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Evaluation of the clearance characteristics of various microspheres in the human nose by gamma-scintigraphy

Mohsen Tafaghodi [a,b,*], S. Abolghasem Sajadi Tabassi [a,b], Mahmood-Reza Jaafari [a,c], S. Rasoul Zakavi [d], Mahdi Momen-nejad [d]

[a] *School of Pharmacy, Mashhad University of Medical Sciences, P.O. Box 91775-1365, Mashhad, Iran*
[b] *Pharmaceutical Research Center, Mashhad University of Medical Sciences, P.O. Box 91775-1365, Mashhad, Iran*
[c] *Biotechnology Research Center, Mashhad University of Medical Sciences, P.O. Box 91775-1365, Mashhad, Iran*
[d] *Nuclear Medicine Department of Imamreza Hospital, Mashhad University of Medical Sciences, P.O. Box 91775-1365, Mashhad, Iran*

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Abstract

The nasal cavity possesses many advantages as a site for drug delivery, such as; ease of administration, applicability for long-term treatments and a large surface area for absorption. One important limiting factor for nasal drug delivery is the limited time available for absorption within the nasal cavity due to mucociliary clearance. Several drug delivery systems including different kinds of microspheres have been tried for encapsulation of drugs and increasing the residence time in nasal cavity. In this study the clearance rate of three kinds of microspheres (Alginate, PLGA, and Sephadex) was determined by gamma-scintigraphy with lactose powder being used as negative control.

^{99m}Tc labeled microspheres were prepared using technetium pertechnetate in the presence of a potent reducing agent, stannus chloride. The labeling procedure was set in a manner that each 3–5 mg of microspheres contained 2 MBq of radioactivity. Labeling efficiency was calculated by paper chromatography using acetone as a mobile phase. Each delivery system containing 2 MBq of activity was administered into right nostril of four healthy volunteers and 1 min static views were repeated each half an hour until 4 h. Clearance rates were compared using two regions of interest (ROIs); the initial site of deposition of particles, and all of the nasopharynx region. The clearance rate of each one of microspheres was calculated after applying the physical decay corrections.

The mean labeling efficiencies for Alginate, PLGA, and Sephadex microspheres were calculated as 60%, 59%, and 74%, respectively. The cleared percent of formulations from nasopharynx region after 4 h was determined as follows: PLGA microspheres 48.5 ± 8.2%; Alginate microspheres 45.0 ± 0.8%; Sephadex microspheres 63.1 ± 3.4%; lactose powder 74.5 ± 4.9%. Alginate and PLGA microspheres showed the lowest clearance rate compared to lactose powder ($P < 0.0001$ and $P < 0.001$, respectively), followed by Sephadex microspheres ($P < 0.01$). The clearance profiles of formulations from deposition ROI and nasopharynx ROI were identical.

This study shows that Alginate and PLGA microspheres have the highest mucoadhesion properties and are suitable nasal delivery systems. Futhermore, this study proves that limiting step for the nasal clearance of nasally administered particulate

* Corresponding author. Tel.: +98 511 8623255; fax: +98 511 8623251.
E-mail address: m-tafaghodi@mums.ac.ir (M. Tafaghodi).

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systems is their dislocation from the initial site of deposition, and their following interactions with mucus layer in the rest of the nasal passage does not significantly affect the clearance time.

Keywords: Mucociliary clearance; Microspheres; Alginate; PLGA; Sephadex; Gamma-scintigraphy

1. Introduction

The nasal cavity possesses many advantages as a site for drug delivery, such as; ease of administration, applicability for long-term treatments and a large surface area for absorption with a sub-epithelial 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; Cornaz and Buri, 1994; Dondeti et al., 1996). Apart from the restricted transport across the epithelial membrane and enzymatic degradation, one of the main reasons for these low bioavailabilities is the limited time available for absorption within the nasal cavity due to mucociliary clearance (Chattaraj et al., 1999; Soane et al., 1999). Mucoadhesive drug delivery systems could be used to increase the contact time of encapsulated drug and absorptive epithelia and thus can increase the time available for absorption (Dondeti et al., 1996).

Alginate is a safe, non-immunogenic and inexpensive natural polymer with high mucoadhesive properties, which has been used for the preparation of microspheres as a nasal delivery system (Gombotz and Wee, 1998). Low toxicity, irritability and immunogenicity of Alginate as well as inert aqueous environment inside the Alginate matrix, introduce it as a safe mucosal delivery system (Gombotz and Wee, 1998; Lemoine et al., 1998). Alginate microspheres have been used in several oral and nasal immunization studies (Wee et al., 1995; Bowersock et al., 1996; Bowersock et al., 1998; Cho et al., 1998; Gombotz and Wee, 1998; Rebelatto et al., 2001; Vandenberg et al., 2001). Alginate as a vaccine delivery system could produce strong antibody responses when soluble antigens were encapsulated in and administered by nasal route (Gombotz and Wee, 1998).

Among microspheres used for nasal drug delivery, the epichlorohydrine cross-linked starch (Spherex®) and dextran (Sephadex®) microspheres are the most frequently used ones (Illum et al., 1987; Maitani et al., 1989; Ryden and Edman, 1992; Pereswetoff-Morath and Edman, 1995; Gill et al., 1998; Illum et al., 2001). These microspheres have increased the nasal absorption of several peptide and proteins including insulin (Bjork and Edman, 1990; Ryden and Edman, 1992; Pereswetoff-Morath and Edman, 1995; Illum et al., 2001), granulocyte-colony stimulating factor (G-CSF) (Gill et al., 1998), biosynthetic human growth hormone (Illum et al., 1990) and octreotide (Oechslein et al., 1996). In addition to mucoadhesion potential of these microspheres (Illum et al., 2001) which has a major contribution to their usefulness as a nasal delivery system, other mechanisms have also been reported for absorption enhancement effects of Spherex® and Sephadex® microspheres, including: effect on tight junctions by reversible shrinking of the epithelial cells and widening of the tight junctions (Bjork and Edman, 1990; Pereswetoff-Morath, 1998) and providing a local high drug concentration in close contact with the epithelial absorptive surface (Illum et al., 2001).

Poly(lactide-co-glycolide) (PLGA) (the copolymer of lactic and glycolic acids) microsphere is another frequently used delivery system in controlled delivery of drugs and macromolecules such as peptides and proteins (Brannon-Peppas, 1995; Cheng and Gupta, 1996; Jain et al., 2000). PLGAs have shown to be biocompatible and they degrade to toxicologically acceptable lactic and glycolic acids that are eventually eliminated from the body (Herrman and Bodmeier, 1995). They have also been approved by the FDA, as controlled drug release microspheres (Herrman and Bodmeier, 1998). PLGA microspheres have also been used as a mucosal delivery system for several drugs and antigens to nasal cavity and GI tract (Chandrasekhar et al., 1994; Tabata et al., 1996; Spiers et al., 2000; Guitierro et al., 2002).

The non-invasive imaging technique of gamma-scintigraphy was developed originally for use in diagnostic tests in nuclear medicine (Newman and Wilding, 1998). Specific radiopharmaceuticals which localize in different organs and which are visualized by gamma camera are used to provide vital information about the structure and function of various body systems. Since about 1980 the technique has been extended to the evaluation of pharmaceutical dosage forms delivered by the oral (Billa et al., 2000), rectal, pulmonary (Saari et al., 1999; Bondesson et al., 2002), nasal (Ugwoke et al., 1999; Soane et al., 1999; Soane et al., 2001), ophthalmic and vaginal (Richardson et al., 1996) routes. This method enables direct visualization and quantification of where the formulation has been delivered, what it is doing, and whether or not it is behaving according to its proposed rationale (Newman and Wilding, 1998). When gamma-scintigraphy is used in the assessment of nasal drug delivery, the formulation is usually labeled with the gamma-ray emitting radionucleide ^{99m}Tc, which has an ideal radiation energy (140 keV) for use with a gamma camera (Newman and Wilding, 1998). The short half-life of ^{99m}Tc (6 h), coupled with a very 'clean' radiation emission profile which contains few beta-particles, results in very low radiation doses, so that satisfactory scintigraphic data can be obtained using only a fraction of the radiation dose required for diagnostic X-ray procedures (Newman and Wilding, 1998).

The primary aim of this study was to investigate the clearance characteristics of various microspheres from the human nasal mucosa. This paper describes the characterization and radiolabeling of three bioadhesive nasal delivery systems: Sephadex, Alginate, and PLGA microspheres. The clearance characteristics of these bioadhesive materials after nasal administration to human volunteers were investigated using the technique of gamma-scintigraphy. In this study lactose powder was used as negative control.

2. Materials and methods

2.1. Materials

Sodium Alginate, Span-80, Sephadex® G-50 superfine were purchased from Fluka (Buchs, Switzerland). PLGA 50:50 co-polymer (inherent viscosity 0.17 dl/g in hexafluroisopropanol) was purchased from Birmingham Polymer Inc. (Birmingham, AL, USA). Polyvinyl alcohol (PVA) (87–89% hydrolyzed, mol. wt. 31,000–50,000 g/mol) was purchased from Merck (Darmschtadt, Germany). ^{99m}Tc-pertechnetate was provided by Atomic Energy Organization of Iran (AEOI).

2.2. Preparation of Alginate microspheres

The preparation method was a modification of emulsification technique described by Cho et al. (1998). Briefly, 1 ml of aqueous solution of sodium Alginate (3%, w/v) was dispersed in an *n*-octanol solution containing 2% (w/v) of a lipophilic surfactant (Span-80). For the primary dispersion, a mechanical homogenizer (Ultra-turrax, Ika Werke, Staufen, Germany) at 8000 rpm was used. Emulsion was prepared by probe sonication (Soniprep-150, MSE, Sussex, UK) in an amplitude of 18 for 90 s. The prepared W/O emulsion was rapidly added to a solution (60 ml) of calcium chloride in octanol (0.33%, w/v) while stirring the whole medium slowly with a magnetic stirrer. After 10 min for further hardening of microspheres, 2 ml of isopropyl alcohol was added dropwise. The microspheres were collected by filtration, washed with 15 ml of isopropyl alcohol and dried in a vacuum desiccator for overnight.

2.3. Preparation of PLGA microspheres

Microspheres were prepared using a W/O/W emulsion and solvent evaporation technique. Briefly, 120 μl phosphate buffer saline (PBS) were emulsified in PLGA solution (600 μl, 30%, w/v) in chloroform for 20 s using a microtip probe sonicator (Soniprep-150, MSE, Sussex, UK) in an amplitude of 18. The resulting primary (W/O) emulsion was then combined with polyvinylalcohol (PVA) solution (4 ml, 7.5%, w/v in Tris–EDTA (TE) buffer) and sonicated for 40 s at an amplitude of 18 to form secondary (W/O/W) emulsion. This secondary emulsion was then added dropwise to a beaker containing PVA solution (16 ml) kept under constant stirring. The emulsion was further stirred for 2 h. Microspheres were collected by centrifugation at 18,000 rpm for 15 min and washed two times with distilled water before freeze-drying.

2.4. Size analysis of microspheres

The volume mean diameters of microspheres (except Sephadex® microspheres) was determined by a particle size analyzer (Zetasizer 2000, Malvern, UK). In the case of Sephadex® microspheres, the diameter of 300 microspheres was determined under the optical microscope (Carl Zeiss, Oberkochen, FRG) equipped with an eyepiece reticule.

Scanning electron microscopy (Leo, Oxford, UK) was also used for studying the surface characteristics of microspheres.

2.5. Radiolabeling procedure of microspheres

The radiolabeling method for microspheres was adopted from procedure described by Illum et al. (1987). The radiolabeling procedure was carried out in the presence of 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. The electron donating functional groups, for example the hydroxyl groups of polymers and phospholipids, are accepting the technetium (Soane et al., 1999).

Fifty milligrams of microspheres (Alginate, PLGA or Sephadex G-50 superfine) were suspended in the labeling medium containing 1.5 ml of normal saline, 1 ml $SnCl_2{\cdot}2H_2O$ (5 mg/ml) and 0.7 ml technetium–99m pertechnetate eluate containing about 6 MCi of activity. The mixture was left under continuous stirring for about 10 min and separated by centrifugation. Microspheres were washed with 2×5 ml sterile distilled water and supernatants were collected. The labeled Alginate and Sephadex microspheres were washed with acetone (2×5 ml). Microspheres were separated by centrifugation and dried by incubation at 60 °C for 30 min. The labeled PLGA microspheres were suspended in sterile PBS and were administered in suspension form (5 mg microspheres/100 μl sterile PBS).

Lactose powder was labeled and used in human studies as a negative control. Lactose powder (50 mg) was dissolved in the above-mentioned labeling media and incubated for 10 min, followed by addition of 10 ml acetone. The labeled lactose was desolvated and precipitated in the presence of acetone. Supernatant was decanted and powder was washed with acetone and dried in 60 °C for 30 min. The activities were adjusted such that each 5 mg of microspheres would have 2 MBq of activity at the time of administration.

2.6. Determination of the labeling efficiency of microspheres

The labeling efficiency was determined by paper chromatography using acetone as the mobile phase. After labeling of microspheres and before washing step, microspheres suspension samples were placed on chromatographic paper. In this system, free pertechnetate migrates to the top of the paper, while microsphere-attached material remains at the application point. The labeling yield was expressed as a percentage of the total amount of radioactivity applied in the testing system.

2.7. In vivo nasal clearance studies

About 5 mg labeled Alginate microspheres, Sephadex microspheres and lactose powder or 100 μl of the PLGA microspheres suspension, containing 2 MBq of radioactivity was administered into the right nostril of four healthy human volunteers. Male volunteers between the ages of 20 and 25 completed a questionnaire about their health. Volunteers were excluded if they smoked, had a history of respiratory allergic conditions or taken any nasal medication within the last month. The study was approved by the regional ethical committee.

The powders were administered intra-nasally using polyethylene tubes, filled with 5 mg of powders. The powders were released from the tubes using a syringe containing 5 ml of compressed air. The suspension samples were sprayed using a specific spraying device. The volunteers were trained to abstain from sneezing and blowing their nose. Data obtained from volunteers that sneezed during the studies, were discarded.

The deposition, distribution and subsequent clearance of microspheres was followed by gamma-scintigraphy, using a single head SMV Gamma Camera (SMV, France) fitted with a low energy collimator. Static right lateral views (60 s duration) of the head were recorded in 30 min intervals for 4 h. The position of the head of the volunteer was fixed on the scintillation bed using a specially designed template. The camera-to-patient distance was standardized by

placing the collimator close to the head of volunteers. Quantification of the data from the volunteers involved defining regions of interest (ROIs) around the desirable areas. Two region of interests were drawn. The deposition ROI around the initial site of deposition of the particles in the nasal cavity and the nasopharynx ROI around all nasopharynx region to throat.

The count rate from each region of interest, 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 formulations from the nasal cavity was evaluated as a decrease in percentage activity against time for each volunteer.

2.8. Statistical analysis

Statistical analysis of the results was carried out using Student's *t*-test.

3. Results and discussion

3.1. Size analysis of microspheres

Volume mean diameters of Alginate and PLGA microspheres was determined using a laser diffraction size analyzer (Table 1). An optical microscope equipped with an eyepiece reticule was used to determine the size of Sephadex G-50 microspheres (Table 1). As it is demonstrated in Table 1, diameters of microspheres (except Sephadex microspheres) was less than 2 μm.

As a nasal drug and antigen delivery system, one of the most important characteristics of microspheres is their particle size. It has been shown that microsphere size influences their uptake by microfold cells (M cells) of mucosa-associated lymphoid tissues (O'Hagan, 1996; Florence, 1997) as well as the character of the immune response. Particles smaller than 5 μm may be transferred to the draining lymph nodes and spleen and stimulate both mucosal and systemic immune responses while particles in the range of 5–10 μm tend to remain in Peyer's patches to stimulate primarily a mucosal immune response (Bowersock et al., 1996). Particles larger than 10 μm are not likely to be taken up at all (Eldridge et al., 1991). In this study the mean size of Alginate and PLGA microspheres was less than 2 μm (Table 1). Pre-formed Sephadex microspheres with the mean size of 24.6 μm were also used because of their absorption enhancement potential on mucosal epithelia (Pereswetoff-Morath, 1998).

Scanning electron microscopy, which used for studying the surface characteristics of microspheres, showed the Alginate, PLGA, and Sephadex microspheres to be smooth and spherical (data were not shown).

Table 1
Mean diameters (±S.D., $n = 3$) and labeling efficiencies of microspheres

Preparations	Microspheres		
	Alginate	PLGA	Sephadex
Mean diameter (μm)	1.3 ± 0.4	1.9 ± 1.1	24.6 ± 8.5
Labeling efficiency (%)	60	59	74

3.2. Labeling efficiency of microspheres

The labeling efficiency of microspheres was determined by paper chromatography using acetone as mobile phase (Table 1).

3.3. In vivo nasal clearance studies

The nasal clearance characteristics of three microsphere drug delivery systems, potentially applicable as nasal drug and antigen delivery systems, were studied. Lactose powder was used as negative control. The averaged clearance data for each formulation from the nasopharynx and deposition ROIs can be seen in Fig. 1.

The percent of the formulations cleared from the nasopharynx and deposition ROIs, in the time course of study (4 h) was shown in Table 2. This data shows that the control lactose powder was cleared rapidly (half-life of nasopharynx clearance was 1.5 h), whereas the bioadhesive delivery systems were retained within the nasal cavity for extended periods of time (half-lives of nasopharynx clearance were >2.5 h). Among microspheres studied, the lowest

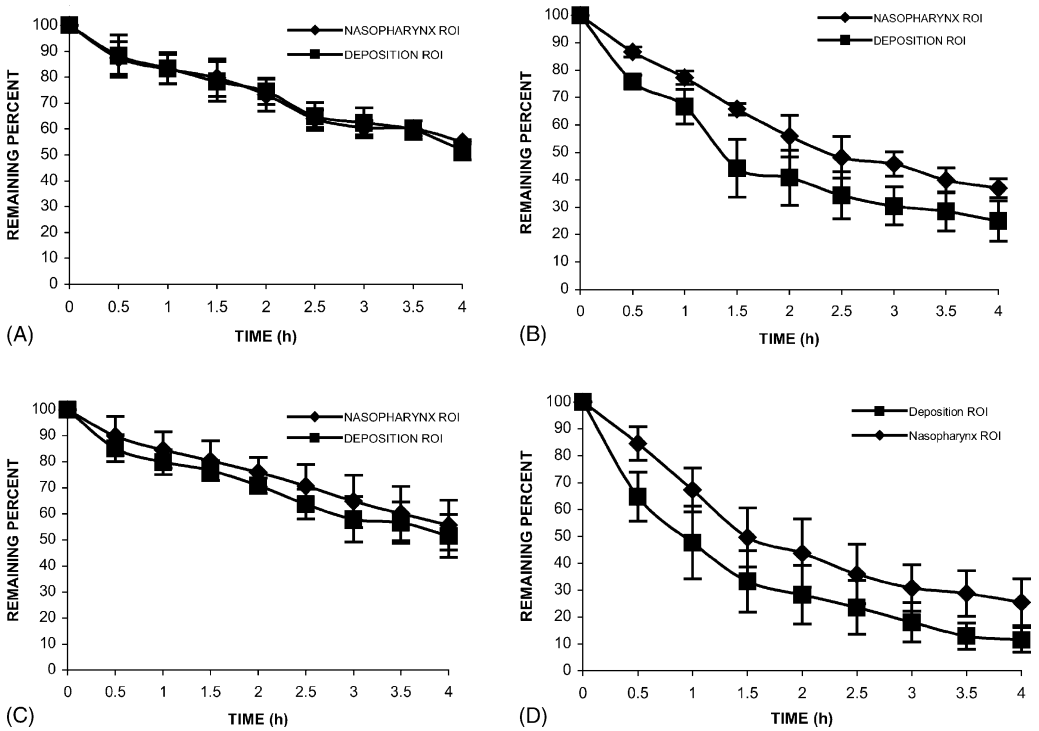

Fig. 1. The clearance characteristics of radiolabeled formulations from nasopharynx region and initial region of deposition of particles in human nose. Alginate microspheres (A), Sephadex microspheres (B), PLGA microspheres (C) and lactose powders (D) as control.

clearance rate and highest mucoadhesion was shown by Alginate and PLGA microspheres followed by Sephadex microspheres. After 4 h, 45.0% of Alginate and 44.3% of PLGA microspheres have been cleared from nasopharynx region while in the same time 63.1% and 74.5% of Sephadex microspheres and lactose powders cleared, respectively. The high mucoadhesion strength of Alginate has also been shown in other in vitro bioadhesion tests. Chickering and Mathiowitz (1995) used a tensile tester apparatus in which the adhesive forces between different polymers and living intestinal epithelium were evaluated. These studies showed that Alginate has the highest mucoadhesive strength when compared to polymers such as polystyrene, chitosan, carboxymethylcellulose and poly(lactic acid).

In mucoadhesion process, both weak and strong interactions (i.e. van der Waals interaction, hydrogen bonding and ionic bonding) can develop between certain types of functional groups on the polymer (e.g. hydroxyl or carboxyl groups) and the glycoprotein network of the mucus layer or the glycoprotein chains attached to the epithelial cells for example in the nose (Illum et al., 1987). In order for strong adhesive bonds to develop, the establishment of intimate molecular contact between the polymer and glycoprotein chains

Table 2
Percent of preparations remained in the nasopharynx and deposition ROIs after 4 h (±S.E., $n = 4$)

Preparations	Microspheres	Control		
	Alginate	PLGA	Sephadex	Lactose powder
Nasopharynx ROI	55.0 ± 0.8	51.5 ± 8.2	36.9 ± 3.4	25.5 ± 4.8
Deposition ROI	51.8 ± 3.7	55.7 ± 9.5	24.9 ± 7.4	11.5 ± 4.6

is essential (Peppas and Buri, 1985). Thus, an important requirement for bioadhesive polymers is their ability to swell by absorbing water (here from the mucous layer in the nasal cavity) thereby forming a gel-like layer in which environment the interpenetration of polymers and glycoprotein chains can take place and the bindings can form rapidly (Illum et al., 1987).

In administration of microspheric powders, the low clearance of the microsphere systems can be probably attributed to the fact that the microspheres undergo a process of taking up water and swelling, which results in polymer/mucus interaction and increased viscosity of polymer/mucus mixture leading to reduced mucociliary clearance (Illum et al., 1987; Ugwoke et al., 2000).

Comparing Alginate and Sephadex microspheres, several explanations could be mentioned for higher mucoadhesion strength and resulting residence time of Alginate microspheres ($P < 0.001$). The larger diameter of Sephadex microspheres and resulting lower contact surface area with mucus layer can decrease the water absorption rate, compared to Alginate microspheres. As it has been shown that rapid water absorption from mucus layer and enabling the polymer chains to penetrate the mucin network and establish adhesive bonds has a key role in mucoadhesion strength (Illum et al., 1987), lower mucoadhesion of Sephadex microspheres could be, in part, explained by this fact.

It has also been reported that the difference between mucoadhesion potential of the microsphere systems can probably be related to the differences in type of bondings (hydrogen and ionic bondings) formed between the gel and mucus. Furthermore, differences in swelling characteristics could also be of importance (Illum et al., 1987).

Another parameter, which can affect the mucoadhesion strength, is the degree of cross-linking of the polymer. Although the cross-linked microspheres will absorb water, they are insoluble and 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 (Peppas and Buri, 1985). Thus, cross-linking effectively limits the length of polymer chains that can penetrate the mucus layer (Illum et al., 1987). Both Alginate and Sephadex microspheres are cross-linked (with calcium cation and epichlorohydrine, respectively) and cross-linking could possibly decrease, though not in the same degree, the mucoadhesion strength.

Alginate and Sephadex microspheres were administered as dry powders, so it is important that these microspheres absorb water from mucus layer, enabling the polymer chains to penetrate the mucin network and establish adhesive bonds. In comparison, adhesion of the PLGA microspheres, which administered in the suspension form, should be rapid, since, the PLGA polymer is already in a hydrated form. However, it is questionable as to whether this is favorable, since any ‘advantage’ gained through the comparatively rapid formation of adhesive bonds may be 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 (Soane et al., 1999). In comparison, the PLGA suspension 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 PLGA suspension. The results of these two effects could possibly explain the similar clearance profiles of the Alginate and PLGA microspheres (the difference between the remaining percent of microspheres in both ROIs in all time points were statistically non-significant ($P > 0.05$)).

Studies have shown that polymers with charge density can serve as good mucoadhesive agents (Park and Robinson, 1984; Chickering and Mathiowitz, 1995). It has also been reported that polyanion polymers are more effective bioadhesives than polycation polymers or non-ionic polymers (Park and Robinson, 1984; Gombotz and Wee, 1998). All of microspheres studied in the present research (Alginate, PLGA, and Sephadex) were polyanionic and showed good mucoadhesive potential. Compared to lactose powder as control, in both nasopharynx and deposition ROIs (Figs. 2 and 3), Alginate and PLGA microspheres showed a significantly higher mucoadhesion ($P < 0.001$ and $P < 0.01$, respectively).

It has been reported that the normal half-life of nasal clearance in man is about 20 min (Schipper et al., 1991). The nasal clearance half-lives of microspheres and lactose powder were extremely higher than normal

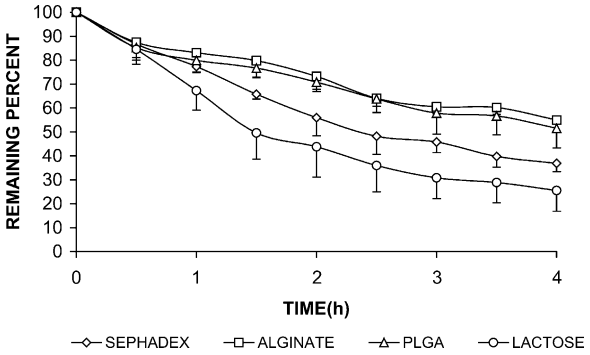

Fig. 2. The clearance characteristics of radiolabeled microspheres (PLGA, Alginate, and Sephadex) from the human nasopharynx region as compared to lactose powders as control.

clearance half-life of human nose (at least four-fold higher), which is representative of high mucoadhesive strength of these particulate systems. It has been shown that the nasal clearance rate of preparations is affected by their deposition site in nasal cavity (Illum et al., 1987; Ugwoke et al., 2000). A drug deposited in the nose posteriorly is cleared more rapidly from the nasal cavity to the nasopharynx than a drug deposited anteriorly, because the mucuciliary clearance is slower in the anterior part of the nose than the more ciliated posterior part (Schipper et al., 1991).

It has been reported that the particle size distribution of droplets or powders administered to the nasal cavity will affect deposition in, and hence clearance from, the nasal cavity (Hardy et al., 1985; Harris et al., 1986; Soane et al., 2001). It has been suggested that 4 μm is a sufficient particle size for intra-nasally administered drugs (Vidgren et al., 1991). On the other hand, Illum et al. (1987) has considered that 10 μm particle size is the most suitable for nasal administration. Particles smaller than 1 μm pass the nasal cavities with the inspired air, whereas particles larger than 10 μm deposit at the anterior parts of the nose and thus avoid ciliated absorption areas (Illum et al., 1987).

In the present study, Alginate, PLGA, and Sephadex microspheres had mean diameters of 1.3 μm, 1.9 μm, and 24.6 μm, respectively. As it shown in Fig. 4, all of microspheres and lactose powder had nearly the same deposition areas.

Factors such as type of formulation (solution versus powder), administration device and aerodynamic properties of the liquid droplets or powders can all affect insufflation and deposition patterns, and ultimately mucociliary clearance, especially with the presence of mucoadhesive polymers (Ugwoke et al., 2000). The site of drug deposition in the nose is also highly dependent on the dosage form. Nasal sprays deposit drugs more anteriorly, resulting in a slower clearance for sprays than that for drops (Hardy et al., 1985).

In this study, two different types of formulations (suspensions and dry powders) and two different devices (pressurized air for powders and mechanical sprayer for suspensions) was used, but resulting deposition areas for both formulations and administration devices were nearly the same.

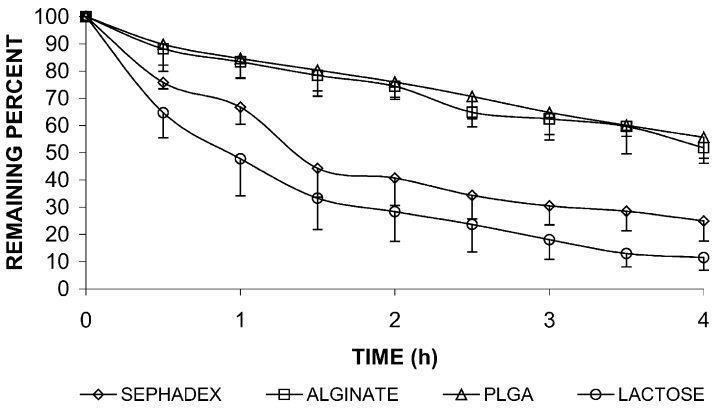

Fig. 3. The clearance characteristics of radiolabeled microspheres (PLGA, Alginate and Sephadex) from their initial region of deposition in human nose as compared to lactose powders as control.

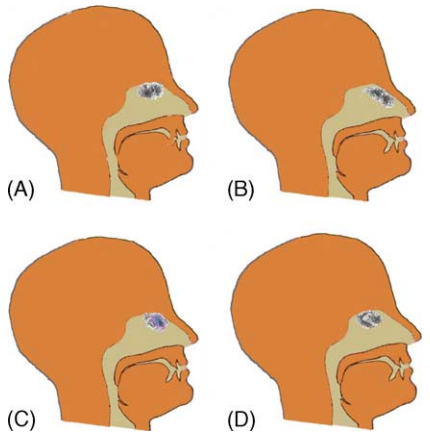

Fig. 4. The deposition sites of microspheres: Alginate (A), Sephadex (B), PLGA (C) and lactose powders (D) in the nasal cavity of volunteers. Each image represents the pictures of deposited particles in the nasal cavity of four volunteers, which are over-layered together and superimposed on a schematic diagram.

In our study, the site of deposition was relatively similar for most formulations (Fig. 4). Therefore, the differences in clearance characteristics observed between formulations were more due to the bioadhesive properties of the formulations than their deposition sites.

Two ROIs were drawn around the initial site of deposition of preparations and the whole nasopharynx region to throat. Comparing the clearance profiles from the two ROIs could result in some ideas about the rate-limiting step in nasal clearance of preparations. As it has been shown in Fig. 1, the clearance patterns of studied particulate systems from both ROIs were nearly identical ($P > 0.05$), indicating that the main time consuming step in the clearance of these particulate systems is their displacement from initial deposition site. As soon as the particles are dislocated from their deposition area, they are passing the remainder of the nose passage very quickly. Therefore, it could be realized that rate limiting step for the nasal clearance of nasally administered particulate systems is their dislocation from the initial site of deposition, and their following interactions with mucus layer in the rest of nasal passage does not significantly affect the clearance time. This interesting observation was not affected by dosage form, administration device or even initial site of deposition.

4. Conclusion

All of particulate delivery systems showed high mucoadhesion strength compared to the normal clearance time. Powders and suspensions were administered using different devices but deposition areas of both kinds of dosage forms and devices were nearly identical. Among preparations, the least clearance rate from nasopharynx region was shown by Alginate and PLGA microspheres. These two microspheres showed similar clearance profiles and their mucoadhesion strengths were more than that of Sephadex microspheres. This is the first time that has been shown the mucoadhesive properties of Alginate and PLGA microspheres using gamma-scintigraphy in human study. The clearance profiles of preparations from initial site of deposition and all nasopharynx region, disregarding dosage form and administration device, were identical. Therefore, it could be concluded that as soon as the deposited particles are dislocated from their deposition area, they are rapidly cleared and their following interactions with mucus layer in the rest of the nasal cavity doesn't have a significant role on the total clearance time.

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