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Formulation of Neostigmine Bromide-Loaded Mucoadhesive Microspheres by Emulsification-Internal Gelation Technique and Evaluation of their Gastro-Retentive Capabilities

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Abstract: The present work was envisaged to reduce the dosing frequency and improve patient compliance by designing and evaluating Sustained Release Mucoadhesive (SRM) microspheres of Neostigmine bromide (NB) for effective control of myasthenia gravis. Microspheres were prepared by emulsification-internal gelation technique using Sodium alginate, Carbopol 934P (CP), and Hydroxyl propyl methyl cellulose K15 M (HPMC) as a mucoadhesive polymers. Microspheres prepared were found discrete, spherical and free flowing. The microspheres exhibits good mucoadhesive properties and showed high drug entrapment efficiency. NB release from these microspheres was slow and extended and dependent on the type of polymer used. The mean particle size decreased and the drug release rate increased at higher Stirring speed of emulsion content. Among all the formulation, formulation F6 containing sodium alginate, 4% & HMPC, 1% and F9 containing sodium alginate, 4% & carbopol, 1% showed the best reproducible results and mucoadhesive profile with good surface morphology. The data obtained thus suggest that mucoadhesive microspheres can successfully design for sustained delivery of NB and to improve patient compliance.

Key words: Mucoadhesive microspheres, Neostigmine bromide, Emulsification-internal gelation technique, HMPC, Carbopol 934P.

INTRODUCTION

Microsphere carrier systems have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control release rate and target drugs to specific site have made an enormous impact for formulation and development of novel drug delivery systems. Microspheres play an important role in novel drug delivery systems (1-3). They have varied applications and are prepared using assorted polymers (4). However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with absorbing membranes (5). This can be achieved by coupling bioadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, much more intimate contact with mucus layer, and specific targeting of drugs to absorption site (6-7).

Neostigmine bromide is a water-soluble drug used in Myasthenia gravis. The previous studies (8-10) reported the mucoadhesive drug delivery systems of Neostigmine bromide in the form of tablets for oral route, nasal route and transdermal patches; however, there is no report on mucoadhesive microspheres for gastro-retentive purpose. Therefore, the objective of the present study was the development and evaluation of gastro-retentive microspheres containing Neostigmine bromide using various mucoadhesive polymers for prolonged gastrointestinal absorption. An attempt was also made to develop microspheres with high incorporation efficiency. The method of microencapsulation is based on emulsification-internal gelation technique involving alginate polymers alone and/or in combination with other mucoadhesive polymers. The use of external gelation process of microencapsulation involving alginate polymers in the aqueous cross-linking agent would minimize the entrapment efficiency due to the diffusion of Neostigmine bromide into the aqueous phase during the curing of the gel beads. The other inconveniences include the limitation in reducing microspheres diameter, the teardrop shape of the microparticles produced and difficulty in industrial scale-up (11). The emulsification-internal gelation technique of microencapsulation uses an external oil phase and thereby may reduce the drug diffusion during incorporation process and improve the drug entrapment efficiency. The gel bead diameter can be easily controlled and that has scale-up potential (12).

MATERIALS AND METHODS

Materials

The following chemicals and solvents were used: Neostigmine bromide (a gift sample from Dr.

Reddy's Laboratories, Hyderabad), Sodium alginate, Hydroxypropyl methyl cellulose (HMPC) and carbopol 934P (Loba Chemical Pvt. Ltd., Mumbai), barium carbonate, chloroform, hydrochloric acid and glacial acetic acid (RanChem, Noida), light liquid paraffin, Span 80 (Central Dug House, New Delhi), sodium hydroxide pellets (Qualigens Fine Chemicals, New Delhi), potassium dihydrogen phosphate (Merck Ltd. Mumbai). All the solvents and chemicals used were of analytical grade satisfying pharmacopoeial standards.

Methods

Preparation of Microspheres

Microspheres containing Neostigmine bromide were prepared employing sodium alginate alone and in combination with HPMC and carbopol 934P. The homogeneous polymer(s) solution was prepared in distilled water stirred magnetically with gentle heat. The drug and cross-linking agent were added to the polymer solution and mixed thoroughly by stirring magnetically to form a viscous dispersion which was then extruded through a syringe with a needle of size no. 23 into light liquid paraffin containing 1.5% span 80 and 0.2% glacial acetic acid being kept under magnetic stirring at 100 rpm. The microspheres were retained in the light liquid paraffin for 30 min to produce rigid discrete particles. They were collected by decantation and the product thus separated was washed with chloroform to remove the traces of paraffin oil. The microspheres were dried at 40 °C under vacuum for 12 hr. The compositions of the microspheres formulations are listed in Table 1.

Table 1. Composition of Neostigmine bromide-loaded various microsphere formulations.

Formulation codes	Polymer entry (% w/v)	Cross-linking agent	Drug Level (% w/w)
F1	Sodium alginate, 2%	$BaCO_3$, 6%	5
F2	Sodium alginate, 3%	$BaCO_3$	5
F3	Sodium alginate, 4%	$BaCO_3$	5
F4	Sodium alginate, 2% + HMPC, 1%	$BaCO_3$	5
F5	Sodium alginate, 3% + HMPC, 1%	$BaCO_3$	5
F6	Sodium alginate, 4% + HMPC, 1%	$BaCO_3$	5
F7	Sodium alginate, 2% + carbopol, 1%	$BaCO_3$	5
F8	Sodium alginate, 3% + carbopol, 1%	$BaCO_3$	5
F9	Sodium alginate, 4% + carbopol, 1%	$BaCO_3$	5

Assay

Neostigmine bromide was estimated by ultraviolet visible spectrophotometric method (Shimadzu UV-1700, Japan). Aqueous solutions of Neostigmine bromide were prepared in phosphate buffer (pH 7.4) and absorbance was measured on UV/Vis spectrophotometer at 261 nm (The United States Pharmacopoeia 2003). The method was validated for linearity, accuracy and precision. The method obeys Beer's Law in the concentration range of 5-50 μg/ml.

EVALUATION OF MICROSPHERES

Percentage yield (w/w)

The dried microspheres were weighed and their percentage yield (w/w) was determined by using following formula (13).

$$\%\ yield = \frac{Amount\ of\ dried\ microspheres\ recovered}{Amount\ of\ drug + Amount\ of\ polymer}$$

Flow properties of microspheres

Angle of repose

Weighed quantity of microspheres was passed through a funnel fixed on a stand at a specific height upon graph paper. A static heap of powder with only gravity acting upon it was tending to form a conical mound. The height of the heap (h) and radius (r) of lower part of cone were measured. The angle of repose was calculated using formula:

$$\mathbf{Tan\ \theta = h/r}$$

Therefore, $\mathbf{\theta = \tan^{-1} h/r}$

Where,

θ = angle of repose,
h = height of cone and
r = radius of cone base

Particle size analysis

Particle size of the microspheres was determined by optical microscopy using stage micrometer and ocular micrometer (14). Microspheres were suspended in distilled water and mounted on a glass slide. A minimum of 200 microspheres per batch were counted for determination of particle size (Table-2).

Shape and surface morphology

The external morphology of microspheres was analyzed by Scanning Electron Microscope (SEM). For scanning electron microscopy samples were prepared by lightly sprinkling microsphere powder on a double adhesive tape, which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of (150-200 Å) using a fine coat ion sputter (JEOL, fine coat ion sputter JFC-1100). The microspheres were examined under Scanning Electron Microscope (JEOL, JSM – 6100 SEM, Japan).

Incorporation efficiency

Accurately weighed amount (50mg) of the microsphere formulations were dispersed in 50ml of phosphate buffer pH 7.4. The sample was ultrasonicated for three consecutive periods of 5 min each, with a resting period of 5 min each. It was left to equilibrate for 24 h at room temperature, and the suspension was then centrifuged at 3000 rpm for 15 min. The supernatant was diluted appropriately with phosphate buffer pH 7.4 and analyzed spectrophotometrically at 261 nm using ultraviolet visible spectrophotometric method (Shimadzu UV-1700, Japan).

Incorporation efficiency was calculated using following formula (15),

$$\text{Encapsulation efficiency} = \frac{\text{Drug Entrapped}}{\text{Theoretical Drug Content}} \times 100$$

Equilibrium swelling studies of microspheres

Swelling index was determined by measuring the extent of swelling of microspheres in phosphate buffer. To ensure complete equilibrium, exactly weighed 100 mg of microspheres were allowed to swell in simulated intestinal fluid pH 7.4 for 24 h. The excess surface adhered liquid drops were removed by blotting and swollen microspheres were weighed by using microbalance. The degree of swelling was then calculated by the following formula (16),

$$\textbf{Degree of swelling} = M_o\text{-}M_t/M_t \times 100$$

Where,

M_t = Initial weight of microspheres,

M_o = Weight of microspheres at equilibrium swelling in the media.

Mucoadhesion testing by *in-vitro* wash-off test

The mucoadhesive property of microspheres was evaluated by *in-vitro* adhesion testing method called as wash-off method (17). A 1 cm piece of rat stomach mucosa was tied onto a glass slide using thread. About 100 microspheres were spread on wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that tissue specimen was given regular up and down movements in a beaker containing a simulated gastric fluid (pH 1.2). After 30 min at the end of 1h, and at hourly intervals up to 12 h, the machine was stopped and the number of microspheres still adhering to the tissue was counted. The results of in vitro wash-off test of batches F1 to F9 are given in Table 3.

***In-vitro* drug release studies**

The *in-vitro* dissolution studies were performed at different pH values: (i) 1.2 pH (simulated gastric fluid) and (ii) 7.4 pH (simulated intestinal fluid). In vitro drug release studies were carried out using US Pharmacopoeia paddle type-II dissolution apparatus at 37 ± 0.5°C with constant stirring rate of 50 rpm. Microspheres equivalent to 10 mg of Neostigmine bromide were used for the test. An accurately weighed sample was responded in dissolution media consisting 900ml of 0.1 N (pH 1.2) HCl containing 0.01% Sodium Lauryl Sulphate and dissolution was done for 2 h. The dissolution medium was then replaced with pH 7.4 phosphate buffer (900 ml) and drug release study was carried out for further 3 h. Finally, the dissolution medium was replaced with phosphate buffer pH 6.8 (900 ml) and dissolution was continued for a further period of 24 h as the average residence time for intestine. A sample volume of 5 ml was withdrawn from each dissolution vessel at regular intervals and replaced with equal volume of fresh dissolution medium. The sample was filtered and analyzed spectrophotometrically at 261nm. All dissolution studies were carried out and standard deviation was applied (18) (Table 4).

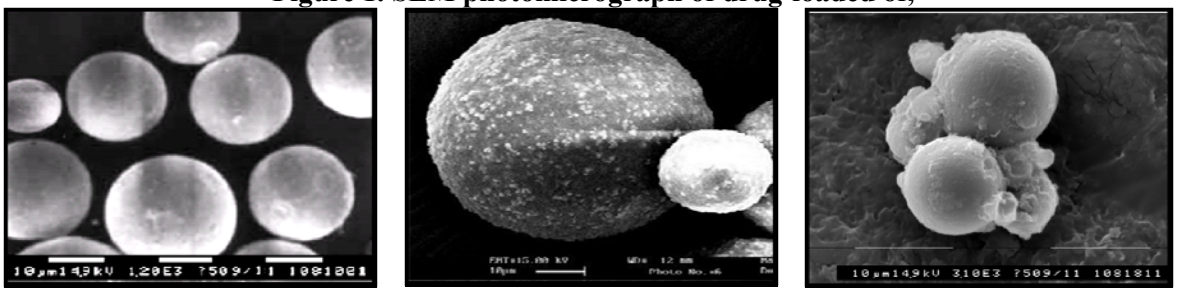
Stability study

The drug-loaded microspheres were stored at various storage conditions (room temperature, 37 °C and 45 °C/75% RH) in airtight sealed vials. The drug content of the microspheres was determined at regular time intervals and the drug release profiles were studied at 0 and 60 days.

RESULTS AND DISCUSSION

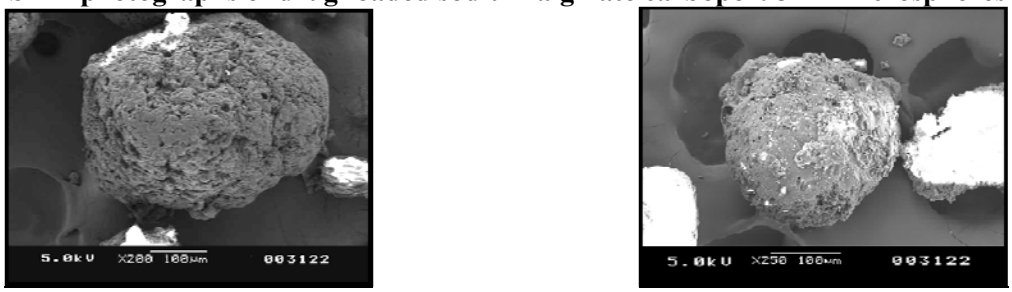
Neostigmine bromide-loaded mucoadhesive microspheres were prepared by emulsification-internal gelation technique. Neostigmine bromide, a hydrophilic drug, can partition out into the aqueous processing phase during the preparation of microspheres by external gelation method. Depending on the processing conditions as much as 80 - 90% of the drug can partition out into the external aqueous processing medium. In this study attempt was made to encapsulate Neostigmine bromide with sufficiently high incorporation efficiency. An external oil phase (liquid paraffin) was used as the harvesting medium with the expectation that for Neostigmine bromide it would be non-favourable to diffuse out of the microspheres before they form as rigid and discrete particles. The emulsification-internal gelation technique use an oil soluble acid (0.2% glacial acetic acid) in the external oil phase, which diffuse through the oil-water interface into the polymeric dispersed globules containing barium carbonate, resulting in the release of free Ba^{2+}. The sodium ion (Na_+) of alginate is exchanged with Ba^{2+} initiating gelation reaction to form barium alginate gel beads.

Figure 1. SEM photomicrograph of drug-loaded of,



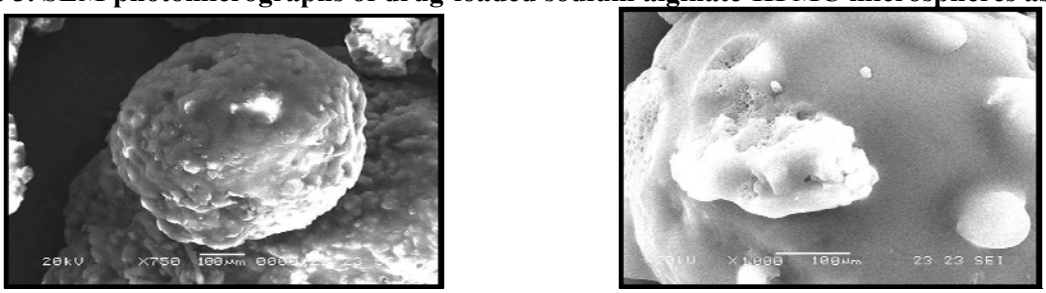
a. Sodium alginate microsphere **b. Sodium alginate-HPMC microsphere** **c. Sodium alginate-carbopol 934 microsphere.**

Figure 2. SEM photographs of drug-loaded sodium alginate carbopol 934 P microspheres as follow,



(a) After dissolution in 0.1 M HCl of pH 1.2 **(b) After dissolution in phosphate buffer of pH 7.4.**

Figure 3. SEM photomicrographs of drug-loaded sodium alginate-HPMC microspheres as follow



(a) After dissolution in 0.1 M HCl of pH 1.2 **(b) After dissolution in phosphate buffer of pH 7.4.**

Table 2. Physical characteristics of mucoadhesive microsphere of Neostigmine bromide.

Code	Particle size µm	% Yield	Incorporation efficiency (%)	Angle of repose	Bulk density (g/cc)	Taped density (g/cc)	Degree of swelling
F1	13.80 ± 0.58	70.61 ± 0.12	69.62 ± 0.86	23.86 ± 0.44	0.33 ± 0.06	0.40 ± 0.04	0.68
F2	15.72 ± 1.09	71.93 ± 0.11	74.61 ± 0.87	17.80 ± 0.11	0.55 ± 0.04	0.66 ± 0.03	0.97
F3	15.80 ± 0.95	56.82 ± 0.57	76.00 ± 0.84	13.72 ± 0.56	0.38 ± 0.01	0.44 ± 0.07	0.94
F4	17.34 ± 1.09	83.60 ± 0.82	65.00 ± 0.56	30.96 ± 0.85	0.45 ± 0.02	0.55 ± 0.08	1.08
F5	20.31 ± 1.75	77.12 ± 0.45	73.61 ± 0.83	25.87 ± 0.99	0.18 ± 0.07	0.19 ± 0.01	1.17
F6	25.63 ± 1.75	66.41 ± 0.37	80.01 ± 1.04	33.15 ± 0.14	0.58 ± 0.15	0.61 ± 0.02	1.13
F7	17.62 ± 0.87	70.50 ± 0.13	67.88 ± 0.44	27.18 ± 0.28	0.51 ± 0.05	0.55 ± 0.10	1.04
F8	21.25 ± 1.19	68.04 ± 0.11	68.75 ± 0.56	21.90 ± 0.13	0.42 ± 0.10	0.46 ± 0.09	0.96
F9	25.91 ± 1.07	70.71 ± 0.56	78.82 ± 0.98	18.08 ± 1.00	0.66 ± 0.13	0.68 ± 0.11	1.20

Neostigmine bromide-loaded mucoadhesive microspheres composed of alginate alone and in combination with HPMC and carbopol were prepared by the emulsification-internal gelation technique. The microspheres were found to be discrete, spherical, free flowing and of the monolithic matrix type. The microspheres were uniform in size with a mean size range of 13.80 ± 0.58 to 25.91 ± 1.07 µm which fall in the arbitrary particle size range of 5-5000 mm (12, 13). The particle size ranges are shown in Table 2. The size of the microspheres was in increasing trend with increasing the alginate concentration. This may be due to the increase in viscosity, which in turn increases in droplet size during addition of the polymer dispersion to the harvesting medium. The use of oil soluble surfactant (Span 80) permits the remarkable reduction in size of alginate gel beads as the result of decreasing the interfacial tension and preventing the droplets coalescence. The SEM photomicrographs (Figure 1) indicated that the microspheres were spherical in shape having particle size of 200 mm and the drug remained dispersed in the polymer matrix at amorphous state. Table 2 and SEM photomicrographs in Figure 1 reveal that the mean microspheres size as observed by optical microscope is significantly higher than that observed under scanning electron microscope. It might be explained by the fact that the incompletely dried microspheres (remaining at swollen state) were observed under optical microscope, whereas the microsphere particles were fully dried when SEM study was performed.

The effects of alginate concentrations and polymer compositions on the drug incorporation efficiency of microspheres are shown in Table 2. The highest incorporation efficiency (80.01 ± 1.04 %) was achieved with 4% w/v sodium alginate in combination with 1% HPMC, which is followed by 4% w/v sodium alginate in combination with 1% w/v carbopol (loading efficiency 78.82 ± 0.98 %). Three different concentrations of sodium alginate (2%, 3% and 4%) were used. The higher incorporation efficiency was observed as the concentration of alginate increased. This may be attributed to the greater availability of active barium binding sites in the polymeric chains and consequently the greater degree of cross linking as the quantity of sodium alginate increased, resulting in the formation of nonporous microspheres. The drug loading efficiency greatly improved when alginate was blended with carbopol at 1% level.

The microspheres consisting of sodium alginate alone and in combination with HPMC and carbopol exhibited good mucoadhesive properties as observed in *in-vitro* wash-off test when compared to a nonmucoadhesive polymer, ethyl cellulose microspheres. The wash-off was slow in the case of microspheres consisting of alginate-mucoadhesive polymers when compared to that of ethyl cellulose microspheres (Table 3).

Table 3. *In-vitro* wash off test observations of Neostigmine bromide-loaded microspheres.

Code	Percentage of microsphere adhered to rat stomach mucosa ± SD (n=3)															
	Time (h)															
	In 0.1 M HCl (pH 1.2)								In phosphate buffer (pH 7.4)							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
F1	100	100	100	98 ± 0.44	97 ± 0.11	90 ± 0.56	84 ± 0.57	78 ± 1	100	98 ± 0.26	96 ± 0.44	93 ± 0.57	85 ± 1.0	82 ± 1.0	79 ± 1.15	72 ± 0.98
F2	100	91 ± 0.98	85 ± 0.04	77 ± 1.08	72 ± 0.44	66 ± 0.57	62 ± 1.1	56 ± 0.15	100	100	95 ± 1.0	89 ± 1.15	81 ± 1.0	75 ± 0.57	66 ± 0.56	56 ± 0.44
F3	100	100	100	97 ± 0.27	95 ± 1.0	88 ± 1.15	86 ± 0.44	86 ± 0.11	100	100	100	100	100	100	92 ± 1.0	76 ± 1.0
F4	100	98 ± 1.0	97 ± 0.57	96 ± 0.57	92 ± 0.56	86 ± 0.44	84 ± 0.11	82 ± 1.0	100	97 ± 1.15	96 ± 0.57	96 ± 1.15	91 ± 0.98	86 ± 0.26	80 ± 0.57	75 ± 1.0
F5	100	94 ± 1.15	92 ± 1.0	90 ± 1.15	88 ± 1.0	86 ± 0.27	82 ±0.28	70 ± 1.15	100	98 ± 1.15	97 ± 0.44	96 ± 0.98	94 ± 0.57	94 ± 1.0	93 ± 0.11	76 ± 0.44
F6	100	98 ± 0.98	92 ± 0.57	90 ± 0.27	84 ± 0.11	82 ± 0.11	78 ± 0.98	72 ± 0.44	100	100	100	100	100	100	98 ± 1.0	90 ± 0.98
F7	100	98 ± 0.98	96 ± 0.44	96 ± 0.11	94 ± 1.15	92 ± 1.0	84 ± 1.15	80 ± 0.57	100	98 ± 1.0	97 ± 0.98	95 ± 0.56	94 ± 0.57	90 ± 0.44	88 ± 0.44	85 ± 0.26
F8	100	100	100	98 ± 0.98	95 ± 0.44	94 ± 1.15	92 ± 0.57	82 ± 0.11	100	100	97 ± 1.0	93 ± 0.57	90 ± 1.0	89 ± 0.98	86 ± 0.11	82 ± 0.57
F9	100	100	97 ± 0.11	96 ± 0.57	94 ± 1.0	92 ± 0.44	84 ± 0.56	80 ± 0.56	100	98 ± 0.98	92 ± 0.57	93 ± 0.44	91 ± 0.26	90 ± 1.0	88 ± 0.56	78 ± 1.0
EC	86	55 ± 0.26	12 ± 0.04	0	0	0	0	0	-	-	-	-	-	-	-	-

The wash-off was faster at simulated intestinal pH (7.4) than that at simulated gastric pH (1.2). Robinson et al. (19) reported that the solubility, hydration and mucoadhesivity of the polymers depend on the pH of the medium. The rapid wash-off observed at simulated intestinal pH may be due to the ionization of carboxyl acid group and other functional groups in the polymers, which increase their solubility and reduce adhesive strength. The results of the wash-off test indicated that the microspheres had fairly good mucoadhesive properties. Our result is supported by the report of Chowdary and Rao (20) who used the microcapsules of glipizide with a coat consisting of alginate and a mucoadhesive polymer-sodium carboxymethyl cellulose, methyl cellulose, carbopol and (hydroxypropyl) methyl cellulose. The mucoadhesive behaviours of various microsphere formulations are shown in Table 3. The developed mucoadhesive microspheres would adhere to the GI walls, thus resisting gastric emptying. It would ensure the prolong residence time at the absorption site to facilitate intimate contact with the absorption surface and thereby improve and enhance the bioavailability (21).

The in vitro drug release studies were carried out in the simulated gastric fluid (0.1 M HCl, pH 1.2) and simulated intestinal fluid (phosphate buffer, pH 7.4). The microspheres were prepared by ionic internal gelation technique using $BaCO_3$ as cross linking agent. The microspheres cross-linked with Ba^{2+} showed delay in disintegration and consequently a slow release of drug was obtained. It can be explained with the fact that the large size of barium ions (1.74) produced hard and nonporous microspheres. Therefore, the exchange of Ba^{2+} ions in the microspheres with Na^{+} ions of the phosphate buffer and their removal as insoluble barium phosphate was obstacled and attributed as delayed swelling of the microspheres and slow release. Our results are in good agreement with the report of Das and Senapati (22) who used the alginate microspheres containing furosemide prepared by the ionic external gelation technique using $BaCl_2$. Sodium alginate at three different concentrations (2%, 3% and 4% w/v) alone and in combination with 1% w/v of HPMC and/or carbopol 934 P was utilized for the preparation of microspheres. The drug release behaviors are tabulated in Table 4. It was observed that the amount of drug release decrease with an increase in the concentration of sodium alginate. It can be attributed to an increase in the densities of the polymer matrix resulting in larger microspheres and this in turn increase the diffusion path length, which the drug molecules have to be traverse (23). It was observed that alginate microspheres had swollen more in phosphate buffer of pH 7.4 than in 0.1 M HCl (pH 1.2). Therefore, the release would depend on diffusion of Neostigmine through the insoluble matrix of alginate polymer in 0.1 M HCl and a sustained drug release behavior was observed.

In contrast, swelling and erosion of the microspheres prepared from alginate polymer was observed in phosphate buffer of pH 7.4. Slow erosion of barium cross-linked alginate microspheres could occur through slight degradation of alginate backbone into smaller fragments. In addition, the exchange of Ba^{2+} ions in the microspheres with Na^{+} ions of the phosphate buffer causes the sustained erosion of the microspheres, which greatly increase the drug release rate in phosphate buffer of pH 7.4. To retard or sustain the drug release from the microspheres, (hydroxypropyl) methyl cellulose and carbopol 934P were blended with the alginate matrix. The microspheres retained their spherical shape after dissolution experiment in 0.1 M HCl. The microspheres composed of only sodium alginate converted to gel form after dissolution experiment in phosphate buffer of pH 7.4, while the microspheres composed of sodium alginate along with HPMC and/or carbopol lose their spherical shape after dissolution experiment. The microspheres were collected from the dissolution medium and dried at 40°C under vacuum for 12 h. The microspheres samples were then completely dried under vacuum and gold coated before performing the SEM study. The surface of the microspheres after dissolution experiment showed pores (SEM photographs, Figure 2 & 3) suggesting that the drug was released through these pores and the mechanism of drug release was diffusion-controlled. The size range of the microsphere samples was smaller than the size range of the microspheres listed in Table 2. The reason has been discussed during physical characterization of the microspheres.

Table 4. Observation for release exponent (n) and coefficient for determination (r^2).

Codes	n	r^2
F1	0.512	0.920
F2	0.879	0.966
F3	0.680	0.998
F4	0.789	0.887
F5	0.792	0.921
F6	0.560	0.975
F7	0.634	0.920
F8	0.884	0.890
F9	0.595	0.923

Table 5. Results of stability testing

Storage conditions	Time (weeks)	Formulation Codes								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
Room Temperature	0	87.20	90.81	90.23	89.36	90.43	90.18	92.10	90.83	90.88
	4	86.78	89.15	88.38	87.41	88.52	88.29	90.62	89.21	88.61
	8	86.20	88.28	86.51	86.02	85.41	86.89	88.15	86.82	86.39
37 °C	0	87.18	90.19	90.19	89.27	90.34	90.09	92.07	90.56	90.75
	4	86.70	88.15	88.18	87.03	88.36	87.91	89.03	88.72	86.78
	8	86.12	86.37	86.72	86.05	86.59	85.32	86.51	86.04	84.62
45 °C/75% RH	0	87.10	89.17	88.52	89.12	90.11	89.87	90.57	90.16	90.09
	4	86.11	85.26	85.61	86.48	88.05	86.52	88.75	88.67	88.09
	8	85.20	82.10	84.21	80.61	85.61	84.81	86.26	86.39	86.53

In order to understand the mode of release of drug from swellable matrices, the release data were fitted to the following power law equation (24): $M_t /M_\mu = Kt^n$, where M_t and M_μ are the amounts of drug released at time t and the overall amount released, respectively, K is the release constant and n is the release exponent indicative of the release mechanism. The value for n is ≤ 0.45 for fickian release, > 0.45 and < 0.89 for non-fickian release, 0.89 for case II release and > 0.89 for super case II type release (25). These values of n and the coefficient of determination (r^2) obtained are listed in Table 4. The values of n fell within the range of 0.512-0.884, indicating non-fickian type release. This kind of release is the characteristics of swelling-controlled system in which the rate of solvent uptake into a polymer is largely determined by the rate of swelling and relaxation of the polymer chains. It is assumed that the drug molecules diffuse out through a dissolving gel-like layer formed around the drug during the dissolving process. Kulkarni et al (26) observed the same type of release behavior of neem seed oil from alginate beads cross-linked with glutaraldehyde. Das and Senapati (22) also observed the non-fickian type release behavior of furosemide from alginate microspheres cross-linked with Ca^{2+}.

As described in Table 5, there was no significant change in drug content of drug-loaded microspheres, stored at room temperature, 37 °C and 45 °C/75% RH, after 8 weeks of study. The cumulative release of Neostigmine bromide from microspheres stored at different storage conditions during weeks 0 and 8 showed that there was no significant effect of temperature of storage on the drug release.

CONCLUSION

Neostigmine bromide-loaded mucoadhesive microspheres were successfully prepared by emulsification-internal gelation technique with a maximum incorporation efficiency of 80.01 ± 1.04 %. The microspheres were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state. The prepared microspheres exhibited good mucoadhesive properties as observed in *in-vitro* wash-off test when compared to a nonmucoadhesive polymer, ethyl cellulose microspheres. The drug release mechanism was non-fickian type controlled by swelling and relaxation of polymer chain. There was no significant change in drug content of drug-loaded microspheres, stored at different storage conditions after 8 weeks of study.

REFERENCES:

1. Woo, B.H., Jiang, G., Jo, Y.W., DeLuca, P.P., Pharm Res.2001, 18, 1600-1606.
2. Capan, Y., Jiang, G., Giovagnoli, S., DeLuca, P.P., AAPS Pharm Sci Tech. 2003. 4(2), article 28.
3. Gohel, M.C., Amin, A.F., J Control Release.1998, 51, 115-122.
4. Vasir, J.K., Tambwekar, K., Garg, S., Int J Pharm.2003, 255, 13-32.
5. Ikeda, K., Murata, K., Kobayashi, M., Noda, K., Chem Pharm Bull.1992, 40, 2155-2158.
6. Rao, S.B., Sharma, C.P., J Biomed Mater Res. 1997, 34, 21-28.
7. Lehr, C.M., Bouwstra, J.A., Schacht, E.H., Junginger, H.E., Int J Pharm.1992, 78, 43-48.
8. Gohel, M.C., Patel, M.M., Amin A.F., Drug Dev. Ind. Pharm.2003, 29, 565-574.
9. Gupta, R., Mukharjee, B., Drug Dev. Ind. Pharm.2003, 29, 495-503.
10. Peh, K.K., Wong, C.F., Drug Dev. Ind. Pharm. 2000, 26(7), 723-730.
11. Lencki, R., Neufled, R., Spinney, T., US Patent 4822534 (1989).
12. Poncelet, D., Lencki, R., Beaulieu, C., Halle, J.P., Neufled, R.J., Fournier, A., Appl. Microbiol. Biotech.1992, 38(1), 39-45.
13. Ziyaur, R., Kanchan, K., Khar, R.K., Mushir, A., Charoo, N.A., Shamsher, AA., AAPS Pharm Sci Tech.2006, 7(2) article 47, E1-E9.
14. Eugene, L., Milling. In: Lachman L, Liberman HA. Varghese Publishing House. India 1991.
15. Rahman, Z., Kohli, K., Khar, R.K., Ali, M., Charoo, N.A., Shamsher, A.A., AAPS Pharm Sci Tech.2006, 7(2), E1-E9.

16. Soppimath, K.S., Aminbhavi, T.M., Eur J Pharm Biopharm.2002, 53, 87-89.
17. Lehr, C.M., Bowstra, J.A., Tukker, J.J., Junginger, H.E., J Control Release.1990, 13, 51-62.
18. Hardy, J.G., Healey, J.N., Reynolds, J.R., Aliment Pharmacol Ther.1987, 1, 273-280.
19. Robinson, J.R., Kelly, P., Park, H., Chíng, H.S., J. Pharm. Sci. 1985, 74, 399-405.
20. Chowdary, K.P.R., Rao, S., Ind J Pharm. Sci.2003, 65, 591-594.
21. Jimenez-Castellanos M.R., Zia A., Rhodes C.T., Drug Dev. Ind. Pharm.1993, 19, 143-194.
22. Das, M.K., Senapati, P.C., Acta Pol. Pharm.2007, 64(3), 253-262.
23. Mishra, B., Jaynath, P., Sankar, C., Ind. Drugs 2003, 40(12), 695-700.
24. Ritger, P.L., Peppas, N.A., J. Control Release 1987, 5, 23-36.
25. Akbuga, J., Int. J. Pharm. 1993, 89, 19-24.
26. Kulkarni, A.R., Soppimath, K.S., Aminabhavi T.M., Dave A.M., Mehta M.H., J. Control Release 2000, 63, 97-105.