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Hydrocolloids and gels of chitosan as drug carriers

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Summary

Chitosan, a natural, biocompatible polymer, is becoming popular in dosage form design. In our study the design factors affecting the release of lidocaine and lidocaine chloride from chitosan hydrocolloids and gels were studied. In hydrocolloids a relatively high viscosity was found at low concentrations of chitosan caused by the increased effective volume of the polymer molecules, due to the reflection of the same charges in the chains. The drug release is slow and sustained, being influenced by the chitosan content. The mechanism of chitosan gel formation is not known exactly, but it is clear that for gel formation the length of the chitosan chains and the degree of reacetylation are responsible. The release profile of gels follows almost zero order kinetics. Also, the degree of reacetylation is responsible for the release behaviour. The rotational motion of nitroxides (Tempol, spin-labeled lidocaine) determined by EPR experiments showed practically equal rotational motion at different degrees of reacetylation. Thus, it was concluded that the free spaces, available for nitroxide rotation, were not changed significantly. The degree of reacetylation affects the translational diffusion more strongly

Introduction

The use of natural polymers as drug carriers has received considerable attention in dosage form design, especially from the viewpoint of safety. Chitin is one of the polysaccharides widely distributed in nature as the principal component of shells of crustaceans and insects and of cell walls of bacteria and mushrooms (Muzzarelli, 1977; Pelletier et al., 1990). Chitin, poly-β-(1-4)-*N*-acetyl-D-glucosamine and chitosan, partially deacetylated chitin (poly(*N*-deacetylglucosamine) (Fig. 1) and their various synthetic derivatives have recently attracted great interest from the standpoint of utilization of natural resources. It has been reported that chitin and chitosan are biocompatible and biodegradable, showing extremely low toxicity (Chandy and Sharma, 1990). Recently, importance has been attached to chitosan derivatives with a defined degree of deacetylation and depolymerization because of their significantly different physico-chemical properties (especially water solubility) (Shiraishi et al., 1990;

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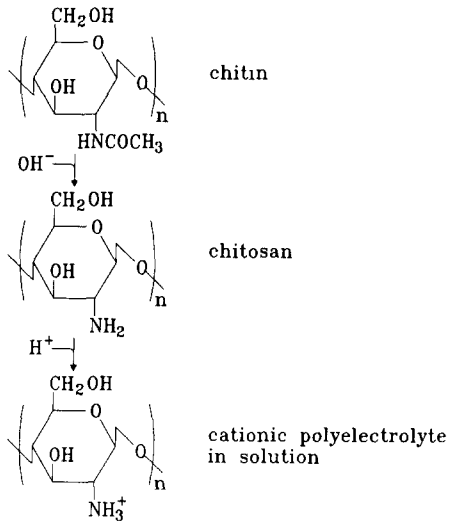


Fig. 1. Chemical structures of chitin, chitosan and its protonated form.

Imai et al., 1991). Considering chitosan as a weak base, a certain minimum amount of acid is required to transform the glucosamine units into the positively charged, water-soluble form. At neutral pH most chitosan molecules will lose their charge and precipitate from solution (Onsoyen and Skaugrud, 1991).

Chitosan is a potential drug carrier, regulating drug release. High molecular mass chitosan is preferrably used for sustained release preparations, partially hydrolysed for the improvement of drug solubility.

With a view towards application of chitosan in pharmaceutical preparations, many investigations have been carried out regarding the following: the possibility of direct compression of tablets with optimal properties; sustained-release preparations containing water-soluble drugs; moderating dissolution properties and bioavailability of poorly soluble drugs from ground mixture; developing microspheres based on chitosan to improve drug release; considering chitosan as a cationic polymer for polyionic complex formation, obtained as a precipitate by mixing it with anionic polymers; and additional possibilities are also in more precisely controlled drug delivery by chitosan (Nagai et al., 1984; Takahashi et al., 1990; Onsoyen and Skaugrud, 1991; Rios et al., 1991).

The physico-chemical properties of chitosan have been reported in our previous publication (Kristl and Šmid-Korbar, 1991). In order to assess the utility of chitosan in pharmaceutical formulations, colloidal dispersions and gels were prepared and investigated.

Materials and Methods

Chitosan is obtained by hydrolysis of the aminoacetyl groups of chitin in aqueous alkaline solutions. Chitosan of various purity grades, degree of deacetylation and average molecular weight is commercially available from several suppliers.

In this study, chitosan supplied by Wella AD (Darmstadt, Germany) was tested. The degree of deacetylation, determined by potentiometric titration, was found to be approx. 70% (Table 1).

The incorporated drugs were lidocaine (Lid) and lidocaine chloride (Lid-Cl) at 1% concentration. Other chemicals used, i.e., acetic acid, acetic anhydride and ethanol, were of analytical grade. For EPR studies, Tempol and spin-labeled lidocaine (sl-Lid) were used (Kristl et al., 1989).

Preparation of chitosan solutions

Chitosan was dispersed in 50 ml of 0.5% acetic acid and stirred until complete solubility was achieved. The solution was prepared by quaternization of the free amino groups on polymer using acetic acid.

Preparation of gels

The gel was obtained by acetylation of chitosan with acetic anhydride in two different ways. The drugs were incorporated into the chitosan

TABLE 1

Degree of deacetylation of chitosan sample

Sample	Degree of deacetylation (%)
Chitosan lm	70.5
Chitosan hm	73.0

hm/lm, high/low molecular mass according to manufacturer's classification.

solution before acetic anhydride addition. The gel was obtained in one step in its final form.

Partially O-acetylated and N-acetylchitosan gel

Gels were obtained by dissolving 1 g of chitosan W hm in 10% acetic acid and adding various amounts of acetic anhydride. The gels were formed in the range of 19.04–31.74 mmol of acetic anhydride (Table 2). The prepared gels were colorless, transparent and elastic. With smaller amounts of acetic anhydride, less than 19.04 mmol, colloidal solutions resulted (no gelation occurred), whilst with greater amounts gelation took place. At 31.74 mmol the gels were rigidly solidified and syneresis occured. The rigidity of gels and their pH values are dependent on the added amount of acetic anhydride (gels of hm chitosan have pH 1.9–1.7 and gels with lm chitosan pH approx. 1.5).

Gelation of chitosan W lm solutions occurs with a larger amount of acetic anhydride (34.38–47.61 mmol). The gels are transparent, not elastic, but very fragile.

N-Acetylchitosan gel

N-Acetylchitosan gel was obtained by dilution of a chitosan solution in 10% acetic acid with methanol or ethanol and subsequent addition of acetic anhydride (Hirano and Yamaguchi, 1976): chitosan W hm (50 mg) was dissolved in 4.0 ml of 10% acetic acid. Then the viscous solution was diluted with 8 ml ethanol and stirred until no further air bubbles were present. Gelation was induced with 0.53–0.79 mmol of acetic anhydride (Table 2). The mixture was stored at room temperature overnight to form a rigidly solidified gel. Syneresis occurred with 1.59 mmol or more acetic anhydride. The gels of hm chitosan were very homogeneous, transparent, and colourless with a pH value of approx. 4.4.

Using the same procedure, gels of chitosan W lm were prepared. The pH values were 4.36–4.79. The gels were also homogeneous and transparent, but very fragile. For *N*-acetylated chitosan gels a smaller amount of acetic anhydride was required and higher pH values resulted. Considering appropriate the pharmaceutical properties, *N*-acetylchitosan hm gels were selected for further examination.

TABLE 2

Amounts of acetic anhydride required for N- and O-acetylchitosan and N-acetylchitosan gels from chitosan

Chitosan	mmol acetic anhydride	Gel formation	
		N- and *O*-acetyl chitosan	*N*-Acetyl chitosan
High molecular mass	0.13		−
	0.26		±
	0.53		+
	0.79		+
	1.59		+(S)
	5.29	−	
	9.52	−	
	10.58	−	
	12.69	−	
	14.81	−	
	15.87	±	
	16.93	±	
	19.04	+	
	21.16	+	
	26.45	+	
	31.74	+	
	37.03	+(S)	
	42.32	+(S)	
	47.61	+(S)	
	52.89	+(S)	
Low molecular mass	0.26		±
	0.42		+
	0.64		+(S)
	1.27		+(S)
	26.45	−	
	29.09	−	
	31.74	±	
	34.38	+	
	42.32	+	
	47.61	+	
	58.15	+(S)	

(+) Gelation occurred; (−) no gelation occurred; (S) syneresis.

Viscosity measurements

The viscosity of chitosan hm colloid dispersions was measured with a rotational viscometer

(Haake Rotovisco RV 20; Haake, Karlsruhe) at room temperature.

In vitro release determination

Drug release profiles were determined using an in vitro flow-through diffusion cell for ointments. The solution or gel was attached to one compartment of the liberation cell, separated from the acceptor medium (aqua purificata at 37°C, flux of 1 ml min^{-1}) by a hydrophilic membrane (Visking dialyzing tube – regenerated cellulose, type 110, Serva Feinbiochemica, Heidelberg, Germany; 38.5 cm^2 area, average pore size 2.4 nm). The samples were withdrawn at predetermined time intervals and the drug assayed spectrophotometrically at a maximum absorbance wavelength of 262 nm (Perkin-Elmer, UV-Vis 554, U.S.A.). The release rate, k, was obtained by plotting the cumulative amount of the drug released vs time.

EPR measurements

EPR spectra were recorded on a Brucker spectrometer operating at X-band frequency (9.5 GHz) with 100 kHz field modulation and at room temperature.

Results and Discussion

Colloidal solutions of chitosan

Colloidal solutions of chitosan show a steep increase in viscosity at concentrations above 1% (Fig. 2). This is due to the increased effective volume of the polymer molecules caused by hydration and mutual electrostatic repulsion of charged functional groups, i.e., NH_3^+ on the chitosan chains and their corresponding extension.

The in vitro release of Lid-Cl from chitosan hydrocolloids is scarsely sustained only at higher polymer concentrations (Fig. 3). High molecular mass chitosan, however, decreases the release more significantly (Fig. 4).

Chitosan gels

The drug loading of chitosan hydrocolloids is less pretentious than that of the gels. The drug molecules must not interfere with the fragile gel

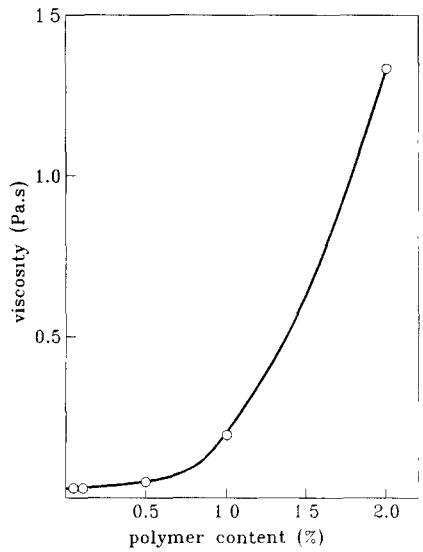


Fig. 2. Viscosity of colloidal solutions of chitosan in 0.2 M acetic acid; shear rate 100 s^{-1}, $T = 20°C$.

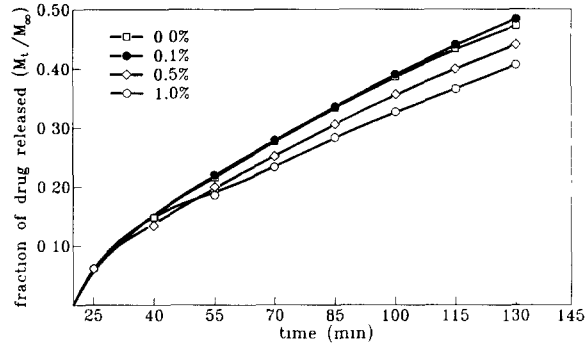


Fig. 3. Effect of chitosan concentration on lidocaine chloride release.

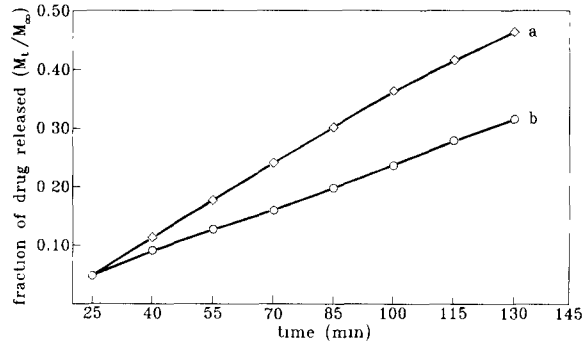


Fig. 4. Effect of chitosan molecular mass (a, low molecular mass; b, high molecular mass) on lidocaine chloride release.

structure because of the possible process of syneresis. The mechanism of gel formation is not exactly known. Chitosan molecules are considered to have disordered conformation in solvents (acetic acid or acetic acid/ethanol) since the amino groups are cationized, forming an amino acetate salt and, therefore repel each other. By *N*-acetylation the number of cations is decreased. At this stage a conformational change of chitosan molecules occurs and molecular aggregation begins to produce gels. Both *N*-acetylation and aggregation occur simultaneously and a three-dimensional structure is formed. In the case of high molecular mass chitosan less acetic anhydride is needed for gelation than with low molecular mass chitosan. Thus, for gel formation the length of polymer chains and a sufficient degree of reacetylation are responsible (Table 2). *N*-Acetylchitosan produced in this way cannot be distinguished from natural chitin by IR spectroscopy (i.e., chemically) but is distinctly different in physical properties, probably due to the various chain conformations.

For determination of the reacetylation effect of chitosan on drug release, the *N*-acetylchitosan hm gel was selected. The effect of acetic anhydride addition on drug release is shown in Fig. 5. In comparison with the gel, the drug release from aqueus solution is significantly greater. The lidocaine release from gels is proportionally sustained as the amount of the reagent is increased. Over a 2 h period at 0.26 mmol acetic anhydride 19%, at 0.5 mmol 13.3% and at 0.79 mmol 9.9%

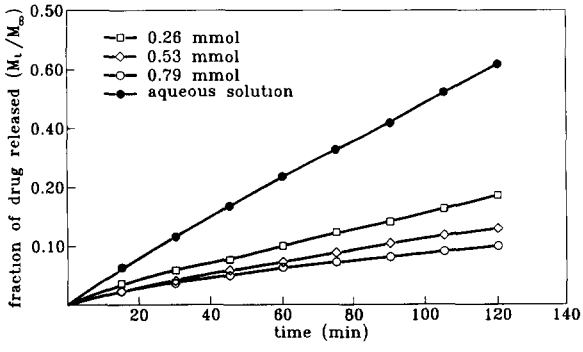


Fig. 5. Effect of acetic anhydride addition to chitosan hm on lidocaine release.

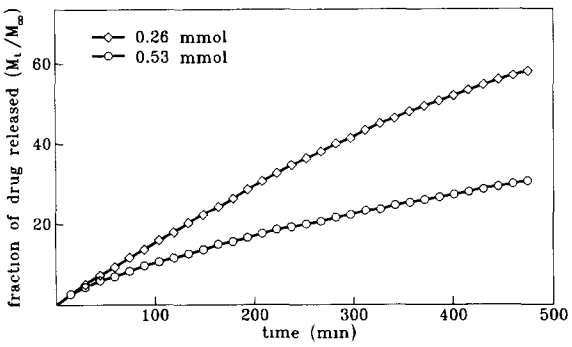
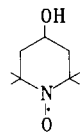


Fig. 6. Prolonged lidocaine release from chitosan hm gels with various amounts of acetic anhydride.

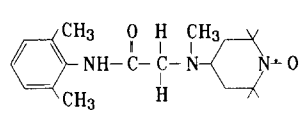
are released. After 15 min almost zero-order release is observed. This drug release tendency holds for more than 8 h (Fig. 6).

An interesting insight into the molecular dynamic phenomena in chitosan dispersions is given by EPR examination. Stable free radicals such as the model nitroxide Tempol and spin-labeled lidocaine (Fig. 7) are dissolved in the system. The rotational motion of these free radicals, expressed as the rotational correlation time (τ_c) is very sensitive to the motional freedom in their local environment. Therefore, the EPR spectral changes (line shape and line width) of these radicals provide information about the molecular dynamics (March, 1989; Kristl et al., 1991). Our studies involved the examination of nitroxide motion as a function of the chitosan molecular mass

$$\tau_c = 6.0 \times 10^{-10} \Delta H_0 \left(\sqrt{\frac{I_0}{I_{-1}}} - 1 \right)$$



Tempol



sl-Lidocaine

Fig. 7. Chemical structure of nitroxides Tempol and spin-labeled lidocaine.

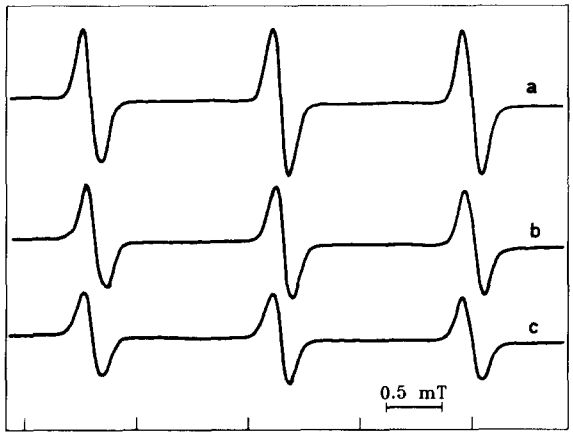


Fig. 8. EPR spectra of Tempol in phosphate buffer solution (a), colloidal dispersion of chitosan lm (b) and colloidal dispersion of chitosan hm (c).

and the degree of reacetylation. Fig. 8 shows the EPR spectra of Tempol probe in phosphate buffer solution and chitosan hydrocolloids. A clearly resolved three-line spectrum is obtained, characteristic for a nitroxide probe undergoing free rotation. As the molecular mass increases, the motionally narrowed spectrum, especially the third peak, becomes broader and lower, representing slower motion of the spin probe. The numerical value of the rotational correlation time is the lowest in phosphate buffer solution, followed by low molecular mass and finally high molecular mass chitosan dispersions (Table 3). Translational mobility changes of the probe correlate with diffusion processes determined through the drug release measurements.

In the microenvironment of chitosan high molecular mass gels the motion of both spin probes is interesting: the correlation times are practically equal at different degrees of reacetylation (Table 3). The experiment has shown that the free spaces, available for nitroxide rotation are not changed significantly. On the other hand, the translational motion (drug release) is strongly affected by the reacetylation degree. Differences in the rotational motion between both nitroxide molecules can be attributed to the spin probe molecule size and shape.

TABLE 3

Motion of nitroxide molecules in chitosan systems expressed as rotational correlation time

Spin-labeled molecule	Medium	Rotational correlation time (s^{-1})
	phosphate buffer solution	40.06×10^{-11}
	chitosan lm solution	2.80×10^{-11}
	chitosan hm solution	2.24×10^{-11}
Tempol	chitosan hm gel (0.26 mmol Ac_2O)	4.19×10^{-11}
	chitosan hm gel (0.79 mmol Ac_2O)	4.13×10^{-11}
Lidocaine	chitosan hm gel (0.26 mmol Ac_2O)	1.01×10^{-10}
	chitosan hm gel (0.79 mmol Ac_2O)	1.01×10^{-10}

Conclusions

Based on our examination it is feasible to conclude that: for the gelation of chitosan hydrocolloids the length of the polymer chains and appropriate degree of reacetylation are responsible; the drug loading of chitosan hydrocolloids is less pretentious than of the gels: drug molecules must not interfere the fragile gel structure; the lidocaine release (almost zero order) from gels is proportionally sustained with the reacetylation degree; and the molecular dynamics phenomena in chitosan dispersions illustrated by EPR can be explained regarding the free spaces for rotational motion of spin probe molecules and the more strongly affected polymer hindrance to translational motion.

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