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# Bioadhesion of hydrated chitosans: an in vitro and in vivo study

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**Abstract**

The (bio)adhesivity of several chitosan chloride samples was screened in vitro and compared with hydroxypropylmethylcellulose (HPMC), Carbopol 934P and polycarbophil by a force of detachment method. This revealed differences between samples, but was judged to be insufficient to describe the bioadhesive behaviour of fully hydrated chitosan. Therefore, an ex vivo method was designed, where freshly excised cattle corneas were treated with tritiated chitosan in solution. The contact time, pH, ionic strength and chitosan molecular weight were investigated by means of factorial design, and were shown to have significant effects on the adsorption. In addition, interactions were seen between the parameters. These effects were not seen when chitosan was incubated with polycarbonate membranes instead of corneas. It is concluded that fully hydrated chitosan has a specific bioadhesive activity towards biological surfaces. In the in vivo study, liposomes and chitosan-coated liposomes containing $^{125}$I-labelled bovine serum albumin (BSA) as a marker were applied to the eyes of anaesthetised rats and their retention at 10, 30 and 90 min compared. Both formulations showed significantly longer retention than a solution of the free $^{125}$I-BSA, but coating the liposomes with chitosan did not significantly improve their retention. It is concluded that the adhesive interaction between chitosan and a biological substrate is dependent on formulation factors as well as the chitosan quality. Copyright © 1997 Elsevier Science B.V.

*Keywords:* Bioadhesion; Chitosan; Corneal adsorption; Force of detachment; In vivo retention

## 1. Introduction

The polysaccharide chitosan has been studied in numerous drug delivery systems, as well as in non-medical applications. This polymer is derived from chitin which occurs in the shells of crustaceans and is available in large amounts as a waste product from the seafood processing industry. The cell-binding activity of chitosan has been well documented. Evans and Kent (1962) observed that chitosan was able to effectively bind

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and agglutinate a wide variety of mammalian cell types. This property appeared to be directly related to its cationic polyelectrolyte structure and the negative charge of cell surfaces (Baran, 1988; Olsen et al., 1989). Other applications have been the immobilisation of cells to solid supports (Champluvier et al., 1988; Goldberg et al., 1990) and the selective flocculation of cell disintegrates with chitosan (Agerkvist, 1992).

A potential pharmaceutical application for chitosan which utilises its bioadhesive activity is the area of drug targeting. Bioadhesion can be defined as the binding of a natural or synthetic polymer to a biological substrate. When this substrate is a mucous layer, the term mucoadhesion is often utilised. The bioadhesive properties of chitosan have been studied in the form of microspheres (Gallo and Hassan, 1988) and solution (Hassan and Gallo, 1990) with complexation and viscometric methods. Takayama et al. (1990) studied the adhesion force of hydrated chitosan/sodium hyaluronate tablets in different buffers, whereas Miyazaki et al. (1995) measured the adhesion force of partly hydrated chitosan/sodium alginate tablets. Other formulations of chitosan that have been tested include films (Lehr et al., 1992), gels (Needleman and Smales, 1995) and liposomes (Takeuchi et al., 1994). These formulations represent differing degrees of hydration, from wetted tablets to chitosan solutions, and have been assessed by widely different methods. The most promising results seem to have been achieved where the polymer was solvated (Hassan and Gallo, 1990; Takeuchi et al., 1994; Needleman and Smales, 1995) or in a film that includes an acid to give a locally low pH environment allowing hydration (Lehr et al., 1992).

Ocular formulations are generally preferred as solutions or suspensions, for reasons of patient compliance. A major problem is that extensive drug loss results from the instillation of solutions into the eye, due to an efficient drainage of solutions via the tear ducts to the nasal mucosa. Hui and Robinson (1985) investigated the use of bioadhesive acrylic acid polymers to deliver progesterone to the eye, and obtained a significantly improved drug bioavailability. Recently, it has been shown that the co-administration of bioadhesive polymers with nanoparticles to the eyes of rabbits gave an improved pharmacological response when compared with other viscous polymers (Zimmer et al., 1995). It was concluded that bioadhesive polymer-coated nanoparticles are ideally suited for ocular delivery: the viscosity of the medium being low and therefore not causing irritation or discomfort. In view of this, the bioadhesive properties of chitosan in solution make this polymer an interesting candidate for ocular drug delivery.

A previous paper dealt with the interaction between chitosan and liposomes (Henriksen et al., 1994). In the present study, the bioadhesive properties of hydrated chitosan and chitosan-coated liposomes were investigated for their potential use in ocular delivery. The study was in three parts: firstly, in vitro measurements on the force of detachment of hydrated tablets/films; secondly, the effect of various factors on adhesion of chitosan molecules to corneas ex vivo; and thirdly, comparing the retention of liposomes and chitosan-coated liposomes instilled into the eyes of rats in vivo.

## 2. Materials and methods

### 2.1. Materials

Chitosan chlorides were kindly provided by Pronova Biopolymer, Norway. The raw materials were in some cases dialysed to remove the excess hydrochloric acid (see Table 1). The chitosan samples were characterised with regard to degree of deacetylation (%DD) by proton nuclear magnetic resonance (NMR) (Vårum et al., 1991) and to molecular weight ($M_w$) estimated from the intrinsic viscosity as described by Anthonsen et al. (1993). Carbopol 934P and polycarbophil Noveon AA1 were supplied from BF Goodrich, USA, while hydroxypropylmethylcellulose (HPMC) was Methocel K100M obtained from Colorcon, England.

$^{3}$H-Labelled acetic anhydride was obtained from Amersham Life Science (TRA 381), while $^{125}$I-labelled bovine serum albumin (BSA), egg L-α-phosphatidylcholine (PC, approx. 99%) and

Table 1
Chitosan qualities used in the study

| Chitosans used (all: chloride salts) | %DD | $\|\eta\|$ (ml/g) | Estim. $M_w$ | % HCl |
|---|---|---|---|---|
| Sea Cure CL 113 | 83 | 84 | —[a] | 15.4 |
| Sea Cure 210 | 92 | 646 | —[a] | 16.0 |
| Sea Cure 210, dialysed | 92 | 728 | 178 000 | [b] |
| Sea Cure 310, dialysed | 78 | 1290 | 369 000 | [b] |
| [$^3$H]Chitosan 1, dialysed | 80 | 660 | 157 000 | [b] |
| [$^3$H]Chitosan 2, dialysed | 80 | 250 | 45 000 | [b] |

%DD, percent degree of deacetylation; $|\eta|$ = intrinsic viscosity; Estim. $M_w$, estimated molecular weight from $|\eta| = K \cdot M^a$ according to Anthonsen et al. (1993); % HCl, HCl content as reported by manufacturer.
[a] Not estimated because of excess HCl present.
[b] Excess HCl removed by dialysis.

L-α-phosphatidyl-DL-glycerol (PG, approx. 99%) were from Sigma, USA. All other chemicals were of analytical grade.

Corneas from freshly killed pigs or cattle were obtained from a local abattoir.

### 2.2. Preparation of $^3$H-labelled chitosans

A chitosan with DD 99% was reacetylated with $^3$H-labelled acetic anhydride as described by Kurita et al. (1989) to a final DD of 80% ([$^3$H]chitosan 1). [$^3$H]Chitosan 2 was prepared from [$^3$H]chitosan 1 by nitrous acid degradation as previously described (Anthonsen et al., 1993). The chitosans were converted to the hydrochloride forms by dialysis towards NaCl, and then towards distilled water before freeze-drying.

The activity was measured with a Packard model 1900 liquid scintillation counter. The activity of the reacetylated chitosans was 127 000 dpm/mg for chitosan 1 and 111 000 dpm/mg for chitosan 2. The %DD and estimated molecular weights of the reacetylated chitosan chlorides are given in Table 1.

### 2.3. Production and coating of liposomes

Liposomes were produced using egg PC and egg PG in a ratio of 10:1. Chloroform solutions of the phospholipids were mixed and evaporated to dryness in a 250-ml round-bottom flask. The lipid film was hydrated with a 1.9% boric buffer/10 mM NaCl (pH = 6.0 ± 0.1) containing 50 μCi $^{125}$I-BSA to give a final lipid concentration of 40 mg/ml. The resulting liposomes were allowed to swell for 2 h before extrusion through two 100-nm polycarbonate membranes. The suspension was extruded 10 times to achieve a narrow unimodal distribution. The liposomes were separated from non-entrapped $^{125}$I-BSA by centrifugation at 247 000 × $g$ for 2 h, the supernatant removed and the liposome pellet resuspended in buffer. This washing procedure was repeated once. The mean diameter of the liposomes was measured by photon correlation spectroscopy (PCS) using a Coulter N4 MD at a 90° angle. The size of extruded and washed liposomes was 111 nm (unimodal analysis) and the polydispersity index 0.1.

Liposomes were coated with chitosan as described previously (Henriksen et al., 1994). The washed liposomes were added to 0.2% chitosan 210 solution (dialysed and filtered) and stirred for 5 min. Excess polymer was removed by centrifugation at 60 000 × $g$ for 15 min and the resulting pellet resuspended in buffer to give a final activity of approximately 500 000 cpm/ml. After coating with chitosan, the mean liposome size was 800 nm and the polydispersity index 0.7. This increase in size is due to some aggregation, as previously described (Henriksen et al., 1994). Coating was confirmed by measuring the change in the zeta potential of the liposomes using a Coulter DELSA 440. Non-coated liposomes had a zeta potential of − 36.4 mV ( ± 1.6, $n$ = 7) after washing, and chitosan-coated liposomes a potential of + 28.6 mV ( ± 1.9, $n$ = 4).

The efficacy of encapsulation of $^{125}$I-BSA was 13%. During the coating process, about 6% of encapsulated marker leaked out and was removed with the supernatant. During storage, non-coated liposomes leaked 17% of their $^{125}$I-BSA content in 24 h compared with 11% leakage from the chitosan-coated liposomes (chitosomes). Both non-coated and chitosan-coated liposomes were used on the day of preparation.

### 2.4. Assessment of adhesion by measuring the force of detachment

Force of detachment was measured with the equipment as described by Smart (1991). The test substance was either compressed to a 50-mg tablet (by applying 1 tonne force for 5 s), and attached to a 1.5-g weight using cyanoacrylate adhesive, or the substance was dissolved in water (2% w/v) and 100 $\mu$l applied to the 1.5-g weight. The films were then dried at 50°C. The force of detachment was measured after prehydration of the film/tablet for 2 or 30 min in 50 mM Tris buffer (pH = 7.2, 37°C) and 2 min adhesion.

The substrate for adhesion was either a dialysis membrane (Visking, size 2, 14.3 mm) or freshly excised pigs cornea. The corneas were used within 24 h after the animal was killed and stored in 0.9% saline solution. Membranes or corneas were cut to a suitable size and mounted in the substrate holder.

### 2.5. Adhesion of dissolved chitosan to cattle corneas ex vivo

Eyes were dissected from freshly killed animals and stored for up to 1 day in saline solution. Corneas were cut to a suitable size and mounted in a holder so that a constant area of tissue (3.75 cm$^2$, anterior surface) would be exposed to the test solution. Then 0.5 ml of 0.5% [$^3$H]chitosan in 10 mM acetic buffer (ionic strength adjusted with 0.15 M NaCl when necessary; see below) was added to the corneal surface for the appropriate time. The test solution was then removed and the tissue washed 10 times with 0.4 ml of the buffer. A circular knife was used to sample $3 \times 0.385$ cm$^2$ of tissue which was then dissolved in 1 ml Soluene-350. Hionic-fluor scintillation liquid (10 ml) was added and activity measured by scintillation counting. Counts were adjusted by a correction factor to give disintegrations per minute (dpm) per treated corneal area (cm$^2$) and calculated as a percentage of original activity in the applied test solution. The washings and the incubate were also counted, and the recovery calculated.

A $2^3$ factorial design (Montgomery, 1991) was used to investigate the effect of selected parameters on the affinity of dissolved chitosan for the corneal surface. A factorial design utilises all the available data in the statistical analysis and is therefore more efficient than the traditional one-factor-at-a-time approach. Furthermore, if interactions exist between some of the factors, a factorial design is necessary to identify them.

The $2^3$ design studies three factors. Because there are only two levels for each factor (high and low value), it must be assumed that the response is approximately linear over the range of the factor levels chosen. The factors to be investigated were contact time (factor A), ionic strength (factor B) and pH (factor C). The levels of the factors are shown in Table 2. As is usual in this design, the test solutions were named with the letter of the factor at a high level, while the test solution with all factors at a low level was named (1). All experiments were run in triplicate. The estimated effects of increasing the factors from a low to a high level were tested for significance by analysis of variance.

In a control experiment, polycarbonate membranes were incubated with test solutions (1) (all factors at a low level) and (c) (factor C at a high level, A and B at a low level).

Table 2
Levels of the factors studied in the $2^3$ factorial design

| Factor | Level | |
|---|---|---|
| | −1 | +1 |
| A: Application time (min) | 10 | 60 |
| B: Ionic strength (mM) | 5 | 150 |
| C: pH | 4.0 | 6.0 |

An experiment with the lower-molecular-weight chitosan 2 (Table 1) was performed with test solution (c).

### 2.6. *In vivo retention of liposomes and chitosan-coated liposomes*

Wistar rats weighing 200–220 g were lightly anaesthetised with sodium thiopentone (50 mg/kg) injected intraperitoneally. Anaesthesia was used to facilitate the instillation of solution into the eye, reducing the risk of accidental damage to the eye due to sudden movement.

Free $^{125}$I-BSA (7 $\mu$l; approx. 300–600 nCi) in 1.9% boric buffer (pH = 6.0), or entrapped in either non-coated liposomes or chitosan-210-coated liposomes was applied to each eye. The eyelids were mechanically blinked five times to ensure even distribution of solution over the corneal surface. The rats were killed by carbon dioxide intoxication 0, 10, 30 or 90 min after application. The eyelids and eyes were excised and the eyes were washed with 1 ml 1% Triton X-100 to remove excess $^{125}$I-BSA on the surface. Retention of radioactivity in the eyelids, eyes and the washings from the eye was measured using a Packard Minaxi 5000 gamma counter.

## 3. Results

### 3.1. *Assessment of adhesion by measuring the force of detachment*

Fig. 1 shows the force of detachment from the dialysis membrane for tablets and films of different chitosan qualities and some control polymers. There were no significant differences between chitosan 210, HPMC or Carbopol 934P tablets because of large standard deviations. The force of detachment for tablets prepared from chitosan 210 was significantly reduced after dialysis ($t$-test, $P < 0.01$). Chitosan 113 showed a low adhesion of about 2 mN (tablets and films). The 2% films of polycarbophil and carbopol did not adhere.

Adhesion of films to porcine corneas is shown in Fig. 2. It was low for chitosan 210 and 113 (3.0 ± 1.03 and 3.6 ± 0.8 mN, respectively; ± S.E.M., $n = 5$), but higher for HPMC (17.6 ± 4.7 mN, $n = 4$).

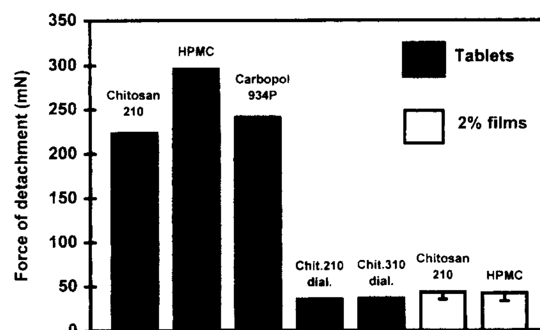


Fig. 1. Force of detachment measured with dialysis membrane as substrate ($n = 4$–6; bars show S.E.M.). Filled bars, polymer tablets; open bars, 2% film of polymer; Chit. 210 dial., dialysed chitosan Sea Cure 210; Chit. 310 dial., dialysed chitosan Sea Cure 310.

Increasing the prehydration time for films from 2 to 30 min increased adhesion of chitosan 210 to dialysis membranes from 43 to 55 mN ($n = 5$), but decreased adhesion for HPMC from 42 to 26 mN ($n = 6$). The difference in tensile force at 30 min was significant ($P < 0.05$).

### 3.2. *Adhesion of dissolved chitosan to cattle corneas ex vivo*

Total recovery of [$^3$H]chitosan incubated with corneas was 98.16 ± 3.41% ($n = 24$), with most of the activity remaining in the test solution and 7.75 ± 1.54% ($n = 24$) in the washing solutions. The activity decreased to zero during 10 washings.

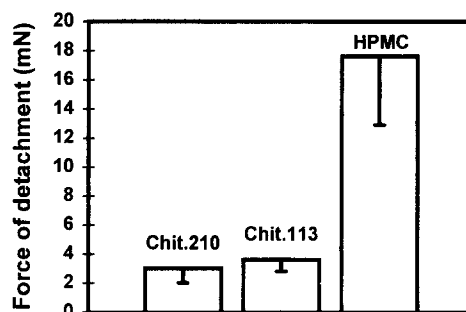


Fig. 2. Force of detachment of 2% films measured with pig corneas as substrate ($n = 4$–5; bars show S.E.M.). Chit. 210, chitosan Sea Cure 210; Chit. 113, chitosan Sea Cure CL 113.

Table 3
% Chitosan recovered from cattle corneas in the $2^3$ factorial design

| Treatment | % Adsorbed to cornea | | |
|---|---|---|---|
| | Parallel 1 | Parallel 2 | Parallel 3 |
| [$^3$H]Chitosan 1 | | | |
| (1) (10 min, 5 mM, pH = 4) | 0.68 | 0.70 | 0.64 |
| (a) (60 min, 5 mM, pH = 4) | 1.50 | 1.40 | 1.13 |
| (b) (10 min, 150 mM, pH = 4) | 0.33 | 0.81 | 0.67 |
| (ab) (60 min, 150 mM, pH = 4) | 1.19 | 0.86 | 1.42 |
| (c) (10 min, 5 mM, pH = 6) | 2.42 | 2.80 | 2.27 |
| (ac) (60 min, 5 mM, pH = 6) | 3.52 | 4.74 | 6.56 |
| (bc) (10 min, 150 mM, pH = 6) | 2.34 | 2.16 | 1.60 |
| (abc) (60 min, 150 mM, pH = 6) | 2.51 | 2.70 | 3.15 |
| $MS_{error} = 0.356$ | | | |
| [H$^3$]Chitosan 2: | | | |
| (c) (10 min, 5 mM, pH = 6) | $4.4 \pm 1.06\%$, $n = 6$ | | |

Each treatment is specified in the brackets as the combination of application time, ionic strength and pH of the test solution

Table 3 shows the percentage of [$^3$H]chitosan 1 adsorbed to the cornea. All three factors had a significant effect ($P < 0.01$), and there was a significant interaction between pH and ionic strength ($P < 0.05$). The average effect (i.e. the mean difference between treatments at low and high level) of factor A was an almost 2-fold increase. The effect of increasing ionic strength (B) was to reduce the percentage adsorbed about 60%, and the effect of increasing pH (C) was to increase the adsorption more than 3-fold. The significant interaction B–C was negative, which means that the positive effect of increasing pH was suppressed when the ionic strength was high.

Incubation of [$^3$H]chitosan 1 with polycarbonate membranes gave $0.47 \pm 0.14\%$ ($n = 6$, $\pm$ S.D.) with treatment (1) and $0.49 \pm 0.11\%$ ($n = 6$) with treatment (c). The difference between the two treatments was not significant. However, both are significantly lower than treatment (c) on the corneas ($2.45 \pm 0.61\%$, $n = 7$, $P < 0.01$).

The lower-molecular-weight [$^3$H]chitosan 2 showed a significantly higher adsorption than chitosan 1 using treatment (c) ($4.4 \pm 1.06\%$, $n = 6$, $P < 0.01$).

### 3.3. Retention of liposomes and chitosan-coated liposomes in vivo

The efficacy of recovery of $^{125}$I-BSA when applied to the eyes of freshly killed rats as a solution was $42.2 \pm 4.2\%$, of which about 70% was associated with the eyelids. There was a progressive decrease in the recovery of $^{125}$I-BSA when it was applied 10, 30 or 90 min prior to killing (Table 4). Retention of the radioactive marker was greatest with chitosomal-entrapped $^{125}$I-BSA, except for the eyelids at 30 min. However, the differences between coated and non-coated liposomes were not significant. Both the coated and non-coated liposomal formulations had a significantly higher retention than $^{125}$I-BSA in solution at 10 and 30 min ($P < 0.05$ and $P < 0.01$, Table 4).

## 4. Discussion

The in vitro screening of chitosans by a force of detachment method had two purposes. Firstly, this method is a convenient way of comparing new (bio)adhesive candidates with established bioadhesive polymers. It has previously been suggested that the general adhesive properties of a test material may be initially screened by using

Table 4
Recovery of free or liposome/chitosome-entrapped $^{125}$I-BSA instilled into the eyes of anaesthetised rats ($n = 6$)

| % $^{125}$I-BSA recovered (± S.E.M.) | | | | | | |
|---|---|---|---|---|---|---|
| Time (min) | Formulation | Eye | Eye-washings | SUM eye+washings | Eyelids | Total |
| 0 | Free BSA | 3.6 ± 0.5 | 8.3 ± 0.5 | 11.9 ± 0.8 | 30.3 ± 2.1 | 42.2 ± 1.7 |
| 10 | Free BSA | 0.5 ± 0.2 | 1.3 ± 0.4 | 1.8 ± 0.7 | 5.0 ± 1.8 | 6.8 ± 2.2 |
| | Liposomes | 1.0 ± 0.2 | 5.1 ± 1.5* | 6.1 ± 1.7* | 11.2 ± 1.9 | 17.3 ± 2.7* |
| | Chitosomes | 1.5 ± 0.2** | 5.0 ± 1.1** | 6.5 ± 1.1** | 17.4 ± 4.5 | 23.9 ± 4.4** |
| 30 | Free BSA | 0.4 ± 0.1 | 1.2 ± 0.3 | 1.7 ± 0.3 | 6.4 ± 2.2 | 8.1 ± 2.5 |
| | Liposomes | 1.0 ± 0.2* | 3.6 ± 0.6** | 4.6 ± 0.8** | 19.1 ± 4.5 | 23.7 ± 5.3* |
| | Chitosomes | 1.5 ± 0.2** | 3.9 ± 0.7** | 5.4 ± 0.9** | 14.8 ± 4.7 | 20.2 ± 4.5* |
| 90 | Free BSA | 0.4 ± 0.2 | 1.1 ± 0.3 | 1.5 ± 0.4 | 4.2 ± 1.1 | 5.4 ± 0.9 |
| | Liposomes | 0.6 ± 0.2 | 1.3 ± 0.5 | 1.9 ± 0.7 | 8.5 ± 3.4 | 10.3 ± 4.0 |
| | Chitosomes | 0.5 ± 0.2 | 2.0 ± 0.8 | 2.5 ± 0.9 | 8.5 ± 4.0 | 11.0 ± 4.9 |

* Significantly different from free BSA ($P < 0.05$).
** Significantly different from free BSA ($P < 0.01$).

standard, inert surfaces (Mortazavi and Smart, 1995). Secondly, it was hoped to obtain some indication as to which chitosan quality would be most suitable for later in vivo studies. The test showed both that chitosan performed comparably to existing adhesives (HPMC and Carbopol 934P) and that there were significant differences between different chitosan qualities. Both tablets and films of chitosan were found to be adhesive, whereas Carbopol 934P films became over-hydrated and lost cohesion. It was also shown that prolonged hydration periods did not reduce the adhesive properties of chitosan 210 films, in contrast to HPMC films. Neither tablets nor films of polycarbophil adhered at pH 7.2. These results can be related to the predicted degree and rate of hydration. Needleman and Smales (1995) also found that Carbopol 934P and polycarbophil did not adhere at neutral pH when applied as a gel to buccal mucosa in organ culture, whereas different chitosan gels showed up to 4 days retention.

Significant differences were seen between chitosan grades. Tablets prepared from non-dialysed chitosan 210 adhered better than chitosan 113 tablets, but dialysis and freeze-drying reduced the adhesion significantly. According to Park and Robinson (1985), physical aspects such as degree and rate of hydration as well as particle size are important to bioadhesion. Several physical aspects may be responsible for the differences seen here: the spray-dried chitosan chlorides from the manufacturer had a different appearance to the freeze-dried dialysed chitosan. It is also possible that a cross-linker is removed during dialysis, or that the excess hydrochloride present in manufactured salts is necessary to promote wetting and/or hydration. Interestingly, Lehr et al. (1992) showed that a low-molecular-weight chitosan glutamate salt had better adhesion than a range of higher-molecular-weight chitosan bases. The trend seen with the bases was that a higher molecular weight gave a higher force of detachment. Likewise, Needleman and Smales (1995) used two hydrochloride salts to achieve good retention of gels, the highest-molecular-weight chitosan showing the greatest retention. This trend was not apparent in the present results, either with the dialysed chitosan tablets on dialysis membrane or with chitosan films on pig corneas. These results emphasise the importance of using well characterised chitosans for comparison with other polymers.

Radiolabelled chitosan was used to study factors that could influence interaction between chitosan and a biological surface, in this case the cornea from cattle. This ex vivo method gave low, but reproducible values for adsorption to the corneas and to the polycarbonate (PC) membranes used as controls. Total recovery of

[$^3$H]chitosan was 98%, 10 washings being sufficient to remove all unadsorbed polymer from the surface. All factors tested had a significant effect on adsorption of [$^3$H]chitosan (Section 3.2.).

The finding of increased adsorption with increased contact time is in agreement with the results of Claesson and Ninham (1992) who found that a few hours were needed for adsorption of chitosan to mica to reach equilibrium. Slow adsorption kinetics may be a problem in drug delivery situations, but the large increase in [$^3$H]chitosan with increasing pH is favourable, considering that tear fluid has a pH of 7.8. This result was surprising if the positive charge of chitosan is important in bioadhesion. Chitosan has a p$K_a$ value of about 6.3, and increasing the pH from 4 to 6 should therefore give a significant reduction in positive charge. However, it is in agreement with general adsorption theory that adsorption is facilitated from poor solvents, chitosan being less charged as the pH increases. The work of Claesson and Ninham (1992) also showed that an increase in pH from 3.8 to 6.2 gave a more compact adsorbed layer due to a less favourable polymer–water interaction and a more favourable polymer–polymer interaction. Increasing the ionic strength had a negative effect on the adsorption and, in addition, there is a significant interaction between pH and ionic strength. A closer analysis of the data showed that the ionic strength had effect only at pH 6.0. The effect coincided with chitosan changing from being a rather strong polyelectrolyte at pH 4 to a weaker polyelectrolyte at pH 6. According to Lyklema (1995), increasing the ionic strength tends to reduce the adsorption of weak polyelectrolytes because the ions may screen the electrostatic attractions. Ion competition may be another explanation. Goldberg et al. (1990) also found a reduced adsorption of chitosan to cells in the presence of $Na^+$. Consistent with the results of Claesson and Ninham (1992) is also the finding that little desorption seems to occur during washing of the incubated tissue. The effect of molecular weight, the most obvious difference between [$^3$H]chitosan 1 and 2, may be related to the respective diffusion coefficients, since the present study was performed at non-equilibrium conditions. Furthermore, these adsorption measurements only represent the 'biological side' of the bioadhesive link. A high molecular weight is probably necessary to make effective bridging between a tissue and a drug device.

Many authors have shown that positively charged liposomes can increase the retention and/or bioavailability of topically instilled drugs in the eye in vivo. Le Bourlais et al. (1995) emphasized that the main object in ocular therapy is to increase the contact time between the carrier and the corneal epithelium. It was, therefore, of interest to test the earlier described chitosan-coated liposomes (Henriksen et al., 1994) in this regard. In the in vivo study, the activity in the eye washings was thought to represent the amount of marker loosely associated with the surface/mucin layer of the eyeball. $^{125}$I-BSA which remains associated with the eyeball may represent binding/adsorption to the corneal surface. Entrapment of $^{125}$I-BSA in liposomes or chitosomes enhanced retention of the marker in the eyelids, the eyes and the eye washings at least 2–3-fold. However, coating the liposomes with chitosan did not appear to increase liposomal $^{125}$I-BSA retention in the eyes significantly following topical application. This contrasts with the results from in vitro studies by Takeuchi et al. (1994) who reported that coating the liposomes with chitosan enhanced their adhesion to isolated rat intestine. However, this group did not remove the excess chitosan in solution and the coated liposomes were diluted 100 times in phosphate buffer (pH = 7.4) before incubation with the tissue. This should theoretically lead to a precipitation of the 85% deacetylated chitosan used, and the technique of counting particles remaining in solution is therefore doubtful. However, it was shown by fluorescence microscopy that chitosan-coated liposomes (but not non-coated liposomes) adhered to the tissue, so this indicates that also in this study chitosan adhered at a neutral pH.

According to Needleman and Smales (1995), chitosan chloride gels show excellent tissue wetting properties. Wetting is an indication of polarity, and it is generally agreed that the presence of hydrogen bonding sites at physiological pH promotes bioadhesion. This will be the case with

chitosan, which at neutral pH has numerous amine and hydroxyl groups. There will also be a number of $NH_3^+$ groups that may increase the interaction with the negative mucin or cell surfaces. Good bioadhesion at a neutral pH has been achieved with chitosan gels (Needleman and Smales, 1995), solutions (Takeuchi et al., 1994) and films (Lehr et al., 1992). It appears that with solid/semi-solid formulations, it is advantageous to include an acid within the device, to ensure a balance between hydration and swelling creating molecular mobility that permits wetting.

The ex vivo study indicated that a liquid chitosan formulation should have a low ionic strength and a low buffering capacity. Because of this, in the in vivo study, boric acid buffer at pH 6.0 was chosen, since at this pH it has a low degree of dissociation ($pK_a = 9.14$), a 1.9% solution being isotonic with the tear fluid. In this study, chitosan was adsorbed to the liposomes, but retention in the eye was not significantly longer than with non-coated liposomes. This suggests that charge alone is not enough to increase retention to the corneas in vivo. Adsorbed chitosan layers may not have the mobility that is necessary to interact closely with corneal mucin, as the layer is probably flat and irreversibly adsorbed (Henriksen et al., 1994). The results of Takeuchi et al. (1994), indicate that it is of advantage to have excess chitosan in solution. All the above factors should be taken into account when chitosan is included in an intended bioadhesive formulation.

## 5. Conclusion

Chitosan is a promising bioadhesive material at physiological pH, as shown in this and earlier reports. However, coating liposomes with chitosan did not significantly improve liposome retention on the eye in vivo. Application of a chitosan solution to the isolated corneas of cattle showed that the polymer had a significantly greater affinity towards corneal tissue than towards a synthetic surface such as polycarbonate. A neutral or slightly alkaline pH is advantageous to adsorption, as is a low ionic strength, a long contact time and a low molecular weight. When chitosan is formulated in a drug delivery system, care must be taken to maximise the bioadhesive properties and to use samples of chitosan which have been well characterised.

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