

Effect of Viscosity on Particle Size, Deposition, and Clearance of Nasal Delivery Systems Containing Desmopressin

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Abstract □ The effect of methylcellulose on the particle size distribution and dosing accuracy of pre-metered spray pump devices containing the peptide desmopressin (DDAVP) was investigated. Using gamma scintigraphy, the influence of methylcellulose on the in vivo deposition and clearance of nasal solutions administered as drops or spray was studied. Nasal formulations containing 0, 0.25, and 0.50% methylcellulose produced a dose-related increase in average particle size from 51 μm for 0% to 81 and 200 μm for 0.25 and 0.50% methylcellulose, respectively. However, no effect was observed on the dosing accuracy of the spray pumps. The addition of methylcellulose gave a more localized in vivo deposition in the anterior region of the nasal vestibule. However, the net effect on clearance followed a biphasic pattern which showed an increase in retention time for the 0.25% solution, followed by a decrease in retention time and faster clearance time for the 0.50% solution. The spray delivers well-controlled doses to the nasal cavity. These findings show that viscosity, particle size, and nasal clearance are important parameters in the design of nasal delivery systems.

Much work has been done recently on the development of delivery systems for nasal administration of drugs, particularly peptides and proteins. Examples are insulin,¹ calcitonin,² and growth hormone releasing factor (GHRH),³ all of which are high molecular weight polypeptides with strikingly poor bioavailability by the nasal, as well as other nonparenteral routes of administration. Against the background of the poor stability and bioavailability of these peptides, much of this work has focused on enhancing their nasal absorption. The formulation tools used for enhancing absorption and bioavailability included the use of viscosity-enhancing agents, such as polyethylene glycol and methylcellulose,⁴ and surfactant material, such as polyoxyethylene 9-lauryl ether and bile salts.⁵ Others have speculated on the value of enzyme inhibitors in restricting the local inactivation of peptides by nasal enzymes.⁶ Parallel to this development, other work has focused on the characterization of delivery systems which are used for nasal administration. For example, it has been demonstrated that factors such as the administration of solutions to the nose by spray or drops affect the local deposition and clearance patterns in the nasal cavity.⁷

In a previous study, the intranasal absorption of the peptide desmopressin (DDAVP) was shown to be clearly enhanced following administration using a metered-dose nasal spray compared with nose drops.⁸ It was also established in this study, as well as in other independent observations,^{7,9} that the nasal spray shows a different deposition pattern in the nasal mucosa which results in a longer retention time and slower clearance along the nasopharynx than nasal drops. Evidence was therefore provided to suggest that the enhancement in absorption and improvement in biological response to desmopressin was directly related to the transit time in the nasal cavity.

Hitherto, most nasal formulations of peptides have been made up of simple aqueous or saline solutions containing a preservative.¹⁰ In some recent formulations, the addition of bile salt conjugates such as sodium deoxycholate, a natural surfactant agent to promote absorption, has been tried.¹¹ However, most solutions are relatively fluid, with a viscosity similar to that of water. The effect of viscosity-enhancing agents has been investigated in animal models using methylcellulose or propylene glycol added to the nonpeptide drug propranolol.⁴ These studies showed that the blood drug profile resulted in different pharmacokinetics compared with nonviscous solutions, with lower initial blood levels, but more sustained action and similar bioavailability. However, no studies have been done in humans to measure the effect of these agents on transport times in the nasal cavity. These findings prompted us to initiate an investigation to characterize the effect of viscosity on particle size distribution, dosing accuracy, and in vivo deposition and clearance of nasal formulations in pre-metered nasal spray pump devices.

Experimental Section

Materials—Desmopressin (Minirin, Ferring Pharmaceuticals, Malmö, Sweden) ^{99m}Tc-labeled human serum albumin (TechneScan, Microspheres 20/40, Mallinckrodt Diagnostica, Holland), and methylcellulose (grade 1500 AKLPM; Id 61084; Apoteksbolaget, Stockholm, Sweden) were used. All reagents were analytical grade.

Nasal Formulations—The nasal solutions were prepared under aseptic conditions. Nasal solutions were prepared by first dissolving the methylcellulose in water for injection at a temperature of 100 °C. On cooling, solutions were made up containing either 0, 0.25, or 0.50% (w/v) methylcellulose in 0.9% sodium chloride (w/v), 0.5% chlorbutanol (w/v), and 1.5 mg/mL desmopressin. Two nasal delivery systems were tested: one a rhinyle delivery device, the other a nasal spray. The rhinyle device was a calibrated plastic catheter designed to give a dose of 100 μL . The nasal spray was a precompression metered-dose spray pump (Pfeiffer GmbH, Radolfzell, West Germany). The pump gave a volume of 100 μL per actuation. Radiolabeling of each device was done by adding 1 mg of human serum albumin radiolabeled with 100–200 MBq ^{99m}Tc. Each dose contained 2–4 MBq ^{99m}Tc.

Viscosity Studies—The viscosity of the two nasal formulations containing 0.25 and 0.50% methylcellulose was studied using a Bohlin VOR rheometer (Bohlin Reologi, Lund, Sweden). Two measurements per concentration were carried out at a temperature of 34 ± 1 °C, which has been reported to be the temperature of the nasal mucosa.¹² As a measuring system, a concentric cylinder with a diameter of 25 mm was used. Shear rates from 1.9×10^{-1} to 1.2×10^{-3} s^{-1} were chosen to characterize the flow properties of the formulations.

Particle Size Analysis—Droplet size analysis of each formulation in the spray pump was carried out by laser diffraction using a Malvern 2600 HSD particle sizer (Malvern Instruments, Malvern, UK). The spray pump was fixed on a support with a distance of 10 cm between the laser beam and actuator head. The spray duration was 15 ms, and the delay trigger varied from 55 to 110 ms.

Dose Accuracy—The dose accuracy of the different nasal formulations in the spray pump was tested. Each pump device was filled with 5.0 mL of solution and weighed using a calibrated analytical balance. After each actuation, the device was reweighed and the difference represented the dose delivered. For each pump, five priming actuations were effected followed by a further 20–65 doses. For the 0% methylcellulose formulation, a total of five pumps were tested; for the 0.25 and 0.50% formulations, one pump per concentration was tested.

Deposition Studies—The nasal solutions were administered to 10 healthy volunteers, all male and of 25 years of age or more. None of the subjects had nasal problems and all were free from colds. Separated by an interval of at least 3 d, all subjects received 0, 0.25, and 0.5% methylcellulose by spray and 0 and 0.5% methylcellulose by a rhinyle delivery device. The treatment sequence was done in a blind, randomized way, using coded, sealed envelopes. In this way, a total of 50 administrations were made.

All nasal solutions were administered, with the subjects sitting in an upright position, into the same nostril on each occasion. A standard dose of 100 μ L of solution was self-administered in every case. The rhinyle dose was administered by first filling 0.1 mL of solution into the tube. One end of the rhinyle was then put into the mouth, the other end was introduced 5–10 mm into the nostril, and delivery was accomplished by blowing. Prior to administration by nasal spray, each device was primed by activating the pump five times. The applicator tip was introduced 5–10 mm into the nostril and a dose of 100 μ L was dispensed during normal inhalation, with the contralateral nostril open.

At the instant of dosing, each subject was seated adjacent to the gamma camera head. Immediately after dosing, the tracer was monitored repeatedly by the gamma camera and a lateral view of the head was recorded for 5 min. Additional images were recorded at 15-min intervals for the first hour and then at 1- or 2-h intervals up to 4 h.

Throughout the study, and at each monitoring period, the subjects continued to breath normally but did not blow their noses or sneeze. Food and drink were withdrawn for the first hour, but were allowed ad libitum thereafter. The study was approved by the hospital ethical committee and radioisotope committee, and each subject gave informed consent prior to entry into the study.

The data were recorded by computer [PDP (11/34), Gamma II V3.1] for subsequent analysis. Each image was displayed on a monitor and regions of interest were demarcated. The count rate at each monitoring period was corrected for radioactive decay. Each count rate in each region of interest was expressed as a percentage of the initial count rate taken immediately after dosing.

Statistical Methods—Unless specified, all results are expressed as mean \pm SD. Friedman's two-way analysis of variance by ranks and the Wilcoxon matched-pairs signed rank test were used where appropriate.

Results and Discussion

Viscosity Studies—The viscosity of both solutions was expressed in terms of flow curves of viscosity versus shear rate (Figure 1). Solutions of methylcellulose are non-Newtonian liquids and exhibit shear thinning or pseudoplasticity properties. At shear rates of 10 s^{-1} or more, the viscosity stabilized at values of $3.5 \pm 0.3 \times 10^{-3}$ and $4.0 \pm 0.06 \times 10^{-3}$ Pas ($p < 0.01$) for 0.25 and 0.50% solutions, respectively. A further experiment on the effect of a sweep in shear rate from low to high to low again gave a hysteresis-loop, indicating a thixotropic character. Unfortunately, no information is available on the shear rate generated by the spray pump during actuation. However, it may be assumed that the thixotropic nature of methylcellulose would mean that the shear rate during actuation of the pump would have little effect on the viscosity of the solution in the nasal cavity.

Particle Size Analysis—The pump device gave spray droplets which were macroparticulate in size. The distribution of particle sizes from each spray pump solution is shown in Figure 2. A clear increase in particle size distribution was seen with increasing concentrations of methylcellulose. The mean diameters of the spray droplets were 51.3 μ m for the

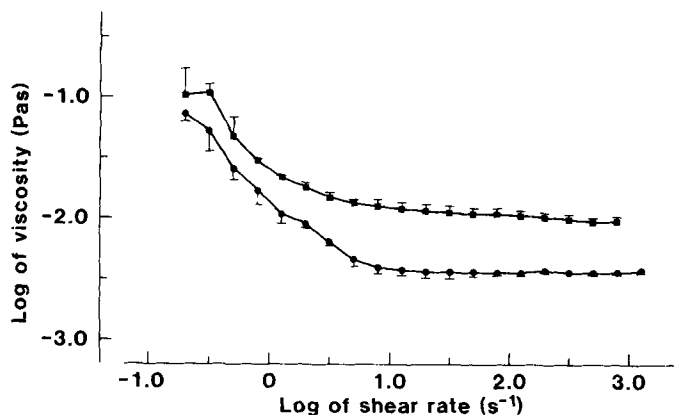


Figure 1—The effect of shear rate on viscosity and rheological properties of nasal solutions containing 0.25% (●—●) and 0.50% (■—■) methylcellulose.

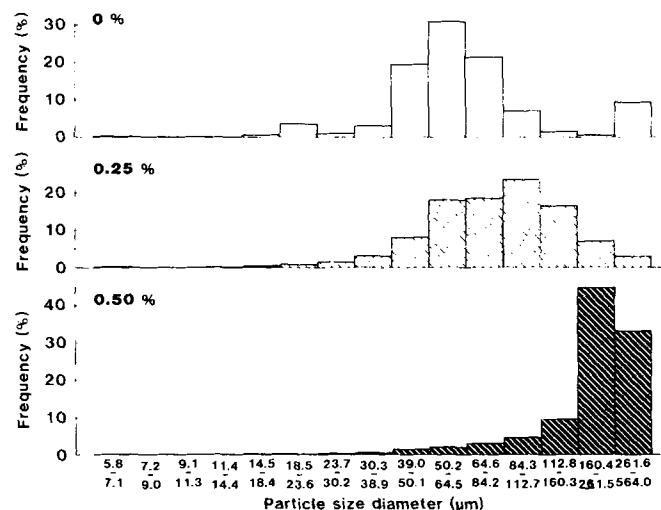


Figure 2—Particle size distribution of spray droplets from a pre-metered spray pump device containing formulations with various concentrations of methylcellulose.

placebo solution (without methylcellulose), and 81.3 and 200 μ m, for the 0.25 and 0.50% methylcellulose solutions, respectively.

Dose Accuracy—No difference was observed between the placebo solution and the formulations containing methylcellulose. On the average, a total of five actuations was required to prime the pump. This was followed by a series of accurate and reproducible doses with a mean weight of 98 ± 2 mg for the placebo solution, and 101 ± 2 and 98 ± 3 mg for the 0.25 and 0.50% methylcellulose solutions, respectively.

Deposition Studies—With the pump device, the spray droplets were deposited in the anterior region of the nasal cavity, mainly in the atrium, whereas the drops delivered by the rhinyle tube appeared to deposit solution more evenly over the nasal cavity, pharynx, and sinuses (Figure 3). This finding agrees with the results of a previous study.⁸ The addition of methylcellulose, however, influenced the deposition of the spray and drops in different ways. With the spray, there appeared to be little difference between the 0 and 0.25% solutions in initial impact and deposition pattern. With the 0.5% solution, however, the initial impact was shifted anteriorly, as evidenced by the rapid clearance, and only a small trace of solution was seen at 30 min (Figure 3). With the rhinyle drops however, an increase in viscosity to 0.5%

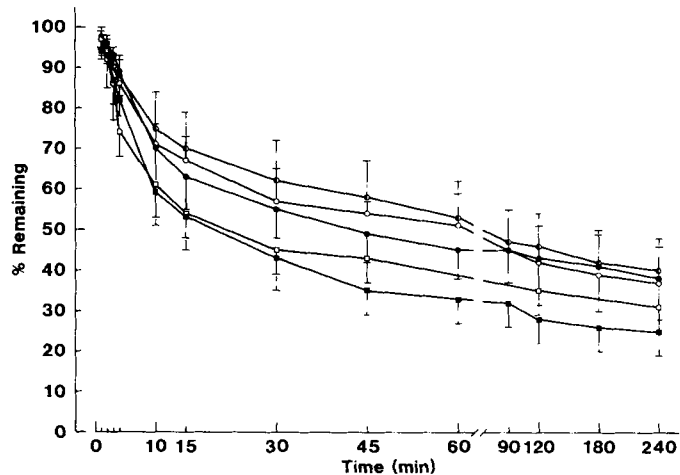
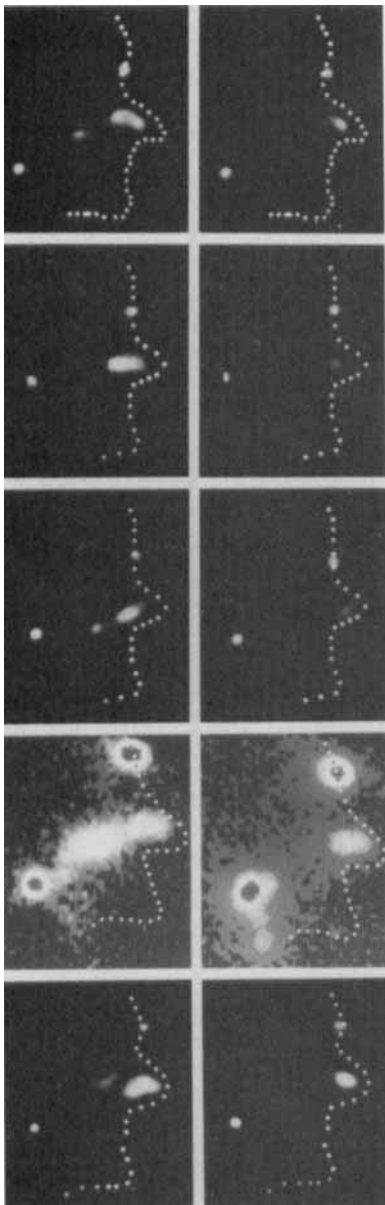


Figure 4—Clearance of the ^{99m}Tc -radiolabeled tracer from the nasal cavity after administration by nasal spray of 0% (○—○), 0.25% (●—●), and 0.5% (●—●) methylcellulose, and drops by a rhinyle catheter of 0% (□—□) and 0.5% (■—■) methylcellulose each to 10 subjects (mean \pm SEM).

viscosity on the retention time in the nasal cavity has a maximum effect which is related to the droplet size generated by the spray pump. The average droplet size of $81.3 \mu\text{m}$ generated by the 0.25% methylcellulose solution gave an enhanced retention time which was clearly reversed when the mean droplet size was increased to $200 \mu\text{m}$ in the 0.5% solution. On the contrary, no difference in 50% clearance rate was seen between the 0 and 0.5% methylcellulose solutions given by rhinyle administration (20 ± 6 and 19 ± 7 min, respectively, NS). This difference is probably related to the fact that the particle size of the drop generated by rhinyle administration is not affected by viscosity, as the rhinyle tube delivers macroparticles in drop form that are much larger than the largest droplet size capable of being measured by the particle sizer ($>1000 \mu\text{m}$). This finding that an optimum mean particle size diameter seems to lie in the region of 59 to $80 \mu\text{m}$ is supported by the work of Illum.¹³ Illum showed that the administration of hydrogels comprising starch or dextran microspheres of mean diameter $60 \mu\text{m}$ gave a prolonged in vivo retention time compared with conventional powder and liquid nasal formulations.

Continual monitoring of the nasal cavity at regular intervals for up to 4 h showed a considerable proportion of the dose to be retained in the vestibular region, with the greatest concentration of tracer at the site of deposition. On imaging the thorax and abdomen at the end of the study, no radioactivity was detected in the lungs. This is thought to be related to the particle size generated by these devices, and it is well established that during normal nose breathing, particles $>10 \mu\text{m}$ in diameter are almost completely deposited in the nose and do not enter the respiratory tract.¹⁴

The use of agents like methylcellulose for increasing contact time with mucous has been investigated previously in other solutions. Mueller and Deardoff¹⁵ showed that the addition of a 1% solution of methylcellulose 4000 to homatropine hydrobromide eye drops increased the effect on the eye. The viscous nature of the preparation was reported to increase the time of contact with the cornea and decrease lachrymation. In another controlled cross-over study,¹⁶ a 2% pilocarpine hydrochloride solution containing 0.5% methylcellulose produced significantly greater miosis and lower intraocular pressure than a pilocarpine solution without methylcellulose.

In a study in dogs, Hussain et al.⁴ found that the addition

Figure 3—Gamma scintigraphy images of sites of deposition and patterns of clearance following administration of nasal formulations by nasal spray pump and rhinyle catheter containing various concentrations of methylcellulose. Each pair of images is from the same subject; however, the five pairs are from different subjects.

tended to give a more localized impact of deposition, while the 0% solution appeared to be widely distributed over the nasal mucosa.

The clearance rate from the nasal cavity is shown in Figure 4. As the images were taken continuously for the first 5 min and thereafter every 15 min, it was possible to quantify the dynamic clearance of each solution from its site of deposition. The results showed that by increasing the viscosity of the nasal solutions, a decrease then an increase in the clearance rate was observed. Thus, the spray pump containing the 0.25% methylcellulose solution showed a 50% clearance at 75 ± 9 min compared with 65 ± 8 min for the placebo solution ($p < 0.05$). On the other hand, the 0.50% methylcellulose solution gave a 50% clearance at 43 ± 7 min ($p < 0.01$). This finding was probably related to the particle size distribution of the three solutions. It appears that the effect of

of 3.0% (w/v) methylcellulose to a nasal solution of propranolol produced maximum blood levels which were much lower than those observed after administration of a solution without methylcellulose. However, blood levels after methylcellulose were sustained over a prolonged period, giving a bioavailability which was identical for both solutions. The administration of the solution to the dogs was done using a micropipette and syringe, and unfortunately no information was available on the viscosity of the solution.

Other viscosity-enhancing agents, such as the polyacrylic gels, have been shown in rat studies to enhance bioavailability.¹⁷ However, this and other agents have been reported to cause irritation of the nasal mucous membranes with some morphological changes as a result of their use.¹⁸ We chose methylcellulose as the viscous agent as earlier work has characterized its bland, nonirritant nature when administered to mucous membranes in ophthalmic solutions.^{15,16} Although these studies are useful in obtaining baseline data, the sustained-release and enhanced bioavailability effects exhibited by methylcellulose and other material remain to be investigated in humans using conventional, standard delivery systems.

However, before any further bioavailability work is initiated, the experience gained from the present study shows that a thorough characterization of the delivery system and formulation is recommended, including a detailed analysis of viscosity, particle size, deposition, and clearance associated with its use. This study shows that the effect of methylcellulose on the retention time of spray formulations in the nasal cavity follows a biphasic pattern, with an enhancement in retention time as viscosity and particle size increase to an optimum level, followed by a decrease in retention time resulting in a faster nasal clearance as viscosity and particle size increase still further. A further investigation into the

optimum particle size and viscosity is warranted, as well as the effect of these factors on in vivo absorption and bioavailability of nasally administered peptides in humans.

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