

DESIGN AND *IN VITRO* EVALUATION OF MUCOADHESIVE MICROCAPSULES OF ACECLOFENAC FOR ORAL CONTROLLED RELEASE

SRINIVASA RAO Y* , VARALAKSHMI SNS TAMMANA, CHANDANA R AND VIJAYA L

Department of Pharmaceutics, Vignan Institute of Pharmaceutical Technology, Beside VSEZ, Duvvada, Visakhapatnam 530046.
Email: yarraguntla@rediffmail.com

Received: 22 Aug, 2012, Revised and Accepted: 06 Oct, 2012

ABSTRACT

The objective of the research is to design and evaluate mucoadhesive microcapsules for oral controlled release. Aceclofenac microcapsules with a coat consisting of alginate and a mucoadhesive polymer sodium carboxymethylcellulose, methyl cellulose, carbopol and hydroxypropylmethylcellulose were prepared by an orifice-ionic gelation process. The resulting microcapsules were discrete, large, and spherical and free flowing. Microencapsulation efficiency was 84.81-99.6%. The microcapsules exhibited good mucoadhesive property in the *in vitro* wash-off test. Aceclofenac release from these mucoadhesive microcapsules was slow and extended over longer periods of time depending on the composition of coat of the microcapsules. Drug release was diffusion controlled and followed first order kinetics. In the *in vitro* evaluation, alginate-sodium CMC and alginate-methyl cellulose microcapsules of Aceclofenac sustained the drug release over a period of 20-24 hours. These mucoadhesive microcapsules are thus, suitable for oral controlled release of Aceclofenac.

Keywords: Mucoadhesive microcapsules, Aceclofenac, Controlled release

INTRODUCTION

Microencapsulation by various polymers and their applications are described in standard text books^{1, 2}. Microencapsulation and the resulting microcapsules have gained good acceptance as a process to achieve controlled release and drug targeting. Mucoadhesion has been a topic of interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs^{3, 6}. Several studies⁷ reported mucoadhesive drug delivery systems in the form of tablets, films, patches and gels for oral, buccal, nasal, ocular and topical routes; however, very few reports on mucoadhesive microcapsules are available⁸⁻¹¹. The objective of this study is to develop, characterize and evaluate mucoadhesive microcapsules of Aceclofenac employing various mucoadhesive polymers for prolonged gastro-intestinal absorption. Aceclofenac, an effective anti-inflammatory drug, which requires controlled release owing to its short biological half-life¹² of 4-4.3 hours, was used as core in microencapsulation. The mucoadhesive microcapsules were evaluated by *in vitro* methods for controlled release.

MATERIALS AND METHODS

Aceclofenac sample was obtained from Yarrow Chem. products, Mumbai. Sodium carboxy methyl cellulose (Sodium CMC, having a viscosity of 1500-3000 cps of 1% w/v aqueous solution at 25°C) was procured from Qualikems Fine chemicals Pvt.ltd, methyl cellulose (having a methoxyl content of 28.32 % w/v and viscosity of 3000-5000 cps in 2% w/v aqueous solution at 20°C) and hydroxy propyl methyl cellulose (HPMC, having a viscosity of 4000 cps in a 2 % by w/v aqueous solution at 20°C) were procured from Yarrow chem. products, Mumbai. Carbopol 934 P was procured from Oxford laboratory, Mumbai. Sodium alginate (Kemphasol, Mumbai), calcium chloride (Qualigens, Mumbai) were procured from commercial sources. All other reagents used were of analytical grade.

Preparation of microcapsules

Microcapsules containing Aceclofenac were prepared employing sodium alginate in combination with four mucoadhesive polymers namely sodium carboxy methylcellulose (sodium CMC), methyl cellulose, Carbopol and hydroxy propyl methyl cellulose (HPMC) as coat materials. Very few methods were reported for microencapsulation by these polymers. An orifice-ionic gelation process^{13, 14} which has been extensively used to prepare large sized alginate beads, was employed to prepare the microcapsules.

Orifice-ionic gelation method

Sodium alginate (1.0 g) and the mucoadhesive polymer (1.0 g) were dissolved in purified water (32 ml) to form a homogeneous polymer solution. The active substance, Aceclofenac (2.0 g) was added to the polymer solution and mixed with a stirrer thoroughly to form a viscous dispersion. The resulting dispersion was then added manually drop wise into calcium chloride (10% w/v) solution (40 ml) through a syringe with a needle of size No. 18. The added droplets were retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce spherical rigid microcapsules. The microcapsules were collected by decantation and the product thus separated was washed repeatedly with water and dried at 45°C for 12 hours. The microcapsules prepared along with their coat composition are listed in Table 1.

Characterization and evaluation of microcapsules

Estimation of Aceclofenac

Aceclofenac content in the microcapsules was estimated by an UV spectrophotometric method¹⁵ based on the measurement of absorbance at 275 nm in phosphate buffer of pH 6.8. The method was validated for linearity, accuracy and precision. The method obeyed Beer's law in the concentration range 5-40 µg/ml. When a standard drug solution was assayed repeatedly (n=6), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.6% and 0.8%, respectively.

Microencapsulation efficiency

Microencapsulation efficiency was calculated using the formula, microencapsulation efficiency = (estimated percent drug content / theoretical percent drug content) × 100.

Scanning electron microscopy

The microcapsules were observed under a scanning electron microscope (SEM-LEICA, S430, and UK). For SEM, the microcapsules were mounted directly onto the SEM sample stub, using double-sided sticking tape, and coated with gold film (thickness 200 nm) under reduced pressure (0.001 torr).

Drug release study

Release of Aceclofenac from the microcapsules was studied in phosphate buffer of pH 6.8 (900 ml) using an USP type II Dissolution Rate Test Apparatus (LABINDIA DS 8000) with a rotating paddle stirrer at 50 rpm and 37 ± 0.5°C. A sample of microcapsules equivalent to 100 mg of Aceclofenac was used in each test. Samples

of dissolution fluid were withdrawn through a filter (0.45 μm) at different time intervals and were assayed at 275 nm for Aceclofenac content using a Shimadzu UV-Visible spectrophotometer. The drug release experiments were conducted in triplicate (n=3).

Mucoadhesion testing by *in vitro* wash-off test

The mucoadhesive property of the microcapsules was evaluated by an *in vitro* adhesion testing method known as wash-off method^{16,17}. The mucoadhesiveness of these microcapsules was compared with that of non-bioadhesive material, ethylene vinyl acetate microcapsules. Freshly excised pieces of intestinal mucosa (2x2 cm) from sheep were mounted onto glass slides (3x1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. About 50 microcapsules were spread onto each wet rinsed tissue specimen and immediately thereafter the support was hung onto the arm of a USP tablet disintegrating test machine. By operating the disintegrating test machine the tissue specimen was given a slow regular up and down movement in the test fluid at 37 °C taken in a 1 l vessel of the machine. At the end of 30 minutes, 1 hour and later at hourly intervals up to 12 hours, the machine was stopped and the number of microcapsules still adhering on to the tissue was counted. The test was performed at both gastric pH (0.1 N HCl, pH 1.2) and intestinal pH (Phosphate buffer of pH 6.2).

RESULTS AND DISCUSSION

Microcapsules of Aceclofenac with a coat consisting of alginate and a mucoadhesive polymer namely sodium CMC, or methyl cellulose, or carbopol or HPMC in 1:1 and 9:1 ratio could be prepared by the orifice- ionic gelation process. Microcapsules with a coat of mucoadhesive polymer alone could not be prepared due to their

water-soluble nature. The microcapsules were found to be discrete, large, spherical, free flowing and monolithic matrix type. The microcapsules were uniform in size with a mean size of 1246 μm . The SEM photographs indicated that the microcapsules were spherical and completely covered with the coat polymer (Fig. 1).

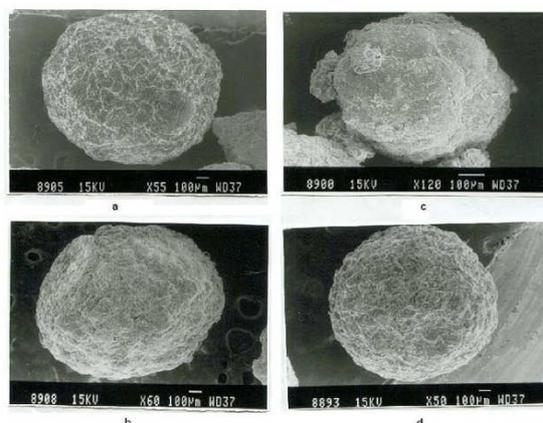


Fig. 1: Scanning electron micrographs of Aceclofenac microcapsules MC1 (a), MC2 (b), MC3 (c) and MC4 (d).

Low C.V. (< 1.0 %) in percent drug content indicated uniformity of drug content in each batch of microcapsules. The microencapsulation efficiency was in the range of 84.81-99.6% (Table 1) and the yield was in the range of 95-98%.

Table 1: Coat Composition, Drug Content and Microencapsulation Efficiency of the Microcapsules Prepared

Micro Capsules	Coat Composition	Percent Drug Content	Microencapsulation Efficiency (%)	Release Rate, K_1 (h^{-1})
MC1	Alginate: Sodium CMC (1:1)	12.30 (0.05) ^a	98.40	0.424 (0.951) ^b
MC2	Alginate: MC (1:1)	11.07 (0.03)	88.56	0.345 (0.959)
MC3	Alginate: Carbopol (1:1)	12.40 (0.03)	99.2	0.483 (0.986)
MC4	Alginate: HPMC(1:1)	11.87 (0.06)	95.00	0.472 (0.981)
MC5	Alginate: Sodium CMC (9:1)	11.62 (0.04)	93.00	0.154 (0.992)
MC6	Alginate: MC (9:1)	11.69 (0.02)	93.52	0.111 (0.995)
MC7	Alginate: Carbopol (9:1)	11.36 (0.02)	90.88	0.235 (0.976)
MC8	Alginate: HPMC(9:1)	12.00 (0.02)	96.00	0.223 (0.930)

a Figures in parentheses are Coefficient of Variation (C. V) values

b Figures in parentheses are Correlation Coefficient (r) values between log% drug undissolved and time in hours.

Microcapsules with a coat consisting of alginate and a mucoadhesive polymer exhibited good mucoadhesive property in the *in vitro* wash-off test when compared to non-mucoadhesive material, ethylene vinyl acetate microcapsules. The wash-off was slow in the case of microcapsules containing alginate-mucoadhesive polymer as coat when compared to that of EVA microcapsules (Table 2). The wash off was relatively rapid at intestinal pH than at gastric pH.

Robinson¹⁸ observed that the pH of the medium was critical for the degree of hydration, solubility and mucoadhesion of the polymers. The rapid wash-off observed at intestinal pH 6.2 is due to ionization of carboxyl and other functional groups in the polymers at this pH increasing their solubility and reducing adhesive strength. The results of wash-off test indicated fairly good mucoadhesive property of the microcapsules.

Table 2: Results of *In Vitro* Wash-Off Test to Assess Mucoadhesive Property of the Microcapsules Prepared

Microcapsules	Percent of microcapsules adhering to tissue at 5 times (h)									
	In 0.1 N HCL, pH 1.2					In Phosphate buffer, pH 6.2				
	1	2	4	6	8	1	2	4	6	8
MC1	75 (1.4) [*]	70 (2.3)	60 (1.2)	55 (1.4)	53 (1.1)	60 (1.3)	17 (1.9)	12 (2.1)	03 (1.6)	-
MC2	65 (1.4)	61 (1.2)	55 (0.8)	53 (0.4)	50 (0.5)	62 (0.6)	43 (1.2)	14 (1.3)	04 (0.8)	-
MC3	83 (1.3)	80 (0.5)	73 (0.9)	68 (0.5)	63 (0.7)	67 (2.0)	59 (1.5)	30 (1.9)	17 (1.3)	13 (1.9)
MC4	79 (1.9)	79 (2.1)	74 (1.4)	74 (1.3)	72 (1.5)	69 (2.1)	56 (1.4)	26 (1.5)	09 (1.3)	03 (0.9)
MC5	86 (0.5)	75 (2.0)	57 (1.6)	36 (1.8)	19 (2.1)	70 (1.9)	54 (2.0)	23 (2.0)	05 (1.8)	-
MC6	83 (2.1)	74 (2.2)	60 (1.5)	31 (2.2)	23 (1.9)	72 (2.0)	54 (1.8)	23 (2.2)	05 (2.1)	-
MC7	73 (2.0)	67 (2.3)	58 (2.4)	46 (2.1)	29 (2.0)	67 (2.0)	63 (1.7)	33 (1.8)	18 (1.5)	15 (1.6)
MC8	80 (1.6)	69 (1.8)	59 (2.1)	33 (1.5)	18 (1.8)	70 (2.1)	56 (1.8)	28 (2.1)	10 (1.6)	04 (2.2)
EVA	53 (1.9)	39 (1.2)	10 (2.0)	-	-	51 (2.1)	40 (2.3)	03 (2.5)	-	-

* Figures in parentheses are Coefficient of Variation (C V) values

Aceclofenac release from the microcapsules was studied in phosphate buffer (pH 6.8) for a period of 24 hours. Aceclofenac release from the microcapsules was slow and spread over extended periods of time and depended on the composition of coat (Fig 2 & 3). Release followed first order kinetics ($r > 0.95$). Microcapsules of alginate-carbopol gave relatively fast release when compared to others. The order of increasing release rate observed with various microcapsules was alginate- methyl

cellulose < alginate- sodium CMC < alginate- HPMC < alginate-Carbopol. The drug release from the microcapsules was diffusion controlled as plots (Fig 4) of amount released Vs \sqrt{t} were found to be linear ($r > 0.94$). Aceclofenac release from microcapsules MC5 and MC6 was slow and extended over a period of 20-24 hours and these microcapsules were found suitable for oral controlled release formulations.

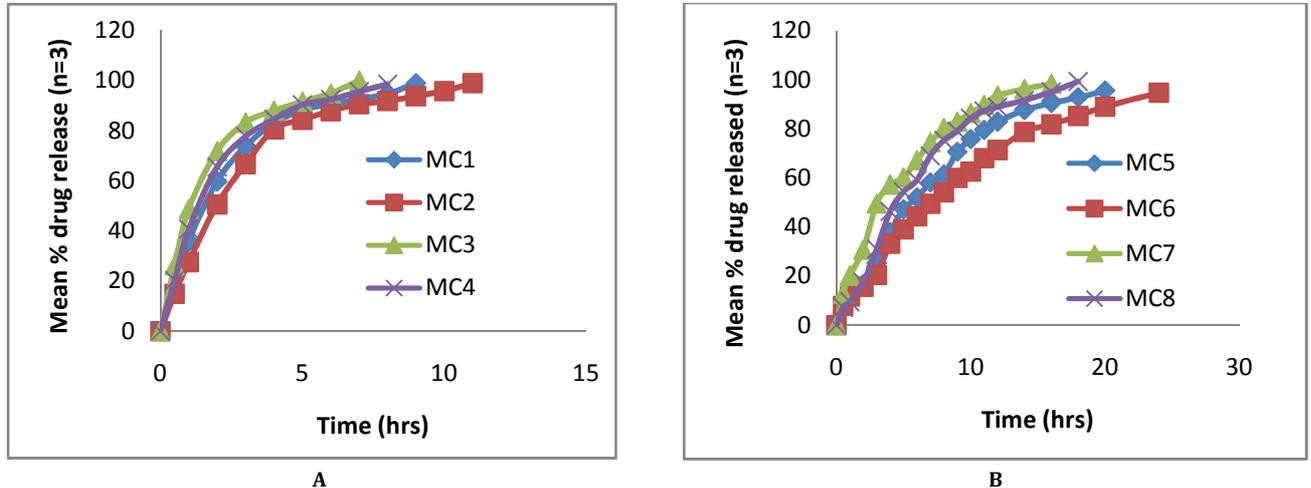


Fig. 2: Release profiles of Aceclofenac microcapsules, (n=3), A: MC1, MC2, MC3 and MC4; B: MC5, MC6, MC7 and MC8.

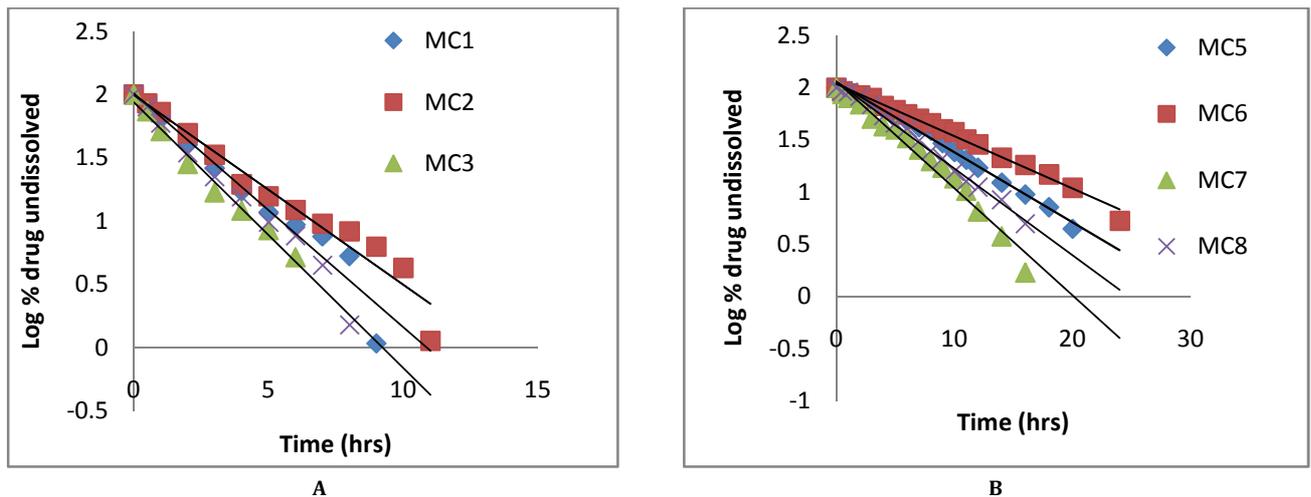


Fig. 3: Log Percent drug undissolved Vs time plots of Aceclofenac microcapsules, (n=3), A: MC1, MC2, MC3 and MC4; B: MC5, MC6, MC7 and MC8

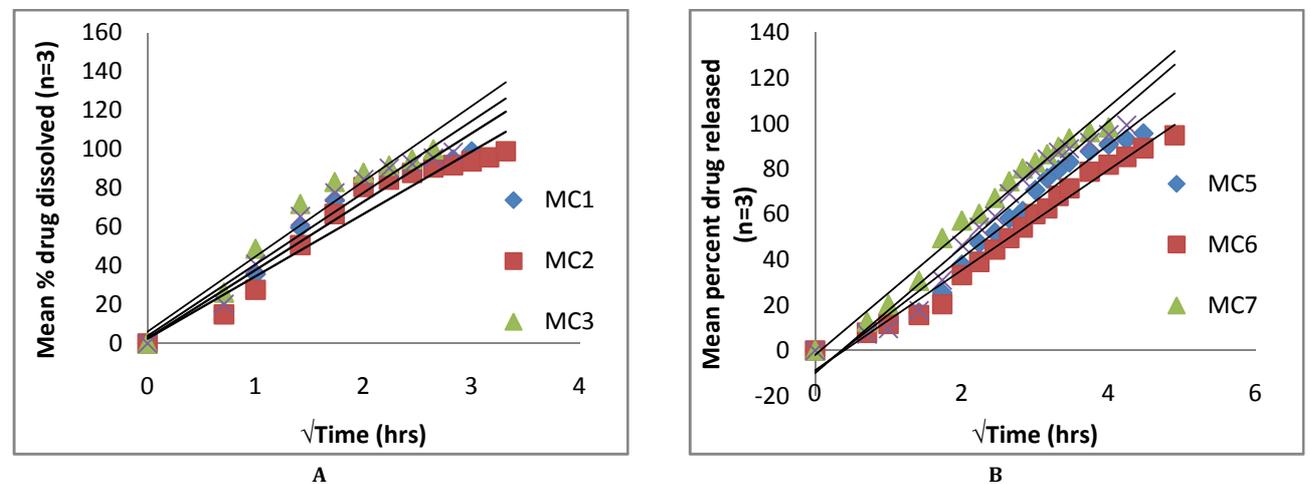


Fig. 4: Percent released Vs square root of time plots of Aceclofenac microcapsules, (n=3), A: MC1, MC2, MC3 and MC4; B: MC5, MC6, MC7 and MC8

CONCLUSION

Thus, large sized spherical microcapsules with a coat consisting of alginate and a mucoadhesive polymer (sodium CMC or methyl cellulose or carbopol or HPMC) could be prepared by orifice-ionic gelation process. The microcapsules exhibited good mucoadhesive property *in vitro* test. Aceclofenac release from these mucoadhesive microcapsules was slow and extended over longer periods of time and depended on composition of the coat. Drug release was diffusion controlled and followed first order kinetics with non-Fickian diffusion. In the *in vitro* evaluation, alginate-Sodium CMC, alginate-methyl cellulose microcapsules of Aceclofenac sustained the drug release over a period of 20-24 hours. These mucoadhesive microcapsules are, thus, suitable for oral controlled release of Aceclofenac.

ACKNOWLEDGEMENTS

The authors are thankful to Dr.L.Rathaiah, Chairman of Lavu Educational society for providing facilities to carry out the above research work.

REFERENCES

1. Kondo A. Eds., In; Microcapsule Processing and Technology. Marcel Dekker, Inc. New York: 1979.p. 18.
2. Gutcho, MH. Eds., In; Microcapsules and Microencapsulation Techniques. Noyes Data Corporation; New Jersey; 1976.p. 236.
3. Ikeda K, Murata K, Kobayashi M, Noda k. Enhancement of bioavailability of dopamine via nasal route in beagle dogs. Chem. Pharm Bull 1992; 40(8): 2155-2158.
4. Nagai T, Nishimoto Y, Nambu N, Suzuki Y, Sekine K. Powder dosage forms of insulin for nasal administration. J Control Release 1984; 1: 15-22.
5. Illum L, Farraj NF, Critcheley H, Davis SS. Nasal administration of gentamycin using a novel microsphere delivery system. Int J Pharm 1988; 46: 261-265.
6. Gavini E, Sanna V, Juliano C, Bonferoni MS, Giunchedi P. Mucoadhesive vaginal tablets as veterinary delivery system for the controlled release of an antimicrobial drug, acriflavine. AAPS Pharm Sci Tech 2002; 3.
7. Chowdary K P R, Srinivas L. Mucoadhesive drug delivery Systems-A status of current review. Indian Drugs 2000; 37: 400-406.
8. Sakagami M, Kinoshita W, Sakon K, Sato JI, Makino Y. Mucoadhesive beclomethasone microspheres for powder inhalation: their pharmacokinetics and pharmacodynamics evaluation. J Control release 2002; 80: 207-218.
9. Takishima J, Onishi H, Machida Y. Prolonged intestinal absorption of cephadrine with chitosan-coated ethyl cellulose micro particles in rats. Biol Pharm Bull 2002; 25: 1498-1502.
10. Lim ST, Forbes B, Berry DJ, Martin GP, Brown MB. In vivo evaluation of novel hyaluronan/chitosan micro particulate delivery systems for the nasal delivery of gentamycin in rabbits. Int J Pharm 2002; 231: 73-82.
11. Cuna M, Alonso MJ, Torres D. Preparation and in vivo evaluation of mucoadhesive micro particles containing amoxicillin-resin complexes for drug delivery to the gastric mucosa. Eur J Pharm Biopharm 2001; 51: 199-205.
12. Sweetman S.C., Martindale the complete drug reference. 32nd edition. London: The pharmaceutical press; 2002. 12.
13. Kim C K, Lee E J. The controlled release of blue dextran from alginate beads. Int J Pharm. 1992; 79: 11-19.
14. Hari, P C, Chandy T, Sharma C P. Chitosan/calcium alginate microcapsules for intestinal delivery of nitrofurantoin. J Microencapsul. 1996; 13: 319-329.
15. Dua K, Ramana M.V., Sara U.V.S., Himaja M., Garg V., Agarwal A., Dissolution enhancement of aceclofenac through solid dispersion, Indian pharmacist 2006;48:70-72.
16. Lehr C M, Bowstra J A, Tukker J, Junginer H E. Intestinal transit of bioadhesive microspheres in an *in situ* loop in the rat. J Control. Release 1990; 13: 51-62.
17. Bhabani S Nayak, Sunil K Ghosh, K Tripathi B Patro. Preparation and characterization of Famotidine microcapsules employing mucoadhesive polymers in combination to enhance gastro retention for oral delivery. International Journal of Pharmacy and Pharmaceutical sciences 2009; vol 1: 112-120.
18. Ch'ing HS, Park H, Kelly P, Robinson JR. For oral controlled delivery II. Synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers. J Pharm Sci 1985; 74: 399-405.