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Preparation and *Invitro* Characterization of Enalapril Maleate Microspheres Prepared by Emulsion Solvent Evaporation Method

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ABSTRACT

The aim of the work was to prepare Enalapril maleate loaded microspheres to achieve sustained release action of Enalapril maleate which is used in treatment of hypertension disorder. Enalapril maleate loaded microspheres were prepared by an emulsion-solvent evaporation method using ethanol/liquid paraffin system. The resultant microspheres were evaluated for average particle size, drug loading, in vitro drug release and release kinetics. FTIR spectrometry, scanning electron microscopy, differential scanning calorimetry and x-ray powder diffraction studies were used to investigate the physical state of the drug in the microspheres. The mean particle size of the microspheres was influenced by varying drug: polymer ratio and emulsifier concentration while drug loading was dependent on drug: polymer ratio. The results of FTIR spectrometry, differential scanning calorimetry and x-ray diffraction studies indicated stable character of Enalapril maleate in drug-loaded microspheres and also revealed absence of drug-polymer interaction. The drug release profiles of the microspheres at pH 1.2 showed poor drug release characteristics while at pH 6.8, drug release was extended over a period of 8 h; release was influenced by polymer concentration and particle size. Drug release followed the Higuchi model. The Enalapril maleate microspheres prepared under optimized conditions showed good sustained release characteristics and were stable under conditions studied.

Key words: Enalapril maleate, Eudragit S100, Microspheres and Sustained Release.

1. INTRODUCTION

Microspheres are one of the particulate delivery systems used to achieve sustained drug delivery, improve the bioavailability, stability and target drug to specific sites. Microspheres also offers advantages such as the limiting fluctuation within therapeutic range, reduction in the side effects, decreased dose frequency and improved patient compliance [1, 2]. The most popular method usedn for encapsulation of drugs within water-insoluble polymers is the emulsion solvent evaporation method. This method offers several advantages and is preferable to the other preparation methods like spray-drying, homogenization as it requires only mild conditions such as the ambient temperature and constant stirring. Thus a stable emulsion can be formed without compromising the activity of the drugs. Eudragit are biocompatible polymers synthesized from the acrylic and meth-acrylic acid esters. These polymers are well tolerated by the skin and have been used in the formulation of dosage forms especially in matrix tablets of oral sustained release (3-5) and tablet coating [6]. They have also been used in the microencapsulation of drugs [7-9]. Eudragit RL and other polymers are insoluble in water but permeable to water and gastric and juices releasing drug by diffusion. Enalapril maleate is an orally absorbed angiotensin converting enzyme inhibitor (ACE) with a short half-life of < 2 hours; the usual oral dosage regimen is 10 mg to be taken once a day. Thus Enalapril maleate is a suitable candidate for oral controlled drug delivery. Therefore, the present study was undertaken to prepare sustained release microspheres of Enalapril maleate using Eudragit S100 by the emulsion solvent evaporation method. The factors affecting particle size, drug loading and drug release behavior of the microspheres were investigated. The physical state of the Enalapril maleate was also studied using FTIR, DSC and X-ray diffraction studies.

2. EXPERIMENTAL

2.1. Materials

The following materials were obtained from the indicated suppliers and used as received. Enalapril maleate was obtained as a gift sample from Qualitech Pharma (Jeedimetla, Hyderabad)

Eudragit S 100, were obtained S D fine chemicals works (Mumbai). Ethanol (99%) and Span 80 (SD-Fine Chemical Works, Mumbai, India); liquid paraffin, di-sodium hydrogen phosphate anhydrous, potassium di-hydrogen phosphate, petroleum ether (40-60º C), hydrochloric acid and acetic acid glacial (Merck, Mumbai, India).

2.2. Methods

2.2.1. Preparation of enalapril maleate microspheres

The enalapril maleate loaded Eudragit S 100 (EURS 100) microspheres were prepared by the emulsion solvent evaporation method. In this procedure, required amount of enalapril maleate and (varying proportions)polymers were dissolved in ethanol, and was emulsified using light liquid paraffin (80 mL) containing the emulsifier, Span 80 (2% v/v). The system was stirred continuously using a propeller stirrer at 2000 rpm and 38 ± 0.5 oC for 5 h to allow the complete evaporation of the solvent. Petroleum ether (40-60 °C), 100 ml, was then added drop wise to the liquid paraffin to harden the microspheres. The paraffin was decanted of; the microspheres were washed repeatedly 4 times with petroleum ether (10 ml), collected by filtration and finally dried in a hot air oven at 40° C for 1 h. The enalapril maleate loaded microspheres were prepared using varying polymer concentrations (1, 2 and 3% w/v) and with different polymers in the dispersed medium (ethanol, 20 ml) with varying drug to polymer ratios (*i.e.,* 1:1, 1:2, 1:3, and 1:4,) while keeping the other parameters constant. The effects of process variables such as type of polymer, polymer concentration, drug to polymer ratio, and emulsifier concentration on the particle size of the microspheres, drug loading efficiency, and drug release were studied and optimized in the preliminary investigations. The optimum concentration of emulsifier was found to be 2% (v/v) and the same concentration was utilized for all the microsphere formulations.

3. RESULTS AND DISCUSSION

3.1. Invitro Characterization

3.1.1. Particle Size Analysis

The mean particle size of the enalapril maleate microspheres were determined by optical microscopy. At least 200 microspheres were analyzed for each preparation and the mean diameter was calculated.

3.1.2. Surface Morphology

The surface morphology and appearance of the microspheres were examined by a Scanning Electron Microscopy (JEOL JSM – 5200, Japan) operating between 5-24 kV. The specimens were mounted on a metal stub (with double-sided adhesive tape) and coated under vacuum with gold in nitrogen atmosphere prior to observation.

3.1.3. Determination of % Drug Loading

10 mg of the microspheres was dissolved in a standard flask containing absolute ethanol (10 ml) and kept for 12 hours for complete digestion at room temperature. The solution was then filtered through a filter disc (particle retention: 11µm) and filtrate was assayed spectrophotometrically for drug content at 410 nm to compute the drug concentration from the standard curve [10, 11]. The drug loading (%) of the microspheres was calculated using the equations given below.

$$\text{Drug loading}(\%) = \frac{\text{M actual}}{\text{weight of powdered microspheres}} X100$$

Where M_{actual} is the actual drug content in sample of microspheres.

3.1.4. In Vitro Drug Release Studies

Drug-release tests on the microspheres were carried out using a USP dissolution rate test apparatus Type II (Electro Lab model TDT- 08L) for 8 h at a stirring speed of 100 rpm and temperature of 37 ± 0.5 °C. An amount of the microspheres equivalent to 10 mg of Enalapril maleate filled in a hard gelatin capsule (Size no.2) were placed in the dissolution medium containing 600 ml of phosphate buffer (pH 6.8) B.P. containing 0.3 % (w/v) of Sodium Do-decyl Sulphate (SDS) to maintain sink condition for the drug. A quantity (5ml) of the dissolution medium was sampled at predetermined time intervals, and fresh dissolution medium was simultaneously used replenish the dissolution medium on each occasion to keep the volume constant. The sample was filtered through filter disc (particle retention: 11µm), the filtrate diluted to 10 ml with fresh dissolution medium and assayed spectrophotometrically (160 – UV – visible Shimadzu spectrophotometer) at 356 nm to determine drug concentration. The same procedure was used to assess drug release pattern in hydrochloric acid (0.1M) buffer solution (pH 1.2). The release kinetics of enalapril maleate from the microspheres was determined using different models, viz, zero order, First order, Higuchi model, Peppas- Korsmeyer and Hixon-Crowell. Kinetic assessment of release data was carried out with a program, PCP Disso v2.08 (Anant Ketkar).

3.1.5. FT-IR Spectroscopic Studies

The IR spectra of the samples were recorded on an FTIR spectrophotometer (Perkin Elmer 1600 series) using KBr pellet (12 mm

disc), compressed in a hydraulic press at 10 tons for 30 seconds.

3.1.6. Differential Scanning Calorimetric Analysis

Differential scanning calorimetry (DSC) analysis was undertaken to characterize the changes, if any, during thermal exposure of samples. The test was carried out using a thermal analysis system (Mettler TA 4000 model). Calibration with the standard (indium) was undertaken prior to subjecting the samples for study (between 30-400 °C), which were heated at 10o C/min in an aluminum pan under a nitrogen atmosphere while using an empty pan as the reference in this instrument. The instrument automatically calculated onsets of melting point and enthalpy of fusion.

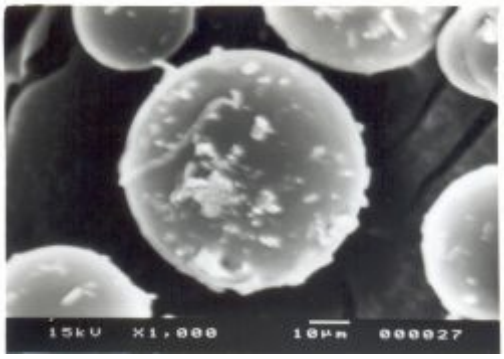
3.1.7. X-Ray Powder Diffraction Analysis

The x-ray diffraction pattern of the samples were obtained using an x-ray diffractometer (Rich Seifert, model 3000 P) at 30 kV, 15 mA over a range of 10-100 2_, using Cu K_ radiation wavelength of 1.5405 Aº. In this technique, the cavity of the metal sample holder of the x-ray diffractometer was filled with ground sample powder and then smoothed out with a spatula.

3.2. Particle Size and Morphological Characteristics

Figure 1 shows the scanning electron photomicrograph of the surface of enalapril maleate loaded microspheres having mean particle size 42.15 ± 12.34 µm. The particle size of the microspheres increased from 14.25 ± 6.85 to 42.15 ± 12.34 µm with increasing polymer concentration. Few drug crystals appeared on the surface of the microspheres. It was also observed that increasing the emulsifier concentration decreased the microsphere size.

Fig -1: Scanning electron micrograph of the enalapril maleate loaded EUS 100 microspheres prepared at 1:4 drug: polymer ratio and **a** polymer concentration of 3% w/v.



4.2. Drug Loading Of the Microspheres

Increase in the concentration of drug in the organic solvent resulted in an increase in the drug loading of the microspheres. The results indicate that the highest enalapril maleate loading of 38.40 ± 0.44 % was observed when the polymer concentration was 3% at a drug: polymer ratio of 1:1 and a stirring rate of 2000 rpm.

3.3. In Vitro Drug Release and Release Kinetic Model Studies

Figure 2 indicates the maximum drug release from enalapril maleate-loaded microspheres at pH 1.2 was about 40% over a period of 4 h. Furthermore, there was no significant difference in drug release characteristics of the EURS microspheres irrespective of the polymer concentration and drug: polymer ratio. Figure 3 illustrates the cumulative percent release of enalapril maleate-loaded microspheres at pH 6.8 over a period of 8 h. The data clearly show that drug release (for microspheres prepared at a drug polymer ratio of 1:1) decreased with increase in the polymer concentration as follows: 1, 2, and 3 % polymer concentrations showed 93.69 ± 0.95%, 89.37 ± 1.01%, 82.65 ± 0.45% drug release, respectively. At other drug : polymer ratios (*i.e.,* 1:2, 1:3, and 1:4) there was only a small retardation of drug release from the microspheres ranging from 86.57 ± 0.84% to 73.40 ± 0.79% (see Figure 3). The EURS microspheres prepared at 3% polymer concentration showed a certain level of sustained release characteristics, especially microspheres prepared with 1:3 drug to polymer ratio (E3d) which released 73.40 ± 0.79% of drug at pH 6.8 over 8 h. An initial 20- 33 % drug release was observed in all the microsphere formulations within the first hour at pH 6.8. Based on correlation coefficient (r2) data, the release pattern of enalapril maleate from Eudragit RL microspheres fitted best to the Higuchi model.

Fig - 2: Cumulative drug release of enalapril maleate at pH 1.2 hydrochloric acid buffer from enalapril maleate loaded Eudragit **S** 100 microspheres prepared with 1% (E1), 2% (E2) and **3 %** (E3) polymer concentrations and at various drug: polymer ratios. (Mean **±** S.D.; n=3). Note: a, b, **c** and **d** represent drug to polymer ratio of 1:1, 1:2, 1:3 and 1:4, respectively.

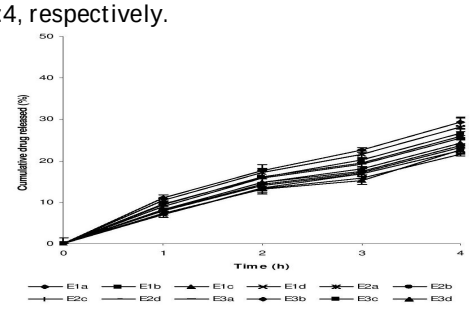


Fig -3: Cumulative drug release of enalapril maleate in pH 6.8 phosphate buffer from enalapril maleate -loaded EUS microspheres prepared with 1% (E1), 2% (E2) and **3 %** (E3) polymer concentrations and at various drug: polymer ratios. Note: a, b, **c** and **d** represent drug to polymer ratios 1:1, 1:2, 1:3 and 1:4, respectively.

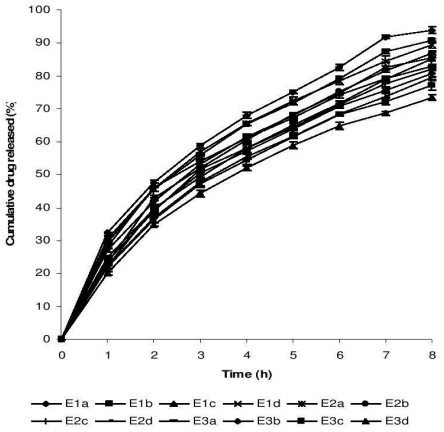


Table -1: Drug loading, particle size, and *in vitro* drug release profiles of enalapril maleate loaded microspheres prepared with different polymers (EU **S** 100) at polymer concentrations of **1** %, 2% and 3% w/v.

Formulation code	Drug: RL ratio (w/w)	Theoretical Drug Content m (%)	Drug Loading (%) ± SD	Mean particle size (µm) ± SD	Cumulative % drug release at pH 1.2 over the period of 4h	Cumulative % drug release at pH 6.8 over the period of 8h
1%						
E1a	1:1	50	34.46 ± 0.45	14.25 ± 6.85	45.73 ± 1.13	93.69 ± 0.95
E1b	1:2	33.3	23.16 ± 0.34	16.94 ± 8.24	43.37 ± 1.16	90.62 ± 1.46
E1c	1:3	25	18.29 ± 0.67	19.38 ± 9.27	41.57 ± 0.98	85.29 ± 1.65
E1d	1:4	20	15.11 ± 0.28	21.46 ± 9.32	40.25 ± 1.34	85.03 ± 1.45
2%						
E2a	1:1	50	36.18 ± 0.34	24.83 ± 10.21	43.16 ± 2.14	89.37 ± 1.01
E2b	1:2	33.3	24.88 ± 0.39	26.10 ± 9.87	41.74 ± 1.82	86.57 ±0.84
E2c	1:3	25	19.09 ± 0.75	28.34 ± 10.28	39.81 ± 1.86	81.93 ± 0.48
E2d	1:4	20	16.30 ± 0.29	33.42 ± 12.16	38.43 ± 2.18	79.28 ± 1.47
3%						
E3a	1:1	50	38.40 ± 0.44	35.94 ± 10.85	48.08 ± 1.64	82.65 ± 0.45
E3b	1:2	33.3	26.53 ± 1.71	38.56 ± 11.78	38.29 ± 1.76	80.66 ± 0.93
E3c	1:3	25	20.61 ± 0.31	41.28 ± 12.18	38.46 ± 0.98	76.97 ± 1.27
E3d	1:4	20	17.27 ± 0.33	42.15 ± 12.34	37.59 ± 0.82	73.40 ± 0.79

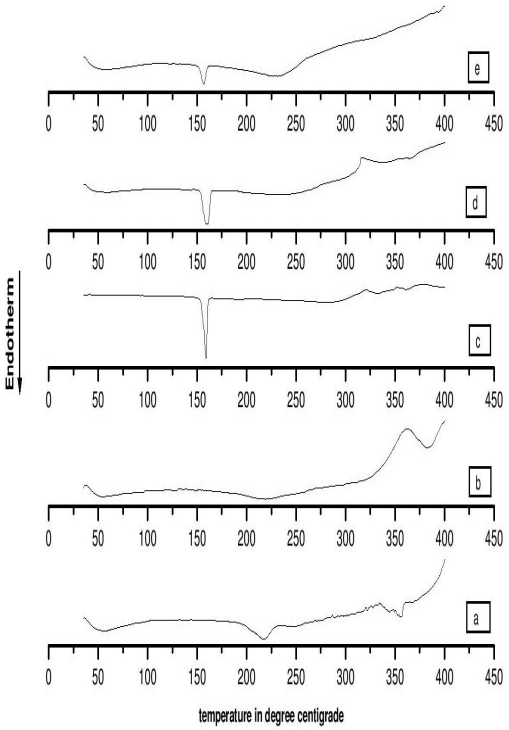
3.4. FTIR- Spectroscopic Studies

FTIR of enalapril maleate shows the principal peaks at the wave numbers of 1020.66 – 1349.17 cm^{-1}, indicating the presence of carboxyl, carboxylate groups, and carbonyl ester vibrations at 1701.64 cm^{-1}, while C-H stretching appeared at 2991.62 – 2902.96 cm^{-1} and NO_2 group appeared between 1349.17 and 1531.18 cm^{-1}. In the IR spectra of the physical mixture of the formulation ingredients as well as those of enalapril maleate microspheres (E3d), the principal peaks for enalapril maleate in the formulation, (E3d), appeared between 1020.66 and 1349.56 cm^{-1} and indicates the presence of carboxyl, carboxylate groups and carbonyl ester vibration at 1701.74 cm^{-1}, as well as NO2 group between 1349.56 and 1532.34 cm^{-1}.Therefore, the possibility of any drug polymer interaction is ruled out.

3.5. Differential Scanning Calorimetric Studies

Figure 4 illustrates the DSC thermo gram of enalapril maleate, physical mixture of enalapril maleate with EURS, and enalapril maleate microspheres (E3d). The DSC thermograms of the physical mixture of enalapril maleate with Eudragit RL, on the one hand, and the microsphere formulation (E3d) on the other, produced almost similar melting endotherms of pure drug at 158.6 oC and 156.0 3 oC, respectively. However, the intensity of the drug fusion peak for the microsphere formulation was lower than that of the pure drug and physical mixture.

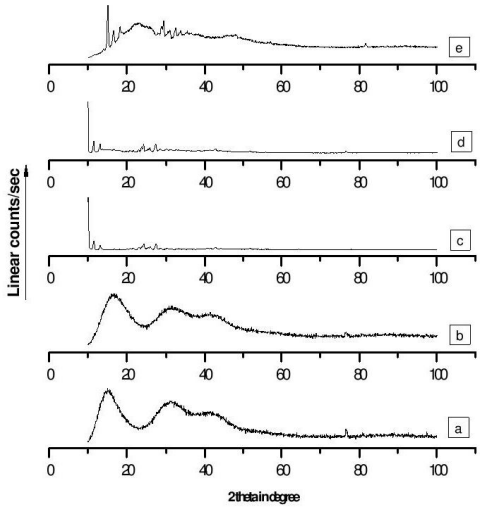
Fig - 4: DSC thermograms of Eudragit S100 (a); Eudragit S100 microspheres without drug (b); enalapril maleate (c); physical mixture of enalapril maleate and Eudragit S100 (d); and enalapril maleate -loaded Eudragit S100 microspheres (e).



3.6. X-Ray Diffraction Studies

Figure 5 illustrates the comparative X-ray powder diffraction pattern of enalapril maleate alone, physical mixture of enalapril maleate with EURS, and enalapril maleate- loaded EURS microspheres (E3d). The x-ray diffraction profile of Eudragit S polymer indicated the presence of a completely amorphous material; pure enalapril maleate showed the classical diffractogram of the crystalline product. No major difference in the XRD patterns of the physical mixture and the drug loaded microspheres was noticed. However, decreases in the peak intensity and the baseline shift of the diffractogram were observed in the case of the enalapril maleate loaded microspheres (E3d) when compared to that of the physical mixture. This is possibly due to the decrease in the degree of crystallinity of the drug following dispersal in the polymer matrix.

Fig- 5: X-ray powder diffraction patterns of Eudragit S100 (a); Eudragit S100 microspheres without drug (b); enalapril maleate (c); physical mixture of enalapril maleate and Eudragit S100 (d); and enalapril maleate - loaded Eudragit S100 microspheres (e).



Enalapril maleate-loaded Eudragit S microspheres can be easily prepared by solvent evaporation method; the microspheres were satisfactory considering their size and shape. The microsphere size increased with increasing polymer concentration; this may be due to increasing polymer concentration that produced a significant increase in the viscosity, thus leading to an increase of the emulsion droplet size and finally a larger microspheres size. On the other hand, microsphere size decreased with increasing emulsifier concentration. The effect of emulsifier concentration on the size of the microspheres can be explained by the higher stability of the dispersed droplets and less tendency of the droplets to coalesce upon collision at higher concentrations of the surfactant. The appearance of drug crystals on the surface of the microspheres may be attributed to high drug concentrations and slow solvent removal as the drug formed a particulate (crystal) dispersion resulting. Drug loading increased with increase in the drug proportion of the preparation. The results of *in vitro* drug release studies showed that the various drug: polymer ratios and different polymer concentrations had a considerable effect on drug release pattern. The poor drug release and insignificant variation in the drug release pattern of enalapril maleate loaded microspheres at pH 1.2 may be attributed to the effect of the dissolution medium on drug dissolution from the microspheres. The microspheres prepared at a polymer concentration 3% showed considerable sustained release characteristics, especially microspheres prepared at a 1:3 drug: polymer ratio (E3d) which released 73.40 ± 0.79% of drug at pH 6.8 over a period of 8 h and showed better sustained release characteristics when compared with other microsphere types (Figure 3). This may be attributed to the higher polymer content which resulted in a larger particle size and a tightened polymer network and thus retarding drug release. The initial 20-33% of drug release from all the microspheres may be attributed to the presence of a small proportion of the drug on the microsphere surface which could have arisen from the diffusion of the drug during preparation and drying. When such microspheres are immersed in dissolution medium, the surface drug is immediately released. The assessment of the release kinetics revealed that drug release from the microspheres at pH 6.8 phosphate buffer followed the Higuchi model. The DSC thermographs of the microspheres showed that the drug fusion peak intensity was less than those for the pure drug and the physical mixture. This may be due to the reduced drug content, (arising from homogeneous dissolution of the drug in the polymer. Other tests indicate that enalapril maleate remained in a crystalline state within the polymer network of the microsphere. It is clearly evident from the XRD pattern, however, that lower peak intensity and a baseline shift were observed for the microsphere when compared to that of the physical mixture. This may be due to a decrease in the degree of crystallinity of the drug.

4. CONCLUSION

From the foregoing investigations it was concluded that the enalapril maleate loaded microspheres under optimized conditions showed some degree sustained release and were stable under the conditions studied. The release kinetics followed the Higuchi model.

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