Advance Research in Pharmaceuticals and Biologicals

(A Peer Reviewed International Journal for Pharmaceutical and Allied Research)

ISSN 2250-0774 USA CODEN: ARPBGZ

NOVEL NASAL MICROCAPSULAR DELIVERY SYSTEM OF GALANTAMINE HYDROBROMIDE

***S. Patel[1], B. Barot[2], P. Parejiya[2], P. Shelat[2] and A. Shukla[2]**

[1]Amneal Pharmaceuticals I. Pvt. Ltd., Rajoda, Dist. Ahmedabad-382220, Gujarat, India

[2] K B Institute of Pharmaceutical Education and Research,Kadi Sarva Vishwavidhyalaya, Gandhinagar, Gujarat, India

Received on 11/08/2012 **Revised on 21/08/2012** **Accepted on 12/09/2012**

ABSTRACT:

The objective of this study was to use spray drying technique to prepare mucoadhesive microcapsules (microparticles) of the antialzheimer drug, Galantamine, in combination with the natural polymer, chitosan, for nasal administration. The effect of different proportion of chitosan on the powder and particle characteristics was also studied. Solutions containing different proportions of chitosans were prepared and spray dried which produced the microparticles having average particle size of 18 µ with the highest production yield of 72%. The size, shape and morphology of the microparticles were also determined by scanning electron microscope. The particle size and particle size distribution of prepared microparticles were determined by projection microscope and the SPAN factor was calculated. The prepared microparticles ware studied for the ex-vivo mucoadhesion and the value found to be more than 180 min. The swelling property of the microparticles was studied and the swelling index was found in the range of 0.68 to 1.42. Solid-state analysis was undertaken using Fourier transform infra-red spectroscopy (FT-IR). The drug release profiles were investigated and the time required to reach maximum solution concentrations (Tmax) was used for comparison. The entrapment efficiency was determined by UV spectrophotometry, with a range of 88-97% drug loading in the microparticles. The microparticles were spherical with a narrow size distribution, irrespective of the formulation. Tmax increased as the proportion of chitosan increased. All the formulation shows the Tmax in the range of 60 to 90 min. Spray drying is a suitable technique for making mucoadhesive microparticles of galantamine and chitosan for nasal administration. The dispersion and release of the drug was affected by the proportion of the chitosan. The nasal administration device for prepared microparticles was successfully developed by the modification of the Rotahaler® device.

KEYWORDS: Nasal drug delivery system, Galantamine, Chitosan, Spray drying.

***Corresponding Author:**
Shantanu Patel
Amneal Pharmaceuticals I. Pvt. Ltd.,
Rajoda, Dist. Ahmedabad-382220,
Gujarat, India
E-mail: santnupatel@gmail.com

INTRODUCTION

Intra Nasal (IN) drug delivery for systemic effects has been practiced since ancient times. In modern pharmaceutics, the nose had been considered primarily as a route for local and systemic delivery of diverse therapeutic compounds[1-5].

Attributes of this approach include a large surface area for delivery due to presence of highly vascularized epithelial layer, rapid onset of drug levels, potential for direct-to-central nervous system delivery, no first pass metabolism, the lower enzymatic activity compared with the gastrointestinal tract and the liver, non-invasiveness to maximize patient comfort and compliance. Owing to these and other factors, marketed IN formulations exist for a variety of low- and high-

molecular weight drugs (e.g., peptides) and many additional products are under development. One of the areas of therapeutic interest in this regard is IN administration of central nervous system (CNS) drugs.
However, rapid mucocillary clearance and low permeability of the nasal mucosa to drugs tend to counteract these advantages somewhat[4,5]. Several attempts have been made to overcome these limitations by incorporating either a penetration enhancer or some form of mucoadhesive material into the formulation to facilitate drug transport through the mucosal membrane or to increase the retention time in the nasal cavity[6,7]. Polymeric microspheres are one of the examples of mucoadhesive nasal delivery systems.
Chitosan, a linear β (1→4) linked monosaccharide produced by a process of alkaline N-deacetylation of chitin, is one such material that has been shown to be mucoadhesive. The free amino groups resultant from the deacetylation process of chitin enable the formation of positively charged chitosan salts with organic and inorganic acids. It is able to interact strongly with the negatively charged nasal epithelial cells and the overlaying mucus layer thereby providing a longer contact time for drug transport across the nasal membrane, before the formulation is cleared by the mucociliary clearance mechanism. In addition, chitosan has been shown (in Caco-2 cell culture studies) to increase the paracellular transport of polar drugs by transiently opening the tight junctions between the epithelial cells. Chitosan may be a good option in nasal delivery as it binds to the nasal mucosal membrane with an increased retention time and it is a good absorption enhancer. Furthermore chitosan is an excipient able to enhance the dissolution rate of low water soluble drugs. The mucoadhesive and viscosity enhancing properties of chitosan may both increase the residence time and intimate contact of drug and nasal mucosa. The type of chitosan most often employed for nasal delivery is a chitosan glutamate salt of a mean molecular weight around 250 kDa and a degree of deacetylation of more than 80%[8,9,10].
Spray drying is well-established method for processing solutions and emulsions in to powders, efficiently controlling the size, morphology of the particles[11]. In addition this method is cheap, fast and one-step process, which is why it has been employed in numerous applications in pharmaceutics, including the preparation of chitosan-based formulations[8,12]. Typically the spray dried powders are amorphous in nature due to rapid solidification.
Galantamine Hydrobromide (GH), 6H-Benzofuro[3a,3,2-ef][2]benzazepin-6-ol,4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-hydrobromide, is tertiary alkaloid and a reversible, competitive acetylcholinesterase inhibitor indicated for symptomatic treatment of Alzheimer's disease and other forms of dementia which improves cognition, function and daily life activities of patients[13,14,15,16]. The decrease of acetylcholine deficit in the brain of patients is a basic effect of the drug in these diseases. It is currently available in the market as conventional tablet form. GH causes several side effects; it affect gastrointestinal tract as abdominal pains[17], nausea and vomiting[18,19,20], diarrhea[21,22]. These effects are connected to a significant degree with the motor function of the stomach and intestines. Their appearance is a consequence of the drug influence on gastrointestinal motility. Alzheimer's disease mainly occurs in elder patients, the inconvenient oral administration and its gastrointestinal side effects are the main causes of the discontinuation of therapy[20]. The development of nasal formulation may overcome all above problems associated with the galantamine therapy.
A sufficient literature is available on the development of a highly soluble formulation suitable for intranasal delivery of galantamine[23]. The said literature dose not discusses about any mucoadhesive properties of the formulation. During the study of said formulation, a significant reduction in emesis and retching was observed with the intranasal administration route in comparison with oral route[24].
All these points strongly support the development of mucoadhesive formulations of galantamine. So, the purpose of the study was to include chitosan (a mucoadhesive polymer) in the preparation of galantamine nasal microparticles using a spray drying technique, with the ultimate intent of enhancing the bioavailability of the nasal galantamine formulations by improving the residence time of the drug in the nose. The prepared microparticles were filled in capsules and can be administered using modified Rotahaler®. To the best of our knowledge, microparticles of galantamine for nasal application have not previously been prepared, characterized and not disclosed for application using any device, infect, no literature is available on such application device for the administration of microparticles via nasal route.
Microparticles of galantamine and chitosan were prepared using a spray drying technique. The proportion of polymer in the formulation was varied. The effect of formulation variables on the physicochemical as well as the dissolution behavior of the formulations was studied.

EXPERIMENTAL

Materials: Galantamine HCl was kindly supplied by Alembic Research Centre, (Baroda, India). Chitosan was received as gift sample from fisheries limited, (Chennai, India). All other chemicals were of analytical reagent grade.

Methods:

Preparation of spray dried chitosan microparticles: The drug and the polymers were accurately measured in various weight ratios as listed in Table 2. Chitosan was added in 0.5% acetic acid solution at 1% (w/v) concentration and stirred until the clear solution. GH was dissolved at 10% (w/v) concentration in purified water. This solution was then mixed with chitosan solution. The final solutions were spray dried using a laboratory scale spray dryer (LU – 228 ADVANCED Laboratory Spray Dryer with N2 inert loop, Labultima, India). Nitrogen was used as the drying medium. The processing conditions were as follows: inlet temperature 160ºC, air flow 290 l/h and solution feed rate 5ml/min. The out-let temperature was 78–82ºC. These conditions were selected following preliminary experimentation. The powders were removed from the collection vessel, sealed in glass vials and placed in desiccators over silica gel in a refrigerator (<4ºC) until analysis. The yields of production were calculated from the theoretical weights and expressed as weight percentages.

Chemical purity: The chemical purity of received galantamine as received was determined by spectrophotometric method at 288 nm.

Assay (active content): The active content in microparticles was determined in phosphate buffer (pH 7.0) and the absorbance was measured on spectrophotometer at 288 nm.

FTIR studies: Fourier transform infrared spectroscopy (FTIR) has been used to assess the interaction between chitosan and drug in the final formulation. The IR spectra for the free drug, chitosan and other excipients were recorded using KBr pellets at moderate scanning speed between 4000-400 cm^{-1} using a FTIR spectrometer (Spectrum - 100, Perkin Elmer).

In vitro dissolution study: The release rate of GH from the formulation (n=3) was determined using dissolution test apparatus USP type I (Evolution 6300, Distek). The dissolution test was performed in 500 ml of 7.0 Phosphate buffer, at 37±0.5°C and 100 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at different predetermined time intervals (10, 20, 30, 60, 90, 120 min). The samples were filtered through a 0.45-µ membrane filter and diluted to a suitable concentration with same medium. The solutions were analyzed for the drug content by UV/VIS Spectrophotometer (Lambda 25, Perkin Elmer) at λmax 288 nm against the same medium as a blank. Cumulative percentage drug release was calculated using an equation obtained from a standard curve.

***Ex vivo* mucoadhesion study (Residence time):** The bioadhesive properties of microspheres were determined by an in vitro wash off test. The principle of this test is based on simulating a biological flow by washing off a mucus membrane covered with the product to be tested. The nasal mucosa of the sheep was obtained from slaughter house and stored in Krebs buffer at 4ºC after collection. A freshly cut 5 cm long piece of the mucosa was cleaned by washing with physiological salt solution. Accurately weighed 20 mg of microspheres were placed on mucosal surface, which was tied onto a glass slide (3 inch by 1 inch) using thread. The prepared slide was hung onto the burette stand at an angle of 45º. The mucus membrane was thoroughly washed with phosphate buffer solution (pH 7.0) at 37ºC at the rate of 5 ml/min using burette. The perfusate was collected on the whatman filter paper. At the end of 3 hrs collected microspheres were dried and weighed. The amount of adhered microspheres was estimated from the difference between the applied microspheres amount and the flowed microspheres amount. The ratio of the applied microspheres to the flowed microspheres was computed as percent mucoadhesion.

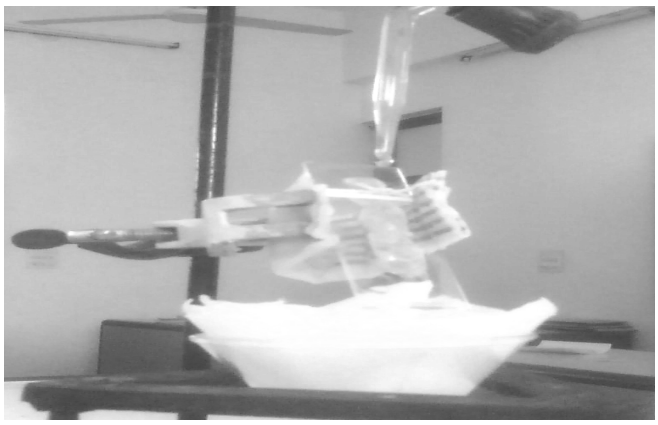

Fig. 1. Ex vivo Mucoadhesion Study

$$\% \text{ Mucoadhesion} = \frac{\text{Amount of microspheres retained on the tissue}}{\text{Amount of microspheres taken}} * 100$$

Size, shape, surface characterization of microparticles: Size, shape, and surface morphology of the GH, chitosan, and microparticles were determined by using scanning electron microscope.

Particle size and particle size distribution: The particle size of the microspheres was determined by using projection microscope. One hundred microspheres were counted for particle size using a calibration table of projection microscope as follows: The particle size was determined using 10X objective.

Table 1: Calibration table of projection microscope

Objectives	Magnification	Least count on Graduated Screen
5X	125X	8 µm
10X	250X	4 µm
20X	500X	2 µm
40X	1000X	1 µm

Span factor: The particle size distribution was expressed in terms of SPAN factor determined as;

SPAN = (d90 – d10)/d50

Where d10, d50 and d90 are the diameter sizes and the given percentage value is the percentage of particles smaller than that size. A high SPAN value indicates a wide particle size distribution.

Entrapment efficiency: Accurately weighed 100 mg of the microspheres were taken into glass mortar and crushed well for about 15 min. Accurately weighed 50 mg of crushed microspheres were added into 100 ml volumetric flask containing about 50 ml of phosphate buffer pH 7.0. The mixture was shaken well for about half an hour and then about 30 ml of phosphate buffer (pH 7.0) was added into the flask. The flask was kept aside for about 3 hrs. The volume was made and shaken well. The solution was filtered through a 0.45-µ membrane filter and the solution was analyzed for determining amount of drug spectrophotometrically by diluting suitably. The % entrapment efficiency was calculated from the following formula.

$$\% \text{ entrapment} = \frac{\text{Amount of drug in microspheres}}{\text{Amount of drug used in formulation}} * 100$$

Swelling index: For calculating swelling index, mean particle size was determined before and after swelling of microspheres. The mean particle size was determined by calculating three hundred particles for particle size. The microspheres were suspended in 7.0 pH phosphate buffer on a glass slide. The mean particle size of swelled microspheres was determined after the period of 4 hrs. The difference of particle size before and after swelling was calculated and computed as swelling index.

RESULTS AND DISCUSSION

Particle characteristics of spray dried powders: The yields of powders produced were in the range 59-72% (Table 2).We were unable to spray dry formulations containing less than 1:1 ratio of chitosan to pure drug as the powders stuck to the cyclone. Increasing the proportion of chitosan in the formulation significantly reduced the tendency of the powders to stick to the cyclone. This could be explained by the anti-plasticising ability of the chitosan in restoring the glassy amorphous state of the powders.

Table. 2. Formulation Components and Evaluation parameters

Formu - lation	CH:GH Ratio	Production Yield (%)	Avg Particle Size µm (± S.D)	% Entrapment Efficiency
SSD1	1:1	59.6	18.15± 0.19	87.53
SSD2	2:1	62.5	18.21 ± 1.0	88.60
SSD3	3:1	68.0	18.94 ± 1.13	92.03
SSD5	4:1	65.2	18.53 ± 1.17	95.25
SSD5	5:1	71.3	18.02 ± 1.3	96.62

Table. 3. Formulation Components and Evaluation parameters

Formulation	CH:GH Ratio	Swelling Index	Sphericity$	Mucoadhesion Time (min)
SSD1	1:1	0.68	++	> 120
SSD2	2:1	0.74	++	> 180
SSD3	3:1	0.85	+++	> 180
SSD5	4:1	1.25	+++	> 180
SSD5	5:1	1.42	+++	> 180

Surface characterization of microparticles: Size, shape, and surface morphology of microparticles of the best batch were determined by using scanning electron microscope (SEM). Morphology of microparticles was investigated and SEM micrographs are illustrated in Fig. 2. SEM photograph indicates that the spray dried microparticles are almost spherical in shape and there is little or no agglomeration observed.

Swelling index: The swelling properties of all the formulations was studied and found in the range of 0.68 to 1.42 as shown in Table 3. The result shows that increase in amount of chitosan in the formulation increases the swelling properties of the microparticles. This is the property of the chitosan polymer and based on the water absorbing phenomenon. Chitosan is linear polysaccharide which is able to absorb water from the site of application. Higher amount of chitosan in the formulation absorbs larger amount of water from the surroundings and thus increases the binding ability and finally increases the residence time of the formulation in the nasal cavity. The absorption of water from the

mucosa opens the tight junctions and thus facilitates the drug penetration.

FTIR studies: The analysis of FTIR spectra of the Galantamine, Chitosan and Galantamine loaded Chitosan microspheres spectra (as shown in Fig. 3, 4, 5) exhibited absence of the prominent peaks of the drug i.e. at 3580, 2620cm^{-1} in the spectra of galantamine microspheres, The absence of characteristic peaks of galantamine in the FTIR spectra of microparticles indicate that the drug is completely entrapped inside the microcapsule formed by the polymer.

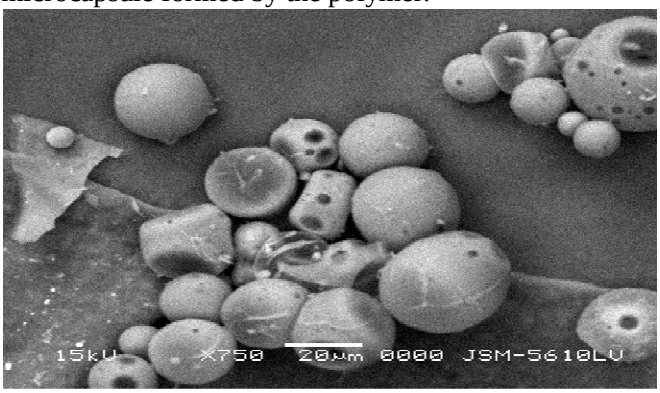

Fig. 2. SEM Photograph of Galantamine Loaded Chitosan Microparticles

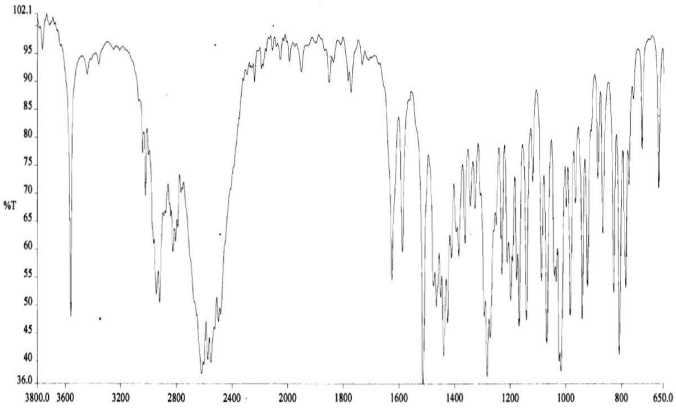

Fig. 3. FTIR Spectra of Galantamine

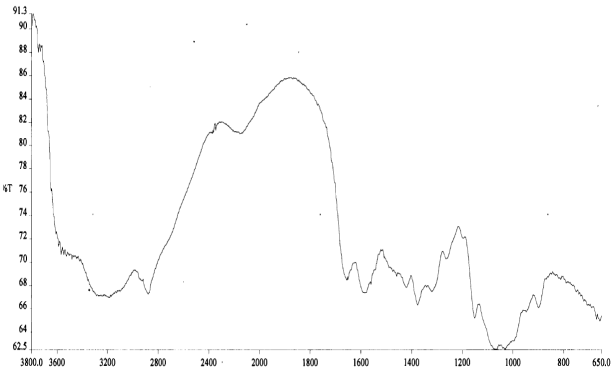

Fig. 4. FTIR Spectra of Chitosan

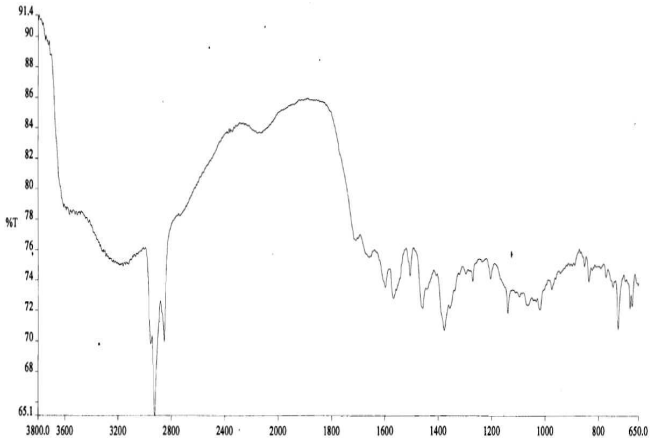

Fig. 5. FTIR Spectra of Galantamine loaded Chitosan microspheres

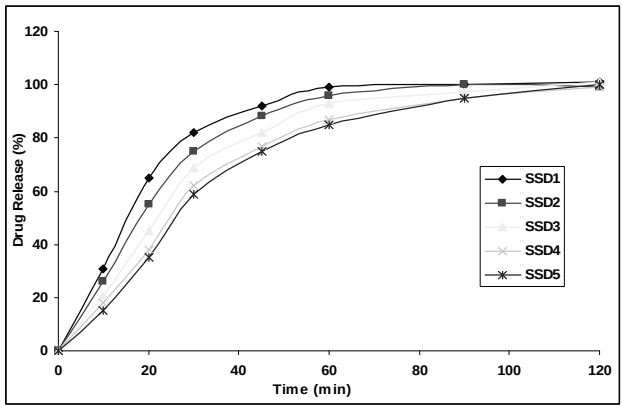

Fig. 6. Comparison of release profile between formulations (Batch # SSD1- SSD5)

In vitro dissolution study: Fig. 6 shows the dissolution profiles of the spray-dried formulations containing various proportions of the polymer. The dissolution or release rate of galantamine from the microparticles was decreased as the proportion of polymer increased, as indicated by the slope of the dissolution profiles. The time to reach maximum solution concentration (Tmax) for formulations with 1:1, 1:2, and 1:3 drug to chitosan ratio was well around 60 min, while for 1:4 and 1:5 drug to polymer ratio shows the (Tmax) between 60 to 90 min. The release of drug from the polymer microparticles was controlled by the formation of a gel which slowed diffusion of the drug across the viscous boundary layer close to the dissolving surface and/or slowed crystallization of the amorphous drug (lower solubility) as a result of supersaturation in the boundary layer[25]. The prolonged release was the result of the gel formation. Thorough mixing of the drug with the chitosan (glassy solid solution) and the development of interactions between them may also have played a role in controlling the release of drug

from the formulations. Chitosans have previously been shown to control the release of highly soluble drugs[26].

Ex vivo mucoadhesion study (Residence time): All the batches (SSD1-SSD5) using 1:1 and 1:5 drug to polymer ratio showed optimum mucoadhesion. Formulations with the ratio of 1:2 to 1:5 imparted greater mucoadhesive properties than the lowest 1:1 drug to polymer ratio. The microparticles prepared with the ratio 1:1 was unable to retain for up to 180 min, while all other formulations were remained adhered to the mucosa for more than 180 min. Thus, the results of residence time study indicated that the increase in amount of chitosan increases the mucoadhesive properties of the microparticles[26].

CONCLUSION

Spray drying is a suitable technique for preparing spherical microparticles of galantamine and chitosan with a narrow particle size range and high drug loading and production yield. The proportion of the chitosan affected drug release. The presence of a chitosan controlled the release of galantamine from microparticles in which the drug was dispersed at a molecular level. The nasal administration device for prepared microparticles was successfully developed by the modification of the Rotahaler® device. These interesting formulations deserve further characterization in terms of long term stability, possibly in vivo studies in the future.

REFERENCES

1. M. I. Ugwoke, R. U. Agu, N. Verbeke, R. Kinget. Nasal mucoadhesive drug delivery: Background, applications, trends and future perspectives, Advanced Drug Delivery Reviews 57: 1640-1665 (2005).
2. H. P. Rang, M. M. Dale, J. M. Ritter, P. K. Moore. Pharmacology, 5th Edition, pp. 181.
3. Martindale, Sean C. Sweetman. The Complete Drug Reference, 33rd edition, pp. 855-856.
4. A. E. Pontiroli, A. Calderara, G. Pozza. Intranasal drug delivery: potential advantages and limitations from a clinical pharmacokinetic perspective, Clin. Pharmacokinet. 17: 299–307 (1989).
5. L. Illum. Transport of drugs fromthe nasal cavity to the central nervous system, Eur. J. Pharm. Sci. 11: 1–18 (2000).
6. S. S. Davis, L. Illum. Absorption enhancers for nasal drug delivery, Clin. Pharmacokinet. 42: 1107–1128 (2003).
7. T. J. Aspden, J. D. T. Mason, N. S. Jones, J. Lowe, O. Skaugrud, L. Illum. Chitosan as a nasal delivery system: the effect of chitosan on in vitro and in vivo mucociliary transport rates in human turbinates and volunteers, J. Pharm. Sci. 86: 509–513 (1997).
8. E. Gavini, A. B. Hegge, G. Rassu, V. Sanna, C. Testa, G. Pirisino, J. Karlsen, P. Giunchedi. Nasal administration of Carbamazepine using chitosan microspheres: In vitro/in vivo studies, International Journal of Pharmaceutics, 307: 9–15 (2006).
9. J. Varshosaz, H. Sadrai and R. Alinagari. Nasal delivery of insulin using chitosan microspheres, Microencapsulation November 2004, vol. 21, no. 7, 761–774.
10. L. Illum. Nasal drug delivery—possibilities, problems and solutions, Journal of Controlled Release 87: 187–198 (2003).
11. J. Elversson, A. Millqvist-Fureby, G. Alderborn, U. Elofsson. Droplet and particle size relationship and shell thickness of inhalable lactose particles during spray drying, J. Pharm. Sci. 92: 900–910 (2003).
12. E. Gavini, G. Rassu, C. Muzzarelli, M. Cossu, P. Giunchedi. Spray-dried microspheres based on methylpyrrolidinone chitosan as new carrier for nasal administration of metoclopramide, Eur. J. Pharm. Sci. 68: 245–252 (2008).
13. L. J. Scott, K. L. Goa. Galantamine: a review of its use in Alzheimer's disease, Drugs 60 (5): 1095– 1122 (2000).
14. J. Corey-Bloom. Galantamine: a review of its use in Alzheimer's disease and vascular dementia, Int. J. Clin. Pract. 57 (3): 219– 223 (2003).
15. M. W. Jann, K. L. Shirley, G. W. Small. Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors, Clin. Pharmacokinet. 41: 719–739 (2002).
16. J. G. Evans, G. Wilcock, J. Birks. Evidence-based pharmacotherapy of Alzheimer's disease, Int. J. Neuropsychopharmacol. 7: 351–369 (2004).
17. A. Nordberg, A. L. Svensson. Cholinesterase inhibitors in the treatment of Alzheimer's disease: a comparison of tolerability and pharmacology, Drug Safety 20 (2): 146 (1999).
18. J. J. Sramek, E. J. Frackiewicz, N. R. Cutler. Review of acetylcholinesterase inhibitor galantamine, Expert Opin. Investig. Drugs 9 (10): 2393– 2402 (2000).
19. J. Poirier. Evidence that the clinical effects of cholinesterase inhibitors are related to potency and targeting of action, Int. J. Clin. Pract., Suppl. 127: 6 – 19 (2002).
20. B. Fulton, P. Benfield. Galanthamine, Drugs Aging 9 (1): 60– 67 (1996).

21. A. Nordberg, A. L. Svensson. Cholinesterase inhibitors in the treatment of Alzheimer's disease: a comparison of tolerability and pharmacology, Drug Safety 20 (2): 146 (1999).
22. J. L. Cummings. Use of cholinesterase inhibitors in clinical practice: evidence-based recommendations, Am. J. Geriatr. Psychiatry 11 (2): 131– 145 (2003).
23. A. Kays Leonard, A. P. Sileno, C. MacEvilly, C. A. Foerder, S. C. Quay, H. R. Costantino. Development of a novel high-concentration galantamine formulation suitable for intranasal delivery, J. Pharm. Sci. 94: 1736–1746 (2005).
24. A. Kays Leonard, A. P. Sileno, G. C. Brandt, C. A. Foerder, S. C. Quay, H. R. Costantino. In vitro formulation optimization of intranasal galantamine leading to enhanced bioavailability and reduced emetic response in vivo, Int. J. Pharm. 335: 138–146 (2007).
25. D. J. V. Drooge, W. L. J. Hinrichs, H. W. Frijlink. Anomalous dissolution behaviour of tablets prepared from sugar glass-based solid dispersions, J. Control. Release 97: 441–452 (2004).
26. E. Gavini, G. Rassu, C. Muzzarelli, M. Cossu, P. Giunchedi. Spray-dried microspheres based on methylpyrrolidinone chitosan as new carrier for nasal administration of metoclopramide, Eur. J. Pharm. Sci. 86: 245–252 (2008).