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Research Article

FORMULATION AND EVALUATION OF CHRONOMODULATED DRUG DELIVERY SYSTEM CONTAINING CAPTOPRIL AND HYDROCHLOROTHIAZIDE

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ABSTRACT

Blood pressures displays circadian rhythm. The objective of present study was to develop chronomodulated drug delivery system containing Captopril and Hydrochlorothiazide to achieve extended retention in upper gastro intestinal tract to enhance absorption and bioavailability. Captopril mucoadhesive microspheres were prepared by emulsification method using sodium alginate and guar gum polymers whereas Hydrochlorothiazide floating microspheres were prepared by non-aqueous solvent evaporation method using ethyl cellulose and hydroxyl propyl methyl cellulose K15 polymers in various ratios. Fourier-transform infrared spectroscopy study showed that drug and polymers were compatible with each other. The effects of polymer concentration on drug release profile were investigated. From the drug and excipients compatibility studies it was confirmed that the drug and excipients used weren't have any interactions. Microspheres obtained were evaluated for percentage yield, micromeritics studies, percentage mucoadhesion, percentage buoyancy, percentage entrapment efficiency, in-vitro percentage drug release after 10 hours, in-vitro kinetic release studies and surface morphology. Formulation M3 (1:3, sodium alginate) showed highest drug entrapment efficiency 72.54 %, 93.09 % mucoadhesion and 74.38 % drug release after 10 hours. Formulation F12 (1:4, ethyl cellulose and hydroxyl propyl methyl cellulose K15) showed higher drug entrapment efficiency 85.75 %, percentage buoyancy 80.42 % and 61.02 % drug release after 10 hours. Scanning electron microscopy showed that microspheres were spherical in shape. In-vitro release studies showed that formulation observed Zero-order and Peppas (Case II transport) pattern. Better results were observed, thereby improving the bioavailability and treating hypertension effectively due to prolong release of drug in stomach.

KEYWORDS: *Chronomodulated drug delivery system, Captopril, Hydrochlorothiazide, Mucoadhesive microsphere, Floating microsphere.*

INTRODUCTION

Various diseases, such as hypertension, rheumatoid arthritis and angina pectoris show circadian rhythm where these diseases show critical conditions during early hours of the day such as inflammations associated with morning stiffness, asthmaand heart attack in early hours of the day. For such diseases conventional drug delivery systems are inappropriate, as they cannot be administered just before the symptoms are worsened, because during this time, the patients are asleep. Such diseases require rationale therapy where drug is released from the dosage forms when the symptoms are worsen particularly during early hours [1].

Hypertension is a chronic medical condition in which the blood pressure in the arteries is elevated. Blood pressures are subjected to circadian rhythms such as vascular reactivity and capillary resistances are higher during day time whereas platelet aggregation is increased in the morning, leading to a state of relative hypercoagulability of the blood. The peak blood pressure is during 4 am and noon. These changes in blood pressure correspond to morning activation in catecholamines, renin and angiotensin [2].

Captopril (CPT) is well absorbed from the proximal small intestine; approximately 70% is absorbed in healthy fasting subjects. CPT is a structural derivative of the amino acid proline and it is, therefore, likely that the drug is absorbed from the small intestine by an active transport process. CPT has a narrow absorption window which limits its absorption from other parts of intestine. Short biological half life of 1-2 hours is one of the most important drawback of captopril. Therefore, the

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development of a once-daily captopril oral formulation would be a significant advantage for patient compliance [3, 4].

Hydrochlorothiazide (HCTZ) is mostly absorbed from the duodenum and first part of Jejunum. Bioavailability of hydrochlorothiazide gets enhanced when given with food by delaying gastric emptying. Bioavailability from sustained release formulation such as pellets was lower compared to an immediate release tablet, because the sustained release formulation passed the drug absorption sites before complete release of the drug. These studies support the absorption window theory for hydrochlorothiazide and therefore a higher bioavailability of the drug in a GRD can be expected if there is a prolonged gastric residence time [5].

Combination of Captoprıl and Hydrochlorothiazide was observed to be more affective than single drug and not a single research was carried out using combination of these drugs as chronomodulated drug delivery system for treatment of hypertension.

MATERIALS AND METHODS

The material used in present work, Captopril and Hydrochlorothiazide was procured from Yarrow Chemicals, Mumbai, India. Hydroxy propyl methyl cellulose (HPMC K15M) and Ethyl cellulose (EC) were procured from Oxfordlab finechem ltd.; Sodium alginate and Guar gum were procured from Medilise chemicals, Kannur, Kerala, India.

1. Compatibility Studies:

The pure drug, polymer mix and formulation were subjected to Fourier transform infrared (FTIR) studies. The pure drug, polymer and formulation was mixed with small quantity of IR grade potassium bromide and scanned in the range of 4000–400 cm^{-1} using an FTIR JASCO instrument (Jasco Corporation, Tokyo, Japan) [6].

2. Formulation of captopril mucoadhesive microspheres:

Microspheres containing CPT a core material were prepared by emulsification method. Weighed amount of CPT (50 mg) was dispersed in aqueous solution of polymer (10 ml). The aqueous phase was emulsified in light liquid paraffin in the ratio 1:10 containing 2% (v/v) Span 80 using a mechanical stirrer at 1500 rpm for 60 min. Five milliliters of 10% w/v calcium chloride dissolved in a mixture of methanol and isopropanol in a ratio of 2:3 was added slowly to the emulsion and stirred to assure efficient cross-linking. Microspheres were collected by filtration, washed with isopropanol thrice, and finally air-dried at room temperature. M1 to M12 were the batches prepared using different ratios of sodium alginate and guar gum polymers as shown in table no. 1 [7].

Table No. 1: Formulation of batches of Captopril mucoadhesive microsphere

Batch code	CPT concentration (%)	Sodium alginate concentration (%)	Guar gum concentration (%)	Stirring speed (rpm)
M1	1	1	-	1500
M2	1	2	-	1500
M3	1	3	-	1500
M4	1	4	-	1500
M5	1	-	1	1500
M6	1	-	2	1500
M7	1	-	3	1500
M8	1	-	4	1500
M9	1	0.5	0.5	1500
M10	1	1	1	1500
M11	1	1.5	1.5	1500
M12	1	2	2	1500

3. Formulation of Hydrochlorothiazide floating microspheres:

Microspheres containing HCTZ as a core material were prepared by a non-aqueous solvent evaporation method. In the preliminary trials, weighed amount of hydrochlorothiazide and polymers were mixed in acetone at various ratios. The slurry was then slowly introduced into a beaker containing 30 ml of liquid paraffin being stirred at 1200 rpm with the help of a mechanical stirrer at room temperature. The solution was continuously stirred for up to 2 hours to allow the solvent to evaporate completely. Microspheres obtained were collected by filtration. Collected microspheres were washed repeatedly with petroleum ether until free from oil and then dried for 1 hour at room temperature and stored in desiccators over fused calcium chloride. F1 to F12 were the preliminary batches prepared using different levels of ethyl cellulose and HPMC K15 as shown in table no. 2 [8].

Table No. 2: Formulation of batches of Hydrochlorothiazide floating microspheres

Batch code	HCTZ (%)	EC concentration (%)	HPMC K15 concentration (%)	Stirring speed (rpm)
F1	1	1	-	1200
F2	1	2	-	1200
F3	1	3	-	1200
F4	1	4	-	1200
F5	1	-	1	1200
F6	1	-	2	1200

F7	1	-	3	1200
F8	1	-	4	1200
F9	1	0.5	0.5	1200
F10	1	1	1	1200
F11	1	1.5	1.5	1200
F12	1	2	2	1200

1. Evaluation of microspheres:

a. Percentage yield:

The percentage yield of all the formulations was determined by weighing the microspheres after drying. The percentage yield of different formulations was calculated as follows [9].

$$\% \text{ Yield} = \frac{\text{Actual weight of microsphere}}{\text{Total weight of drug and polymer}} \times 100$$

b. Micromeritic studies:

Particle size analysis: Particle sizes of all the formulations were determined by optical microscopy with the help of ocular and stage micrometer. Sizes of around 100 particles were measured, and their average particle size was determined. The mean particle size of all formulations was determined by using the Edmondson's equation: [10, 11].

$$\text{D mean} = \frac{\Sigma \text{ nd}}{\Sigma \text{ n}}$$

Where, n = number of microspheres checked; d = mean size range.

Bulk density: The prepared microspheres was weighed and introduced into a graduated measuring cylinder of 10 ml capacity. The volume of the sample was taken, and bulk density was calculated using the formula given below: [10, 11]

$$\text{Bulk density} = \frac{\text{Weight of the microsphere}}{\text{Bulk volume of the microsphere}}$$

Tapped density: The prepared microspheres was weighed and introduced into a graduated measuring cylinder of 10 ml capacity. The initial volume was noted and the cylinder was allowed to fall under on to a hard surface from the height of 2.5 cm at 2-second intervals. Tapping was continued until no further change in volume was noted: [10, 11]

$$\text{Bulk density} = \frac{\text{Weight of the microsphere}}{\text{Volume of the microsphere after tapping}}$$

Carr's Compressibility Index: The percentage compressibility index was calculated according to the following formula: [10, 11]

$$\% \text{ Compressibility Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio: Hausner's ratio of microspheres was determined by comparing the tapped density to the bulk density using the following formula: [10, 11]

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Angle of Repose: It was determined fixed funnel method whose tip was fixed at a constant height (h) of 2cm from the horizontal surface. The microspheres were allowed to freely pass through the funnel until the tip of the pile touches the tip of the funnel. The radius of the base of the pile was measured (r cm). The angle of repose was determined using the formula: [10, 11]

$$\text{Angle of repose} = \tan^{-1}\frac{\text{h}}{\text{r}}$$

c. Percentage mucoadhesive:

A freshly excised piece of intestinal segment from rat was mounted on to glass slides. A weighed amount of microsphere sample was added over a fresh rat intestinal segment, mounted on a tilted glass slide with an angle of 45 degree and allowed to rest for 3 hour. The effluent was run over the intestinal segment. The effluent was collected in a whattman filter paper and weight of detached microsphere particles was determined. By using the following equation percentage mucoadhesive can be calculated [12] (Figure 1). To carry out the procedure on rat intestinal segment approval was given by Institutional Animal Ethics Committee (IAEC) of Shree Devi College of Pharmacy:

$$\text{Percentage mucoadhesive} = \frac{\text{Weight of sample} - \text{Weight ofdetached particles}}{\text{Weight of sample}} \times 100$$

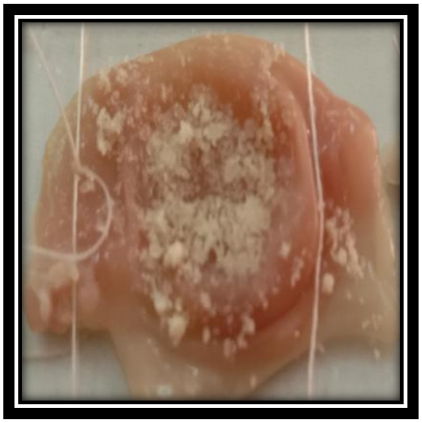


Fig. 1: Percentage mucoadhesive test using fresh rat intestinal segment

d. Percentage buoyancy:

About 50 mg of the microspheres was weighed and placed in 900 ml of simulated gastric fluid (pH 1.2). The microspheres were stirred at 100 rpm in a dissolution apparatus for 7 hours. After 7 hours, the layer of buoyant microspheres was separated by filtration. Microsphereswere dried indesiccators until constant weight was obtained. Microspheres were weighed and buoyancy was determined by using following formula: [13]

$$\text{Percentage Buoyancy} = \frac{\text{Weight of floating microspheres}}{\text{Total weight of microspheres}} \times 100$$

e. Drug entrapment efficiency:

Microspheres equivalent to 10 mg of drug were accurately weighed, triturated and digested in 10 ml simulated

gastric fluid (pH 1.2)and kept overnight for extraction of drug. The digested homogenate was centrifuged and supernatant was collected. After appropriate dilution of supernatant with same buffer solutions, aliquots were assayed by UV spectrophotometer at λmax 223 and 275 nm for CPT and HCTZ respectively. Corresponding drug concentrations in the sample was calculated from the standard calibration curve.

Efficiency of drug entrapment for each formulation was calculated in terms of percentage drug entrapment as per the following formula: [14, 15]

$$\text{Drug Entrapment efficiency} = \frac{\text{Practical Drug Content (mg)}}{\text{Theoretical Drug Content (mg)}} \times 100$$

The theoretical drug content was determined by calculation assuming that that the entire drug present in the solution gets entrapped in microspheres and no loss occurs at any stage of preparation of microspheres.

f. In vitro Drug Release Studies:

The dissolution studies of microspheres (equivalent to 50 mg of drug) were carried out using USP dissolution type I apparatus (basket type) and 900ml of simulated gastric fluid (pH 1.2), maintained at 37±0.5 ºC. The speed of stirrer was maintained at 100 rpm. An aliquot of 5 ml of the solution was withdrawn at predetermined time intervals, and replaced by an equivalent volume of fresh dissolution medium to maintain perfect sink condition. The sample solution was filtered through whatman No.1 filter paper and analyzed to determine the amount of drug releasedusing a UV spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan) at λmax 223 and 275 nm for CPT and HCTZ respectively. All experiments were performed in triplicate and average values were plotted: [16, 17]

g. Surface morphology:

Scanning electron microscopy was used to study surface topography, texture and to examine the morphology of fractured or sectioned surface of the mucoadhesive microspheres. The best formulation were mounted using a double-sided sticking tape and coated with gold (200 Ao) on the scanning electron microscopy (SEM) sample stab, under reduced pressure (0.001 torr) for 5min using ion sputtering device (Jeol JFC-1100E, Tokyo, Japan). The gold-coated samples were observed under the scanning electron microscopy (SEM-Jeol JSM-840A, Tokyo, Japan) and photomicrographs of suitable magnification were obtained: [18, 19]

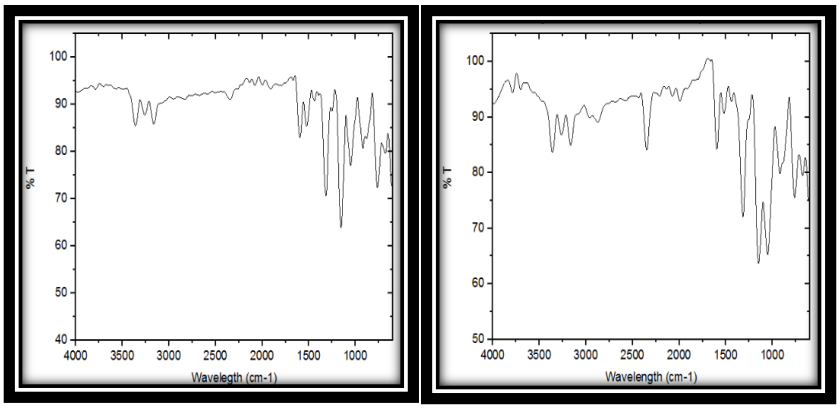
h. Kinetic studies:

The *in vitro* drug release data of the best formulation was evaluated to check the goodness of fit to the zero-order kinetics, first-order kinetics, higuchi's model and Korsmeyer-Peppas model for quantifying the phenomena controlling the release from mucoadhesive microspheres: [20]

RESULTS AND DISCUSSIONS

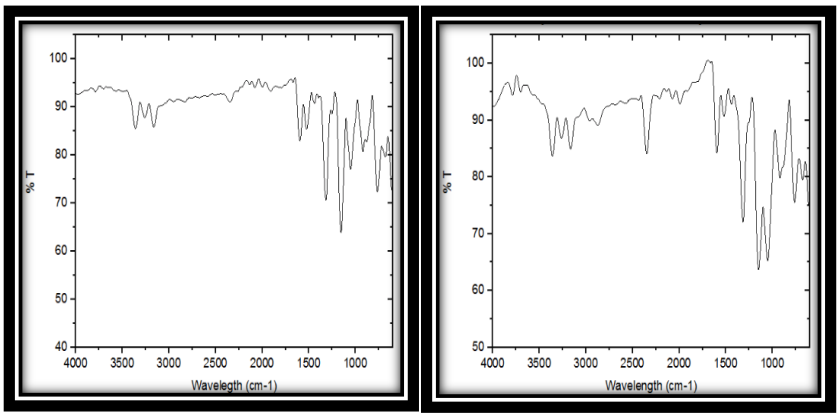
1. Drug-excipient compatibility studies:

FT-IR studies revealed that the drug and excipients used weren't have any interactions as shown in figure 2 and 3.



A **B**

Fig. 2: FTIR spectra of pure drug captopril (A) and mixture of Captopril and polymers



A **B**

Fig. 2: FTIR spectra of pure drug Hydrochlorothiazide (A) and mixture of Hydrochlorothiazide and polymers

2. Percentage yield:

It was found that as the amount of sodium alginate was increased from 1 % to 4 % the percentage yield of microspheres increased from 71.50 % to 88.86 %, as the amount of guar gum was increased from 1 % to 4 % the percentage yield of microspheres increased from 66.98 % to 85.73 % and in case of combination of polymers the percentage yield of microspheres increased from 68.65 % to 86.86 %. Batches consisting sodium alginate gave higher yield compared to other formulations. The cause may be due of higher molecular weight of sodium alginate than guar gum as shown in table no. 3.

It was found that as the amount of EC was increased from 1 % to 4 % the percentage yield of microspheres increased from 48.6 % to 83.2 %, as the amount of HPMC K15 was increased from 1 % to 4 % the percentage yield of microspheres increased from 46.3 % to 80.2 % and in case of combination of polymers the percentage yield of microspheres increased from 50.9 % to 84.6 %. Batches consisting combination gave higher yield compared to other formulations. Decrease in the polymer concentration has resulted in a decrease in the percentage yield. This may be due to the fact that as the concentration of polymer decreases the quantity of polymer becomes less to cover drug particles completely. As more amount of polymer is available, therefore as the polymer concentration increases percentage yield increases as shown in table no. 3.

Table No. 3: Percentage yield of formulations

Batch code	Percentage yield	Batch code	Percentage yield
M1	71.50±1.17	**F1**	48.6±1.48
M2	79.08±1.43	**F2**	73.3±1.54
M3	87.98±1.65	**F3**	79.5±1.04
M4	88.86±1.13	**F4**	83.2±1.43
M5	66.98±1.05	**F5**	46.3±1.90
M6	72.71±1.07	**F6**	70.8±1.31
M7	80.20±1.05	**F7**	75.1±1.56
M8	85.73±1.07	**F8**	80.2±1.34
M9	68.65±1.02	**F9**	50.9±1.36
M10	75.08±1.10	**F10**	76.5±1.32
M11	83.50±1.11	**F11**	80.4±1.41
M12	86.86±1.07	**F12**	84.6±1.38

3. Micromeritic studies:

a. Particle size analysis:

Particle size increased from 52.98 µm to 89.93 µm as the sodium alginate amount increased from 1 % to 4 %. As the guar gum amount increased from 1 % to 4 %, particle size increased from 46.39 µm to 83.88 µm and in case of combination of polymers the particle size increased from 49.48 µm to 87.58 µm. Batches consisting sodium alginate had higher particle size compared to other formulations as shown in table no. 4.

Particle size increased from 121.6 µm to 165.8 µm as the EC amount increased from 1 % to 4 %. As the HPMC K15 amount increased from 1 % to 4 %, particle size increased from 109.3 µm to 140.4 µm and in case of combination of polymers the particle size increased from 118.7 µm to 158.4 µm. Batches consisting combination of polymers had higher particle size compared to other formulations as shown in table no. 5.

The result indicates that as the polymer concentration increase it results in high viscosity of polymer solution thereby increasing the size of the particle. As the polymer concentration increases, there is an increase in the frequency of collision, which results in the fusion of semi-formed particles and production of an overall increase in the size of the microspheres

b. Bulk density, Tapped density, Carr's Compressibility Index, Hausner's ratio and Angle of Repose:

The bulk density, tapped density, hausner's ratio of formulation M1 to M12 ranges from 0.72±0.02 to 1.04±0.22, 0.76±0.83 to 1.38±0.39 and 1.05±0.19 to 1.32±0.25 respectively. The carr's compressibility index ranges between 5.26±0.11 to 24.63±0.50 %. The angle of repose ranges from 16.00±0.55 to 25.81±0.27. The carr's index and angle of repose values showed great flow properties of microspheres as shown in table no. 4.

The bulk density, tapped density, hausner's ratio of formulation F1 to F12 ranges from 0.70±0.56 to 0.63±0.53, 0.72±0.48 to 0.67±0.37 and 1.02±0.20 to 1.02±0.66 respectively. The carr's compressibility index ranges between 2.77±0.29 to 5.97±0.94 %. The angle of repose ranges from 33.12±0.42 to 18.12±0.21. The carr's index and angle of repose values showed great flow properties of microspheres as shown in table no. 5.

Table No. 4: Micromeritic studies of Captopril mucoadhesive formulations

Batch code	Particle size (µm)	Bulk density	Tapped density	Hausner's ratio	Carr's index	Angle of repose
M1	52.98±3.43	0.79±0.27	0.85±0.43	1.07±0.03	7.05±0.45	19.88±0.31
M2	61.57±3.05	0.85±0.37	1.05±0.23	1.23±0.37	19.04±1.43	20.04±0.15
M3	86.32±3.09	0.95±0.26	1.12±0.31	1.17±0.83	15.17±0.23	23.27±0.21
M4	89.93±3.83	0.97±0.74	1.19±0.55	1.22±0.19	18.48±0.65	25.81±0.27
M5	46.39±3.50	0.72±0.02	0.76±0.83	1.05±0.19	5.26±0.11	16.00±0.55
M6	56.25±3.30	0.82±0.04	0.89±0.85	1.08±0.21	7.86±0.07	18.02±0.31

M7	81.72±3.30	0.93±0.80	1.10±0.84	1.18±0.24	15.45±0.11	20.23±0.23
M8	83.88±3.65	0.95±0.69	1.12±0.94	1.17±0.36	15.17±0.10	21.67±0.43
M9	49.48±4.45	0.82±0.67	0.89±0.92	1.08±0.34	7.86±0.02	18.10±0.45
M10	59.72±3.43	0.93±0.55	1.10±0.30	1.18±0.37	15.45±0.07	19.67±0.65
M11	82.61±3.23	0.97±0.20	1.04±0.37	1.22±0.23	18.48±0.30	21.66±0.55
M12	87.58±3.31	1.04±0.22	1.38±0.39	1.32±0.25	24.63±0.50	25.20±0.80

Table No. 5: Micromeritic studies of Hydrochlorothiazide floating formulations

Batch code	Particle size (µm)	Bulk density	Tapped density	Hausner's ratio	Carr's index	Angle of repose
M1	121.6±4.69	0.70±0.56	0.72±0.48	1.02±0.20	2.77±0.29	33.12±0.42
M2	125.2±3.38	0.72±0.34	0.76±0.73	1.05±0.45	5.26±0.93	28.22±0.91
M3	130.5±2.89	0.63±0.35	0.70±0.44	1.11±0.42	10.00±0.73	26.20±0.12
M4	165.8±3.50	0.67±0.54	0.75±0.82	1.11±0.76	10.66±0.49	25.10±0.92
M5	109.3±5.60	0.64±0.34	0.69±0.47	1.07±0.96	7.24±0.29	28.67±0.63
M6	115.4±4.32	0.68±0.32	0.73±0.62	1.07±0.40	6.84±0.38	21.66±0.43
M7	120.2±3.90	0.52±0.56	0.55±0.73	1.05±0.20	5.45±0.58	20.23±0.32
M8	140.4±3.34	0.58±0.54	0.61±0.72	1.05±0.42	4.91±0.73	17.00±0.52
M9	118.7±4.18	0.68±0.65	0.69±0.44	1.05±0.22	1.44±0.85	32.22±0.36
M10	124.3±3.45	0.70±0.43	0.72±0.22	1.06±0.54	2.77±1.20	25.20±0.34
M11	127.2±3.82	0.58±0.22	0.61±0.28	1.01±0.02	4.91±0.92	21.66±0.38
M12	158.4±4.04	0.63±0.53	0.67±0.37	1.02±0.66	5.97±0.94	18.12±0.21

4. Drug entrapment efficiency:

It was found that as the amount of sodium alginate was increased from 1 % to 3 % the percentage drug entrapment of microspheres increased from 57.54 % to 72.54 %, but as the amount was increased to 4 % the percentage drug entrapment decreased to 60.00 %. The cause may be due to more concentration of sodium alginate present. In case of guar gum, the percentage drug entrapment of microspheres increased from 50.02 % to 69.47 % as the concentration increased from 1 % to 4 %. Similarly in case of combination of polymers the percentage drug entrapment of microspheres increased from 52.74 % to 67.52 % as shown in table no. 6.

It was found that as the amount of EC was increased from 1 % to 3 % the percentage drug entrapment of microspheres increased from 67.25 % to 79.75 %. In case of HPMC K 15 the percentage drug entrapment of microspheres increased from 70.08 % to 82.47 % as the concentration increased from 1 % to 4 %. Similarly in case of combination of polymers the percentage drug entrapment of microspheres increased from 79.02 % to 85.75 % as shown in table no. 6.

Table No. 6: Drug entrapment efficiency of formulations

Batch code	Percentage yield	Batch code	Percentage yield
M1	57.54±1.1	**F1**	67.25±0.8
M2	68.59±1.4	**F2**	68.49±2.4
M3	72.54±1.0	**F3**	72.29±1.4
M4	60.00±1.6	**F4**	79.75±1.7
M5	50.02±1.4	**F5**	70.08±2.8
M6	55.57±1.2	**F6**	75.57±1.1
M7	62.25±1.5	**F7**	79.25±1.1
M8	69.47±2.1	**F8**	82.47±1.7
M9	52.74±1.2	**F9**	79.02±1.4
M10	60.08±1.1	**F10**	81.32±1.6
M11	69.57±0.2	**F11**	81.68±1.2
M12	67.52±1.9	**F12**	85.75±1.4

5. Percentage mucoadhesive:

It was found that as the amount of sodium alginate was increased from 1 % to 4 % the percentage mucoadhesive of microspheres increased from 76.84 % to 94.80 %, as the concentration of guar gum was increased from 1 % to 4 % the percentage mucoadhesive of microspheres increased from 67.70 % to 89.61 % and in case of combination of polymers the percentage mucoadhesive of microspheres increased from 70.29 % to 92.89 % as shown in table no. 7. It was noticed that among all the formulations formulation consisting sodium alginate showed highest percentage mucoadhesive compared to other batches. The mucoadhesive property of these microspheres resulted in prolonged release in the gastric mucosa.

Table No. 7: Percentage mucoadhesive of Captopril mucoadhesive formulations

Batch code	Percentage mucoadhesive
M1	76.84±1.5
M2	84.45±1.8
M3	93.09±1.4
M4	94.80±1.6
M5	67.70±1.9
M6	72.89±2.1
M7	83.11±1.0
M8	89.61±2.3
M9	70.29±0.5
M10	82.61±1.4
M11	89.29±2.6
M12	92.89±1.0

6. Percentage buoyancy:

It was found that as the amount of EC was increased from 1 % to 4 % the percentage buoyancy of microspheres increased from 62.54 % to 76.51 %, as the concentration of HPMC K15 was increased from 1 % to 4 % the percentage buoyancy of microspheres increased from 65.81 % to 79.56 % and in case of combination of polymers the percentage buoyancy of microspheres increased from 75.18 % to 80.42 % as shown in table no. 8. It was observed that among all the formulations formulation consisting combination of polymers showed highest percentage buoyancy compared to other formulations. The buoyancy property of these microspheres showed prolonged retention in the gastric mucosa.

Table No. 8: Percentage buoyancy of Hydrochlorothiazide floating formulations

Batch code	Percentage mucoadhesive
F1	62.54±1.0
F2	67.25±1.3
F3	72.55±2.7
F4	76.51±1.1
F5	65.81±0.8
F6	68.31±1.8
F7	73.84±1.7
F8	79.56±1.9
F9	75.18±1.2
F10	78.13±1.3
F11	78.26±1.4
F12	80.42±1.2

***7. In-vitro* Drug Release Studies:**

It was seen that as the amount of polymers was increased, the percentage drug release of drug decreased. As sodium alginate amount was increased from 1 % to 4 %, the percentage drug release decreased from 84.29 % to 70.23 % whereas the percentage drug release decreased from 93.24 % to 77.42 % in case of guar gum. In case of combination of polymers, the percentage drug release decreased from 86.43 % to 74.91 % as amount of polymers increased. Figure no. 4 represents the plot of cumulative percentage drug release v/s time graph for the 12 formulations. It was seen that sodium alginate formulations exhibited lowest percentage drug release after 10 hrs compared to other formulations (Table no. 9).

It was seen that as the amount of polymers was increased, the percentage drug release of drug decreased. As EC amount was increased from 1 % to 4 %, the percentage drug release decreased from 98.47 % to 79.48 % whereas the percentage drug release decreased from 95.74 % to 70.27 % in case of HPMC K15. In case of combination of polymers, the percentage drug release decreased from 88.25 % to 61.02 % as amount of polymers increased. Figure no. 5 represents the plot of cumulative percentage drug release v/s time graph for the 12 formulations. It was seen that formulations containing combination of polymers showed lowest percentage drug release after 10 hrs compared to other formulations. (Table no. 9).

8. Surface morphology:

The morphology of the best formulations was observed using scanning electron microscopy. The view of the microspheres presented a spherical structure with a smooth surface morphology and within batches showed a range of sizes of microspheres (Figure no. 6 and 7).

Table No. 9: *In-vitro* Drug Release Studies of formulations

Batch code	Percentage drug release after 10 hours	Batch code	Percentage drug release after 10 hours
M1	84.29±0.37	**F1**	98.47±0.37
M2	78.62±0.88	**F2**	96.89±0.88
M3	74.38±0.28	**F3**	88.94±0.28
M4	70.23±0.74	**F4**	79.48±0.74
M5	93.24±0.19	**F5**	95.74±0.19
M6	87.52±0.21	**F6**	91.73±0.21
M7	82.80±0.10	**F7**	80.74±0.10
M8	77.42±0.23	**F8**	70.27±0.23
M9	86.43±0.05	**F9**	88.25±0.05
M10	81.71±0.14	**F10**	84.17±0.14
M11	76.33±0.26	**F11**	74.04±0.26
M12	74.91±0.10	**F12**	61.02±0.10

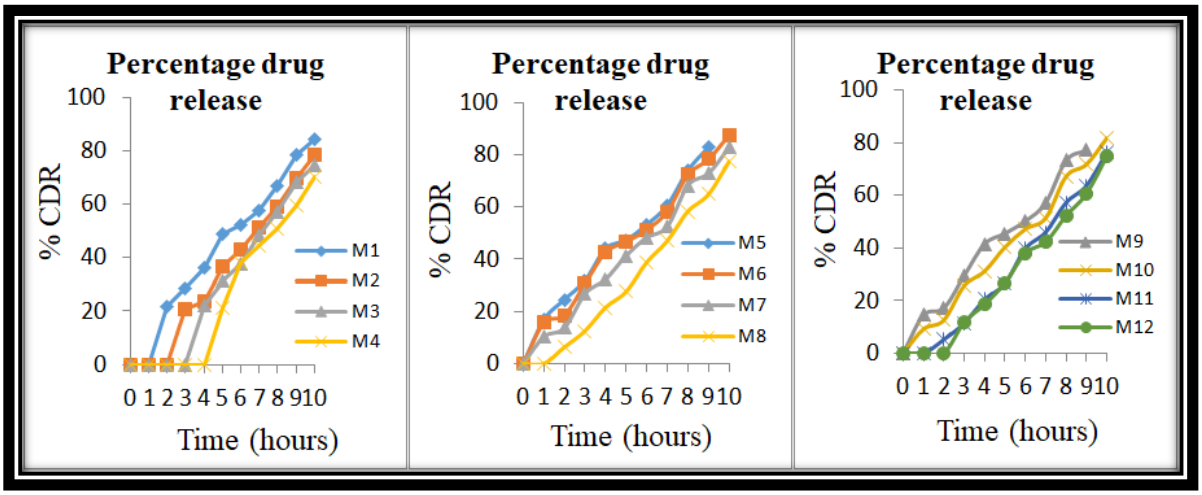


Fig. 4: Percentage drug release of Captopril mucoadhesive formulations

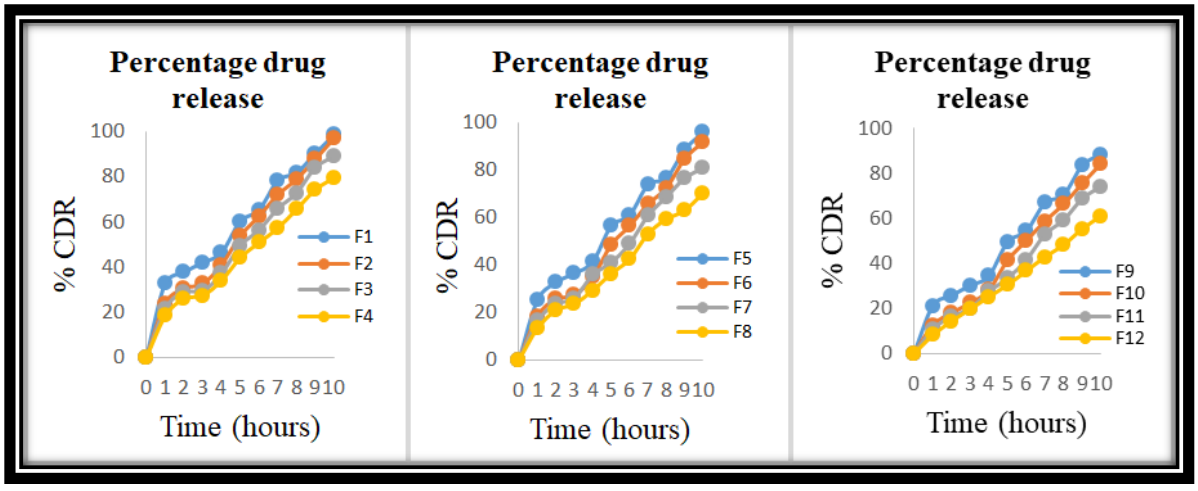


Fig. 5: Percentage drug release of Hydrochlorothiazide floating formulations

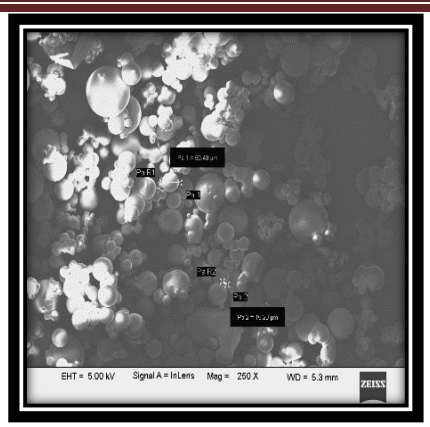


Fig. 6: Scanning electron microscopy of M3 (best formulation)

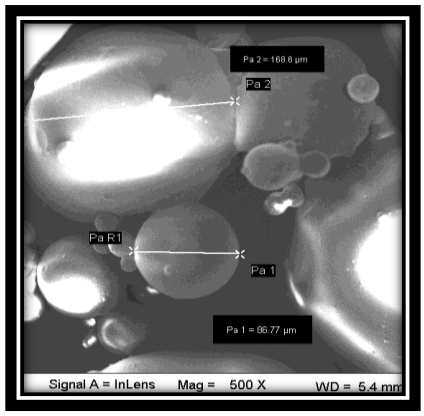


Fig. 7: Scanning electron microscopy of F12 (best formulation)

9. Drug Release Kinetics Studies:

Calculated regression co-efficient for various best formulation are shown in table no. 10. These values of *in-vitro* were fitted into various numerical models, zero order, first order, higuchi matrix and peppas. These values were related with each other for model fitting equation. Depending on highest regression values (r), the perfect fit model was Korsmeyer and Peppas.

Further Korsmeyer and Peppas equation showed the values of n > 0.89 confirming that drug release from microspheres was by Case II transport. This model is used to figure out the release of drug when the release system is controlled by the erosion and swelling of the polymer.

Table No. 10: Kinetics release study of Captopril mucoadhesive formulations

Code	Zero Order	First Order	Higuchi	Peppas Plot	n- Value	Best fit model
M3	0.9927	0.9233	0.9001	0.9641	0.892	Zero order Peppas (Case II transport)
F12	0.9986	0.9809	0.8079	0.9276	1.282	Zero order Peppas (Case II transport)

CONCLUSION

The chronomodulated drug delivery system is a promising approach to achieve controlled release using polymers. The present study of captopril mucoadhesive microspheres and hydrochlorothiazide floating microspheres, proved to be an ideal formulation as it released the drug in controlled fashion for extended period of time and thereby improving the bioavailability of drug. The best formulation M3 and F12 showed better release profile and therefore can be considered as the best formulation. Thus, the aim of this study was achieved. Further preclinical and clinical studies are required to evaluate the efficacy of these formulations.

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