Design and Characterization of Benazepril Containing Emulsomes Vescicular Drug Delivery Systems

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Abstract

The proposed work was to formulate benazepril emulsomes for percutaneous administration for sustaining the drug release. The formulation containing nanolevel particle size was used for better therapy. The compatibility studies of drug with other excipients i.e. cholesterol (CH) and Lecithine (LN) and Stearylamine (STL) and the physical mixture were conducted using I.R spectroscopy. The results revealed that the peaks obtained for benazepril pure samples and physical mixture were to be compatible and without changes in the functionalities indicating their compatibility. Benazepril emulsomes were formulated by varying the concentrations of cholesterol (CH) and Lecithine (LN) and Stearylamine (STL) by employing thin film hydration technique followed by emulsification followed and high speed homogenization. In-vitro permeation studies of benazepril emulsomes revealed that the drug release from all the formulations followed First-order kinetics and ascertained peppas mechanism & ascertained higuchi's mechanism respectively. Application of Korsmeyer-peppas equation to the data of all the formulations revealed that the mechanism of benazepril emulsomes was governed by predominant non-Fickian diffusion and case –II transport. Based on the above statement the formulation F6 was considered as better formulation for the effective management of cardiovascular diseases.

Keywords: Emulsomes, benazepril, cholesterol (CH) and Lecithine (LN) and Stearylamine (STL), thin film hydration method.

Introduction

Benazepril, an angiotensin-converting enzyme (ACE) inhibitor, is a prodrug which, when hydrolyzed by esterases to its active Benazeprilat1, is used to treat hypertension and heart failure, to reduce proteinuria and renal disease in patients with nephropathies, and to prevent stroke, myocardial infarction, and cardiac death in high-risk patients. Benazeprilat, the active metabolite of Benazepril, competes with angiotensin I for binding at the angiotensin-converting enzyme, blocking the conversion of angiotensin I to angiotensin IILabel. Inhibition of ACE results in decreased plasma angiotensin IILabel. As angiotensin II is a vasoconstrictor and a negative-feedback mediator for renin activity, lower concentrations result in a decrease in blood pressure and stimulation of baroreceptor reflex mechanisms, which leads to decreased vasopressor activity and to decreased aldosterone secretion label. Emulsomes are lipid based drug delivery systems, which are poorly water soluble. Emulsome particles are basically consisting of microscopic lipid assembly with a polar core. These systems are prepared by lipid formation method, Sonication method, Microfludization method, Cast film method, Ethanol injection method, High pressure extrusion technique, Detergent removal method and Reverse phase evaporation method. Emulsomes is a novel lipoidal vesicular system with an internal solid fat core surrounded by a phospholipid bilayer. Emulsome is an advance nanocarrier technology for poorly aqueous soluble drugs. It possess both emulsion and liposomes features. Emulsome represents lipid-based drug delivery systems with broad variety of therapeutic applications particularly for drugs that are poor aqueous soluble. Emulsomes consist of microscopic lipid assembly with a polar core, which contains water insoluble drugs in the solution form without requiring any surface active agent or co-solvent.

MATERIALS AND METHODS:

Benazepril was procured as gift sample from Deccan pharmaceutical Pvt. Ltd., Goa. India; cholesterol (CH) and Lecithine (LN) and Stearylamine (STL) and span 60, were purchased from Sigma Aldrich; Butylated hydroxy toluene, Oleic acid were purchased from S.D. Fine chemicals, Mumbai or Himedia Laboratories, Mumbai, India; and all other chemicals used throughout the study were of analytical grades.

Drug –excipients compatibility studies:

Compatibility study of drug, cholesterol (CH) and Lecithine (LN) and Stearylamine (STL) and span 60 by IR spectroscopy. The physicochemical compatibility among all excipients used in the research were carried out by IR Spectral studies using Perkin ElmerFourier transform infrared spectrophotometer, Bruker, Germany, in the wavelength region between 4000cm^{-1} to 400cm^{-1}. The spectra obtained for allexcipients were compared.

Formulation of Emulsomes:

Benazepril were formulated by employing thin film hydration technique. For this accurately weighed quantities of drug, cholesterol (CH) and Lecithine (LN) and Stearylamine (STL) and span 60 were dissolved in 10 ml of Chloroform. The resultant solution was placed in 250 ml evaporating flask and subjected to rotation using rotary vacuum flask evaporator at 50 rpm maintaining at 60^0C temperature until the solvent was evaporated, thus thin film of lipid layer was showed on the walls of the evaporating flask. 10 mL solvent Phosphate buffer saline pH 7.4 in a separate beaker with Dimethyl sulphoxide (DMSO) as a cosolvent (0.1 mL) was incorporated into the flask for the hydration of the thin film under similar conditions, probe sonicator used for themultilammenar vescicles as emulsomes.

Evaluation

Determination of percentage entrapment efficiency:

The prepared 10 ml of emulsomes was placed in two centrifugal tubes separately for centrifuged at 15,000 rpm at 4^0C temperature using cooling centrifuge machine for 1hr. The resultant clear supernatant was decanted and the resultant precipitate was added with 5ml 7.4 phosphate buffers for 30 minutes under similar conditions. The suspension was decanted and the process was repeated again by adding 5ml phosphate buffer to ensure complete removal of unentrapped drug. the drug content was determined using UV-Vis spectroscopy at 305 nm. The percentage efficiency was determined by following equation:

$$\text{Percentage drug entrapment efficiency} = \frac{\text{Amount of entrapped drug}}{\text{Total amount of drug}} \times 100$$

SEM analysis:

One drop of diluted Benazepril emulsomes solution was placed on a stub covered with a clean glass and subjected to SEM analysis. Shape and surface morphology of emulsomes was determined by Transmission Electron Microscope (TEM) technique.

Determination of average particle size and size distribution:

The average particle size and size distribution of the benazepril emulsomes formulation was estimated using Zetasizer Nanoseries (Malvern Master Sizer, Malvern Instruments Ltd., Malvern, UK) using a flow-through cell. The number of particles present in the size range was considered and the average particle size was determined.

Determination of zetapotential:

The zeta potential of the Benazepril emulsomes formulation was estimated using Zetasizer Nanoseries (Malvern Master Sizer, Malvern Instruments Ltd., Malvern, UK) using a flow-through cell.

In-vitro diffusion studies:

The *in vitro* release of benazepril was carried out in the modified Franz diffusion cell. It consists of two chambers. The upper chamber/donor compartment holds the formulation while the lower one carries receptor or buffer medium. The dialysis membrane was placed in between the two compartments and clamped. The receptor medium is stirred by a stainless steel pin at a constant speed of 100 rpm. Physiological temperature 37±1º c has to be maintained throughout the experiment. Aliquots (1mL each) were periodically withdrawn at suitable time intervals from the sampling port and replaced with equal volume of diffusion medium to maintain the constant receptor volume. The samples were analyzed spectrophotometrically at a wavelength of 305nm for determining the concentration of the drug.

Results and discussion

The present research is focused on the formulation of cholesterol (CH) and Lecithine (LN) and Stearylamine (STL) based nanolevel emulsomes for percutaneous administration of drug for sustaining the drug release and reduction in the particle size leading to better therapy. It also emphasis on the minimization of the side effects when the drug is administered topically. Benazepril emulsomes are formulated by varying concentrations of cholesterol (CH) and Lecithine (LN) and Stearylamine (STL) as key excipients. The compatibility studies of cholesterol (CH) and Lecithine (LN) and Stearylamine (STL) and their physical mixture were conducted using I.R spectroscopy. The results revealed that the peaks obtained for drug pure samples and physical mixture were found to be compatible and without changes in the functionalities indicating their compatibility. The optimization of the particle size and entrapment efficiency was done on the basis of phospholipid to total lipid ratio. The various emulsomal formulations possessed a zeta potential value in the range of -20.03 mv to -24.21 mv leading to better stability indicating that smaller particle size and higher zeta potential are favorable for stability of formulations. The concentration of the Triglycerides (Stearylamine) had direct impact on the entrapment efficiency of the formulation as the entrapment efficiency values were increased with increase in the concentration of Stearylamine. Further increase in the concentration of cholesterol more than sudden increase in the particle size. In-vitro diffusion studies of the formulations revealed that the benazepril emulsome followed zero-order kinetics. Application of Korsmeyer-peppas equation with data of the formulations revealed that the mechanism of the benazeprilemulsomes was governed by predominant non-Fickian diffusion and case –II transport for all the formulations.. Based on the above statement BEM3 was considered as better formulation.

Conclusion:

Thin film hydration technique followed by emulsification and high speed homogenization was employed for the formulation of benazepril emulsomes. The zeta potential value was increased and the particle size of the various formulations was decreased by increased in the concentration of cholesterol (CH) and Lecithine (LN) and Stearylamine (STL) up to certain concentrations. Further rise in the concentrations of cholesterol (CH) and Stearylamine (STL) lead to tremendous increase in particle size leading to increase in the particle size. SEM analysis report revealed that the particles were well within the nanosomal range. In-vitro diffusion studies of the formulations revealed that the benazepril emulsome followed zero-order kinetics. Application of Korsmeyer-peppas equation with data of the formulations revealed that the mechanism of the benazeprilemulsomes was governed by predominant non-Fickian diffusion and case –II transport for all the formulations. Based on the above statements the formulations BEM3 was considered as a better formulation for the effective management of hypertension and cardiovascular diseases.

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Table 1: Composition of various formulation of emulsomes

Formulation Code	Lipid content (X_1)			Amount of surfactant (X_2) (%) (Span 60)	Addition of sonication time (X_3) (Min.)
	Cholestrol (CH) (mg) X_a	Phospholoipids (Lecithine) (LN) (mg) X_b	Triglycerides (Stearylamine) (STL) (mg) X_c		
BEM1	25	75	50	2.5	5
BEM2	50	75	25	2.5	5
BEM3	25	75	50	5	10
BEM4	50	75	25	5	10
BEM5	25	75	50	7.5	15
BEM6	50	75	25	7.5	15

Table 2: Characterization of Emulsomes

Formulation Code	Particle size (nm)	Layers	PDI	Zeta potential (mV)	PDI	Drug Entrapment (%)
BEM1	130.12±1.06	Single	0.228±0.11	-20.03±0.91	0.228±0.11	62.75±0.2
BEM2	130.21±1.12	Single	0.224±0.07	-22.12±1.08	0.224±0.07	71.81±0.9
BEM3	127.21±1.11	Single	0.216±0.95	-24.21±1.09	0.216±0.95	81.37±0.8
BEM4	126.11±1.09	Double	0.215±0.02	-23.01±1.03	0.217±0.35	79.98±1.2
BEM5	128.01±0.91	Double	0.226±0.95	-23.01±0.96	0.226±0.95	82.97±0.8
BEM6	123.03±1.04	Double	0.215±0.02	-20.18±1.05	0.215±0.02	79.78±1.1

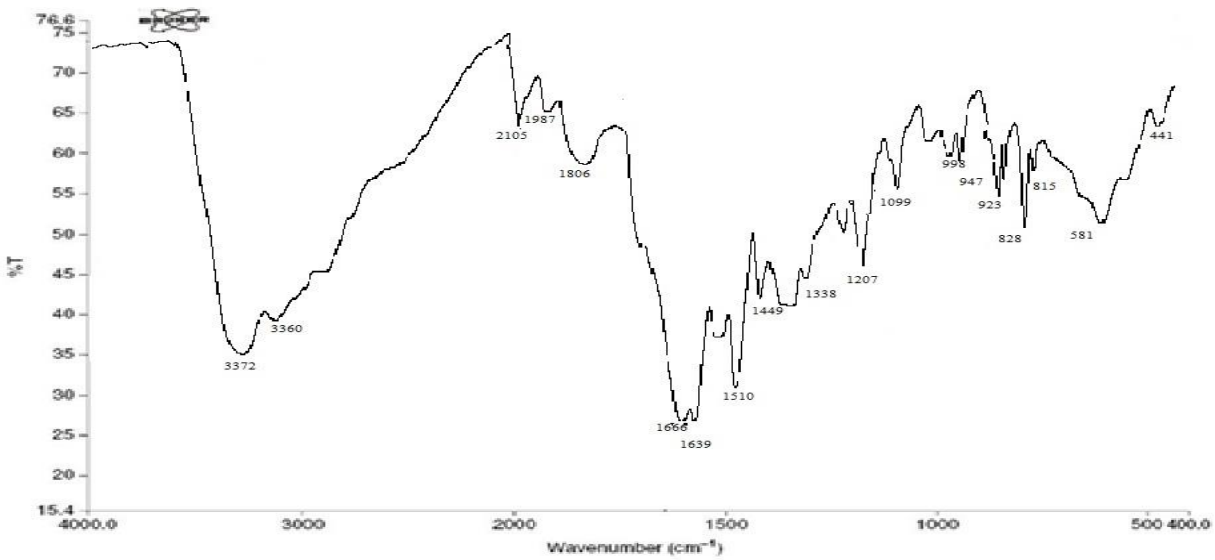

Figure 1: The I. R. Spectrum of drug samples and all excipients

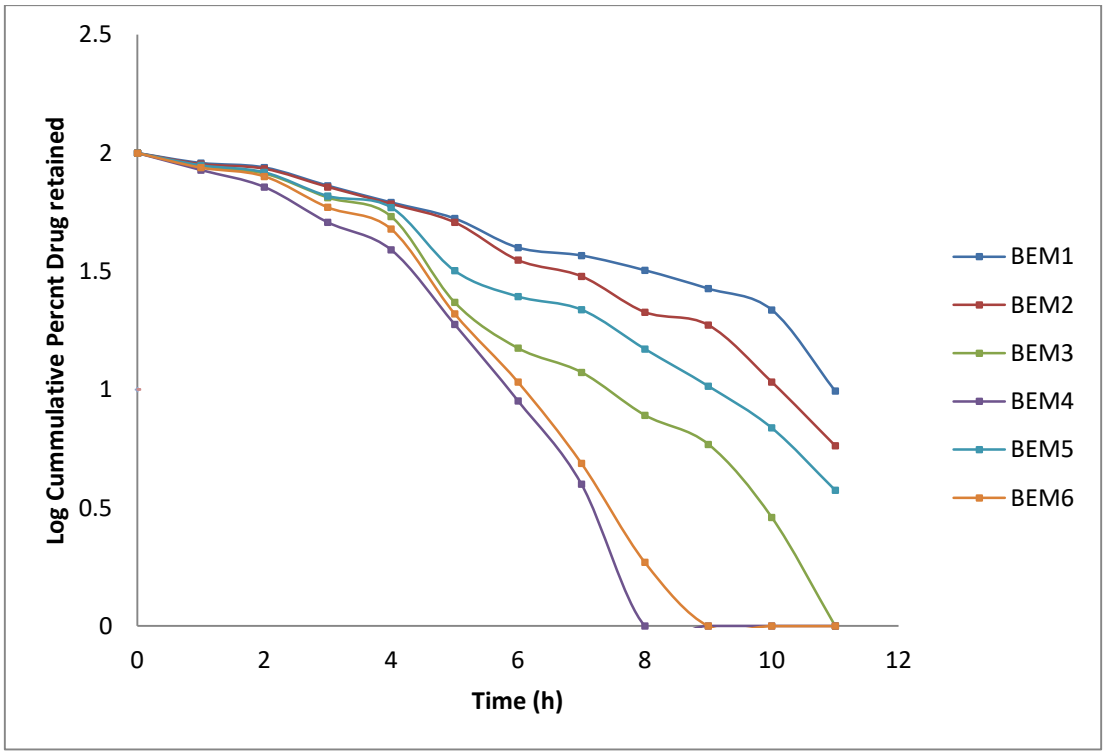

Figure 2: in-vitro drug permeation study of emulsomes