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RESEARCH ARTICLE

Formulation Development and Evaluation of Alginate-Based Bromhexine Pellets Prepared by Extrusion/Spheronization Containing Hydroxy Propyl Methyl Cellulose

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ABSTRACT

The study was conducted to develop a stable floating pellet as a multiparticulate system and analysis of the different parameter and in vitro evaluation of the preparation, containing Bromhexine, a mucolytic expectorant. Extrusion/spheronization is a well-known technique which aid in the formation of spherical pellets with regular shape and size, allow the application of a release retarding membrane was utilized in this purpose. Hydroxy propyl methyl cellulose (HPMC) as HPMC K4M and HPMC K100LV and Sodium Alginate polymers at different ratio were incorporated and mixed well with Bromhexine for providing floating properties. Different ratio of these polymers leads to the development of 9 batches. A number of factors such as contraction ratio, moisture content, buoyancy test, swelling index of the formulated preparation and in vitro evaluation at pH 3 were evaluated. Results showed that pellets of all batches are spherical in shape with poor size distribution, remain buoyant in the simulated gastric fluid, water and 0.9 % NaCl solution, whereas Batch CX showed low swelling index (22.65). Scanning Electron Microscopy provides proper idea about pellet morphology and drug distribution in the polymer network. In vitro release study reveal poor release profile up to two hours. Thus stable Bromhexine floating pellet could be formulated by considering the parameter studying in this project work.

KEYWORDS

Floating pellet, Mucolytic, Extrusion/Spheronization, Polymers, *In vitro*, Scanning Electron Microscopy

INTRODUCTION

In order to achieve systemic effects of therapeutic agents, oral route is the most suitable and choosable. Due to the advantages like patient acceptance, suitability and cheap formulation process, oral route is regarded as the first approach in case of new kind of drug discovery and development.

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Conventional immediate release formulations could maintain the safety level in addition with desired pharmacokinetic and pharmacodynamic balance for some drug substances to the patient[1], although fluctuation in drug plasma level is documented from conventional dosage form, could be avoided through sustain release dosage form.[2] Sustained/controlled release formulations could prolong the effects of drug without the fluctuation of drug levels and hence minimize side effects.[3] To enhance the bioavailability of

drug with effective drug concentration in the blood for prolong period, drug delivery systems like controlled / sustain release have been employed in the pharmaceutical dosage forms.[4,5]

Different factors affect the bioavailability of oral formulations; one is gastric residence time (GRT). Parameters like onset of floating, floating duration of a gastro retentive dosage form could help to evaluate the floating characteristics and floating strength, whereas greater floating strength increases the probability of floating capability of oral medication and consequently lower the effects of food on gastric retention.[6]

Floating systems are sufficiently buoyant as they are low density system to remain afloat for a longer period over the gastric contents in the stomach. Drug is released from the floating system slowly during their floatation and thus results in rising the retention time, in addition lower the fluctuation in plasma drug concentration.[7]

Due to numerous advantages like significant distribution characteristics, transit time consistency and decreased probability of gastric irritation; focus on multiparticulate systems are raising rather than single-unit dosage forms. Though certain technologies are now available for preparing multiparticulate system but still spray-drying, spheronization, and film-coating technology consist the mainstream technologies.[8]

The aim of the present study is to develop alginate gel microsphere of Bromhexine sodium using sodium alginate and hydroxyl propyl methyl cellulose as the hydrophilic carrier and calcium chloride as cross-linking agent. Further, different parameters of the prepared beads like particle size analysis, buoyancy test, swelling study, *in vitro* evaluation etc. were examined.

MATERIALS AND METHOD

Materials

Bromhexine HCl was a gift sample from Ibna Sina Pharmaceuticals Ltd., Bangladesh. Sodium alginate and Calcium chloride was purchased from LOBA Chemicals Pvt. Ltd., India and Merk, India respectively. HPMC (Methocel K100 LV CR Premium USP and Methocel K4M Premium USP) were bought from Aircon Asia Pvt. Ltd. India. All other reagents and solvents used were of analytical grade satisfying pharmacopoeial specifications were commercially available.

Methods

Method for Pellets Preparation

Spheronization techniques were employed to prepare pellets and different batches of pellets were prepared by varying the concentration of Sodium Alginate and HPMC. For this reason firstly, Sodium Alginate gel of different concentrations (1%, 1.5 %, 2% w/w) was prepared by overnight soaking in 250 ml of distilled water and after stirring with electronic stirrer (4000rpm) for half an hour. Then specified amount of HPMC K4M & HPMC K100LV for identical Sodium Alginate gel were added and followed by homogenized for 30 minutes. Later, allocated amount of Bromhexine were added to the obtained mixture and stirred for further 45 minutes before adding drop wise via a needle fitted with a 5ml syringe into 200ml of calcium chloride solution (1% w/v), placed above the magnetic stirrer. The droplets instantaneously gelled into discrete matrices upon contact with the solution of calcium chloride and stirred in the solution for half an hour prior to removal. Finally, gelled beads were isolated through cotton, washed with 4 × 50ml volumes of distilled water and dried in the room temperature for approximately 12 hours.[9]

Buoyancy of the Preparation

Buoyancy was observed visually, after placing the pellets (ten beads) from each batch in 50ml of individual test solution. The pellets were considered to be buoyant when they float on the fluid.[10]

Study of Particle Size and Contraction Ratio of Bromhexine Pellet

The particle sizes (n=20) were measured firstly with a digital slide calipers (Fisher Brand) and followed by Scanning Electron Microscopy and the contraction ratio of the beads was calculated

by dividing the mean volume of dried gel (dried pellet) by that of the hydrogel (wet pellet).[10]

Surface Morphology Study

With the aid of Scanning Electron Microscopy (SEM) (Hitachi, S-3400N) surface morphology of Bromhexine pellets was studied at Bangladesh Center of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh.[11]

Swelling Study

Here, percent (%) of weight gained by the beads after keeping in p^H 1.2 chloride buffers for two hours were evaluated to determine the extent of swelling and 10 mg pellets of each batch were used for this study. Finally, after 2 hours the beads were withdrawn, soaked with tissue paper and weighed. Following formula is used to calculate the percent weight gained[12] -

$$\text{Swelling ratio} = \{(Mt-Mo) / Mo\} \times 100$$

Where,

Mt = weight of beads at time 't' and

Mo = weight of beads at time, t = 0.

In Vitro Dissolution of Pellets

In vitro drug release were carried out by USP apparatus 2 (rotating paddle method) at 50 rpm rotation speed. Dissolution medium (900 ml) was p^H 3 chloride buffer and maintained at 37 ± 0.5º C. 10 ml sample from each basket was withdrawn at time intervals of 10min, 15min, 25min, 40min, 55min, 70min, 85min, 100 minutes and replaced with an equal volume of test medium to maintain the total volume constant. Quantity of drug release was measured by taking the absorbance at 245nm in UV spectrophotometer.

Determination of Drug Release Kinetics

With the help of statistical software (SPSS 15), the value of correlation coefficient (r^2) was measured for Higuchi, Zero order and first order model. To understand the models, the correlation coefficients with better statistical fit were used as the main criteria and hence equation with maximum correlation coefficient was considered to be the most suitable model for each system.[13]

RESULTS AND DISCUSSION

Evaluation of Pellet Preparation Method

In extrusion/spheronization, it is desirable for the wet mass to remain homogeneous at the time of extrusion and the extrudates should devoid of any adherence sign. For this purpose, the wet mass should be properly formulated to form a plastic cohesive mass. Furthermore, adequate mechanical properties and brittle behavior of extrudates is necessary to form discrete pellets rather than large amount of fine particles.[14]

In this study Na-alginate and HPMC were used as polymer and formulation of all batches showed enough extrudability properties without creating any problem in spite of variation in polymer concentration during extrusion. The use of Na-alginate and HPMC as polymer and binder to formulate pellets through extrusion/spheronization was shown in previous studies.[10,14] Bromhexine was trapped in sodium alginate beads as a chelate structure, egg box junction between alginate and calcium ions, was formed.[15] The composition of all formulations is described in table 1.

Buoyancy of Pellets

When Bromhexine loaded Alginate – Calcium (Alg-Ca) was steeped in water, 0.9% NaCl or GI fluid that mimics gastric juice, it floats in the solution and similar pattern appeared for all the batches. Similar result in case of high amount of drug loaded Alg-Ca beads was documented in previous studies.[10]

Particle Size and Contraction Ratio of Bromhexine Pellets

The diameter and contraction ratio of Bromhexine pellets at different concentration of Sodium Alginate and HPMC are shown in Table 2. In the formulation of pellets it is standard that optimal formulation and process conditions are employed to obtain narrower size distribution as much as possible. The mean particle size of the various formulations (AX – CZ) was obtained in the range of 1.5693 ± 0.1625 to 1.961 ± 0.106.

Table 1: Formulations of pellets

Batch No.	Ingredients used				
	Sodium Alginate		HPMC K4M: HPMC K100LV		Bromhexine
	Percent used	Amount (gm)	Ratio used	Amount (gm)	Amount (gm)
AX	1	2.5	2:1	5 : 2.5	1
BX	1	2.5	1:2	2.5 : 5	1
CX	1	2.5	1.5:1.5	3.75 : 3.75	1
AY	1.5	3.75	2:1	5 : 2.5	1
BY	1.5	3.75	1:2	2.5 : 5	1
CY	1.5	3.75	1.5:1.5	3.75 : 3.75	1
AZ	2	5	2:1	5 : 2.5	1
BZ	2	5	1:2	2.5 : 5	1
CZ	2	5	1.5:1.5	3.75 : 3.75	1

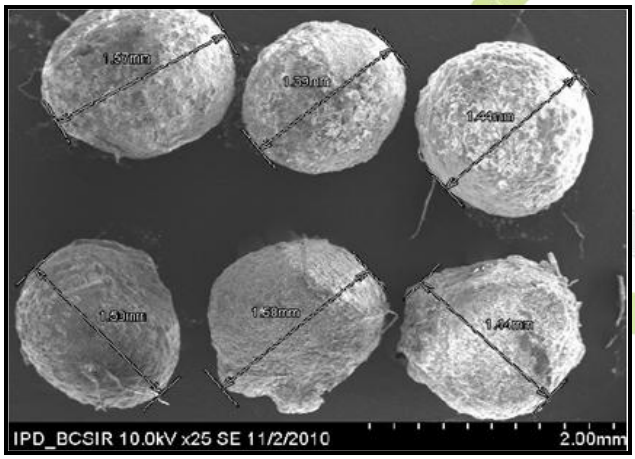

Figure 1: Diameter of Pellets

Figure 2: Rough Surface and Spherical Shape of Pellets

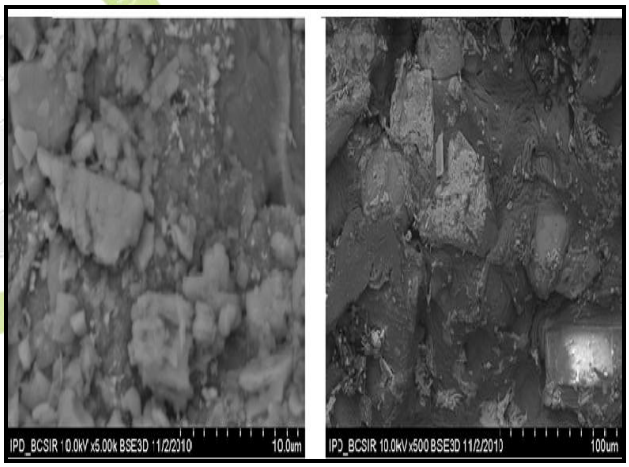

Figure 3: Dispersion of Drug Crystals on Pellet Surface

The pellet particle size distribution was within a narrow range and was not significantly varied by differences with concentration and amount of sodium alginate and HPMC. Though, amount of sodium alginate changed but amount of HPMC was constant in nine batches. Equal amount of HPMC (though different ratio of HPMC K4M and HPMC K100 LV) makes the formulation denser as HPMC has binder and adhesive property, led to the formation of larger particles in all batches. Evidence of increasing size distribution with rising amount of HPMC K4M,

HPMC K100 LV and HPMC 6 cp was proposed in earlier studies.[14] Contraction ratio of the pellets is also within narrow range like the particle diameter, ranges from 0.17 to 0.31. Insignificant effect of sodium alginate on contraction ratio was reported in former work.[10]

Scanning Electron Microscopy of Bromhexine Pellets

To observe differences in atomic number, unlike secondary electron imaging, back-scattered electron imaging employs high-energy flexible electrons. Elements which have lower atomic number absorb more electrons and create dark appearance on SEM photographs, whereas elements with higher atomic number reflect or highly deflect more electrons along the primary electron axis, as for instance, the order of brightness for elements like C, N and Ca is C < N < Ca on the micrographs.[9]

SEM (HITACHI, Model: S-3400N) was performed on the prepared Bromhexine pellet after drying to access their surface and morphological characteristics. "SEM" Micrographs were taken using different magnifications encompasses 10-3500. It is clear from the figures that magnifications provide the morphology of single pellet with diameter (Figure 1) which ranges from 1.39 to 1.57 nm and the pellet is roughly spherical (Figure 2) in shape, a densely gel structure was also shown. Pellets were free flowing with a sandy appearance because of drug crystal association on the surface whereas variation of sodium alginate and HPMC (two types) concentration didn't bring any significant differences on the surface. Through the use of scanning electron microscope, images of Bromhexine loaded Alg-Ca based pellets containing different amounts of the polymers and dispersion of the drug (Figure 3) could be observed. Drug crystals appeared on the surface of the no aggregated pellets probably formed as a result of drug migration along with water to the surface during drying. The evidence of soluble components migration during drying are in accord with the examination executed earlier.[9]

Swelling Behavior

The result of swelling index is present in table 2. As the rate of hydration increased, weight gain with higher swelling index is illustrated in case of all batches, though no linear relationship between polymer concentration and swelling index value is predicted. Higher swelling index is also attributable due to the lack of erosion of the outermost gelled layer in the chloride buffer. The increase rate of hydration with swelling index up to 3 hours is reported in study executed by PG Yeole.[12]

Table 2: Physical properties of Bromhexine pellets

Batch No.	Diameter of Dried pellets [a]	Contraction ratio(CR)	Swelling index (%) (at 2 hours)
AX	1.569 ± 0.162	0.24	33.742
BX	1.412 ± 0.164	0.17	25.012
CX	1.961 ± 0.106	0.31	22.65
AY	1.582 ± 0.120	0.18	30.22
BY	1.459 ± 0.136	0.21	35.673
CY	1.752 ± 0.201	0.27	27.65
AZ	1.543 ± 0.246	0.17	28.175
BZ	1.475 ± 0.115	0.18	35.095
CZ	1.780 ± 0.083	0.31	42.29

[a] The data are presented as mean value ± SD (n=20).

Table 3: *In vitro* drug release profile

Batch No	Release Rate (%mg/hr)	r^2 of Higuchi Plot	r^2 of first order plot	r^2 of zero order plot
AX	4.5	0.883	-0.762	0.874
BX	4.17	0.374	-0.141	0.144
CX	3.34	0.506	-0.295	0.299
AY	2.56	0.581	-0.397	0.465
BY	3.06	0.417	-0.172	0.177
CY	3.18	0.385	-0.176	0.177
AZ	1.45	-0.077	0.190	-0.205
BZ	1.8	-0.318	0.489	-0.502
CZ	1.03	0.330	-0.179	0.172

Drug Release from Bromhexine Pellets

The drug release rate (mg/hr) and correlation coefficients value of pellets are shown in table 3 and figure 4 depicts drug release profile prepared by plotting amount of drug release per hour (mg/hr) against batches. Release of Bromhexine from the formulated pellets is very low. Although the total amount of HPMC (though different ratio of HPMC K4M and HPMC K100LV) is consistent in case of all the formulated batches, concentration of sodium alginate is different and it could be observed that with the increase of sodium alginate amount, drug release decreased. Similar effect of HPMC and sodium alginate in delaying the release from pellets has been reported previously.[13,16,17] Such result revealed the protection of bromhexine from gastric juice.

Drug release profile of the batches AX to CY shows maximum linearity with values between 0.883 and 0.374 by Higuchi's model which is employed for diffusion release of drug through water filled pores in the matrix. On the other hand, batches AZ to CZ with regression values from 0.489 to -0.179, best fit the first order kinetic model.

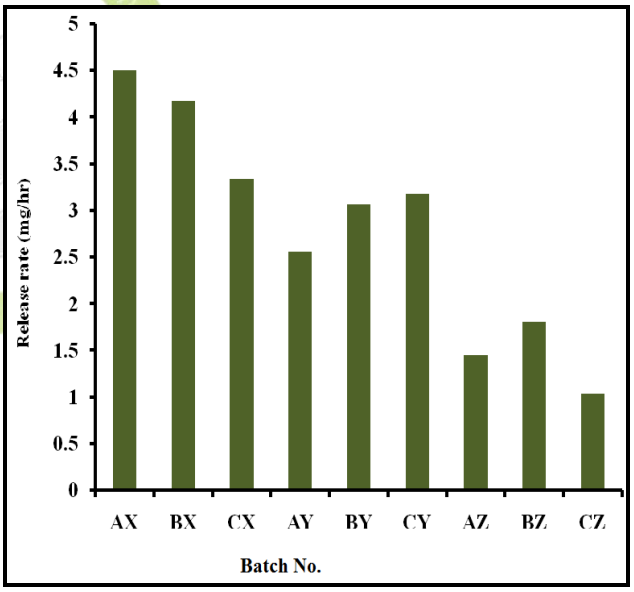

Figure 4: Drug Release Rate

CONCLUSION

Bromhexine loaded pellets were prepared here through extrusion / spheronization technique, with polymers Sodium alginate and Hydroxy propyl methyl cellulose (HPMC) K100LV and K4M. Then properties of the pellets were evaluated and results revealed relationship between pellets particle size and contraction ratio with sodium alginate and HPMC

concentrations. Surface morphology was studied by Scanning Electron Microscopy and dissolution studies by USP dissolution tester (Apparatus-II), different kinetic models were used to explain the release pattern which showed a good fit with Higuchi model. This study could help to establish a successful formulation and extensive human study will focus light on compliance.

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