Characterization of Gamma Irradiated Concentrated Aqueous Solutions of Chitosan/Sodium Alginate Blends and Their Drug Uptake-Release Characters

Horia M. Nizam El-Din,[1] Abdel Wahab M. El-Naggar[2]

[1]*Polymer Chemistry Department, National Center for Radiation Research and Technology, Cairo, Egypt*
[2]*Radiation Chemistry Department, National Center for Radiation Research and Technology, Cairo, Egypt*

Received 26 December 2010; accepted 8 February 2011
DOI 10.1002/app.34320
Published online 17 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Blends films based on different ratios of concentrated aqueous solutions of chitosan (CS) and sodium alginate (AG) in the presence of 1% of glutaraldehyde, as a cross-linking agent for chitosan, were prepared by solution casting and then exposed to gamma irradiation. The formed blends were characterized by IR spectroscopic analysis, differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). The uptake-release properties of CS/AG blends, taking ketoprofen as an example for drug, were also investigated. DSC thermograms of CS/AG blends revealed good miscibility was sustained between CS and AG. The water uptake and gel content of CS/AG blends was found to decrease by increasing the ratio of AG in the initial solution. The IR spectra indicated the formation of cross-linking and hydrogen bonding, while the TGA study showed that the CS/AG blends displayed higher thermal stability than pure CS polymer. Based on Fick's law, it was demonstrated that the main parameters affecting the release of ketoprofen drug from the CS/AG blend hydrogels were composition and pH. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 2383–2390, 2011

Key words: chitosan; alginate; gamma irradiation; blends; controlled release of ketoprofen

INTRODUCTION

There has been an increasing interest in blending different homopolymers to obtain new products having some of the characteristic properties of each component. The demand for compatible blends may constitute one of the most important attractive trends in the field of polymer blends. This is because a wide variety of properties can be tailored from the simple physical combination of two compatible polymeric components. Radiation technique may be considering a very convenient tool for the modification of polymer materials through cross-linking, grafting, or degradation. In this regard, the miscibility of poly(vinyl alcohol) (PVA)/polyacrylamide blends before and after gamma irradiation was investigated by different methods.[1]

Chitosan, the deacetylated derivative of chitin, is one of the most abundant naturally occurring polysaccharides and has good film forming properties, excellent biodegradability, biocompatibility, antimicrobial activity, and accelerated wound healing properties.[2–4] On the other hand, sodium alginate is a naturally occurring nontoxic polysaccharide found in all species of brown algae and is widely used in food and pharmaceutical industries. It contains two uronic acids, β-(1-4)-linked D-mannuronic acid (M), and α (1-4) linked L-guluronic acid (G), and is composed of homopolymeric blocks M–M or G–G, and blocks with an alternating sequence of M–G blocks.[5]

A suitable delivery carrier system must be characterized by: (1) the drug is released at the right site, in the right dose and for the required time, (2) they are biocompatible or biodegradable, and (3) the drug is transformed into nontoxic natural or synthetic polymers that are eliminated harmlessly from the body.[6–10] Films of alginate and gelatin, cross-linked with Ca^{2+}, with ciprofloxacin hydrochloride as model drug incorporated in different concentrations, were obtained by a casting/solvent evaporation method.[11] The results of controlled release tests showed that the amount of ciprofloxacin hydrochloride released decreased with an increase in the proportion of gelatin present in the film. Moreover, the release rate of drug decreased as the amount of drug loaded in the film increased. A series of pH-sensitive composite hydrogel beads composed of chitosan-g-poly (acrylic acid)/attapulgite/sodium alginate (CTS-g-PAA/APT/SA) was prepared as drug delivery matrices cross-linked by Ca^{2+} owing to the ionic gelation of SA.[12] The results showed that the composite hydrogel beads had good pH-sensitivity. The

Correspondence to: Horia M.M. Nizam El-Din (nizam_eldin@yahoo.com).

cumulative release ratios of diclofenac sodium (DS) from the composite hydrogel beads were 3.76% in pH 2.1 solution and 100% in pH 6.8 solutions within 24 h, respectively. The effect of compatibility on the structure of the microporous membrane prepared by selective dissolution of chitosan polymer blends membrane with poly(vinyl pyrrolidone) (PVP) and poly(ethylene glycol) (PEG) was investigated.[13] Results of Fourier transform infrared (FTIR) characterization, differential scanning calorimeter (DSC) analysis, wide angle X-ray diffraction (WAXD) measurements showed that there are special interactions between chitosan and the counterpart polymers. Films of chitosan and PEG, with ciproXoxacin hydrochloride as model drug incorporated at different concentrations, were obtained by a casting/solvent evaporation method.[14] The results of controlled release tests showed that the amount of ciproXoxacin hydrochloride released increased with an increase in the proportion of PEG and decreased as the amount of drug loaded in the film increased; however, the cumulative release amount of the drug increased. The chitosan/PEG films were also found sensitive to pH and ionic strength. The diffusion of acetaminophen in alginate gels cross-linked with calcium or zinc ions was studied.[15] Recently, chitosan based hydrogels sensitive to changes of external conditions such as pH [16] temperatures,[17] and electric currents [18] are receiving increasing attention as drug delivery carriers. The effect of preparation method on the release behavior of brilliant blue from calcium alginate gel beads coated by chitosan was investigated.[19]

Polysaccharides such as cellulose, starch, chitin/chitosan and their water-soluble derivatives have been known as degradable type polymers under action of ionizing radiation.[20] It was found that water-soluble polysaccharides derivatives such as carboxymethyl cellulose (CMC), carboxymethyl starch (CMS), and carboxymethyl chitin (CMCT), carboxymethyl chitosan (CMCTS) lead to radiation cross-linking at high oncentrated aqueous solution (more than 10%, paste-like state). The effect of temperature and pH on the degree of swelling in water was also studied. A series of excellent hydrogels were prepared from PVA and carboxymethylated chitosan (CM-chitosan) with electron beam (EB) at room temperature.[21] Electron spectroscopy analysis of the blend hydrogels revealed that good miscibility was sustained between CM-chitosan and PVA. Blend hydrogels based on the CMC and carboxymethyl chitosan (CMCts) were prepared by γ-irradiation of a high concentrated CMC/CMCts aqueous solution.[22] Properties of the hydrogels, such as gel fraction, swelling ratio, gel strength, and metal adsorption for Pb and Au were investigated.

In previous studies, we were interested in hydrophilic hydrogels based on natural polymers. In this respect, hydrogels of PVA and AG were produced by exposure to EB irradiation.[23] Hydrophilic hydrogels based on poly(acrylic acid) as synthetic polymer and sodium alginates as natural polymer (AG) were prepared by gamma irradiation.[24] Recently, the swelling and drug release properties of acrylamide/CMC networks formed by gamma irradiation have been studied.[25] The objective of this work was to explore the formation of hydrogels by gamma irradiation of aqueous concentrated solutions of blends based on chitosan and sodium alginate. The structural properties of blend hydrogels were characterized by different spectroscopic techniques. In addition, the uptake-release characters of ketoprofen as a model drug were studied.

EXPERIMENTAL

Materials

Sodium alginate (AG) used in this study was a laboratory grade chemicals high viscosity obtained from Aldrich Chemical Co. (Milwaukee, WI) and used as received. Chitosan with 85% degree of deacetylation and molecular weight of 30,000 was purchased from Koyo Chemicals, Japan. Glutaraldehyde, pure grade chemicals was used as a cross-linking agent for chitosan.

Preparation of CS/AG blends films

The CS/AG blends films, in the presence of glutaraldehyde (1 wt %) as a cross-linking agent for CS, was prepared by solution casting as follows: Concentrated aqueous solution of CS (20%) was dissolved in equal ratios of methanol and distilled water, whereas AG was dissolved in distilled water. The CS and AG solutions were mixed with continues stirring until homogenous solutions were obtained. The different CS/AG blends were prepared such that to obtain ratios of 80/20, 60/60, and 50/50 wt %. The blend solutions were poured into Petri dishes and dried at 37°C. The films were washed thoroughly with distilled water and dried again at 37°C. The sample films were put in polyethylene pages, made air free by purging nitrogen for 5 min at least and sealed. The blends samples were gamma irradiated at a dose rate of 10 kGy/h at room temperature. The blends films were washed with excess distilled water to remove unreacted polymers. Gamma irradiations were carried out using ^{60}Co γ-ray source at the National Center for Radiation Research and Technology, Cairo, Egypt.

Gel content determination

Dry samples of the prepared blends were accurately weighed (W_o) and then extracted with distilled water using soxhlet system for 6 h. After extraction,

the samples were removed and dried in vacuum oven at 80°C to constant weight (W_g). The gel content was calculated according to the following equation:

$$\text{Gel content } (\%) = (W_g)/W_o) \times 100$$

FTIR spectroscopic analysis

The IR spectra of the different polymers were performed over the range 500–4000 cm^{-1}. The IR spectra were acquired by transmission FTIR with a Mattson 5000 FT-IR spectrometer. The spectra were taken with resolution of 4 cm^{-1} and were averaged over 16 scans.

Differential scanning calorimetry

DSC measurements were performed using a Shimadzu DSC calorimeter (Kyoto, Japan) equipped with data station. A heating rate of 10°C/min was utilized and the scans were carried out under a flowing nitrogen gas at a rate of 20 mL/min.

Thermogravimetric analysis

The TGA thermograms were performed on a Shimadzu instrument (Kyoto, Japan) at a heating rate of 10°C/min under flowing nitrogen (20 mL/min) from room temperature to 500°C.

Swelling studies

Swelling studies were conducted on CS/AG blends films as a function of time, in which a dry weight of insoluble blend (W_1) was immersed in water at 25°C and pH of 7 for different intervals of time durations up to 24 h. After each time interval, the sample was withdrawn and blotted on filter paper to remove excess water and weighed (w_t), in which the swelling was calculated according to the following equation:

$$\text{Swelling } (\%) = [(W_t - W_1)/W_1] \times 100$$

Loading and release of ketoprofen drug

Ketoprofen was used as a model drug for the drug controlled release studies. Dry weight of gels films (0.5 g) of CS/AG blends were immersed in different aqueous solutions (0.2–0.8 g/L) of ketoprofen at pH 7 and at room temperature for 72 h until saturation. The drug-loaded gels were dried at room temperature. The uptake of ketoprofen was estimated in terms of g drug/g gel (%). Ketoprofen drug was allowed to release in 50 mL buffer solutions of different pH values[2–8] at room temperature. At various time intervals, aliquots of 3 mL were drawn from the medium to follow the release of ketoprofen and returned into the vessel so that the solution volume is kept constant. Ketoprofen release was determined by a spectrophotometric method using a Unicam 8625 UV/visible spectrophotometer at λ_{max} 272 nm.

RESULTS AND DISCUSSION

Formation of CS/AG blend gels

Different substances have been used to cross-link chitosan; the most usual compounds are glutaraldehyde, formaldehyde, and other dialdehydes.[26–28] Glutaraldehyde has two aldehyde groups, separated by a flexible chain of three methylene bridges (OHC—$(CH_2)_3$—CHO). The mechanism of cross-linking of chitosan by glutaraldehyde is well-documented; the aldehyde groups form covalent imine bonds with the amino groups of chitosan, due to the resonance established with adjacent double ethylenic bonds [27] via a Schiff reaction. In this work, gamma irradiation was used *in situ* with glutaraldehyde for two reasons; to avoiding using concentrated solutions and to minimize irradiation dose and hence avoiding the degradation of alginate component. Preliminary experiments were carried out to explore the most suitable conditions to obtain homogenous blend hydrogels based on different ratios of CS/AG: (1) By using gamma irradiation individually up a dose of 40 kGy, cross-linked CS/AG based on different ratios of AG up to 50 wt %, transparent films were obtained with low gel contents, (2) when the same experiment was carried out in the presence of 3 wt/vol % of glutaraldehyde *in situ* of gamma irradiation, cross-linked CS/AG blend films were obtained. This finding indicates that the cross-linking agent glutaraldehyde enhanced the cross-linking of CS by gamma irradiation. The cross-linking enhancers during irradiation is reported, in which these materials do not directly enter into cross-linking reactions but enhance secondary reactions that lead to the formation sites.[29] The formation of free radicals on the backbone of alginate upon gamma irradiation will eventually leads to oxidative degradation to some extents, however, it cannot leach out due the formation of CS network structure. Thus it may suppose that CS/AG blends form semi-interpenetrating network structure, in which AG polymer chains were included inside the cross-linked CS.

The gel content and swelling in water of different ratios of CS/AG blend gels prepared at different doses of gamma irradiation is shown in Figure 1. It is clear that the gel content decreases with increasing the ratio of AG in initial solutions and increases with increasing irradiation dose. However, the swelling was found to increase with increasing AG ratio and decreases with increasing irradiation dose.

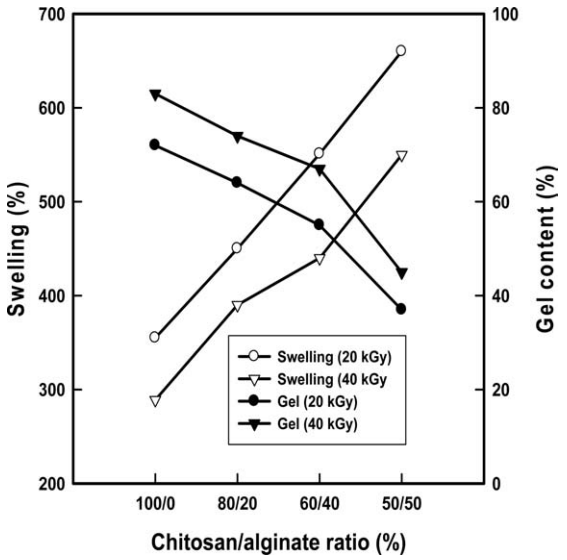


Figure 1 Gel content and swelling in water at room temperature (pH 7) of different ratios of CS/AG blends prepared at different doses of gamma irradiation.

IR spectroscopic

To determine the formation of the CS/AG (CS/AG) blend hydrogels, the IR spectra of the formed gels at a dose of 20 kGy of gamma irradiation, after extraction of sol part, were studied as shown in Figure 2. For the hydrogel based on pure chitosan, the sharp band at 1560 cm^{-1} corresponds to the carboxyl group and $-NH_3$ [20] confirms the contribution of chitosan into the gel structure. Pure alginate was reported to show two characteristic absorption bands at 1627 and 1409 cm^{-1} attributed to the asymmetric and symmetric stretching vibration of COO group, respectively,[11], while wide absorption band around 3421 cm^{-1} was due to the stretching vibration of O—H bonded to *N*—H. As shown in Figure 2, the IR spectra of pure chitosan and the different (CS/AG) blends showed intensive bands with increased intensity within the rang 2865–2957 cm^{-1} due to the stretching absorption band of C—H groups, like almost organic compounds. This band was shifted to 2976–2883 cm^{-1} and 2957–2865 cm^{-1} in the IR spectra of CS/AG blends containing 20 and 50% AG, respectively. It can be also noticed that band at 1560 cm^{-1} was shifted to 1606 cm^{-1} in the IR spectra of CS/AG (80/20%) blend and to 1569 cm^{-1} in the CS/AG (50/50%) blend due to the formation of hydrogen bonding between CS and AG. The stretching vibration of *N*—H group bonded to O—H group, was shifted from 3282 to 3338 cm^{-1} in the IR spectrum of pure CS to a lower wavenumbers within the rang 3374–3430 cm^{-1} and 3485 cm^{-1} in the IR spectra of the blends containing 20 and 50% AG, respectively. This finding suggests an increase in the hydrogen bonding. All those changes show a strong evidence of the intermolecular interactions and good molecular compatibility between AG and CS.

Differential scanning calorimetry

Figure 3 and Table I show the DSC curves and kinetic parameters of gels based on pure CS and CS/AG of different ratios prepared by gamma irradiation at a dose of 20 kGy, respectively. The curves of all gels showed an endothermic peaks with onset around 150°C corresponding to the crystalline melting temperature (T_m). It can be seen that the aria under these peaks (heat of fusion ΔH_f) increases with increasing AG ratio up to 50%. However, the T_m was found to decrease with increasing AG ratio. The decrease of T_m and ΔH_f values may be due to the stiff molecular chains of AG, which have a significant effect on the overall chain mobility in the mixture and retards the rate of crystal growth.[30] In these DSC scans, the glass transition temperature (T_g) of neither pure CS or AG can be detected indicating the compatibility between them in their blends. In addition, the decrease in T_m and increase in ΔH associated with increasing the ratio of AG indicates that CS and AG form a strong hydrogen-bonding interaction between them.

Thermogravimetric analysis

The dissociation energies of the covalent bonds C—H, C—C, C=O, C—O, C—N, C=N, and O—H were reported to be 414, 347, 741, 351, 293, 615, and

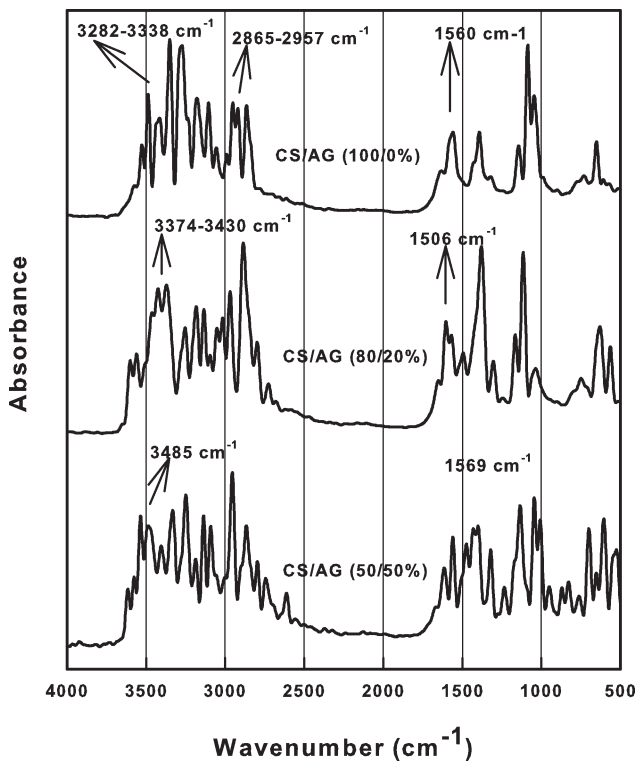


Figure 2 IR spectra of different ratios of CS/AG (CS/AG) blend hydrogels formed by gamma irradiation at a dose of 20 kGy.

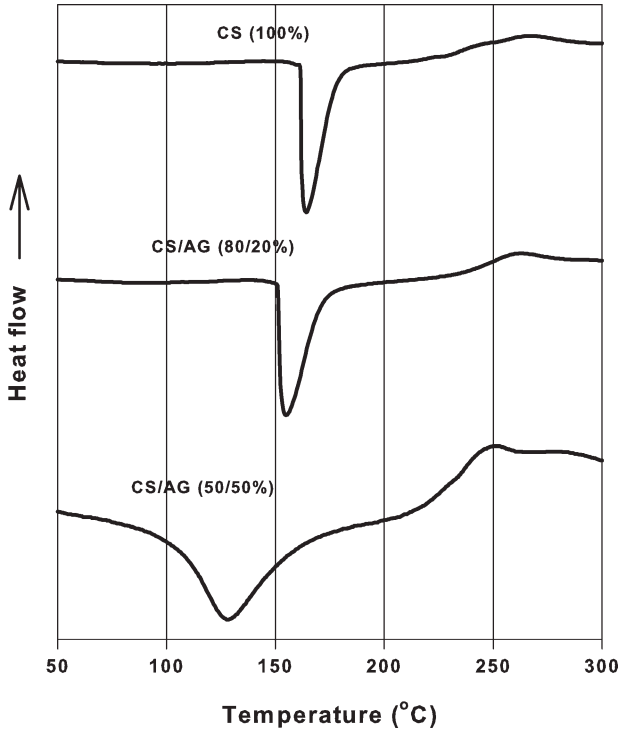


Figure 3 DSC curves of gels based on pure CS and CS/AG of different ratios prepared by gamma irradiation at a dose of 20 kGy.

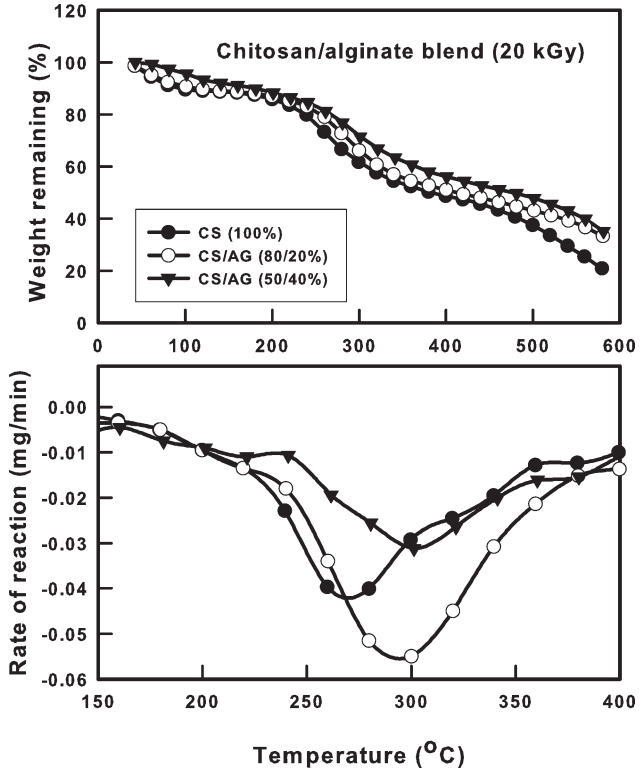


Figure 4 TGA thermograms and the corresponding rate of thermal decomposition reaction curves of hydrogels based on pure CS and CS/AG blends of different ratios prepared by gamma irradiation at a dose of 20 kGy.

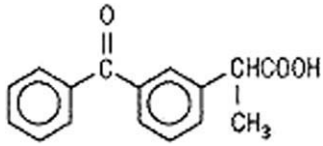
464 kJ/mol, respectively.[31] According to these values, the average complete dissociation energy of sodium alginate and chitosan polymers is calculated to be 487.7 and 403.1 kJ/mol, respectively. These values indicate that alginate possesses higher dissociation energy than chitosan. Thus, it may be expected that the formation of blends based on different ratios of chitosan and sodium alginate will results in materials with higher thermal stability than pure chitosan component. Thermogravimetric analysis (TGA) was used to investigate experimentally the thermal stability of CS/AG blend hydrogels formed by gamma irradiation at a dose of 20 kGy as shown in Figure 4. The different TGA kinetic parameters taken from these curves are shown in Table II**.** It can be seen that the thermal decomposition against heating temperatures for the hydrogels based on pure CS and CS/AG blends displayed similar behavior, in which CS/AG based blends showed higher thermal stability than pure CS hydrogel. According to the derivative of the TGA thermograms (DTGA) curves and the different temperatures (T_{onset}, T_{endset}, and T_{peak}), the thermal stability of blends are higher than the CS hydrogel, in accordance with the theoretical calculations based on the average complete dissociation energies. The existence of single T_{peak} in the rate of reaction curves of pure CS and CS/AG blends indicates the occurrence of compatibility between the two components in their blends.

TABLE I
DSC Kinetic Parameters of CS/AG Blends Prepared by Gamma Irradiation at a Dose of 20 kGy

Chitosan/alginate blends	T_m (°C)	ΔH (J/g)
CS (100%)	164.2	34.83
CS/AG (80/20%)	154.9	39.14
CS/AG (60/40%)	124.7	444.48
CS/AG (50/40%)	127.9	403.23

Ketoprofen uptake and release studies

Ketoprofen is a nonsteroidal anti-inflammatory, analgesic, and antipyretic drug. The chemical name for ketoprofen is 2-(3-benzoylphenyl)-propionic acid with the following structural formula:



First, a standard curve representing the UV absorbance of different concentrations of ketoprofen (at λ_{max} = 272 nm) was constructed and the relation

TABLE II
TGA Kinetic Parameters of the Thermal Decomposition of CS/AG Blends Prepared by Gamma Irradiated at a Dose of 20 kGy

Chitosan/alginate blends	T_{onset} (°C)	T_{endset} (°C)	T_{peak} (°C)
CS (100%)	210	336	280
CS/AG (80/20%)	236	347	300
CS/AG (50/50%)	238	379	301

correlating this curve (not shown) can be written as: Concentration (mg/L) = absorbance/0.0147.

From this relation, the concentration of unknown sample can be determined. Before the release experiment, the uptake (%) of different concentrations of ketoprofen was measured as shown in Figure 5. In general, it can be seen that the uptake increases with increasing the concentration of ketoprofen. However, the uptake was found to decrease with increasing the ratio of alginate in the initial solution. As shown in Figure 1, the swelling was found to increase with increasing the ratio of AG in the blends. It seems that the increase swelling of solutions impedes the uptake of ketoprofen drug.

The accumulated release (%) profile of ketoprofen at different pH values as a function of time from CS/AG blend hydrogels, prepared by gamma irradiation at a dose of 20 kGy, is shown in Figures 6 and 7. It can be seen that the release from the hydrogel increases substantially with time up to 100 min and then tends to level off, in which the release at pH 8 is almost ~ 2 times at pH 2.

The nature of ketoprofen transport mechanism from CS/AG blend hydrogels was analyzed according to the Fick's law according to the following equation[32]:

$$F = M_t/M_e = Kt^n$$

or

$$\mathrm{Ln}\ F = \ln K + n \ln t$$

where M_t/M_e is the fraction of drug released (mg drug/g gel) at time t (min) and drug released at equilibrium, K is a constant related to the drug

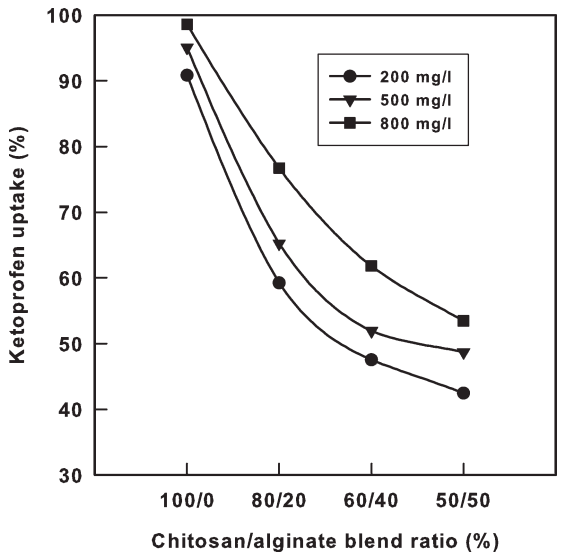


Figure 5 Uptake of different concentrations of ketoprofen drug by different ratios of CS/AG blends, gamma prepared at a dose of 20 kGy.

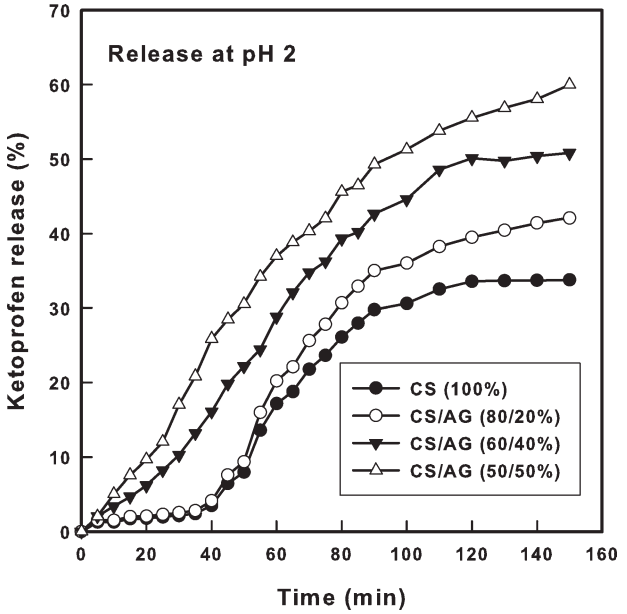


Figure 6 Release profile of ketoprofen drug at pH 2 from different ratios of CS/AG blends, prepared by gamma irradiation at a dose of 20 kGy.

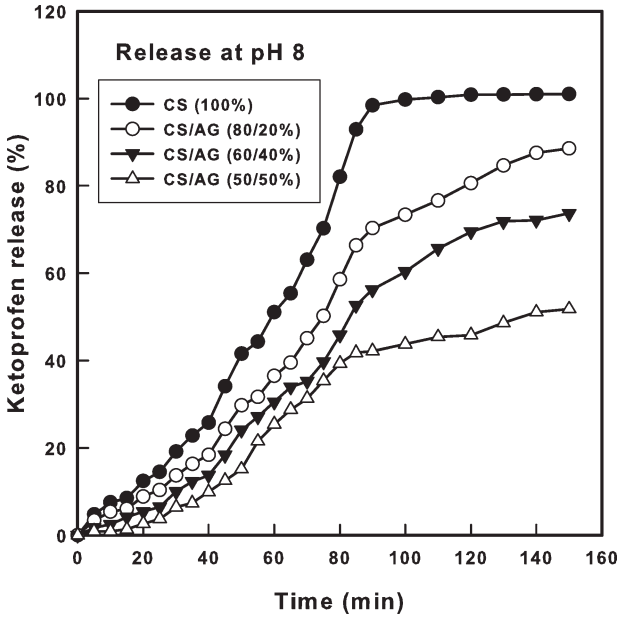


Figure 7 Release profile of ketoprofen drug at pH 8 from different ratios of CS/AG blends, prepared by gamma irradiation at a dose of 20 kGy.

TABLE III
Kinetic Parameters of Ketoprofen Release at Different pH Values from Pure Chitosan (CS) and Different Ratios of Chitosan/Alginate (CS/AG) Blends Prepared by Gamma Irradiation at a Dose of 20 kGy (by applying Fick's law)

	pH = 2			pH = 8		
CS/AG blend (%)	*K*	*n*	r^2	*K*	*n*	r^2
100/0	0.0126	0.92	0.9456	0.0133	0.91	0.9240
80/20	0.0947	0.74	0.9662	0.0033	1.18	0.9565
60/40	0.0027	1.22	0.9478	0.0022	1.25	0.9812
50/50	0.0204	0.77	0.9519	0.0400	0.64	0.9771

release and "*n*" is the diffusion exponent describing the drug release mechanism. When ln *F* is plotted against ln *t*, it gives a straight line from which the intercept determines the constant *K* and the slope gives the number *n*. In this regard, a value of $n = 0.5$ indicates a Fickian diffusion mechanism, in which the release is diffusion controlled, whereas a value of 0.5 n n > 1 indicate Super Case II transport mechanism, implying swelling and relaxation of hydrophilic polymer chains help to transport.

Fick's equation was plotted for the Ketoprofen release from pure CS and different compositions of CS/AG blend gels at different pH values (not shown). However, the kinetic parameters calculated from the release plots are shown in Table III. The release of Ketoprofen at pH of 2 showed the non-Fickian mechanism. The increase of AG ratio could be accompanied with an increase in pH-responsive character of the blend gels and this in turn increases the electrostatic interactions between the hydrogel and ketoprofen. At pH of 8, the release process showed "*n*" values greater than 1 indicating Super Case II transport mechanism, implying that the swelling and relaxation of hydrophilic polymer chains enhances the transport. On the other hand, the release from pure CS the value of "n" was not affected by change in pH values. At low pH, most carboxylate groups in AG are in the form of COOH, no dissociation occurs, the gel structure is devoid of charge, and collapsing is observed because of hydrogen-bonding formation. As the pH of the medium increases, the carboxylic groups become ionized, and the resulting electrostatic repulsion in the network causes the hydrogel to swell.[10] Therefore, it may be concluded that the diffusion of ketoprofen from the hydrogel networks at low pH value is not controlled but it depends on sorption process, which in turn depends on the structure and pathways through the networks.

CONCLUSIONS

Polysaccharides such as cellulose, starch, chitin/chitosan, and their water-soluble derivatives and sodium alginate have been known as degradable type polymers under action of ionizing radiation. In this work, it was tried to prepare blend hydrogels based on CS/AG by gamma irradiation of concentrated aqueous solutions (20%) of different ratios of chitosan (CS) and sodium alginate (AG) in the presence of glutaraldehyde, as a cross-linking agent. It was proposed that the formation of blend hydrogels were based on semi-interpenetrating network structure, in which AG polymer was enclosed in cross-linked CS. The CS/AG blend hydrogels exhibited high water swelling due to the presence of hydrophilic groups along the structure and displayed higher thermal stability than the individual CS hydrogel. Also, CS/AG blend hydrogels showed pH responsive release character of ketoprofen drug, in which the release in higher pH values was greater than in lower pH values.

References

1. Nizam El-Din, H. M.; El-Naggar, A. M.; Ali, F. I. Polym Int 2003, 52, 225.
2. Hirano, S.; Seino, H.; Akiyama, Y. Chitin and Chitosan: Ecologically Bioactive Polymers. In Biotechnology and bioactive polymers, Gebelein, C. G., Carraher, C., Eds.; New York: Plenum Press, 1994, pp 43–54.
3. Qurashi, M. T.; Blair, H. S.; Allea, S. J. J Appl Polym Sci 1992, 46, 255.
4. Wel, C. Y.; Hudson, S. M.; Mayer, J. M. J Polym Sci Part A: Polym Chem 1992, 30, 2187.
5. Yotsuyanagi, T.; Yoshioka, I.; Segi, N.; Ikeda, K. Chem Pharm Bull 1991, 39, 1072.
6. Aguzzi, C.; Cerezo, P.; Viseras, C.; Caramella, C. Appl Clay Sci 2007, 36, 22.
7. Haznedar, S.; Dortunc, B. Int J Pharm 2004, 269, 131.
8. Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M. Chem Rev 1999, 99, 3181.
9. Adriano, V. R.; Marcos, R. G.; Osvaldo, A. C.; Adley, F. R.; Edvani, C. M. Polymer 2006, 47, 2023.
10. Wach, R. W; Mitomo, H.; Naotsugu, N.; Yoshii, F. Radiat Phys Chem 2003, 68, 771.
11. Dong, Z.; Wang, Q.,Du, Y. J Membr Sci 2006, 280, 37.
12. Wang, Q.; Zhang, J.; Wang, A. Carbohydr Polym 2009, 78, 731.
13. Zeng, M.; Fang, Z.; Chengwei Xu, C. J Membr Sci 2004, 230, 175.
14. Wang, Q.; Dong, Z.; Du, Y.; Kennedy, J. F. Carbohydr Polym 2007, 69, 336.
15. Aslani, P.; Kennedy, R. A. J Control Release 1996, 42, 75.
16. Krishna Rao, K. S. V.; Naidu, B. V. K.; Subha, M. C. S.; Sairam, M.; Aminabhavi, T. M. Carbohydr Polym 2006, 66, 333.
17. Alvarez-Lorenzo, C.; Concheiro, A.; Dubovikb, A. S.; Grinbergb, N. V.; Burovab, T. V.; Grinberg, V. Y. J Control Release 2005, 102, 629.
18. Liu, K. H.,Liu, T. Y.; Chen, S. Y.; Liu, D. M. Acta Biomater 2007, 3, 919.
19. Shu, X. Z.; Zhu, K. J. Eur J Pham Biopharm 2002, 53, 193.
20. Yoshii, F.; Zhao, L.; Radoslaw, A.; Wach, Nagasawa, N.; Mitomo, H.; Kume, T. Nucl Instrum Methods Phys Res B 2003, 208 320.
21. Zhao, L.; Mitomo, H.; Zhai, M.; Yoshii, F.; Nagasawa, N.; Kume, T. Carbohydr Polym 2003, 53, 439.
22. Hiroki, A.; Tran, H. T.; Nagasawa, Yagi, N. T.; Tamada, M. Radiat Phys Chem 2009, 78, 1076.

23. Nizam El-Din, H. M.; Abd All, S.G.; El-Naggar, A. M. J Macromol Sci Part A: Pure Appl Chem 2007, 44, 291.
24. Nizam El-Din, H. M.; Abou Taleb, M. F.; El-Naggar, A. M. Nucl Instrum Methods Phys Res B 2008, 266, 2607.
25. Nizam El-Din, H. M.; Abd All, S. G.; El-Naggar, A. M. Radiat Phys Chem 2010, 79, 725.
26. Kumari, K.; Raina, K. K.; Kundu1, P. P. J Appl Polym Sci 2008, 108, 681.
27. Bergera, J.; Reista, M.; Mayera, J. M.; Feltb, O.; Peppasc, N. A.; Gurny, R. Eur J Pharm Biopharm 2004, 57, 19.
28. Wittaya-areekul, S.; Prahsarn, C.; Sungthongjeen, S. AAPS Pharm Sci Tech 2006, 7, E1.
29. Shultz, A. R. Crosslinking with Radiation "in EPST Ist Ed, 1985, Vol.4, pp 398–414.
30. Caykara, T.; Demirci, S.; Eroglu, M. S.; Guven O. Polymer 2005, 46, 10750.
31. Whitten, K. W.; Gailelt, K. D. General Chemistry with Quantitative Analysis; Saunders College Publishing: Philadephia, 1981, p 372.
32. Peppas, N. A.; Franson, N. F. J Polym Sci Polym Phys Ed 1983, 21, 983.