A NOVEL CORE-COAT CHRONOTHERAPEUTIC TECHNIQUE FOR EFFECTIVE DELIVERY OF ANTIHYPERTENSIVE DRUG

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ABSTRACT

Objective: The objective of the work was to formulate coated microbeads using core-coat technique for chronotherapeutic delivery of Carvedilol (cd).

Methods: Sodium alginate microbeads containing cd was formulated by ionic gelation method using calcium chloride as a gelling agent. Microbeads were then coated with acrylcoat L 100 (acL 100), acrylcoat S 100 (acS 100), ethyl cellulose (EC), and acrylcoat E30 D (acE 30D) in different ratio. Formulated batches were evaluated using FTIR, particle size measurement, percent entrapment efficiency, micrometric properties, swelling studies, loose surface crystal studies, percentage moisture loss. The formulations were then subjected to *in vitro* dissolution studies. Cumulative release data was then subjected to different dissolution models reported in the literature.

Results: Formulated batches of microbeads were within acceptable particle size range with good to fair flow properties. Entrapment efficiency was in the range of 64% to 99.52%. Loose surface crystal study revealed successful drug entrapment within a range of 2.11 to 9.93%. Swelling studies revealed maximum swelling in alkaline phosphate buffer pH 7.4 solution and percentage moisture loss was within 0.24% to 7.55%. SEM showed microbeads to be spherical in nature and FTIR study exhibited compatibility among the drug and polymer. *In vitro* release study was carried out for first 3 h in 0.1N HCl and subsequently for 7 h in phosphate buffer solution pH 7.4. Formulation code AC3, AC4, LA4 and LA6 were found in line with our objective of chronotherapeutic drug delivery.

Conclusion: Sodium alginate beads loaded with cd and coated with different ratios of coating solutions were successfully formulated and evaluated to optimize the best possible combinations of the coat-core ratio for chronotherapeutic delivery.

Keywords: Chronotherapeutic drug delivery, Carvedilol, Core-coat technique, Microbeads

INTRODUCTION

With the expansion of biopharmaceutics and pharmacokinetics knowledge, many new drug delivery systems have been introduced over the last 25 y including controlled, sustained, and prolonged drug delivery systems. One of the newer drug delivery systems which have attracted many researchers is based on the concept of chronotherapeutic which can deliver the drug when it is mostly needed [1, 2]. Chronotherapeutic drug delivery is the one that delivers the drug molecule in a rapid and transient manner within a short time period immediately after a predetermined lag period [3]. The rationale for the use of this system for our work was to deliver drug at a time when the disease condition is in the most morbid and mortal state during one circadian cycle of 24 h. Particular rhythmic onset of symptoms is regularly observed in diseases like asthma, ulcers, diabetic conditions, hypertension, myocardial infraction, angina pectoris and cardiac attacks and sometimes in attention deficit syndrome and many others [4]. Several chronotherapeutic release formulation have been developed recently wherein tablets and capsules are the dosage form developed to release the drug after a lag time period for the match the circadian cycle of the disease [5]. The symptoms of hypertension are pronounced predominantly during the early hours of the morning as renin angiotensin–aldosterone system is activated in the morning and therefore blood pressure rises rapidly, and this rise in blood pressure has been associated with increased risk of cardiovascular events such as stroke and myocardial infarction [6]. Thus treatment of such disease occurring in the early hours is not convenient using the existing conventional marketed tablets and capsules [7]. Hence, a newer dosage form which can release the drug after a lag time causing a maximum concentration of drug in plasma to match the symptom of the disease when it is worse to fatal may help to minimize the rate of such attacks and death [8]. The other advantages of chronotherapeutic drug delivery systems include convenience, reduced dosing frequency, decreased fast pass metabolism, reduced tolerance as exposure to drug is not constant like conventional dosage forms, reduced toxicity and decreased total required therapeutic or instantaneous preventive drug dose level matching exactly the biological and physiological needs to treat the disease at each point in time. Ultimately, all these lead to improved patient compliance. cd, an antihypertensive drug was used as a model for this work as it is widely used for reducing hypertension, chronic heart failure, angina pectoris and kidney problems etc. Also the fact that no chronotherapeutic dosage form of cd is available in the market with reference to above mentioned conditions which needs the drug at its peak (C_{max}) during early hours of morning though immediate release and controlled released formulations are available from long time. This had made us to take up this drug for the preparation and evaluation of coated microbeads of cd for chronotherapeutic delivery which will release the dug after a lag time causing a maximum concentration of drug in plasma to match the symptom of the disease when it is worse to fatal.

MATERIALS AND METHODS

Materials

CD was obtained as a gift sample from Mylan Laboratories, Hyderabad. Sodium alginate, EC, acL 100, acS 100, acE 30D and calcium chloride was purchased from SD Fine chem., Mumbai. All other chemicals and reagents used were of analytical grade.

Methods

Preparation of CD micro beads by ionic gelation method

The weighted quantity of sodium alginate was dissolved slowly in 100 ml of deionized water in a beaker using a mechanical stirrer untill all the alginates dissolves to give a viscous solution. In this solution, cd was added with constant stirring to get a dispersion of water-insoluble drug. This mixture is then taken in a syringe fitted with a needle of 21 gauges and drop by drop poured in a 250 ml calcium chloride solution (10% w/v) to obtain the beads by ionic exchange mechanism. After the formation of the beads, these were

kept in calcium chloride solution for two hour time for complete ionic exchange, to get nearly spherical rigid microbeads. Microbeads were then washed several times with distilled water and dried at 40 °C for 12 h for complete drying [9, 10].

Fourier transformed infrared spectroscopy

IR spectroscopy was performed on Fourier transformed infrared spectrophotometer (840, Shimadzu, Japan) to ascertain the compatibility between sodium alginate and cd. The pellets of cd, micro-beads prepared, and potassium bromide was prepared by compressing the powders at 20 psi for 10 min on KBr-press and measured in the range the spectra were scanned in the wave number range of 3000-400 cm[-1][11].

Coating of microbeads

Microbeads prepared with sodium alginate and drug by ionic gelation technique was coated with a rate controlling polymers namely acL100, acS 100, and EC, acE30 D and their combination in different ratios as given table 1. Coating solutions were prepared using acetone and ethanol in 2:1 ratio. 4 % acL100 and 15 % acS 100 solutions were first prepared using coating powders. These solutions were then mixed in different ratios viz 1:1, 1:2, 1:3, 2:1 and 3:1. EC was dissolved in a mixture of acetone and ethanol 2:1. acE 30D solution was used in the range of 0.4 to 0.7% as given in the references of the manufacturer. Dip coating technique was employed for coating of all microbeads and then left to air dry for 24 h and then dried in a hot air oven, packed and stored in self-sealable plastic packets until further use [12].

Evaluation of microbeads

Particle size measurement study

Particle size analysis was done by sieving method using Indian Standard sieves ≠ 10, 12, 16, 20, 22, 40, 44. Average particle size was calculated using the formula d avg =Σdn/Σn where n is frequency weight and d is the mean diameter [13].

Scanning electron microscopy (SEM)

Scanning electron microscopy (Stereo scan S250 MK III, Cambridge, UK) was carried out to study the morphological characteristics of cd microbeads. Dried microbeads were coated with gold (100A °) under an argon atmosphere in a gold coating unit and scanning electron micrographs of both higher and lower resolutions were observed [14].

Drug content estimation and drug entrapment efficiency (DEE %)

For drug content estimation accurately weighed the amount of microbeads equivalent to 100 mg of cd, was suspended in 50 ml of methanol to dissolve the polymer as well as the polymer coat. The drug was extracted with 50 ml of methanol in separating funnel and analyzed by using UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) after suitable dilution. The total drug content was determined by employing absorbance obtained in the linear regression equation of the calibration curve. The entire test was performed in triplicate [15, 16].

Entrapment efficiency of the microbeads was calculated by using the formula

$$DEE\% = Practical\,drug\,loading(Drug\,content)/Theoritical\,drug\,loading\,X\,100$$

Micromeritic studies

Flow of microbeads was investigated by determining the angle of repose, bulk density, Carr's index and Hausners ratio. The angle of repose was determined by fixed funnel method. Microbeads were tapped using bulk density apparatus (Electro lab) for 300 taps in a cylinder and the change in volume was measured. Carr's index and Hausner ratio were calculated by the formula $CarrsIndex(\%) = [(Df - D0)X100]/Df$ and $HausnerRatio = Df/D0$, where, D_f is tapped density; D_0 is poured density. All the experimental units were studied in triplicate (n=3) [17].

Loose surface crystal study

Loose surface crystal study was done to observe the excess drug present on the surface of microbeads. From each batch, 100 mg of microbeads was shaken in 100 ml of 0.1N HCl solution for 5 min in a volumetric flask

and then filtered through Whitman filter paper 41. The amount of drug in the filtrate was determined spectrophotometrically at 241 nm and calculated as a percent of total drug content. This estimates the surface entrapment of the drug by the microbeads [18].

Swelling index

Each 100 mg of the prepared microbeads were weighted initially (W_1) and was dipped in glass petridishes containing 05 ml 0.1 N HCl and phosphate buffer pH 7.4 solution. After an hour, the beads were removed wiped with butter paper, and reweighed (W_2). The swelling index was calculated as follows [19].

$$Sw = (W1 - W2)/W1\,X\,100$$

Percentage of moisture loss

The CD loaded microbeads were evaluated for percentage moisture loss, which gives an idea of their nature. The weighed amount of microbeads (10 G, W_1) was initially kept in a desiccator containing calcium chloride at 37 °C for 24 h. The final weight (W_2) was noted when no further change in weight of sample was observed. Finally the percent moisture content was determined by Equation

Percent moisture loss= $(W1 - W2/W1)X100$

In vitro dissolution studies

As the microbeads were formulated for chronotherapeutic release of the drug mostly in the intestinal region of GIT, therefore before entering the intestinal area, these microbeads have to pass through the acidic region of the stomach which takes around 3 to 4 h. Therefore dissolution studies were conducted on two mediums, taking 25 mg equivalent of drug three hours in 0.1N HCl which mimics the acidic region of the stomach and gastric transit time and subsequently in phosphate buffer pH 7.4 which mimic the intestinal alkaline pH for next 7 h. The overall dissolution study was carried out for 10 h (Electro lab TDT-08L) and then after suitable dilutions analyzed using UV spectroscopy (UV-1700, Shimadzu, Japan) [20].

Drug release mechanism and kinetics

In order to establish the mechanism and kinetics of drug release from the microbeads, experimental data obtained from the *invitro* dissolution study was fitted with different kinetic model like zero order (%cumulative release vs time), first order (log % cumulative amount unabsorbed vs time), Higuchi's model (%cumulative release vs √t), Korsmeyer and Peppas model (log Mt/M∞ vs log t) etc. Korsemeyer's model is widely used when the release mechanism is not well known or when more than one type of release phenomena could be involved [21, 22].

Korsmeyer and Peppas equation: $Q = K\,.tn$

Where Q-is functional drug release in time t

K–Constant incorporating of structural and geometric characteristics of controlled release device.

n–Diffusional release exponent indicative of release mechanism.

The n value could be used to characterize different release mechanism as per the table below.

Characterization of drug release mechanism

"n" value	Release mechanism
n<0.4	Case I anomalous transport
0.4<n<0.5	Diffusion predominantly
n=0.5	Fickian diffusion (Higuchi matrix)
0.5<n>1	Anomalous transport (Non-diffusional release)
n=1	Case II transport (Ideal zero order release)
n>1	Super case II transport

RESULTS

Microbeads loaded with cd were prepared by ionic gelation technique using sodium alginate and calcium chloride was nearly spherical and rigid in shape and strength. The drug-polymer (sodium alginate) compatibility study was performed using FTIR study to ascertain if any interaction was there between the drug and polymer (fig. 1).
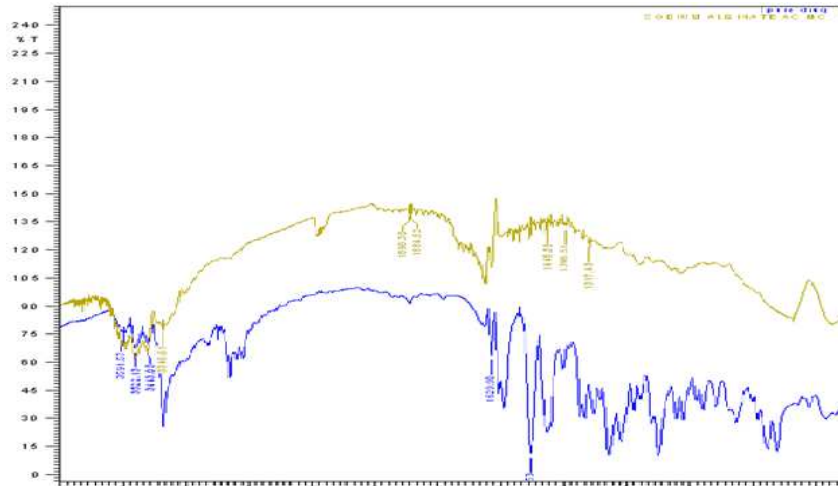
Fig. 1: FTIR spectra of cd and microbeads containing cd

Particle size measurement of cd microbeads

The average particle size was found to be within a narrow range of 402.85 to 510.03μm.

Coating of microbeads

The microbeads prepared were then coated with a different rate controlling coating polymer solutions and their combinations in the different ratio as shown in table 1. This was done keeping in mind the objective of getting a rate delayed the chronotherapeutic release, predominantly to the intestinal part of the GIT. As the concept of the

current study was not to vary the cd: sodium alginate ratio but to vary the ratio of the coating solution to get the desired type of release, therefore, this concept was named as a coat-core ratio and not a drug-polymer ratio.

Drug content estimation and drug entrapment efficiency (DEE %)

The drug content and drug entrapment efficiency of all the coated microbeads were determined. The results are summarized in table 2. The drug entrapment efficiency of the cd microbeads was found to be in the range of 64% to 99.52 %.

Table 1: Microbeads coating parameters using different coating solutions and their combinations

FC	Drug (mg)	Coating polymer	Ratio of polymers	Type of coating
LA1	CD	acL 100 and acS 100 mixture	1:1	Microbeads
LA2	CD	Do	1:2	Microbeads
LA4	CD	Do	1:3	Microbeads
LA6	CD	Do	2:1	Microbeads
LA7	CD	Do	3:1	Microbeads
EA1	CD	ECand acL 100 mixture	1:1	Microbeads
EA2	CD	Do	1:2	Microbeads
EA3	CD	Do	1:3	Microbeads
EA4	CD	Do	3:1	Microbeads
EA5	CD	Do	2:1	Microbeads
AC1	CD	acE 30 D	0.4%	Microbeads
AC2	CD	Do	0.5%	Microbeads
AC3	CD	Do	0.6%	Microbeads
AC4	CD	Do	0.7%	Microbeads

(FC=Formulation code)

Table 2: Entrapment efficiency of the microbeads

FC	Practical drug loading (drug content) (mg)	Theoretical drug loading (mg)	% drug entrapment efficiency
LA1	2.90±1.20	3.125	92.89±0.88
LA2	2.89±0.39	3.125	92.76±1.62
LA4	3.0±0.15	3.125	96.00±0.12
LA6	2.71±0.12	3.125	86.72±1.62
LA7	3.03±1.62	3.125	97.14±1.90
EA1	2.87±0.12	3.125	91.84±0.39
EA2	3.11±0.28	3.125	99.52±0.15
EA3	2±0.25	3.125	64.00±1.15
EA4	2.8±0.93	3.125	89.60±0.93
EA5	2.7±0.15	3.125	86.40±0.25
AC1	2.56±0.28	3.125	81.92±0.28
AC2	2.31±0.93	3.125	73.92±0.19
AC3	2.8±0.88	3.125	89.60±1.91
AC4	2.84±0.19	3.125	90.88±2.08

*All the results are expressed mean±SD (n=3)

Micromeritic studies

The rheological study of the prepared microbeads was performed which reflects the physiochemical properties of the microbeads and tabulated in table 3. Rheological study revealed angle of repose for all the microbeads were within the range of good flowability (<25). The bulk density was found in a range of 0.3471 to 0.3890. Carr's indexes of microbeads were excellent except LA7 and AC1 and the Hausner's ratio of almost all formulations was good except LA6, LA 7 and AC4.

**Table 3: Rheological behavior of cd microbeads**

FC	Angle of repose(θ)*	Bulk density (gm/cm³)	Carr's index	Comment	Hausner's ratio	Comment
LA1	28.14±0.12	0.3711	13.11	Excellent	1.20	Good
LA2	24.19±0.17	0.3478	10.31	Excellent	1.11	Good
LA4	29.47±0.20	0.3479	9.42	Excellent	1.14	Good
LA6	24.71±0.43	0.3254	10.01	Excellent	1.27	Poor
LA7	27.67±0.37	0.3518	16.21	Good	1.30	Poor
EA1	27.58±0.39	0.3201	13.41	Excellent	1.19	Good
EA2	25.84±0.44	0.3348	12.20	Excellent	1.21	Good
EA3	24.35±0.91	0.3358	11.82	Excellent	1.23	Good
EA4	28.87±0.94	0.3577	10.63	Excellent	1.26	Poor
EA5	25.78±0.71	0.3478	09.41	Excellent	1.18	Good
AC1	27.48±0.57	0.3456	18.11	Good	1.20	Good
AC2	29.10±0.46	0.3587	13.30	Excellent	1.22	Good
AC3	28.75±0.58	0.3678	12.76	Excellent	1.24	Good
AC4	28.50±0.73	0.3741	10.42	Excellent	1.27	Poor

* All the results are expressed mean±SD (n=3)

Loose surface crystal study

Loose surface crystal studies lend a hand to estimate the excess amount of drug attached on the surface of microbeads after a successful drug entrapment. The study was executed with variously coated microbeads and the results were presented in fig. 2. The percentage of drug content in the surface was found to be within the range of 2.11 to 9.938%.
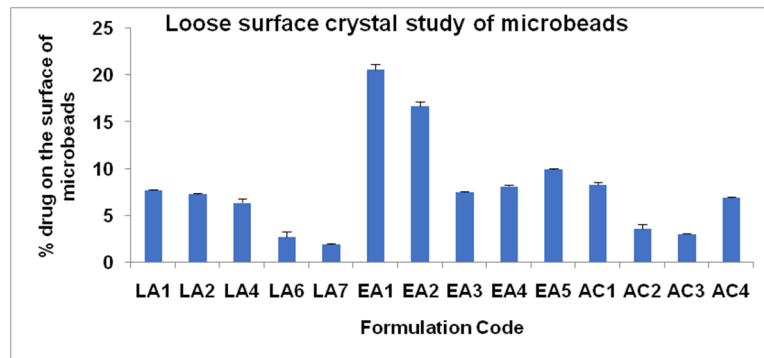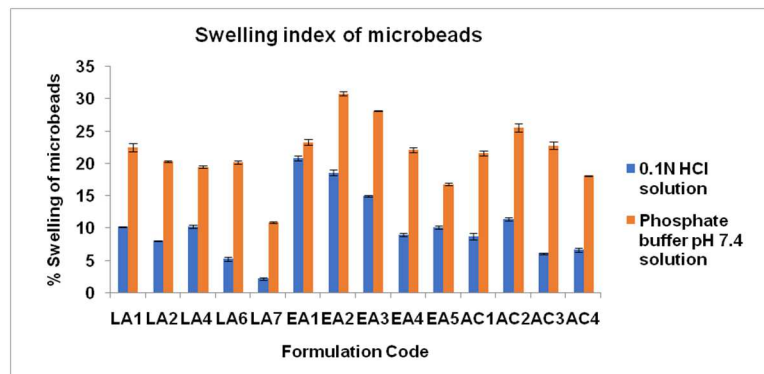

Fig. 2: **Percentage of cd on the surface of the microbeads (All the values were calculated as mean±standard deviation; n=3)**

Swelling Index

Swelling studies for coated microbeads was performed in two pH solutions viz. 0.1N HCl providing an acidic pH 1.2 of stomach and phosphate buffer pH 7.4 simulating intestinal alkalinity and the results are shown in fig. 3. As seen from the fig. the swelling of the microbeads were more in alkaline media than acidic media due to the solvation of coat and permeation of alkaline media into the microbeads than in acid media.


Fig. 3: Swelling index of cd microbeads in 0.1N HCl and phosphate buffer pH 7.4 solution (All the values were calculated as mean±standard deviation; n=3))

Percentage of moisture loss

The percentage moisture loss of the formulated microbeads was evaluated and the results are shown in fig 4. Moisture loss was found to be in the range of 0.24% to 7.55%.
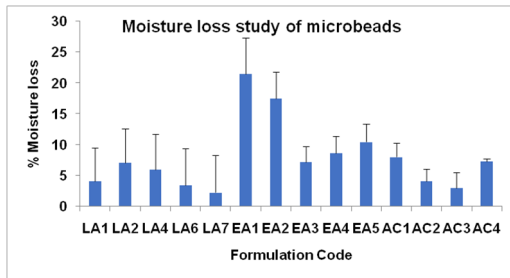
Fig. 4: Percentage moisture loss of cd microbeads (All the values were calculated as mean±standard deviation; n=3))
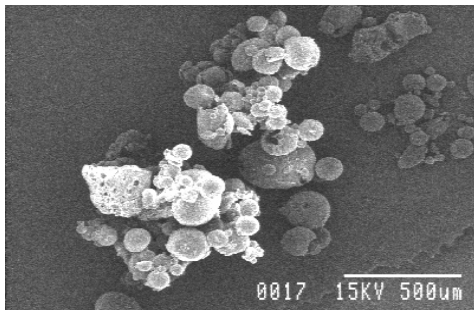
**Fig. 5: SEM of cd microbeads under low resolution of X 30**

Fig. 6: SEM of cd microbeads under high resolution of X 300

Scanning electron microscopy

Microbeads formulated were found to be nearly spherical without much aggregation, (as revealed in SEM studies), discrete and free-flowing, which are shown in fig. 5 and fig. 6.

***In vitro* drug release and kinetic studies of coated microbeads**

Invitro drug release studies of the coated microbeads were carried out for three hours in acidic buffer pH 1.2 using 0.1N HCL and subsequently for 7 h in phosphate buffer solution pH 7.4. Since the objective of the current work was to formulate microbeads which will release cd profoundly after 5 to 6 h after administration, therefore microbeads were coated with polymers which mainly dissolves in the intestinal pH which will delay the drug release for 5 to 6 h due to the gastric emptying time of the microbeads. The zero order release of the dissolution studies conducted for all the batches of coated microbeads are depicted in fig. 7, 8 and 9. The proposed mechanism for release along with the n values of microbeads coated with acS 100 and acL 100, EC and acL 100, and acE 30D, are shown in table 4, 5 and 6 respectively.
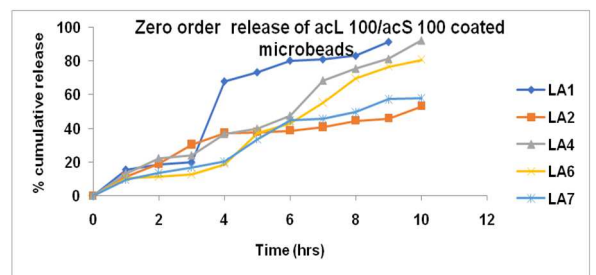
Fig. 7: Zero order *in vitro* drug release kinetic studies of acL100/acS 100 coated microbeads
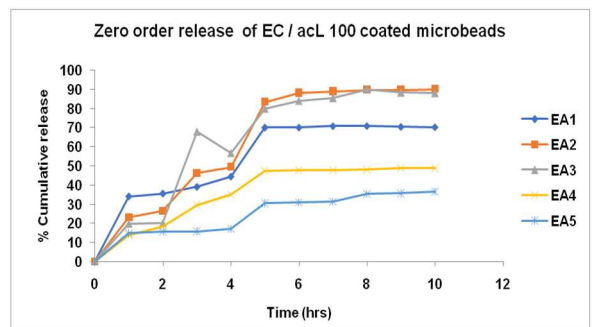
Fig. 8: Zero order *in vitro* drug release kinetic studies of EC/acL 100 coated microbeads

Table 4: Release kinetics of microbeads coated with acS 100 and acL 100 mixtures

FC	Release kinetics						
	Medium	Zero-order	First order	Higuchi	Accepted	"n-Value"	Proposed mechanism for release
LA1	0.1N HCl	0.783	0.964	0.999	Higuchi	0.234	Anomalous super case I
	7.4 buffer	0.942	0.762	0.944	Higuchi	0.366	Anomalous super case I
	Overall	0.883	0.786	0.893	Higuchi	0.98	Very near to ideal zero order
LA2	0.1N HCl	0.994	0.999	0.928	First order	0.895	Firstly swelling then diffusion
	7.4 buffer	0.967	0.930	0.972	Higuchi	0.269	Anomalous super case I
	Overall	0.861	0.737	0.962	Higuchi	0.631	Very near to diffusion based
LA4	0.1N HCl	0.905	0.841	0.983	Higuchi	0.561	Diffusion based release pattern
	7.4 buffer	0.931	0.917	0.961	Higuchi	0.082	Anomalous super case I
	Overall	0.795	0.752	0.954	Higuchi	0.532	Purely diffusion based release
LA6	0.1N HCl	0.849	0.860	0.930	Higuchi	0.547	Diffusion based release pattern
	7.4 buffer	0.902	0.970	0.925	First order	1.364	Anomalous super case Π
	Overall	0.917	0.962	0.879	First order	0.922	Very near to ideal zero order
LA7	0.1N HCl	0.997	0.954	0.997	unpredictable	0.293	Anomalous super case I
	7.4 buffer	0.663	0.853	0.973	Higuchi	0.689	Firstly swelling then diffusion
	Overall	0.780	0.849	0.900	Higuchi	1.21	Anomalous super case Π

Table 5: Release kinetics of microbeads coated with EC and acL 100 mixtures

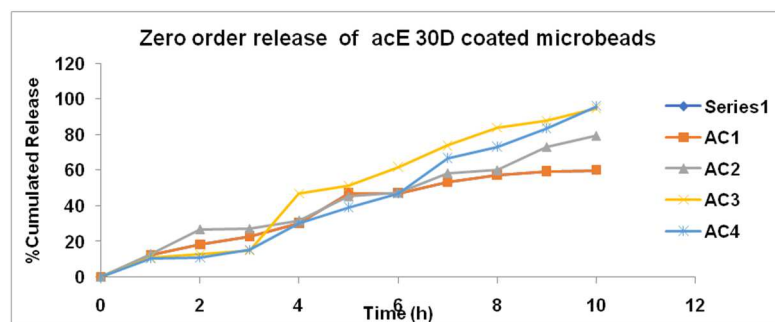| FC	Release kinetics						
	Medium	Zero order	First order	Higuchi	Accepted	"n-Value"	Proposed mechanism for release
EA1	0.1N HCl	0.843	0.901	0.909	Higuchi	0.178	Anomalous super case I
	7.4 buffer	0.932	0.823	0.940	Higuchi	0.006	Anomalous super case I
	Overall	0.783	0.822	0.911	Higuchi	0.405	Predominantly diffusion based
EA2	0.1N HCl	0.914	0.943	0.997	Higuchi	0.598	Diffusion based release pattern
	7.4 buffer	0.907	0.921	0.981	Higuchi	0.095	Anomalous super case I
	Overall	0.861	0.893	0.915	Higuchi	0.700	Firstly swelling then diffusion
EA3	0.1N HCl	0.905	0.841	0.983	Higuchi	0.920	Firstly swelling then diffusion
	7.4 buffer	0.917	0.910	0.972	First order	0.149	Anomalous super case I
	Overall	0.826	0.923	0.910	First order	0.745	Firstly swelling then diffusion
EA4	0.1N HCl	0.809	0.827	0.960	Higuchi	0.689	Diffusion based release pattern
	7.4 buffer	0.912	0.905	0.970	Higuchi	0.042	Anomalous super case I
	Overall	0.822	0.848	0.932	Higuchi	0.602	diffusion based
EA5	0.1N HCl	0.928	0.954	0.997	Higuchi	0.100	Anomalous super case I
	7.4 buffer	0.763	0.813	0.983	Higuchi	0.301	Anomalous super case I
	Overall	0.884	0.906	0.932	Higuchi	0.483	Predominantly diffusion based

Fig. 9: Zero order *in vitro* drug release kinetic studies of acE 30D coated micro beads

Table 6: Release kinetics of the microbeads coated with varying percentage of acE 30D

FC	Release kinetics						
	Medium	Zero order	First order	Higuchi	Accepted	"n-Value"	Proposed mechanism for release
AC1	0.1N HCl	0.967	0.980	0.979	First order	0.630	Diffusion based release pattern
	7.4 buffer	0.925	0.935	0.933	First order	0.409	Predominantly Diffusion based
	Overall	0.945	0.968	0.947	First order	0.759	Firstly swelling then diffusion
AC2	0.1N HCl	0.881	0.896	0.960	Higuchi	0.635	Diffusion based release pattern
	7.4 buffer	0.656	0.762	0.695	First order	0.506	Predominantly diffusion based
	Overall	0.923	0.928	0.897	First order	0.876	Firstly swelling then diffusion
AC3	0.1N HCl	0.766	0.716	0.621	Zero order	0.861	Firstly swelling then diffusion
	7.4 buffer	0.889	0.890	0.912	Higuchi	0.103	Anomalous super case I
	Overall	0.805	0.824	0.848	Higuchi	0.855	Firstly swelling then diffusion
AC4	0.1N HCl	0.839	0.853	0.965	Higuchi	0.353	Anomalous super case I
	7.4 buffer	0.836	0.984	0.867	First order	0.968	Very near to zero order release
	Overall	0.936	0.939	0.845	First order	01.041	Anomalous super case Π

DISCUSSION

Characteristic peaks observed in cd after FTIR studies viz. 3443.05 for-N-H str, 3522.13 for-O-H str (free) 3591.57 for O-H str (bound) 1629.90 for N-H bending and 1099.46 for-C-O str were also observed for the microbeads confirming no interaction between the drug and the polymer. Drug entrapment efficiency of the cd microbeads was found to be in the range of 64% to 99.52%. The cd content of all formulations was good and this may happen due to the proper mixing of sodium alginate and drug during mixing using mechanical stirrer which didn't allow the drug to settle down at the bottom of the beaker and remain undisclosed. The flow properties of the microbeads were found to be good which shows that microbeads are non-aggregated. The bulk density and the Carr's index of formulated microbeads were excellent except LA7 and AC1 and Hausner's ratio was good except LA6, LA 7 and AC 4. Swelling studies revealed that almost all the microbeads showed maximum swelling in alkaline phosphate buffer pH 7.4. The swelling of alginate beads in phosphate buffer can be accounted for the exchange of

Ca^{2+} and Na^+. The sodium ions present in phosphate buffer exchange with calcium ions, bound to COO- groups of mannuronic blocks.

Thus, an electrostatic repulsion between negatively charged COO- groups increases resulting in gel swelling. Exchanged Ca^{2+} ions precipitate in the form of insoluble calcium phosphate reflecting in slight turbidity of swelling medium. Moisture loss was found to be in the range of 0.24% to 7.55%. The lesser proportion of water in the microbeads may be attributed to proper drying (before and after coating) and instant hardening due to quick gelation occurring between calcium chloride and sodium alginate. The microbeads with higher moisture content may be due to the involvement of water during microbeads formation and hydrophilic property of polymers like EC used for coating.

In vitro dissolution study conducted in subsequently, two media showed acS-100 and acL 100 solutions and their combination in different ratios exhibited satisfactory results. acL-100 is insoluble in gastric pH and soluble from pH 6 and above whereas acS 100 is also

gastric insoluble and is soluble from pH 7 and above, in a combination of both, the drug release may take place throughout the length of intestine and not so much in stomach. Limited drug release was seen in almost all the LA coded formulations in acidic medium whereas as the release was much higher and faster in alkaline medium as the coating dissolved in alkaline surrounding. LA6 showed 12% drug release in 3 h in an acidic medium and 81% overall release in 10 h. Similarly LA4 showed 24% release in 3 h and 92.43% overall release in the next 7 h. Formulation LA1 though releases the drug in similar fashion of LA4 and LA6 in overall 10 h, but the release of drug jumped from 20% in 3 h to 68% in 4th hours which may cause dose dumping as well as it does not meet our objective of releasing the maximum portion of drug after 5 to 6 h. Formulation LA2 and LA7 showed release of 52% and 53% respectively in 10 h. This indicated that higher concentration of acS 100 may interfere with the release of drug even in alkaline pH. In batch of microbeads coated with acL100 and acS100 polymers, almost all formulations followed timed based release pattern and the mechanism of release after comparison of R^2 values was found to be Higuchi diffusion kinetics which was supported by Peppas "n-value". Only in case of LA6 the overall mechanism of release was found to be first order release that is concentration depended release.

The batch of microbeads coated with EC and acL 100 and their combination did not match our expectation as much of drug release was observed in the acidic medium in the first phase of dissolution where according to our objectives, delayed drug release and not controlled or sustained release was expected. The first three sets of microbeads i.e. EA1, EA2 and EA3 releases drug in the range of 39% to 68% in acidic medium and last two ratios i.e. EA4 and EA5 which contains higher percentage of EC was capable of delaying the release in the acidic medium as they released only 29.71% and 17.92% respectively as shown in fig. 8. The basic mechanism for release of the drug in this batch mostly was found to be Higuchi with exception of EA3 which was found to be first order release. In EA4 and EA5, the delayed drug release may have been due to the thick coating of EC (3:1 and 2:1) which may have delayed the release as the dissolution medium took more time to make pores in the coating thereby delaying the drug release from the microbeads.

The last set of microbeads was coated with different percentage (0.4% to 0.7%) of coating solution of acE 30D. The microbeads showed good control of release in the first half of dissolution study as the drug release was minimum in acidic pH that may be attributed to the low permeability of the coating film around the microbeads. Though acrylcoat has the property to allow drug to permeate at any pH, but it has to first swell and then permeation may happen through the pores formed in the coating layer which takes a lag time. This delay may have helped out objective of delayed drug release in the stomach pH. By the time these microbeads entered the alkaline medium, sufficient pores may have formed for drug to diffuse out of the microbeads profoundly. The range of release in acidic medium was 15% to 27.14% in first 3 h and after these were put in to alkaline medium the total drug release in 10 h was found in the range of 59.95% to 95.97% as depict in fig. 9. It was also observed that higher the concentration of coating solution as in case of AC3 and AC4, lower the drug release in acidic medium which is in accordance with out earlier proposed mechanism of delayed release duo to relationship between thickness of coating and time taken for pore formation. The mechanism of drug release for most of the formulations was found to be first order release with the exception of AC3 which showed Higuchi kinetics. According to n value most of the formulations showed swelling and diffusion of drug from the microbeads with exception of AC4 which showed Anomalous super case Π transport. Case II transport occurs when the sorption is entirely controlled by stress-induced relaxations taking place at a sharp boundary separating an outer swollen shell, essentially at equilibrium penetrate concentration, from an unpenetrated glassy core. Ideally, this sharp boundary moves through the polymer at a constant velocity during Case II transport.

If we compare all the batches of formulations coated with different coating materials and their combination, formulation AC3 and AC4 which was coated with 0.6% and 0.7% of acE 30D showed minimum drug release in acidic pH and in alkaline pH release was around 95%

in 10 h. Although formulation EA4 and EA5 coated with combination of ethyl acetate and acL 100 also showed less drug release in acidic medium but the overall drug release was also very low in 10 h 48.92% and 36.71% which may not provide sufficient drug in the systematic circulation for showing therapeutic activity. Formulation code LA4 and LA6 coated with acL 100 and acS 100 mixture was also in line with our objective of delayed drug release as they showed 24 % and 12.5 % release in acidic medium and overall 92.4% and 80.89% of drug release. Since the period of observation was restricted to 10 h, we were unable to quantify the precise duration of release of drug extended antihypertensive efficacy. These formulation need to be further studied in respect to other parameters and the best formulation may be chosen for *invivo* studies in future.

CONCLUSION

Among the 14 formulations designed and evaluated, four formulations were in line with our objective of being potentially effective dosage form suitable for chronotherapy of early morning hypertension and cardiac attacks for which drug products needs to be taken at bedtime and able to act in the early morning. But before that, further processing parameters needs to be optimized and best-formulated micro bead need to be taken for *in vivo* clinical studies in suitable dosage form to ascertain its ultimate effectiveness.

CONFLICT OF INTERESTS

None

AUTHORS CONTRIBUTIONS

All author have contributed equally

REFERENCES

1. Bellamy N, Sothern RB, Campbell J. Rhythmic variations in pain perception in osteoarthritis of the knee. J Rheumatol 1990;17:364-72.
2. Ghosh T, Ghosh A, Kumar A. Chronotherapeutic drug delivery: a way forward to treat rhythm guided diseases. J Pharm Sci Res 2017;9:1894-8.
3. De Geest BG, Mehuys E, Laekeman G, Demeester J, De Smedt SC. Pulsed drug delivery. Expert Opin Drug Delivery 2006;3: 459-62.
4. Youan BB. Chronopharmaceutics: gimmick or clinically relevant approach to drug delivery? J Controlled Release 2004;98:337–53.
5. Shidhaye SS, Lotlikar VM, Ghule AM, Phutane PK, Kadam VJ. Pulsatile delivery systems: an approach for chronotherapeutic diseases. Syst Rev Pharm 2010;1:55–61.
6. Pasic J, Shapiro D, Motivala S, Hui KK. Blood pressure morning surge and hostility. Am J Hypertens 1998;11:245–50.
7. Ura J, Shirachi D, Ferrill M. The chronotherapeutic approach to pharmaceutical treatment. California Pharmacist 1992;23: 46-53.
8. Smolensky MH, Labreque G. Chronotherapeutic. Pharm News 1997;2:10-6.
9. Dhalleine C, Assifaoui A, Moulari B, Pellequer Y, Cayot P, Lamprecht A, et al. Zinc-pectinate beads as an in vivo self-assembling system for pulsatile drug delivery. Int J Pharm 2011;414:28-34.
10. Kunjachan S, Jose S. Understanding the mechanism of ionic gelation for the synthesis of chitosan nanoparticles using qualitative techniques. Asian J Pharm 2010;4:148-3.
11. Mattam J, Sailaja K. Preparation and evaluation of sulfasalazine loaded sodium alginate microbeads for sustained delivery. Asian J Pharma Clin Res 2016;9:72-6.
12. Andrea C, Giulia A, Pasquale DG, Francesca S, Rita PA, Paola R. Novel core-shell chronotherapeutic system for the oral administration of ketoprofen. J Drug Delivery Sci Technol 2016;32:126-31.
13. Mazumder R, Nath LK, Haque A, Maity T, Choudhury PK, Shrestha B, *et al.* Formulation and *in vitro* evaluation of natural polymers based microspheres for colonic drug delivery. Int J Pharm Pharm Sci 2010;2:211-9.
14. Girhepunje KM, Piillai K, Pal RS, Gevariya HB, Thirumoorthy N. Celecoxib loaded microbeads: a targeted drug delivery for colorectal cancer. Int J Curr Pharma Res 2010;2:46-55.

15. Saparia B, Murthy RSR, Solanki. Preparation and evaluation of chlorquine phosphate microspheres using cross-linked gelatin for long-term drug delivery. Indian J Pharm Sci 2002;64:48-52.
16. Sangeetha S, Sakthisaravanan V, Komala M, Harish G, Sivakumar V. Design and evaluation of gastro retentive beads of theophylline by Ionotropic gelation. Int J Pharm Pharm Sci 2010;2:99-101.
17. Ghosh A, Biswas S, Ghosh T. Preparation and evaluation of silymarin β-cyclodextrin Molecular inclusion complexes. J Young Pharmacists 2011;3:205-10.
18. GN Vishal, Gowda D, Balamuralidhara V, Khan SM. Formulation and evaluation of olanzapine matrix pellets for controlled release. Daru 2011;19:249-56.
19. Lachman L, Lieberman HA. Joseph LK. editors. The theory and practice of industrial pharmacy. 3rd Edition. Philadelphia: Lea and Febiger; 1986.
20. Ghosh A, Saha A, Das S, Ghosh T. Formulation and *in vitro* evaluation of zidovudine loaded E C microcapsules. Int J Pharma Sci Res 2011;2:289-97.
21. Varshosaz J, Jaffarian A, Golafshan S. Colon-specific delivery of mesalazine chitosan microspheres. J Microencapsulation 2006;23:329-39.
22. Dandagi PM, Mastihomath VS, Patil MB, Manvi FV, Gadad AP, Sharma R. Development and evaluation of theophyllin and salbutamol sulphate sustained release matrix tablets. Indian J Pharm Sci 2005;67:598-602.