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DEVELOPMENT OF CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM OF TRIMETAZIDINE HYDROCHLORIDE FOR ANGINAPECTORIS

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ABSTRACT

The aim of the present study is to develop colon targeted drug delivery systems for Trimetazidine Hcl using sodium alginate as a carrier. In this study, investigation of an oral colon specific, pulsatile device to achieve time or site specific release of Trimetazidine, based on chronopharmaceutical considerations. The basic design consists of an insoluble hard gelatin capsule body, filled with sodium alginate microsphere of trimetazidine and sealed with a hydrogel plug. The entire device was enteric coated, so that the variability in gastric emptying time can be overcome and a colon-specific release can be achieved. The trimetazidine microsphere were prepared, evaluated for the FTIR study, surface morphology, particle size, drug content, and from the obtained results one better formulation was selected for further fabrication of pulsatile capsule. Different concentration of the hydrogel polymers were used as plugs, to maintain a suitable lag period and it was found that the drug release was controlled by the proportion of polymers used. FTIR study confirmed that there was no interaction between drug and polymer, the shape of microsphere was found to be spherical by SEM studies. In vitro release studies of pulsatile device revealed that, increasing the hydrophilic polymer content resulted in delayed release of trimetazidine from microspheres.

Key words: *Pulsatile, Chronopharmaceutics, Anginapectoris, Trimetazidine Hydrochloride,*

1. INTRODUCTION.

Colon-specific drug delivery has gained increased importance in the delivery of drugs for the treatment of local diseases associated with the colon, such as Crohn's disease, ulcerative colitis, colorectal cancer and amoebiasis.Control Drug Delivery System (CDDS) has been developing as one of the site-specific drug delivery systems. This delivery system, by means of combination of one or more controlled release mechanisms, hardly releases drug in the upper part of the gastrointestinal (GI) tract, but rapidly releases drug in the colon following oral administration. The necessity and advantage of CDDS have been well recognized and reviewed recently[1]. In view of CDDS specifically delivering drug to the colon, a lot of benefits would be acquired in terms of improving safety and reducing toxicity when treating local or systemic chronic diseases.

CDDS would be advantageous when a delay in absorption is desirable from a therapeutic point of view, as for the treatment of diseases that have peak symptoms in the early morning and that exhibit

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circadian rhythms, such as nocturnal asthma, angina pectoris and rheumatoid arthritis[2].

Various approaches have been proposed for targeted colon drug delivery, namely pH- and time-dependent systems, pressure-controlled release systems, osmotic systems, prodrugs and polysaccharide-based delivery systems[3].

The pH approach has been shown to lack site-specificity because of inter/intra subject variation and the similarity of the pH between the small intestine and the colon. Timed-release systems depend on the relative consistency of the small intestinal transit times, but the high variability in gastric retention times makes prediction of the accurate location of drug release difficult[4]. Prodrugs and polysaccharide-based delivery systems depend on the enzymatic degradation carried out by the inherent bacterial flora present in the colon, thereby resulting in drug release. The enzyme trigger mechanism in such delivery systems makes them highly site-specific[5].

Among the different approaches to achieve target drug release to the colon , the use of polymers especially biodegrable by colonic bacteria holds great promise. Polysaccharide are bacterial enzymes that are available in sufficient quantity to be exploited in colon targeting of drugs. Based on approach polysaccharide have been investigated for colon specific drug release. These polysaccharide include pectin, alginate, guar gum, amylase, insulin, dextran, chitosan and chondroitin sulphate[6].

Trimetazidine hydrochloride, an anti anginal drug which has biological half life of 6±1.4h and pKa values 4.32 and 8.95, with good gastrointestinal absorption, which makes it an ideal candidate for chronopharmaceutical drug delivery system, to achieve time and site specific delivery of trimetazidine[7]. A therapeutic system that will synchronize the drug delivery with the circardian variation in periods of increased risk which is highly desirable for an angina regimen. This can be achieved by a bed time administration of a drug delivery system, with a delayed start of drug release can provide adequate protection in the early morning[8].

2. MATERIALS AND METHODS

2.1 MATERIALS

Trimetazidine hydrochloride (TMH) was received as a gift samples from Nivedita chemicals, IPCA, ratlam. Sodium alginate, HPMC and Guar gum was from SD Fine Chem. Limited, Mumbai. All other chemicals and solvents used were of analytical grade. Paddle stirrer (Remi Motors), dissolution apparatus (Electrolab TDL-08L) and UV-visible spectrophotometer (Shimadzu) were the equipments used in this study.

2.2 PREPARATION OF MICROSPHERES[9]

Microspheres were prepared by the water-in-oil (w/o) emulsification solvent evaporation technique. TMH was dissolved in Sodium alginate polymer aqueous solutions. The solutions were poured in to 200 g of Sesame oil containing 0.5 % span – 20 as an emulsifying agent. The aqueous phase was emulsified into the oily phase by stirring the system in a 500 ml beaker. Constant stirring at 2000 rpm was carried out using mechanical stirrer. The beaker and its content were heated by an on the hot plate at 80˚. Stirring and heating were maintained for 4-5 h until the aqueous phase was completely removed by evaporation. The Sesame oil was decanted and collected microspheres were washed three times with 100 ml aliquots of n-hexane, filtered through Whatman filter paper, dried in an oven at 50˚ for 2 h and stored in desiccators at room temperature. Three different formulations with drug: polymer ratios (1:1, 1:2, 1:3) are prepared, named A1, A2 and A3 respectively.

2.3 EVALUATION OF MICROSPHERES

A} Drug-excipient interaction study

FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm-1. FTIR study was carried on TMH, physical mixture of TMH and polymer, TMH microspheres and blank microspheres.

B} Particle size and external morphology

Determination of average particle size TMH microspheres was carried out by optical microscopy. External morphology of TMH microspheres was carried out by Scanning electron microscopy (SEM). SEM studies were carried out by using JEOL JSM T-330A Scanning microscope (Japan). Dry TMH microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of TMH microspheres were taken by random scanning of the stub.

C} Micromeritic properties

Flow properties of the microspheres were evaluated by determining the angle of repose and the compressibility index. Static angle of repose was measured according to the fixed funnel and free standing cone method of Banker and Anderson. A funnel with the end of the stem cut perpendicular to the axis of symmetry is secured with its tip at a given height (1 cm), H, above graph paper placed on a flat horizontal surface. The microspheres were carefully poured through the funnel until the apex of the conical pile so formed just reached the tip of the funnel. Thus, the R being the radius of the base of the microspheres conical pile:

$\tan \theta = H/R$ or $\theta = \tan^{-1}(H/R)$

Where θ = the angle of repose.

Compressibility index (I) values of the microspheres were determined by measuring the initial volume (V0) and the final volume (V) after subjecting to 100 tappings in a graduated measuring cylinder using the equation

$I = [1- (V/V0)] \times 100$

D} Determination of Percentage Drug Entrapment (PDE)

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula;

$$PDE = \frac{Practical\ drug\ loading}{Theoretical\ drug\ loading} \times 100$$

Theoretical drug loading

Theoretical drug loading was determined by calculation assuming that the entire drug present in the polymer solution used gets entrapped in microspheres, and no loss occurs at any stage of preparation of microspheres

Practical drug loading

Weighed amount of TMH microspheres equivalent to 100mg of Trimetazidine hydrochloride was dissolved in 100ml of distilled water. This solution was kept overnight for the complete dissolution of the TMH in water. This solution was filtered and further diluted to make a concentration of 10µg/ml solution. The absorbance of the solutions was measured at 269 nm using double beam UV-Visible spectrophotometer against distilled water as blank and calculated for the percentage of drug present in the sample.

E} *In vitro* release studies

In vitro dissolution profile each formulation was determined by employing USPXXIII dissolution test apparatus by rotating basket method in 900 ml of phosphate buffer pH 7.4, at 100rpm, 37 ±0.5 ºC . Microspheres equivalent to 40 mg of TMH were filled into dialysis bag and loaded into the basket of the dissolution apparatus. 5ml of sample withdrawn from the dissolution media at suitable time intervals and the same amount was replaced with fresh

buffer. The absorbance of the filtrate was determined at wave length of 269 nm by using UV-vis spectrometer, against pH 6.8 as blank. The amount of drug present in the filtrate was then determined from the calibration curve and cumulative percentage of drug release was calculated. Data obtained was also subjected to kinetic treatment to understand release mechanism.

2.4 PREPARATION OF CROSS-LINKED GELATIN CAPSULES[10]

10-20 ml of formaldehyde was taken into dessicator and a pinch of potassium permanganate was added to it, to generate formalin vapours. The wire mesh containing the empty bodies of the 100 mg capacity hard gelatin capsules was then exposed to formaldehyde vapors. The dessicator was tightly closed, the reaction was carried out for 12 hours Then they were removed and kept on a filter paper and dried for 48h to ensure completion of reaction between gelatin and formaldehyde vapors, afterwards the capsules were kept in an open atmosphere, to facilitate removal of residual formaldehyde. These capsule bodies were capped with untreated cap and stored in a polythene bag.

Various physical tests such as, identification attributes, visual defects, dimension changes, solubility studies were carried out.

2.4 FORMULATION OF PULSATILE DRUG DELIVERY SYSTEM[10]

The microspheres equivalent to 40 mg of the drug were incorporated into treated bodies of empty capsule shells using a manual hand filling machine and plugged with different hydrogel polymers like HPMC, Guar gum at different concentration (20mg and 40mg for each polymer). The joint of the capsule body and cap was sealed with a small amount of the 5% ethyl cellulose ethanolic solution. The treated capsules were completely coated with 5% cellulose acetate phthalate (CAP) to prevent variable gastric emptying. Coating was repeated until an 8-12% increase in weight obtained. %weight gain of the capsules before and after coating was determined. (5%w/v solution of CAP was prepared by using acetone: ethanol (8:2) as solvent.)

2.5 EVALUATION OF DESIGNED PULSATILE CAPSULE

***A} In vitro* release profile**

In vitro dissolution profile of each formulation was determined by employing USP XXIII apparatus by rotating basket method in different media like stimulated gastric fluid pH 1.2 buffer for 2 hours (since the average gastric emptying time is 2hrs), stimulated intestinal fluid pH 7.4 buffer for 3hours (average small intestinal transit time is 3hrs) and colonic fluid pH 6.8 buffer for subsequent hours. The dissolution media were maintained at a temperature of 37± 1ºC, the speed rotation of basket maintained were 50rpm. Microsphere equivalent to 40 mg TMH modified pulsing capsules were placed in basket in each dissolution vessel to prevent floating.5ml of the samples withdrawn from dissolution media at suitable intervals and same amount was replaced with fresh buffer. The absorbance was measured at 269 nm.

3. RESULTS AND DISUSSION

As indicated in introduction, the aim of the work described here was to design a new pulsatile, colonic drug delivery device, for better treatment of angina pectoris.

Drug-excipient interaction study

From the spectra of TMH, combination of TMH with polymer, TMH microspheres and blank microspheres, it was observed that all characteristic peaks of TMH were present in the combination spectrum, thus indicating compatibility of the drug and polymer. IR spectra are shown in figure 1.

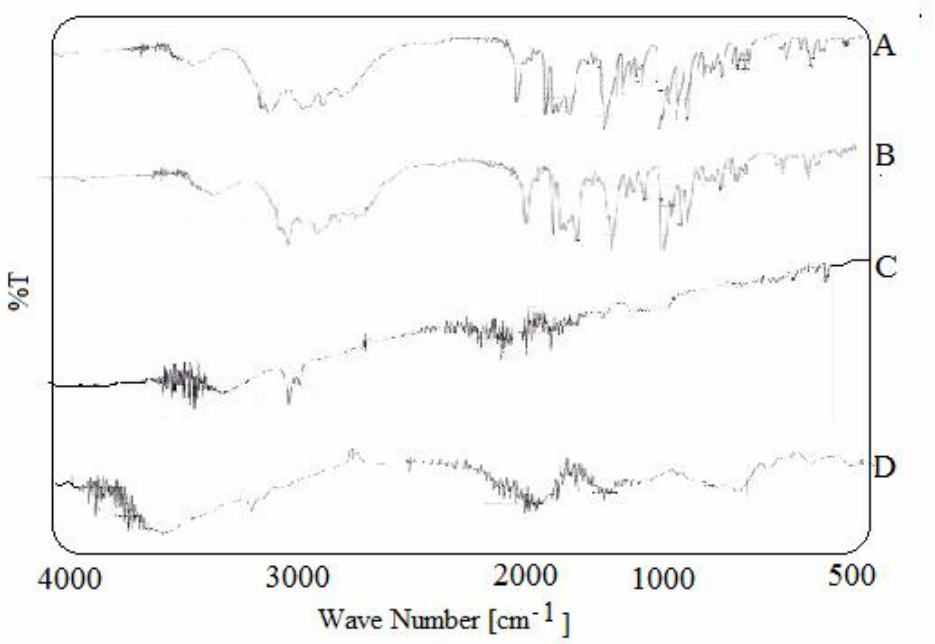


Figure 1: IR Spectrum of A) TMH B) TMH and polymer C) TMH microspheres D) Blank microspheres

Particle size and external morphology

The results normal frequency distribution of microspheres shown in figure 2. As the drug to polymer ratio was increased, the mean particle size (MPS) of TMH microspheres was also increased (Table 1). The surface morphology of TMH loaded microspheres were studied by scanning electron microscopy (Fig. 3). Surface smoothness of MS was increased by increasing the polymer concentration, which was confirmed by SEM. At lower polymer concentration rough and wrinkled surface of MS was obtained (Fig. A1) and at higher polymer concentration the MS with smooth surface was obtained (Fig. A3).

Table 1: Average diameter of TMH microspheres

Sl. No	Formulation code	Average size (µm)±SEM
1	A1	521±9.18
2	A2	651±6.27
3	A3	714±7.31

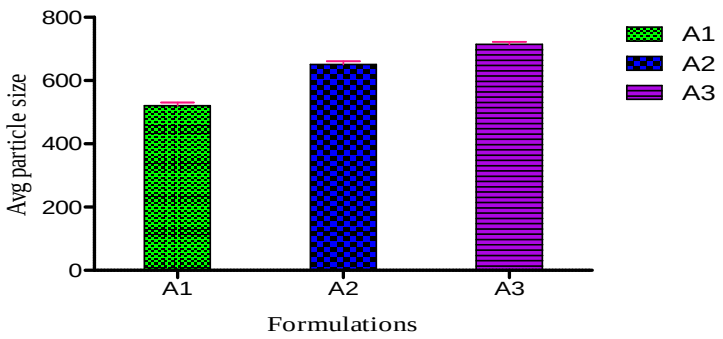
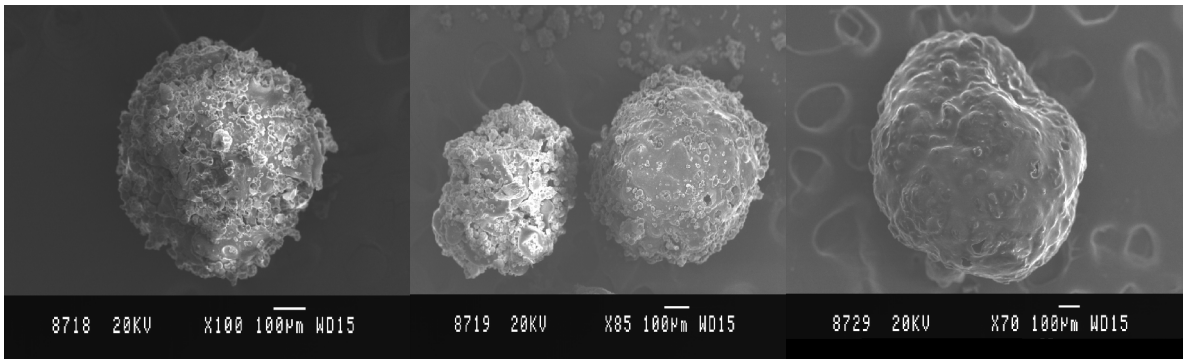


Figure 2: Average diameter of TMH microspheres



A1 **A2** **A3**

Figure 3: SEM photographs of microspheres of different formulation

Micromeritic properties

The results of the micrometric studies carried out in drug loaded microspheres are given The values of angles of repose were in the range of 21.90± 0.55 degrees to 29.33 ± 0.90 and the values of compressibility indices were in the range of 15.67 ± 1.2 % to 22.66 ± 1.1 % for Sodium alginate microspheres which indicate an overall good free flowing nature of microspheres of all batches. Values of angle of repose ≤ 30° usually indicate a free flowing material, while values of compressibility index below 25 % give rise to good flow characteristics.

Percentage Drug Entrapment

A maximum of 97.56 % of drug entrapment efficiency was obtained by the method employed. By increasing the polymer concentration the encapsulation efficiency was increased (Table 2). Amount of microspheres to be taken for invitro release studies and further development of pulsatile capsule was calculated based on the content of drug present in each formulation.

Table 2: Drug entrapment efficiency of TMH microspheres

Sl. No	Formulation code	Percentage Yield	Drug content (%)	Entrapment Efficiency (%)
1	A1	74.37	56.29	63.74
2	A2	82.91	41.96	79.92
3	A3	88.48	32.52	97.56

***In vitro* release studies for TMH microspheres**

In vitro release studies were carried out using USP XXIII dissolution assembly. The release profile obtained for all the three formulations were shown in figure 4. It was observed that the drug release from formulation decreased with increase in the amount of polymer added in each formulation. The release of drug from polymer matrix take place after complete swelling of the polymer and as the amount polymer in the formulation increase the time required to swell also increase thereby decrease in the drug release. However, the release showed a bi-phasic release with an initial burst release with an initial burst effect. In the first 60 min drug release was 28.14%, 25.27% and 20.12% for A-1, A-2 and A-3 respectively. The mechanism for the burst release can be attributed to the drug loaded on the microspheres or imperfect entrapment of drug. The overall cumulative % release for the A1, A2, and A3

were found to be 93.12%, 88.44% and 81.12% at end of 12th hour.

In vitro release study was analyzed using various mathematical models. The slopes and the regression co-efficient of determinations (R^2) were listed in table 3. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism.

Additional evidence for the diffusion controlled mechanism was obtained by fitting the Korsemeyer-Peppas equation to the release data. The diffusion exponent 'n' values were found to be in the range of 0.5 to 1 for the TMH microspheres indicating Non-Fickian diffusion.

Figure 4: Comparative *in vitro* release profile of TMH microspheres

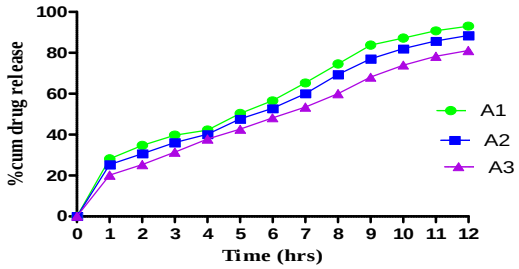


Table 3: Regression co-efficient (R^2) values of different kinetic models and diffusion exponent (n) of Peppas model for TMH microspheres.

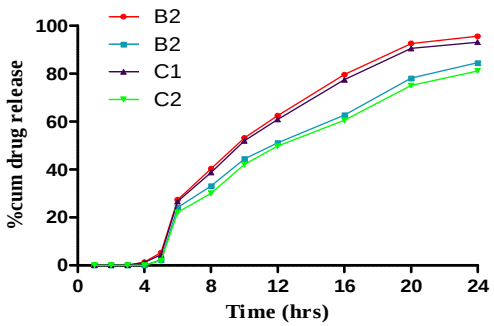
Formulation	Zero order	First order	Higuchi Matrix	Peppas plot	
				R^2 value	'n' value
A1	0.9844±0.0003	0.9358±0.02	0.9577±0.0002	0.9460±0.005	0.5290 ± 0.039
A2	0.9908±0.0004	0.9513±0.04	0.9639±0.0005	0.9559±0.007	0.5512 ± 0.038
A3	0.9965±0.0003	0.9660±0.02	0.9741±0.0003	0.9768±0.004	0.5986 ± 0.030

EVALUATION OF MODIFIED PULSATILE CAPSULES

***In vitro* release studies of modified pulsatile capsules**

On the basis of the drug encapsulation efficency, particle size morphology, *invitro* release formulation A3 was selected as better formulation for designing pulsatile device. The release profile obtained for all the four formulations were shown in the figure 5. The results obtained for all four formulations B1 (HPMC 20%)-B2 (HPMC 40%) and C1 (Guar gum 20%)-C2 (Guar gum 40%) are shown in the figure, indicating the plots of comparative in vitro release profile for formulation. During dissolution studies, it was observed that, the enteric coat of the cellulose acetate phthalate was intact for 2h in pH in 1.2, but dissolved in intestinal pH, leaving the soluble cap of the capsule, which also dissolved in pH 7.4, then the polymer plug absorbed the surrounding fluid, swelled and release through the swollen matrix. After complete wetting of the plug, it formed a soft mass, which was then easily ejected out of the capsule body, releasing the alginate microspheres into stimulated colonic fluid. With all the formulations, there was absolutely no drug release in pH 1.2, thus indicating the efficiency of 5% CAP for enteric coating.

Figure 5: Comparative *in vitro* release profile of TMH modified pulsincap formulations



Formulation with HPMC and Guar gum as hydrogel plug

With formulations B1 and C1 at the end of the 5th hour 5.31 % and 4.41% end of 24th hour 95.61% and 93.12% drug was released respectively.

In case B2 and C2 at the end of the 5th hour 2.20% and1.98% end of 24th hour 84.52% and 81.12% drug was released respectively shown in figure 5.

4. CONCLUSION

The study demonstrates that trimetazidine microsphere could be successfully targetted to colon by design of time dependent and polysaccharide based chronopharmaceutical formulation. In conclusion, drug release over a period of 4-24h, can be achieved from insoluble gelatin capsule, in which microsphere was sealed by means of a hydrogel plug. The release of TMH from the formulation is proportional of hydrogel. As the concentration of polymer was increased, the drug release rate decreased.

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