Preparation and Evaluation of Photocrosslinkable Chitosan as a Drug Delivery Matrix

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ABSTRACT: Epichlorohydrin (1-chloro-2,3-epoxypropane) was reacted with sodium azide in the presence of a phase transfer catalyst to obtain 1-chloro-2-hydroxy-3-azidopropane, which was further coupled onto chitosan to prepare a photocrosslinkable derivative of the biopolymer. Elemental analysis and infrared (IR) spectroscopy confirmed the incorporation of azide groups onto chitosan. Films were cast from an aqueous acetic acid solution of azidated chitosan containing a model drug, such as theophylline. Irradiation of the film with ultraviolet (UV) light led to crosslinking of the drug incorporated film. IR spectra indicated complete surface crosslinking within 2 h of irradiation. Release of theophylline from uncrosslinked and crosslinked films was examined in simulated gastric and intestinal fluids without enzymes at 37 °C. The release of the drug from the crosslinked films was slower than the release from uncrosslinked films. Although the system is far from being optimized to obtain sustained release of a pharmacologically active agent for long periods, the data obtained indicate the possibility of developing photocrosslinkable matrices of biopolymers, such as chitosan, for sustained drug delivery with many advantages over chemical crosslinking. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 1873–1877, 2002

Key words: drug delivery systems; microencapsulation; polysaccharides; photocrosslinking; chitosan; theophylline; sustained release

INTRODUCTION

Chemical crosslinking is an important method to control drug release from diffusion-controlled polymeric drug delivery matrices. Thus, hydrogels, proteins, and polysaccharides have been crosslinked using a number of crosslinking agents to manipulate the diffusion of an entrapped pharmacologically active agent from such polymeric matrices.[1–4] Although bifunctional vinyl monomers, such as ethyleneglycol dimethacrylate and *N*,*N*′-methylene bisacrylamide, have been used as crosslinking agents for the preparation of synthetic hydrogels from polyacrylamide and poly(2-hydroxyethyl methacrylate), etc., crosslinking agents, such as glutaraldehyde, formaldehyde, terephthaloyl chloride, 2,3-butanedione, epichlorohydrin, dicyclohexyl carbodiimide, etc., have been used for crosslinking drug delivery matrices derived from proteins and polysaccharides.

Chitosan is a deacetylated derivative of chitin, a biopolymer second in abundance to cellulose. Many biomedical applications of chitosan have been envisaged in the literature.[5,6] Among them is the possibility of using this polysaccharide as a matrix for sustained drug delivery. In a series of papers from this laboratory, it was demonstrated that glutaraldehyde crosslinking of chitosan reduces the lysozyme susceptibility of chitosan to biodegradation and therefore could be used as a vehicle for prolonged delivery of drugs spanning weeks or months.[7–11]

Chemical modification of chitosan have been extensively carried out because of the presence of active amino and hydroxyl groups in this biopolymer. There are, however, only very few references in the literature on the preparation of a photocrosslinkable chitosan derivative. Thus, a photoactive chitosan derivative has been synthesized using 4-azido pyridine derivatives for protein immobilization.[12] Covalent immobilization of chitosan onto polymeric film surfaces was carried out by attaching a photosensitive reagent 4-azidobensoimidate by Aiba et al.[13] Recently, Ono et al.[14] reported the preparation of a photocrosslinkable chitosan for wound dressing application by incorporating aromatic azides onto the polymer.

Although photocrosslinking is a technique widely used in the coating industry,[15] there is very little information in the published literature on the use of this technique for controlled drug delivery. Anderson et al.[16] used photosensitive ammonium dichromate to crosslink poly(2-hydroxyethyl methacrylate) films containing hydrocortisone succinate by UV radiation. If the drug carrier polymer itself contained photolabile functions, it may offer many advantages over chemical crosslinking or crosslinking induced by photosen-

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sitizers. For example, activities of drugs such as salbutamol, epinephrine, etc., are affected by aldehyde crosslinking of protein matrices such as albumin.[17] Thus, drugs with active functions that enter into reaction with crosslinking agents cannot be employed when the matrix is chemically crosslinked. If drug release can be manipulated as a function of crosslinking density of the matrix, then photocrosslinking offer the advantage of controlling the release as a function of irradiation dose. The matrix could be crosslinked to the desired extent after preparation by simply irradiating with the appropriate wavelength of light for the desired amount of time.

In this communication, we report on the preparation of a photocrosslinkable chitosan by attaching an aliphatic azide onto the polymer and the possibility of employing such a polymer matrix for sustained release applications.

EXPERIMENTAL

Materials

Chitosan with a viscosity average molecular weight of 3.15×10^5 Da and degree of deacetylation of 74% was from Central Institute of Fisheries and Technology, Cochin, India, and was used without further purification. Theophylline and sodium azide were from Sigma Chemical Company, St. Louis, MO. Epichlorohydrin was from S.D. Fine Chemicals Ltd., Bombay, India, and tetrabutylammonium bromide was from Spectrochem Ltd., Bombay, India. All other reagents and solvents were of the highest purity commercially available.

Methods

Azidation of epichlorohydrin

Sodium azide (16 g, 0.25 mol) was dissolved in 40 mL of water containing 0.196 g (6×10^{-4} mol) tetrabutylammonium bromide as the phase transfer catalyst in a 150-mL Erlenmeyer flask, and then 20 mL (0.25 mol) epichlorohydrin was added, protected from light, and stirred overnight using a magnetic stirrer at room temperature (27 °C).The nonaqueous layer was then separated using a separating funnel after adding dichloromethane and dried over anhydrous sodium sulfate. The dichloromethane was then removed under vacuum at room temperature in a rotovapor to yield the azidated product in 90% yield.

Preparation of azidated chitosan

Chitosan 1.2 g was dissolved in 30 mL of 5% acetic acid. To this was added 5 mL of 5% hydrochloric acid and 5 mL of azidated product. The solution was protected from light and stirred magnetically at room temperature for 24 h. The azidated chitosan was then precipitated in methanol containing ammonia; filtered; washed several times with methanol, once with water, and finally with acetone; vacuum dried at room temperature; and kept in a desiccator in the dark until use. The yield was $> 90\%$.

Preparation of chitosan film

One gram of azidated chitosan was dissolved in 25 mL of 5% acetic acid. The solution was then cast on a glass plate and allowed to dry for 4 days, protected from light at room temperature, to obtain a film of thickness $\sim$0.1 mm. Theophylline-loaded film was prepared in the same manner after mixing a definite amount of the drug with the chitosan solution.

Photocrosslinking

Crosslinking the film was carried out using UV radiation. A piece of film (1×1 cm) was taken in a quartz tube and exposed to radiation from a 125 W UV lamp (Phillips, Bombay, India) at a distance of 15 cm from the center of the lamp.

Attenuated total reflection-fourier transform infrared (ATR-FTIR) spectra

Spectra of the chitosan films were recorded using a FTIR spectrophotometer (Nicolet, Model 410, Madison, WI) using baseline horizontal ATR accessory (Nicolet). The IR spectra of theophylline were recorded in the same instrument using KBr pellets.

Thermal and elemental analysis

Thermal analysis of various samples were carried out using a thermal analyzer (TA Instruments Inc., SDT 2960, New Castle, DE) at a heating rate of 20 °C/min in nitrogen atmosphere whereas elemental analyses were carried out using a Perkin Elmer, CHNS analyzer (Series II, Model 2400, Norwalk, CT).

Estimation of epoxy content

Estimation of epoxy group in azidated epichlorhydrin was done using hydrochlorination method[18] Briefly, 10 mL of hydrochlorinating agent (prepared by mixing 6.5 mL of conc. HCl with 250 mL of pure dioxane) was added to 0.1 g of the compound taken in a stoppered Erlenmeyer flask and shaken well. The solution was then allowed to stand at room temperature for 15 min. It was then titrated against 0.1 N methanolic NaOH using phenolphthalein as indicator. A blank titration was also carried out.

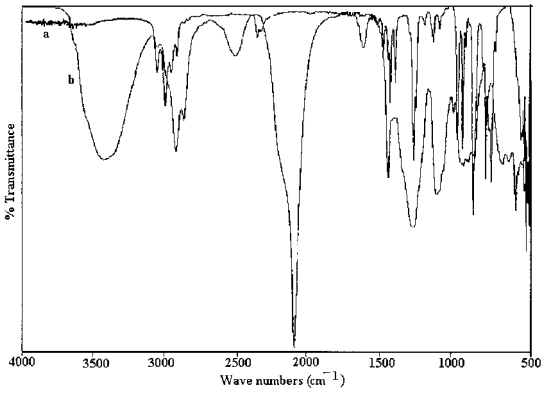


Figure 1 IR spectra of (a) epichlorohydrin and (b) azidated epichlorhydrin.

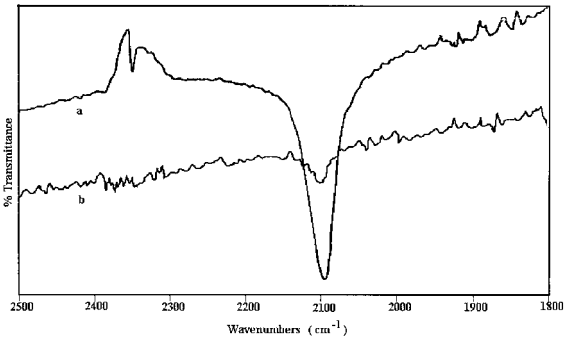


Figure 2 ATR-FTIR spectra of azidated chitosan film before UV irradiation showing (a) the strong azide peak at 2100 cm^{-1} and (b) after 2 h of irradiation showing almost complete disappearance of the peak at 2100 cm^{-1}.

In vitro release

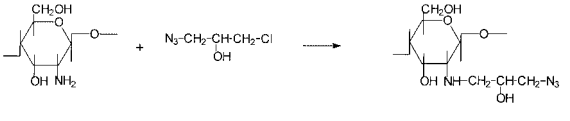
Crosslinked and uncrosslinked chitosan films containing theophylline (~1 × 1 cm) were cut and introduced into 100 mL of simulated gastric or intestinal fluid without enzymes in a USP paddle type assembly and stirred at 125 rev/min at 37 °C. The simulated fluids were prepared according to US Pharmacopeia.[19] Aliquots of 0.5 mL were withdrawn at various time intervals and the medium was replenished with the same amount of fluid. Theophylline released was assayed at 274 nm in a UV-vis spectrophotometer (Milton Roy, Genesys 2, Rochester, NY). Values plotted are average of three determinations. The amount of drug initially present in the film was computed from the ratio of the weight of drug to the weight of chitosan in the casting solution.

RESULTS AND DISCUSSION

Azidated chitosan was prepared by reacting azidated epichlorohydrin with chitosan. Azidation of epichlorohydrin was carried out by reacting epichlorhydrin with sodium azide in the presence of a phase transfer catalyst. Under the experimental conditions described, the azidation of epichlorhydrin proceeded presumably as reported in the literature with the attack of the azide ion on the epoxide resulting in the opening of the ring and giving rise to the product 1-chloro-2-hydroxy-3-azido propane. The epoxide is the by far the most reactive site and a variety of nucleophiles have been used to open the ring in the C-3 position.[20] The IR spectra (neat) of epichlorohydrin and azidated compound are shown in Figure 1a and b, respectively. The azidated compound shows a sharp peak at 2100 cm^{-1} which is due to azide and a broad band at 3500 cm^{-1} due to the hydroxyl group, thus confirming the opening up of the epoxy ring. The opening up of the epoxy group was further confirmed by epoxy titration of the product. Taking epichlorohydrin as the standard with epoxy content 100%, the azidated product showed an epoxy content of only 1.6%. This result clearly demonstrated that the azide nucleophile attacks the epoxy group leading to its opening as reported in the literature.

The ATR-FTIR spectrum of azidated chitosan film before and after UV irradiation is shown in Figure 2. The unirradiated film shows the strong azide peak at 2100 cm^{-1}. Azidated chitosan has been subjected to extensive washings using methanol and therefore the azide peak in the modified polymer is not due to the residual azide compound, but due to covalently bound azide. This peak disappears almost completely after 2 h of irradiation, demonstrating complete surface crosslinking. The azide group on photoirradiation decomposes into highly reactive nitrenes, which react via several nonselective reactions, including insertion into the C—H bond and hydrogen atom abstraction. All such reactions lead to crosslinking.[21]

Elemental analysis showed an increase in the percentage of nitrogen in the modified biopolymer equivalent to 2.34% substitution. The extent of incorporation of azide is rather low. The present substitution reaction which is akin to the reaction between an alkyl halide and an amine that takes place more efficiently in an organic reaction medium is presumably not efficient enough in an aqueous medium especially when the nucleophilic amine is a polymer of very high molecular weight. Ono et al.[14] found that when *p*-azido benzoic acid was reacted with chitosan, the extent of substitution was only ~2.5%. However, such a low degree of substitution was sufficient enough for the polymer to undergo crosslinking when irradiated with UV light. The reaction is shown in Scheme 1.



Scheme 1

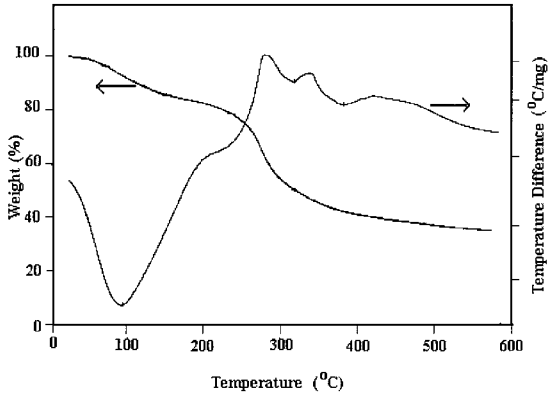


Figure 3 DTA-TGA trace of chitosan film casted from 5% acetic acid.

Because azide groups are thermolabile, the incorporation of azide onto chitosan may decrease the thermal stability of chitosan. The thermal stability of azidated chitosan compared to chitosan was evaluated using thermal analysis. The results are shown in Figures 3 and 4. Thermogravimetric analysis (TGA) showed that chitosan azide is less stable compared with chitosan. For chitosan, 50% weight loss occurred at 326.8 °C, whereas for chitosan azide, a corresponding weight decrease occurred at a lower temperature of 312.2 °C. Differential thermal analysis (DTA) showed fewer peaks for chitosan in the temperature range examined, except the endothermic peak at 95 °C, which is most probably due to the loss of water (Figure 3). Chitosan azide, on the other hand, shows marked difference from that of chitosan. The endotherm at 89 °C, similar to chitosan, is due to loss of water. The spectrum however, shows a characteristic sharp exothermic peak at 220 °C, which is attributable to the decomposition of azide (Figure 4). Such exothermic peaks are characteristic of the decomposition of azide as reported in the literature.[22]

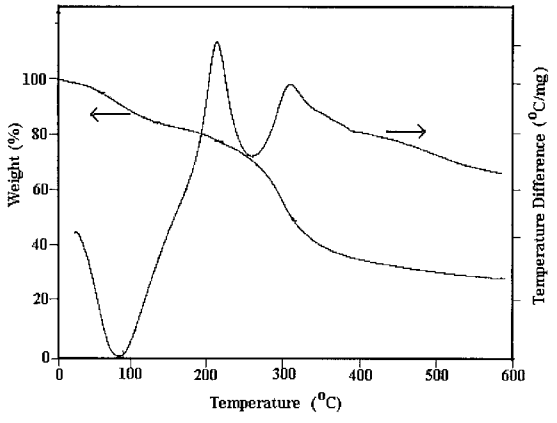


Figure 4 DTA-TGA trace of chitosan azide film casted from 5% acetic acid.

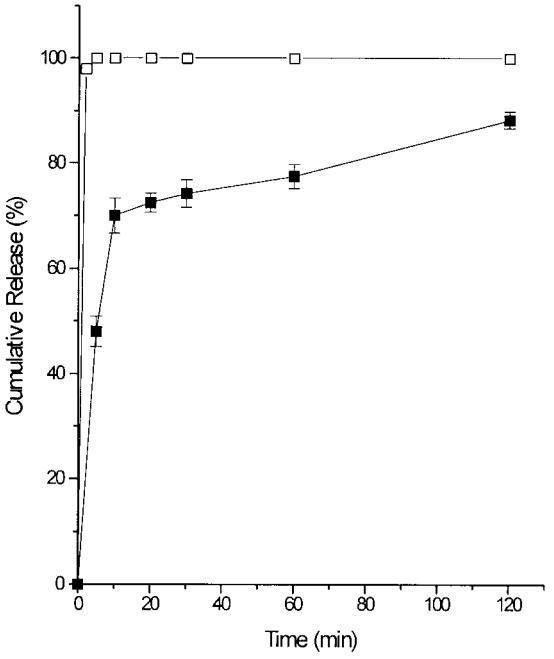


Figure 5 Release of theophylline from azidated chitosan films with 10% drug payload into simulated gastric fluid at 37 °C before and after photocrosslinking. Key: (□) before crosslinking; (■) after photocrosslinking.

To examine the possibility of using the azidated chitosan for sustained chemical delivery, theophylline was used as a model drug. Before incorporation of the drug into the polymer matrix, its photostability was ensured by irradiating the drug in a quartz tube with UV light for 2 h and examining its UV and IR spectra before and after irradiation for any change due to irradiation. The UV and IR spectra of theophylline before and after irradiation were exactly identical (data not shown).

The *in vitro* release of theophylline into gastric fluid from azidated chitosan films before and after photocrosslinking is shown in Figure 5. The unirradiated film dissolved in gastric fluid rather quickly (2–3 min), thereby releasing all the incorporated drug into the medium. From the irradiated films, there was only 70% release in 10 min, and thereafter there was a steady increase in the release rate with time. It took nearly 2 h before 90% of the drug was released from the irradiated film. No dissolution of the film occurred in the medium, demonstrating that UV irradiation leads to crosslinking of the polymer matrix and makes it insoluble.

In simulated intestinal fluid, however, the release from the unirradiated film was not as rapid as in simulated gastric fluid (Figure 6). Complete release was seen in ~20 min. The pH of the medium being slightly alkaline (pH 7.5), the film did not undergo complete dissolution in the intestinal fluid, unlike in gastric fluid. The less rapid release seen in intestinal

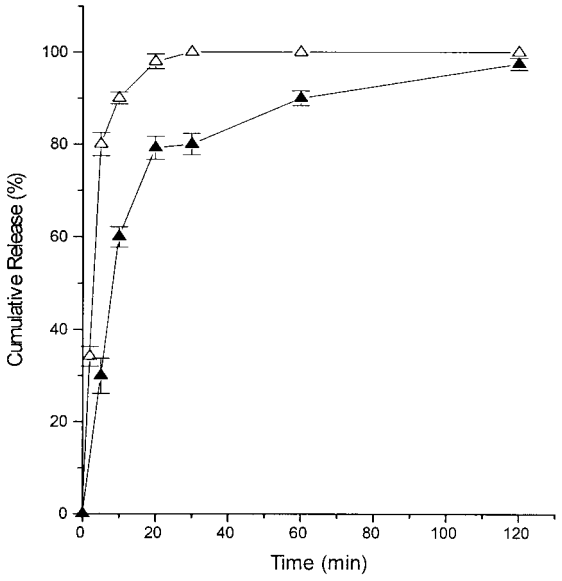


Figure 6 Release of theophylline from azidated chitosan films with 10% drug payload into simulated intestinal fluid at 37 °C before and after photocrosslinking. Key: (△) before crosslinking ; (▲) after photocrosslinking.

fluid compared with the release seen in gastric fluid is because of the lower solubility of the film in the former. From the irradiated film, the release was less rapid; 80% of the drug diffused out in ~20 min and thereafter there was a steady increase in the rate with time. Complete release was seen in ~2 h.

The results of the release experiments as seen in the present study do not seem spectacular, at least for a drug molecule such as theophylline. It was seen that the extent of azidation of chitosan was rather low, although such low concentration of the azide was sufficient enough to crosslink the polymer on irradiation. Although the photocrosslinking made the polymer insoluble, the density of crosslinking was obviously not sufficient enough to control the diffusion of the drug in an efficient manner for prolonged periods. It was shown in many of our earlier studies that glutaraldehyde crosslinking of chitosan can effectively control the release of the entrapped chemical entity spanning weeks to many months.[9–11]

The present system is, therefore, far from being optimized to deliver an entrapped drug for prolonged periods. The preliminary data obtained in this study, however, show that photocrosslinking will be an interesting method to control drug diffusion from a polymer matrix. Theophylline is a small drug molecule and therefore the diffusion is more facile through the crosslinked network. With large drug molecules, such as steroids or macromolecules, the diffusivity through the crosslinks may be retarded and a prolonged release may be possible. Many protein-based biologically active molecules cannot be incorporated into drug carriers, such as albumin or gelatin, when the latter is stabilized by chemical or thermal crosslinking because the protein also enters into crosslinking or suffers thermal denaturization. Incorporation into the carrier matrix with photolabile groups would therefore be an interesting way of incorporating such molecules in their native form and controlling their diffusion. It should also be possible to derivatize the polymer with a higher density of photolabile groups, which would result in extensive crosslinking of the matrix on irradiation, thereby controlling the drug diffusion to the desired extent.

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