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DESIGN AND EVALUATION OF TIME AND pH DEPENDENT DELIVERY SYSTEMS OF LERCANIDIPINE HYDROCHLORIDE FOR CHRONOMODULATED THERAPY

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ABSTRACT: The present work deals with the development and characterization of lercanidipine hydrochloride pulsatile drug delivery system for chronomodulated therapy for improvement in therapeutic index and efficacy. The lercanidipine hydrochloride chitosan microspheres were prepared by emulsion cross linking method by varying drug to polymer ratio and evaluated for the particle size, drug content and in vitro release profiles. Optimised formulations were selected and enteric coated with Eudragit S-100 and Eudragit L-100. The two enteric coated formulations were selected for further fabrication of pulsatile capsule. Different hydrogel polymers were used as plugs, to maintain a suitable lag period. The entire device was enteric coated with cellulose acetate phthalate, so that colon-specific release can be achieved *In vitro* release studies of pulsatile device revealed that, increasing the hydrophilic polymer content resulted in delayed release of lercanidipine Hcl from microcapsules. The microspheres remained stable during the stability period without any significant changes in their physico-chemical properties.

Key words: Lercanidipine Hcl; Chitosan microspheres; *In-vitro* characterization; p^H dependent.

INTRODUCTION:

Hypertension (HTN) or high blood pressure is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated above the normal value i.e. (140/90).The prevalence of hypertension increases with advancing age; for example, about 50% of people between the ages of 60 and 70 years old have hypertension, and the prevalence is further increased beyond age 70. Hypertension is classified as either primary (essential) hypertension or secondary hypertension about 90–95% of cases are categorized as "primary hypertension," which means high blood pressure with no obvious medical cause. Elevated arterial pressure causes pathological changes in the vasculature and hypertrophy of the left ventricle. As a consequence, hypertension is the principal cause of stroke, is a major risk factor for coronary artery disease and its attendant complications myocardial infarction and sudden cardiac death, and is a major contributor to cardiac failure, renal insufficiency and dissecting aneurysm of the aorta (Tripathi, 2003)

Lercanidipine hydrochloride, a calcium-channel blocker, which is chemically *2-[(3,3-diphenylpropyl)methylamino]- 1,1-dimethylethylmethyl- 2,6-dimethyl-4-(3-nitrophenyl) 1,4-dihydropyridine-3,5-dicarboxylate*, is a relative new drug which belongs to the well-known pharmacological active compound series classified as 1,4-dihydropyridine calcium channel blockers. This drug is used for treating angina pectoris and hypertension, based on its selectivity and specificity on the smooth vascular cells. This molecule corresponds to a new molecular design in which its liposolubility has been increased to obtain a long action. It is an amphypatic drug which is transported quickly across the cellular barrier, arriving inside to both hydrophilic and hydrophobic sites in spite of its highest solubility in the lipophyllic bilayer. This fact explains the differences observed in both the clinical and the pharmacokinetic profiles compared with other type of drugs. For example, a long action of amlodipine in connection with a long plasma half life, in contrast lercanidipine exhibits a short plasma half-life compared with a long pharmacological effect. The drug is orally administered in dose of 10- 20 mg daily as its hydrochloride salt reducing significantly the blood diastolic pressure after a single dose. It is rapidly absorbed from gastrointestinal tract, widely distributed and undergoes an extensive first pass metabolism generating mainly inactive metabolites. Its half-life of elimination ranges from 2 to 5 h, but the therapeutic action is increased about 24 h due to it high liposolubility (Martindale 2005).

The objective of this study was to develop a capsule system based on chronopharmaceutical approach for the treatment of hypertension using lercanidipine hydrochloride as a model drug. The aim was to have a lag time of 4 h, i.e., the system is to be taken at bed time (10 pm) and is expected to release the drug after a period of 4 h, i.e., at 2 am.

MATERIALS AND METHODS

Lercanidipine hydrochloride drug was obtained as a gift sample from Hetero Pharma Ltd, Hyderabad, India. Chitosan was obtained as a gift sample from EM Bios Ltd Mumbai and Primex Ltd, Iceland respectively. All other chemicals were purchased from SD Fine Chemicals Mumbai.

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Method of preparation:

Lercanidipine HCl loaded chitosan microspheres were prepared by emulsion cross linking technique using glutaraldehyde as a cross-linking agent. Accurately weighed quantity of chitosan (2%w/v) was dissolved in 1%v/v acetic acid solution by stirring overnight on a magnetic stirrer. Thereafter, known quantity of drug was dissolved in the polymeric solution using magnetic stirrer and then ultra-sonicated for 15 minutes to remove the air bubbles. The resulting solution was added drop wise through syringe (needle no: 20) into 50 ml of dispersion medium of light liquid paraffin containing 1% w/w of tween-80 and the dispersion was stirred at 1000 rpm using digital overhead stirrer. The system was allowed for emulsification for 30 minutes and then known volume of glutaraldehyde (25% v/v aqueous solution) was added and stirring was continued for the specified time period. Microspheres thus obtained were filtered and washed several times with petroleum ether to remove traces of oil and then they were finally washed with acetone to remove excess amount of glutaraldehyde. The microspheres were then dried at room temperature for 24 hrs (Kotadiya et al, 2009).The formulation details are given in Table 1.

Enteric coating of the drug loaded CHT microspheres:

The drug-loaded Chitosan microspheres were enteric coated with Eudragit L-100 and S-100 in the ratios of 1:2 following the same technique. The prepared microspheres were suspended in 5 ml of 10% w/v ethanolic solution of Eudragit L-100 and S-100 (1:2) and emulsified into 100 ml of light liquid paraffin containing 1% w/w of tween-80. Emulsification was maintained using an overhead stirrer at 500 rpm to allow complete solvent evaporation. The coated microspheres formed were filtered, washed with n-hexane, and dried for 48 hours in a vacuum oven (Shivakumar et al, 2006). The formulation details are given in Table No.2.

Formulation of pulsatile drug delivery system:

The bodies and caps of formaldehyde treated hard gelatine capsules were separated manually. Microcapsules equivalent to 20 mg of Lercanidipine HCl were accurately weighed and filled into the treated bodies by hand filling. The capsules containing the microcapsules were then plugged with different amounts (15, 30 and 45 mg) of various polymers, i.e., Guar gum, HPMC K100M and Xanthan gum. The joint of the capsule body and cap was sealed with a small amount of the 5% ethyl cellulose ethanolic solution.

Thermograms were obtained by using a differential scanning calorimeter (DSC Q20 V24.4 Build 116, Japan.) at a heating rate of 10^{o}C/min over a temperature range of 0-200°C The sample was hermetically sealed in an aluminum crucible. Nitrogen gas was purged at the rate of 10 ml/min for maintaining inert atmosphere.

The sealed capsules were completely coated by dip coating method with 5% Cellulose acetate pthalate in 8:2 (v/v) mixture of acetone: ethanol plasticized with dibutylphthalate (0.75%), to prevent variable gastric emptying. Coating was repeated until an 8–12% increase in weight is obtained. Increase in the weight of the capsules after coating was determined (Mastiholimath et al, 2007). Composition of the pulsatile drug delivery system is given in Table No.3.

Evaluation:

Size analysis:

The particle size of microspheres was determined by optical microscopy method. The microscope eyepiece was fitted with micrometer and then the calibration of the eye piece micrometer was done by stage micrometer and then the microspheres were uniformly spread on a slide which was mounted on a mechanical stage. Approximately 100 microspheres were counted for the particle size (Subramanyam, 2005).

Scanning electron microscopy

The particle shape and surface morphology of microspheres were examined by scanning electron microscopy. Microspheres were fixed on aluminum studs and coated with platinum using a sputter coater SC 402, under vacuum (0.1 mm Hg). The microspheres were then analyzed by SEM (JSM-6360, JOEL, London, U.K.).

Encapsulation efficiency

Crushed microspheres equivalent to 10 mg of Lercanidipine hydrochloride were dispersed in 50 ml of phosphate buffer of pH 7.4. The content was stirred for 24hrs and filtered. After suitable dilution, the drug content was analyzed spectrophotometrically at 351.5 nm against blank. The estimation was carried out in triplicate using calibration curve constructed in the same media. Polymers did not interfered at this wavelength (Bodmeier et al, 1994).

The encapsulation efficiency was calculated using the formula:

$$\text{Encapsulation efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \times 100$$

Fourier transform infrared spectroscopy (FT-IR) study

The compatibility between drug and polymer was detected by IR spectra obtained on Shimadzu 8400 instrument, Japan. The pellets were prepared on KBr-press (spectra lab, India). The spectra were recorded over the wave number range of 4000 to 400 cm^{-1}.

Differential scanning calorimeter (DSC) study

In- vitro dissolution studies

A. Chitosan core microspheres;

The drug release from the microspheres was studied, by employing USP XXIV rotating basket type apparatus (Model TDT-08L-Electrolab). Accurately weighed microspheres equivalent to 10mg of Lercanidipine Hcl were filled into muslin cloth and

loaded into the basket of dissolution apparatus. Dissolution studies were carried in phosphate buffer of p^H 7.4. Five ml of the sample was withdrawn from the dissolution media at suitable time intervals and the same amount was replaced with fresh buffer and the study was continued up to 24 hrs. The absorbance of the filtrate was determined at 351.5 nm using UV-Visible spectrophotometer, against the phosphate buffer of pH 7.4 as blank. The amount of drug present in the filtrate was then determined from the calibration curve and cumulative percent of drug release was calculated.

B. Eudragit coated chitosan core microspheres:

In vitro dissolution profile of lercanidipine hydrochloride from eudragit coated chitosan coarse was carried out in phosphate buffer of p^H 7.4 till the end of the study using the same procedure. The dissolution conditions and the method for the analysis of the drug in the withdrawn sample remained same as mentioned above.

Stability Studies:

The stability study of the optimized microsphere formulation was carried out according to ICH guidelines at 40±2°C/75±5%RH for three months by storing the microspheres in stability chamber (Lab-care, Mumbai). The formulations were evaluated for physical attributes (size, shape, colour etc.), encapsulation efficiency (%) and in vitro drug release after stability period.

Preparation of cross-linked gelatin capsules:

25 ml of 15% (v/v) formaldehyde was taken into dessicator and a pinch of potassium permanganate was added to it, to generate vapors. The wire mesh containing the empty bodies of the 100 mg capacity hard gelatin (about 100 in number) capsule was then exposed to formaldehyde vapours. The caps were not exposed leaving them water-soluble. The dessicator was tightly closed. The reaction was carried out for 12 h after which the bodies were removed and dried at 50 ◦C for 30 min to ensure completion of reaction between gelatin and formaldehyde vapors. The bodies were then dried at room temperature to facilitate removal of residual formaldehyde. These capsule bodies were capped with untreated caps and stored in a polyethylene bag.

Qualitative chemical test for free formaldehyde:

Standard formaldehyde solution used is formaldehyde solution of 0.002 %w/v and sample solution is formaldehyde treated bodies (about 25 in number), which were cut into small pieces and taken into a beaker containing distilled water. This was stirred for 1 h on a magnetic stirrer, to solubilize the free formaldehyde. The solution was then filtered into a 50 ml volumetric flask, washed with distilled water and volume was made up to 50 ml with the washings. In brief, to 1 ml of sample solution, 9 ml of water was added. One ml of resulting solution was taken into a test tube and mixed with 4 ml of water and 5 ml of acetone reagent. The test tube was warmed in a water bath at 40 °C and allowed to stand for 40 min. The solution was not more intensely colored than a reference solution prepared at the same time and in the same manner using 1 ml of standard solution in place of the sample solution. The comparison was made by examining tubes down their vertical axis (Mastiholimath et al, 2007).

C. In vitro release profile of pulsatile capsule:

Dissolution studies were carried out by using USP XXIV rotating basket type apparatus (Model TDT-08L-Electrolab). Capsule was placed in the basket; the capsule should be immersed completely in dissolution media. In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used, referred to as sequential pH change method. When performing experiments, the pH 1.2 medium was first used for 2 h (since the average gastric emptying time is 2 h), then removed and the fresh pH 7.4 phosphate buffer saline (PBS) was added. After 3 h (average small intestinal transit time is 3 h), the medium was removed and fresh pH 6.8 dissolution medium was added for subsequent hours. 900 ml of the dissolution medium was used each time. Rotation speed was set at 100 rpm and temperature was maintained at 37±0.5 ◦C. 5 ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 351.5 nm, by UV absorption spectroscopy and the cumulative percentage release was calculated over the sampling times (Khan et al, 1999).

Release kinetics

Data obtained from the in vitro release studies were fitted to various kinetic models such as zero order, first order, Higuchi model and Kosmeyer peppas model.

RESULTS AND DISCUSSION:

Particle size

The emulsion cross linking method resulted in the formation of discrete, spherical microspheres for chitosan with particle size range of 67.63±0.03 to 91.32±0.03µm. Proportionate increase in particle size was observed with increased core: coat ratio. This could be due to greater amounts of coat material contained in a same volume of liquid droplet. This can be ascribed to an increase in the viscosity resulting in enhanced interfacial tension and demised shearing efficiency. A more viscous solution would require higher shear force for emulsification and lead to larger droplets which would result in larger particles.

Encapsulation efficiency

The encapsulation efficiency of the microspheres was in the range of 70.68±0.08 to 89.12±0.02% which was largely affected by formulation and process variables. The encapsulation efficiency proportionately increased with increase in core: coat ratio. It was observed that, as the amount of coat material increased, more efficient entrapment of the drug within the polymeric matrix of the microspheres occurred thereby leading to higher encapsulation efficiency. The encapsulation efficiency was also increased with higher level of glutaraldehyde concentration and cross-

linking extent. This could be due to more denser cross-linking of polymeric chains that prevented the migration of drug into the dispersion medium resulting in higher encapsulation efficiency. However, the encapsulation efficiency reduced when the surfactant concentration was increased in the microspheres which could due to the reduction in the particle size of the emulsion droplets. The miscibility of solvent with liquid paraffin oil also increases with increasing emulsifier concentrations, which might increase the diffusion of drug into the continuous phase resulting into reduced encapsulation efficiency. The results are shown in Table No.4.

Scanning electron microscopy analysis

The SEM photographs revealed that, the chitosan microspheres were discrete, very spherical with smooth surface. The smooth surface could be due to formation of relatively strong covalent bonds between aldehyde group of glutaraldehyde and the amine groups of chitosan. The microspheres also exhibited some inward dents that might have occurred due to aggregation of particles before washing and drying process and the SEM photographs are depicted in Fig 1.

Fourier transformer infrared spectroscopy (FT-IR) study

Any sign of interaction would be reflected by changes in the characteristic peaks of drug in the formulation depending on the extent of interaction. The characteristic absorption bands of pure drug were compared with that of pure drug present in physical mixture and optimised formulation. From the spectra it was clear that all the characteristic absorption bands in the physical mixture as well as in the optimised formulation remained same without any significant changes. Thus the study revealed the compatibility of drug with the excipients both in physical mixture and also in the formulation shown in Fig 2.

Differential scanning calorimeter (DSC) study

The DSC thermogram obtained for pure drug shows an intense significant endothermic peak at about 194ºC. This melting range is almost in agreement with the reported melting point of the drug in the literature (190-195ºC). Similarly DSC thermogram of physical mixture shows an endothermic peak at temperature 191.4ºC and is held within the melting point range of drug. The polymer has shown a broad endothermic peak at 52.3ºC. The DSC thermogram of formulation shows the very broad endothermic peak at 193^{o}c and which is also almost in the range of the pure drug.

Since in all the three DSC thermograms though the peak nature is changed the temperature of the endothermic peak does not change appreciably. The melting point range in all the three cases remains same, clearly indicating that there is no interaction of the drug with the polymer. The results are depicted in Fig 3.

It may be concluded from the IR spectral study and DSC thermogram study that, there is absence of any possible interaction and the drug remains in the same normal form before and after the formulation.

In vitro drug release

To ascertain the effect of core: coat ratio on drug release, chitosan microspheres (A-D) were prepared and subjected for dissolution studies. The release pattern of chitosan microcapsules of A, B, C and D were to be found 95.14, 97.46, 98.40 and 99.05% at the end of 7th, 8th, 9th and 12th hr respectively. The dissolution studies indicated that microspheres were characterized by initial burst effect during first hour which could be due to release of surface adhered drug or improper coating. The burst effect was reduced with increasing chitosan levels in microspheres. Further the chitosan microspheres were coated with methacrylic acid copolymers (Eudragit L-100 and Eudragit S-100soluble at pH above 6 and 7, respectively). The release profile of the Eudragit coated Chitosan core microspheres (F) was studied in acid buffer of pH 1.2 for 2 hours and phosphate buffer of pH 6.8 till the end of the study. The drug release from coated microspheres (F) was found to be 99.42 at 16h of dissolution study. The dissolution study of the enteric coated microspheres revealed a strong drug release control (1.3% at the end of 2 hours) in pH 1.2 indicating the complete coating of the chitosan core microspheres by the adopted technique and also the efficiency of Eudragit L-100 and S-100 as enteric coating polymers. In vitro drug release from the pulsatile drug delivery system was found to be 84.23, 78.47, 69.90 % with FX1, FX2, FX3 formulations of xanthan gum, 83.77, 73.57, 61.04% with FX4, FX5, FX6 formulations of guar gum, and 91.54, 90.07, 78.09% with FX7, FX8, FX9 formulations of HPMC K 100M as plugging materials. However, when compared to other formulations containing Xanthan gum and Guar gum as plugging materials, more sustained drug release was observed with HPMC K100M as a plugging material. This could be due to higher viscosity of the HPMC than the other two polymeric plugging materials. The lag period and the drug release could be efficiently modulated by altering the concentration of plugging material to a certain extent. Thus, the study conclusively demonstrated the efficacy and suitability of these natural polymers as plugging materials in the design of pulsatile drug delivery system of lercanidipine Hcl. In vitro release profiles are shown in Fig 4-6.

Stability Studies:

The optimised formulations were subjected for three months stability study by exposing the microspheres to 40±2°C temperature and relative humidity 75±5% in programmable environmental test chamber. The stability studies revealed the absence of any considerable changes in physical attributes (size, shape, colour etc.), encapsulation efficiency (%) and in vitro drug release. The Lercanidipine Hcl loaded chitosan microspheres were found to be stable during three months stability studies. Results are shown in Table No.5.

Mechanism of drug release

Drug release mechanisms from the formulations (FX1-FX9) were determined by fitting its drug release data to various kinetic models. High regression values were observed with Krosmayer-peppas model followed by first order and zero order. The formulations showed the n value between 0.5to1 with high regression value of 0.997 which suggests non-Fickian diffusion controlled with first order kinetics. The results are shown in Table No.6.

CONCLUSION

Enteric coated pulsatile capsule remained intact in pH 1.2 for 2 hrs, plugging materials further maintained lag period for 2-3 hrs and resulted in sustained drug release over 24 hrs from microspheres as desired for the chronotherapeutic treatment of hypertension. The mechanism of drug release of pulsatile drug delivery systems was diffusion controlled first order kinetics. Hence, time and pH dependent pulsatile drug delivery systems of Lercanidipine hydrochloride showed promising results and there exists a scope for further *in vivo* evaluation using suitable animal models.

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Table 1

Formulation of Chitosan microspheres of Lercanidipine hydrochloride.

CODE	A	B	C	D
Core: Coat	1:1	1:2	1:3	1:4
Ingredients Lercanidipine hydrochloride (mg)	200	100	66.6	50
Chitosan (mg)	200	200	200	200
Tween 80 (% w/w)	1	1	1	1
Glacial acetic acid (2%w/v)	10	10	10	10
Methanol (ml)	2	2	2	2
Glutaraldehyde(ml)	2	2	2	2
Speed (rpm)	500	500	500	500

Table 2

Formula of Enteric coated microspheres of Lercanidipine hydrochloride

Code	Core microspheres batch	Core: Coat	Amount of core microspheres (mg)	Coating material (mg	
				Eudragit S-100 (mg)	Eudragit L-100 (mg)
F	C	1:5	100	333.33	166.67

Table 3

Composition of pulsatile drug delivery system

Code	Weight of empty body (mg)	Weightof Microspheres* (mg)	Plugging material	Weight of Plugging material (mg)	Total weight of capsule (mg)	Weight after HPMCP Coating (mg)
FX1	78	362	Xanthan gum	15	503	538
FX2	79	362	Xanthan gum	30	519	558
FX3	78	362	Xanthan gum	45	533	570
FX4	80	362	Guar gum	15	505	541
FX5	81	362	Guar gum	30	521	559
FX6	80	362	Guar gum	45	535	573
FX7	80	362	HPMC K100M	15	505	541
FX8	79	362	HPMC K100M	30	519	559
FX9	81	362	HPMC K100M	45	536	574

Table 4

Effect of core: coat ratio of Lercanidipine hydrochloride loaded microspheres on particle size and encapsulation efficiency.

Code	Core: Coat	Particle size (µm)	Encapsulation Efficiency (%)
A	1:1	67.63 ± 0.03	70.68 ± 0.08
B	1:2	73.21 ± 0.21	75.52 ± 0.13
C	1:3	85.58 ± 0.17	82.92 ± 0.13
D	1:4	91.32 ± 0.03	89.12 ± 0.02

Table 5

Stability studies of optimised microspheres loaded with Lercanidipine hydrochloride

Formulation code	Drug content (mg)		Encapsulation efficiency (%)		In-vitro release (%)	
	Before	After	Before	After	Before	After
C	8.29± 0.35	8.29± 0.49	82.92± 1.13	85.52 ±1.94	98.40± 0.38/9h	98.18± 0.79/9h

Table 6
In-vitro release kinetics of microspheres and pulsatile drug delivery systems containing Lercanidipine hydrochloride

Code	Zero order		First order		Higuchi		Korsmayer-Peppas	
	n	r^2	n	r^2	n	r^2	n	r^2
FX1	4.033	0.952	-0.035	0.986	14.82	0.813	0.809	0.984
FX2	3.762	0.944	-0.03	0.984	13.94	0.814	0.803	0.978
FX3	3.432	0.941	-0.024	0.976	12.61	0.806	0.850	0.967
FX4	3.915	0.956	-0.033	0.984	14.53	0.822	0.862	0.980
FX5	3.562	0.947	-0.026	0.982	13.06	0.808	0.834	0.978
FX6	2.905	0.961	-0.018	0.984	10.45	0.803	0.732	0.992
FX7	4.450	0.882	-0.049	0.967	18.52	0.852	0.852	0.927
FX8	4.157	0.950	-0.042	0.990	16.64	0.873	0.806	0.979
FX9	3.734	0.890	-0.030	0.960	15.58	0.861	0.848	0.922

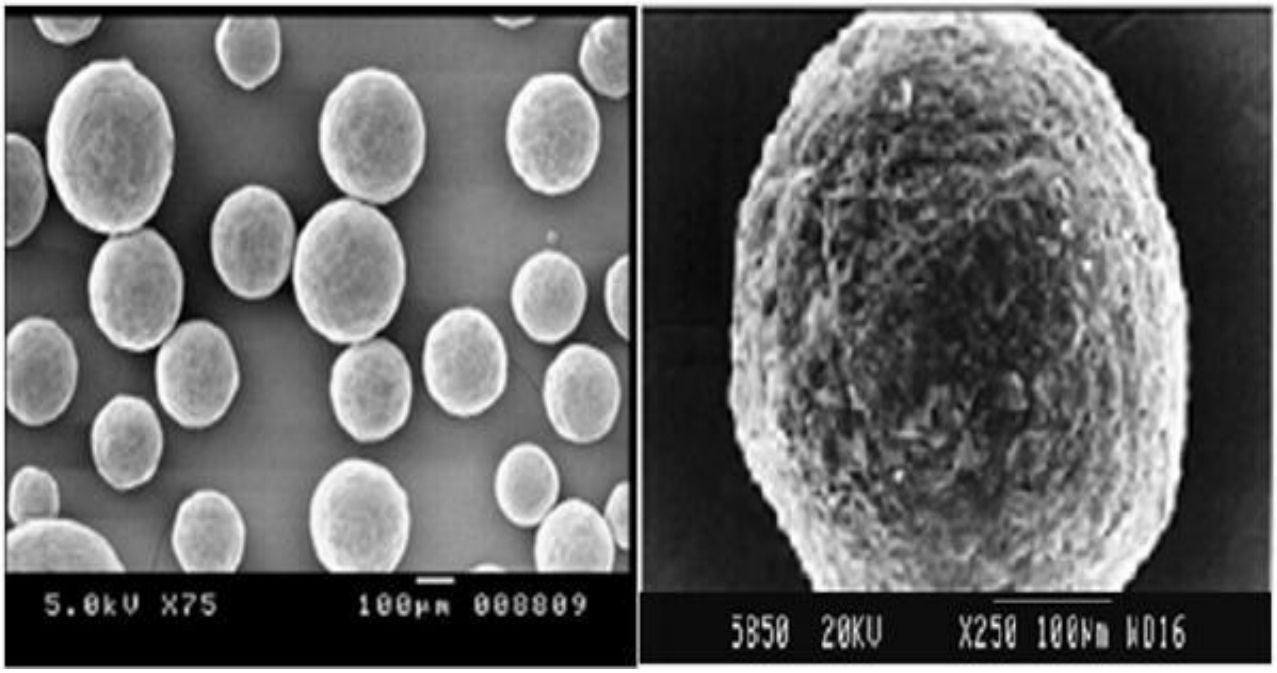

Fig 1 SEM photographs of eudragit coated chitosan microspheres

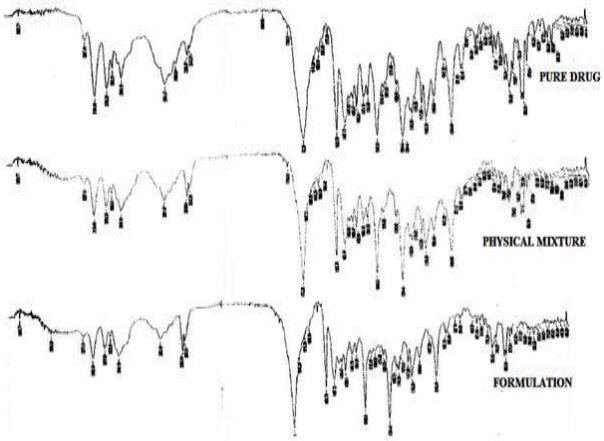

Fig 2 FT-IR Spectra of pure drug, physical and mixture and optimized formulation

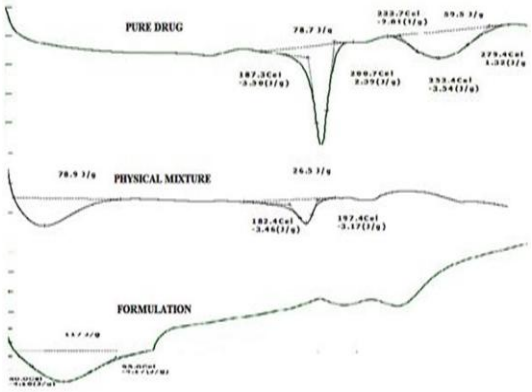

Fig. 3 DSC thermograms of drug, physical mixture and optimised formulation

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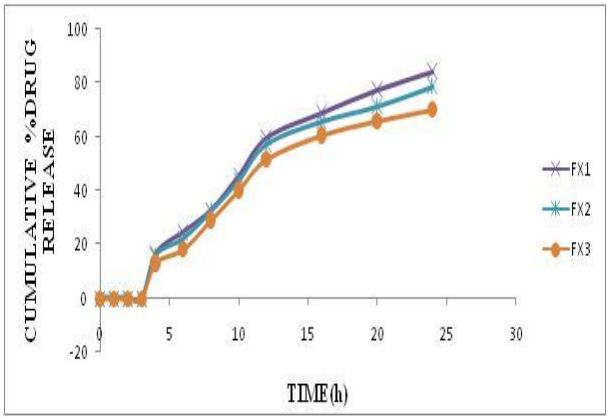

Fig. 4 *In-vitro* release of Lercanidipine hydrochloride from pulsatile device in pH 1.2, 7.4 and 6.8.

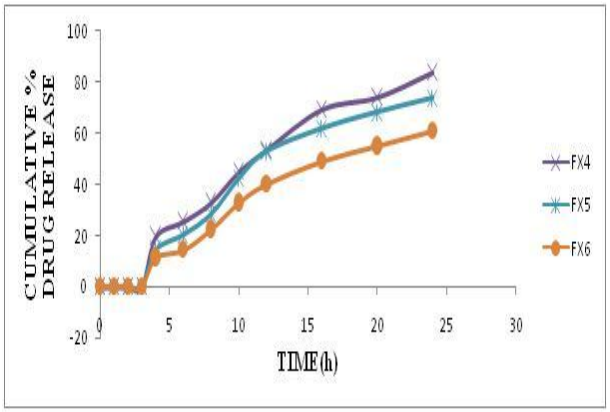

Fig 5: *In-vitro* release of Lercanidipine hydrochloride from pulsatile device in pH 1.2, 7.4 and 6.8.

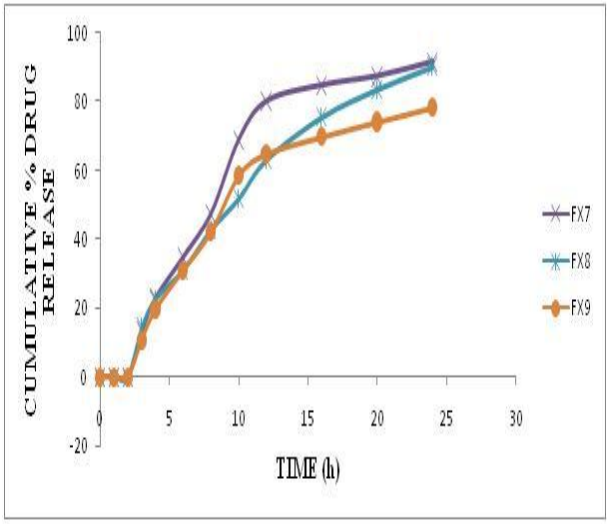

Fig 6: *In-vitro* release of Lercanidipine hydrochloride from pulsatile device in pH 1.2, 7.4 and 6.8.

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