

Formulation and evaluation of mucoadhesive microspheres of propranolol hydrochloride for sustained drug delivery

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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 microspheres of Propranolol hydrochloride (PH) for effective control of hypertension. Microspheres were prepared by emulsification solvent evaporation method using Sodium carboxy methyl cellulose (SCMC), Carbopol 934P (CP) and Hydroxyl propyl methyl cellulose K4M (HPMC) as mucoadhesive polymers. Microspheres prepared were found discrete, spherical and free flowing. The microspheres exhibits good mucoadhesive properties and showed high drug entrapment efficiency. PH 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 PH1 containing SCMC and PH2 containing CP showed the best reproducible results and mucoadhesive profile with good surface morphology. The data obtained thus suggest that mucoadhesive microsphere can successfully design for sustained delivery of PH and to improve patient compliance.

Keywords: Mucoadhesive microspheres, Propranolol hydrochloride (PH), Mucoadhesion, Carbopol 934P, Hydroxyl propyl methyl cellulose K4M.

INTRODUCTION

Substantial efforts have recently been focused upon placing a drug or drug delivery system in a particular region of the body for extended period of time. From a technological point of view, an ideal Sustained Release Mucoadhesive (SRM) dosage form must have three properties. It must maintain its position in the mouth for a few hours, release the drug in a controlled fashion and provide the drug release in a unidirectional way towards the mucosa. Microspheres form an important part of such novel drug delivery systems. 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 the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres.

Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site. Mucoadhesive microspheres that are retained in the stomach would increase the drug absorption and decrease dosing frequency which provides better patient compliance as compared to conventional dosage forms. Gastric emptying studies revealed that orally administered controlled release dosage forms are subjected to basically two complications: that of short gastric residence time and unpredictable gastric emptying rate. Propranolol is a nonselective beta-adrenergic receptor blocking agent possessing no other autonomic nervous system activity. It specifically competes with beta-adrenergic receptor agonist agents for available receptor sites. It is used as antihypertensive, antianginal, antiarrhythmic, and in treatment of migraine. Propranolol is reported to be of value in cardiovascular disorders, many of which are associated with central nervous system. Propranolol is

highly lipophilic and almost completely absorbed after oral administration. However, it undergoes high first-pass metabolism by the liver, and on average, only about 25% of propranolol reaches the systemic circulation. Approximately 90% of circulating propranolol is bound to plasma proteins. Propranolol is extensively metabolized with most metabolites appearing in the urine.

Literature survey revealed that SCMC, CP and HPMC are the polymer which shows good mucoadhesive properties, high drug entrapment efficiency and release the drug in sustained release manner. Therefore in the present study PH is selected as a model drug and SCMC, CP and HPMC are chosen as a mucoadhesive polymer for design and evaluation SR Microspheres to increase the bioavailability of the drug.

MATERIALS AND METHODS

Material

PH was a gift sample from IPCA Laboratories Ltd., Mumbai, India. CP was gifted from Colorcon Asia Pvt. Ltd, Goa. HPMC was received as gift sample from Zydus Cadila Healthcare Ltd, Ahmadabad, India. SCMC, n-Hexane and span 20 were procured from central drug house, New Delhi. Liquid paraffin was procured from Loba Chemie Pvt. Ltd., Mumbai. All the reagents were used of analytical grade.

Methods

Preparation of microspheres

Mucoadhesive microspheres of PH were prepared by emulsification solvent evaporation method using various ratios of SCMC, CP and HPMC. For this, aqueous solution of drug and polymer is prepared. Then drug and polymer solution was added drop wise to the liquid paraffin containing 0.5 % span 20 as an emulsifying agent with constant stirring. The constant stirring was carried out using magnetic stirrer. The beaker and its content were heated at 80⁰C with constant stirring for 4 hrs until the aqueous phase was completely removed by evaporation. The liquid paraffin was decanted and collected microsphere were washed 5 times with n-hexane, filtered through whattman's filter paper and dried in hot air oven at 50⁰C for 2 hours. Table I shows composition of various formulations of microspheres.

Surface morphology

The surface morphology and structure were visualized by scanning electron microscopy (SEM). The samples were prepared by lightly sprinkling the microspheres powder on a double side adhesive tape which already stucked to on aluminum stubs. The stubs were then placed into fine coat ion sputter for gold coating. After gold coating samples were randomly scanned for particle size and surface morphology.

Particle Size

Particle size analysis of drug-loaded microspheres was performed by optical microscopy using a compound microscope (Erma, Tokyo, Japan). A small amount of dry microspheres was suspended in n-hexane (10 mL). The suspension was ultrasonicated for 5 seconds. A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and 300 particles were measured using a calibrated ocular micrometer. The average particle size was determined by using the Edmondson's equation:

$$D_{\text{mean}} = \sum nd / \sum n$$

Where, n= number of microspheres observed and d=mean size range. The process was repeated 3 times for each batch prepared.

Table 1. Composition of drug loaded microspheres

Formulation	Drug (mg)	SCMC (mg)	CP (mg)	HPMC (mg)	Stirring Speed (rpm)
PH1	100	900	--	--	500
PH2	100	--	900	--	500
PH3	100	--	--	900	500
PH4	100	450	450	--	500
PH5	100	450	--	450	500
PH6	100	--	450	450	500
PH7	100	300	300	300	500
PH8	100	300	300	300	1000

*Values are represented as mean \pm standard deviation (n=3). All formulations were prepared at 2% polymer concentration.

Drug entrapment efficacy

50 mg of microsphere were taken and drug was extracted from microspheres by digesting for 24 hours with 10 ml of simulated gastric fluid (pH 1.2). During this period the suspension was agitated. After 24 hours, the solution was filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated using the following formula:

$$\text{Entrapment efficiency} = (\text{Practical drug content} / \text{theoretical drug content}) \times 100$$

The results are given in the Table 2.

***In vitro* mucoadhesivity**

The mucoadhesive properties of the microspheres were evaluated by *in vitro* wash-off test as reported by Lehr et al. A 1x1 cm piece of rat stomach mucosa was tied onto a glass slide (3-inch by 1-inch) using thread. Microspheres were spread (~50) onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the grooves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid (pH 1.2). At hourly intervals up to 10 hours, the number of microspheres still adhering onto the tissue was counted. Percent mucoadhesion was given by the following formula:

$$\% \text{ mucoadhesion} = (\text{no. of microspheres remains} / \text{no. of applied microspheres}) \times 100$$

The observations are shown in Table 2 and expressed in figure 2, 3, 4.

***In vitro* drug release**

In vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using simulated gastric fluid (pH 1.2) as dissolution medium, volume of dissolution medium was 900 ml and bath temperature was maintained at $37 \pm 1^\circ\text{C}$ throughout the study. Paddle speed was adjusted to 50 rpm. An interval of 1 hour, 10 ml of sample was withdrawn with replacement of 10 ml fresh medium and analyzed for drug content by UV Visible spectrophotometer at 290 nm. All the experimental units were analyzed in triplicate (n=3). Cumulative percentage drug release was calculated using an equation obtained from a standard curve. The observations are expressed in figure 5, 6, 7.

RESULTS AND DISCUSSION

Surface morphology

Surface morphology of the mucoadhesive microspheres was examined by scanning electron microscopy (SEM). The SEM showed that the microspheres obtained from all the formulations are spherical with smooth surface. The SEM showed that the blend of SCMC and CP produced spherical with smooth surface microspheres due to their high solubility in water. The SEM of microspheres of formulation F2 is shown in Figures 1.

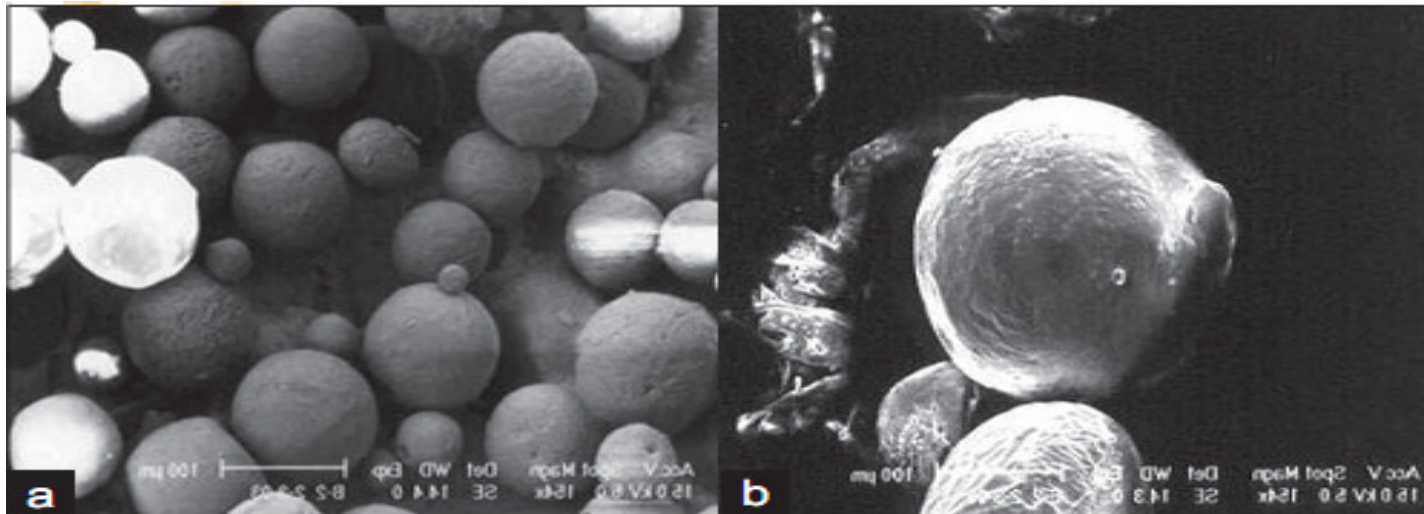


Figure 1. Scanning electron microscopy (SEM) image of microspheres

Particle size analysis

Particle size analysis of different formulations was done by optical microscopy. The average particle size was found to be in the range of 30.44 ± 3.21 to $53.54 \pm 3.22 \mu\text{m}$. The mean particle size was significantly varied according to type of polymer used for the preparation of microspheres; this may be due to fact that difference in the viscosity of the polymer solution. Since high viscosity of polymer solution requires high shearing energy for breaking of droplets of the emulsion. Microspheres containing HPMC are larger as compared to SCMC microspheres because HPMC solution has more viscosity at the same concentration. Particle size decreased with increasing stirring speed due to the fact that increased in stirring speed, produce high energy, which leads to further decrease in droplets size. Results of particle size analysis are shown in Table 2.

Table 2. % yield, % drug entrapment, Particle size, % mucoadhesion and R^2 value of microspheres

Formulation Code	% Yield	% Drug Entrapment	Particle size (μm)	% Mucoadhesion after 1 hour	R^2 for in-vitro drug release
PH1	69.82 ± 3.10	72 ± 2.55	39.53 ± 2.13	94.21 ± 2.11	0.9843
PH2	67.96 ± 2.70	82 ± 3.21	37.32 ± 1.32	93.33 ± 2.45	0.9949
PH3	75.21 ± 2.98	65 ± 2.92	53.54 ± 3.22	91.45 ± 2.33	0.9977
PH4	72.66 ± 2.77	79 ± 2.88	44.64 ± 2.34	85.64 ± 1.90	0.9829
PH5	68.34 ± 3.23	68 ± 2.33	49.22 ± 2.56	97.12 ± 2.13	0.9847
PH6	70.22 ± 2.49	63 ± 3.21	47.85 ± 3.41	85.23 ± 2.54	0.9963
PH7	71.33 ± 2.32	62 ± 2.55	43.56 ± 2.34	89.65 ± 2.71	0.9985
PH8	66.72 ± 2.67	59 ± 2.87	30.44 ± 3.21	88.49 ± 3.27	0.9983

*Values are represented as mean \pm standard deviation (n=3). All formulation were prepared at 2% polymer concentration

Drug entrapment efficiency

Drug content in different formulations was estimated by UV Spectrophotometric method. Percent drug loading efficiency of microspheres was found in the range of 62 ± 2.55 to 82 ± 3.21 (Table 2). Formulation PH2 containing CP showed maximum % drug loading about 82 % whereas formulation PH8 containing Blend of SCMC, CP and HPMC showed minimum % drug loading about 59% because these microspheres are small in size which results more loss of drug from surface during washing of microspheres.

In vitro mucoadhesivity test

To assess the mucoadhesive property of microspheres, *in vitro* wash-off test was performed for all the formulations. Adhesion of polymer with the mucus membrane is mediate by hydration in the case of hydrophilic polymer. Upon hydration these polymers becomes sticky and adhere to mucus membrane. Formulation PH1 containing SCMC showed the highest mucoadhesivity. The greater mucoadhesivity of SCMC microspheres were due to anionic nature of the polymer which is desirable characteristics of adhesion to the mucus layer. Formulation PH8 containing Blend of SCMC, CP and HPMC showed the shortest mucoadhesion time to the small size of microsphere which take short time for solubilization. The order of % mucoadhesivity for all the formulations, after 8 hours was found to be as follows (Figure 2, 3, 4 and Table 2):

PH1 > PH5 > PH2 > PH3 > PH7 > PH8 > PH4 > PH6

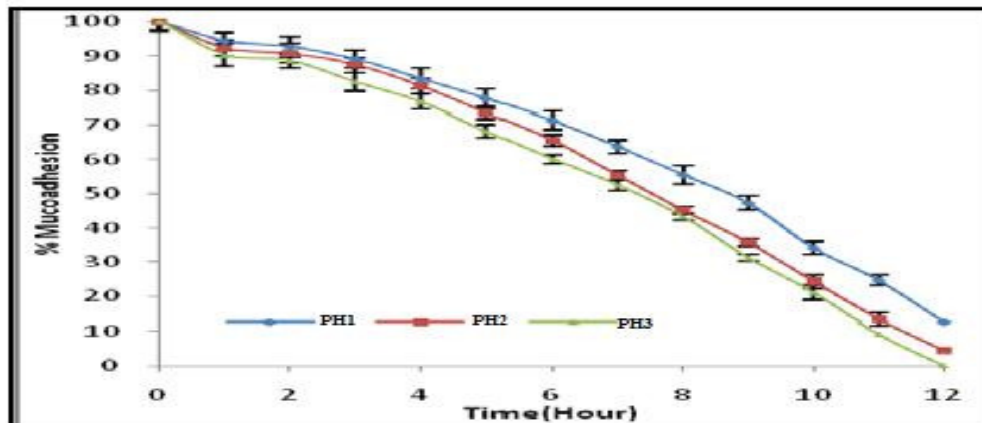


Figure 2. Comparative % mucoadhesion of microspheres of formulations PH1-PH3

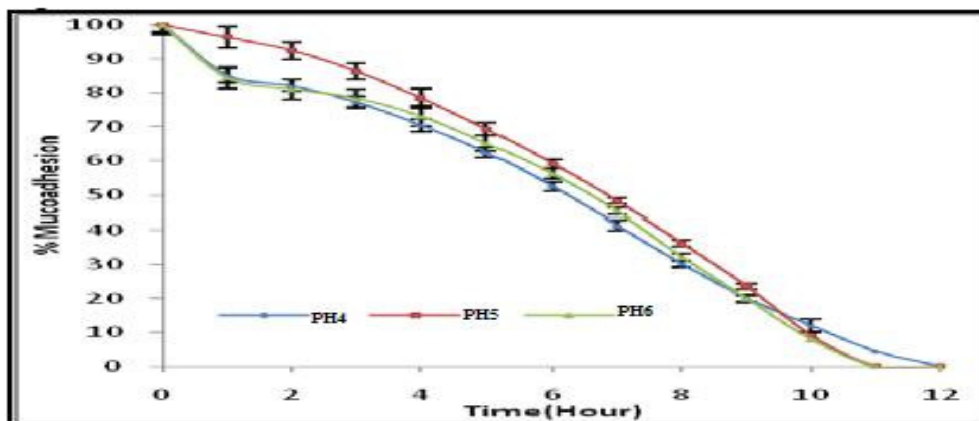


Figure 3. Comparative % mucoadhesion of microspheres of formulations PH4-PH6

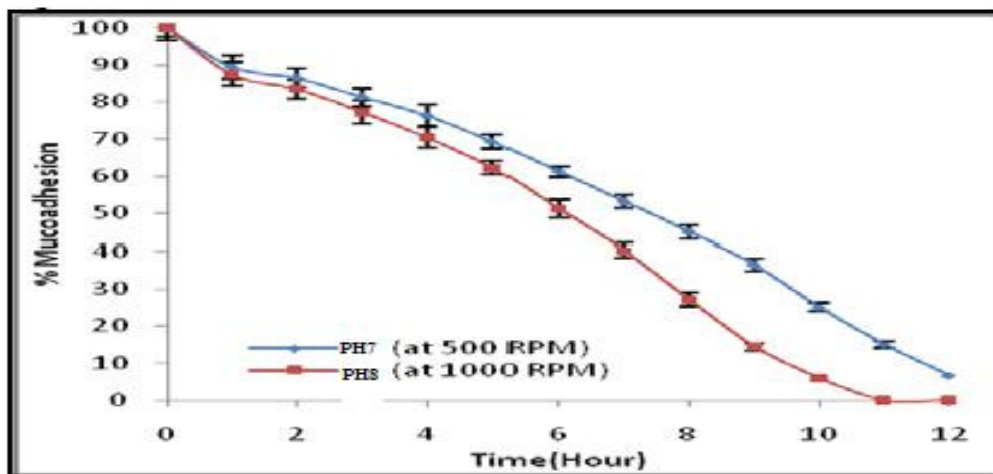


Figure 4. Comparative % mucoadhesion of microspheres of formulations PH7 and PH8

Drug release study

Drug release study of all the formulations containing drug were performed in simulated gastric fluid (pH 1.2) at $37\pm 1^\circ\text{C}$. Drug release from these microspheres were slow, extended and dependent on the type of polymer and concentration of polymer used. Formulation PH1 containing SCMC showed the maximum release due to rapid swelling property and high dissolution of SCMC in dissolution environment (0.1 N HCl). Dissolution medium permeation into the microspheres is facilitated due to high swelling action of the SCMC which leads to more medium for the transport of the drug is available. While HPMC microspheres showed the least drug release. Drug release is significantly affected by the size of microspheres. Formulation PH8 shown fastest drug release among all the formulation due to fact that these microspheres are small in size. After the end of 10 hrs, the drug release were found to be 95.45 ± 2.55 , 91.43 ± 2.55 , 72.23 ± 2.10 , 86.22 ± 3.33 , 82.54 ± 3.44 , 76.76 ± 2.33 , 78.54 and 96.22 % for formulations PH1, PH2, PH3, PH4, PH5, PH6, PH7 and PH8 respectively (Figure 5, 6 and 7).

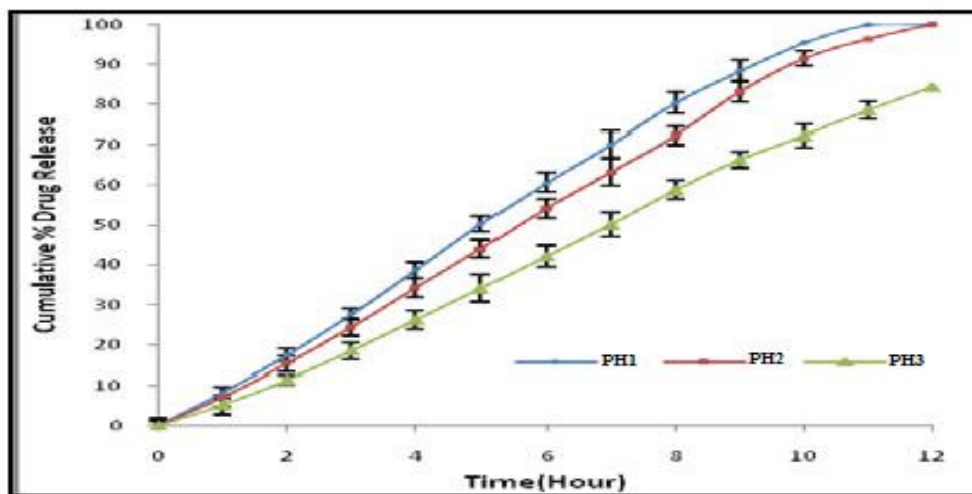


Figure 5. Cumulative % drug release from microspheres of formulation PH1-PH3

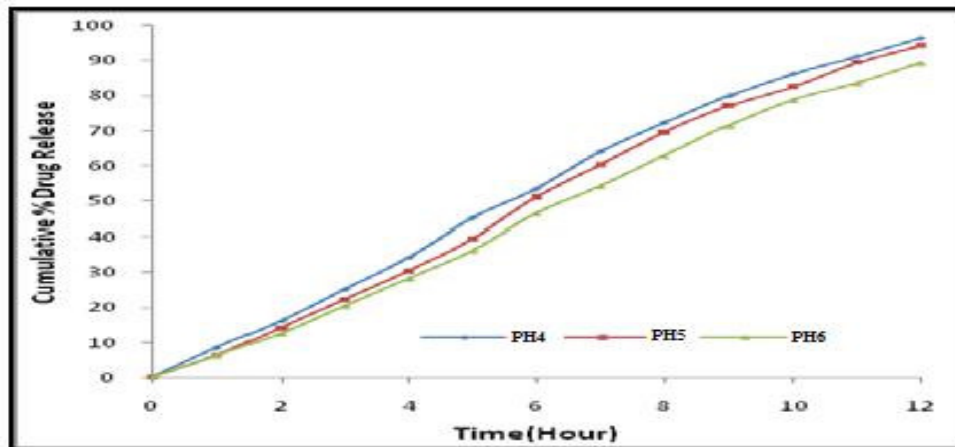


Figure 6. Cumulative % drug release from microspheres of formulation PH4-PH6

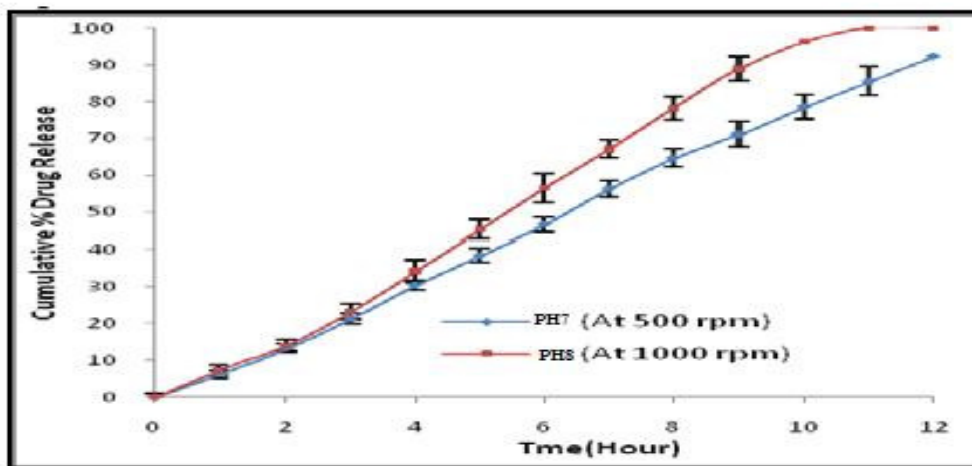


Figure 7. Cumulative % drug release from microspheres of formulation PH7 and PH8

FUTURE PROSPECTS

While the control of drug release profiles has been a major aim of pharmaceutical research and development in the past two decades, the control of GI transit profiles could be the focus of the next two decades and might result in the availability of new products with better therapeutic possibilities and substantial benefits for patients. Mucoadhesive microspheres would become the promising candidate for delivery various drugs in sustained release manner. Dosing frequency and loss of drug also reduced by use of such type of formulations. Thus SRM microspheres of PH would become a promising candidate to increase the bioavailability of the drug.

ACKNOWLEDGEMENTS

The authors are very grateful to College of Pharmacy, IPS Academy, Indore, for providing all the necessary facilities for carrying out this study. The authors also wish to thank Dr. Praveen Malviya, Indian Institute of Technology (IIT), New Delhi, India, for assistance with SEM facilities.

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