Design and Development of Oral Mucoadhesive Multiparticulate System Containing Atenolol: *in Vitro–in Vivo* Characterization

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The aim of the present study was to prepare mucoadhesive multiparticulate system for oral drug delivery using ionic gelation technique. Microspheres of different mucoadhesive polymers including hydroxypropyl methylcellulose (HPMC) K15M and carbopol 971P were prepared. In this technique cross linking of sodium alginate with calcium chloride was done which retarded the release of drug from the mucoadhesive polymer. In the present work atenolol was used as model drug. Interaction studies performed using FT-IR spectroscopy revealed that there was no drug to polymer interactions. Multiparticulates so prepared were discrete, bulky, free flowing and showed an average encapsulation efficiency ranging from 23—74%. Particle size of the multiparticulates as determined by the scanning electron microscopic analysis (SEM) studies was found to be between 561—831 μm. The prepared formulations also exhibited a good mucoadhesive strength which was determined in *in vitro* conditions through falling film technique. The multiparticulate so prepared also exhibited a good swelling index which confirmed the strong mucoadhesive property of the formulation. Atenolol release from the multiparticulate system was regulated and extended until 12 h and exhibited a non-Fickian anomalous transport from the swellable microspheres, as evident from the release rate exponent values which varied between 0.569—0.622. The stability studies performed on the optimized batch at 40 °C/75% RH for 90 d indicated no significant change in the physicochemical properties. *In vivo* radioimaging studies in rabbits showed the residence of mucoadhesive microspheres for 6-8 h in upper part of gastrointestinal tract (GIT).

Key words gastrointestinal delivery; mucoadhesion; ionic gelation; multiparticulate system; radio imaging

Natural hydrophilic polymers owing to their characteristic biocompatibility and biodegradability properties are widely exploited in the pharmaceutical industry for the development of novel drug delivery system. Among these polymers, alginate is one that has been widely used in numerous biomedical applications, used in sutures and dressing materials with characteristic features such as mucoadhesion, bioadhesion and modifying drug release profile. In order to develop oral drug delivery systems, it is necessary to optimize both the residence time of the system in the gastrointestinal (GI) tract and the release rate of the active ingredient from the system. One of the most extensively studied methods for prolonging the residence time in the GI tract is using mucoadhesive polymers that adhere to the mucus layer and release the loaded drug in a sustained manner.[1)] Moreover mucoadhesive dosage forms have also been reported to improve the absorption and systemic bioavailability of the drugs that are normally poorly absorbed.[2)]

When the mucoadhesive dosage form is administered in either tablet or capsule form, they may or may not adhere to the mucous surface due to the weight of the dosage form and the vigorous movement of the GI tract, resulting in a large variation. However, the mucoadhesive microspheres have some advantages which include a light weight and a smaller dose variations due to the large number of microspheres administered.

Oral controlled release system continues to be the most popular ones among all the drug delivery systems. It offers several advantages over the conventional systems like better plasma level profile, lower dosing and toxicity. The problem frequently encountered with controlled release dosage form is its inability to increase the residence time of the dosage form in the stomach and proximal portion of the small intestine.[3)] This may be due to the rapid gastrointestinal transit phenomenon of the stomach which may diminish the extent of absorption of many drugs since most of drug entities are mostly absorbed from the upper part of the intestine. Therefore it would be beneficial to develop a sustained release formulation which remains at the absorption site for an extended period of time. Mucoadhesive multipaticulates of metoprolol tartarate using various mucoadhesive polymers were prepared and studied.[4)] Several approaches have been reported to prolong the gastric residence time of the dosage forms at the absorption site and one of these is the development of oral controlled release bioadhesive system.[5)]

Calcium-induced alginate gel beads have been developed in recent years as a unique vehicle for drug delivery system. These beads have been used in formulations as single or multiple units, with or without the addition of other hydrogels, nanospheres, polycations, and many more dosage forms for achieving temporal and spatial drug release.

Atenolol (ATN) is a beta-adrenergic receptor blocking agent without membrane stabilizing or intrinsic sympathomimetic activities and it has been used for the treatment of hypertension, either alone or with other antihypertensive such as thiazide diuretics.[6)] It is poorly absorbed from the lower GIT. The oral bioavailability of atenolol has been reported to be 50%.[7)] The drug is slightly water soluble and has elimination half life after an oral dose 6—7 h. It is prescribed widely in diverse cardiovascular diseases, *e.g.* hypertension, angina pectoris, arrhythmias, and myocardial infarction.[8)] The human jejunal permeability and extent of absorption is also low.[9)] Thus, it seems that an increase in GRT may increase the extent of absorption and bioavailability of the drug. Administration of conventional tablets of atenolol has been reported to exhibit fluctuations in the plasma drug levels, results either in manifestation of side effects or reduction in drug concentration at the receptor site.[10,11)]

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Hence the objective of the study was to develop and optimize gastrointestinal mucoadhesive multiparticulate system of ATN using various mucoadhesive polymers like HPMC K15M and carbopol 971P. This will increase the residence time of the drug at the absorption site, increasing the bioavailability of the drug and thus decreasing the dosing frequency of the drug. In the present study multiparticulate system was preferred as a formulation over conventional tablet or capsule formulations as it has several advantages like it increases the surface area of the formulation exposed to the absorption site thus increasing the absorption of drug and decreasing the dosing frequency of the drug.

Experimental

Materials Atenolol (ATN) was obtained as a gratis sample from Zyduscadila, Ahmedabad, India. Hydroxypropyl methylcellulose (HPMC K15M) was generous gift from Colorcon Asia Pvt. Ltd. Goa, India. Carbopol 971P (CP) was a kind gift from Noveon Pharmaceuticals Pvt. Ltd. (Cleveland, U.S.A.). Barium sulfate ($BaSO_4$), extra pure quality for X-ray diagnosis, sodium alginate (Alg) and calcium chloride ($CaCl_2$) were obtained from S.D. Fine Chemicals, Mumbai. All the other reagents used were of analytical grade.

Method. Preparation of Microspheres by Ionic Gelation Technique In this technique[12] sodium alginate is cross linked with $CaCl_2$ solution to release the drug in a controlled manner. Chemically, alginates are anionic block copolymer consisting monomers of d-mannuronic acid joined together by 1—4 glycosydic linkages. Bivalent alkaline earth metals like Ca^{2+} undergoes ionic interaction with –COOH moiety of sodium alginate and results in cross linking of sodium alginate. Microspheres were prepared by using the technique in which sodium alginate in varied quantities was dissolved in 25 ml of deionized distilled water. Mucoadhesive polymer was slowly added to the above solution with continuous stirring to form a homogenous solution which was then sonicated for 15 min to get bubble free solution. The drug substance ATN was then added to the above polymer–alginate mixture and stirred thoroughly to form the clear solution. The drug–polymer bubble free mixture was then added drop wise into the $CaCl_2$ solution using an insulin syringe. The added droplets were then retained in the $CaCl_2$ solution for 15—30 min to complete the curing reaction and to produce spherical and rigid microspheres. The microspheres so prepared were collected by decantation technique, washed repeatedly with deionized water and dried at 45 °C for 12 h. Different formulations prepared using varied alginate to mucoadhesive polymer ratios in order to sustain the release of the drug for 12 h are listed in Table 1. The same formulation batches (B1—B15, Data shown for B1—B5 only) were prepared using the mucoadhesive polymer CP 971P. The effect of formulation parameters on drug entrapment efficiency, namely concentration of alginate as well as of $CaCl_2$, the curing time with hardening agent and the addition of mucoadhesive polymers like HPMC K15M, CP 971P to alginate were investigated. The effect of different mucoadhesive polymers on the *in vitro* release behaviour of ATN from alginate multiparticulates was examined.

Characterization of Multiparticulates. Production Yield[13] The production yields of microspheres of various batches were calculated using the weight of finally dried particles with respect to the initial total quantity of the drug and polymer used for preparation. Percent production yields were calculated as per the formula mentioned below:

$$\text{production yield} = \frac{\text{practical mass (microsphere)}}{\text{theoretical mass (polymer + drug)}} \times 100$$

Actual Drug Content and Entrapment Efficiency Actual drug content and entrapment efficiency of the multiparticulates were determined by direct method. In a 100 ml volumetric flask accurately weighed 25 mg of microspheres were taken and volume was made up to mark with pH 6.8 phosphate buffer solution. The flask was shaken for 12 h using an orbital shaker incubator (CIS-24, Remi Instruments Ltd.).Then the solution was filtered and from the filtrate appropriate dilutions were made and absorbance was measured at 224 nm by using UV spectrophotometer. The quantity thus obtained was subjected to the formula given below to find the entrapment efficiencies of various formulations.

Table 1. Formulation Composition of ATN Containing Alg-Ca-HPMC K15M

Sr. No.	Formulation	ATN (mg)	HPMCK15M (mg)	Sod. alginate	$CaCl_2$ (% w/v)	Curing time (min)
1	A1	100	100	250	5	30
2	A2	100	100	500	5	30
3	A3	100	100	750	5	30
4	A4	100	100	1000	5	30
5	A5	100	—	1000	5	30
6	A6	200	100	1000	5	30
7	A7	300	100	1000	5	30
8	A8	100	100	1000	1	30
9	A9	100	100	1000	3	30
10	A10	100	100	1000	7	30
11	A11	100	100	1000	9	30
12	A12	100	100	1000	5	15
13	A13	100	100	1000	5	45
14	A14	100	100	1000	5	60
15	A15	100	100	1000	5	120

$$\text{\% encapsulation efficiency} = \frac{AQ}{TQ} \times 100$$

where AQ is the actual drug content of microspheres and TQ is the theoretical quantity of drug present in microspheres.

Size Analysis ATN containing Ca-Alg-HPMC K15M and Ca-Alg-CP971P microspheres were evaluated for particle size. The microspheres sizes ($n=50$) were taken for particle size analysis and average particle size was determined. Particle size of the microspheres was measured using a Motic DMWB2-223 digital microscope (Canada) fitted with 1/3 CCD Camera Imaging accessory and using Motic Images 2000 (1.3 Version) image analysis software.

Surface Topography. Scanning Electron Microscopic Analysis (SEM) The microstructure and surface topography of the microspheres were investigated and photographed using scanning electron microscope (JSM 6390, JEOL, Japan). Microspheres were mounted on metal grids using double-sided tape and gold coated under vacuum at 10 kV accelerating voltage.

Drug-Excipient Compatibility Study. Fourier Transform Infrared Spectroscopy (FT-IR) The crushed microspheres were mixed with potassium bromide (Merck) in 1 : 100 proportions and dried at 40 °C. The mixture was compressed to a 12 mm semi transparent disk by applying a pressure of 10 tons (KBr press, tsi, Mumbai) for 2 min. The FT-IR spectra over the wavelength range 4000—400 cm^{-1} were recorded using a FT-IR spectrometer (FTIR-8400S, Shimadzu, Japan).

Differential Scanning Calorimetry (DSC) Analysis. DSC Thermograms Were Recorded on a General Thermal Analyzer (Perkin Elmer Cyris-DSC, U.S.A.). The DSC studies were performed on pure ATN, ATN loaded microspheres and blank microspheres. The thermograms of samples were obtained at a scanning rate of 10 °C/min conducted over range of 30—300 °C in nitrogen atmosphere.

Determination of the Flow Property of ATN Loaded Ca-Alginate Microspheres The flow properties of the microspheres were evaluated from the changes in the volume due to rearrangement and packing occurring during tapping in a graduated measuring cylinder and was expressed as :

1. Carr's compressibility index[14]:

$$\text{Carr's index} = \frac{\text{TBD} - \text{LBD}}{\text{TBD}} \times 100$$

2. Hausner ratio[15]:

$$\text{Hausner's ratio} = \frac{\text{TBD}}{\text{LBD}}$$

where LBD and TBD are loose and tapped bulk density resp.

Swelling Determination of Microspheres Amount of swelling incurred by the microspheres in HCl solution (pH 1.2) and phosphate buffer pH 6.8 was determined by using the method reported by[16] where the microspheres were allowed to settle on the glass slide and the diameter (initial diameter) of

the same was determined using the Motic DMWB2-223 digital microscope (Canada) fitted with 1/3 CCD Camera Imaging accessory and using Motic Images 2000 (1.3 Version) image analysis software. Particles were allowed to immerse in phosphate buffer pH 6.8 over glass slide and the diameter was redetermined after 60 min. Dynamic swelling was followed by determining the change of the particle diameter as a function of time. The percent swelling was then calculated using the formula mentioned below:

$$\% \text{ swelling} = \frac{\text{final diameter} - \text{initial diameter}}{\text{initial diameter}} \times 100$$

***In Vitro* Mucoadhesive Strength Determination. Falling Liquid Film Technique**[17] In this technique male Albino rats (200—250 g) were sacrificed and their intestine region was isolated. Then from the intestine region, jejunam part was separated and cut longitudinally. This separated portion was placed on the semi cylindrical plexiglass support and washed with saline solution for 30 min at the rate of 30 ml/min. Then 25 number (N_0) of counted microspheres were hydrated with little amount of water and were dispersed on the mucosal tissue and left on it for 20 min for interaction with mucosal surface. During this period, whole system was placed in a constant humidity chamber which was adjusted to 90% relative humidity. At the end the system was washed with phosphate buffer pH 6.8 for 20 min at the rate of 22 ml/min and the number of microspheres remaining on the mucosal surface (N_s) was counted. The adhesive strength was determined using the formula given below.[18,19]

$$\% \text{ adhesive strength} = \frac{(N_0 - N_s)}{N_0} \times 100$$

***Ex Vivo* Mucoadhesive Strength Determination** In this technique which is previously reported[20] four number of Albino rats were fasted for overnight and 25 number of microspheres were administered to these rats through oral feeding needle, then these rats were sacrificed at an interval of 0, 4, 8, 12 h respectively. Then after dissection their stomach and intestine regions were isolated and cut opened longitudinally to note the number of microspheres adhering to the stomach and intestine region, which gave their adhesive strength using the formula given below.

$$\% \text{ adhesive strength} = \frac{(N_0 - N_s)}{N_0} \times 100$$

Histological Examination of Gastrointestinal Mucosa Histological eamination of gastrointestinal mucosa was studied using paraffin embedding technique.[21] The histological evaluation of intestinal tissue incubated in phosphate buffer (pH 6.8) for more than 6 h after collection was compared with tissue incubated with microsphere formulation. Tissue was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Sections were cut on glass slides and stained with hematoxylin and eosin. Sections were examined under a light microscope to detect damage to the tissue.

***In Vitro* Drug Release Studies** Microspheres equivalent to 50 mg of ATN were taken and the drug release was determined using USP XXVI paddle (Type II) apparatus.[4] The microspheres were enclosed in the muslin cloth and the cloth was tied with the paddle. The paddle was then immersed in the phosphate buffer of pH 6.8 maintained at 37±5 °C and was rotated at the speed of 100 rpm. Sample aliquots of 5 ml were withdrawn at every hour up to 12 h and the withdrawn sample was estimated spectrophotometrically at 224 nm.

Kinetic Modelling of Release Data In order to understand the drug release mechanisms from the polymer system, the power law (Peppas equation) is used to analyse the results.

$$M_t/M_\infty = kt^n$$

Where M_t/M_∞ is the fractional release of the drug at time t, k the constant related to the structural and geometric characteristic of the device, and n is the swelling exponent, indicative of the drug release mechanism. The diffusional exponent, n, specifies the mechanism of release. For spheres, values of n between 0.43 and 0.85 are an indication of both diffusion controlled drug release and swelling controlled drug release (anomalous transport). Values above 0.85 indicate case-II transport which relate to polymer relaxation during gel swelling.[22,23]

Stability Studies and Storage Conditions Stability studies were carried out for optimized formulation as per ICH guidelines. Microspheres of optimized batch was placed in sealed vial which was then stored at 40 °C/75% RH for 90 d in environmental test chamber (CHM 10S, Remi Motors, Mumbai). The physicochemical properties as well as release profile of the optimized batch was determined after stability studies.

Table 2. Production Yield, Entrapment Efficiency and Particle Size of Microspheres

Sr. No.	Formulation	% yield	Entrapment efficiency	Mean size (μm)±S.D.
1	A1	85.33±0.52	32.86±2.29	561±14
2	A2	86.14±1.48	43.13±4.63	607±15
3	A3	83.7±0.74	56.8±3.85	664±20
4	A4	80.14±1.23	60.38±4.48	710±18
5	A5	85.3±0.61	23.6±2.42	703±12
6	B1	71.41±1.34	36.28±3.82	682±16
7	B2	70.83±0.63	48.18±2.01	744±11
8	B3	76.44±0.89	57.15±3.16	790±22
9	B4	73.82±1.43	65.2±4.59	816±14
10	B5	79.09±0.56	21.79±2.87	831±10

Values are mean±standard deviation (S.D.).

***In Vivo* Radio Imaging Study** The protocol for *in vivo* study was approved by the Institutional Animal Ethics Committee (IAEC) of R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur and is in accordance with guidance of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Three adult male New Zealand white strain rabbits weighing approximately 2.0—2.5 kg were used for this study. The rabbits were fasted overnight before the start of the study. The microparticles excluding drug and containing barium sulphate as well as mucoadhesive polymer (HPMC K15M), $CaCl_2$ (5%), curing time 30 min were formulated using 21 guage needle. The amount of $BaSO_4$ (100 mg) that allows visibility by X-ray, but does not preclude the mucoadhesion of microspheres was used. The dried particles were administered through plastic tubing followed by flushing of 25—30 ml of water. During the entire study, the rabbits had free access to water only. Photographs were taken at 0, 1, 2, 4, 6 and 8 h.

Results and Discussion

Production Yield The production yields of microspheres prepared by ionic gelation technique were found to be between 77—86% for HPMC K15M and 70—81% for CP 971P containing microspheres (Table 2). The production yield for HPMC K15M containing microspheres was greater than for CP 971P. This may be due to the high viscosity of the carbopol solution which decreased its syringeability resulting in blocking of needle and wastage of drug polymer solution which ultimately decreased the yield.

Actual Drug Content and Encapsulation Efficiency Encapsulation efficiency of different batches of microspheres was found to be in the range of 23—74% and 21—70% for HPMC K15M and CP971P microspheres respectively (Table 2).

It was observed that by increasing the concentration of sodium alginate and mucoadhesive polymer the encapsulation efficiency of the microspheres also increases. It was found that with the increase in polymer amount, more intact matrix network is formed which slows down the diffusion of drug in aqueous $CaCl_2$ solution in which the microspheres are formed. Thus the amount of drug leached during formulation is decreased and encapsulation efficiency is increased. Moreover, the encapsulation efficiencies of water soluble drugs are in general lower than that for slightly soluble or insoluble drugs.[24] It was also found that raising the amount of drug taken initially, entrapment efficiency increases.

Effect of Sodium Alginate Concentration Four differ-

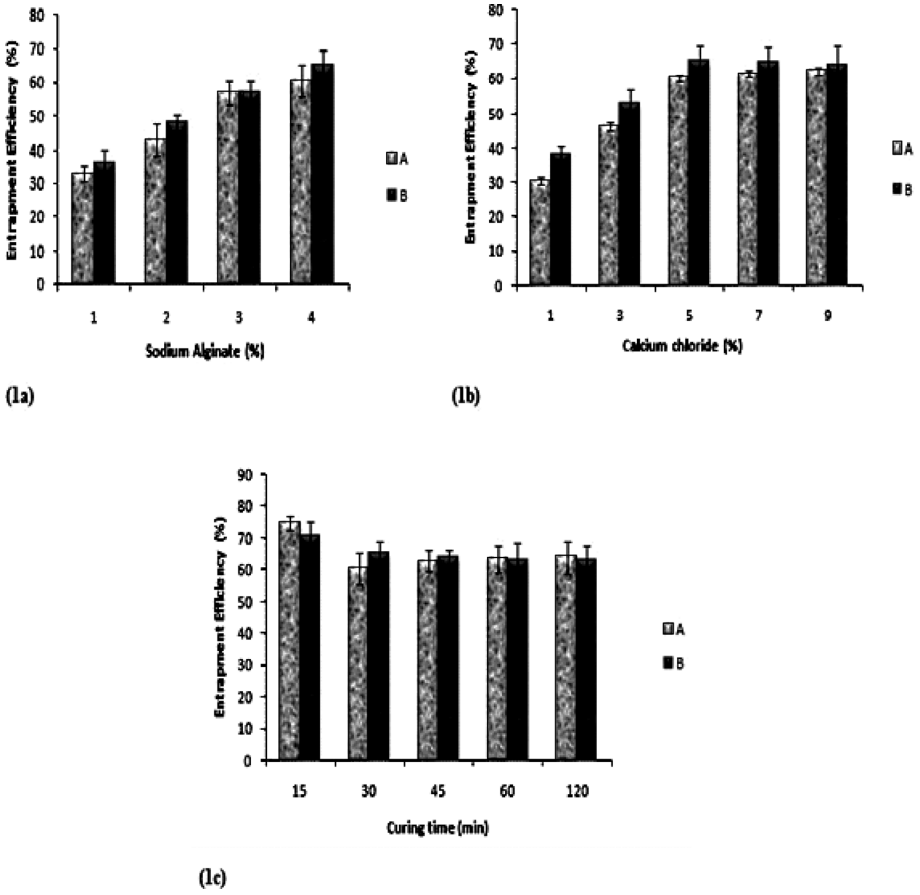

Fig. 1. Effect of (a) Sodium Alginate Concentration, (b) $CaCl_2$ Concentration, (c) Curing Time on Entrapment Efficiency of Alginate Microspheres
(A) ATN-Ca-Alg-HPMCK15M, (B) ATN-Ca-Alg-CP 971P microspheres.

ent concentration (conc.) of sodium alginate (1—4%) were examined using $CaCl_2$ (5%) as a cross linking agent for 30 min. The optimum polymer conc. was found to be 4% (w/v) for both polymers, higher conc. of polymer dispersion could not be dropped from the injector due to its high viscosity. Increasing sodium alginate concentration from 1 to 4% increased ATN loading from 32.86 to 60.38% and 36.28 to 62.21% for HPMC K15M and CP 971P microspheres respectively. Higher loading efficiency was obtained as the concentration of alginate increased (Fig. 1a). This may be attributed to the greater availability of active calcium binding sites in the polymeric chains and also the greater degree of cross linking as the quantity of sodium alginate increases.

Effect of $CaCl_2$ Concentration Increasing $CaCl_2$ concentration from 1 to 9% increased ATN loading from 30.4 to 62.3% and 38.3 to 64.2% for HPMC K15M and CP 971P microspheres (Fig. 1b) prepared using 4% alginate and 30 min curing time. This may be due to the increase in the gel strength as the calcium ions increased. It is observed that increasing $CaCl_2$ concentration increases the cross linking of the polymer and compactness of the insoluble matrices resulting in more drug entrapment. These results are in accordance with Takka *et al.* 1998[25] and Mirghani *et al.* 2000.[26] It was also found that further increase in $CaCl_2$ conc. up to 9% did not enhance the drug loading. This could be due to possible saturation of calcium binding sites in the glucoronic acid chain, preventing further calcium ion entrapment and hence cross linking was not altered with higher concentrations of $CaCl_2$ solution.

Effect of Curing Time on Entrapment Efficiency It was found in both HPMC K15M and CP 971P microspheres (Fig. 1c) that as the curing time increases from 15 to 120 min, the entrapment efficiency initially decreases up to 30 min and later remains constant. The initial higher loss of loaded drug could be due to high water solubility and rapid diffusion of ATN through the weakly cross linked alginate microspheres. However, constant drug loading was achieved at 60—120 min; this could be due to the formation of tight junction zones between the calcium ions and the active sites on the guluronic acid chain. The tight junctions may have produced high strength and an inflexible polymeric chain. Consequently, the drug was entrapped in a highly bound calcium alginate matrix from which no further drug release occurred. These results are in accordance with the previous reports. Previous investigators have also reported similar results.[27]

Size Analysis Microscopical characteristic indicate that the ATN-Ca-Alg-HPMCK15M microspheres have the size between 561 ± 14 to 703 ± 12 μm and ATN-Ca-Alg- CP 971P microspheres have the size 682 ± 16 to 831 ± 10 μm (Table 2). It was found that as the amount of sodium alginate increases, microspheres size also increases. Other researchers have reported[28] a similar relationship between the polymer concentration and the mean size.

Surface Topography Scanning electron micrographs of microspheres and their surface morphology are shown

in (Fig. 2). As it can be seen from the SEM images the microspheres presented a rough surface with characteristic large wrinkles. The surface morphology appears to have interconnected micropores or open channels as it is evident from the photomicrographs. It was observed from Table 3 that the of alginate microspheres shows excellent flowability as compared to the drug. All microspheres possessed Carr's compressibility indices in the range of 5—15 and Hausner's ratio (HR) of less than 1.25.

Drug-Excipient Interaction Studies. FT-IR Spectroscopy The results indicated that ATN binds to alginate with hydrogen bonds. This was consistent with earlier report.[29] IR spectra of the plain drug showed the major peaks at wavenumbers 3354, 3172, 2963, 1638, 1516, 1413, 1381, 1242 which were compared with the IR spectra of the microsphere formulation containing drug matrixed in various mucoadhesive polymers. It was observed from the spectra of the plain drug and formulations that there was no differentiable shift in the wavenumber of the peaks, however, the intensity of the peaks was diminished due to the molecular dispersion of the ATN in polymer matrix (Fig. 3).

Differential Scanning Calorimetry DSC thermograms of ATN, blank Ca-Alg microparticles and ATN loaded Ca-Alg-HPMC K15M microspheres are shown in (Fig. 4). ATN shows sharp melting endotherm at 156.38 °C. Blank alginate microspheres shows broadened endothermic peak at 134.06 °C and ATN loaded Ca-Alg microparticles shows peak at 121.57 °C indicating the presence of sodium alginate. The principal peak of the drug in the microspheres was completely disappeared due to molecular dispersion of the drug in the polymer matrix suggesting no drug polymer interaction.

Swelling Studies The swelling behavior of alginate polymer is the major factor controlling the release of the drugs from the microparticulate systems. Alginate shows swelling properties that are sensitive to pH. The microspheres exhibited higher swelling rate in pH 6.8 (Fig. 5),

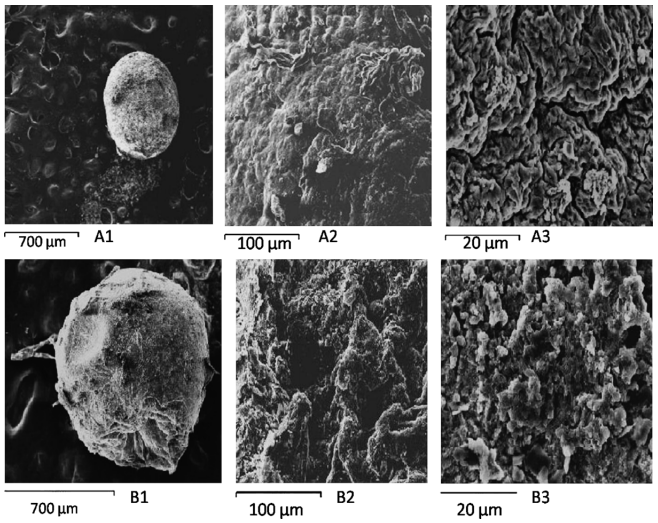

Fig. 2. Scanning Electron Micrographs of Mucoadhesive Microspheres

A1, A2, A3; ATN-Ca-Alg-HPMCK15M microspheres at 40×, 500×, 2000× (batch A4). B1, B2, B3; ATN-Ca-Alg-carbopol 971P microspheres at 80×, 500×, 2000× (batch B4).

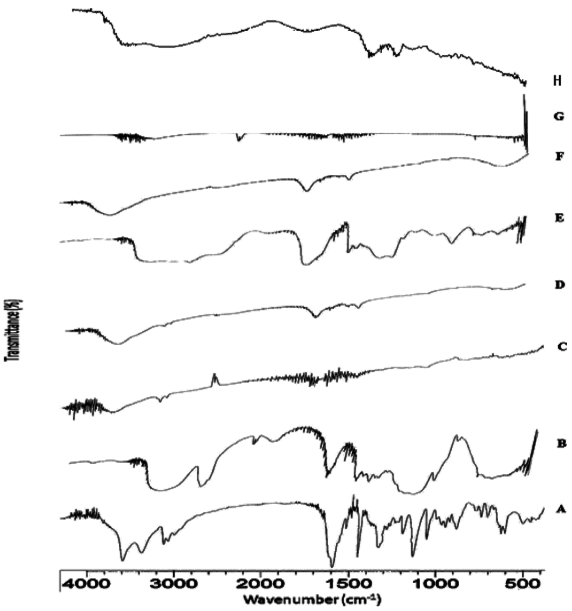

Fig. 3. FT-IR Spectra of Pure Drug (A), HPMCK15M (B), ATN-Ca-Alg-HPMCK15M (C), Blank Ca-Alg-HPMCK15M (D), CP 971P (E), ATN-Ca-Alg-CP 971P (F), Blank Ca-Alg-CP971P Microspheres (G), ATN-Ca-Alg Microspheres (H)

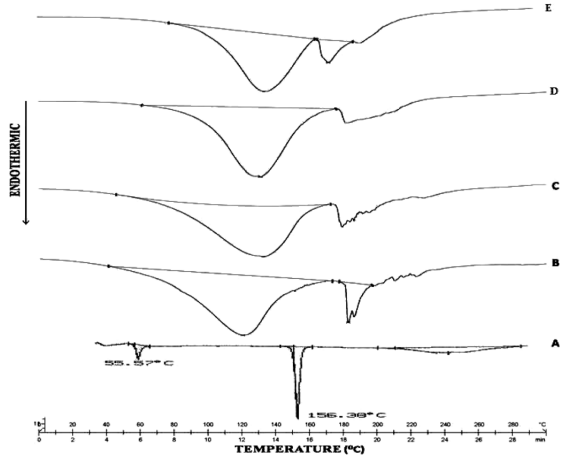

Fig. 4. DSC Thermograms of Pure ATN (A), ATN-Ca-Alg-HPMCK15M Microspheres (B), Blank Ca-Alg-HPMCK15M Microspheres (C), ATN-Ca-Alg-Carbopol 971P Microspheres (D), Blank Ca-Alg-CP 971P Microspheres(E)

Table 3. Micromeritic Properties of ATN Loaded Microspheres

Batch	Angle of repose (θ)	LBD (g/ml)	TBD (g/ml)	Carr's index (%)	Hausner's ratio
Atenolol	34.5±1.2	0.384±0.02	0.471±0.017	13.15±0.31	1.17±0.54
A4	26.44±1.3	1.00±0.04	1.06±0.05	5.80±0.14	1.07±0.11
B4	28.74±1.4	1.08±0.07	1.16±0.08	6.30±0.16	1.06±0.16

Values are mean±standard deviation (S.D.).

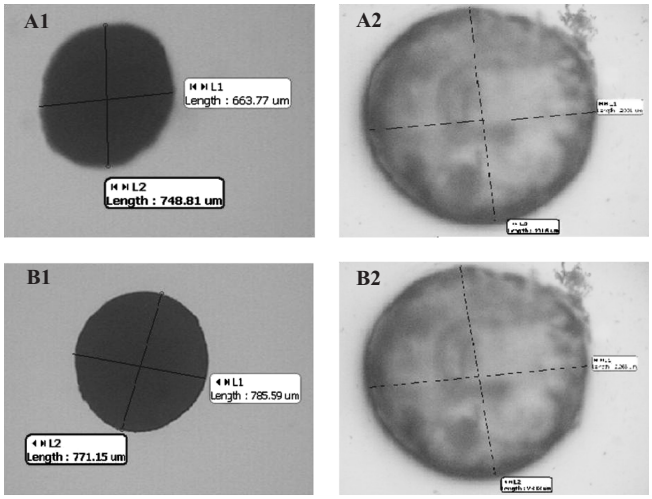

Fig. 5. Swelling Behaviour of ATN-Ca-Alg-HPMCK15M Microspheres (A1, A2) and ATN-Ca-Alg-Carbopol 971P (B1, B2) at pH 6.8

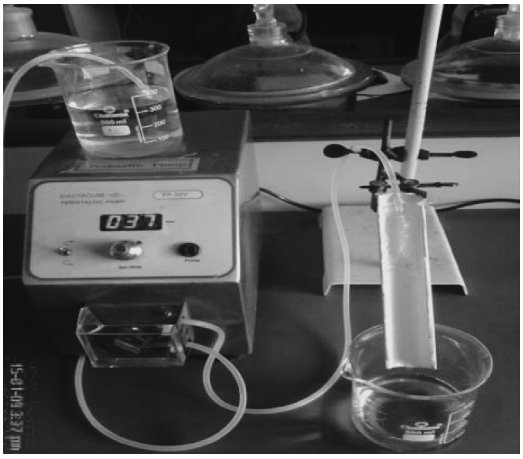

Fig. 6. Test Apparatus Designed for *in Vitro* Mucoadhesive Strength Determination of Microspheres

while the lowest swelling rate was noticed in pH 1.2. In 0.1 N HCl, the ratio of water uptake by the microparticles was low and independent of time relative to that obtained at pH 6.8, after which erosion and breakdown of particles occurred. The water uptake and swelling of the microspheres in pH 6.8 phosphate buffer was higher than those in 0.1 N HCl because calcium ions cross-linked with alginate were rapidly exchanged with sodium, phosphate ions in phosphate buffer. These results suggests that the dried gel particles will swell in the stomach and as they are subsequently transferred to upper part of intestine, the particles will begin to swell more and behave as matrices for controlled release of incorporated drug. The ion exchange with phosphate buffer which resulted in swelling and erosion of the beads[30] and formation of the solute Ca phosphate all have led to increasing the drug release rate. However they are subject to erosion in the lower intestine.

Mucoadhesive Strength Determination Studies It was found that mucoadhesive strength determined by falling liquid film technique (Fig. 6) is greater than that determined by *ex vivo* technique (Fig. 7, Tables 4, 5). This may be due to the reason that in *ex vivo* studies microspheres were ingested to rats and peristaltic movements in stomach forces

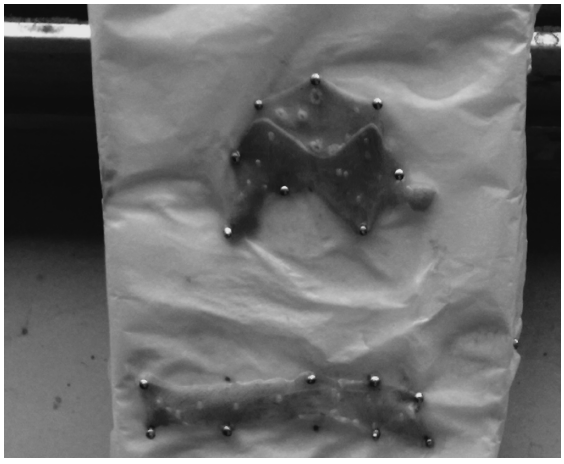

Fig. 7. *Ex Vivo* Mucoadhesion Strength Determination

Table 4. *In Vitro* Mucoadhesive Strength Determination

Formulation	No. of microspheres adhered to mucosa initially	No. of microspheres adhered to mucosa after 60 min			Percent mucoadhesion (mean±S.D.)
A4	25	24	23	25	96±1%
B4	25	23	22	22	89.33±0.58%

Table 5. *Ex Vivo* Mucoadhesive Strength Determination of Optimized Formulations

	No. of microspheres adhered to stomach				No. of microspheres adhered to intestine				Total	Percent mucoadhesion (%) (mean±S.D.)
Time (h)	0	4	8	12	0	4	8	12		
A4	15	07	06	04	09	17	18	19	23	92±1.15
B4	14	06	06	04	09	16	17	18	22	88±0.57

the microparticles to lower GIT. This reduces the contact of microspheres and mucin layer and hence lowers the mucoadhesive strength. Such condition do not prevail in the *in vitro* studies. It was found that HPMC had greater mucoadhesive strength than that of carbopol. This may be due to the greater swelling rate of HPMC, which results in larger surface of polymer that is exposed to the mucosal layer, resulting in the increase in number of hydrogen bonding between the polymer and mucosal layer and thus increase in the mucoadhesive strength of the polymer.

Histological Examination Photomicrographs of rat intestinal mucosa after the incubation with microsphere formulations were observed for histopathological changes in comparison with the untreated mucosa. Microphotographs were taken of intestinal mucosa following incubation with microsphere formulations for more than 6 h. The section of mucosa treated with formulation (ATN-Ca-Alg-HPMC) showed very slight degeneration of intestinal epithelium along with slight erosion. Results are depicted in Fig. 8. There was no sign of remarkable destructive effect of formulations on the treated intestinal mucosa. The examination of

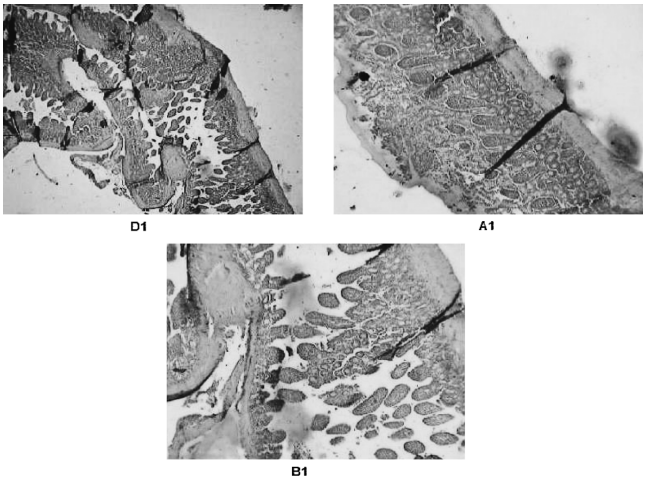

Fig. 8. Photomicrograph of Histological Examination of Rat Intestinal Mucosa

D1, Control or untreated mucosa; A1, mucosa treated with Ca-Alg-HPMCK15M (batch A4); B1, mucosa treated with Ca-Alg-CP971P (batch B4).

tissue showed columnar epithelium and mucus secreting normal goblet cell are interspaced between the columnar cells. None of the severe signs such as appearance of epithelial necrosis, sloughing of epithelial cells was detected.

***In Vitro* Drug Release Studies** The *in vitro* release profiles of ATN from microspheres in phosphate buffer of pH 6.8 are shown in Fig. 9. In an attempt to retard or sustain ATN release, mucoadhesive polymers like HPMC K15M and Carbopol 971P were added to the alginate matrix. It was found that increase in alginate conc. decreases the drug release. This is due to the reason that increase in the amount of alginate increases the number of COOH groups which are crosslinked by Ca^{2+} ions resulting in a formation of more intact matrix which makes the drug release more difficult. Moreover, incorporation of mucoadhesive polymers like HPMC K15M and CP 971P to alginate matrix increases the matrix integrity and entrapment efficiency while retards the ATN release up to 12 h while in case of batch A5 which was formulated without using mucoadhesive polymer showed complete drug release within 3—4 h.

Increasing the concentration of $CaCl_2$ solution also increases the crosslinking of the COOH groups of alginate molecule and thus retards the release of the drug from the alginate matrix.[31] All batches of microspheres showed burst release initially followed by constant release of drug. The initial burst release through alginate microspheres may be due to the quick diffusion of molecules through their water swollen microporous structures. The drug release data of these optimized batches was then explored for the type of release mechanism that they followed. The data were treated with zero order, first order, Higuchi and Korsmeyer–Peppas equations as shown in Table 6. It was evident that ATN followed non Fickian drug release kinetics where diffusion and swelling play a part in the drug release mechanism.

Kinetic Modelling of Release Data The results shown in Table 6 indicate that the drug release mechanism was found to be anomalous transport from the swellable microspheres. These results agreed with the previous values reported by Peppas *et al.* with regard to spheres.[32] Values of the exponent *n* lying between 0.5—1 for all formulations indicate a

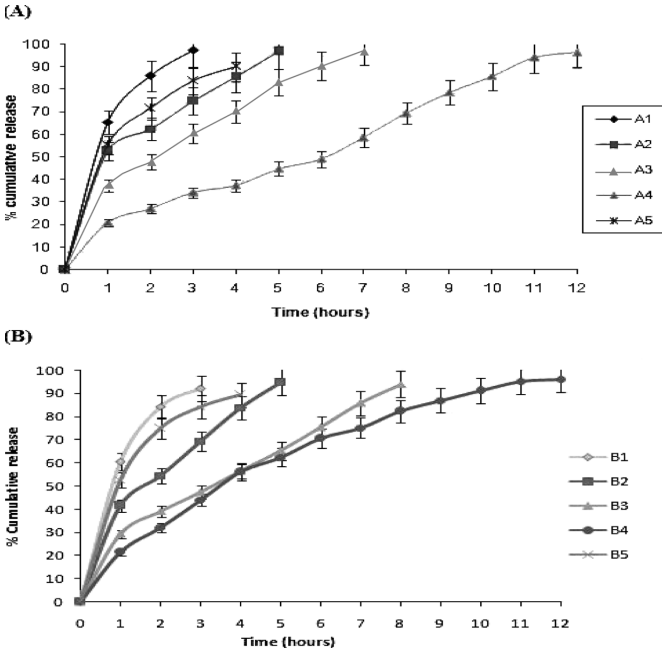

Fig. 9. Cumulative Release of ATN from (A) Ca-Alg-HPMCK15M Microspheres (B) Ca-Alg-CP 971P Microspheres

Values are mean±standard deviation (S.D.) of three experiments.

Table 6. Kinetic Modeling of Drug Release from Mucoadhesive Microspheres

Formulation code	Zero order	First order	Higuchi	Korsmeyer–Peppas	Korsmeyer–Peppas
	R^2	R^2	R^2	R^2	*n* (release exponent)
A4	0.9843	0.8492	0.9961	0.9406	0.569
B4	0.9338	0.9663	0.9929	0.9921	0.622

R^2: Correlation coefficient.

non-Fickian transport controlled by diffusion and relaxation of the polymer. The dried alginate microspheres swelled slightly in 0.1 N HCl. However, they swelled more at pH 6.8 and underwent erosion. So the release of drug from micro spheres took place by both diffusion through the swollen matrix and relaxation of the polymer in 0.1 N HCl. However, at pH 6.8, the release was due to both diffusion and erosion mechanisms. These results are in accordance with those obtained by other researchers.[31]

The formula containing a mixture of HPMCK15M and alginate (A4) was chosen on the basis of production yield, entrapment efficiency, *in vitro* and *ex vivo* mucoadhesive strength and *in vitro* release profile for further *in vivo* performance and stability studies.

Stability Study and Storage Conditions From the stability studies of the optimized batches it was concluded that the microspheres remained stable even after exposing to high temperature and moisture conditions. The dissolution rates of ATN from the microspheres of optimized batches showed no significant change even after 90 days.

***In Vivo* X-Ray Imaging Study** ATN-Ca-Alg-HPMC K15M microspheres (A4) formulation showed good mucoadhesion *in vitro* and *ex vivo* mucoadhesion study. Same opti-

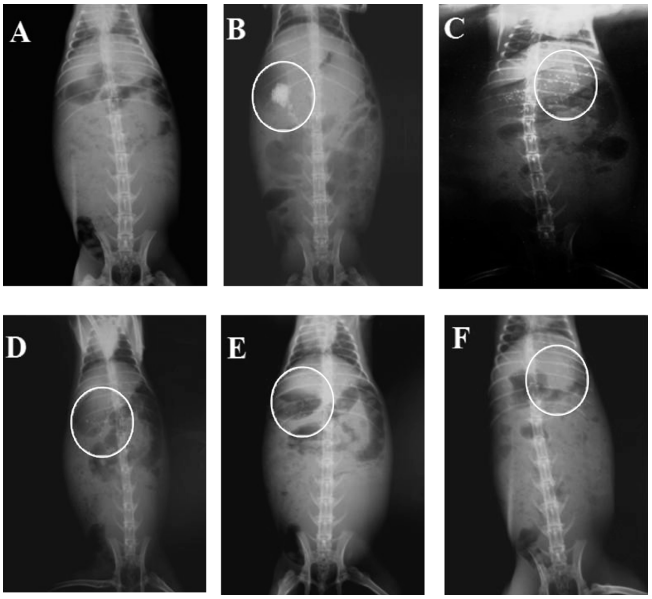

Fig. 10. X-Ray Photographs of the Mucoadhesive Microspheres in the Upper Part of GIT at (A) 0 h, (B) 1 h, (C) 2 h, (D) 4 h, (E) 6 h and (F) 8 h after Administration to the Rabbits

mized formulation is studied for *in vivo* X-ray imaging study to establish the product performance in rabbits. X-ray photographs are shown in Fig. 10. Photomicrographs were taken immediately after 1, 2, 4, 6 and 8 h respectively. The presence of microspheres in stomach can be clearly noticed and the microspheres remain in the stomach not being subjected to disintegration in rabbits. *In vivo* X-ray imaging study clearly indicated that the prepared microspheres of ATN remained adhered to mucosa for at least 8 h in upper part of rabbit GIT and that they had good *in vivo* performance.

Conclusion

Calcium alginate mucoadhesive microspheres encapsulated with atenolol can function as an excellent gastrointestinal mucoadhesive delivery system in a sustained fashion. Thus discrete free flowing mucoadhesive microspheres were prepared using cross linking of alginates. The prepared microspheres exhibited good mucoadhesive properties *in vitro* and *ex vivo* tests. ATN release from mucoadhesive micro spheres was found to be slow, controlled and extended over a period of 12 h. The drug release was found to be non Fickian and anomalous type. Thus, present study indicates a promising potential of mucoadhesive multiparticulate system in the delivery of drugs with lower half lives and less bioavailability.

Acknowledgments Authors are thankful to Zydus-cadila, Ahmedabad, India, Colorcon Asia Pvt. Ltd., Mumbai, India, Noveon Pharmaceuticals Pvt. Ltd., Cleveland, U.S.A. for providing gratis samples of drug atenolol and polymers like HPMC K15M, Carbopol 971P respectively. Authors also wish to thank Mrs. B. A. Chalke, Department of CMP and MS, Tata Institute of Fundamental Research, Mumbai for providing facility to carry out the SEM studies. Authors are also grateful to Indira Gandhi Memorial Hospital, Shirpur, India, for help in carrying out the radio imaging study.

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