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EUDRAGIT S-100 BASED NANOPARTICLES OF DILTIAZEM HYDROCHLORIDE FOR CHRONOTHERAPEUTIC DELIVERY

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ABSTRACT

The objectives of present investigation were to prepare & evaluate Eudragit S-100 based nanoparticles of diltiazem HCl by solvent evaporation technique. The naonoparticles were prepared by solvent evaporation technique by using eudragit S100 as a polymer with different concentrations, methanol and ethyl acetate as a solvent and antisolvent respectively. Nanoparticles were characterized for Drug content, FTIR, Zeta potential, Particle size distribution, DSC, SEM and In-vitro release study and Stability study. All the batches showed drug content in the range 94.50% to 99.07%. Particle size was found in the range of 189.8- 144.5 nm. Zeta potential values of formulations were from -2.58 mV to -24.70mV & formulation comply with requirement of Zeta potential for stability. Nanoparticles exhibited increased dissolution rate, compared to raw material due to reduction in particle size, increased surface area & solubility in lower gastrointestinal conditions. The prepared nanoparticles by solvent evaporation method of diltiazem HCl and different concentrations of Eudragit S-100 will prevent premature drug release in upper gastrointestinal tract will be useful for prevention of cardiovascular diseases as chronotherapeutic drug delivery.

Keywords: Nanoparticles, Chronotherapeutic, diltiazem hydrochloride, Solvent Evaporation, Eudragit S-100

INTRODUCTION

Targeted drug delivery to colon is highly desirable for local treatment of variety of bowel disease such as rheumatoid arthritis, osteoarthritis, ulcerative colitis, cronh's disease, amebiosis, colonic cancer and for local treatment of local colonic pathologies and systemic delivery of protein and peptide drugs. The colon specific drug delivery system should be capable of protecting the drug en route to colon and to allow drug release only in the colon **[1- 2]**. Drug targeting to colon is useful when a delay in drug absorption is desired from therapeutic point of view. Drugs are degraded and poorly absorbed in upper gut may be preferentially absorbed from colon because of lower level of luminal and mucosal digestive enzyme ,as compared to small intestine. Lower dose may be adequate and so side effect may be reduced. These delivery system when taken orally, allow drugs to release drug from the delivery system once the delivery system arrives into the colon. These delayed mechanisms are designed to improve the efficacy of the drug concentrating the drug molecules where they are need most, and minimize the potential side effects and drug instability issues associated with premature release of drug in the upper parts of the GIT, namely stomach and small intestine **[3]**.

Particulate drug delivery systems have become important in experimental pharmaceutics and clinical medicine. Particulate drug delivery system includes micro/nano technologies. The distinction is often made between micro and nano-particles respectively. In micronization particle size reduces to 1-10 micrometer. Particulate delivery system as a carrier for small and large molecules. Particulate systems like micro/nanoparticles have been used physical approach to alter and improve the pharmacokinetics and Pharmacodynamics properties of various types of drug molecules. The reduction of particle size up to nano scale increase dissolution velocity, which leads to improve bioavailability **[4]**. In literature different technologies are reported like Contin, Chronotopic, Ceform, Pulsincap, TIMERx, Codas, Diffucaps, Egalet, Tablet in capsule device, Core- in – cup tablet technology, a coated drug – core tablet matrix, Bilayered tablet, Enteric coated microspheres and Chronset **[5]**.

In case of hypertension, cardiovascular events occur more frequently in the morning and ambulatory blood pressure (BP) exhibits a diurnal variation with increase in morning (morning BP surge). Studies have shown that angina attacks occur in a diurnal

cycle, and their occurrence is common in the hours shortly after an individual commences activity after waking. The morning BP surge was reported to be associated with high risk of cardiac death, ischemic and hemorrhagic stroke. Thus antihypertensive medication more specific for morning BP in addition to 24 hrs would be useful for the prevention of cardiovascular events in hypertensive patient **[5-6]**. Currently, there are antihypertensive products in the market that are chronotherapeutic medications with novel drug delivery systems, releasing drug during the vulnerable period of 6 am to noon upon administration of medications at 10 pm. Some of these are InnoPran XL, Cardizem LA, Verelan PM and Covera HS **[7]**.

Diltiazem hydrochloride is an Antihypertensive drug also used in the management of angina pectoris. The initial dose of Diltiazem HCl is 120 -240 mg daily may be increased up to 540 mg.. It is widely used calcium channel blocking agent. DTZ when given orally is well absorbed from the gastrointestinal tract and is subject to an extensive first-pass effect **[8]**. Extended release tablets and capsules are currently marketed for DTZ. In literature Bovine Serum Albumin and Gelatin - Pluronic F-6 based nanoparticles are reported **[9-10]**. Also in one of the study the pH dependent multiparticles (Pellets coated with Eudragit S-100) are prepared and evaluated[11]. Eudragit S-100 is pH dependent polymer which is used to prevent premature drug release in upper GI Tract. Since the single unit forms are known to exhibit variable gastric emptying time and lower colon residence time, The multi-particulates are reported to exhibit higher colonic residence time], more predictable gastric emptying with gastric emptying being less dependent on the state of nutrition. The objectives of present investigation were to formulate nanoparticles of diltiazem HCl by solvent evaporation technique, characterize the same for solid state properties and evaluate for particle size, *in vitro* drug release and stability studies.

MATERIALS AND METHOD

Materials;

Diltiazem hydrochloride obtained as a gift sample form Micro-Labs Ltd, Mumbai, India and Eudragit S-100 from Degussa Pharma Pvt. Ltd, Mumbai, India. All Other reagents were of analytical grade were procured from Loba Chemicals .Pvt. Ltd (Mumbai, India)

Preparation Eudragit S-100 based nanoparticles of Diltiazem hydrochloride;

Eudragit S-100 based nanoparticles were prepared by solvent evaporation technique.

Methanol and ethyl acetate was used as solvent and antisolvent respectively for diltiazem HCl. The drug was dissolved in polymer (Eudragit-S 100) which was previously dissolved in methanol antisolvent (Ethyl Acetate) was added dropwise into diltiazem HCl methanol solution with a vigorous stirring at 1000rpm by using homogenizer, solution solidified into particles by solvent evaporation and desiccated at room temperature for 24 hrs **[8]**. The composition is mentioned in **Table 1**.

Table 1: Composition of nanoparticles

Batch Code	Diliazem Hydrochloride	Eudragit S100	Solvents(methanol:Ethyl acetate) (2:8) ratio
A_1	500 mg	500mg	10ml
A_2	500mg	1000mg	10ml
A_3	500mg	1500mg	10ml
A_4	500mg	2000mg	10ml
A_5	500mg	2500mg	10ml
A_6	500mg	3000mg	10ml
A_7	500mg	3500mg	10ml

FT IR spectroscopy;

Prepared optimized formulations were characterized by Fourier Transform Infrared spectrophotometer (Shimadzu IR–8300S) and the spectrum was recorded in the wavelength region of 4500-400cm^{-1} with resolution of 4 cm^{-1} and 45 scans. The procedure consist of a sample in excess was dispersed in potassium bromide nearly at ratio 1:100,mixed well and then mixture kept into sample holder for analysis.

Differential Scanning Calorimetry;

The DSC analysis was carried out using SDT Q 600 V 20.9 build 20 TA Instrument, samples were placed in crucible and thermo-grams were recorded at a heating rate of 10 ^{0}C/min in the range of 0^o to 400^{0}C at a nitrogen flow of 20 ml/min. Empty Aluminum pan was used as reference.

Drug Content Determination;

Drug content of the drug particles were determined by weighing 10 mg of particles from each formulation and transferred to a volumetric flask and added it 100ml of phosphate buffer pH 7.4.The resultant solution was sonicated for 10min.From this solution 1ml was transferred to another 10 ml volumetric flask and volume was made with phosphate buffer pH 7.4. The amount of drug present was analyzed spectrophotometrically (SHIMADZU UV-1601, Japan) at 236nm. Each sample analyzed in triplicate. Actual drug content was calculated for all batches using the equation as follows **[10]**:

$$\text{Drug Content} = \frac{O_{act}}{O_{ss}} \times 100$$

O_{act}: Actual diltiazem HCl content in weight quantity of particles.

O_{ss}: Theoretical amount of diltiazem HCl particles.

Particle Size Determination;

Mean particle size and size distribution of prepared particles were determined by dynamic light scattering (PSS-Nicomp, Particle sizing systems Santa Barbar, California USA.) The sample were diluted with three times of water and sonicated to create a homogenous suspension this diluted suspension was added to the sample cell (quartz cuvettes) and put into the sample holder unit measurement was carried out in triplicate **[12-14]**.

Zeta Potential Analysis;

Zeta potential of the prepared particles was determined by dynamic light scattering (PSS-Nicomp, Santa Barbara, California USA.) The samples were diluted with water three times and sonicated to create a homogeneous suspension this diluted suspension was added into the specialized zeta (electrophoretic cell) and put into the sample holder unit were an electric field was applied. Measurement was carried out in triplicate **[14, 15]**.

Scanning Electron Microscopy;

The surface characteristics of pure drug, Eudragit S-100 as excipients and prepared particles were studied by scanning electron microscopy [SEM] (JEOL, JSM 50 A, Tokyo, Japan.) at 2000x. The samples or a glass slide with a small drop of the suspension were fixed on an SEM stub using double sided adhesive tape and coated with Au at 50 mA for 6 min through a sputter-coater (KYKY SBC-12, Beijing, China). A scanning electron microscope with a secondary electron detector was used to obtain digital images of the samples at an accelerating voltage of 10Kv **[9]**.

In-vitro release study of Eudragit S-100 based Nanoparticles in Simulated GI condition;

The release study was carried out by using USP type II dissolution test apparatus. The accurately weighed nanoparticles, equivalent to 150 mg of diltiazem HCl were added to 900ml of dissolution medium at 37± 0.5^0C at 50rpm. Perfect sink conditions prevailed during the drug dissolution study period. The simulation of GI pH variations was accomplished by modifying the pH of the dissolution medium at various time intervals. The pH of dissolution medium was kept at 1.2 for 2 hrs with 0.1N HCl. After 2hrs pH of dissolution medium adjusted to 6.8 with 1.0M NaOH. The release rate analysis was

run for another 2hrs. After 4hrs, the pH of dissolution medium was adjusted to 7.4 with 1.0M NaOH and maintained up to 12hrs. A sample volume of 5ml was withdrawn from the medium at predetermined time intervals and replaced with fresh dissolution medium and further subjected to UV analysis; the absorbance was measured at 236nm. The effects of drug-polymer ratio on drug release of Eudragit S-100 based nanoparticles of diltiazem HCl were also evaluated.

Stability study;

Short-term stability studies were performed on optimized formulations. Stability study was carried out at room temperature (RT) for the period of 3 months. At the end of 3 months the nanoparticles were examined for physical appearance and in *in-vitro* drug release studies.

RESULTS AND DISCUSSION

Fourier Transform Infrared spectroscopy (FTIR)

IR spectrum of pure Diltiazem HCl and IR spectrum formulation A_7 (optimized batch) shown in **Figure 1**. As shown if figure 1a diltiazem HCl showedaromatic C-H stretch at 3055.24 cm^{-1},aliphatic C-H stretch at 2966.52cm^{-1} ,O-CH_3 C-H stretch at 2835.36cm^{-1},amine HCl N-H stretch at 2387.87cm^{-1},acetate C=O stretch at 1741.72cm^{-1},benzene ring C=C stretch at 1510.26cm^{-1}, C-N stretch at 1215.15cm^{-1} and C-O stretch at 1055.06cm^{-1} It was found that there were no considerable changes in the FTIR peaks of the formulation when compared to pure diltiazem HCl **[16]**.

Differential Scanning Calorimetry;

The DSC curve of pure DTZ exhibited a sharp endothermic peak at 212.96^{0}C corresponding to the melting of drug. As shown in **Figure 2**, Formulation A_7 showed endothermic peak at 210.86^0 C it indicates that drug was present in crystalline form in formulation which is the most stable solid state of the drugs **[17]**.

Drug Content;

The drug content in each formulation was determined by the UV spectroscopy method. The drug content of formulation (A_1, A_2, A3,A_4, A_5, A6andA_7) was found to be 98.24, 96.99, 99.07, 95.87, 94.50, 98.96 and 95.77 percentages respectively with no significant effect of polymer concentration on drug content **[18-19]**.

Particle Size Determination;

Particle size analysis of all formulation was done using dynamic light scattering. Polydispersity index is the measure of width of distribution. PDI below 0.5 indicates narrow particle size distribution. PDI index of DTZ particles A_1,A_2,A_3,A_4,A_5,A_6,A_7 was 0.412,0.548,0.591,0.520,0.576, 0472,0.285

respectively as shown in **Table 2** which indicates narrow particle size distribution of formulation A_7 as compared to other formulations. The results of particle size distribution by DLS showed that all formulations were found in the range of nanometer. The particle size was found to decrease with increase in polymer concentration this might be due to the tight surface formed by polymer chains at high concentration also polymer may coalesce at high concentration. The increased amount of polymer provided an additional space for drug molecules to get entrapped, thus decreasing the total surface area **[20-21]**.

Zeta Potential Analysis;

The stability study of the nanoparticles was evaluated by measuring the zeta potential.A measurement in distilled water gives information about the covering of the particle surface. The zeta potential of the formulation was found to be A_1, A_2, A_3, A_4, A_5, A_6, and A_7 (-2.58, -8.90,-10.45, -12.10, -18.09, -20.22 and -24 .70 respectively). So, negative zeta potential was attributed for prepared formulations. It is a very good index of the magnitude of the electrostatic repulsive interaction between the particles. It is an indication for the long-term stability of particulate system. For a physically stable particles zeta potential of approximately ±30mV is required as minimum. Absolute zeta potential value higher than -60 mV is considered extremely stability, -30mV means good stability and -20mV shows acceptable short term stability **[22-23]**.

Scanning Electron Microscopy;

SEM images of DTZ, Eudragit S-100 and Formulation A_7 (Optimized batch) as shown in **Figure 3**. The image of DTZ indicated that it was smooth doughnut shaped crystalline solid .The formulation image indicated that shape of particles was regular, hexagonal with a smooth surface due to coating of polymer onto the surface of drug particle, which enable them to flow very easily **[24]**.

In-Vitro Dissolution Study;

In-Vitro drug release data obtained for all formulation and pure Diltiazem HCl is shown in **Figure 4**. The % drug released of pure Diltiazem HCl and A_1 to A_7were (69.89, 90.57, 91.15, 93.50, 94.42, 94.47, 94.63, and 95.28 %) respectively at the end of 12 hrs. From results of in vitro dissolution studies it was observed that the formulation A7 exhibited better dissolution in phosphate buffer pH 7.4 after 4 hrs.The formulation A7 showed better control over the drug release for first 4 hrs. This is due to use of high concentration of Eudragit S-100.

Stability study;

The optimized batch A_7 was evaluated for stability study. Various changes in Physical properties like colour, drug content was studied. The study conducted for 3 months, no statistically significant differences in dissolution studies **(Figure 5)** and drug content were found after 3 months.

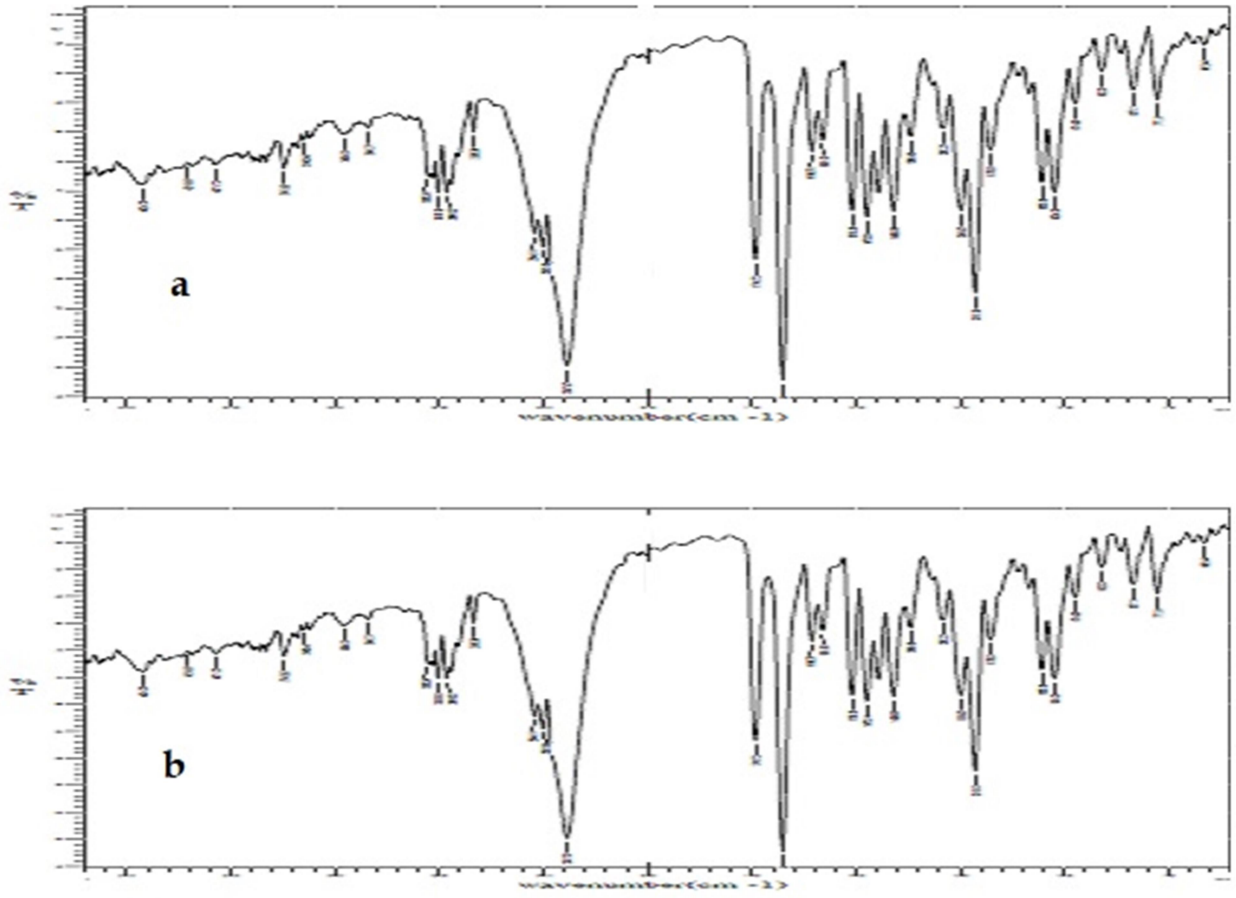

Figure 1. FTIRspectras of (a) Pure Diltiazem HCl and (b) formulation A_7(optimized batch).

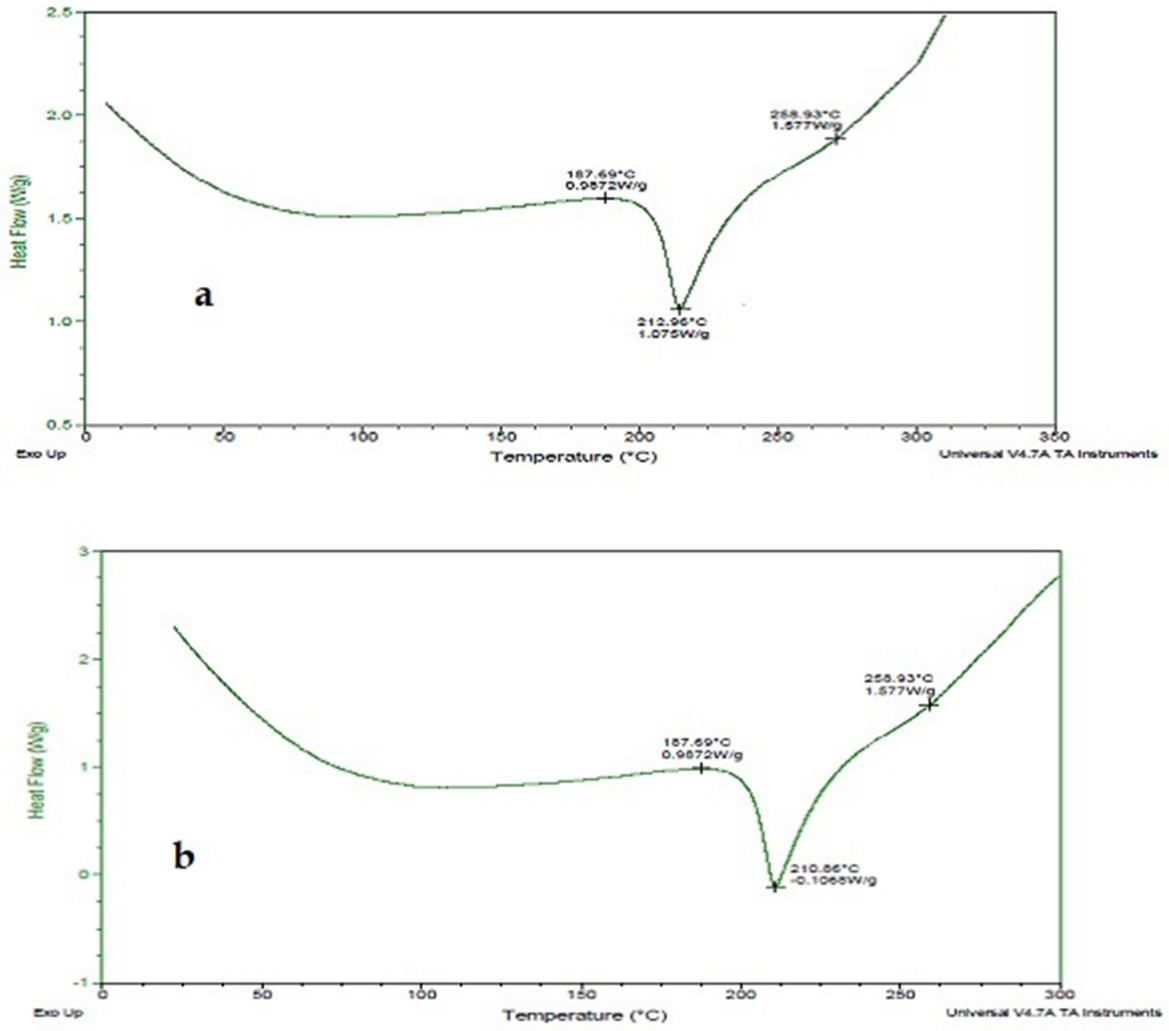

Figure 2. DSC Curve of (a) Pure Diltiazem HCl (b) Formulation A_7 (optimized batch)

Table 2: Particle size of all Formulations

Sr. No.	Formulation Code	Mean Particle size (nm)	PDI
1	A_1	189.8	0.412
2	A_2	188.1	0.548
3	A_3	187.9	0.591
4	A_4	174.8	0.520
5	A_5	169.2	0.576
6	A_6	155.7	0.472
7	A_7	144.5	0.285

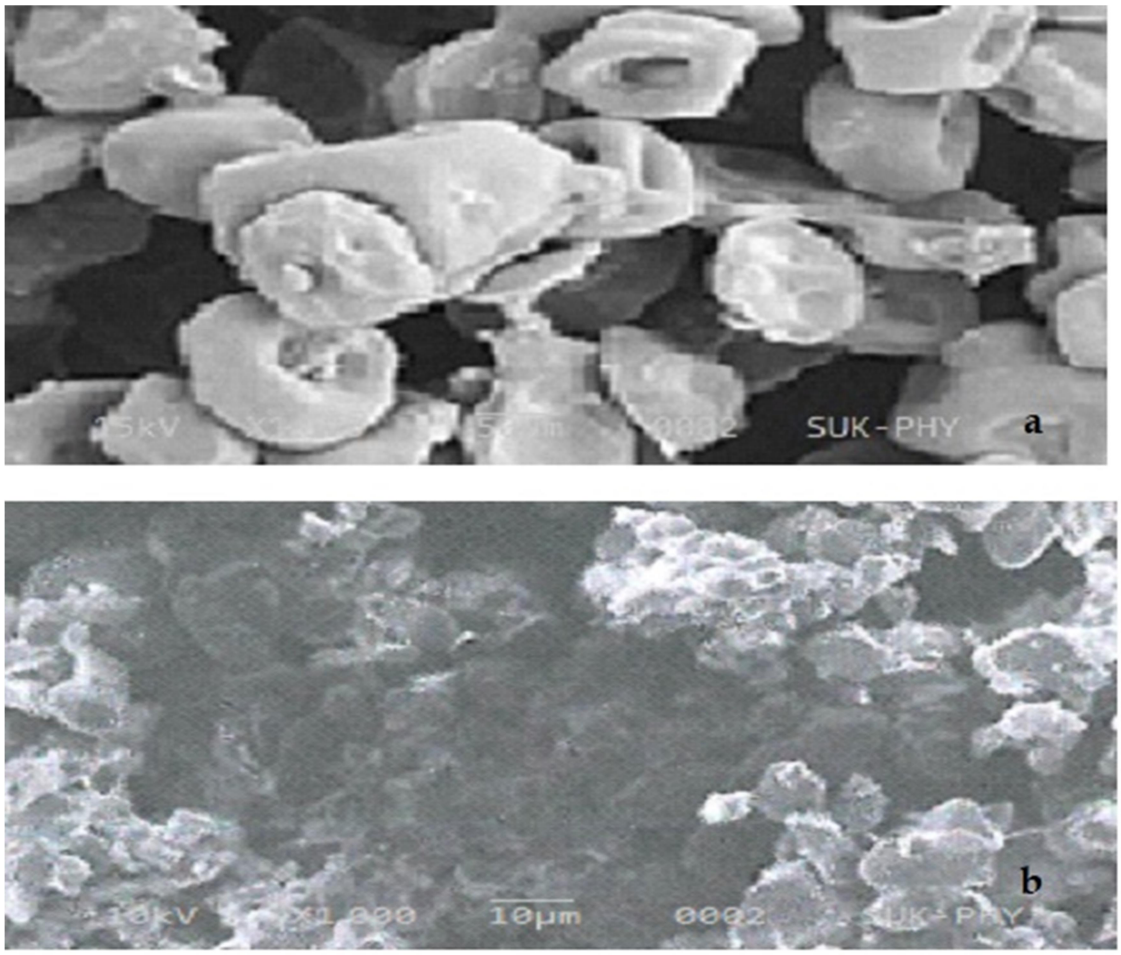

Figure 3. SEM images of (a) Diltiazem HCl and (b) Formulation A_7 (optimized Batch)

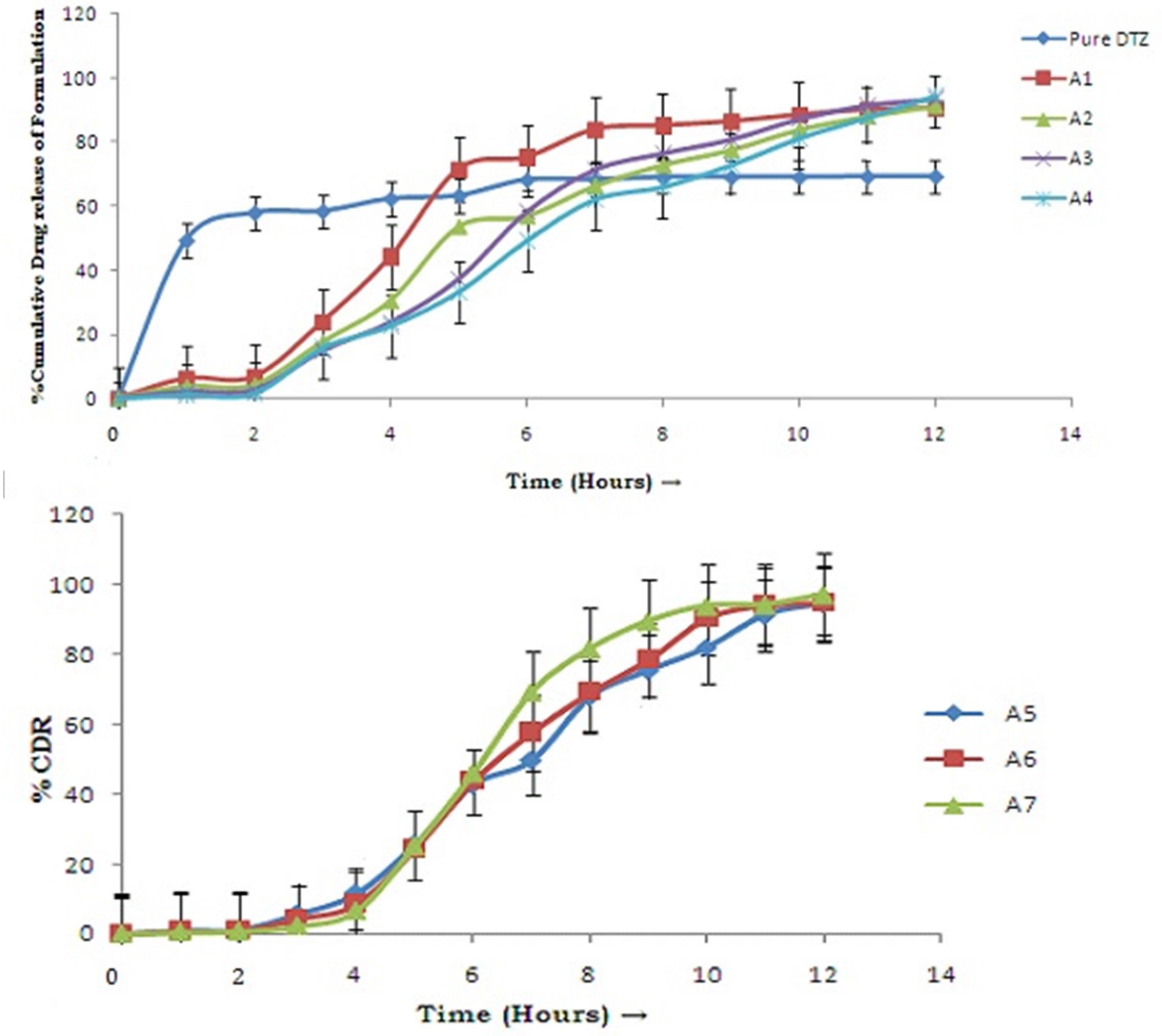

Figure 4. In-Vitro Release Profile of (a) Formulation (A_1,A_2,A_3,A_4) ,Pure DTZ and (b)(A_5,A_6,A_7) in 0.1 N HCl, Phosphate Buffer pH 6.8 and Phosphate Buffer pH 7.4

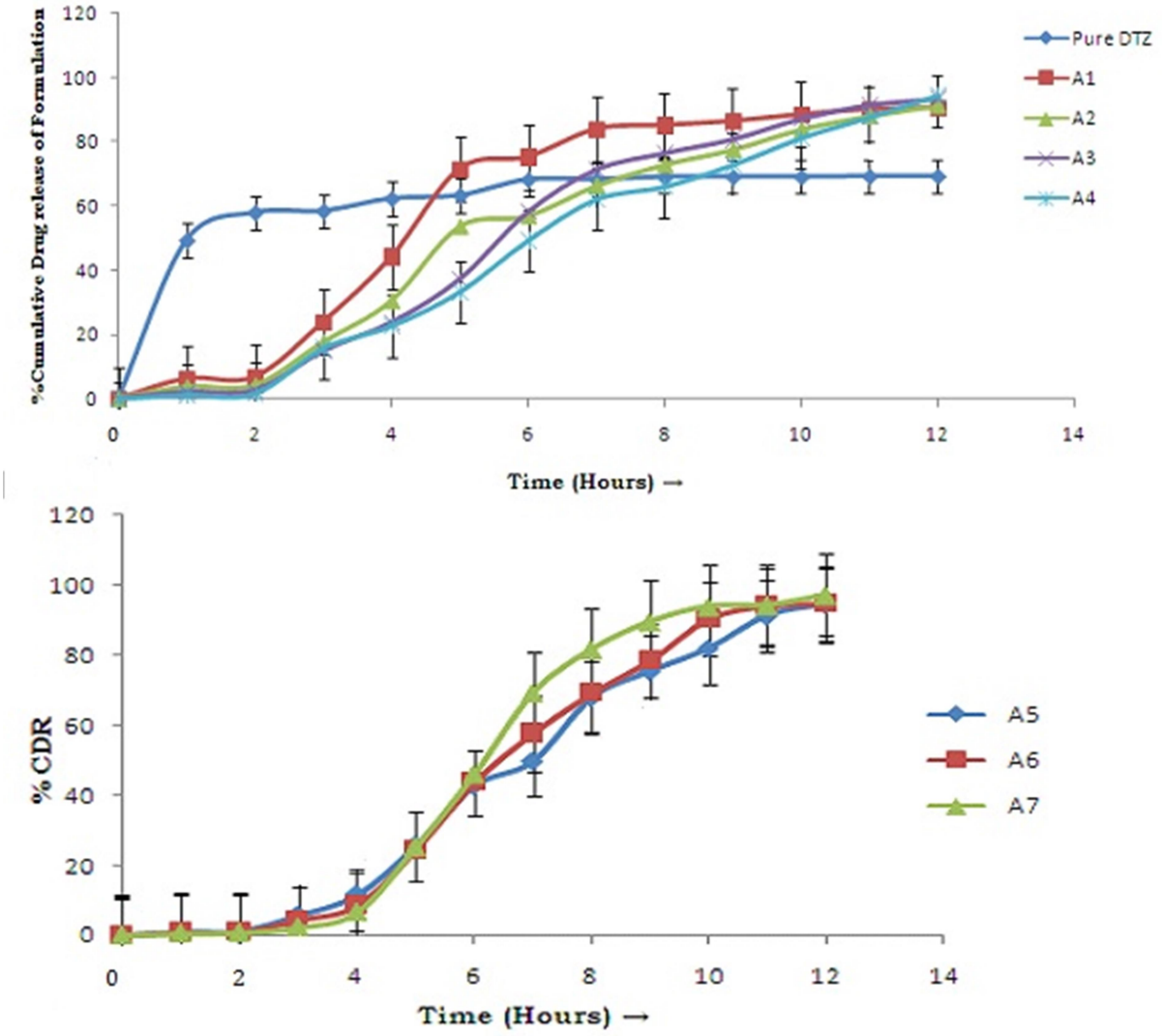

Figure 4. In-Vitro Release Profile of (a) Formulation (A_1,A_2,A_3,A_4) ,Pure DTZ and (b)(A_5,A_6,A_7) in 0.1 N HCl, Phosphate Buffer pH 6.8 and Phosphate Buffer pH 7.4

CONCLUSIONS

Prepared nanoparticles by solvent evaporation method of Diltiazem HCl and different concentrations of Eudragit S-100 will prevent premature drug release in upper GI. Eudragit S-100 based Nanoparticles add the advantage of rapid dissolution in lower GI tract and may enhance the bioavailability which will be useful for the prevention of cardiovascular events in hypertensive patient early in the morning.

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