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Evaluation of the clearance characteristics of bioadhesive systems in humans

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Abstract

This paper describes the characterisation, radiolabelling and clearance characteristics of three bioadhesive nasal delivery systems; starch microspheres, chitosan microspheres and chitosan solution. The time taken for 50% of these bioadhesive materials and a control to be cleared from the nasal cavity, after nasal administration to human volunteers, was evaluated using gamma scintigraphy. The data show that the control was cleared rapidly, with a half life of 21 min, whereas the bioadhesive delivery systems had much longer half lives. The clearance of the chitosan solution almost doubled to 41 min, whilst the half life of clearance for the starch microspheres more than tripled to 68 min and for the chitosan microspheres the half life of clearance quadrupled to 84 min. From the results reported in this study it is possible to determine that both chitosan systems and the starch microspheres have good bioadhesive characteristics. The results have supported the hypothesis that chitosan delivery systems can reduce the rate of clearance from the nasal cavity, thereby increasing the contact time of the delivery system with the nasal mucosa, providing the potential for increasing the bioavailability of drugs incorporated into these systems.

Keywords: Nasal drug delivery; Chitosan; Starch; Bioadhesive polymers; Mucoadhesion and gamma scintigraphy

1. Introduction

Most peptide and protein drugs are poorly absorbed following oral administration due to substantial degradation in the gastrointestinal tract and/or poor permeability of the intestinal

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epithelial barrier. Furthermore drugs delivered orally are susceptible to first pass metabolism. Consequently, most peptide and protein drugs are administered by parenteral routes and increasing attention has been paid to the nasal route of delivery as a means to increase drug absorption and patient compliance.

The nasal cavity possesses many advantages as a site for drug delivery such as; a large surface area for absorption with a subepithelial layer that is highly vascularised. In addition, blood is drained directly from the nose into the systemic circulation, thereby avoiding first pass metabolism by the liver. However, most peptide and protein drugs are not well absorbed from the nasal cavity when administered as simple solutions; the bioavailabilities normally achieved being of the order of less than 1% (O'Hagan and Illum, 1990). One of the main reasons for these low bioavailability figures, apart from the restricted transport across the epithelial membrane, is the limited time available for absorption within the nasal cavity due to mucociliary clearance.

One strategy for increasing absorption is to prevent the rapid clearance of the delivery system from the nasal cavity and thereby prolong the contact between the drug and the nasal mucosa. Nasally administered bioadhesive materials are capable of slowing down the rate of mucociliary clearance through interactions with the nasal mucosa and thus can increase the time available for drug absorption to take place.

Chitosan, a linear polysaccharide comprised of two monosaccharides: *N*-acetyl-D-glucosamine and D-glucosamine linked together by B(1–4) glucosidic bonds, is one such material that has been shown to be mucoadhesive (Lehr et al., 1992; Henriksen et al., 1996). The term chitosan is a generic term that refers to a family of polymers, individually characterised by their molecular weight and ratio of acetylated to deacetylated units. Chitosan salts are positively charged and soluble in water. Chitosan is produced by the alkaline hydrolysis (deacetylation) of chitin which can be obtained from the waste outer shells of crustaceans, for example crabs and shrimps, the exoskeletons of which consist of 15-20% chitin (Mathur and Narang, 1990).

In addition to mucoadhesion, chitosan has also been shown to enhance drug absorption via the paracellular route through neutralisation of fixed anionic sites in the tight junctions between mucosal cells (Artursson et al., 1994; Borchard et al., 1996). As a consequence, chitosan has great potential as a nasal delivery system, facilitating the passage of large hydrophilic molecules such as insulin and salmon calcitonin across the nasal mucosa (Illum et al., 1994). Chitosan has also been shown to facilitate the transport of decapeptides and hydrophilic marker molecules across intestinal mucosa and Caco-2-cell lines, respectively (Rentel et al., 1993; Artursson et al., 1994). Furthermore, chitosan is considered to be non-toxic with an oral LD_{50} in mice of more than 16 g/kg (Arai et al., 1968) and does not cause significant changes in the histology of the nasal mucosa at therapeutic concentrations (Illum et al., 1994).

The concept of the bioadhesive starch microspheres as a nasal delivery system was first introduced by Illum et al., 1987, in a study that examined human nasal clearance rates. Since then starch microspheres have been shown to enhance the nasal absorption of a number of drugs including; the polar antibiotic gentamicin (in sheep; Illum et al., 1988), human monocomponent insulin (in rats; Bjork and Edman, 1988), biosynthetic human growth hormone (in sheep; Illum et al., 1990), semisynthetic zinc-human insulin (in sheep; Farraj et al., 1990) and desmopressin (in rats and sheep; Critchley et al., 1994).

A conceivable hypothesis for the mechanism of action of starch microspheres is that, due to water absorption in the gelling process, starch is able to dehydrate the epithelial mucosa causing a reversible shrinkage of the epithelial cells. This shrinkage could lead to the physical separation of the intracellular junctions and thus enhance paracellular absorption. This hypothesis was supported by a study that reported focal dilations in the tight junctions between human intestinal epithelial Caco-2 cells after administration of starch microspheres (Bjork and Edman, 1990).

An extended toxicological evaluation of the starch microspheres still has to be performed. However, an 8-week study of twice daily nasal administration of starch microspheres to rabbits

caused no effect on mucociliary clearance and gave no evidence of inflammation or toxicity (Edman et al., 1992).

Although there has been much research in the last few years evaluating chitosan as a potential intranasal drug delivery system no study has yet described the nasal clearance characteristics of chitosan solution or microspheres in humans in vivo. The primary aim of this study, was therefore, to investigate the clearance rate of chitosan formulations from the human nasal mucosa. Starch microspheres were evaluated at the same time so that the clearance characteristics of the two bioadhesive materials could be compared.

This paper describes the characterisation and radiolabelling of three bioadhesive nasal delivery systems; starch microspheres, chitosan microspheres and chitosan solution. The clearance characteristics of these bioadhesive materials after nasal administration to human volunteers were investigated using the non-invasive technique of gamma scintigraphy. Gamma scintigraphy is particularly useful for this purpose, since, unlike most conventional medical imaging techniques such as X-rays, ultrasound and magnetic resonance, which provide high resolution anatomical definition, gamma scintigraphy provides a functional map of physiological processes (Perkins and Frier, 1996).

2. Materials and methods

2.1. Materials

Chitosan glutamate (Seacure UP G210, batch no. 902-572-05) was purchased from Pronova (Drammen, Norway). Chitosan glutamate is, as part of the production process, spray dried and thereby formed into microspheres. Starch microspheres (Eldexomer, lot no. 019) were purchased from Perstorp AB (Perstorp, Sweden). The starch microspheres were crosslinked with epichlorohydrin and thus rendered insoluble but gelling in water.

99m-Technetium, as sodium pertechnetate, and labelled diethylenetriaminepentaacetic acid (99m Tc-DTPA) were obtained from the Department of Medical Physics, Queens Medical Centre (Nottingham, UK). Metered dosage devices were purchased from Pfeiffer (Kingston-Upon-Thames, UK) and single powder dose devices were purchased from Bespak (King's Lynn, UK). All other chemicals were of analytical grade.

2.2. Characterisation procedures

2.2.1. Microsphere size and surface morphology

Scanning electron microscopy was used to study the size and surface morphology of the chitosan and starch microspheres. For each sample a sample holder was coated with Liet-C glue, a small amount of sample microspheres were then sprinkled as evenly as possible onto the holder whilst the glue was still wet. Once the glue had dried the samples were gold coated for 4 min using a 030 Gold Sputter Coater (Balzers Union). The gold plated samples were visualised using a 505 Scanning Electron Microscope (Phillips) in cryogenic mode. The instrument was kept at −180°C, the other variables were as follows; 25.0 keV, 20 nm spot size and 0°C tilt.

2.2.2. Intrinsic viscosity of chitosan

The chitosan glutamate was dissolved in acetate buffer pH 4.4 to give concentrations between 0.25 and 5 mg/ml. Once the chitosan had dissolved, the solutions were filtered through a 0.5-μm membrane filter. The intrinsic viscosity of chitosan was determined using a 2-ml viscometer (Ostwald). After filling, the viscometer was placed in a thermostatically controlled water bath (Scott-Gerate) set at 25°C. The flow times were recorded electronically using photoreceptors mounted on the viscometer stand, which detected the movement of the solution meniscus.

In order to calculate intrinsic viscosity, values for relative (η_{rel}) and reduced (η_{red}) viscosity were calculated first. The ratio between the flow times of the chitosan solution (η) and pure solvent (η_0) is referred to as relative viscosity ($\eta_{rel} = \eta/\eta_0$). The relative viscosity values for each chitosan concentration (c) were then used to calculate reduced viscosity ($\eta_{red} = \eta_{rel} - 1/c$). Finally, intrinsic viscosity ($[\eta]$) was determined by plotting a graph of reduced viscosity against chitosan concentration

and extrapolating to the interception point of the *y*-axis, corresponding to infinite dilution and consequently intrinsic viscosity (Molyneux, 1984).

2.2.3. Apparent viscosity of chitosan

Chitosan solutions (5 mg/ml) were prepared by dissolving chitosan glutamate in distilled water. The pH of the solutions was adjusted to 4.4 with hydrochloric acid. These solutions were then filtered through a sterile 0.5-μm filter and left to stand for at least 1 h. Aliquots of the solutions (500 μl) were evaluated at 25, 33 and 37°C (room, nasal cavity and body temperature, respectively). Each solution was allowed to equilibrate at the desired temperature for 5 min before evaluation. Finally, after the evaluation at 37°C the samples were cooled to 25°C and their viscosity was re-measured.

The viscosity measurements were made using a DVIII Programmable Rheometer (Brookfield). Calibration of the rheometer was achieved through the use of an oil of known viscosity. The instrument was programmed to spin at 5 rpm, producing shear rates of 37.5/s for 60 s before viscosity measurements were made.

2.3. Radiolabelling procedures

The three radiolabelling procedures described here were carried out under reducing conditions produced by the powerful reducing agent stannous chloride. The stannous ion reduces 99m-technetium from the $^{+7}$ oxidation state to the more reactive $^{+5}$ oxidation state to promote binding. This binding will normally occur at the electron donating functional groups of bioadhesive systems, for example the hydroxyl functional groups of chitosan, since 99m-technetium prefers ligands that are able to compensate for the high positive charge of the central atom (Johannsen, 1987). Due to the short physical half-life of 99m-technetium all activities have been corrected for decay to the time of dosing.

2.3.1. Starch microspheres

Starch microspheres (200mg) were suspended in 2.5 ml 0.9% saline, 1 ml 5 mg/ml stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$) in 1 M HCl, 2.5 ml sodium acetate (NaAC) 100 mg/ml and 1 ml sodium pertechnetate containing about 20 MBq of activity. The suspension was left under continuous stirring for 5 min and then centrifuged at 3000 rpm for 20 min. The supernatant was removed and the microspheres washed with 0.9% saline and centrifuged again. This washing stage was repeated twice. The microspheres were rapidly frozen using liquid nitrogen and then placed into a freeze-drier overnight. The activity was calculated such that a 10-mg dose of microspheres would have 1 MBq of activity at the time of administration.

2.3.2. Chitosan microspheres

Chitosan microspheres (200 mg) were suspended in 5 ml 5 mg/ml $SnCl_2 \cdot 2H_2O$ (in acetone) and 200 μl sodium pertechnetate containing about 20 MBq of activity. The suspension was left under continuous stirring for 5 min and then centrifuged at 3000 rpm for 20 min. The supernatant was removed and the microspheres washed with acetone and centrifuged again. This washing stage was repeated twice. The microspheres were then left in a fumehood to dry overnight. The activity was calculated such that a 10-mg dose of microspheres would have 1 MBq of activity at the time of administration.

During the chitosan and starch microsphere radiolabelling procedures all of the washings were retained for analysis using a 1282 Compugamma CS Universal gamma counter (LKB Wallac). The activity of the radiolabelled microspheres was calculated using an Isocal radionuclide assay calibrator (Vinten). The radiolabelling process was found to be 97% (± 6) efficient for both the starch and the chitosan microspheres.

2.3.3. Chitosan solution

One millilitre of a solution of chitosan glutamate in distilled water (10 mg/ml) was added to 800 μl of a 5-mg/ml $SnCl_2 \cdot 2H_2O$ solution (in 1 M HCl, filtered through a sterile 0.5 μm filter) and 200 μl sodium pertechnetate containing about 20 MBq of activity. The solution was left under continuous stirring for 5 min before being placed into a preactivated dialysis tubing. The solution was then left to dialyse against distilled water

overnight. The activity was calculated such that a 200-μl dose of solution would have 1 MBq of activity at the time of administration.

During the radiolabelling process aliquots were taken from the distilled water dialysate at predetermined intervals and assayed for activity using a 1282 Compugamma CS universal gamma counter (LKB Wallac). At the end of the procedure the dialysis tube was emptied and the activity in the chitosan solution and the membrane were assayed using an Isocal Radionuclide Assay Calibrator (Vinten). Analysis of the dialysate, dialysis membrane and the chitosan solution showed the experiment to be 74% (± 9) efficient. The remaining 26% activity was accounted for as 9% (± 6) in the dialysate and 17% (± 3) still in the dialysis membrane.

2.4. Volunteer recruitment

Prospective male and female volunteers between the ages of 18 and 30 completed a questionnaire about their health and underwent medical screening before being recruited to the study. Only volunteers with no medical abnormalities, as assessed by the questionnaire and screening, were recruited to the study. Prospective volunteers were also excluded if they; smoked, had taken any nasal medication within the last 6 months, used any recreational drugs or had taken part in another medical trial within the previous 3 months.

2.5. Nasal clearance studies

In order to study the clearance characteristics of the nasal delivery systems, radiolabelled chitosan microspheres, starch microspheres, chitosan solution and DTPA were administered into the right nostril of eight healthy human volunteers in a cross-over design with a week between each treatment. No attempt was made to identify the stage of the nasal cycle for the right nostril at the time of administration (Lund, 1996). The study was designed as a four-way cross-over study in eight volunteers with 7 days wash out period between the study legs.

The chitosan and starch microspheres were administered using single dose powder devices (Bespak). The volunteer took two deep nasal inspirations whilst holding the device in the tip of the right nostril. Hence, the contents of the device (10 mg, approximately 1 MBq) was delivered to the mucosal surface of the right half of the nose. The chitosan solution and DTPA solution were filled into metered dosage devices (Pfeiffer) and 100 μl was administered into the right nostril, followed immediately by a second dose in the same nostril of 100 μl making a total of 200 μl; approximately 1 MBq.

The volunteers were trained in the use of the administration devices. However, in some cases the dose was not administered correctly resulting in either some formulation being left in the devices or being administered to the outside of the nose and face. Data obtained from these volunteers, and from volunteers that sneezed during the studies, were discarded. At least six volunteers successfully completed the trial for each formulation.

The deposition and subsequent clearance of the different nasal delivery systems was followed by gamma scintigraphy, using a Maxi Camera II Gamma Camera (General Electric). The position of the nose of the volunteer was fixed on the collimator of the gamma camera using a specially designed template. Dynamic lateral views of the head were recorded for 10 min (60-s frames) directly after administration followed by static lateral views (60 s duration) at appropriate time intervals for 180 min after administration. The images were recorded for subsequent analysis and quantification.

Quantification of the data from the volunteers involved defining regions of interest around the nasal cavity and throat. The count rate from each region of interest (ROI), corrected for radioactive decay and background, was then expressed as a proportion of the highest 1 min count rate, typically the image recorded in the nasal cavity ROI immediately after dosing. That is, the highest count rate was assigned a 100% value, which was then used to calculate the percentage remaining for the other time points.

In this way the clearance of the four formulations from the nasal cavity was evaluated as a decrease in percentage activity against time for

each volunteer. The study had approval from the University of Nottingham Medical School Ethical Committee and was covered by an ARSAC licence from the Department of Health. The whole body effective dose equivalent was calculated to be 0.05 mSv per volunteer for the entire study.

3. Results and discussion

Scanning electron microscopy of the chitosan microspheres used in this study showed the microspheres to be smooth, spherical and variable in size within a range of 5–40 μm. The starch microspheres were found to be smooth, spherical, discrete in nature and variable in size within a range of 45–90 μm.

The reduced and intrinsic viscosity values determined by experimentation and extrapolation for chitosan glutamate are shown in Table 1 and Fig. 1. The intrinsic viscosity of the chitosan was calculated to be 871 ml/g. It can be seen from Table 2 that an increase in temperature caused a decrease in the apparent viscosity of the chitosan solutions. The chitosan samples that were cooled from 37 to 25°C regained apparent viscosities comparable to the aliquots originally tested at 25°C. This implies that the decrease in solution viscosity at a higher temperature is the result of an increase in the flexibility of the chitosan chains and not chitosan degradation. This is important since at nasal cavity temperatures of 33–34°C the chitosan systems will posses increased flexibility, giving enhanced penetration of the mucin network.

Table 1
The flow times for the solvent and chitosan solutions used to calculate the reduced viscosity values

Sample identification	Average flow times (s)	Reduced viscosity
Acetate buffer	81.19	–
0.25 mg/ml chitosan	99.64	909
0.5 mg/ml chitosan	122.37	1914
3 mg/ml chitosan	434.71	1451
5 mg/ml chitosan	872.78	1950

For the purposes of deposition the nose can crudely be considered to be made up of two parts; an anterior region which is non-ciliated and relies on traction from contiguous mucus for clearance, and a ciliated region from which clearance is rapid in comparison. Studies have shown that particles deposited within the anterior region of the nasal cavity can remain static for up to 2 h (Newman et al., 1987a). Therefore, for any formulation the proportion of the dose deposited within the anterior region of the nose is a major factor in the clearance characteristics observed (Newman et al., 1987b).

Upon administration, the formulations evaluated in this study were deposited into both the anterior and turbinate regions of the nose. The proportions that were delivered into each region appeared to be dependent on the administration technique of individuals rather than the size of the microspheres or the administration device used. That is, the proportion of the dose deposited in the anterior region varied within the volunteer group for each formulation. However, the site of deposition was relatively similar for all four formulations for each volunteer. Therefore the differences in clearance characteristics observed between formulations will be due to the bioadhesive properties of the formulations and not to the site of deposition.

The averaged clearance data for each formulation from the nasal cavity ROI can be seen in Fig. 2. In addition, using the averaged clearance data, the time taken for 50% of the formulation to be cleared form the nasal cavity ROI was calculated (Table 3). This averaged data shows that the control DTPA solution was cleared rapidly, with a half-life of 21 min, whereas the bioadhesive delivery systems had much longer half-lives.

The time taken for 50% clearance of the chitosan solution almost doubled to 41 min, whilst the half-life of clearance for the starch microspheres more than tripled to 68 min and for the chitosan microspheres half-life of clearance quadrupled to 84 min. The rapid clearance of the DTPA control solution was not unexpected since similar results for Lomudal powder, Lomudal solution and albumin solutions have been previously reported (Hardy et al., 1985; Illum et al., 1987).

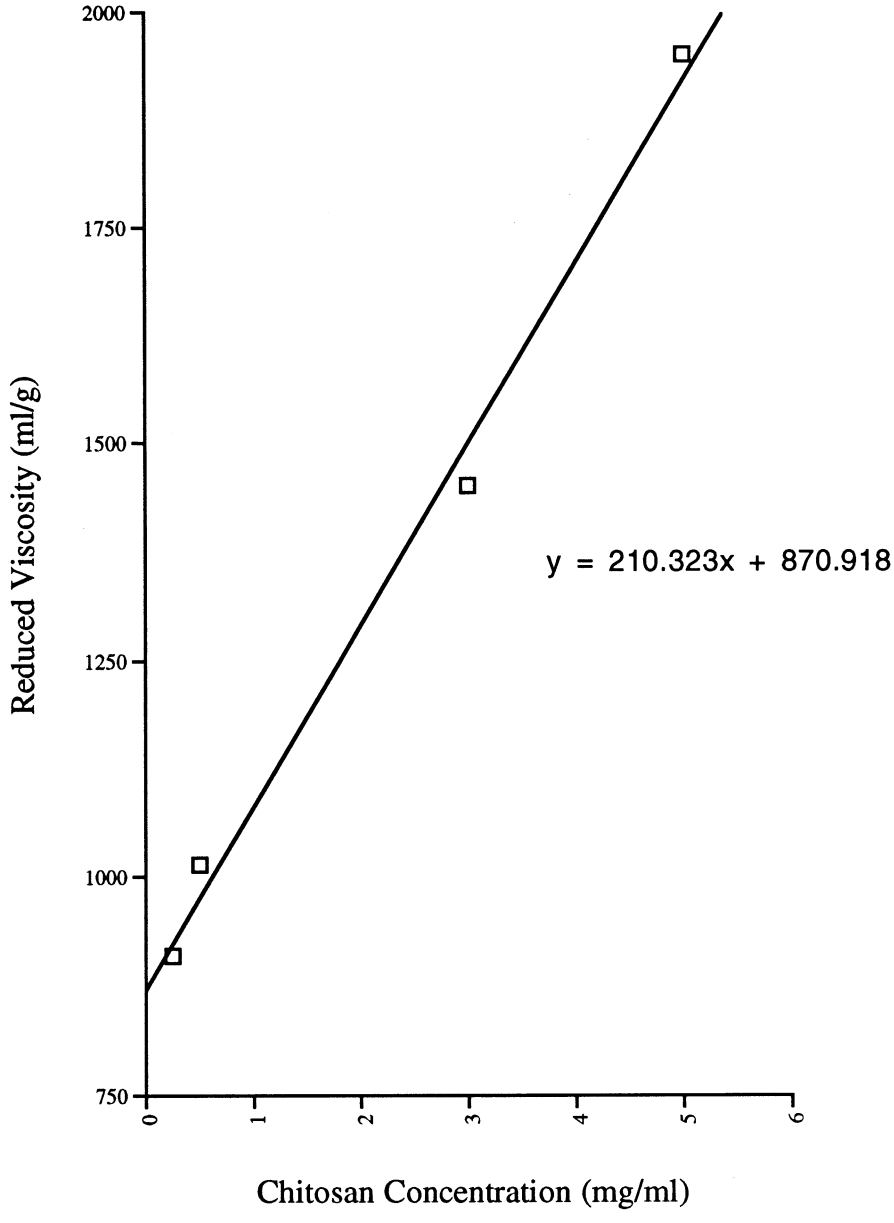

Fig. 1. Reduced viscosity of chitosan versus chitosan concentration (Huggins Plot).

The slow clearance of the chitosan and starch systems can be explained by their bioadhesive properties.

The mechanism of interaction between bioadhesive polymers and the mucus layer has been discussed previously (Ahuja et al., 1997). Briefly, the mucus layer is made up of a network of mucin molecules which are linear, flexible and negatively charged due to the sialic acid and sulphate residues present on the mucin molecules. The network is cross-linked and highly hydrated (Leung and Robinson, 1988). It is essential that intimate molecular contact exists between the bioadhesive systems and the mucins so that strong bonds may develop.

Table 2
The effect of temperature on the apparent viscosity of 0.5% chitosan glutamate solutions

Temperature (°C)	Viscosity of chitosan glutamate samples (cP)				Average	S.D.
25	24.5	21.3	28.3	26.3	25.1	±2.97
33	18.3	17.7	21.3	19 9	19 3	±1 62
37	16.4	16.2	17.8	18.9	17.3	±1.27
37–25	24.9	26.7	29 2	27 0	26 9	±1.76

It is important that the two microsphere systems absorb water from this hydrated layer, enabling the polymer chains to penetrate the mucin network and establish adhesive bonds. In comparison, adhesion between the chitosan solution and the mucins should be rapid, since, the chitosan solution is already in a hydrated form. However, it is questionable as to whether this is favourable since any 'advantage' gained through the comparatively rapid formation of adhesive bonds may be more than eclipsed by the loss of the mucosal dehydrating effects.

As the microsphere systems absorb water they may dehydrate the mucous layer forming areas of concentrated bioadhesive gel/mucus with an increased viscosity. In comparison, the chitosan solution may further hydrate the mucosa. These areas of increased viscosity, due to both dehydration of the mucosa and adhesive bond formation, may impart increased resistance to cilia beat frequency when compared to the sole resistance produced by the chitosan solution. This would explain the differences observed between clearance of the two chitosan systems. The chitosan microspheres had a half-life approximately double that of the chitosan solution.

The variation in clearance characteristics observed between the chitosan and starch microsphere systems can also be, in part, explained by variations in water absorption and molecular contact. Although the cross-linked starch microspheres will absorb water they are insoluble and in contrast to the chitosan microspheres will not form a liquid gel on the nasal epithelium but rather a more solid gel-like structure. This decrease in flexibility imposed upon polymer chains by the cross-linking makes it more difficult for cross-linked polymers to penetrate the mucin network (Pepas and Buri, 1985). Thus, cross-linking effectively limits the length of polymer chain that can penetrate the mucus layer.

This effect contrasts markedly with the soluble chitosan microspheres whose flexibility in the liquid gel state is further enhanced due to the ambient temperature of the nasal cavity. Therefore, it is likely that the starch microspheres are not capable of the degree of intimate molecular contact and thus the strength of adhesive bonds with the mucin network that chitosan is capable of.

In addition to the differences in flexibility and thus molecular contact, chitosan and starch also possess different charges. Chitosan is strongly cationic whilst starch is anionic. These differences in surface charge will affect the molecular interactions; such as the electrostatic, van der Waals and hydrogen bonding forces, between the interpenetrating bioadhesive polymer chains and the mucin network. Both starch and chitosan posses the electronegative groups associated with hydrogen bonding. However, the anionic starch microspheres will also induce repulsive electrostatic forces with the negatively charged mucin network. These repulsive electrostatic forces could reduce the overall mucoadhesive strength.

In contrast, it has been suggested that one of the main reasons for the strong mucoadhesive characteristics displayed by chitosan is the electrostatic interactions between the positively charged amino groups in chitosan and the negatively charged sialic acid residues present in mucins (Aspden et al., 1995). Therefore, charge differences, in conjunction with the differences in molecular contact and flexibility, would explain the differences observed between clearance of the two microsphere systems. The half-life of clearance was 25% greater for the chitosan micro-

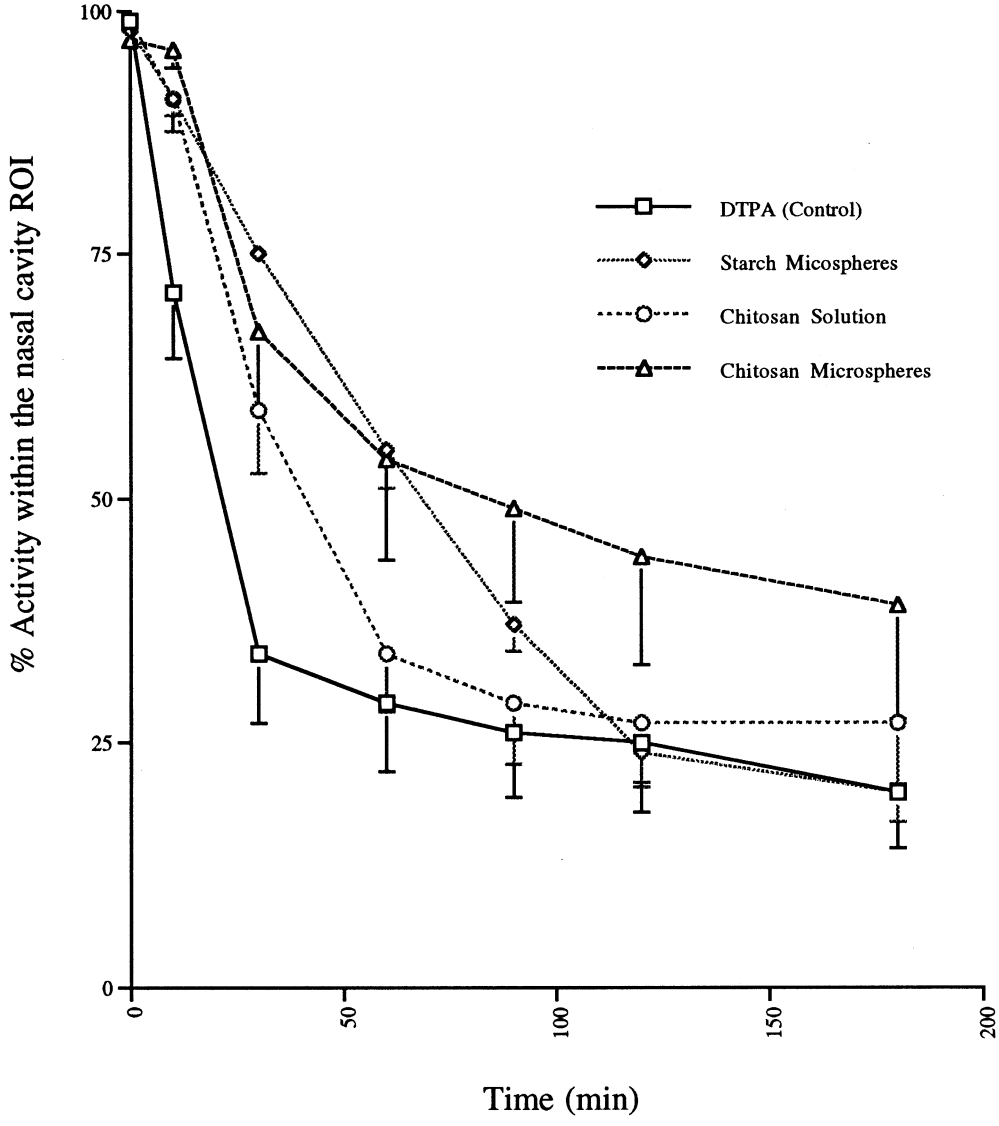

Fig. 2. The nasal clearance of bioadhesive formulations and a control in human volunteers ($n = 6$, $\pm$ S.E.).

sphere formulation than that of the starch microspheres.

Chitosans inhibitory effect on mucociliary clearance has been evaluated in previous studies. One study assessed the effect of chitosan on mucociliary transport velocity in the frog palate model and another on ex vivo human nasal turbinates (Aspden et al., 1995, 1997a). In both studies the effect was found to be transient with the transport velocity returning to normal after the removal of chitosan. This transient effect was also observed in a study that evaluated the effect of chitosan on cilia beat frequency in guinea pigs (Aspden et al., 1997b). Cilia beat frequency was evaluated after daily administration of chitosan solutions for 28 days. The authors reported that none of the animals dosed with chitosan had significantly different cillia beat frequencies to those animals dosed with the control solution.

In addition to these studies, a small scale clinical trial assessed the effect of daily application of chitosan over 7 study days in human volunteers. The mucociliary clearance of volunteers participating in this study was assessed on three occasions. Clearance rates were first assessed before and 1 h after chitosan application on the first study day and then again on the seventh study day 2 h after chitosan application. The results showed that daily application of chitosan solution for 7 days to human volunteers had no effect on clearance times (Aspden et al., 1997a).

The observed variations between the three bioadhesive systems evaluated in this study can probably be explained by differences in intimate molecular contact, water absorption and charge properties of the bioadhesives and their resulting effects on the nasal mucosa. With these factors in mind, it is interesting to note the varying clearance times reported for starch microsphere systems in this and previous studies (Ridley et al., 1985; Illum et al., 1987). Illum's group reported the half-life for the clearance of starch microspheres to be 240 min, whilst Ridley's group reported the clearance of starch microspheres to be 90 min. Although both groups used the same variety and quantity of starch microspheres different administration devices were employed to deliver the microspheres.

Ridley's group reported that the rhinyle catheters they employed deposited the starch microspheres over a significantly larger area than a DTPA control solution that was delivered by nasal spray pump devices. Although no further description of the actual site of deposition is given in Ridley's study, it is known that nasal pump sprays deposit their contents mainly into the anterior part of the nose (Newman et al., 1987b). Thus, the rhinyle catheters probably deposited starch into both the anterior and turbinate regions of the nose since they were deposited over a significantly larger area. In contrast Illum's group reported that their Lomudal nasal insufflator devices deposited the starch microspheres mainly in the anterior part of the nose. Consequently, it is likely that the major variations in clearance characteristics observed for the starch microspheres in these different studies was their site of deposition.

Table 3
The half-lives of clearance for each formulation administered to the nasal cavity of human volunteers as calculated from the average clearance data

Formulation	Half-life (min)
DTPA	21
Starch microspheres	68
Chitosan solution	41
Chitosan microspheres	84

Comparisons between the starch clearance characteristics reported here and those previous studies are difficult since, different varieties of starch microsphere and differing administration devices were used. However, it is interesting to note that the half-life for starch reported here was similar to the half-life reported by Ridley's group. The sites of deposition for the starch microspheres used in these studies were also similar.

In conclusion, from the results reported in this study it is possible to determine that both chitosan systems and the starch microspheres have good bioadhesive characteristics. The results have strongly supported the hypothesis that chitosan delivery systems can control the rate of clearance from the nasal cavity, thereby increasing the contact time of the delivery system with the nasal mucosa, providing the potential for increasing the bioavailability of drugs incorporated into the systems.

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