ELSEVIER

International Journal of Pharmaceutics 129 (1996) 175–183

international journal of pharmaceutics

In vitro characteristics of nicotine microspheres for transnasal delivery

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Received 22 June 1995; accepted 17 August 1995

Abstract

A new transnasal formulation containing nicotine was developed and characterized. Sephadex® G25 and G50, crosslinked dextran microspheres, were loaded with nicotine base (NB) and nicotine hydrogenotartrate (NHT). Their high swelling capacity, which is believed to enhance the bioavailability of nicotine by acting on the nasal mucosa, was not altered by the loading process. The in vitro kinetics were measured by the means of a special diffusion chamber which mimics the hydration conditions of the nasal cavity. 90% of the nicotine were released in a time similar to the nasal residence time (15 min). The delivery from a regular nasal device is described and related to the porosity of the microspheres.

Keywords: Nicotine; Dextran microspheres; Transnasal delivery; In vitro characterization

1. Introduction

Nicotine replacement therapy offers a new approach to help giving up smoking. In this therapy, the physiological dependence on smoking is transfered to a safer and more therapeutically manageable nicotine system (Jarvis and Henningfield, 1988; Pomerleau et al., 1992).

The use of nicotine in polacrilex gum or in transdermal therapeutic systems as a substitute for cigarettes has proved effective in reducing withdrawal symptoms and increasing success in giving up smoking (Russel et al., 1983; Jarvis et al., 1987; Perkins et al., 1992). But nicotine is absorbed rather slowly and an alternative route which could ensure a more rapid delivery would be useful to help smokers overcome the urge to smoke (Svensson, 1987). Sutherland et al. (1992) observed that the absorption of nicotine from nicotine nasal sprays was very rapid the first 2.5 min, and reached a peak after 7.5 min (West et al., 1984). The nicotine nasal spray may have more potential for the treatment of dependency than gum, because of its speed and ease of administration (Schneider, 1994).

In fact, nicotine is well absorbed nasally and reaches a peak concentration after a single snuff

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SSDI 0378-5173(95)04289-M

(powder tobacco) in a time similar to that of a cigarette puff (Russel et al., 1980; West et al., 1984). Accordingly, a nicotine powder formulation for transnasal delivery which allows a better physical, chemical and microbiological stability of the active principle during storage than a solution was developed. Sephadex® microspheres were chosen for this purpose, since they are not rapidly cleared from the nasal cavity and therefore prolong the drug nasal residence time (Illum et al., 1987).

Sephadex® microspheres take up water very rapidly and have a considerable swelling capacity. Their ability to absorb water from the mucus layer probably affects the epithelial cells in such a way that the paracellular passage of drug is enhanced (Edman et al., 1992).

Nasal powder delivery requires that the dose be quantitatively released and distributed in the nasal cavity. The deposition pattern may certainly affect drug bioavailability (Vidgren et al., 1988), and thus it is important to investigate the behaviour of the microspheres during their delivery from a commercial device (Provasi et al., 1993a; Provasi et al., 1993b; Provasi et al., 1994).

The objective of this work is to load microspheres at 10% equivalent nicotine base, to characterize them by their physical aspect, their release and delivery ability.

2. Materials and methods

2.1. Materials

Free nicotine base (NB) and nicotine hydrogen tartrate salt (NHT) were purchased from Sigma, Buchs, Switzerland.

Sephadex® G-25 Fine (G25) and Sephadex® G-50 Fine (G50) (Pharmacia, Uppsala, Sweden) are dextran microspheres crosslinked by epichlorohydrin. These two types of Sephadex® microspheres have different degrees of crosslinking, G25 being more crosslinked than G50.

All other ingredients were of reagent grade unless stated otherwise.

2.2. Characterization of microspheres

The equivalent diameter of loaded, unloaded, dry and swollen microspheres was determined by the means of an image analysis system Microscale TM/TC (Digithurst, Nünnberg, Germany), a microscope Optiphot-2® (Nikon, Küsnacht, Switzerland) and a video camera DXC-101P (Sony, Japan).

The true density, ρ, was measured by means of an air comparison picnometer (Beckman 930, Irvine, CA).

The apparent poured density, ρ_a, was determined by using a 10-ml graduated cylinder to measure the volume occupied by 3 g of powder.

The total porosity, ϵ, was calculated according to the expression:

$$\epsilon\ (\%) = (\rho - \rho_a) \times 100/\rho$$

2.3. Loading of microspheres

Microspheres G25 and G50 were soaked in an aqueous solution of either NB or NHT during 2 h with frequent agitation, filtered under vacuum and freeze-dried until a light powder was obtained (Fig. 1).

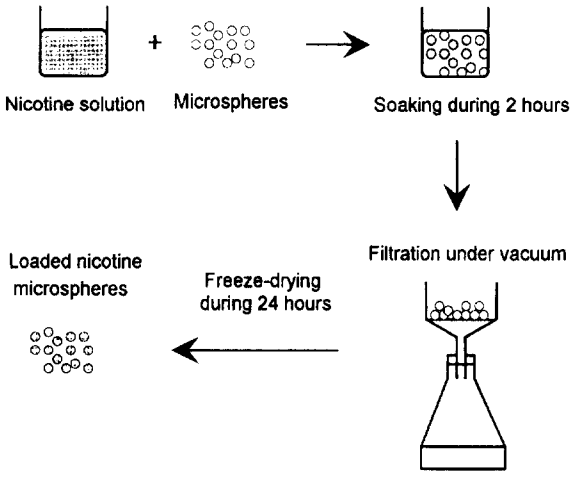

Fig. 1. Loading microspheres method.

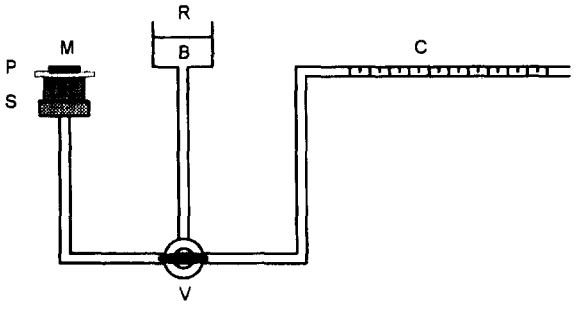

Fig. 2. U-shaped glass tube modified by Nogami et al. (1969). B, buffer solution; C, capillary tube; M, microspheres; P, paper filter; R, reservoir; S, Swinnex; V, three-way valve.

2.4. Determination of nicotine loading

Twenty mg of nicotine-loaded microspheres were soaked in 2 ml of water, and regularly vortexed at intervals during 2 h. The microspheres were allowed to sediment by gravity, and the supernatant was removed and analyzed with a diode array spectrophotometer HP8452A (Hewlett Packard, Waldbronn, Germany) at 260 nm.

In order to check that nicotine is not trapped by the microspheres, the same process was applied with conc. HCl which dissolves the microspheres by hydrolyzing the dextran glycosidic bonds.

2.5. Determination of liquid uptake

The ability of microspheres to absorb different liquid media was evaluated using a liquid uptake apparatus: the U-shaped glass tube modified by Nogami et al. (1969) (Fig. 2).

The apparatus consists of a U-shaped tube connected at one end to a graduated capillary tube, the other one being closed by the male part of a Swinnex® filter (Millipore SX 0001300, F-Molsheim). The filling of the device between the zero graduation of the capillary tube and the upper part of the Swinnex® filter is ensured from a reservoir containing the liquid tested, via a three-way valve.

Microspheres lie on a paper filter placed at the top of the Swinnex® filter and absorb the medium tested by capillarity.

The measurements were performed at 3.5 min, which is a time interval well-suited for nasal applications. The swelling capacity of loaded and unloaded microspheres was determined with distilled water, phosphate buffer, pH 7.4 (with an ionic strength of 0.46 M), and Tween® 80 0.13% aqueous solution (surface tension = γ = 42.5 mN/m), in order to determine whether a change in ionic strength or in surface tension could cause differences in the swelling capacity of microspheres.

2.6. In vitro release studies

The release kinetics of nicotine were studied with a special diffusion chamber, which mimics the hydration conditions of the nasal mucosa (small volume of dissolution medium) (Fig. 3).

The diffusion chamber is composed of two parts: a donor compartment containing air saturated with water; a receiver compartment containing phosphate buffer, pH 7.4 (USP), at 37°C.

The microspheres lie on a paper filter which is maintained just in contact with the liquid phase of the receiver compartment. A gentle agitation takes place in the receiver compartment by the means of a magnetic stirrer.

Two hundred-μl samples were drawn from the receiver compartment 1, 3, 5, 10, 15, 20, 30 and 45 min after the experiment started, and immediately replaced with the same amount of fresh buffer.

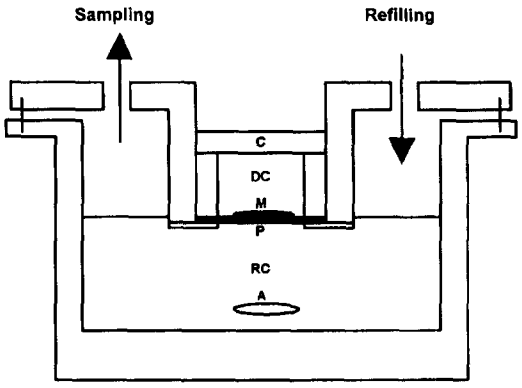

Fig. 3. The diffusion chamber. A, agitator; C, cover; DC, donor compartment; M, microspheres; P, paper filter; RC, receiver compartment.

Table 1
Size characterization of microspheres (M) before and after soaking in water (mean ± S.D.)

Sephadex®	Without nicotine(mean diameter ± S.D. (μm))		NB(mean diameter ± S.D. (μm))		NHT(mean diameter ± S.D. (μm))	
	dry M	soaked M	dry M	soaked M	dry M	soaked M
G25 M	45.0 ± 9.0	79.6 ± 12.5	48.1 ± 7.4	82.8 ± 11.7	47.7 ± 9.1	77.6 ± 11.8
G50 M	44.4 ± 14.1	95.6 ± 28.0	57.3 ± 16.8	127.2 ± 38.4	51.4 ± 16.3	117.6 ± 34.2

Measurement of the dosage of nicotine was performed by UV spectroscopy at 260 nm.

2.7. Characterization of microspheres delivered from a nasal device

Twenty-mg doses of the microsphere formulation were loaded into hard gelatine capsules (# 3) and delivered through a MIAT® nasal insuflator (MIAT® S.p.A, Milan, Italy). The microspheres were delivered in standardized conditions (air pressure, 0.3 bars; impulsion time, 0.2 s). Actuations were repeated 10 times, and mean values of fraction dose delivered were calculated.

The appearance of the puffs during the delivery was recorded using a video camera (Panasonic F15 and a video recorder Panasonic S-VHS AG 7350) and analyzed by means of Adobe Photoshop computer software (Adobe Systems Inc., Montain View, CA), and characterized in terms of the area and shape of the powder clouds (Provasi et al., 1993b; Provasi et al., 1994).

3. Results and discussion

The size characterization of loaded and unloaded microspheres before and after soaking in water is necessary in order to determine if the presence of nicotine changes their swelling capacity. The results in Table 1 show that the mean diameter of loaded and unloaded microspheres before and after soaking in water remains in the same range. Sephadex® microspheres swell very rapidly and the amount of liquid absorbed reaches a 'plateau' after only 1 min. The swelling coefficient calculated as the ratio of the volume of the swollen microspheres to that of the dry microspheres is of about 5.0 for G25 and 10.0 for G50. This difference was anticipated since G50 is less crosslinked than G25 and consequently absorbs more water. The loading of Sephadex® with either NB or NHT does not seem to affect the swelling capacity of microspheres.

Scanning electron micrographs (Fig. 4a–d) show that the loading of microspheres with NB and NHT produces a change in the shape of the spheres, making them polyhedric in the dry state. This change can be due to the lyophilization process requiring the freezing of the spheres, as well as to the nicotine itself, which is located at the surface and probably inside of the spheres. The microspheres seem to be porous after loading. Anyway, the swollen microspheres have the same appearance before and after loading with nicotine.

Another way to determine if the swelling capacity of microspheres is retained after loading, is to measure their liquid uptake capacity (Table 2). The media tested were of different ionic strength (distilled water, phosphate buffer, pH 7.4) and surface tension (Tween® 80, aqueous solution).

The amount of liquid uptake does not change with the different media used for the types of microspheres, except in the case of NHT loaded microspheres, which were found to have a lower swelling capacity compared to the NB microspheres. A possible explanation is that the NHT microspheres are loaded with almost 28% NHT (in order to achieve a concentration of 10% NB), and NB microspheres only with 10% of NB. Thus a determined weight of NHT Sephadex® contains fewer microspheres than an equivalent amount of NB Sephadex®and consequently absorbs less water.

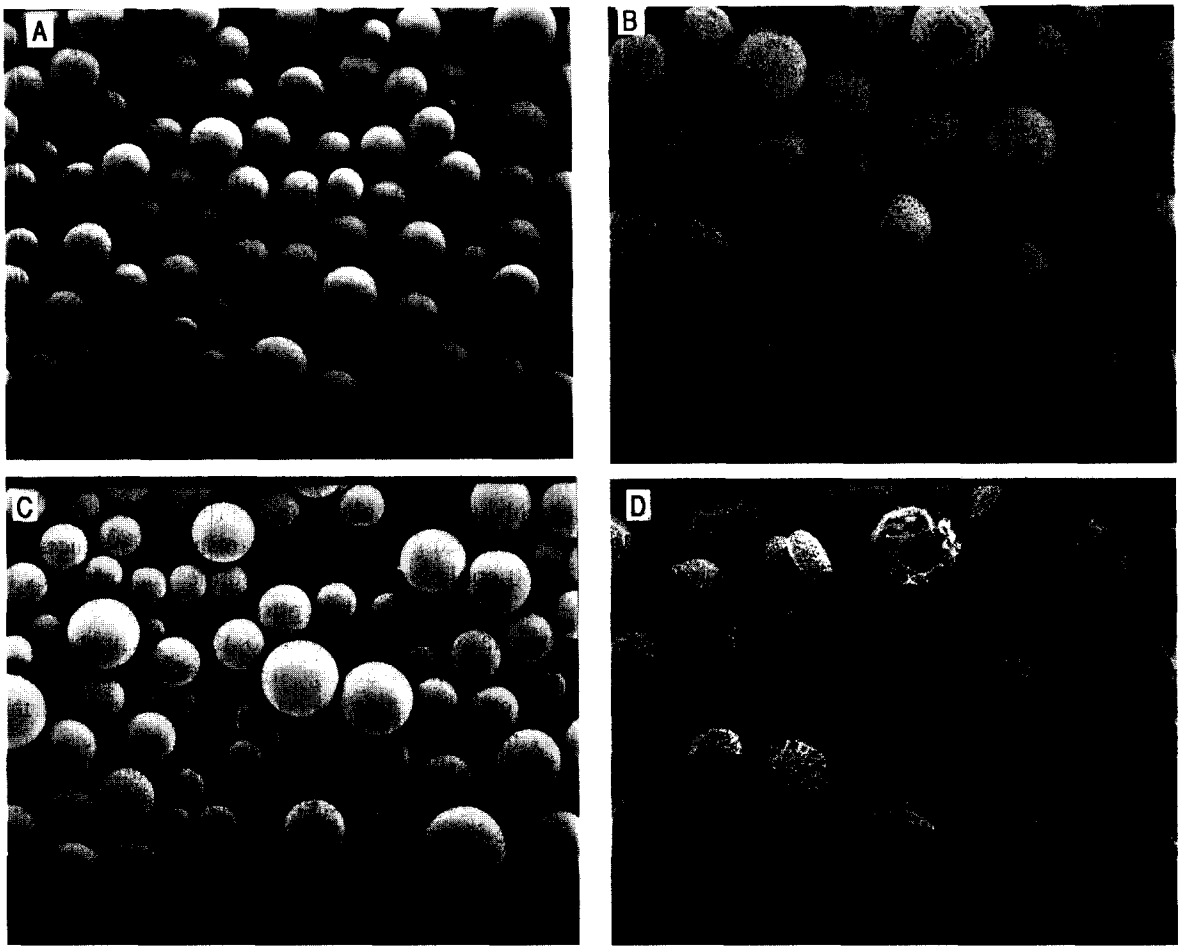

Fig. 4. Scanning electron micrographs (SEM): (a) Sephadex® G25; (b) Sephadex® G25 loaded with NHT; (c) Sephadex® G50; (d) Sephadex® G50 loaded with NHT.

In conclusion, the liquid uptake capacity of microspheres does not seem to be altered by the presence of nicotine.

It is interesting to note that the water absorption by the microspheres is so rapid that it was not possible to measure water uptake kinetics (the absorption was complete in less than 1 min).

Since the nicotine release from Sephadex® microspheres is very fast in water, a special diffusion chamber was developed in order to simulate the hydration conditions of the nasal mucosa (Fig. 3). Microspheres lie on a paper filter which is placed at the interface air-medium of the receiver compartment. The microspheres swell, by absorbing water from the receiver compartment through the paper filter. Nicotine dissolves in contact with the medium and is released from the microspheres.

Nicotine release kinetics (Figs. 5 and 6) were measured in phosphate buffer, pH 7.4 (USP), during 45 min. Solutions of NB and NHT were used in order to check the rapid passage of nicotine through the membrane.

Approximately the same release kinetics were found for both Sephadex® G25 and G50, containing either NB or NHT. The swelling capacity of the microspheres does not seem to affect the release of nicotine. G50, which is less crosslinked than G25 and needs more water to swell, was expected to produce a slower release kinetic than G25, as was found by Björk and Edman (1990),

Table 2
Absorption of different media by microspheres, expressed as ml of medium per g of microspheres (mean ± S.D.)

Sephadex®	Water	Phosphate buffer pH 7.4 (USP)	Tween® 80 solution (0.13%)
G25 M	4.6 ± 0.2	4.5 ± 0.1	4.5 ± 0.1
G25 M + NB	4.6 ± 0.1	4.5 ± 0.2	4.3 ± 0.2
G25 M + NHT	3.5 ± 0.1	3.6 ± 0.3	3.5 ± 0.1
G50 M	11.2 ± 0.3	11.8 ± 0.1	12.3 ± 0.3
G50 M + NB	12.0 ± 0.3	11.8 ± 0.3	12.2 ± 0.1
G50 M + NHT	9.7 ± 0.1	10.0 ± 0.2	10.2 ± 0.1

but no significant difference of nicotine release was noted. In this model, the time required to release 90% of nicotine (15 min) is acceptable for a transnasal delivery, because the nasal clearance time is approximately 20 min. Ryden and Edman (1992) obtained almost the same release profile with Sephadex® G25 loaded with insulin.

Edman et al. (1992) hypothetized that microspheres can act on the nasal epithelium to promote the passage of a drug through the nasal mucosa. This phenomenon could be due to the swelling capacity of microspheres (Fig. 7).

In fact, when the microspheres are in contact with the mucus layer, they swell rapidly and take up liquid from the mucus layer. In order to restore the mucus layer, the epithelial cells loose water and shrink, which provokes an opening of tight junctions, allowing the drug to be absorbed.

According to this mechanism of enhancement, G50 could be the most suitable microspheres for promoting the absorption of nicotine through the nasal mucosa, due to their high water uptake capacity acting strongly on the mucosa. Nevertheless, this assertion has to be confirmed by in vivo studies.

The study of the powder clouds delivered from a nasal device gives quantitative and qualitative information about the delivery of the microspheres (Provasi et al., 1993a) (Table 3).

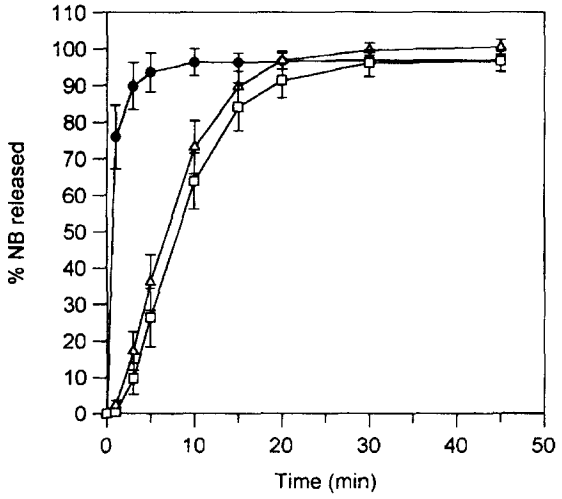

Fig. 5. In vitro nicotine base release kinetics. ●, solution (2.1%); △, G25 (10.1%); □, G50 (11.2%).

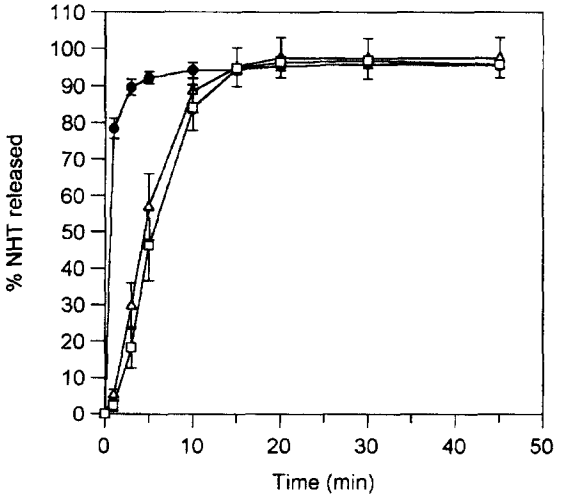

Fig. 6. In vitro nicotine hydrogenotartrate release kinetics. ●, solution (5.6%); △, G25 (27.8%); □, G50 (28.7%).

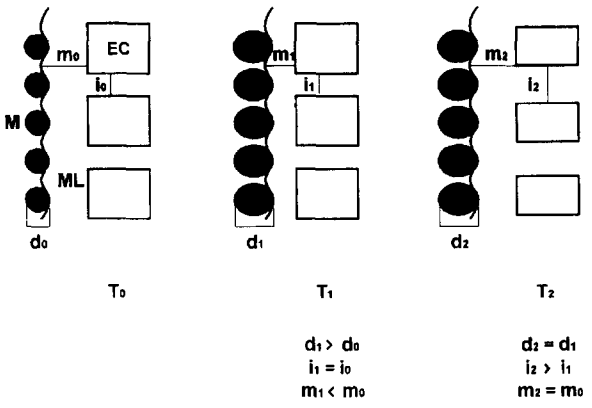

Fig. 7. Action of swellable microspheres on the nasal mucosa. T0, the microspheres (M) are in contact with the nasal mucosa; T1, the microspheres swell and take up water from the mucus layer; T2, the cells shrink and restore the water content of the mucus layer; M, microspheres; ML, mucus layer; d, microspheres diameter; i, intercellular space; m, mucus thickness.

The fraction of dose actually delivered by the device is an important quantitative information. We can observe that the amount delivered is higher with loaded particles than with unloaded ones. Considering that no significative difference exists in terms of particle size, it is possible that the lyophilization of loaded particles produces a light and porous powder, easily ventilated during the actuation.

The shape and area of the clouds give qualitative information about the delivery ability of the powder (Fig. 8a–d).

The puff area of loaded particles are smaller than those of unloaded particles (Table 3). This could be due to the fact that the porosity of loaded particles is higher, which implies a difference in air resistance, the highly porous microspheres offering a bigger resistance. These results are in agreement with those of Provasi et al. (1993a), who observed with different pharmaceutical excipients that the more the porosity increases, the less expanded the powder clouds are. The loaded particles seem to produce the most homogenous clouds, and can be expected to provide an effective distribution pattern.

It is interesting to note that there are no significant differences in cloud delivery between G25 loaded with NB or NHT, and G50 loaded with NB or NHT. The degree of cross-linking does not seem to play a role in the delivery of these microspheres through the insufflator.

4. Conclusion

Different characteristics of Sephadex® G25 and G50 transnasal dosage form of nicotine were investigated. These microspheres can be loaded either by NB or by NHT. The morphological as well as the physicochemical properties of loaded and unloaded particles were assessed in order to define the conditions of a transnasal administration of nicotine. The loading of Sephadex® G25 and G50 with nicotine does not alter their swelling capacity.

The in vitro kinetics have shown a rapid release of nicotine from the microspheres, which is certainly due to the high and rapid swelling of both Sephadex® G25 and G50 investigated in this study. Ninety percent of the nicotine is released in 15 min, which is compatible with a transnasal administration.

The quantitative and qualitative aspects of the microsphere clouds seem to be related to their porosity, showing a good dispersion ability for the different dosage forms.

The mechanism of action of microspheres has to be confirmed by in vivo investigations, but according to the enhancement mechanism described in this study, G50 could provide the most suitable microspheres for promoting the absorption of nicotine, due to its high water uptake ability. Nevertheless, the hydration conditions on the nasal mucosa could be different compared to an in vitro system and could consequently alter the swelling and release characteristics of nicotine-loaded microspheres.

Accordingly, our findings have to be verified by further in vivo investigations on human volunteers.

Table 3
Physical characteristics of microspheres

Sephadex®	ρ_a (g/cm^2)	ρ (g/cm^2)	ϵ (%)	% delivered	puff area (cm^2)
G25 M	0.79	1.52	48.0	79.5	55.7
G25 M + NB	0.75	1.53	51.0	87.5	31.4
G25 M + NHT	0.65	1.60	59.4	84.6	30.9
G50 M	0.79	1.52	48.0	68.9	66.9
G50 M + NB	0.54	1.51	64.2	94.9	32.5
G50 M + NHT	0.54	1.60	66.3	94.6	31.0

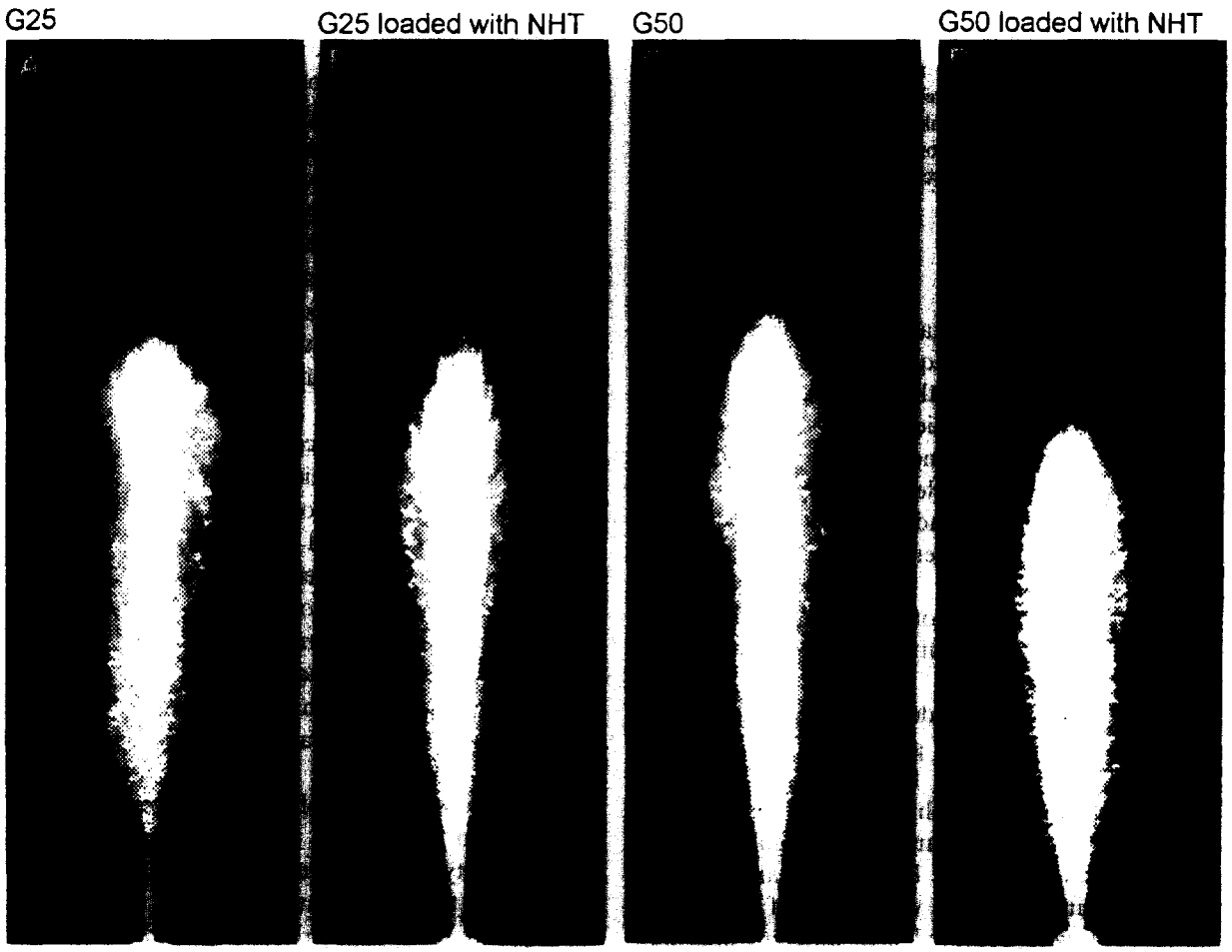

Fig. 8. Pictures of microsphere clouds at 16/100 s. (a) Sephadex® G25; (b) Sephadex® G25 loaded with NHT; (c) Sephadex® G50; (d) Sephadex® G50 loaded with NHT.

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