

Biodegradable Microspheres for Captopril Drug Delivery

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Abstract: This paper presents formulation and evaluation of Poly (L-Lactide) microspheres for captopril delivery. Microspheres were prepared using water in oil in water (W/O/W) solvent evaporation method. The microspheres were evaluated for the yield, particle size and surface morphology using scanning electron microscopy (SEM). Entrapment efficiency and drug loading were assayed using UV-visible spectrophotometric system. In-vitro drug release was conducted in phosphate buffer (pH 7.4) and in 0.1 N HCl (pH 1.2) as dissolution medium. FTIR spectroscopy was used to study any interaction between the drug and components of the microparticles. Results showed that the method was suitable for formulating microspheres with an average particle size of $3.62 \pm 0.35 \mu\text{m}$ having entrapment efficiency of captopril up to 98.74 %. SEM characterization results showed that the microspheres have spherical shape and smooth surface. Drug-polymer ratio was found to alter the microspheres yield and entrapment efficiency. FTIR study revealed the absence of any interaction between the components of the microspheres. The drug release study showed that microspheres were able to sustain the release of captopril up to 6 hrs in acidic media (pH 1.2) and up to 30 min in phosphate buffer (pH 7.4). The mechanism of drug release of captopril in phosphate buffer is suggested to be via diffusion process and to follow Korsmeyer-Peppas model. It is possible to sustain the release of captopril using poly (L-Lactide) microspheres.

Keywords: Biodegradable-Polymeric microspheres, Poly (L-Lactide), Captopril, Mechanism of drug release.

I. INTRODUCTION¹

Captopril (CP) is classified as an antihypertensive drug. The plasma half-life of CP is 2-3 hour and only 40 % of the drug reaches the systemic circulation due to hepatic first pass metabolism. CP has a short half life and low bioavailability in the upper part of GIT hence it is suitable for gastro-retentive system. [1]. The bioavailability of captopril is reduced to 55% in presence of food [2]. Clinical use requires a daily dose of 37–75 mg to be taken three times in divided doses. [3] Microspheres are defined as “Monolithic spheres or therapeutic agents distributed throughout the matrix either as a molecular dispersion of particles”. They were also defined as a structure made up of continuous phase of one or more polymers in which drug particles are dispersed at the molecular or macroscopic level. [4] Microspheres range in size is from 1-1000 nm. [5] The use of microspheres for drug formulation has attracted much interest in recent years. [6] They have been used as a protective measure against oxidation and hydrolysis of active components and, more recently, sustained release of several drugs [7] Different polymers have been investigated for the preparation of microspheres [8] Synthetic polymers such as poly(lactic acid) and poly(glycolic acid).[9] natural polymers such as albumin and gelatin have been used in microsphere construction. In order to obtain a sustained release formulation, the polymer used to construct the microspheres must be biodegradable over the intended time to give a suitable therapeutic effect. The intention of this work is preparation of biodegradable microspheres containing captopril.

II. MATERIALS AND METHODS

1. Chemicals

Captopril (Mw 217.29 g/mol.), Poly (L-lactide) Mw 5,000 and Poly (vinyl alcohol) were all obtained from Sigma-Aldrich Ltd., USA. All solvents were of HPLC grade and supplied by BDH Ltd., Poole, England, UK.

2. Preparation of microspheres

Microspheres of poly (L-lactide) (PLLA) as matrix were prepared using solvent evaporation double emulsification method. Briefly, PLLA with weight ratio from 1 to 6 to drug were dissolved in dichloromethane (5 ml) followed by the addition of captopril solution in water (1 ml). The mixture is then stirred at 13 000 rpm using Ultra-turrax T25, IKA-WERKE (Germany) for 5 min. Poly (vinyl alcohol) (PVA) (1% w/v, 20 ml) in deionized water was subsequently added to the mixture under same speed of stirring for further 5 min. The resulted w/o/w double emulsion was then stirred with magnetic stirrer at room temperature for 5 hours to allow evaporation of the organic solvent. Microspheres were isolated by centrifugation at 3000 rpm for 5 min and washed in triplicate with deionized water. Microspheres were freeze dried at -60°C for 24 h and stored in desiccator until use. Blank PLLA microspheres with omission of captopril were prepared using the same procedure.

3. Microspheres Characterizations:

3.1. Percentage Yield:

The resulted microsphere powder were accurately weighed. The percentage yield then calculated by dividing the weight of dry microspheres powder by the theoretical total amounts of non-volatile component used in the preparation according to the following equation:

$$\text{Percentage yield (\%)} = \frac{\text{Mass of microspheres obtained}}{\text{Sum of mass of non volatile component}} \times 100\% \quad (1)$$

3.2. Particle size and surface morphology:

Particle size and surface morphology of blank and drug loaded microspheres were done using Scanning electron microscopy (SEM), LEO 1430VP, Germany and UK. Sample of microsphere powder was mounted on metal stubs with double-sized conductive adhesive tape and coated with a thin gold film (approximately 60 nm in thickness) using an acceleration potential procedure. Pictures of micro particles were taken using optical microscope by random scanning of the stub. from which the diameters of about 50 micro- particles of each batch were measured. The diameters of about 50 micro- particles of each batch were measured from which the average mean diameters were calculated.

3.3. Loading and Entrapment efficiency:

Sample (5 mg) of the freeze dried microspheres was dispersed in 0.5 ml dichloromethane in a volumetric flask and the volume is adjusted to 5 ml with water. The sample was transferred into test tubes and centrifuged at 3000 rpm for 5 min. One ml of the supernatant was diluted to 10 ml with water and assayed in triplicate for captopril using spectrophotometric measurement at λ_{max} of 220 nm. Drug content was calculated using a calibration curve equation made of several concentrations of captopril in water versus absorbance. The loading efficiency percentage (LE %) and encapsulation efficiency percentage (EE %) were calculated using the following equations

$$\text{LE (\%)} = \frac{\text{Actual amount of Drug in microspheres}}{\text{Theoretical amount of Drug in microspheres}} \times 100 \quad (2)$$

$$\text{EE (\%)} = \frac{\text{Actual amount of Drug in microspheres}}{\text{Amount of Drug for microspheres}} \times 100 \quad (3)$$

3.4. In-vitro drug release studies

In-vitro release of captopril from microspheres was studied in phosphate buffer (pH 7.4) and in 0.1N HCl (pH 1.2) as dissolution medium. Briefly, 5 mg freeze dried microspheres were dispersed in 25 ml of the dissolution medium using 50 ml conical flasks. The flasks were left to shake for 24 hrs using shaking water bath (SWB20, medingen, P-D industriegesellschaft mbH; Germany) maintained at 37 °C and 50 rpm. At specific times, 1ml samples were withdrawn and replaced by an equivalent volume of fresh dissolution medium. The removed samples were suitably diluted with dissolution medium, centrifuged at 3000 rpm for 5 min and filtered through 0.45 μm membrane filter. The absorbance was determined spectrophotometrically at $\lambda_{\text{max}} = 220 \text{ nm}$ ($n = 3$). The drug released % was calculated according to the following equation:

$$\text{Drug release (\%)} = \frac{\text{mass released}}{\text{actual drug loaded}} \times 100 \quad (4)$$

The calculated cumulative amount of drug released was then plotted versus time.

3.5. IR Studies:

Fourier transformed infrared spectrophotometer (IRPrestige-21; Shimadzu, Japan) was used in the study. Briefly, Pellets of pure drug and potassium bromide were prepared by compressing the powders at 80 kilo-newton for 3 min on KBr-press and the spectra were scanned in the wavelength range from 4000- 400 cm^{-1} with 32 number scans and 4 cm^{-1} spectral resolution. The same procedure was carried out on pure polymer, empty and drug loaded microspheres.

3.6. Drug release kinetics:

The drug release kinetics and mechanism for captopril were tested using: Zero order ($Q \text{ v/s } t$), First order ($\text{Log } (Q_0 - Q) \text{ v/s } t$), Higuchi's square root of time ($Q \text{ v/s } t^{1/2}$) and Korsemeyer- Peppas ($\text{Log } Q \text{ v/s } \text{Log } t$), Where Q is the cumulative percentage of drug released at time t and $(Q_0 - Q)$ is the cumulative percentage of drug remaining after time t .

III. Results and discussion

1. Yield and Entrapment efficiency of captopril:

Percentage yield and entrapment efficiency percentage of all formulation prepared using PLLA as matrix are shown in Table 1. The percentage yield found to be directly proportional to the amount of PLLA used as matrix. As PLLA ratio increased from 1 to 6 relative to the drug, an increase in the percentage yield from 48% to 79 % w/w was attained. The entrapment efficiency was also increased from 30% to 99% respectively. The results suggested that the amount of polymer used as matrix able to contain the drug leading to increase in the entrapment efficiency percentage of captopril.

2. Particle size and surface morphology

Figure 1 shows the morphology of blank and captopril-PLLA microspheres obtained with scanning electron microscopy (SEM) technique. The average particle size was ranged from $3.62 \pm 0.35 \mu\text{m}$ to $6.46 \pm 0.90 \mu\text{m}$ for the formed microspheres. The microspheres were with uniform, regularly spherical, smooth surface, and no evidence of collapsing indicating complete entrapment of captopril molecules within PLLA matrix. The smooth surface also indicates complete washing of non-entrapped drug.

3. In-vitro drug release

The release profile of captopril from PLLA microsphere in buffer pH 7.4 as dissolution medium is shown in Figure 2. The cumulative percentage release of captopril in 30 min was ranged from 81.73% to 100% respectively. The release profile for captopril from PLLA microspheres in 0.1N HCl solution as dissolution was lasted up to 6 hrs (Figure 3). The cumulative percentage release of captopril was ranged from 90 % to 100% respectively. As the ratio of polymer to drug increased, the release of captopril was delayed. The results can be explained by increased thickness of the matrix leading to longer time for the drug molecule to cross the matrix and dissolve in 0.1N HCl. The results also indicate that the type of dissolution media can affect the release of captopril from microspheres.

4. IR studies:

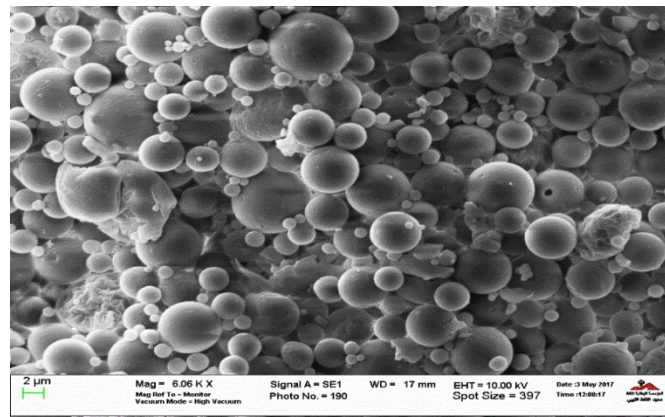
The FTIR spectra of captopril, Blank PLLA microspheres and PLLA-captopril loaded microspheres are shown in Figure 4. IR spectrum for pure captopril shows peaks at 1748.55, 1589.41, 1473.68 and 1383.98 cm^{-1} (Figure 4a). Blank PLLA microspheres scan is shown in Figure 4b. No change in position of peaks compared to pure powder of PLLA was observed. IR spectrum for PLLA-captopril microspheres is shown in Figure 4c. No new peaks appeared in the scan indicating absence of any interaction between the captopril and PLLA.

5. Discussion

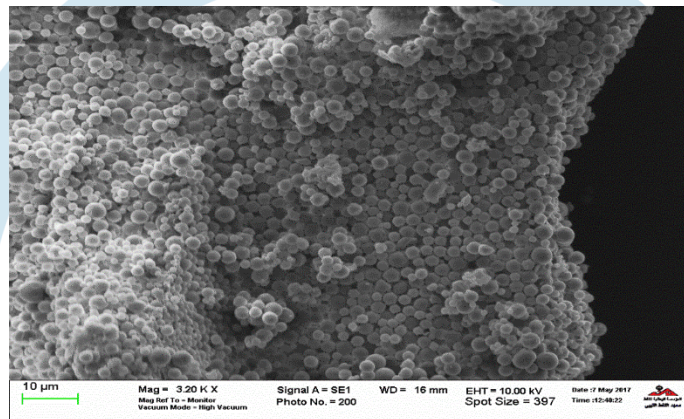
PLLA microspheres containing captopril were prepared by solvent evaporation double emulsification method. In the process of mechanical stirring, the energy transferred to suspension medium was increased and the organic phase can be dispersed into smaller droplets and the size was reduced. The microspheres showed uniform and regularly spherical shape with smooth surface and no evidence of collapsing, this may be due to the fact that captopril was easier to be accommodated into PLLA matrix. It was observed that drug entrapment efficiency showed an increase with an increase in the ratio of polymer relative to the drug reaching captopril encapsulation efficiency of 98.74. The type of dissolution media seems to affect the release of captopril. The release of captopril from PLLA microspheres in phosphate buffer (pH 7.4) was in 30 min and lasted up to 6 hrs in 0.1N HCl. The finding can be explained by the solubility of captopril in water compared to 0.1 N HCl ($\text{pK}_a = 9.8$). The hydrophobic property of PLLA may also affected captopril in vitro release from microspheres. The release media had difficulty entering the PLLA microspheres due to the strong hydrophobicity of PLLA, which leading to slower release rate. Infrared spectra results showed no peaks for the drug within microspheres thermograms indicating the absence of any interaction between the drug and the polymer. The results also indicate that the polymer matrix contained all the drug molecules and support the entrapment efficiency results. The release kinetic study using different mathematical models conducted on captopril-loaded PLLA microspheres in phosphate buffer showed values of correlation coefficients (R^2) of > 0.95 % using Higuchi kinetics indicating that the release of captopril from microsphere matrix in phosphate buffer is via diffusion. The release kinetic of captopril-loaded PLLA microspheres in 0.1 N HCl were according to Korsmeyer-Peppas model.

IV. Conclusion:

Emulsification technique can be an appropriate method to microencapsulation of captopril into the poly (L-lactide) microspheres. Microspheres with good size distribution can be obtained. Entrapment efficiency, yield, and drug release can be controlled mainly by drug polymer ratio. Poly (L-lactide) microspheres can be promising drug delivery system for administration of captopril and may provide a convenient dosage form.



(a)



(b)

Figure (1): Scanning electron photographs of (a) PLLA blank microspheres and (b) PLLA-captopril microspheres.

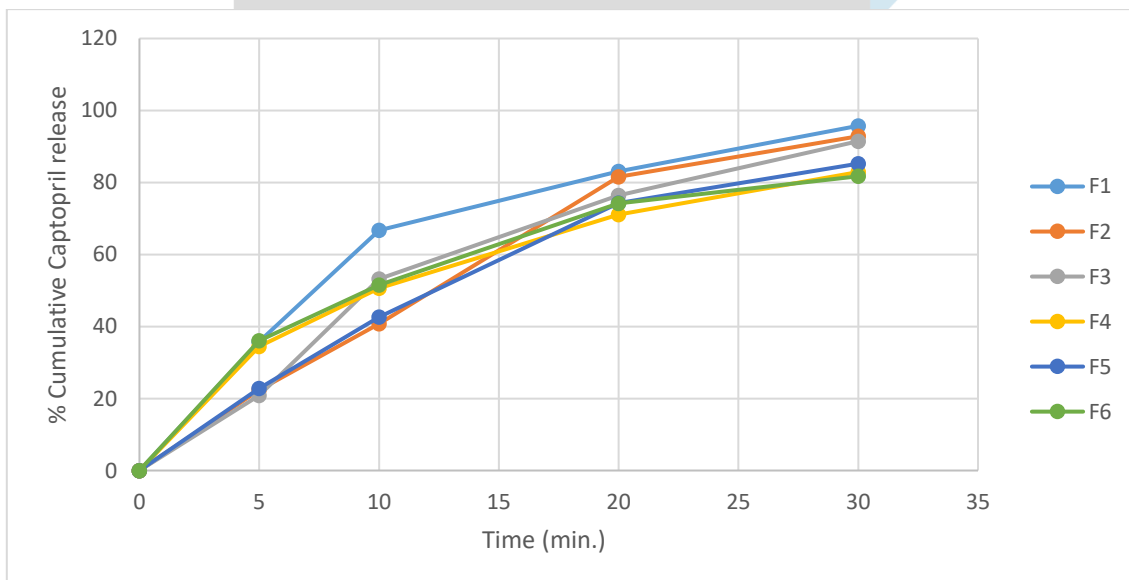


Figure (2): In vitro release profile of captopril from PLLA microspheres in phosphate buffer having different drug: polymer ratios.

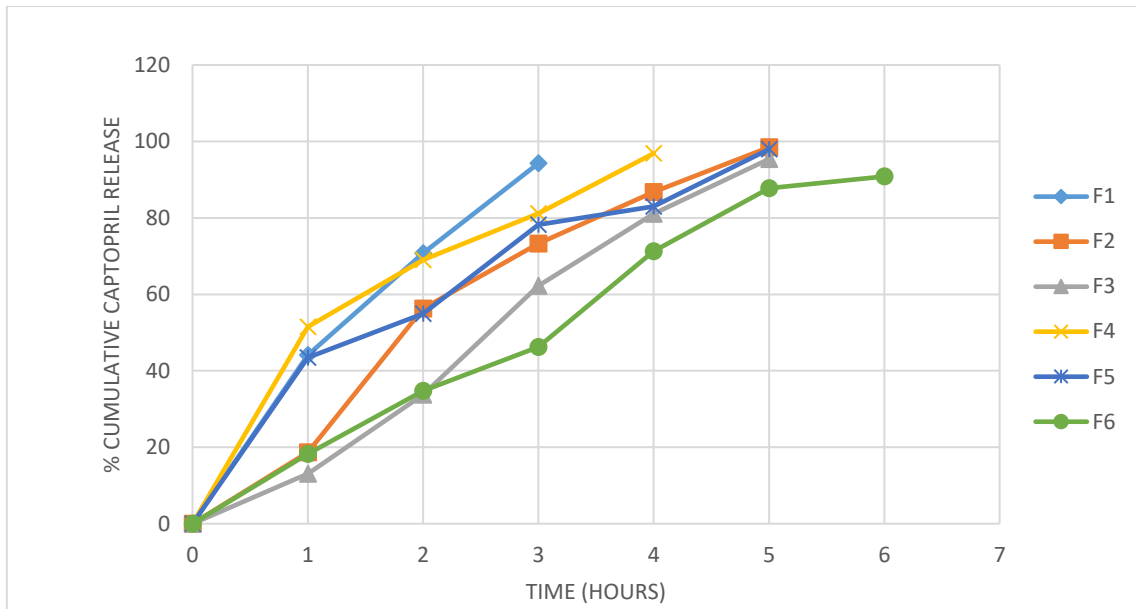


Figure (3): In vitro release profile of captopril from PLLA microspheres in 0.1N HCl. having different captopril: PLLA ratios.

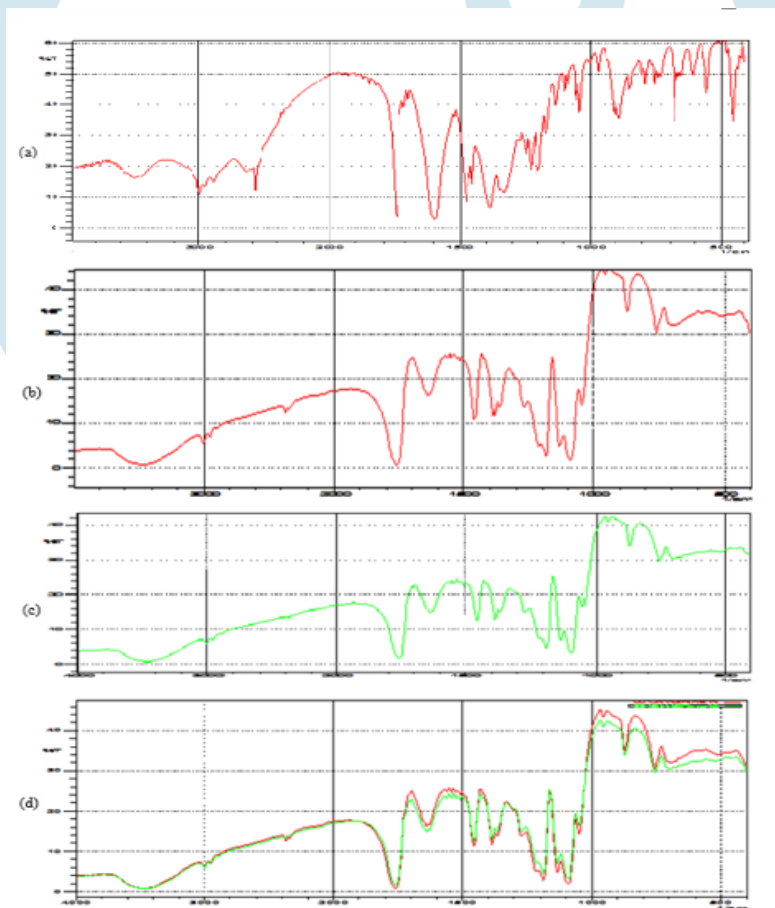


Figure (4): IR spectra of (a) Captopril, (b) Blank PLLA microsphere, (c) Captopril loaded microsphere and (d) Comparison of loaded and blank microspheres.

Table 1: The percentage yield and entrapment efficiency of captopril loaded PLLA microspheres using different drug: polymer ratio.

Formulation	Ratio D:P	Yield (%)	Entrapment Efficiency (%) \pm S.D	Loading (%) \pm S.D
F1	1:1	48.2	29.99 \pm 0.46	14.99 \pm 0.23
F2	1:2	57.73	41.23 \pm 3.98	13.74 \pm 1.33
F3	1:3	68.00	49.59 \pm 1.50	12.40 \pm 0.38
F4	1:4	75.04	66.57 \pm 2.70	13.31 \pm 0.54
F5	1:5	77.00	85.05 \pm 5.50	14.18 \pm 0.92
F6	1:6	78.74	98.74 \pm 2.79	14.11 \pm 0.54

The results are presented as Average \pm S.D (n = 3), F is formulation

Table 2: The release kinetics of captopril from PLLA microspheres of the different models in phosphate buffer as dissolution medium.

Formulation	Model							
	Zero order kinetics		First order kinetics		Higuchi kinetics		Korsmeyer-Peppas kinetics	
	R ²	slope	R ²	Slope	R ²	slope	R ²	Slope
F 1	0.8567	2.9707	0.585	0.004	0.9788	18.081	0.8843	1.3415
F 2	0.9574	3.1866	0.6492	0.0532	0.9488	18.062	0.9493	1.3464
F3	0.9299	3.0404	0.021	0.009	0.9619	17.607	0.9402	1.3485
F4	0.8891	2.5508	0.1087	0.020	0.9979	15.387	0.88212	1.2873
F5	0.9486	2.8605	0.0669	0.0162	0.9686	16.458	0.9384	1.3186
F6	0.8652	2.5271	0.1156	0.0204	0.9917	15.405	0.8757	1.288

Table 3: The release kinetics of captopril from PLLA microspheres of the different models using 0.1N HCl as dissolution medium.

Formulation	Model							
	Zero order kinetics		First order kinetics		Higuchi kinetics		Korsmeyer-Peppas kinetics	
F1	0.9762	30.951	0.9308	-0.401	0.9849	53.281	0.9999	0.6902
F2	0.9601	20.387	0.8696	-0.333	0.9396	46.518	0.9383	1.0178
F3	0.9893	20.265	0.8805	-0.254	0.8814	44.119	0.9895	1.2677
F4	0.8978	22.343	0.911	-0.342	0.9972	47.626	0.9938	0.4454
F5	0.9191	18.041	0.8625	-0.295	0.9904	43.196	0.9691	0.5119
F6	0.9821	16.085	0.9337	-0.182	0.9181	39.773	0.9883	0.9379

F, formulation

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