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PREPARATION AND EVALUATION OF GASTRORETENTIVE MUCOADHESIVE MICROSPHERES OF CAPTOPRIL

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ABSTRACT

The aim of the present investigation was to evaluate the potential use of mucoadhesive microspheres for gastroretentive delivery of captopril. Chitosan was used as mucoadhesive polymers. The release of drug was prolonged to 12 h (94.61±0.939) when incorporated into mucoadhesive microspheres. The batches were prepared by using different volumes of the cross-linking agent, and the cross linking time was also varied. From the resulting batches, the volume of the cross linking agent was optimized to 6 ml. and the cross-linking time was optimized to 2 hours, and the polymer and drug concentration was varied. FTIR shows that there is no interaction with. Pure drug, pure chitosan and physical mixture while taken by preparing KBr pellets (Disk method). From the entrapment and dissolution study, it was concluded that batch CM-4 showed sustained release for 12 hrs. The batch was optimized for the sustained release microspheres of Captopril by using Chitosan. Overall, the result indicated prolonged delivery with significant improvement in oral bioavailability of captopril from mucoadhesive microspheres due to enhanced retention in the upper GI tract.

Key Words:- Captopril, Mucoadhesion, Oral drug delivery systems, Microspheres, Chitosan.

INTRODUCTION

Captopril is an orally active inhibitor of angiotensin converting enzyme and it is widely used in the treatment of hypertension and congestive cardiac failure. The bioavailability of captopril is approximately 65 % have relatively short half-life of 3 hours and requires frequent administration of dose 25 – 50 mg, 2-3 times daily [1]. Hence it is necessary to develop sustained release formulation to overcome this draw back. Studies showed that, prolonged inhibition of ACE activity of captopril could be achieved by control release dosage form, using oily matrix formulation filled in gelatin capsules [2]. Because of oily vehicles gastric emptying time was delayed and decreased GI motility, thereby retaining the drug for longer period of time at the site of absorption. Captopril is freely water soluble drug and has site specific absorption from GIT and on other hand, the drug is unstable in the alkaline pH of the intestine, whereas stable in acidic pH and specifically absorbed from stomach. Based on the above reasons there is a clear need to localize the developed formulation at the target area of the GIT [3].

Reacting to chitosan with controlled amounts of multivalent anion results in cross linking between chitosan molecules. The cross linking may be achieved in acidic, neutral or basic environments depending on the method applied. This cross linking has been extensively used for the preparation of chitosan microspheres. Many factors affect the entrapment efficiency of the drugs in the chitosan microspheres, e.g. nature of the drug, chitosan concentration, drug polymer ratio, stirring speed, etc [4,5]. Generally a low concentration of chitosan shows low encapsulation efficiency. However, at higher concentrations, chitosan forms highly viscous solutions, which are difficult to process.

MATERIALS AND METHODS

Materials

Captopril Obtained as a gift sample from Panchasheel Organics Ltd, Indore. Chitosan Obtained as a gift sample from Mahtani Chitosan Pvt. Ltd., Veraval, Heavy liquid paraffin (Loba Chemic Pvt. Ltd.), Light liquid Paraffin (E. Merck India Ltd.), Glutaraldehyde (Loba Chemic Pvt. Ltd.), Hexane (E. Merck India Ltd.), Dioctyl sodium sulphosuccinate (Loba Chemic Pvt. Ltd.).

Methods

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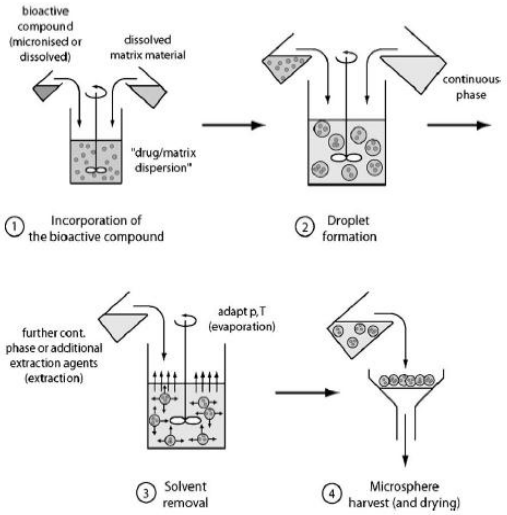
Preparation of Microspheres

Chitosan 4% w/v solution was prepared in 5% v/v acetic acid in which Captopril (100 mg) was dissolved. This was then dispersed in 150ml (1:1) heavy and light liquid paraffin containing dioctyl sodium sulphosuccinate (0.2% w/v). While dispersing the stirring rate was kept constant at 1000 rpm. Glutaraldehyde was then added. Initially, 2ml. of aqueous 25% glutaraldehyde was added to the liquid paraffin before adding the drug polymer dispersion. Then after 30 minutes and 60 minutes, 2ml each of 25 % glutaraldehyde were added and stirring was continued for the total of 2 hrs. The liquid paraffin was decanted; the microspheres were filtered, washed with n- hexane to remove traces of oil and ice then with water to remove excess of glutaraldehyde. The collected microspheres were air dried [6-10].

Fig 1. Four important steps in microsphere in preparation



Formulation of Batches

The batches were prepared by using different volumes of the cross-linking agent, and the cross linking time was also varied. From the resulting batches, the volume of the cross-linking agent was optimized to 6 ml and the cross-linking time was optimized to 2 hours and the polymer and drug concentration was varied [11-14] as follows.

Table 1. Formulations were prepared by using different volumes of polymers

Formulation Code	Drug(mg)	Polymer Chitosan (mg)
CM-1	100	100
CM-2	100	200
CM-3	100	300
CM-4	100	400
CM-5	100	500
CM-6	100	600

RESULT & DISCUSSION

The procured sample of Captopril was tested for its identification. The drug sample showed compliance with the data given in British pharmacopoeia, which reflected its quality and purity. The polymer chitosan and all excipients provided by the supplier confirmed by their identification test official in BP and EP. All the excipients showed results in compliance with standard specifications [15-27].

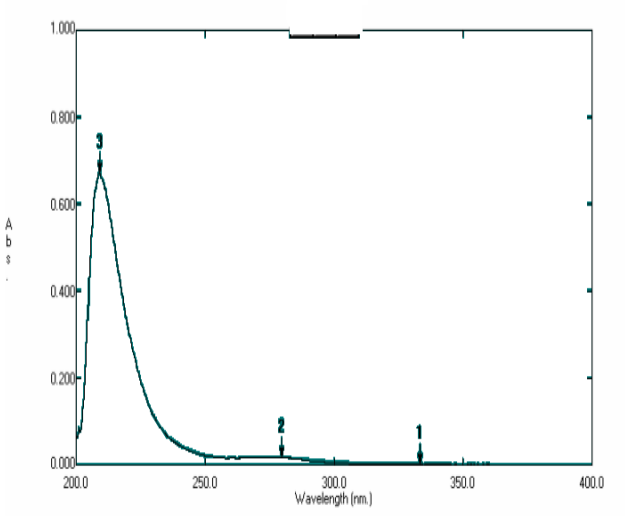
PREFORMULATION STUDY

A) Melting Point

The melting point of the drug sample was determined by capillary method and found to be 104-110 ^{o}C, which compiles with melting point reported in B.P. (106^{o}C).

B) UV Scanning

Fig 2. Scanning of Captopril in Simulated gastric fluid



C) Solubility

Captopril (0. 5g) was dissolved in 25 ml of water and shaken vigorously. A clear and colorless solution was obtained, which compiles with that described in IP/BP.

D) pH

Captopril (0. 5g) was dissolved in 25 ml of water and shaken vigorously and the pH was recorded using digital pH Meter, Model no. 335. The pH of the resultant solution was found to be between 2.0 to 2.6, which compiles with the standards given in IP/BP.

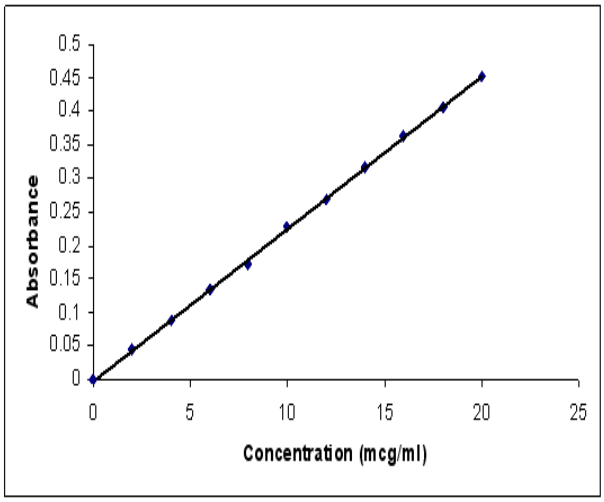
Standard Calibration Curve of Captopril

The standard calibration curve of drug in simulated gastric fluid pH 1.2 is as depicted in Fig. No. 10. Standard calibration curve followed Beer-Lambert's law in the range y=0.0227x-0.0024 with the highcorrelation coefficients of (R^2) 0.9996.

Table 2. Standard Calibration Curve of Captopril in Simulated Gastric Fluid (pH 1.2)

S.No	Concentration	Absorbance
1	0	0
2	1	0.045±0.001
3	2	0.089±0.002
4	3	0.134±0.009
5	4	0.173±0.002
6	5	0.228±0.005
7	6	0.269±0.004
8	7	0.318±0.011
9	8	0.363±0.007
10	9	0.406±0.004
11	10	0.452±0.003

Fig. 3. Standard calibration curve Captopril in Simulated gastric fluid (pH 1.2)



FT-IR

The FT-IR spectra of pure drug, pure chitosan and physical mixture were taken by preparing KBr pellets (Disk method).

Scanning Range: 4000 – 500 cm^{-1}

Observation: The observations are as shown in Fig. 4.

EVALUATION OF MICROSPHERES

Drug Content

Accurately weighed microspheres (100mg) were crushed in glass mortar-pestle, and the powdered microspheres were suspended in 20ml of simulated gastric fluid (pH 1.2).After 24 hours the solution was filtered using Wattman filter paper. Of this 1 ml of the filtrate was taken and diluted to 10 ml. The absorbance was measured at 238 nm.

Dissolution Studies

The dissolution studies were performed using Dissolution Apparatus USP Type II (Rotating Paddle Model no. DA-3).using simulated gastric fluid (pH1. 2) – 900 mL hours and at 100 rpm at temp, 37 ºC ± 0. 50C. During dissolution study 5 mL aliquot was withdrawn at different time intervals of 1 to 12 hrs and same was replaced with equal volume of a fresh medium. The withdrawn samples were filtered through Whatman filter paper no.42 and absorbances were measured at 238 nm for simulated gastric fluid. Cumulative percent drug dissolved was found out at each time interval and graph was plotted between cumulative % drug dissolved and time in minutes, shown in table No. 3-8 and fig. No. 5-10.

Table 3. Percentage drug content of prepared batches

Sr. No.	Batch	%drug content
1	CM-1	50.206
2	CM-2	63.034
3	CM-3	89.113
4	CM-4	95.621
5	CM-5	90.346
6	CM-6	91.773

Table 4. Cumulative percent drug dissolved of batch CM-1

S.No	Time in hrs	% cumulative drug release
1	0	0
2	1	26.73±1.585
3	2	42.11±1.866
4	3	59.48±1.506
5	4	70.09±2.021
6	5	81.22±1.557
7	6	90.69±2.234

Table 5. Cumulative percent drug dissolved of batch CM-2

S.No	Time in hrs	% cumulative drug release
1	0	0
2	1	21.97±1.519
3	2	36.13±0.628
4	3	49.39±1.959
5	4	57.16±1.004
6	5	65.55±1.687
7	6	78.48±2.098
8	7	89.07±2.180
9	8	91.94±0.575

Table 6. Cumulative percent drug dissolved of batch CM-3

S.No	Time in hrs	% cumulative drug release
1	0	0
2	1	20.41±1.820
3	2	32.94±2.030
4	3	46.37±2.373
5	4	54.08±0.252
6	5	70.29±0.765
7	6	77.14±0.434
8	7	85.69±1.133
9	8	93.04±1.125

Table 7. Cumulative percent drug dissolved of batch CM-4

S.No	Time in hrs	% cumulative drug release
1	0	0
2	1	17.89±1.729
3	2	22.26±1.585
4	3	31.37±1.620
5	4	39.48±2.184
6	5	43.11±2.062
7	6	50.62±1.176
8	7	68.91±2.024
9	8	72.00±1.688
10	9	78.65±1.462
11	10	82.19±1.109
12	11	89.93±1.582
13	12	94.61±0.939

Table 8. Cumulative percent drug dissolved of batch CM-5

S.No	Time in hrs	% cumulative drug release
1	0	0
2	1	16.41±1.083
3	2	21.93±1.100
4	3	28.69±1.109
5	4	36.04±0.324
6	5	45.33±1.127
7	6	57.72±0.596
8	7	62.88±0.425
9	8	79.09±0.699
10	9	88.95±1.483
11	10	92.46±0.806

Table 9. Cumulative percent drug dissolved of batch CM-6

S.No	Time in hrs	% cumulative drug release
1	0	0
2	1	18.12±0.751
3	2	23.46±1.110
4	3	30.77±1.059
5	4	41.69±1.339
6	5	56.99±0.805
7	6	63.24±1.152
8	7	78.56±0.739
9	8	87.81±1.513
10	9	95.06±0.812

Determination of particle size

The particle size was determined using the stage micrometer. The diameters of about 275 microspheres were measured, and the average particle size determined.

***In vitro* Wash-off Test**

The *in-vitro* adhesion testing was carried out using USP Tablet Disintegrating Apparatus. A freshly excised rat stomach mucosa ($1cm^2$) was stuck on a piece of glass slide. Approximately, 100 microspheres were spread onto the wet rinsed tissue specimen, and the prepared slide was put into one of the grooves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated whereby the tissue specimen was given regular up and down movements in the beaker of the disintegration apparatus, which contained the gastric fluid (pH 1.2). The numbers of microspheres still adhering to the tissue were counted at an interval of 1, 2, 4, 8, 10 and 12 hours.

Table 10. Particle diameter and frequency

S.No	Time in hrs	% cumulative drug release
1	400-410	12
2	410-420	31
3	420-430	46
4	430-440	56
5	440-450	42
6	450-460	24
7	460-470	10
8	470-480	8
9	480-490	20
10	490-500	29

Fig. 5. *In-vitro* release profile of Captopril from formulation CM-1

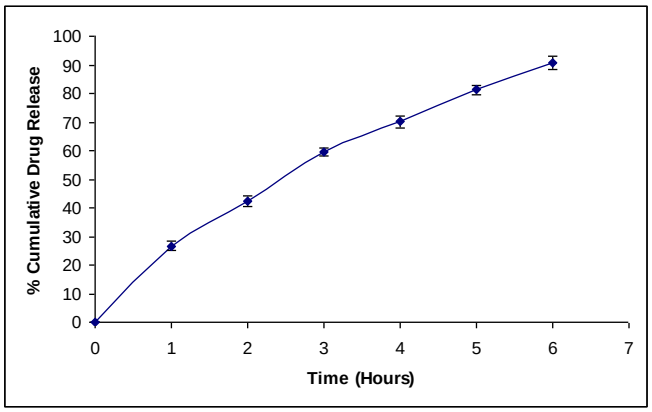


Fig. 6. *In-vitro* release profile of Captopril from formulation CM-2

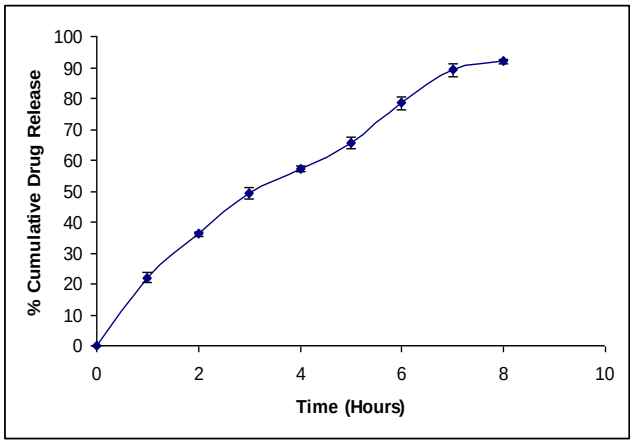


Fig. 4. FT-IR of (a) Captopril, (b) Chitosan, (c) Physical Mixture

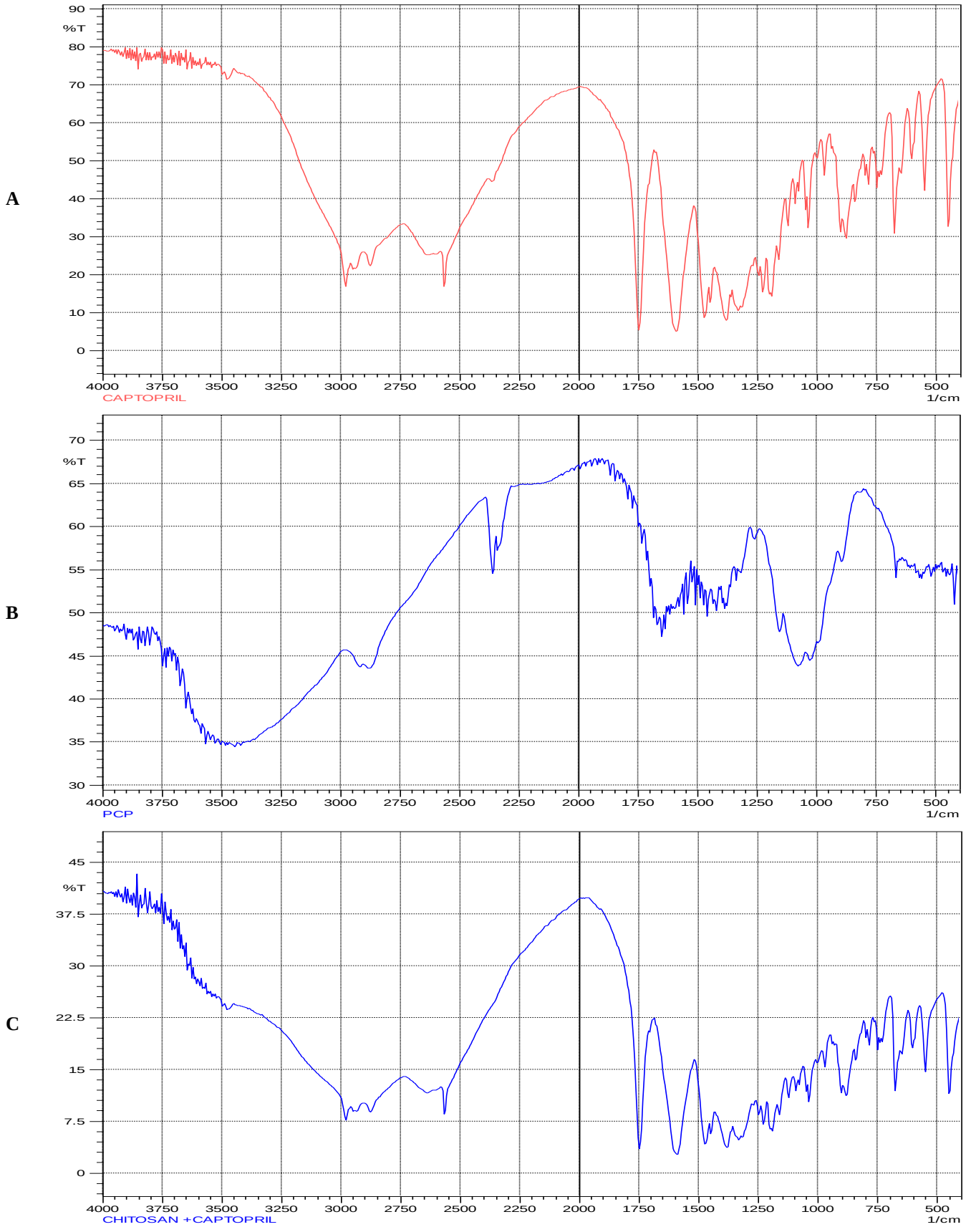


Fig. 7. *In-vitro* release profile of Captopril from formulation CM-3

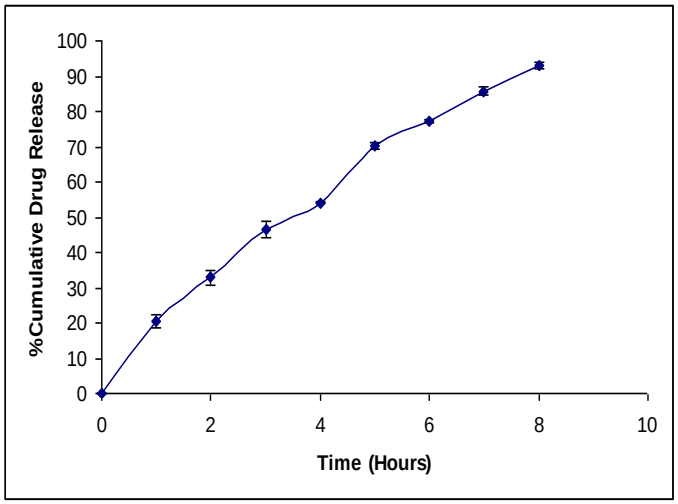


Fig. 8. *In-vitro* release profile of Captopril from formulation CM-4

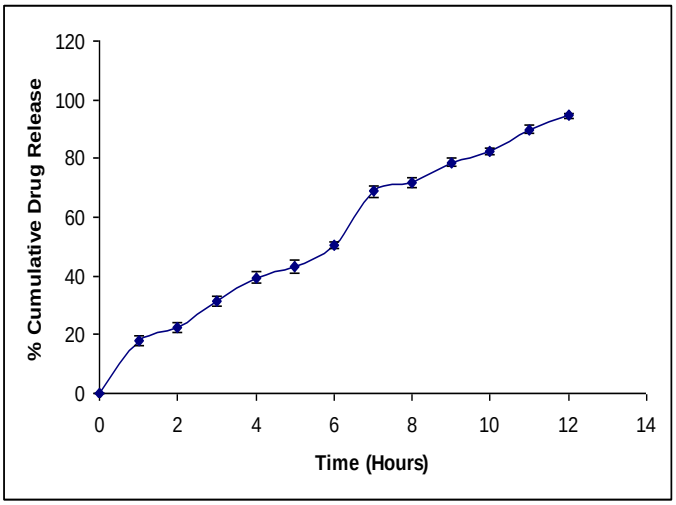


Fig. 9. *In-vitro* release profile of Captopril from formulation CM-5

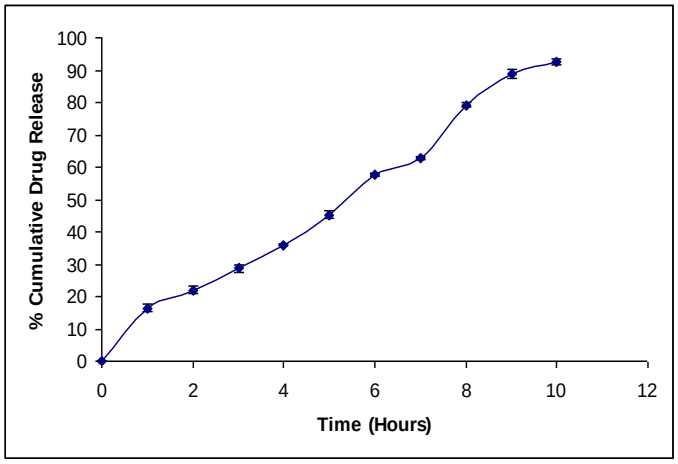


Fig. 10. *In-vitro* release profile of Captopril from formulation CM-6

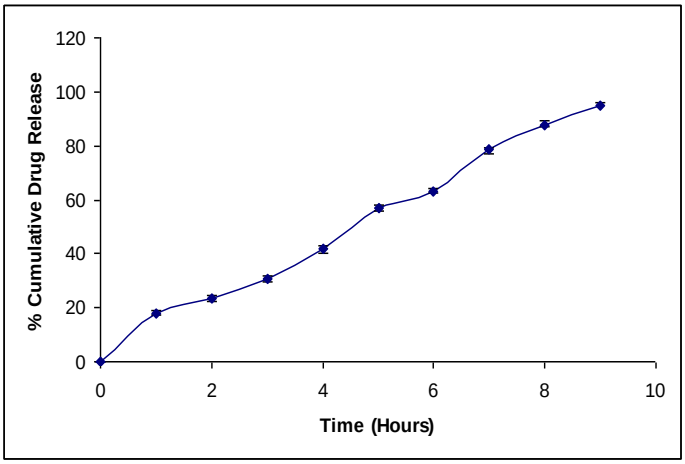


Fig.11. Particle size analysis CM-4

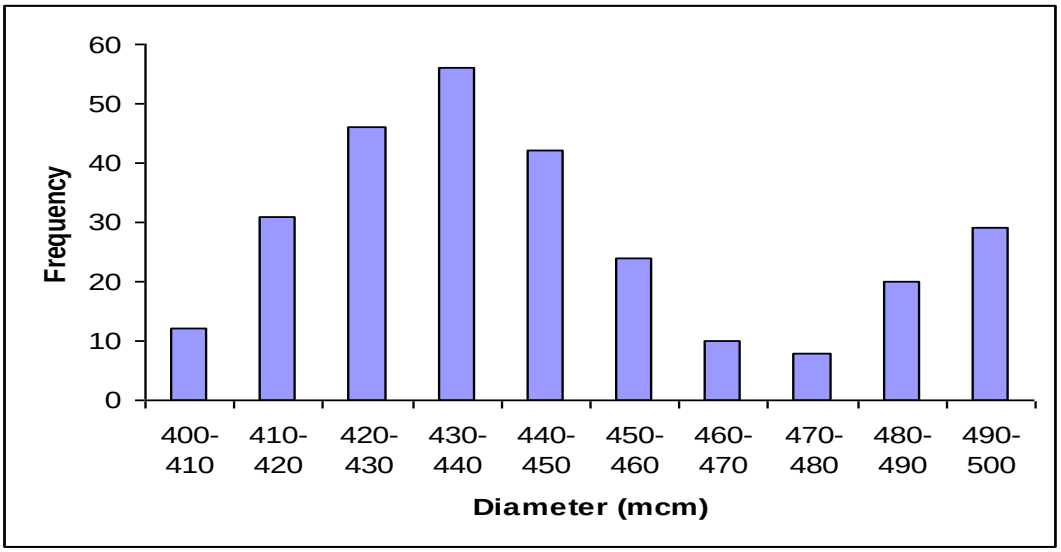


Table 11. *In-vitro* mucoadhesion testing

Time in hours	% Mucoadhesion after					
	1	**2**	**4**	**8**	**10**	**12**
CM-1	68	46	39	12	-	-
CM-2	72	53	40	17	04	-
CM-3	83	67	49	45	40	31
CM-4	89	85	79	76	68	57
CM-5	85	63	51	43	30	22
CM-6	78	57	43	29	15	09

CONCLUSION

From the entrapment and dissolution study, it was concluded that batch CM-4 showed sustained release for 12 hrs. The batch was optimized for the sustained release microspheres of Captopril by using Chitosan.

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