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**PESQUISA/RESEARCH**

# Development, optimization and evaluation of alfuzosin hcl mucoadhesive microspheres

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## ABSTRACT

The purpose of this research was to formulate and systematically evaluate in vitro and in vivo performances of mucoadhesive microspheres of alfuzosin HCL for the potential use in the treatment of benign prostatic hyperplasia (BPH). Alfuzosin HCL mucoadhesive microspheres containing PEO 301 as mucoadhesive polymer were prepared by an emulsion-solvent evaporation technique. Microspheres were discrete, spherical, free flowing and showed a good percentage of drug entrapment efficiency. Preliminary trial batches, F1 to F6 batches of microspheres were prepared by using different volume of polymer-to-drug ratio. From these batches of each polymer, the optimized formulation is selected based on the percentage of mucoadhesion, drug entrapment efficiency and sphericity of microspheres. The morphological characteristics of the mucoadhesive microspheres were studied under a scanning electron microscope. In vitro and Ex vivo mucoadhesion studies has been performed. From the mucoadhesive strength determination studies of the optimized batches it was found that mucoadhesive property of optimized formulations was observed to be greater in *in vitro* studies than *ex vivo* studies. In vitro drug release of optimized batch shown 92.75% drug release after 12 hours.

**Keywords**: Benign prostatic hyperplasia, alfuzosin HCL, ex vivo mucoadhesion, alpha 1-adrenergic receptors

## INTRODUCTION

Alfuzosin Hydrochloride is anantagonist for alpha 1-adrenergic receptors in the lower urinary tract, which cause smooth muscle in the bladder neck and prostate to relax, resulting in improved urine flow and a reduction in symptoms of benign prostatic hyperplasia (BPH) (British Pharmacopoeia, 2005).It can cause few cardiovascular adverse effects. The extended release formulations showed lower frequency of cardiovascular adverse effects reported when compared with immediate-release formulation (Colombo, 1993).

Alfuzosin is freely soluble in water (Costa, 2001; Debruyne, 1998) and thus readily absorbed after administration. The oral absorption is significantly aided by the presence of food. Recently 10 mg once daily extended release formulation has become available in the market (Dortune et al., 1997) which is more convenient for older patients. Marketed alfuzosin formulation is a three layered Geomatrix tablet that requires special facilities, high cost, more time and complex operation than conventional formulations (Indian Pharmacopoeia, 1996). PEO as a mucoadhesive polymer and HPMC is widely used in matrix formulations as a release retardant polymer(Kees el al., 1991; Kerrebroeck et al., 2000; Lee, 2003) that controls drug release by quickly forming a gel barrier. When a drug is formulated with gel forming hydrocolloids such as HPMC, it swells in the gastric fluid affording a prolonged gastric residence time.

The present research endeavor was directed towards the development of mucoadhesive microspheres of Alfuzosin hydrochloride in the form of simple and cost effective. Different polymers viz. Hydroxypropyl methylcellulose (HPMC), Polyethylene Oxide and its combinations were tried. The microspheres were evaluated for different parameters such as micromeritics properties, % yield, % entrapment efficiency, In-vitro and Ex vivo mucoadhesion, SEM and In vitro drug release.

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## MATERIAL AND METHODS

### Materials

Alfuzosin hydrochloride was gift sample from Dr. Reddy's Laboratories Ltd, Hyderabad. Kollidon SR was donated by BASF chemical (Shreveport, LA). HPMC K4M, Eudragit S 100, Acrycoat S 100, PEO 301, carbopol were provided by Colorcon Inc (WestPoint, PA).

### Preparation of mucoadhesive Alfuzosin HCL microspheres

Mucoadhesive microspheres loaded with alfuzosin HCL were prepared by emulsion solvent evaporation (Lehr et al., 1992; Liu et al., 2008)). Formulations were formulated using different polymers Eudragit S 100, Kollidone SR, cellulose acetate, Acrycoat S 100, PEO 301, carbopol and HPMC K4M. Drug and polymer in proportion 1:2, (drug: polymer) were dissolved in organic solvent (Acetone, Ethanol and dichloromethane) (Liu et al, 2005; Lunstedt, 1998; Martin, 1993; Nair et al., 2007) ). This clear solution was poured slowly as a thin stream in aqueous phase; about 100 mL of polyvinyl alcohol solution and in oil phase; about liquid paraffin containing 0.01% tween 80 with continuous stirring at a speed of 500 rpm using remi stirrer at room temperature until complete evaporation of solvent took place. The mucoadhesive microspheres were collected by decantation, while the non mucoadhesive microspheres were discarded along with any polymer precipitates. The microspheres were then washed with the n- hexan and dried over night at 40$^{o}$C. The microspheres were weighed and stored in desiccators until further analysis. Overall twelve formulations were formulated using different polymers with different continuos phase.
Overall six formulations were formulated using different polymers PEO 301 (code F1), carbopol (code F2), HPMC K4M (code F3), Kollidone SR (code F4), Acrycoat S100 (code F5) and Cellulose acetate (code F6) as shown in Table 1.

Table 1. Composition of mucoadhesive microspheres of alfuzosin HCL

| Sr. No. | Formulation code | Drug : polymer Ratio | Organic solvent | Continuous phase |
|---|---|---|---|---|
| 1 | F1 | 1:2 | Dichloromethane | Liquid paraffin containing 0.01% tween 80 |
| 2 | F2 | 1:2 | Dichloromethane | Liquid paraffin containing 0.01% tween 80 |
| 3 | F3 | 1:2 | Dichloromethane | Liquid paraffin containing 0.01% tween 80 |
| 4 | F4 | 1:2 | Dichloromethane | Liquid paraffin containing 0.01% tween 80 |
| 5 | F5 | 1:2 | Dichloromethane | Liquid paraffin containing 0.01% tween 80 |
| 6 | F6 | 1:2 | Dichloromethane | Liquid paraffin containing 0.01% tween 80 |

### Selection of polymers

It was done by various evaluation parameters like micromeritics properties, drug entrapment efficiency and %yield of microspheres. The results are shown in Tables 3 and 4.

### Micromeritics properties of mucoadhesive microspheres (Nazzal et al., 2002)

### Particle size determination :

The particle size was determined using an optical microscope under regular polarized light, and mean particle size was calculated by measuring 100 particles with the help of a calibrated oculometer.

### Bulk density

Bulk density was determined by three tap method, after filling the weighed quantity of microspheres in a graduated cylinder, the volume occupied by microspheres was determined.

### Tapped density

The tapping method was used to calculate tapped densities. The volume of weighed quantity of microspheres was determined after 100 taps as well as 1000 taps using tapped density apparatus.

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}} \quad (1)$$

### Compressibility index and hausner ratio

Compressibility index and hausner ratio was calculated from the values of bulk density and tapped density by using following formulas:

$$\% \text{ Compressibility Index} = \frac{\text{Tapped density} - \text{Bulk Density}}{\text{Tapped Density}} \times 100 \quad (2)$$

$$\text{Hausner Ratio} = \frac{\text{Tapped density}}{\text{Bulk Density}} \quad (3)$$

### Angle of repose

The angle of repose $\phi$ of the microspheres, which measures the resistance to particle flow, was calculated as

$$\tan \phi = 2H / D \quad (4)$$

Where 2H/D is the surface area of the free standing height of the microspheres heap that is formed after making the microspheres flow from the glass funnel.

### Yield of microspheres

The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

% Yield = (Actual weight of product / Total weight of excipients and drug) x 100 (5)

### Optimization of the selected formulations

Modification of all above formulations were affected by preparing the microspheres using different ratio of drug and polymer, combine effect of solvent system also investigated. The mucoadhesive microspheres were prepared according to the method given in the section. They are designated as PEO 301 for $P_1$, $P_2$, $P_3$, HPMC K4M for $H_1$, $H_2$, $H_3$, combination of the both polymer for $HP_1$, $HP_2$, and $HP_3$. The detailed composition was given in the Table 2.

Table 2. Compositions of optimized formulations of mucoadhesive microspheres

| Sr. No. | Formulation code | Drug: polymer Ratio | Organic solvent system (1 : 1) | Stirring speed |
|---|---|---|---|---|
| 1 | $H_1$ | 1:1 | methanol | 600 |
| 2 | $H_2$ | 1:2 | methanol | 600 |
| 3 | $H_3$ | 1:3 | Methanol | 850 |
| 4 | $P_1$ | 1:1 | Dichloromethane: methanol | 600 |
| 5 | $P_2$ | 1:2 | Dichloromethane: methanol | 600 |
| 6 | $P_3$ | 1:3 | Dichloromethane: methanol | 850 |
| 7 | $HP_1$ | 1:1 | Dichloromethane: methanol | 600 |
| 8 | $HP_2$ | 1:2 | Dichloromethane: methanol | 600 |
| 9 | $HP_3$ | 1:3 | Dichloromethane: methanol | 850 |

**Evaluation of prepared mucoadhesive microspheres**

*In vitro* performance was evaluated by the usual pharmacopoeial and other tests such as micromeritics properties (Table 5), drug entrapment efficiency, flow properties, *In Vitro* Mucoadhesive studies, *ex vivo* mucoadhesive studies, *in vitro* drug release studies, stability studies etc.

**Determination of drug loading and encapsulation efficiency** (Nicholas et al., 2011)

The alfuzosin HCL content in the microspheres was determined by pulverizing the alfuzocine HCL -loaded microspheres (10mg) followed by immersing them in 100mL 0.1 N HCL with agitating at room temperature for 12 h. After filtration through a 0.45 um membrane filter (Millipore), the drug concentration was determined spectrophotometrically at the wavelength of 244 nm. The filtered solution from the empty microspheres (without alfuzocine HCL) was taken as blank. All samples were analyzed in triplicate and the drug loading (DL) and encapsulation efficiency (shown in Table 6) (EE) was calculated according to the following equation:

$$DL\ (\%) = W_D/W_T \times 100 \quad (6)$$

DL: drug loading; $W_D$: the weight of the drug loaded in the microspheres; $W_T$: the total weight of the microspheres.

$$EE\ (\%) = W_A/W_T \times 100 \quad (7)$$

EE: encapsulation efficiency; $W_A$: actual drug content; $W_T$: theoretical drug content.

**In *vitro* drug release studies**

The drug release studies were carried out using six basket dissolution apparatus USP type II. The microspheres were placed in a non reacting mesh that had a smaller mesh size than the microspheres. The mesh was tied with a nylon thread to avoid the escape of any microspheres. The dissolution medium used was 900 mL of 0.1 N hydrochloric acid at 37°C. At specific time intervals, 5 mL aliquots were withdrawn and analyzed by UV spectrophotometer at the respective λmax value 244 nm after suitable dilution against suitable blank. The withdrawn volume was replaced with an equal volume of fresh 0.1 N hydrochloric acid. The results are shown in Figures 3 (a,b,c).

***In Vitro* Mucoadhesive Strength Determination** (Patel et al., 2005)

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 x 1 inch) using thread. Microspheres were spread (~50) onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that 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 5 hours, the number of microspheres still adhering onto the tissue was counted. Percent mucoadhesion was given by the following formula. Results are reported in Table 16 and figure 1.

$$\%\ \text{adhesive strength} = (\text{no. of microspheres remains/no. of applied microspheres}) \times 100 \quad (8)$$

**Figure 1.** Schematic presentation *of In Vitro* Wash Off Test to Asses Mucoadhesive Properties

***Ex Vivo* Mucoadhesive Strength Determination** (Rosa et al., 1994)

In this technique four number of Albino rats were fasted overnight and then 50 numbers of microspheres (N0) were ingested to these rats through an oral feeding needle. These rats were then sacrificed at an interval of 0, 4, 8, 12 hours respectively to isolate their stomach. The stomach regions were then cut opened longitudinally to note the number of microspheres adhering to these regions (NS). This ultimately gave the adhesive strength of the formulation which was calculated using the formula given below.

Results are reported in Table 17 and figure 2.

$$\%\ \text{adhesive strength} = (NS / N0) \times 100 \quad (9)$$

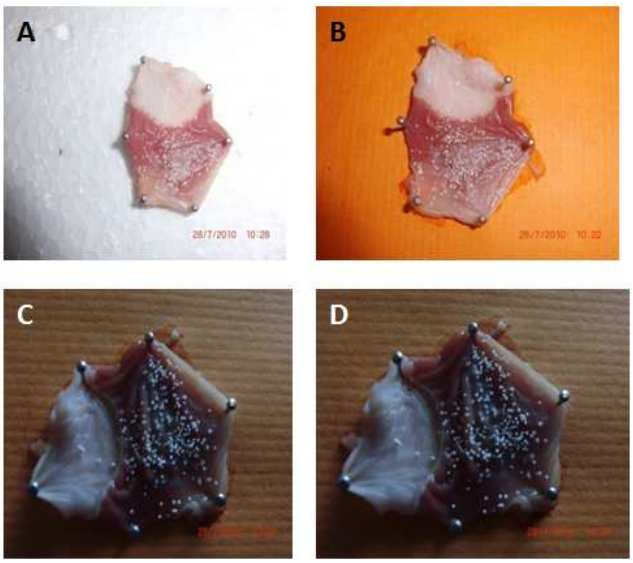


**Figure 2.** Schematic presentation of mucoadhesive microspheres in stomach at (A) 0 hour (B) 4 hour (c) 8 hour and (D) 12 hour after administration to the rats

**Surface topography (SEM)** (Silvina et al., 2002)

The surface morphology, shape and to confirm the hollow nature, microspheres were analyzed by scanning electron microscopy for selected batches (Leo, VP-435, Cambridge, UK). Photomicrographs were observed at required magnification operated with an acceleration voltage of 15 kV and working distance of 19 mm was maintained. Microspheres were mounted on the standard specimen-mounting stubs and were coated with a thin layer (20 nm) of gold by a sputter-coater unit to make the surface conductive. (VG Microtech, Uckfield, UK). The results are shown in Figures 4 to 6.

**Kinetic modeling of drug dissolution profiles** (Soppimath et al., 2001; US pharmacopoeia, 2005; Vyas et al., 1989)

Costa et al., suggested that the dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions are obtained) could be represented by zero order kinetic equation. Colombo et al.,suggested that the quantity of drug released from matrix tablets is often analyzed as a function of the square root of time, which is typical for systems where drug release is governed by pure diffusion. However, the use of this relationship in swellable system is not justified completely as such systems can be erodible. Therefore, analysis of drug release from swellable matrices must be performed with a flexible model that can identify the contribution to overall kinetics, an equation proposed by Ritger and Peppas. For finding out the mechanism of drug release from mucoadhesive hydrophilic matrix tablet, the dissolution data obtained from the above experiments were treated with the different release kinetic equations.

Zero order release equation:

$$Q = K_0 t \qquad (10)$$

Higuchi's square root of time equation:

$$Q = KH\, t^{1/2} \qquad (11)$$

Korsmeyer and Peppas equation:

$$F = (Mt/M) = Km\, t^{n} \qquad (12)$$

Where, Q is amount of drug release at time t, Mt is drug release at time t, M is total amount of drug in dosage form, F is fraction of drug release at time t, $K_0$ is zero order release rate constant, KH is Higuchi square root of time release rate constant, Km is constant depend on geometry of dosage form and n is diffusion exponent indicating the mechanism of drug release, where for cylinder value of n is 0.45 indicate Fickian diffusion, between 0.45 and 0.89 indicate anomalous transport and 0.89 indicate case-II transport.

The dissolution profile of all the batches was fitted to Zero order, First order, Higuchi and Korsemeyer and Peppas models to ascertain the kinetic modeling of drug release. The method of Bamba et al. was adopted for deciding the most appropriate model. The results are shown in Tables (7- 15 and 18) and Figures 7to 9.

**Stability study of the optimized batches**

With the recent trend towards globalization of manufacturing operation, it is imperative that the final product be sufficiently rugged for marketing worldwide under various climatic conditions including tropical, sub tropical and temperate. Stability studies were carried out as per ICH guidelines. The mucoadhesive microspheres were placed in a screw capped glass containers and stored at room temperature, (25 ± 2°C), Humidity chamber (40°C, 75 % RH), and in Refrigerator (2-8°C) for a period of 90 days. The samples were assayed for drug content at regular intervals. The graph of percent drug content versus time (in days) was plotted. The results are shown in Figure 10.

## RESULTS AND DISCUSSION

**Preliminary screening of the formulations**

From the results of evaluation of the mucoadhesive microspheres for preliminary screening it was found that the microspheres prepared with carbopol (code F2), Kollidone SR (code F4), Acrycoat S100 (code F5) and Cellulose acetate (code F6) were not showing the satisfactory results. The micromeritics properties of the prepared mucoadhesive microspheres with carbopol (code F2), Kollidone SR (code F4), Acrycoat S100 (code F5) and Cellulose acetate (code F6) shown that the flow properties of the microspheres were poor. The value of angle of repose of formulationF2, F4, F5 and F6 were found to be, 25.44º ± 0.31, 31.22º ± 0.03, 27.59º ± 0.28 and 31.66º ± 0.60 respectively which indicates poor flow properties. % yield of the formulation F2, F4, F5 and F6 were found to be 62.10 ± 1.74, 43.86 ± 8.82, 47.53 ± 5.11and 54.72 ± 5.78 respectively. Drug entrapment efficiency of the formulation F2, F4, F5 and F6 were found to be 70.1 ± 3.15, 49.59 ± 3.12, 59.31 ± 4.61and 63.38 ± 2.82 respectively. % yield and Drug entrapment efficiency of the formulation F2, F4, F5 and F6 were found to be comparatively less than formulation F1 and F3. So formulation F1 and F3 were selected for further studies with modifications.

**Table 3.** Micromeritics properties of mucoadhesive microspheres

| Formulation Code | Angle of repose* | Carr's Index (%) * | Hausner ratio * | Mean particle size (µm) * |
|---|---|---|---|---|
| F1 | 22.65º ± 0.20 | 13.32 ± 2.92 | 1.12 ± 0.08 | 296.77 ± 6.23 |
| F2 | 25.44º ± 0.31 | 18.88 ± 0.24 | 1.23 ± 0.03 | 385.83 ± 4.44 |
| F3 | 22.86º ± 0.44 | 14.92 ± 2.79 | 1.14 ± 0.08 | 302.08 ± 1.82 |
| F4 | 31.22º ± 0.03 | 26.43 ± 1.45 | 1.24 ± 0.04 | 574.66 ± 5.90 |
| F5 | 27.59º ± 0.28 | 28.24 ± 1.27 | 1.28 ± 0.02 | 602.73 ± 2.33 |
| F6 | 31.66º ± 0.60 | 30.30 ± 1.62 | 1.26 ± 0.09 | 618.39 ± 4.73 |

* Mean ± standard deviation, n=3

**Table 4.** Characterization of prepared mucoadhesive microspheres (preliminary batches)

| Formulation Code | % Yield* | % Drug Entrapped* |
|---|---|---|
| F1 | 86.90 ± 3.70 | 81.4 ± 3.12 |
| F2 | 62.10 ± 1.74 | 70.1 ± 3.15 |
| F3 | 78.65 ± 3.22 | 86.7 ± 1.88 |
| F4 | 43.86 ± 8.82 | 49.59 ± 3.12 |
| F5 | 47.53 ± 5.11 | 59.31 ± 4.61 |
| F6 | 54.72 ± 5.78 | 63.38 ± 2.82 |

* Mean ± standard deviation, n=3

**Selection of the formulations for further studies**

The screening of microspheres formulations were based on different physicochemical and evaluation parameters. The optimistic formulations from above all are F1 and F3. These formulations shows satisfactory results of different physicochemical parameters and evaluation parameters therefore modifications in these three formulations were made by varying the drug: polymer ratio and solvent systems investigated.

**Optimization of the selected formulations (F1 and F3)**

Modifications of all above formulations were affected by preparing the microspheres using different ratio of drug and polymer, combine effect of solvent system also investigated. The mucoadhesive microspheres were prepared according to the method given in the section 2.3.1. They are designated as PEO 301 for $P_1$, $P_2$, $P_3$, HPMC K4M for $H_1$, $H_2$, $H_3$, combination of the both polymer for $HP_1$, $HP_2$, and $HP_3$. The detailed composition was given in the Table 2.

**Characterization of optimized formulations**

**Micromeritics properties**

Micromeritics properties and evaluations like Yield of microspheres and Drug entrapment efficiency of the optimized formulations were done according to the methods given in the Tables 3 and 4.

The prepared microspheres were evaluated for the micromeritics properties. The average of three readings was taken. The mean particle size, flow properties and standard deviation were calculated. The low standard deviation of the measured mean particle size, % compressibility, Hausner ratio and angle of repose of all the 9 formulations ensures the uniformity of the microspheres prepared by emulsion solvent evaporation method. The mean particle size was found to be in the range of 208.75 ± 4.31 µm to 382.50 ± 3.09µm. The variation in mean particle size could be due to variation in drug-polymer ratio. The % compressibility of all the microspheres was found to be in the range of 12.92 ± 1.42 to 24.86 ± 2.92. The hausner ratio of all the microspheres was found to be in the range of 1.11 ± 0.016 to 1.24 ± 0.028. The angle of repose of all the microspheres was found to be in the range of 24.48 ± 0.68 to 31.63 ± 0.60. For the all formulations, % drug entrapped was found to vary from 69.3 % to 84.6 % and it shows that the drug entrapment is higher in microspheres containing combination of PEO 301 and HPMC K4M and lower in microspheres containing PEO 301 and HPMC K4M. For the all formulations, % yield was found to vary 55.93 % to 94.60 % and it shows that the yield is higher in microspheres containing combination of PEO 301 and HPMC K4M and lower in microspheres containing PEO 301 and HPMC K4M.

Table 5**.** Micromeritics properties of mucoadhesive microspheres

| Formulation code | Mean Particle Size (µm)* | Flow Properties | | |
|---|---|---|---|---|
| | | % Compressibility* | Hausner ratio* | Angle of repose* |
| $H_1$ | 302.70 ± 1.21 | 17.86 ± 0.26 | 1.17 ± 0.041 | 25.42 ± 0.67 |
| $H_2$ | 342.61 ± 2.20 | 16.30 ± 0.62 | 1.13 ± 0.007 | 28.42 ± 0.03 |
| $H_3$ | 382.50 ± 3.09 | 19.43 ± 0.23 | 1.16 ± 0.017 | 30.89 ± 0.55 |
| $P_1$ | 278.45 ± 2.43 | 21.25 ± 1.59 | 1.24 ± 0.028 | 28.83 ± 0.31 |
| $P_2$ | 289.33 ± 3.17 | 24.86 ± 2.92 | 1.21 ± 0.028 | 31.63 ± 0.60 |
| $P_3$ | 249.00 ± 2.34 | 17.78 ± 0.56 | 1.21 ± 0.07 | 29.88 ± 0.07 |
| $HP_1$ | 208.75± 4.31 | 12.92 ± 1.42 | 1.11 ± 0.016 | 24.46 ± 0.58 |
| $HP_2$ | 230.64 ± 2.68 | 14.36 ± 2.10 | 1.14 ± 0.017 | 25.23 ± 0.28 |
| $HP_3$ | 211.34 ± 4.35 | 14.55 ± 1.88 | 1.12 ± 0.041 | 26.48 ± 0.68 |

* Mean ± standard deviation, n=3

Table 6. Characteristics of alfuzosin HCL mucoadhesive microspheres

| Formulation code | % Yield | % Drug Entrapped |
|---|---|---|
| $H_1$ | 78.40 | 73.8 % |
| $H_2$ | 74.85 | 75.7 % |
| $H_3$ | 55.93 | 81.6 % |
| $P_1$ | 77.14 | 76.9 % |
| $P_2$ | 75.15 | 69.3 % |
| $P_3$ | 73.59 | 72.6 % |
| $HP_1$ | 94.60 | 84.6 % |
| $HP_2$ | 88.60 | 80.8 % |
| $HP_3$ | 89.00 | 82.9 % |

**In vitro drug release studies**

In the present study, *in vitro* release studies of the mucoadhesive microspheres were carried out in 0.1 N hydrochloric acid at 37°C for a maximum period of 12 hr. At different time intervals, samples were withdrawn and cumulative % drug release was calculated. The percentage drug release of all the formulations is presented in Figure 3 (a,b,c). Out of 9 formulations tried, the formulation $HP_1$ containing HPMC K4M and PEO 301was found to be satisfactory; since it showed prolonged and complete release with 92.75 % at end of 12 hr.

***In Vitro* and *Ex Vivo* Mucoadhesive Strength Determination Studies**

From the mucoadhesive strength determination studies of the optimized batches it was found that mucoadhesive property of optimized formulations was observed to be greater in *in vitro* studies than *ex vivo* studies. This may be

due to the reason that in *ex vivo* studies microspheres were ingested to rats and thus the peristaltic movement of the stomach forces the microspheres in the lower G.I.T. This reduces the time of contact between the microsphere and mucin layer and thus reduces the mucoadhesive strength of microspheres. While such peristaltic movements are absent in *in vitro* tests so the formulations showed a greater mucoadhesive property during this test.

Table 7. Dissolution data of formulation $HP_1$

| Time(hr) | Root t | Cumulative % release | Log Cumulative % release | Cumulative % retained | Log cumulative % retained |
|---|---|---|---|---|---|
| 1 | 1.000 | 28.1 | 1.449 | 71.9 | 1.857 |
| 2 | 1.414 | 42.15 | 1.625 | 57.85 | 1.762 |
| 3 | 1.732 | 44.96 | 1.653 | 55.04 | 1.741 |
| 4 | 2.000 | 48.15 | 1.683 | 51.85 | 1.715 |
| 5 | 2.236 | 59.13 | 1.772 | 40.87 | 1.611 |
| 6 | 2.449 | 62.99 | 1.799 | 37.01 | 1.568 |
| 7 | 2.645 | 69.16 | 1.840 | 30.84 | 1.489 |
| 8 | 2.828 | 72.03 | 1.858 | 27.97 | 1.447 |
| 9 | 3.000 | 78.49 | 1.895 | 21.51 | 1.333 |
| 10 | 3.162 | 86.31 | 1.936 | 13.69 | 1.136 |
| 11 | 3.316 | 89.18 | 1.950 | 10.82 | 1.034 |
| 12 | 3.464 | 92.75 | 1.967 | 7.25 | 0.860 |

Table 8. Dissolution data of formulation $HP_2$

| Time(hr) | Root t | Cumulative % release | Log Cumulative % release | Cumulative % retained | Log cumulative % retained |
|---|---|---|---|---|---|
| 1 | 1.000 | 19.37 | 1.287 | 80.63 | 1.906 |
| 2 | 1.414 | 34.29 | 1.535 | 65.71 | 1.818 |
| 3 | 1.732 | 40.69 | 1.609 | 59.31 | 1.773 |
| 4 | 2.000 | 45.2 | 1.655 | 54.8 | 1.739 |
| 5 | 2.236 | 52.23 | 1.718 | 47.77 | 1.679 |
| 6 | 2.449 | 57.57 | 1.760 | 42.43 | 1.628 |
| 7 | 2.645 | 64.94 | 1.813 | 35.06 | 1.545 |
| 8 | 2.828 | 68.12 | 1.833 | 31.88 | 1.504 |
| 9 | 3.000 | 75.14 | 1.876 | 24.86 | 1.396 |
| 10 | 3.162 | 80.32 | 1.905 | 19.68 | 1.294 |
| 11 | 3.316 | 84.16 | 1.925 | 15.84 | 1.200 |
| 12 | 3.464 | 89.69 | 1.953 | 10.31 | 1.013 |

Table 9. Dissolution data of formulation $HP_3$

| Time(hr) | Root t | Cumulative % release | Log Cumulative % release | Cumulative % retained | Log cumulative % retained |
|---|---|---|---|---|---|
| 1 | 1.000 | 19.56 | 1.291 | 80.44 | 1.905 |
| 2 | 1.414 | 39.15 | 1.593 | 60.85 | 1.784 |
| 3 | 1.732 | 41.86 | 1.622 | 58.14 | 1.764 |
| 4 | 2.000 | 44.35 | 1.647 | 55.65 | 1.745 |
| 5 | 2.236 | 49.61 | 1.696 | 50.39 | 1.702 |
| 6 | 2.449 | 55.87 | 1.747 | 44.13 | 1.645 |
| 7 | 2.645 | 58.72 | 1.769 | 41.28 | 1.616 |
| 8 | 2.828 | 62.3 | 1.794 | 37.7 | 1.576 |
| 9 | 3.000 | 72.13 | 1.858 | 27.87 | 1.445 |
| 10 | 3.162 | 76.13 | 1.882 | 23.87 | 1.378 |
| 11 | 3.316 | 82.38 | 1.916 | 17.62 | 1.246 |
| 12 | 3.464 | 87.2 | 1.941 | 12.8 | 1.107 |

Table 10. Dissolution data of formulation $H_1$

| Time(hr) | Root t | Cumulative % release | Log Cumulative % release | Cumulative % retained | Log cumulative % retained |
|---|---|---|---|---|---|
| 1 | 1.000 | 32.93 | 1.518 | 67.07 | 1.827 |
| 2 | 1.414 | 41.48 | 1.618 | 58.52 | 1.767 |
| 3 | 1.732 | 49.2 | 1.692 | 50.8 | 1.706 |
| 4 | 2.000 | 58.03 | 1.764 | 41.97 | 1.623 |
| 5 | 2.236 | 68.35 | 1.835 | 31.65 | 1.500 |
| 6 | 2.449 | 72.45 | 1.860 | 27.55 | 1.440 |
| 7 | 2.645 | 85.49 | 1.932 | 14.51 | 1.162 |
| 8 | 2.828 | 90.3 | 1.956 | 9.7 | 0.987 |

Table 11. Dissolution data of formulation $H_2$

| Time(hr) | Root t | Cumulative % release | Log Cumulative % release | Cumulative % retained | Log cumulative % retained |
|---|---|---|---|---|---|
| 1 | 1.000 | 28.33 | 1.452 | 71.67 | 1.855 |
| 2 | 1.414 | 36.76 | 1.565 | 63.24 | 1.801 |
| 3 | 1.732 | 44.24 | 1.646 | 55.76 | 1.746 |
| 4 | 2.000 | 49.34 | 1.693 | 50.66 | 1.705 |
| 5 | 2.236 | 61.83 | 1.791 | 38.17 | 1.582 |
| 6 | 2.449 | 68.23 | 1.834 | 31.77 | 1.502 |
| 7 | 2.645 | 76.11 | 1.881 | 23.89 | 1.378 |
| 8 | 2.828 | 82.49 | 1.916 | 17.51 | 1.243 |
| 9 | 3.000 | 88.35 | 1.946 | 11.65 | 1.066 |

Table 12. Dissolution data of formulation $H_3$

| Time(hr) | Root t | Cumulative % release | Log Cumulative % release | Cumulative % retained | Log cumulative % retained |
|---|---|---|---|---|---|
| 1 | 1.000 | 18.06 | 1.257 | 81.94 | 1.913 |
| 2 | 1.414 | 25.8 | 1.412 | 74.2 | 1.870 |
| 3 | 1.732 | 31.76 | 1.502 | 68.24 | 1.834 |
| 4 | 2.000 | 44.24 | 1.646 | 55.76 | 1.746 |
| 5 | 2.236 | 52.46 | 1.720 | 47.54 | 1.677 |
| 6 | 2.449 | 64.24 | 1.808 | 35.76 | 1.553 |
| 7 | 2.645 | 72.66 | 1.861 | 27.34 | 1.437 |
| 8 | 2.828 | 84.42 | 1.926 | 15.58 | 1.193 |

Table 13. Dissolution data of formulation $P_1$

| Time(hr) | Root t | Cumulative % release | Log Cumulative % release | Cumulative % retained | Log cumulative % retained |
|---|---|---|---|---|---|
| 1 | 1.000 | 26.08 | 1.416 | 73.92 | 1.869 |
| 2 | 1.414 | 31.27 | 1.495 | 68.73 | 1.837 |
| 3 | 1.732 | 38.34 | 1.584 | 61.66 | 1.790 |
| 4 | 2.000 | 43.23 | 1.636 | 56.77 | 1.754 |
| 5 | 2.236 | 49.06 | 1.691 | 50.94 | 1.707 |
| 6 | 2.449 | 53.12 | 1.725 | 46.88 | 1.671 |
| 7 | 2.645 | 57.56 | 1.760 | 42.44 | 1.628 |
| 8 | 2.828 | 63.78 | 1.805 | 36.22 | 1.559 |
| 9 | 3.000 | 68.42 | 1.835 | 31.58 | 1.499 |
| 10 | 3.162 | 72.45 | 1.860 | 27.55 | 1.440 |
| 11 | 3.316 | 79.47 | 1.900 | 20.53 | 1.312 |
| 12 | 3.464 | 82.89 | 1.919 | 17.11 | 1.233 |

Table 14. Dissolution data of formulation $P_2$

| Time(hr) | Root t | Cumulative % release | Log Cumulative % release | Cumulative % retained | Log cumulative % retained |
|---|---|---|---|---|---|
| 1 | 1.000 | 9.25 | 0.966 | 90.75 | 1.958 |
| 2 | 1.414 | 12.14 | 1.084 | 87.86 | 1.944 |
| 3 | 1.732 | 19.26 | 1.285 | 80.74 | 1.907 |
| 4 | 2.000 | 24.98 | 1.398 | 75.02 | 1.875 |
| 5 | 2.236 | 31.95 | 1.504 | 68.05 | 1.833 |
| 6 | 2.449 | 34.84 | 1.542 | 65.16 | 1.814 |
| 7 | 2.645 | 48.56 | 1.686 | 51.44 | 1.711 |
| 8 | 2.828 | 57.05 | 1.756 | 42.95 | 1.633 |
| 9 | 3.000 | 60.62 | 1.783 | 39.38 | 1.595 |
| 10 | 3.162 | 62.06 | 1.793 | 37.94 | 1.579 |
| 11 | 3.316 | 66.25 | 1.821 | 33.75 | 1.528 |
| 12 | 3.464 | 68.72 | 1.837 | 31.28 | 1.495 |

Table 15. Dissolution data of formulation $P_3$

| Time(hr) | Root t | Cumulative % release | Log Cumulative % release | Cumulative % retained | Log cumulative % retained |
|---|---|---|---|---|---|
| 1 | 1.000 | 8.84 | 0.946 | 91.16 | 1.960 |
| 2 | 1.414 | 14.06 | 1.148 | 85.94 | 1.934 |
| 3 | 1.732 | 19.72 | 1.295 | 80.28 | 1.905 |
| 4 | 2.000 | 24.56 | 1.390 | 75.44 | 1.878 |
| 5 | 2.236 | 27.78 | 1.444 | 72.22 | 1.859 |
| 6 | 2.449 | 32.82 | 1.516 | 67.18 | 1.827 |
| 7 | 2.645 | 42.94 | 1.633 | 57.06 | 1.756 |
| 8 | 2.828 | 48.28 | 1.684 | 51.72 | 1.714 |
| 9 | 3.000 | 51.66 | 1.713 | 48.34 | 1.684 |
| 10 | 3.162 | 56.18 | 1.750 | 43.82 | 1.642 |
| 11 | 3.316 | 64.48 | 1.809 | 35.52 | 1.550 |
| 12 | 3.464 | 68.18 | 1.834 | 31.82 | 1.503 |

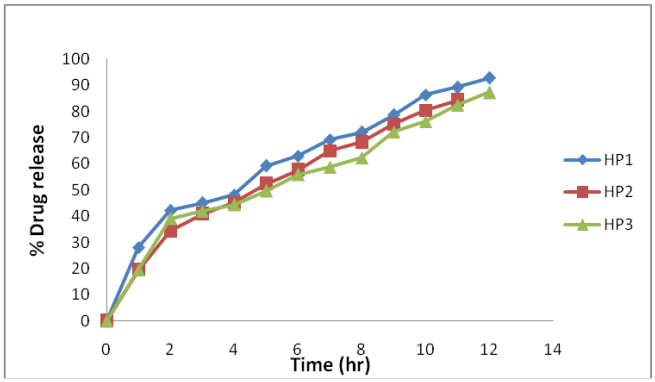


Figure 3. (a) Release rate profiles of PEO 301 and HPMC K4M containing mucoadhesive microspheres

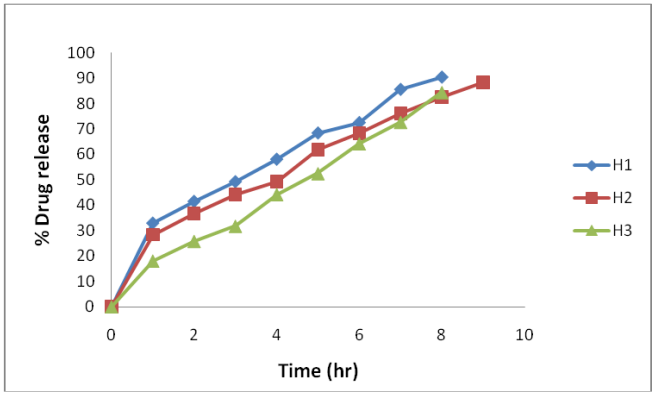


Figure 3. (b) Release rate profiles of HPMC K4M containing mucoadhesive microspheres

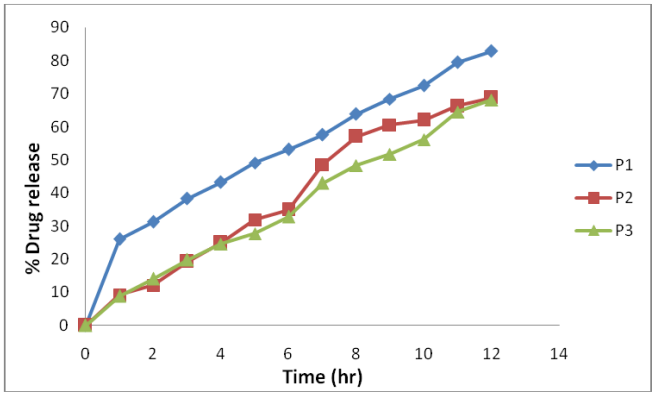


Figure 3. (c) Release rate profiles of PEO 301 containing mucoadhesive microspheres

Table 16. Results of *In Vitro* Wash Off Test to Assess Mucoadhesive Properties

| Batch | Number of microspheres adhered to the mucosa initially (No ) | Number of microspheres adhered to mucosa (NS ) | | | | | | Percent bioadhesion |
|---|---|---|---|---|---|---|---|---|
| | | 1 hour | 2 hour | 3 hour | 4 hour | 5 hour | Average | |
| $H_1$ | 50 | 45 | 43 | 42 | 42 | 41 | 43 | 86 |
| $P_1$ | 50 | 47 | 45 | 44 | 44 | 43 | 45 | 90 |
| $HP_1$ | 50 | 49 | 49 | 48 | 48 | 47 | 48 | 96 |

Table 17. Results of *Ex Vivo* Test to Assess Mucoadhesive Properties

| Batch | Number of microspheres adhered to the mucosa initially (No ) | Number of microspheres adhered to mucosa (NS ) | | | | | Percent bioadhesion |
|---|---|---|---|---|---|---|---|
| | | 0 hour | 4 hour | 8 hour | 12 hour | Average | |
| $H_1$ | 50 | 42 | 41 | 40 | 40 | 41 | 82 |
| $P_1$ | 50 | 43 | 42 | 41 | 41 | 42 | 84 |
| $HP_1$ | 50 | 45 | 45 | 44 | 42 | 44 | 88 |

## Surface Topography

Morphological examinations of microspheres were determined by scanning electron microscopy which was used to obtain the photographs of the microspheres. The scanning electron microscopy images of loaded microspheres of PEO 301 and HPMC K4M, PEO 301 and HPMC K4M are presented in Figures 4.33 to 4.35. All the images show spherical shapes of microspheres. The surface of PEO 301 containing microspheres has pores and smooth. The surface of PEO 301 and HPMC K4M containing microspheres are smooth with no avidence of drug crystallization on the surface. However, the surface of HPMC K4M containing microspheres contains both smooth and rough. Shape and surface differences between the formulations occurred due to how homogeneous the droplets in the emulsion were solidify.

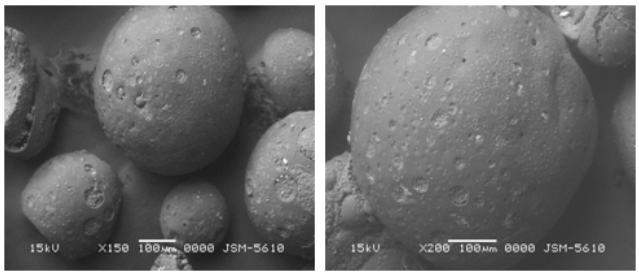

Figure 4. SEM photographs of mucoadhesive microspheres of PEO 301 and HPMC K4M($HP_1$)

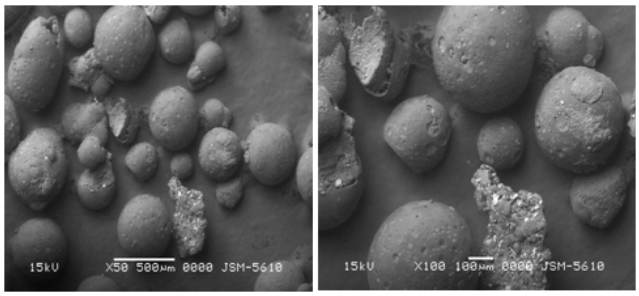

Figure 5. SEM Photographs of mucoadhesive microspheres of PEO 301 ($P_1$)

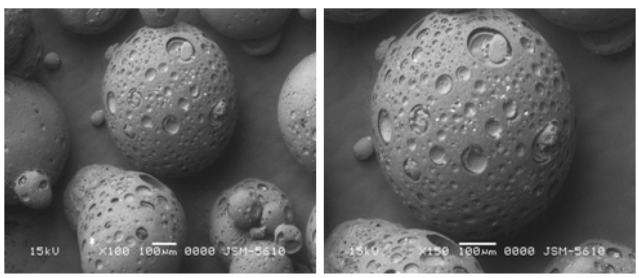

Figure 6. SEM photographs of mucoadhesive microspheres of HPMC K4M($P_1$)

**Kinetic modeling of drug dissolution profiles**

The drug release kinetic data is compiled in Table 18. In all the cases, the $R^2$ values of Higuchi matrix model were close to 1. The diffusion coefficient (n) values ranged between 0.467 to 0.870. Since the $R^2$ values of Higuchi matrix were close to 1, the drug release follows matrix diffusion kinetics and the plot shown in (Figure 8) revealed linearity; hence it was concluded that diffusion was the main mechanism of drug release from the mucoadhesive microspheres. Further, the observed diffusion coefficient values are indicative of the fact that the drug release from the formulation follows non-Fickian transport mechanism (Anomalous).

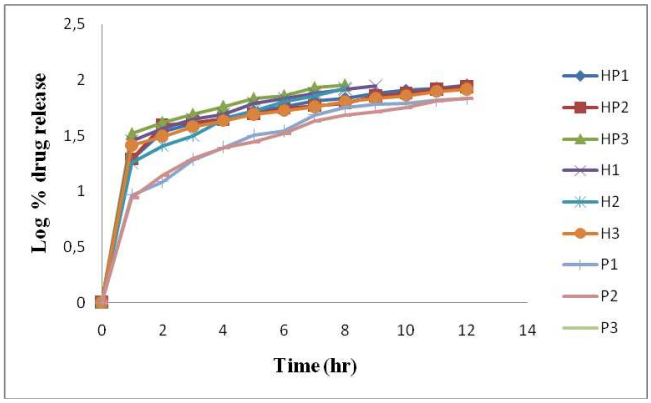


Figure 7**.** First order kinetic plot of different formulations

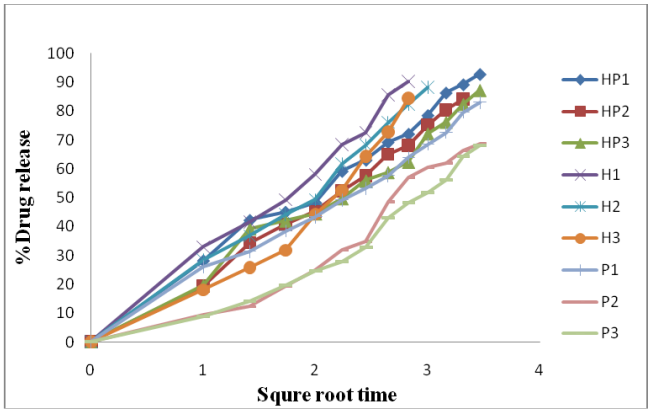


Figure 8. Higuchi plot of different formulations

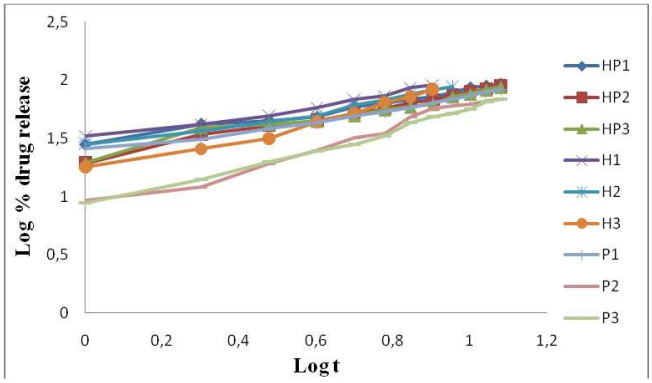


Figure 9. Korsemeyer-Peppas plot of different formulations

**Table 18.** Kinetic data of drug release from various formulations

| Zero order | | First order | | Higuchi's kinetics | | Peppas double log Plots | |
|---|---|---|---|---|---|---|---|
| Rate Constant (K) mg. min$^{-1}$ | Regression coefficient ($R^2$) | Rate Constant (K) mg. min$^{-1}$ | Regression Coefficient ($R^2$) | Rate constant (K) mg. min$^{-1}$ | Regression coefficient ( $R^2$) | Slope *(n)* | Regression coefficient ( $R^2$) |
| 5.322 | 0.9762 | 0.039 | 0.9400 | 24.059 | 0.9806 | 0.526 | 0.9740 |
| 4.755 | 0.9245 | 0.039 | 0.8770 | 21.796 | 0.9550 | 0.467 | 0.9716 |
| 5.929 | 0.9807 | 0.058 | 0.9777 | 26.042 | 0.9501 | 0.600 | 0.9535 |
| 5.840 | 0.9818 | 0.048 | 0.9801 | 25.783 | 0.9409 | 0.497 | 0.9583 |
| 5.907 | 0.9731 | 0.083 | 0.9314 | 26.393 | 0.9550 | 0.870 | 0.9717 |
| 5.416 | 0.9384 | 0.039 | 0.9082 | 24.807 | 0.9679 | 0.582 | 0.9708 |
| 5.941 | 0.9737 | 0.047 | 0.8854 | 26.903 | 0.9815 | 0.513 | 0.9751 |
| 6.097 | 0.9853 | 0.054 | 0.8887 | 27.990 | 0.9926 | 0.589 | 0.9905 |
| 5.538 | 0.9656 | 0.049 | 0.8608 | 24.944 | 0.9629 | 0.540 | 0.9529 |

**Stability studies of the optimized batches of hydrochlorothiazide**

The stability study was performed for optimized batch $HP_1$. The results were shown in Figure 9. The data shown in figure indicates that the amount of drug content in the optimized batch are constant through the stress conditions such as room temperature, (25 ± 2°C), Humidity chamber (40°C, 75 % RH), and in Refrigerator (2-8°C) for a period of 90 days. This ensures that the optimized batch is stable in different environmental condition which confirms the official criteria of ICH stability studies guidelines.

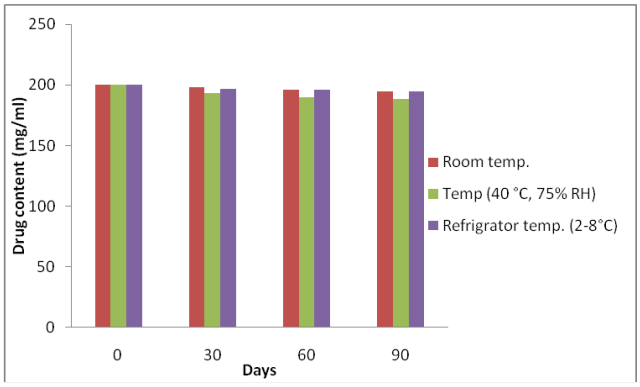


Figure 10. Graphical representation of stability studies of prepared mucodhaesive microspheres (Formulation Code $HP_1$)

**CONCLUSIONS**

Alfuzosin HCL mucoadhesive microspheres were prepared successfully using PEO as a mucoadhesive polymer. Preformulation studies of Alfuzosin HCL were done initially and results directed for the further course of formulation. Based on the preformulation studies different batches were prepared using selected excipients. Prepared microspheres were evaluated for the percentage yield, entrapment efficiency, particle size determination, *in-vitro* wash- off test, Ex vivo mucoadhesion and *in-vitro* dissolution test. The percentage yield and entrapment efficiency were good. Among all the formulations $HP_1$ showed better results. Mucoadhesive property of $HP_1$ was better than the other formulations. From the mucoadhesive strength determination studies of the optimized batches it was found that mucoadhesive property of optimized formulations was observed to be greater in *in vitro* studies than *ex vivo* studies. The scanning electron microscopy images of loaded microspheres of PEO 301 and HPMC K4M, PEO 301 and HPMC K4M has been done. All the images show spherical shapes of microspheres. Dissolu-

tion was carried out in 0.1N HCL at 244 nm. All the formulations were evaluated using different kinetic models i.e. Zero order kinetics, First order kinetics, Krosmeyer peppa`s model and Higuichi kinetics. All the formulations exhibited Non-Fickian diffusion mechanism. The drug release was diffusion controlled as the plot of Higuichi model was found to be linear. The formulation F5 was selected as an optimized formulation with 92.75 % of drug release in 12 hours. Data for the stability study indicate that there was no change in residual drug content for the selected formulation $HP_{1.}$

## REFERENCES


British Pharmacopoeia. Vol. I. London: Her Majesty's Stationary Office, British Pharmacopoeia Commission, 73: 86-89, 2005.

Colombo P. Swelling-controlled release in hydrogel matrices for oral route. Adv Drug Deliv Rev, 11: 37–57, 1993.

Costa P, Lobo MS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci, 13: 123–133, 2001.

Debruyne FMJ, Jardin A and Colloi D. Sustained-release alfuzosin, finasteride and the combination of both in the treatment of benign prostatic hyperplasia. Eur Urol, 34: 169–175, 1998.

Dortune B and Gunal N. Release of acetazolamide from swellable HPMC matrix tablets. Drug Dev. Ind. Pharm, 23: 1245-1249, 1997.

http://wwwbrookfieldengineeringcom/products/rheometers /laboratory-dv-rotationalasp.Accessed June 3, 2007.

Indian Pharmacopoeia, the Controller of Publications:Vol-II, Delhi,734-736, 1996.

Kees F, Lukassek U, Naber KG and Grobecker H. Comparative investigations on the bioavailability of cefuroxime axetil. Drug Res, 41: 843-846, 1991.

Kerrebroeck VP, Jardin A, Laval KU and Cangh VP. Efficacy and safety of a new prolonged release formulation of alfuzosin 10 mg once daily versus alfuzosin 2.5 mg thrice daily and placebo in patients with symptomatic benign prostatic hyperplasia. Eur Urol, 37: 306–313, 2000.

Lee M, American Journal of Health – System Pharmacy, 60: 1426-1439, 2003.

Lehr CM, Bouwstra JA, Schacht EH and Junginger HE. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int J Pharm, 78: 43-48, 1992.

Liu Q, and Fassihi R. Zero-order delivery of a highly soluble, low dose drug alfuzosin hydrochloride via gastro-retentive system. Int J Pharm, 348: 27–34, 2008.

Liu Z, Lu W, Qian LX, Zhang X, Zeng, P and Pan J. In-vitro and in-vivo studies on mucoadhesive microspheres of amoxicillin. J. Control. Release, 102: 135–144, 2005.

Lunstedt T et al. Experimental design and optimization. Chem. Intell. Lab. Sys, 42: 3-40, 1998.

Martin A, Physical Pharmaceutics, fourth edition. Lea Febiger, Philadelphia, 431-432, 1993.

Nair A, Gupta R and Vasanti S. *In vitro* controlled release of Alfuzosin hydrochloride using HPMC-based matrix tablets and its comparison with marketed product. Pharm Dev Tech, 12: 621–625, 2007.

Nazzal S and Khan MA. Response surface methodology for the optimization of ubiquinone self-nanoemulsified drug delivery system. Pharm Sci Tech, 3: 1-9, 2002.

Nicholas ME, Karunakar R, Pavan Kumar K and Raghunadha Gupta C. Journal of Pharmacy Research, 4: 1436-1437, 2011.

Patel VF, Patel NM and Yeole PG. Studies on formulation and evaluation of Ranitidine floating tablets. Indian J. Pharm. Sci, 67: 703-709, 2005.

Rosa M, Zia H and Rhodes T. Dosing and testing in vitro of a bioadhesive and floating drug delivery system for oral application. Int. J. Pharm, 105: 65-70, 1994.

Silvina AB, Maria CL and Claudio JS. *In vitro* studies of diclofenac sodium controlled-release from biopolymeric hydrophilic matrices. J Pharm Pharmaceut Sci 5: 213–219, 2002.

Soppimath KS, Kulkarni AR and Aminabhavi TM. Development of Hollow Microspheres as Floating Controlled Release Systems For Cardiovascular Drugs. Drug Dev. Ind. Pharm, 27: 507-515, 2001.

US pharmacopoeia 28, United state pharmacopoeial convention, Rockville,M.D., USA, Asian edition, 67: 265-272, 2005.

US pharmacopoeia 28, United state pharmacopoeial convention, Rockville,M.D., USA Asian edition,205(4): 557-560, 2005.

Vyas SP, Jain NK and Khana S. Formulation and performance evaluation of controlled-release diclofenac sodium tablets. J Control Release, 10: 219–223, 1989.