Available online at www.pharmacie-globale.info ISSN 0976-8157

Research Article

PHARMACIE GLOBALE

INTERNATIONAL JOURNAL OF COMPREHENSIVE PHARMACY

DESIGN AND EVALUATION OF pH SENSITIVE MULTIPARTICULATE SYSTEM FOR CHRONOTHERAPEUTIC DELIVERY OF CARVEDILOL

Sruthy N V*, Sajeeth C I and K Santhi

Department of Pharmaceutics, Grace College of Pharmacy, Kodunthirapully, Palakkad, Kerala, India.

Received: 20 January 2011; Revised: 20 April 2011; Accepted: 28 April 2011; Available online: 5 May 2011

ABSTRACT

A pH sensitive multiparticulate system intended to approximate the chronobiology of angina pectoris is proposed for site specific release to the colon. The multiparticulate system consisting of drug loaded chitosan microspheres encapsulated within Eudragit S-100 microcapsules was designed for chronotherapeutic delivery of Carvedilol. Drug loaded chitosan microspheres were prepared by emulsion cross linking method in different drug to polymer ratios (5, 10, 15, 20, 30 mg drug / 120 mg of polymer). *In-vitro* drug release studies in simulated gastrointestinal fluids showed a burst release pattern in the initial hours necessitate microencapsulation around chitosan microspheres. The optimized formulation was then subjected to microencapsulation with Eudragit S 100 by emulsion solvent evaporation technique. The effect of core/ coat ratio on particle size, percentage drug loading, flow properties, swelling properties and *in-vitro* drug release were tested. Formulation which contain 1:6 core/coat ratio released lesser amount of drug in the upper gastro intestinal conditions and so selected as the best formulation and filled in a capsule shell an amount equivalent to antianginal dose.

Keywords: Chitosan Microspheres, Eudragit S 100, Chronotherapeutics, Angina pectoris, Carvedilol.

INTRODUCTION

Chronotherapeutics refers to a clinical practice of synchronising drug delivery in a manner consistent with the body's circadian rhythm including disease states to produce maximum health benefits and minimum harm. It is now recognized that episodes of angina pectoris, asymptomatic ischemia, acute coronary syndromes, sudden death, ventricular ectopic activity, and stroke all exhibit an increased incidence in the early mornings. This is due to the day-night pattern differences in the sympathetic drive, blood coagulation, blood pressure, heart rate, coronary blood flow, and myocardial oxygen supply versus demand. The morning triggers include the change from supine to upright posture, increase in physical exertion, and rise of mental and emotional load due to work onset and engagement in other activities.[1] A therapeutic system that would synchronize the drug delivery with the circadian variation in periods of increased risk is highly desirable for an antianginal regimen. This can be achieved by a bed time administration of a drug delivery system which with a delayed start of drug release can provide adequate protection in the early mornings.

Conventional drug at night may bring blood pressure down too low at night and end up having little effect for the rest of the day. A timed, pulsatile delivery system, capable of providing one or more rapid release pulses at predetermined lag times or at specific sites, results in better absorption of the active, and thereby provides more effective plasma concentration-time profile. Chronotherapeutic strategy constitutes a new option to optimize blood pressure control and to reduce the risk which is capable of releasing drug after predetermined time delay and maintain constant drug levels throughout the day. In this context colon specific drug delivery systems has been utilized for chronotherapeutic drug administration.

Methods based on pH sensitive delivery are simple and practical means for colon targeting. Possible variation in the GI transit time risking incomplete carrier disintegration and a subsequent therapy failure is thought to be reduced with this approach.[2]

Several acrylic polymers particularly Eudragit S 100, Eudragit™ S, Eudragit FS and Eudragit P-4135F have been investigated for colonic delivery. Since the pH of the colon in normal subjects varies from 6.4±0.6 to 7.5±0.4, these polymers have been designed to be soluble at pH values higher than 7, keeping in mind the pH prevalent in the large intestine.[3]

Due to the advantages of multiparticulate dosage forms over single unit preparations, such as more uniform dispersion in the GI tract, more uniform drug absorption, less inter- and intra-individual variability, and more flexible formulation process, interest in multiparticulates as oral drug delivery systems has been growing steadily. Because of their small particle size, multiparticulates can pass through the upper GI tract easily and can reach the colon quickly, with more predictable gastric emptying and are retained longer in the ascending colon. Therefore, a multiparticulate system would be a desired dosage form for colon targeting.[4,5]

***Corresponding Author:**
Sruthy N V
Grace College of Pharmacy,
Kodunthirapully-678004, Palakkad, Kerala, India.
Contact no: +91-9746198318; Email: sruthyjithu@gmail.com

The aim of the present study was to investigate the feasibility of a multiparticulate system consisting of chitosan microspheres encapsulated in Eudragit S 100 microcapsules for the chronotherapeutic delivery of Carvedilol which is used in angina and hypertension and to evaluate some of the physico-chemical characteristics of drug and polymers, particle size analysis, mucoadhesive properties, swelling properties, flow properties, drug loading efficiency and *in-vitro* drug release pattern of the prepared multiparticulate system. Following a bed time administration these coated microspheres are intended to maintain a low plasma drug concentration during the night when the cardiovascular events are reported to be minimum and optimal therapeutic concentrations when it is maximum.

Carvedilol is a non-selective and adrenergic antagonist with no intrinsic sympathomimetic activity widely used to treat essential hypertension and angina pectoris. The short biological half-life (7-10 hours) and low dose (6.25 - 25mg) of carvedilol coupled with good colonic absorption makes it an ideal candidate for colon targeting.

MATERIALS AND METHODS

Chemicals and Reagents

Carvedilol, (Ranbaxy laboratories,Delhi, India), Chitosan (Central Institute of Fisheries Technology, Cochin, India), Eudragit S 100 (Yarrow Chem Products, Mumbai, India) were obtained as gift samples. Glacial acetic acid, Hydrochloric acid, Gluteraldehyde 25%, Acetone, Sodium hydroxide was purchased from Nice Chemicals Pvt Ltd, Cochin, India. Petroleum ether and Light liquid paraffin were procured from Prowess Lab Chemicals, Ottapalam, India. Ethanol and methanol were purchased from Jiangsu Huxai International Trade Co. Ltd, China, and Span 80 from SD Fine Chemicals Limited, Mumbai, India.

Method

Infrared Spectroscopy

Drug excipient compatibility can alter the physicochemical properties and bioavailability of the drugs. This incompatibility there by affects its safety and/or efficacy. Infrared Spectroscopy was done to find out the possible interaction between the selected polymers, Chitosan and Eudragit S100 and the drug Carvedilol and to identify the compatibility between the drug and polymers.

Formulation of chtosan microspheres[6]

The chitosan microspheres were prepared by emulsion crosslinking method. Chitosan solution 2% was prepared in aqueous glacial acetic acid. Chitosan was dissolved in 4% acetic acid by overnight stirring in a magnetic stirrer. The drug was dispersed in this solution and mixed well. 6ml of this mixture was added to 40ml liquid paraffin containing 3ml of span 80 and stirring was performed in a magnetic stirrer to form w/o emulsion. After 30minutes homogenisation, 2ml of gluteraldehyde 25% was added. It was left for stabilisation and cross linking for a period of 3hrs. Microspheres thus obtained were centrifuged at 2000 rpm and sediment was then collected and washed with petroleum ether for three times and acetone for three times and then dried in hot air oven.

Analysis of Particle size, Shape and Surface morphology[7]

The particle size and particle size distribution of microspheres was evaluated using optical microscope. The freshly prepared microspheres were spread on a clean and dried glass slide and examined on an optical microscope and size of the microspheres was measured by using the pre-calibrated ocular micrometer and stage micrometer. About 100 particles of each formulation were observed and counted. The average particle size was determined by Edmondson's equation. Scanning Electron Microscopy (SEM) was performed for morphological characterization of microspheres and also to determine particle size.

Mucoadhesive property[8,9]

The mucoadhesive property of the microspheres was evaluated by using phosphate buffer, pH 7.4. The freshly excised pieces of intestinal mucosa (2x3 cm) from goat were mounted onto glass slides. About 30 nos. of microspheres from formulations D1 to D5 were spread onto each wet rinsed tissue specimen and immediately thereafter the slides with suitable support were hung onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up and down movement in the test fluid at 37°C contained in a one litre vessel. At different time intervals up to 7 hours the machine was stopped and the number of microspheres still adhering to the tissue was counted.

Percentage Drug Loading[10]

20mg microspheres were accurately weighed and triturated with methanol and kept overnight for complete extraction of drug for the determination of entrapment efficiency. This was centrifuged at 2000rpm for 3minutes. After proper dilution of supernatant with methanol the absorbance was measured at 241nm with UV/Vis spectrophotometer by keeping methanol as blank. The percentage drug loading (PDL) was determined as:

$$PDL = \frac{Practical\ Drug\ Content}{Theoretical\ Drug\ Content} \times 100$$

In-vitro drug release studies in simulated gastrointestinal fluids[11]

Chitosan microspheres were evaluated for drug release studies in simulated gastrointestinal fluids. In order to simulate the pH changes along the GIT, four dissolution media with pH 1.2, 6.0, and 7.4 were sequentially used referred to as the sequential pH change method. An accurately weighed amount of chitosan microspheres equivalent to 5mg was added to 900ml dissolution medium and the release of Carvedilol from the microspheres were investigated using USP rotating paddle dissolution apparatus at 100rpm at 37±0.5°C. The simulation of gastrointestinal transit conditions was achieved by altering the pH of dissolution medium at various time intervals. The pH of the dissolution medium was kept at 1.2 with 0.1 N HCl. The pH of medium was then adjusted to 6 by adding KH_2PO_4 and sufficient quantity of 1M NaOH. The release rate study was continued for another 2 hours. The pH of dissolution medium was further adjusted to 7.4 by adding 1M NaOH. Five millilitres of samples were withdrawn in various time intervals for a period of 24hours and replaced with fresh medium. The samples were then subjected to UV analysis at 241nm. The effects of drug-polymer ratio on drug release of chitosan microspheres were evaluated.

Microencapsulation of chitosan microspheres[11]

The chitosan microspheres were microencapsulated by emulsion- solvent evaporation technique. 100mg of chitosan microspheres were suspended in 5ml ethanol solution of Eudragit S-100 (10%) and emulsified into 40ml liquid paraffin containing 1%w/v of span 80. Emulsification was maintained with a mechanical stirrer for 3 hours to allow complete solvent evaporation. The microencapsulated chitosan microspheres were collected

and washed with petroleum ether for 3 times and dried in a hot air oven.

After the microencapsulation the Eudragit S 100 microencapsulated chitosan microspheres were evaluated for percentage yield, particle size, shape, surface morphology, percentage drug loading flow properties, swelling properties, and in vitro drug release in simulated gastro intestinal fluids.

Micromeritics properties[12]

The flow properties of microspheres were characterized in terms of angle of repose, Carr's index (CI) and Hausner's ratio (HR). For determination of angle of repose, Eudragit S100 coated microspheres were poured through the walls of a funnel, which was fixed at a position such that its lower tip was at a height of exactly 2.0 cm above hard surface. The microspheres were poured till the time when upper tip of the pile surface touched the lower tip of the funnel. The $\tan^{-1}$ of the height of the pile (h)/ radius of its base (r) gave the angle of repose (θ).

$$\theta = tan^{-1}\frac{h}{r}$$

Eudragit S100 coated microspheres microspheres were poured gently through a glass funnel into a 10ml graduated cylinder. The cylinder was then tapped from a height of 2.0 cm until the time when there was no more decrease in the volume. Bulk density (ρb) and tapped density (ρt) were calculated.

$$HR = \frac{\rho t}{\rho b}$$

$$CI = \frac{\rho t - \rho b}{\rho t} \times 100$$

Swellability study of coated and uncoated chitosan microspheres[13]

A known weight (100 mg) of chitosan microspheres and Eudragit S 100 coated microspheres were placed in simulated gastric fluid (pH 1.2) and allowed to swell for the required period of time at 37±0.5°C in the dissolution apparatus. The microspheres were periodically removed and blotted with filter paper, and then their change in weight was measured until attainment of equilibrium. The swelling ratio (SR) was then calculated using the following formula:

$$SR = \frac{Wg - Wo}{Wo}$$

Where, *SR*= Swelling ratio; W_g = Final weight of microspheres; W_0 = Initial weight of microspheres.

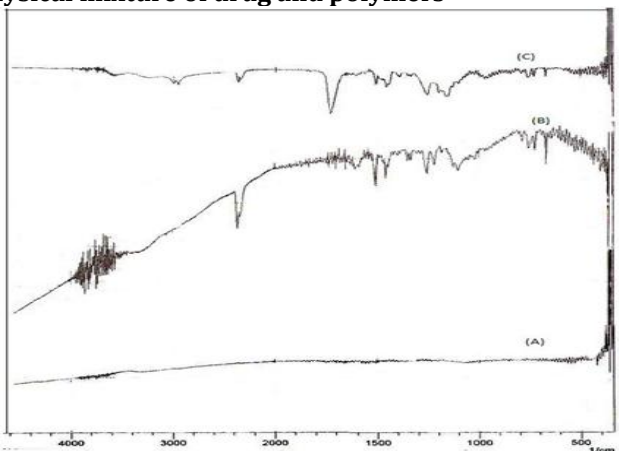
Filling of microencapsulated chitosan microspheres in capsule shell and *in vitro* drug release study[14]

As EC3 formulation was found to be the best formulation, it was filled in the hard gelatine capsule of size 1. A quantity of 270.77mg of Eudragit S100 coated chitosan microspheres equivalent to 12.5 mg Carvedilol, which is indicated for treatment of hypertension and prophylactic treatment of stable angina was filled in each capsule and evaluated for drug release studies in simulated gastric fluids.

RESULTS AND DISCUSSION

The procured sample of Carvedilol and polymers were tested for their identification. IR spectra of the physical mixture exhibited absorption peaks similar to those in pure samples of drug and polymers. The results of IR analysis indicated that there was no chemical interaction between the drug and the excipients in the microsphere formulation. IR spectra of carvedilol alone and its combination with polymers are shown in figure **1**. IR spectrum of pure Carvedilol showed the peaks at 3345.89 cm^{-1} due to N-H stretching vibrations and at 1106 cm^{-1} due to C-O stretching vibrations. These peaks can be considered as characteristic peaks of carvedilol and were not affected and prominently observed in IR spectra of carvedilol along with polymers, indicated no interaction between carvedilol and polymers.

Figure 1. IR spectra of A) chitosan, B) Carvedilol and C) physical mixture of drug and polymers



The plain chitosan microspheres were successfully prepared in different chitosan concentrations by emulsion cross-linking method. Microscopic analysis was performed to determine the nature of chitosan microspheres. By increasing concentration from 0.5% to 2%, more discrete spherical microspheres of uniform size were obtained. But the chitosan solution was too viscous to pass through a needle with 3% polymer concentration. Percentage yield was also found to be increased with concentration, 0.5% to 2% (table **1**). Hence 2% chitosan solution was selected for further study.

Table 1. Effect of concentration of chitosan solution on microsphere formation

Formulation code	Chitosan concentration (% w/v)	Percentage yield (%)	Description of microspheres
C1	0.5	88.75	No discrete spheres, clumpy masses formed.
C2	1	95	Irregular, aggregated.
C3	1.5	97.5	Small spheres with few crystalline particles.
C4	2	99.23	Discrete spherical spheres of uniform size.

The size analysis study of drug loaded chitosan microspheres has shown that average particle size of all the batches is around 13.314µm. With increasing amount of drug incorporated in polymer (D1 – D5), mean diameter is also increased from 8.470 to 17.71 (table **2**).

Table 2. Effect of amount of drug loaded in chitosan microspheres

Formution code	Amount of drug/120mg chitosan	%Yield	Average particle size	% drug loading	description of microspheres
D1	5	98.4	8.470 µm	6.53 ± 0.02	Few, irregular spheres.
D2	10	93.07	11.97 µm	17.74 ± 0.016	Few spheres.
D3	15	99.25	13.86 µm	22.09 ± 0.008	Discrete uniform spheres.
D4	20	92.86	14.56 µm	11.93 ± 0.014	Discrete, more irregular spheres.
D5	30	94.67	17.71 µm	8.745 ± 0.008	Irregular spheres.

It was evident from SEM photographs that the chitosan microspheres were discrete, spherical and with a smooth surface (figure **2**). Mucoadhesive study showed that chitosan microspheres exhibited good mucoadhesive property (figure **3**). The percentage drug loading of different batches of drug loaded chitosan microspheres were found to be increased with increase in drug concentration. The saturation capacity of the carrier occurs at 15mg per 120mg of polymer (formulation D3). After this concentration there is a decrease in percentage drug loading.

Figure 2. Scanning Electron Micrographs of chitosan microspheres

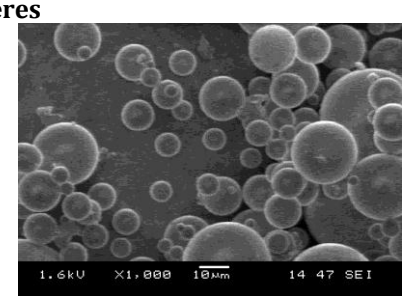
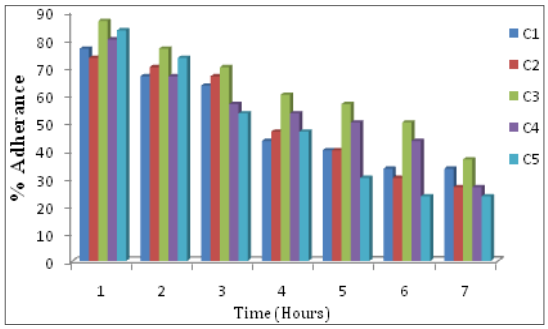
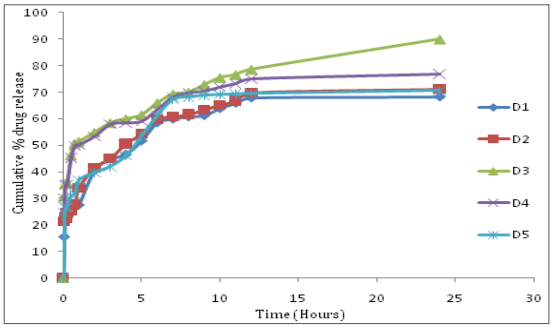


Figure 3. Percent of chitosan microspheres adhering to tissue at different time intervals



The *in-vitro* drug release studies of D1 to D5 formulations in simulated gastrointestinal fluids showed a burst release pattern in the initial hours. A high burst release of 30.816% was observed from the formulation D3, whereas a least burst release of 15.58% was observed from D1. Within 4hours 40–60% of drug was released from formulations D1 – D5 (figure **4**).

Figure 4. Percent cumulative *in vitro* drug release profile of Carvedilol from chitosan microspheres containing different amount of drug, in simulated gastrointestinal fluids



The burst release in acidic pH is due to the pH sensitive property of chitosan. Chitosan dissolves easily at low pH. Chitosan exhibits a pH-sensitive behaviour as a weak polybase due to the large quantities of amino groups on its chain. The mechanism of pH sensitive swelling is due to the protonation of amine groups of chitosan under low pH conditions. This type of burst release in stomach and intestine is not satisfactory for the formulation, which is supposed to be release its contents in colon. In order to prevent the drug release in stomach and small intestine, these chitosan microspheres were microencapsulated with Eudragit S100, which is supposed to be release in colon. Since D3 formulation showed high drug loading and good drug release pattern, it is selected for further microencapsulation process.

Chitosan microspheres (D3) were microencapsulated with Eudragit S 100 in different coat/core ratios by emulsion solvent evaporation technique to achieve colon targeted delivery of Carvedilol. The effect of core – coat ratio of Eudragit S 100 coated microspheres on particle size was studied and the size analysis study has shown that average particle size of all the batches is around 89.3µm. Particle size was increased from 63.98 to 115.64 µm with increasing core – coat ratio from 1:2 to 1:8 (table **3**). This increase in particle size may be due to corresponding increase in polymer concentration that results in larger emulsion droplets. Scanning Electron Micrographs of microencapsulated chitosan microspheres exhibited smooth surface and spherical shape (figure **5**). Angle of repose of different batches varies from 26.57º to 30.96º, Hausner's ratio and Carr's index from 0.746 to 0.876 and 12.24 to 25.37 respectively, indicating a good flow property (table **3**).[15]

Figure 5. Scanning Electron Micrographs of Eudragit S100 coated chitosan microspheres

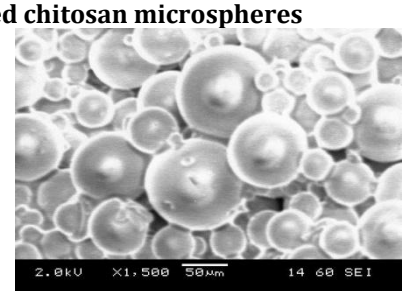


Table 3. Micromeritic study of of different batches Eudragit S100 coated chitosan microspheres

Formulation code	Angle of repose (θ)	Hausner's ratio	Carr's index
EC1	29.75	0.807	19.28
EC2	30.96	0.746	25.37
EC3	26.57	0.875	12.46
EC4	28.30	0.876	12.24

The drug loading efficiency of formulations (EC1-EC4) varied between 94% – 96% with increasing core – coat ratio (table **4**). When the core – coat ratio was increased into 1:8 a slight fall in drug loading efficiency was observed. With the increase in the polymer concentration there was increase in the encapsulation efficiency of the drug which can be attributed to the increased availability of the polymer for encapsulating the drug.

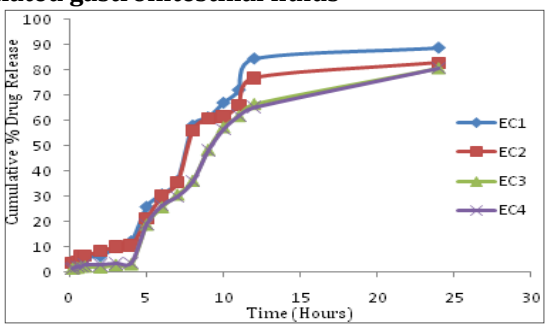
Table 4. Effect of core - coat ratio on Eudragit S 100 coated chitosan microspheres

Formulation code	% Yield	average particle size(µm)	% drug loading	description of coated microspheres
EC1	94.2	63.98	96.30 ± 0.141	Small discrete spheres of uniform size.
EC2	95.7	82.18	94.33 ± 0.763	Discrete uniform spheres.
EC3	96.8	95.62	96.76 ± 0.673	Discrete uniform spheres.
EC4	97.5	115.64	95.88 ± 0.964	Large discrete spheres.

The *in-vitro* drug release studies of various Eudragit S100 coated chitosan microspheres were performed in simulated gastrointestinal fluids as shown in figure **6**. The effects of core – coat ratio on the in vitro drug release were studied. The drug release studies indicated that Eudragit S100 coating around the chitosan microspheres

offers a high degree of protection from premature release in the stomach and small intestine.

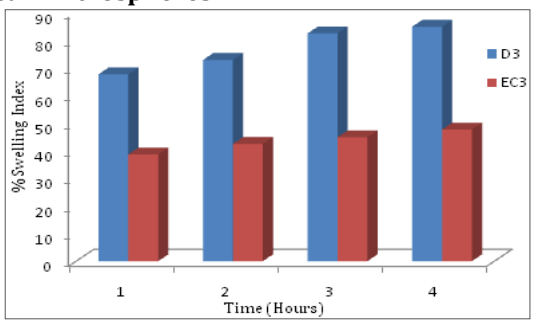
Figure 6. Percent cumulative *in-vitro* drug release profile of Carvedilol from Eudragit S 100 coated chitosan microspheres in different core coat, in simulated gastrointestinal fluids



Results showed that 12.26% to 10.50% of drug was released within initial 4 hours from the formulations EC1 and EC2 respectively. The drug release rate was increased after 4h, at that time formulations were exposed to pH 7.4, which is above the solubility of the Eudragit S 100. EC3 formulation containing 1:6 core – coat ratio releases only 3.18% of Carvedilol within 4 hours. A similar drug release pattern was observed for EC4 formulation. An increase in coat thickness of Eudragit S shows a decrease in the dissolution rate of Carvedilol which can be attributed to the greater binding of the drug with polymer.

By swelling study it was found that no significant swelling was observed with Eudragit coated chitosan microspheres (EC3) as compared to uncoated chitosan microspheres (D3) (figure **7**).

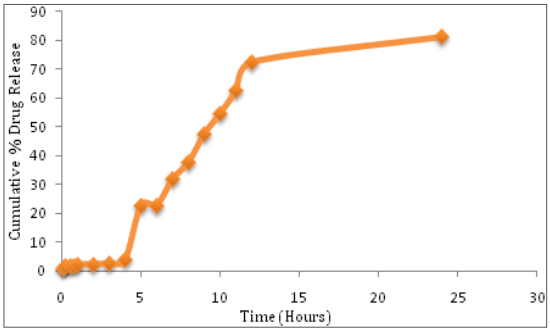
Figure 7. Degree of swelling of coated and uncoated chitosan microspheres



Swellability study indicated the better resistance of Eudragit coated chitosan microspheres in upper GI tract to swelling and subsequent drug releasing at non targeted site. From the swellability study it is also predicted that chitosan microspheres were properly coated with Eudragit S 100.

As EC3 formulation was found to be the best formulation,it is filled in a capsule size of 1 by hand filling by considering the antianginal dose. Capsule size was selected based on the powder density of coated microspheres which is about 0.625 and the best suited capsule size is capsule size 1. Results showed that 3.98% of drug was released within initial 4 hours from the capsule (figure **8**). The drug release rate was increased after 4h, at that time formulations were exposed to pH 7.4, which is above the solubility of the Eudragit S 100.

Figure 8. Percent cumulative *in-vitro* drug release profile of Carvedilol from capsule in simulated gastrointestinal fluids



CONCLUSION

The formulated Eudragit coated Chitosan microspheres would be a promising chronotherapeutic drug delivery system for Carvedilol for the treatement of angina pectoris and hypertension. The microspheres were designed to release the drug after a predetermined time delay and maintain controlled drug levels throughout the day.

ACKNOWLEDGMENT

The authors are indebted to Ranbaxy Laboratories, Delhi, India for gift sample of Carvedilol. They are also thankful to Principal and staff of Grace College of Pharmacy, Kodunthirapally, Palakkad, Kerala.

REFERENCES

1. Kitamura T, Onishi K, Dohi K, Okinaka T, Ito M, Isaka N and Nakano T; Circadian rhythm of blood pressure is transformed from a dipper to a non-dipper pattern in shift workers with hypertension. Journal of hypertension. March 2002; 16(3), 193-197.
2. Shivakumar H N, Sarasija Suresh, Desai B G; Design and evaluation of multiparticulate system foor the chronotherapeutic delivery of diltiazem hydrochloride. Indian journal of pharmaceutical sciences. 2006; 66(6):781-787.
3. Akhgari A, Afrasiabi Garekani H, Sadeghi F, Azimaie M; Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. International Journal of Pharmaceutics. 2005; 305, 22–30.
4. Hua Zhang, Ibrahim A Alsarra and Steven H Neau; An *in-vitro* evaluation of a chitosan-containing multiparticulate system for macromolecule delivery to the colon. International Journal of Pharmaceutics. 2002; 239, 197-205.
5. Laila Fatima Ali Asghar and Sajeev Chandran; Multiparticulate Formulation. Approach to Colon Specific DrugDelivery: Current Perspectives. J Pharm Pharmaceut Sci. 2006; 9 (3): 327-338.
6. Roy S, Panapalia S G, Nandy B C, Rai V K, Tyagi L K, Dey S, Meena K C; Effect of method of preparation on chitosan microspheres of mefenamic acid. International Journal of Pharmaceutical Sciences and Drug Research. 2009; 1(1), 36-42.
7. Jain S K, Rai G, Saraf D K, Agrawal G P; The preparation and evaluation of albendazole microspheres for colonic delivery. Pharmaceutical Technology. 2004; 28(12): 66-71.
8. Sakchai Wittaya-areekul, Jittiporn Kruenate, Chureerat Prahsarn; Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. International Journal of Pharmaceutics. 2006; 312:113–118.
9. Koradia H D, Patel V A, Patel H V, Patel K N; Preparation and Elvaution Of Thermally Cross-Linked Chitosan

Clarithromycin Mucoadhesive Microspheres. International Journal of Pharmaceutical Research. 2009; 1(3):60-65.

10. Jos S, Dhanya K, Cinu T A, Alleykutty A; Multiparrticulate system for colon targeted delivery of ondansetrone. Indian journal of pharmaceutical sciences. Indian J Pharm Sci. 2010; 72(1):58-64.
11. Gang Cheng, Feng An, Mei-Juan Zou, Jin Sun, Xiu-Hua Hao, Yun-Xia He; Time- and pH-dependent colon-specific drug delivery for orally administered diclofenac sodium and 5-aminosalicylic acid. World J Gastroenterol. 2004; 10(12):1769-1774.
12. Tanwar Y S, Naruka P S, Ojha G R; Development and evaluation of floating microspheres of verapamil hydrochloride. Revista Brasileira de Ciências Farmacêuticas, Brazilian Journal of Pharmaceutical Sciences 2007; 43(4):529-534.
13. Ji Zhang, Zhaoli Du, Shengjun Xu, and Shengtang Zhang; Synthesis and Characterization of Karaya Gum/Chitosan Composite Microspheres. Iranian Polymer Journal. 2009; 18(4)307-313.
14. Tropac website; Mini Capsules for Laboratory rats and guinea pigs. http://www.torpac.com/Torpac%20Rat%20Gpig%20Catalog.pdf, Accessed on 28 December 2010.
15. Escubed Limited. Particle & powder density Hausner index and Carr ratio; http://www.escubed.co.uk/sites/default/files/density_measurement_%28an006%29_carrs_index_and_hausner_ratio.pdf, Accessed on 12 December 2010.