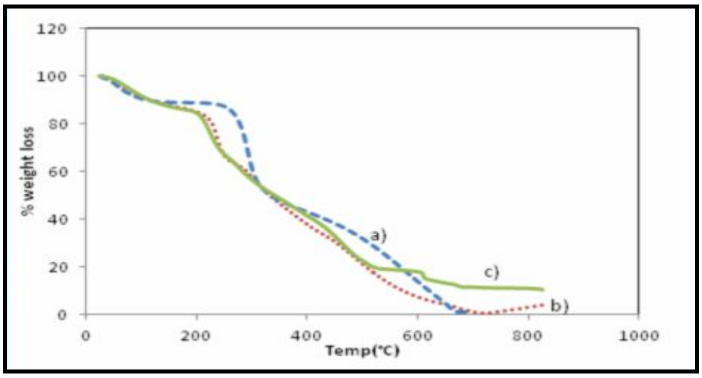
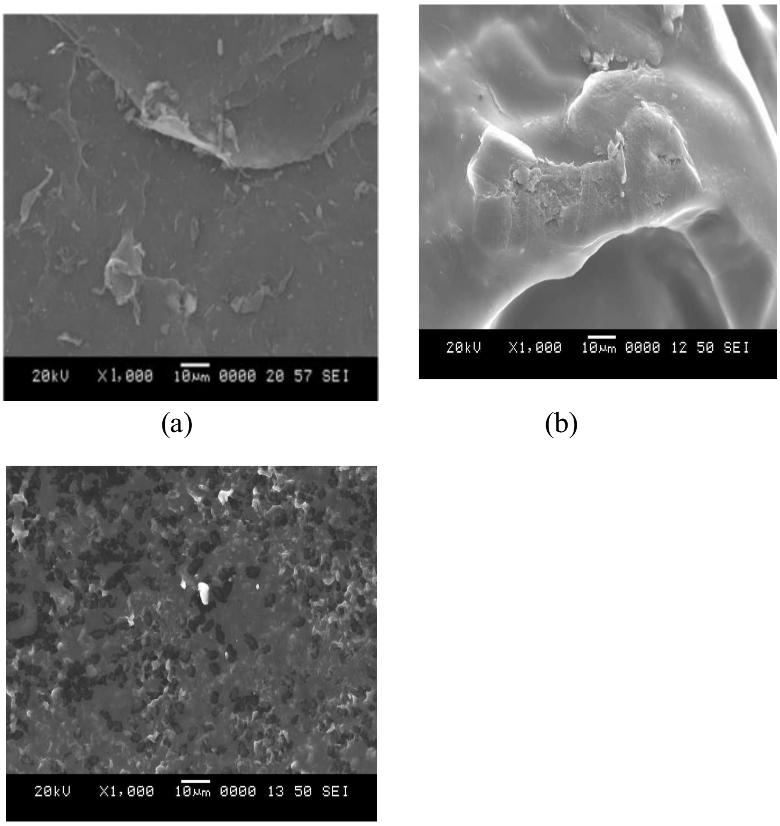


Fig.2. TGA curves of a) Cts, b) NaAlg-g-Am and c) ((NaAlg-g-Am)-Cts)

TGA of NaAlg-g-Am, Cts and PEC samples are presented in Figure 2. In case of PECs the degradation pattern is intermediate of NaAlg-g-Am and Cts was observed. The initial weight loss due to loss of water contained in PECs occurred in the range, 25-220°C. At the second stage ranging from 220 to 550°, degradation of the grafted chains occurs. At the third stage of degradation ranging from 550 to 800°C, the weight loss is attributed to the degradation of the polysaccharide. At 700°C complete weight loss occurs in the component polymers, whereas about 10% of residual remains in the PEC.

3.3. SEM Analysis

Figure 3 shows scanning electron micrographs of Cts (a), NaAlg-g-Am (b) and ((NaAlg-g-Am)-Cts) (c). The Cts sample shows fibrillar surface as reported in literature.[21] NaAlg-g-Am copolymer appears to have smooth surface. PEC shows porous, irregular morphology which is entirely different from component polyelectrolytes.



(a) (b)

(c)

Fig.3. SEM micrographs of (a) Cts, (b) NaAlg-g-Am and (c) ((NaAlg-g-Am)-Cts)

3.4.0 Swelling behaviour of PEC

The swelling behaviour of ((NaAlg-g-Am)-Cts) has been studied under different pH and ionic strength conditions and the results are discussed below.

3.4.1 Effect of pH on swelling

The formation of PEC is based up on the electrostatic interaction between the oppositely charged polymers. Thus, the degree of ionization of polymers can dictate the efficiency of PEC formation. Once the PEC is formed, the charge balance inside the PEC could change with the pH; the degree of interaction between the two polymers would modify accordingly and swelling may occur because of dissociation of the complex.[9]

The swelling data obtained for the PEC in distilled water and in media of pH 1.2 and 7.4 are shown in Figure 4. The PEC shows an initial stage of quick swelling followed by a gradual increase leading to equilibrium situation. The equilibrium swelling ratio is observed to be highly dependent on the swelling conditions. The initial fast-swelling behavior of the PEC observed here agrees with other reports.[22] At the initial equilibrium (maximum swelling), the Cts and surrounding graft copolymer are in meta-stable equilibrium due to sufficient interactions as well as interlocking between them. On further exposure of samples to the sorption medium, the solvation energy dominates on the interactions between the graft polymer and Cts, thus leads to the second-stage swelling (equilibrium swelling), which is more time dependent than the first-stage swelling.

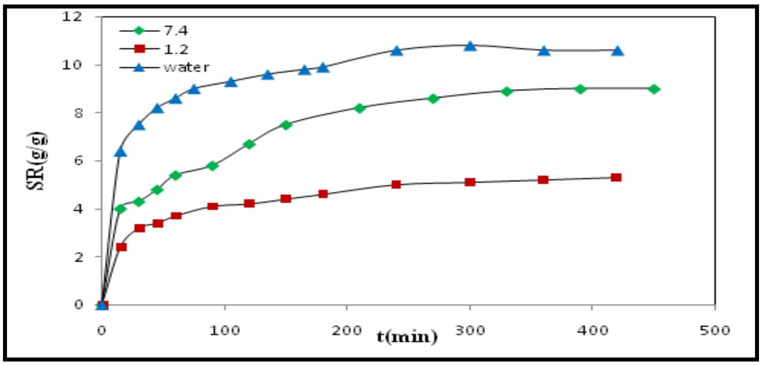


Fig.4. Swelling behaviour of PEC

The swelling behavior of the ((NaAlg-g-Am)-Cts) system is shown in Figure 4. It is observed that the complex has a lower degree of swelling at pH 1.2. Alginate at pH 1.2 is in its unionized form while Cts is fully protonated at this pH, as the pKa of Cts is 6.0. This explains the low degree of swelling of the complex. The swelling process is controlled by the Donnan potential, [23] i.e. the process depends on the number of ionized groups in the complex. Therefore, it is expected that the complexe show a lower degree of swelling at this pH. The swelling degree of complex increased to a maximum at pH 7.0(water) and then decreased due to disintegration and dissolution of complex chain. At pH 7.4, deprotonation of Cts weakens the extent of ionic interactions leading to dissociation of complex.

3.4.2 Effect of ionic strength on swelling

The swelling behaviour of PEC was investigated with salt solution of various concentration and the results are displayed in Figure 5**.** It is evident that swelling is depended on concentration of salt added to the swelling medium. The swelling of the complex gradually increases with increase in the ionic strength of the solution. Under low ionic strength condition, the PECs tend to be in an extended and uncoiled form due to the intramolecular repulsive interactions between the charged units. Further increase in ionic strength above 1.0 result in lowering of swelling attributed to the screening effect of the electrolyte resulting in contraction of the complex structure.

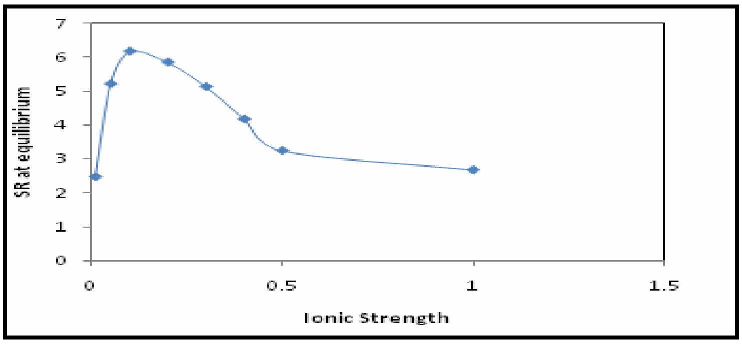


Fig.5. Effect of ionic strength on swelling of ((NaAlg-g-Am)-Cts)

3.4.3 Kinetics of swelling

The swelling and diffusion kinetic parameters, namely, initial swelling rate (ri, g (water)/g (PEC) min^{-1}), swelling rate constant (K_s, g PEC/g water min^{-1}), maximum equilibrium swelling ratio (S_{max}, g water/g PEC) ,diffusion constant (n) and diffusion coefficients (D, cm^2 s^{-1}) were calculated using the dynamic swelling data. The various plots for the sample ((NaAlg-g-Am)-Cts) are shown in Figure 6,7 and 8. The swelling and diffusion parameters obtained using standard methods [24] are listed in Table 4. The linearity of the plot 't/SR' vs 't' (Fig. 6) indicates that the swelling process follows second-order kinetics. It is confirmed that the S_{eq} calculated from the slopes are in good agreement with the ratio determined experimentally by swelling measurements. The diffusion coefficients 'D' was calculated from the slope of the lines displayed in Figure 7. Swelling exponents 'n' was calculated from the slopes and intercepts of the lines of 'ln(SR)' vs. 'ln(t)' plots (Fig. 8) were less than 0.5, indicating the mechanism of water diffusion is to be fickian.

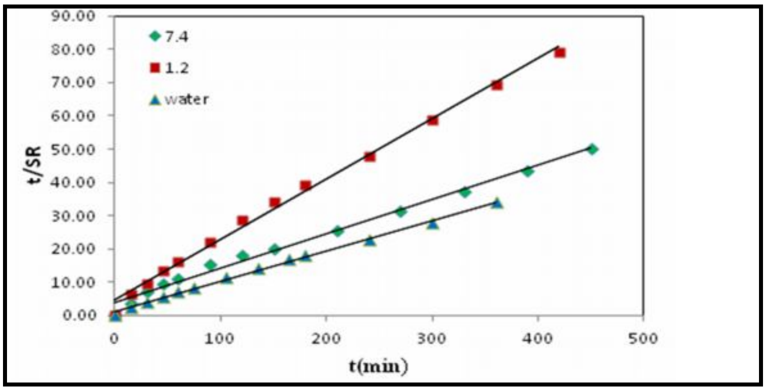


Fig.6. Plot of t/SR vs t for ((NaAlg-g-Am)-Cts)

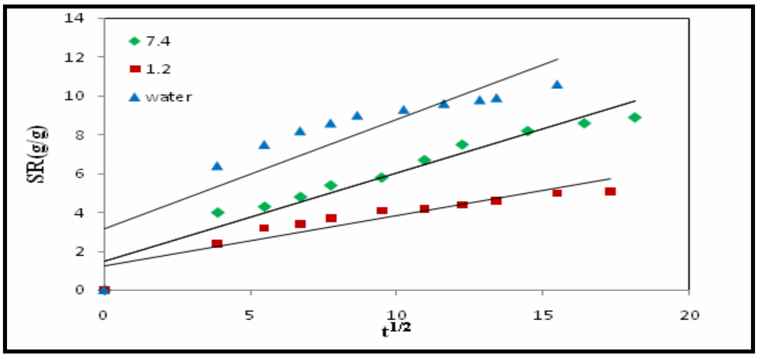


Fig.7. Plot of SR vs $t^{1/2}$ for ((NaAlg-g-Am)-Cts)

Table.4. Swelling parameters for ((NaAlg-g-Am)-Cts)

Medium	EWC(%)	Equilibrium Swelling ratio(g/g)	Theoretical S_{eq}(g/g)	r_i(g/g)/ min	K_s(g/g)/min in (10^{-3})	Swelling Exponent (n)	D(cm^2/sec)
Water	91.5	10.6	11.0	0.79	7.4	0.404	0.563
pH-1.2	84.6	5.3	5.5	0.22	2.9	0.284	0.259
pH-7.4	90.3	9.0	9.7	0.26	6.6	0.366	0.453

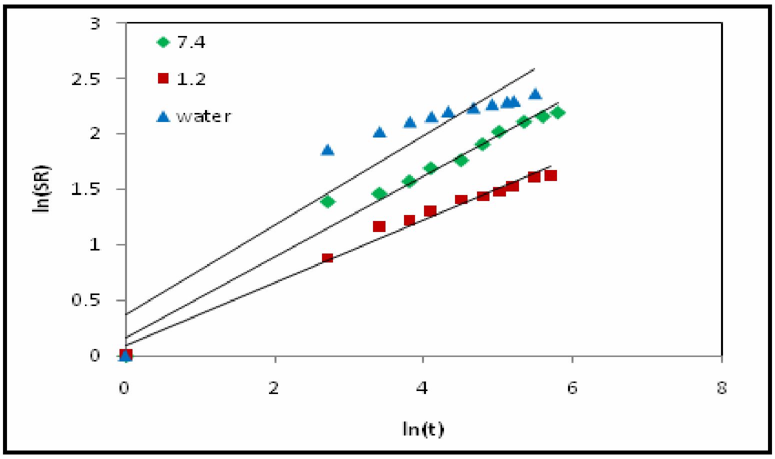


Fig.8. Plot of ln(SR) vs ln(t) for ((NaAlg-g-Am)-Cts)

4. Conclusion

This present investigation confirms the formation of PEC between NaAlg-g-Am and Cts. The maximum amount of the complex was formed at pH 5.5. The stoichiometry of the complex was established to be 1:2 (Cts and NaAlg-g-Am). The PEC exhibited pH dependent swelling with maximum swelling at neutral condition and minimum at acidic pH (pH 1.2). Increasing the ionic strength above 1.0 M resulted in the decomposition of the complex.

The kinetic analysis of the swelling of the complex indicated the mechanism of water diffusion to be fickian with n being less than 0.5. The swelling process is observed to follow second-order kinetics. The pH responsive swelling behaviour of the complex makes it an ideal matrix material for controlled release of drugs.

References

1. Michaels A.S., Polyelectrolyte complexes, Ind. Eng. Chem. Res., 1965, 57, 32-40.
2. Tsuchida E and Abe K., Interactions between macromolecules in solution and intermacromolecular complexes, Adv. Polym. Sci., 1982, 45, 1-119.
3. Philipp B., Dautzenberg H., Linow K.J., Koetz J and Dawydoff W., Polyelectrolyte complexes: Recent development, open problems, Prog. Polym. Sci., 1989, 14, 91-172.
4. Vishalakshi B., Ghosh S and Kalpagam V., The effects of charge density and concentration on the composition of polyelectrolyte complexes, Polymer, 1993, 34, 3270-3275.
5. Berger J., Reist M., Mayer J.M., Felt O and Gurny R., Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications, Eur. J. Pharm. Biopharm., 2004, 57, 35–52.
6. Muzzarelli R.A.A., Chitin. Pergamon Press, Oxford, U.K., 1977.
7. Rinaudo M., Chitin and chitosan: properties and applications, Prog. Polym. Sci., 2006, 31, 603–632.
8. He Z and Wang K., Alginate-konjac glucomannan-chitosan beads as controlled release matrix, Int. J. Pharm., 2002, 244, 117–126.
9. Sankalia M.G., Mashru R.C., Sankalia J.M., and Sutariya V.B., Reversed chitosan-alginate polyelectrolyte complex for stability improvement of alpha-amylase: optimization and physicochemical characterization, Eur. J. Pharm. Biopharm., 2007, 65, 215–232.
10. Maricarmen R., Luisa L.S., Carlos P., Francisco M.G., Marguerite R., Julio S.R and Waldo M.A., Thermo- and pH-responsive polyelectrolyte complex membranes from chitosan-g-N-isopropylacrylamide and pectin, Carbohydr. Polym., 2011, 86, 1336– 1343.
11. Krishna Rao K.S.V., Ildoo C and Chang-Sik H., Synthesis and characterization of poly(acrylamidoglycolicacid) grafted onto chitosan and its polyelectrolyte complexes with hydroxyapatite, React. Funct. Polym., 2008, 68, 943–953.
12. Deshmukh S.R., Sudhakar K and Singh R.P., Drag-reduction efficiency, shear stability, and biodegradation resistance of carboxymethyl cellulose-based and starch-based graft copolymers, J. Appl. Polym. Sci., 1991, 43, 1091-1101.
13. Sanchita M., Ray R., Basu S.K., and Biswanath S., Evaluation of a Matrix Tablet Prepared with Polyacrylamide-g-Sodium Alginate Co-polymers and Their Partially Hydrolyzed Co-polymers for Sustained Release of Diltiazem Hydrochloride, J. of Biomaterials Science, Polym. Ed., 2010, 21, 1799-1814.
14. Tripathya T., Pandeya S.R., Karmakara N.C., Bhagat R.P and Singh R.P., Novel flocculating agent based on sodium alginate and acrylamide, Eur. Polym. J., 1999, 35, 2057-2072.
15. Saether H.V., Hilde K.H., Gjertrud M., Olav S and Bjorn T.S., Polyelectrolyte complex formation using alginate and chitosan, Carbohydr. Polym., 2008, 74, 813–821.
16. Deshmukh S. R., Ph.D. Thesis, 1986, IIT Kharagpur, India.
17. Lee J.W., Kim S.Y., Kim S.S, Lee Y.M., Lee K.H and Kim S.J., Synthesis and characteristics of interpenetrating polymer network hydrogel composed of chitosan and poly(acrylic acid), J. Appl. Polym. Sci., 1999, 73, 113–120.
18. Denuziere A., Ferrier D., Damour O and Domard A., Chitosan-chondroitin sulfate and chitosan-hyaluronate polyelectrolyte complexes: biological properties, Biomaterials, 1998, 19, 1275–1285.
19. Chavasit V and Torres J.A., Chitosan-poly (acrylic acid): mechanism of complex formation and potential industrial applications, Biotechnol. Prog., 1990, 6, 2–6.
20. Chethan P. D and Vishalakshi B., Synthesis of ethylenediamine modified chitosan and evaluation for removal of divalent metal ions, Carbohydr. polym., 2013, 97, 530–536.
21. Vandana S., Ashutosh T., Devendra N.T., Rashmi S., Microwave enhanced synthesis of chitosan-graft-polyacrylamide, Polymer, 2006, 47, 254-260.
22. Peniche C., Monal W.A., Davidenko N., Sastre R., Gallardo A and Roman J.S., Self-curing membranes of chitosan/PAA IPNs obtained by radical polymerization: preparation, characterization and interpolymer complexation, Biomaterials, 1999, 20, 1869–1878.

23. Tapia C., Costa E., Moris M., Sapag-Hagar J., Valenzuela F and Basualto C., Study of the influence of the pH media dissolution, degree of polymerization, and degree of swelling of the polymers on the mechanism of release of diltiazem from matrices based on mixtures of chitosan/alginate, Drug Dev. Ind. Pharm., 2002, 28, 217–224.
24. Keshava Murthy P. S., Murali Mohan Y., Sreeramulu J and Mohana Raju K., Semi-IPNs of starch and poly(acrylamide-co-sodium methacrylate): Preparation, swelling and diffusion characteristics evaluation, React. Funct. Polym., 2006, 66, 1482-1493.

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