Formulation and evaluation of mucoadhesive microspheres of lisinopril, an antihypertensive drug

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Abstract

The objective of this research work is to develop and evaluate mucoadhesive microspheres of an anti-hypertensive drug for sustained release. Mucoadhesive microspheres were prepared by emulsification method using sodium alginate, HPMC K4M, HPMC K100M, Na CMC, carbopol 974P and chitosan in the various drug-polymer ratios. Six formulations were formulated and evaluated for possible drug polymer interactions, percentage yield, micromeritic properties, particle size, drug content, drug entrapment efficiency, drug loading, swelling index, in vitro wash off test, in vitro drug release, surface morphology and release kinetics.

The results showed no significant drug polymer interaction in FTIR studies. Among all the formulations, F4 containing HPMC K100M showed 99.51% drug release in 24 hrs. Amongst the developed mucoadhesive microspheres, F4 formulation containing HPMC K100M exhibited slow and sustained release in a controlled manner and it is a promising formulation for sustained release of Lisinopril.

Keywords: Mucoadhesive Microspheres**,** HPMCK100M, Formulations**,** Evaluations, Sustained release.

Introduction

Mucoadhesive microspheres is one category of microspheres offering advantages of increasing the residence time, efficient absorption, enhanced bioavailability, much more intimate contact with the mucus layer and reduction in frequency of drug administration. Hence, in this study, an effective attempt was made to formulate the mucoadhesive microspheres of lisinopril as a model drug whose half-life less than 8hrs with poor bioavailability of 25% due to first pass metabolism. The drug was chosen with an objective to sustain the drug action and to enhance the bioavailability.

In order to improve the bioavailability, localization of the active component to a specific site, mucoadhesive drug delivery systems have been utilized for the designing of microspheres using mucoadhesive polymers.

Mucoadhesive polymers are water-soluble and water insoluble polymers, which have swellable network joined by cross linking agents. These polymers possess optimal polarity to make sure that they permit sufficient wetting by the mucus and optimal fluidity that permit the mutual adsorption and interpenetration of polymer and mucus.[1-3]. In this study, Lisinopril mucoadhesive microspheres are formulated and evaluated.

Material and Methods

Materials: Lisinopril was obtained as gift sample from Ranbaxy Labs. Ltd. (Haryana, India). Sodium alginate, HPMC K4M, HPMC K100M, Na CMC, carbopol 974P, chitosan, calcium chloride were obtained from Loba Chem Pvt Ltd (Mumbai, India) and all reagents used were of analytical grade.

Pre-formulation studies: Pre-formulation is considered as important phase where researcher characterizes the physical, mechanical and chemical properties and other derived properties of the drug powder to be determined for new drug substance which helps to develop stable, effective and safe dosage forms. Not only for drug, but also they check possible interaction with various excipients.

Organoleptic properties:

- **Colour:** A small quantity of lisinopril was taken in a butter paper and viewed in well- illuminated place.
- **Taste and Odour:** Very less quantity of Lisinopril was used to get the taste with the help of tongue as well as smell to get the odour.

Solubility studies of Lisinopril[4-6]: The solubility of lisinopril was determined in distilled water, 0.1 N HCl, phosphate buffer pH 6.8. and other organic solvents. 1 mg of lisinopril was soluble in 10 mL of distilled water, 0.1 N HCl, phosphate buffer pH 6.8 and organic solvents

Analysis of Lisinopril[7]: 100mg of Lisinopril is accurately weighed and transferred into a 100ml volumetric flask which contains 50ml 0.1 N HCl, buffer pH 6.8 solution separately and the volume is made up to the mark by using buffer solution. From the stock solution, different concentrations of solutions t 10µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL, 60 µg/mL, 70 µg/mL, 80 µg/mL, 100 µg/mL, 120 µg/mL, 150µg/mL and 200 µg/mL are made and absorbance is measured using UV visible spectrophotometer at respective λmax 231nm.

Compatibility study between drug and polymer[8,9]: The FTIR spectra of the drug (alone), polymers (alone) and the drug-polymer (mixture) were recorded by the potassium bromide pellet method. The pellets were scanned over a wave number range of 4000–400 cm^{-1} in a Thermo scientific, FTIR instrument.

Preparation of mucoadhesive microsphere by ionic gelation technique: The ion tropically-gelled microspheres containing lisinopril were prepared using calcium chloride ($CaCl_2$) as cross-linker. Aqueous dispersions of sodium alginate were prepared separately using distilled water by heating at 60°C using magnetic stirrer (Remi Motors, India). On the other hand, polymer aqueous dispersions were prepared separately using distilled water at room temperature using magnetic stirrer. Both the dispersions were well mixed together with stirring for 10 min at 1000rpm using magnetic stirrer to prepare sodium alginate dispersion mixtures containing 400mg different selected polymer concentration in all formulations. Afterwards, required quantity of drug was added to the dispersion mixture maintaining the ratio of drug to polymer 1.25:1 in all formulations.

The final polymer-blend dispersion mixture of alginate-polymer containing drug was homogenized for 20 min at 1000 rpm using a homogenizer (Remi Motors, India) and ultra-sonicated for 5 min for de-bubbling. The resulting dispersion was then added via a 14-gauge needle. The added droplets were retained in the $CaCl_2$ solution for 15 min to complete the curing reaction and to form rigid microspheres. The wet microspheres were collected by decantation and washed two times with distilled water and dried at 40°C for 24h. The prepared dried microspheres containing drug were stored in a desiccator until used.

Characterization of mucoadhesive microspheres

Percent yield: The prepared microspheres are evaluated for percentage yield. The percentage yield is calculated as per equation:

$$\text{Percentage yield} = \frac{\text{Practical mass(microspheres)}}{\text{Theoretical mass(polymer + drug)}} \times 100$$

Particle size analysis: The mucoadhesive microspheres are examined by optical microscope. The freshly prepared suspension of microspheres is examined on an optical microscope and size of the microspheres is measured by using a pre- calibrated ocular micrometer and stage micrometer.[6]

Drug entrapment efficiency:[10-12] Drug loaded microspheres (100 mg) are powdered and transferred into 100 ml volumetric flask dissolved in 10 ml of solvent and the volume is made up with suitable dissolution medium. The resultant dispersion was kept for 24 hrs for complete dissolution and filtered through a 0.45µm membrane filter. The drug entrapment efficiency is determined spectrophotometrically after appropriate dilutions at respective λ max. The drug entrapment efficiency is calculated by the following equation:

$$\text{Entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Determination of drug content in microspheres: Drug loaded microspheres (100 mg) are powdered and transferred into 100 ml volumetric flask dissolved in 10 ml of solvent and the volume is made up with suitable dissolution medium. The drug content is determined spectrophotometrically after appropriate dilutions at respective λmax. The drug content is calculated by the following equation:

$$\text{Drug content} = \frac{\text{Amount of drug in microspheres}}{\text{Amount of microspheres}} \times 100$$

Determination of drug loading in microspheres: The drug loading in the microspheres is estimated by using the formula:

$$L = Qm/Wm \times 100$$

where L= Percentage of drug loading in the microspheres, Wm= Weight of microspheres in grams and Qm = Quantity of drug present in Wm grams of microspheres.

Micromeritic properties: The microspheres are characterized for micromeritic properties such as true density, tapped density, compressibility index and flow properties. The tapped density and compressibility index are determined by tapping method.

Bulk density: True density of microspheres is determined by pouring sample through a glass funnel into a graduated cylinder. The volumes occupied by the microspheres are recorded. True density is calculated as follows:

$$\text{Bulk density} = \frac{\text{Mass}}{\text{Bulk Volume}}$$

Tapped density: Tapped density of microspheres is determined by pouring sample through a glass funnel into a graduated cylinder. The tapped volume occupied by the microspheres is recorded. Tapped density is calculated by using the formula:

$$\text{Tapped density} = \frac{\text{Mass}}{\text{Tapped volume}}$$

Angle of repose: Flow ability of the prepared microspheres is determined by calculating angle of repose by fixed funnel method. A funnel with 10 mm inner diameter of stem is fixed at a height of 2 cm. over the platform. About 10 gm of sample is slowly passed along the wall of the funnel till the tip of the pile is formed and touches the steam of the funnel. A rough circle is drawn around the pile base and the radius of the powder cone is measured. Angle of repose is calculated by using the following formula:

$$\theta = \tan^{-1}(h/r)$$

where θ = Angle of repose, h = Height of the pile and r = Average radius of the powder cone.

Carr's Index: It is also one of the simple methods to evaluate flow property of powder by comparing the bulk density and tapped density. A useful empirical guide is given by the Carr's compressibility.

$$\text{Carr's Index} = 1 - \frac{\text{Bulk Density}}{\text{Tapped Density}} \times 100$$

Swelling index: [13]The swelling index is a property measured to know the behaviour of polymer in physiological solution. It is determined by keeping the microspheres in buffer solution for 24 h and then washed. The swelling index is calculated using formula:

$$\text{Swelling Index(Sw)} = \frac{\text{Wt} - \text{W0}}{\text{W0}} \times 100$$

where Sw is swelling index, W0 is weight of microspheres before swelling and Wt is weight of microspheres after swelling.

***In vitro* wash off test (mucoadhesion test):** The mucoadhesive properties of the microspheres were evaluated by in vitro wash off test. A 4cm x 4cm piece of goat intestinal mucosa was tied onto the paddle bottom of a USP dissolution test apparatus - II using a thread. A weighed amount of microspheres i.e. 100mg were spread onto the wet, rinsed tissue specimen. The dissolution test apparatus was operated such that the tissue specimen was rotated at a speed of 25 rpm in phosphate buffer (pH 7.4).

At the end of 6th hour, the amount of microspheres still adhering onto the tissue was scrapped and weighed. The percentage mucoadhesion of the microspheres was determined using the following formula:

$$\% \text{ Mucoadhesion} = \frac{\text{Number of microspheres adhering to the tissue specimen at time 't'}}{\text{No. of Initial microspheres taken}} \times 100$$

***In vitro* drug release study:** The drug release is studied by using USP type II apparatus at 37 ± 0.5^{0}C and at 100 rpm in phosphate buffer pH6.8. Five ml of the sample solution is withdrawn at predetermined time intervals, filtered, diluted suitably and analyzed spectrophotometrically. Equal amount of the fresh dissolution medium is replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals is calculated using the Lambert-Beer's equation. The result is obtained in triplicate and the average value is reported.[10]

***In vitro* drug release study of selected mucoadhesive microspheres of Lisinopril and marketed conventional tablets:** The *in vitro* drug release values of selected mucoadhesive microspheres of Lisinopril were compared with the marketed conventional tablet.

Surface topography by Scanning Electron: The surface morphology and structure are visualized by scanning electron microscopy (SEM)[10].

Drug release pattern from microspheres: In order to understand the mechanism and kinetics of drug release, the results of the *in vitro* drug release study are fitted with various kinetic equations like zero order, first order and Higuchi model.

1. Zero – order model: Drug dissolution from dosage forms that do not disaggregate and release the drug slowly, can be represented by the equation:

$$Q_t = Q_0 + K_0 t$$

where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution, K_0 is the zero order release constant and t is time in hours.

2. First order model: The release of the drug which followed first order kinetics can be expressed by the equation:

$$\text{Log } Qt = \log Q_0 + Kt / 2.303$$

where Q0 is the initial concentration of drug, Qt is cumulative amount of drug released per unit surface area and k is the first order rate constant and t is the time.

3. Higuchi model: Higuchi model describes the drug release from several typed of matrices initially conceived for planar systems, then extended to different geometrics and porous systems. It was derived by Higuchi in 1961. For higuchi release kinetics equation is:

$$Q = KH\ t_0$$

where Q is amount of drug released per unit surface area of the dosage form and KH is Higuchi release rate constant.

4. Korsmeyer – Peppas model: Koresmeyer derived a simple relationship which describes drug release from a polymeric system. To find out the mechanism of drug release, first 60% drug release data was fitted in Koresmeyer – Peppas model equation:

$$\frac{Mt}{M\alpha} = K\text{tn}$$

where $\frac{Mt}{M\alpha}$ is the fractional drug release in time't' and K= constant incorporating structural and geometric characteristics of controlled release device.

Results and Discussion

Pre Formulation Studies: Preformulation studies were performed for the drug to rule out the interaction with the polymers used for formulating mucoadhesive microspheres.

The various preformulation parameters like organoleptic characteristics, analysis of API and compatibility studies were studied and results were shown below.

Organoleptic properties:

- **Colour:** White to off white crystalline powder.
- **Taste and odor:** Bitter taste and Odorless.

Standard curve of Lisinopril in pH 0.1NHCl: The UV spectrophotometric method of analysis showed linearity range from 0-200 µg/ml for Lisinopril in phosphate buffer pH 0.1NHCl at 231nm wavelength. The regression coefficient (R^2) of lisinopril in the solution was found to be 0.999 and was within the limits as shown in table 1 and in fig. 1.

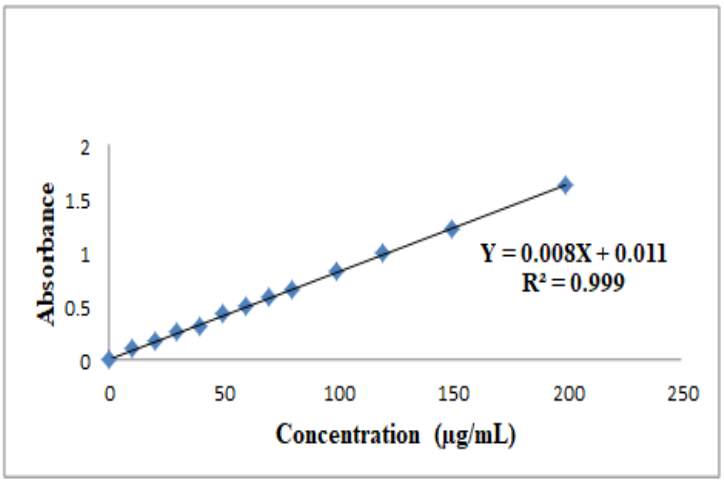


Figure 1: Calibration Curve of Lisinopril in 0.1 N HCl at λmax 231 nm

Table 1
Absorbance of Lisinopril In pH 0.1NHCl

S.N.	Concentration (µg/mL)	Absorbance at λ_{max} 231nm
1	0	0
2	10	0.1
3	20	0.177
4	30	0.255
5	40	0.310
6	50	0.427
7	60	0.506
8	70	0.579
9	80	0.661
10	100	0.820
11	120	0.989
12	150	1.225
13	200	1.622

Table 2
Absorbance in phosphate buffer pH 6.8

S.N.	Concentration (µg/mL)	Absorbance at λ_{max} 231nm
1	0	0
2	10	0.09
3	20	0.182
4	30	0.290
5	40	0.408
6	50	0.503
7	60	0.618
8	70	0.690
9	80	0.783
`10	90	0.915
11	100	1.03

Compatibility study between drug and polymer by FTIR: The FTIR spectrophotometric method reveals that there was no interaction between drugs and its combination with sodium alginate and other mucoadhesive polymers such as chitosan, carbopol974P, HPMC and sodium CMC. IR spectra of pure drug showed the major peaks at wave number which were compared with the IR spectra of physical mixtures of pure drugs with combination of sodium alginate with mucoadhesive polymers. It was observed from the spectra of pure drug and their physical mixture: neither remarkable shift in the wave number of the peaks nor the intensity of peaks of drug between graphs which proved that there was no interaction between the drug and with their physical mixtures with other mucoadhesive polymers.

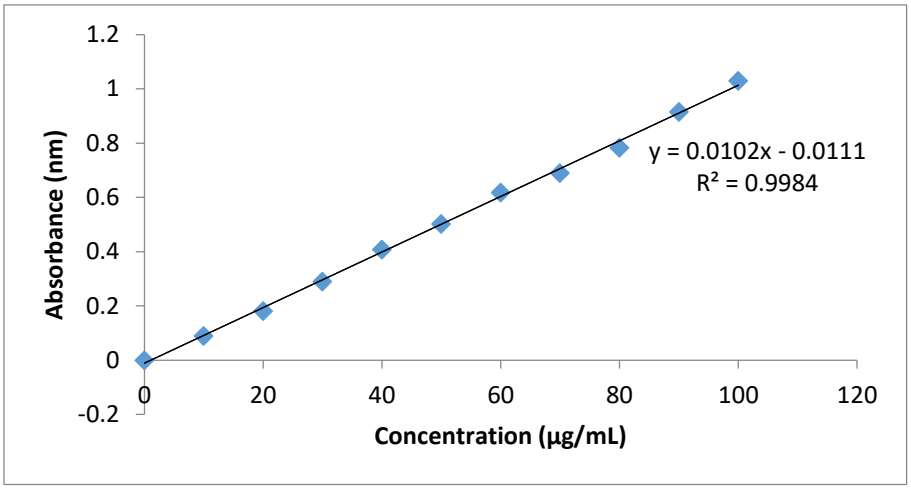


Figure 2: Calibration curve of Lisinopril in phosphate buffer pH 6.8

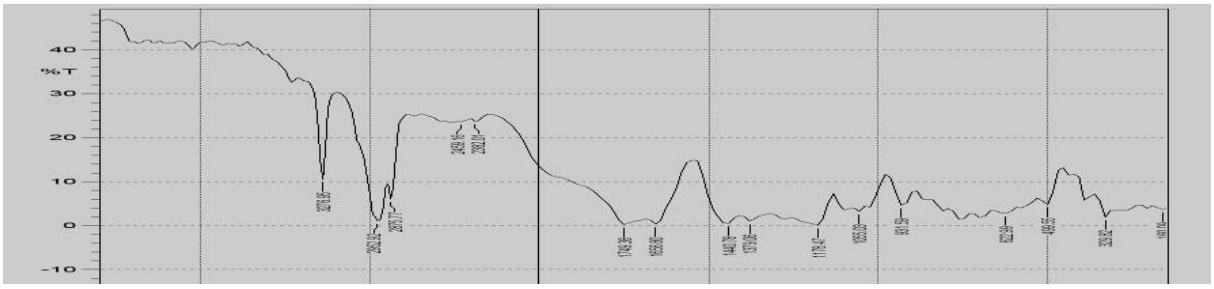


Figure 3: FTIR spectra of Lisinopril

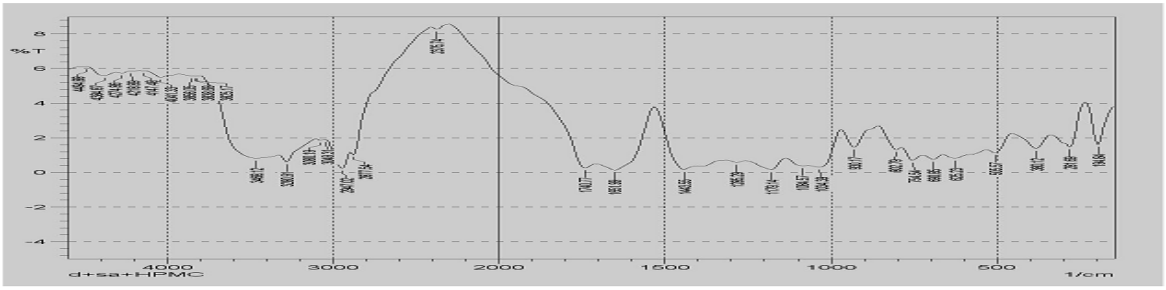


Figure 4: FT-IR spectra of Sod. Alginate

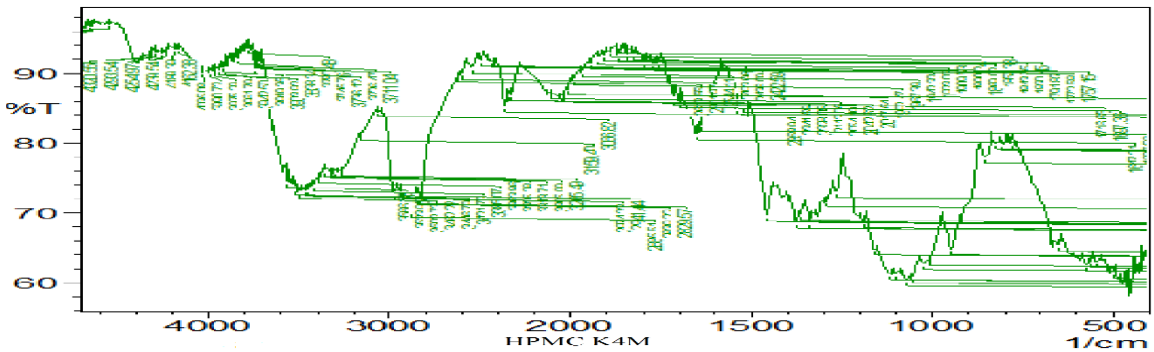


Figure 5: FT-IR spectra of HPMC K4M

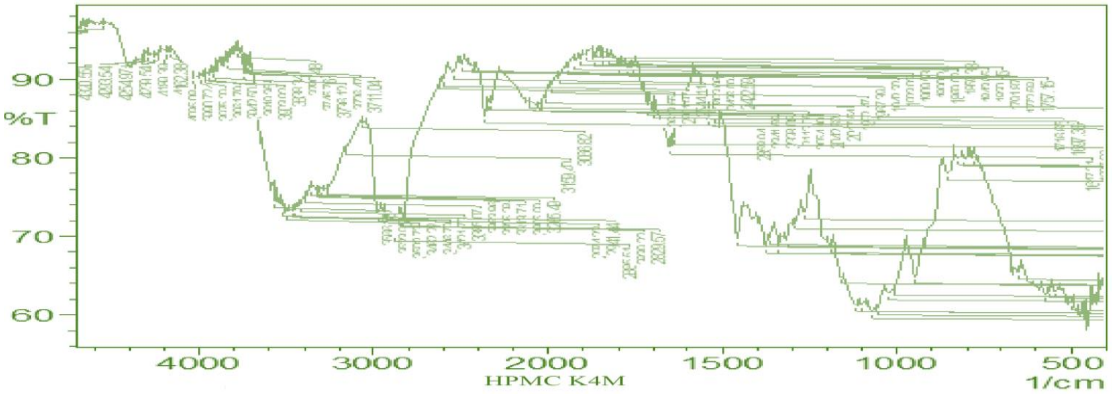


Figure 6: FT-IR spectra of HPMC K100M

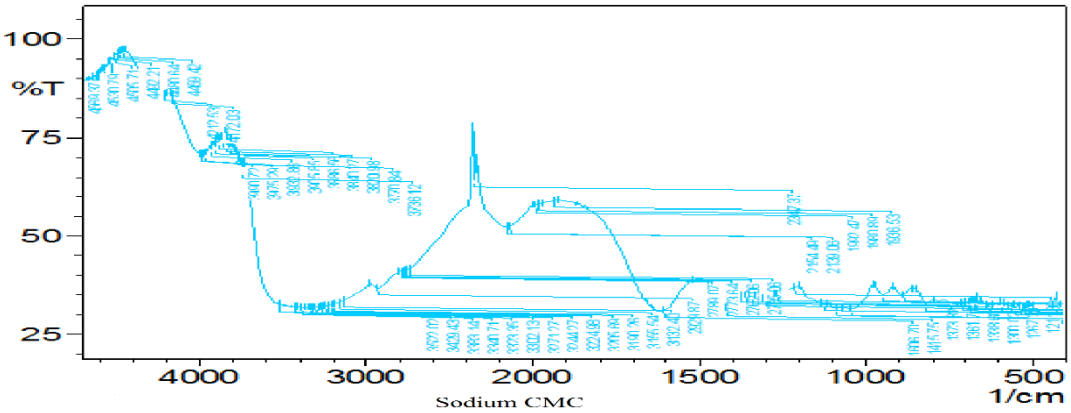


Figure 7: FT-IR spectra of Sodium CMC

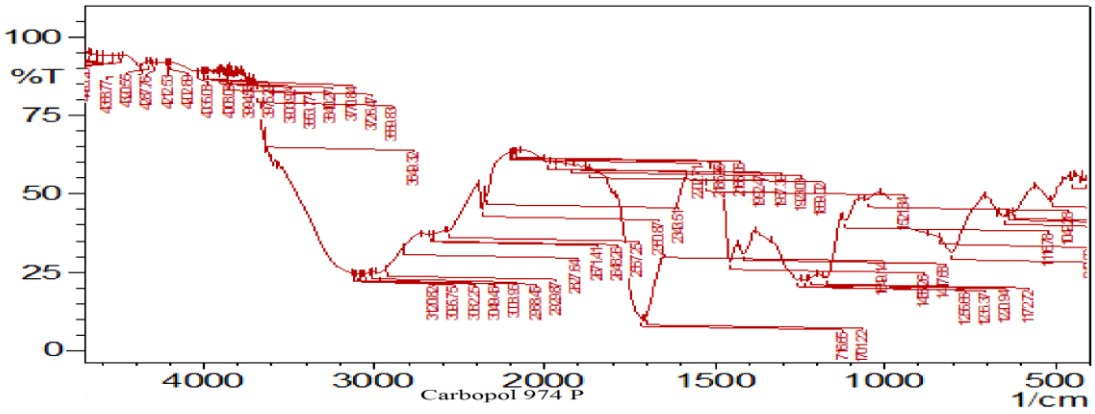


Figure 8: FT-IR spectra of Carbopol 974 P

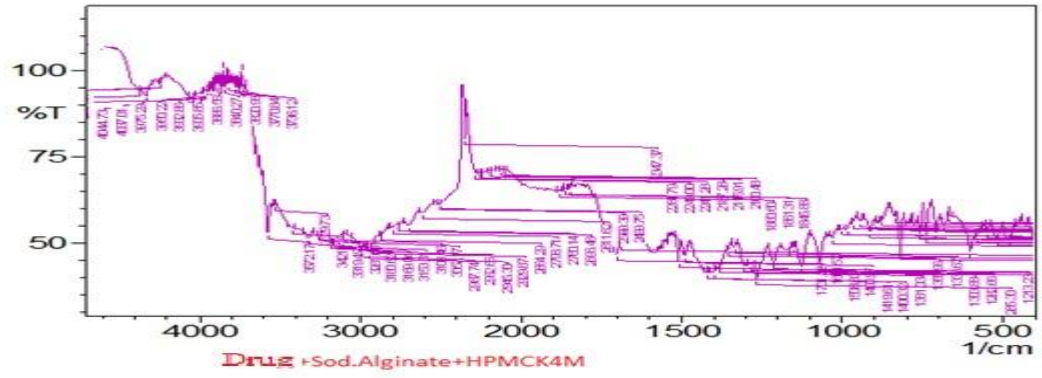


Figure 9: FT-IR spectra of Drug + sodium alginate + HPMCK4M

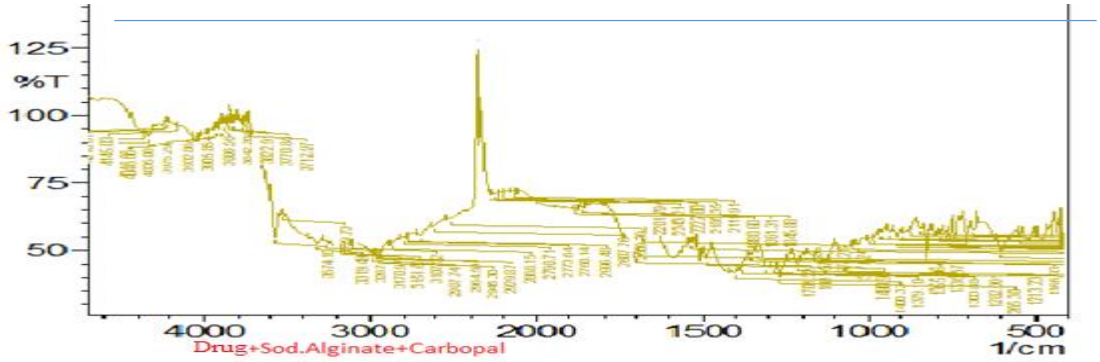


Figure 10: FT-IR spectra of Drug + sodium alginate + Carbopol 974 P

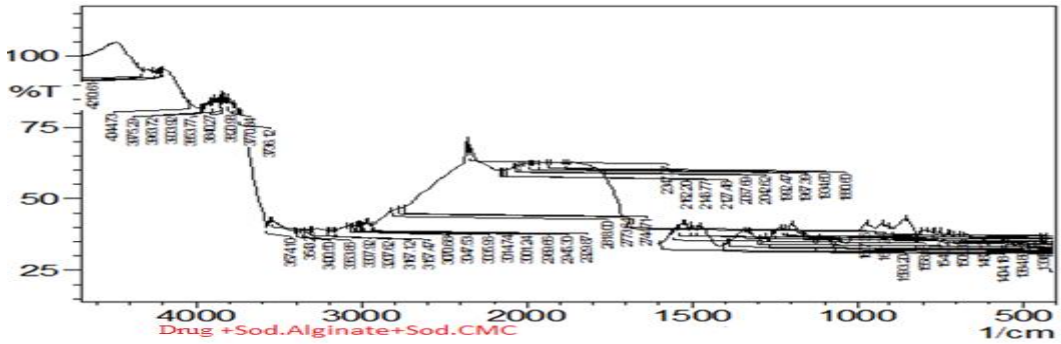


Figure 11: FT-IR spectra of physical mixture of Lisinopril + Na Alginate+ sodium CMC

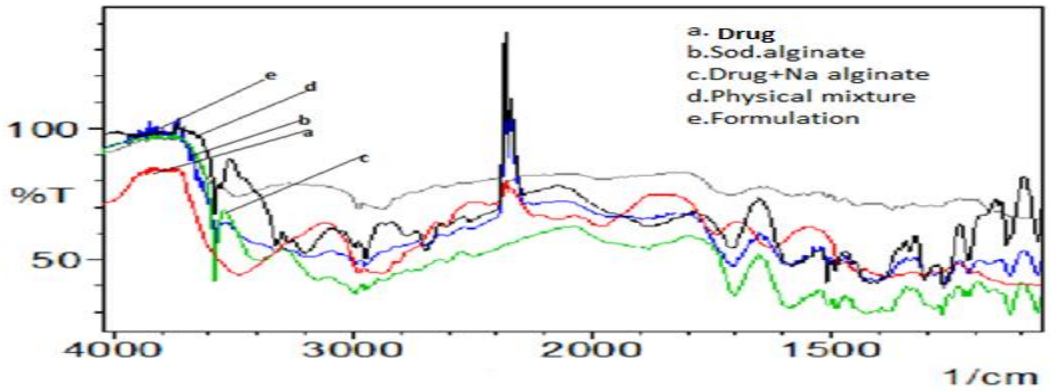


Figure 12: FT-IR spectra of pure drug (a), Na alginate (b), physical mixture of drug+ Na alginate (c), physical mixture of drug+ Na alginate + carbopol 974 P (d) and selected formulation (e)

Figure 13: Preparation of mucoadhesive microspheres

Table 3
Formulation of Mucoadhesive Microspheres

Ingredients	Formulation code					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Lisinopril(mg)	500	500	500	500	500	500
Sodium Alginate(mg)	1600	1600	1600	1600	1600	1600
Carbopol 974 P(mg)	-	400	-	-	-	-
HPMC K4M(mg)	-	-	400	-	-	-
HPMC K 100M(mg)	-	-	-	400	-	-
Na CMC(mg)	-	-	-		400	-
Chitosan(mg)	-	-	-	-	-	400
Calcium chloride(gm)	10	10	10	10	10	10
Distilled water(ml)	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient	Quantity sufficient

Table 4
Characterization of Lisinopril mucoadhesive microspheres

S.N.	Formulation code	Percentage yield (%)	Mean particle size (µm)	Drug Content (mg)	Drug entrapment efficiency (%)	Drug loading Capacity (%)
1	F1	33.65±0.02	670±0.03	55.87±0.01	58.28±0.02	35.74 ±1.32
2	F2	48.35±0.03	242.5±0.01	60.93±0.02	69.73±0.03	23.09 ±1.54
3	F3	44.51±0.04	432.4±0.02	70.26±0.03	51.39±0.04	16.61 ±1.25
4	F4	47.45±0.05	540±0.04	68.43±0.04	56.14±0.01	43.59 ±1.06
5	F5	45.36±0.05	642.5±0.03	81.23±0.05	66.49±0.02	28.61 ±1.15
6	F6	41.87±0.01	1007.5±0.02	93.86±0.06	59.87±0.03	20.45 ±1.39
7						38.37 ±1.71
8						24.44 ±1.62
9						17.55 ±1.34

Mean±standard deviation (n=3)

Particle Size Analysis: All the formulations were subjected to particle size analysis by optical microscopic method and the results are tabulated in the table 3. From the study, it was observed that the mean particle size significantly increased with increase in the polymer concentration. The small particle size was observed with mucoadhesive microspheres of lisinopril with F2 when compared with the other polymers will may be due to the formation of unstable nuclei.

Drug content, Drug Entrapment Efficiency and Drug loading of mucoadhesive microspheres of Lisinopril: The results of drug content, drug entrapment efficiency and drug loading of mucoadhesive microspheres of lisinopril are enlisted in table 3. The percentage of drug content, drug entrapment efficiency and drug loading for all the formulations Drug content ranged from 55.87 to 93.86 and drug entrapment efficiency from 51.39 to 45.95 respectively. 66.46 %to 87.18% and 16.61% to 43.59% respectively. From the study, it was observed that an increase in the concentration of polymer in all the formulations results in decrease in the percentage of drug content, drug entrapment efficiency and drug loading. The reason may be due to loss of drug during washing, adherence of drug on the walls of the beaker and stirrer, addition of insufficient amount of cross linking agent and duration of stirring. Higher percentage of drug content, drug entrapment efficiency and drug loading was observed with F2 mucoadhesive microspheres of lisinopril which may be due to its hydrophilic nature.

Micromeritic properties: The micromeritic properties such as bulk density, tapped density, Hausner's ratio, compressibility index and angle of repose were carried out and the results are shown in the table 4. From the study, it was observed that the bulk and tapped density, Hausner's ratio, Carr's index and angle of repose of all the preparations were within the range. Further, it was observed that the values of bulk density and tapped density in all the formulations were within the limit. An increase in the Hausner's ratio was observed with mucoadhesive microspheres of Lisinopril and polymer than with the other polymers which may be due to its hydrophilic nature. The low values of angle of repose and Carr's index was observed in all the formulations of mucoadhesive microspheres of lisinopril which may be due to more fineness of the formulation and mucoadhesive nature of the polymer.

Swelling index: The swelling index demonstrated the ability of the mucoadhesive microspheres to get swell at the absorbing surface by absorbing fluid at the site of absorption. It is also used to check the water absorption property of the polymers. The swelling index for all the formulation was

calculated and results are shown in table 5. From the study, it was observed that the swelling index value was in the order of F4>F3>F5>F2>F6>F1, indicating that an increase in the concentration of polymer produced an increase in the swelling property of microspheres. Among the polymers, swelling index value was high with HPMCK4M formulations than with the other polymers. It may be due to more water absorbing nature of sodium alginate which absorbs water within its porous structure.

SEM analysis: Mean±standard deviation (n=3) was calculated using Scanning electron microscopy (SEM) and the morphological analysis of the mucoadhesive microspheres of lisinopril was carried out as shown in fig. 7. The SEM photographic result reveals that the microspheres were almost spherical in shape having rough surface.

***In-vitro* wash off test (mucoadhesion test):** *In vitro* wash off test is used to determine the mucoadhesion behaviour of the polymers. The test was carried out for all the formulations and the results are enlisted in table 5. From the result, it was found that the percentage of mucoadhesion for all the formulations was in the range of 22 to 96% showing good mucoadhesion nature and the values also indicated that an increase in the concentration of polymer resulted in an increase in the percentage of mucoadhesion of microspheres. The microspheres consisting of sodium alginate in combination with various mucoadhesive polymers exhibited good mucoadhesive properties as observed in the *in vitro* wash-off test when compared to non mucoadhesive polymer.

The result of the wash-off test indicated that formulation F4 showed maximum mucoadhesion. This might be to due to high molecular weight, linear, unbranched structure and viscosity of HPMCK4M as on enhancing agent in selected medium.

Table 5
Micromeritic properties of Lisinopril mucoadhesive microspheres

Formulation code	Hausner's ratio	Carr's index (%)	Angle of repose (°)
F0	2.36	25.69	36.29
F1	1.1	12.45	16.20
F2	1.02	13.23	15.52
F3	1.04	8.42	20.30
F4	1.02	14.65	16.25
F5	1.01	12.26	18.12
F6	1.3	10.12	22.20

Mean±standard deviation (n=3)

Table 6
Swelling index and percentage mucoadhesion of Lisinopril mucoadhesive microspheres

Formulation code	% Swelling Index*						
	1 h	2 h	3 h	4 h	6 h	18 h	24 h
F1	35	40	45	47	50	62	73
F2	50	53	55	61	63	70	80
F3	51	53	55	57	61	76	88
F4	55	57	59	61	65	70	90
F5	32	35	44	48	53	44	82
F6	52	55	59	61	62	64	76

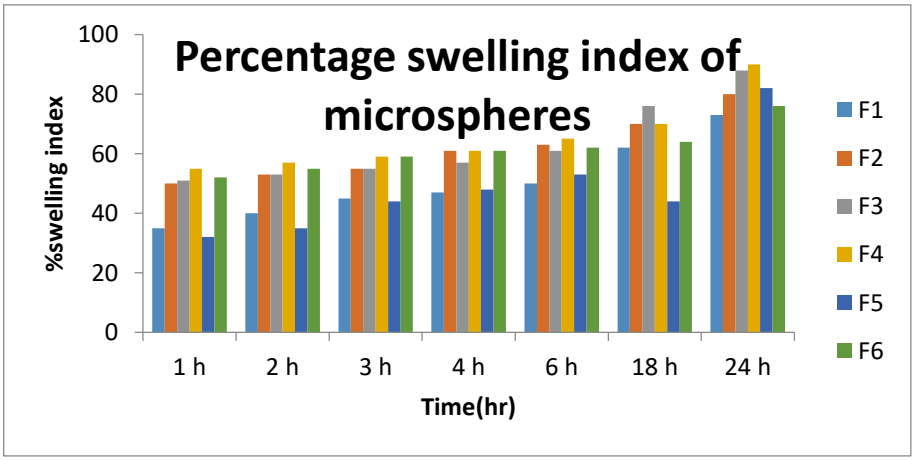


Figure 14: Bar diagram showing swelling index of the microspheres

Figure 15: Schematic pictures for swelling of microspheres

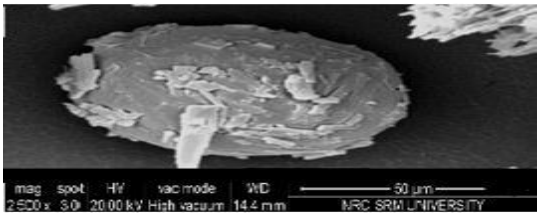


Figure 16: Scanning electron microscopy of mucoadhesive microspheres of Lisinopril

Table 7
***In vitro* wash-off test of the mucoadhesive microsphere in phosphate buffer pH 6.8**

Formulation Code	% Microspheres adhering to tissue at various time intervals*							
	0.5 h	1 h	2 h	3h	4h	5h	6h	F cal= 35.045 Fcrit= 4.383 at P<0.001
F1	80	78	69	62	39	17	-	
F2	84	78	83	69	48	23	20	
F3	85	89	84	72	49	26	22	
F4	96	90	87	78	55	39	30	
F5	87	81	76	73	44	30	24	
F6	80	70	65	60	48	21	18	

*Each reading is an average of three determinations (Mean ± S.D.) (n=3) n=No of observation.

The one way ANOVA without replication was applied to percentage mucoadhesion to find out the significant effect of different polymers with different viscosity grades after 6 h at P<0.001 level. Analysis of Variance (ANOVA) showed a highly significant difference in percentage mucoadhesion in different polymers with different viscosity grades in 6 h as $F_{cal.}$ Value > $F_{crit.}$(35.045> 4.677).

***In vitro* drug release studies:** The percentage cumulative drug release was calculated and the values are shown in table 6 and fig. 8. At 8th hr, the percentage cumulative drug release for F1, F2, F3, F4, F5 and F6 formulations was found to be 78.96%, 79.56%, 78.56%, 44.26%, 78.53%, 78.72% respectively.

Among all the formulations, HPMCK100M microspheres showed increased and sustained drug release. Among all formulations, F4 showed increased amount of percentage drug release due to increased drug polymer ratio and the mechanism of drug release is due to swelling and erosion.

From the *in vitro* dissolution data and plot, it is seen that after 24 hr study, formulation F4(HPMCK100M) showed better drug release retardation. The drug release in case of HPMCK100M might be 94%. Viscosity of the mucoadhesive polymer has role in both bioadhesion and sustained action. Hence, among all the formulations, F4 was chosen for further study due to its increased drug release.

***In vitro* drug release of mucoadhesive microspheres of Lisinopril (F4) with marketed conventional tablets:** The percentage cumulative drug release for mucoadhesive microspheres of lisinopril and marketed conventional tablets values are shown in fig. 9. The percentage cumulative drug release for the marketed tablets was 99% at 12 hr while F4 formulation showed 99.51% drug release at 24th hr.

Kinetics of Drug release: The kinetics of *in vitro* drug release for mucoadhesive microspheres of lisinopril (SF3) was determined by applying the drug released data to various kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas. The result obtained is represented in table 7 and in fig. 10. In the present study, the release profile of the F4 formulation follows Korsmeyar-Peppas equation with the 'R^2' value-0.984. Further the 'n' values of Korsmeyar-Peppas was 0.90. Therefore, the most probable mechanism of drug release was super case II transport.

Conclusion

Varying degrees of sustained release were obtained from lisinopril mucoadhesive microspheres prepared from sodium alginate, carbopol 974, HPMC K4M, HPMC K 100M and

chitosan by ionic gelation technique. Among all the formulations developed, sodium alginate mucoadhesive microspheres showed the most drug sustaining and it is promising for sustained release of lisinopril. Hence, it was conducted that lisinopril is a suitable drug candidate to formulate a mucoadhesive drug relative system (microspheres) for better therapeutic treatment of hypertension by reducing frequency of drug optimization.

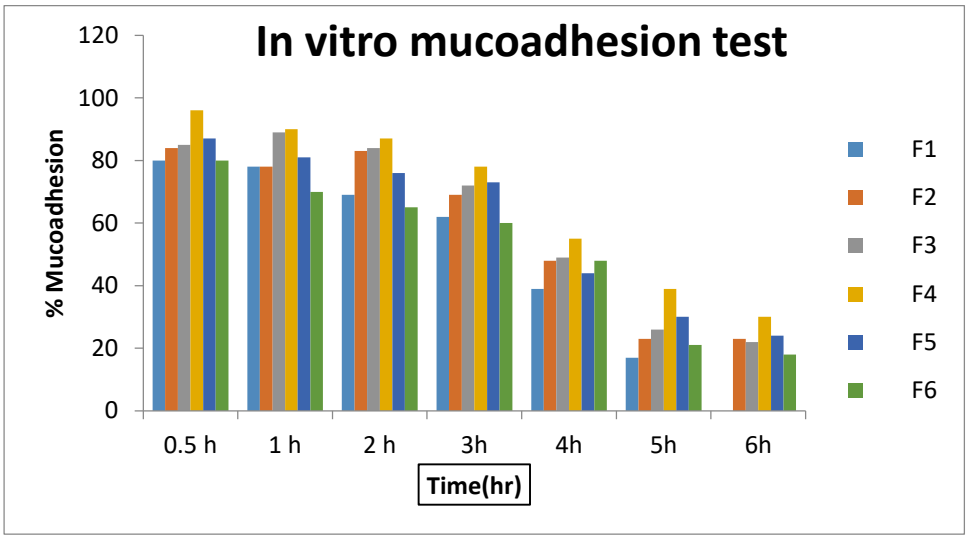
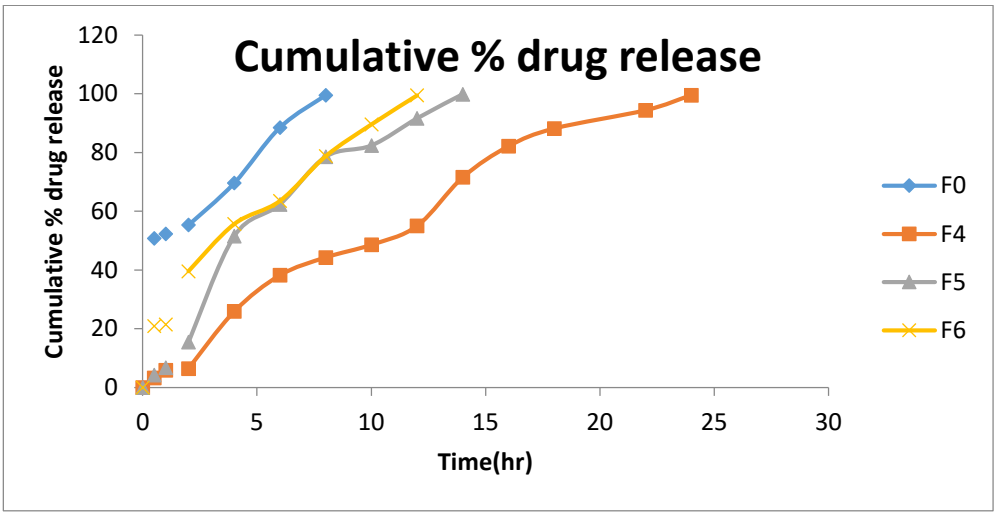


Figure 17: Bar diagram showing *in vitro* wash-off test of the mucoadhesive microspheres in phosphate buffer pH 6.8



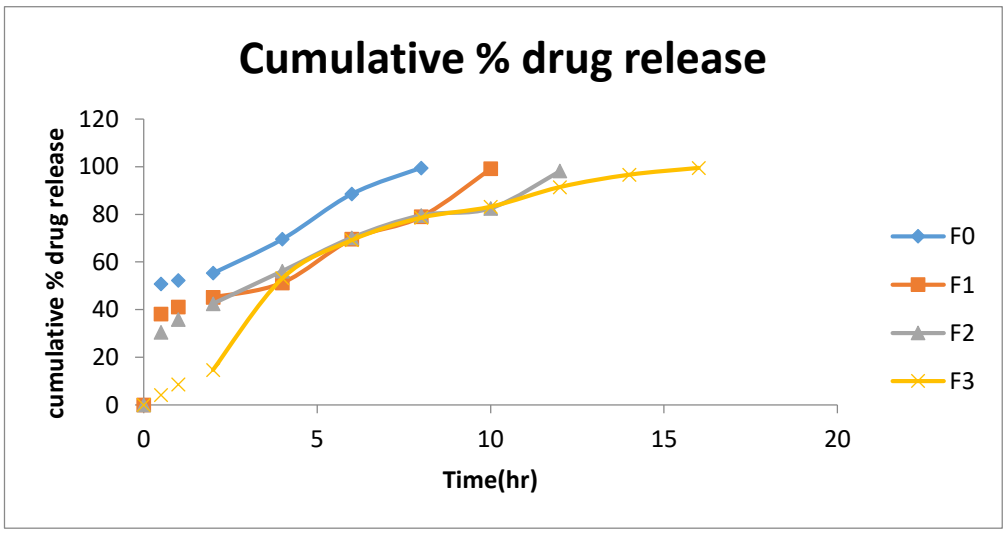


Figure 18: Cumulative percentage drug release for mucoadhesive microspheres of Lisinopril (F0-F6)

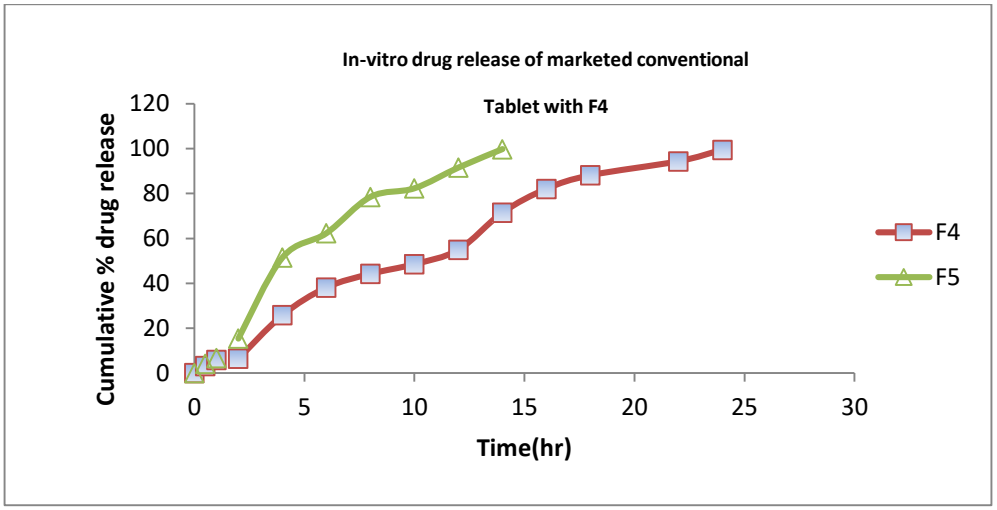


Figure 19: *In vitro* drug release of mucoadhesive microspheres of Lisinopril (F4) with marketed conventional tablet

Table 8
Cumulative percentage drug release for mucoadhesive microspheres of Lisinopril

Time (hr)	Formulation Code						
	Pure drug (F0)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0	0
0.5	50.73	38.15	30.50	4.16	3.21	4.23	20.91
1	52.32	41.14	35.82	8.53	5.91	6.71	21.44
2	55.32	45.16	42.46	14.68	6.44	15.44	39.56
4	69.58	51.23	56.16	53.15	25.86	51.51	55.62
6	88.51	69.54	70.16	69.14	38.19	62.36	63.51
8	99.46	78.96	79.56	78.56	44.26	78.53	78.72
10	-	99.12	82.51	83.16	48.56	82.36	89.56
12	-	-	98.16	91.42	54.98	91.59	99.46
14	-	-	-	96.59	71.53	99.81	-
16	-	-	-	99.46	82.12	-	-
18	-	-	-	-	88.15	-	-
22	-	-	-	-	94.38	-	-
24	-	-	-		99.51	-	-

Figure 20: Zero order plot for formulations F0 & F4

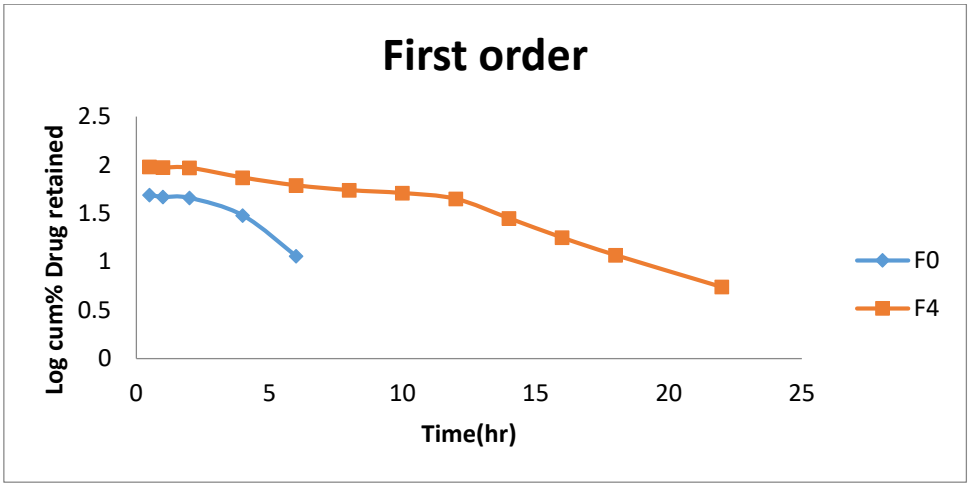


Figure 21: First order plot for formulations F0 & F4

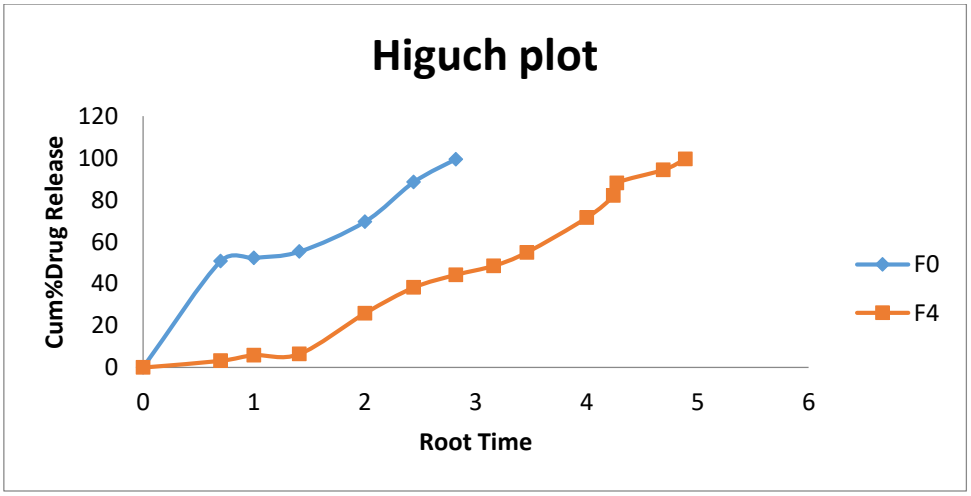


Figure 22: Higuchi plot for formulations F0 & F4

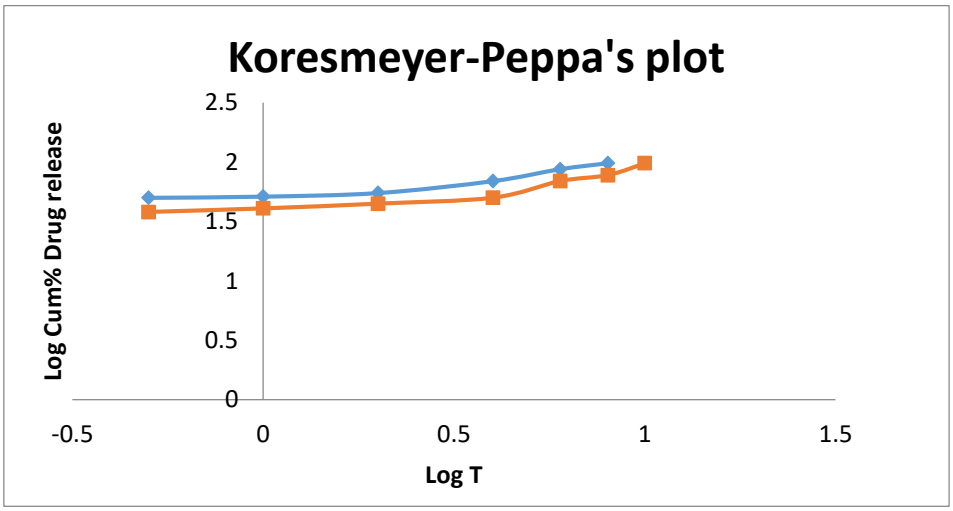


Figure 23: Koresmeyer peppa's plot for formulations F0 & F4

Table 9
Drug release kinetics data for mucoadhesive microspheres of Lisinopril (F4)

Formulation Code	Zero-order R^2	First-order R^2	Higuchi diffusion kinetics R^2	Korsmeyer-peppa's R^2n
F4	0.97	0.96	0.92	0.90

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(Received 18th September 2024, accepted 20th November 2024)