Design and Characterization of Microspheres of Anti Hypertensive Drug Using Biodegradable Natural Polymers

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ABSTRACT

Present investigation describes preparation of microspheres by solvent evaporation followed by in vitro characterization of microspheres to evaluate the effect of method of preparation on physical properties and drug release profile of microspheres. The microspheres were found to be discrete, spherical with free flowing properties. The morphology (Scanning Electron Microscopy), particle size distribution, entrapment efficiency and their release profiles were investigated. The yield was found to be maximum in case of solvent evaporation method. The microsphere prepared by solvent evaporation method was found in ranges of 250-50 μm, respectively. The microspheres formulation prepared by solvent evaporation method the drug carrier interactions were investigated in solid state by Fourier Transform Infrared (FT-IR) spectroscopy study. In vitro drug release rate for A microsphere was found to be sustained over 12 hours. Hence, it can be concluded that the Formulation prepared by solvent evaporation method, has potential to deliver Losartan Potassium in a controlled manner in a regular fashion over extended period of time in Comparison to all other formulations and can be adopted for a successful oral delivery of Losartan potassium for safe management of hypertension.

Keywords: Losartan potassium; sustained drug delivery; sodium alginate microspheres; natural polymers; solvent evaporation technique.

INTRODUCTION

Sustained release systems include any drug delivery system that achieves slow release of drug over an extended period of time. More precisely, sustained drug delivery can be defined as "Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects. The efficiency of any drug therapy can be described by achieving desired concentration of the drug in blood or tissue, which is therapeutically effective and non toxic for a prolonged period. This goal can be achieved on the basis of proper design of the dosage regimen. Microspheres have potential to deliver drug in a controlled fashion. Losartan potassium is an effective antihypertensive drug but is extensively bound to plasma proteins and also causes gastrointestinal disorders, neutropenia, acute hepatotoxicity, migraine and pancreatitis. It may therefore be more desirable to deliver this drug in a sustained release dosage form. The present study was focused on development of sustained release Losartan microspheres using solvent evaporation method and to study the effect of method of preparation on physical properties and drug release profile of Lorsatan potassium microspeheres.[1] Biodegradable natural polymers offer certain advantages over synthetic one in respect to toxicity, availability and cost. Biodegradable natural polymers remain attractive primarily because they are biodegradable natural products of living organisms, and capable of a multitude of chemical modifications. Various natural gums like agar, guar gum, chitosan, gelatin, carboxy methyl cellulose, xanthan gum, sodium alginate and lotus bean gum etc., for potential pharmaceutical and biomedical applications[2]. Chitosan has been considered for pharmaceutical formulation and drug delivery application which has been focused on its absorption-enhancing, controlled release and bio-adhesive properties. Gelatin is a biocompatible and biodegradable protein and has a unique characteristic of dependent sol-gel Change[6]. Albumin includes its reported biodegradation into natural products, its lack of toxicity and its non-antigenicity. It has been used as a carrier for targeting drugs to tumors, an also to target drugs to the inflamed joint. Sodium alginate promising strategy for cell microencapsulation and used as a coating polymer[3].

The present study was focused on development of Losartan potassium microspheres by using biodegradable natural polymers and to study the effect of method of preparation on physical properties and drug release profile of Losartan potassium microspheres.

MATERIALS AND METHODS

Losartan potassium was procured as a gift sample from Karnataka pharmaceutical Ltd, (India). Sodium alginate was obtained from SD fine chemicals, Mumbai. Span 80, light liquid paraffin.

Preformulation studies

Selection of vehicle

The solubility of Losartan potassium was checked in various solvents like water and methanol, ethanol, chloroform and ethyl acetate, ether and n-hexane. Studies revealed that Losartan potassium was found to be freely soluble in water and methanol. The solubility was confirmed by analyzing the sample by quantitative determination by UV spectroscopy. Wavelength scan was done from 400-200 nm and maximum absorbance was found at 206nm.

Melting point determination

Melting point of LP was determined by Open capillary method. The melting point of LP was found to be 183.5-184.5^{o} C.

Determination of λ_{max}

A solution of LP containing the concentration 10 μg/ ml was prepared in PH 7.4 and UV spectrum was taken using Shimadzu (UV-1800) double beam spectrophotometer. The solution was scanned in the range of 200 – 400 nm. Wavelength of maximum absorption of Losartan potassium in was found to be 206 nm.

Drug polymer interaction (FTIR) study [4]

FTIR spectroscopy was performed on Fourier transformed infrared spectrophotometer (IR-Affinity-1, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm-1.

Formulation studies

❖ **Preparation of microspheres of Losartan potassium using natural polymers**

Method used**:** Solvent evaporation method

The Sodium alginate microspheres were prepared by solvent evaporation method reported by sahoo *et al*[5] with some modifications. A 1% w/v solution of Sodium alginate was prepared in distilled water. LP is dispersed in above solution. This solution was dispersed in 100 ml of liquid paraffin light containing 0.5ml Span 80 in a 250 ml beaker. The dispersion was stirred at 1000 rpm for 30 min. After the stirring time, microspheres were centrifuged, washed several times with n-hexane ether and finally with acetone. The microspheres were dried at 50˚C and stored in desiccator.

Formulation design

Table 1: Formulation composition for Losartan potassium microspheres using Sodium alginate.

Sr.no	Batch code	Drug: Polymer Ratio
1.	LP-1	1:1
2.	LP-2	1:2
3.	LP-3	1:3
4.	LP-4	1:4
5	LP-5	1:5
6.	LP-6	1:6

EVALUATION OF LOSARTAN POTASSIUM LOADED SODIUM ALGINATE MICROSPHERES

❖ **Drug polymer interaction (FTIR) study [6]**

FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm^{-1}. FTIR study was carried on LP, physical mixture of LP and polymer, LP microspheres and blank microspheres.

❖ **Surface morphology (SEM) [7]**

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry LP microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of LP microspheres were taken by random scanning of the stub.

❖ **Frequency distribution analysis**

Determination of average particle size of LP microspheres was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of LP microspheres was spread on a clean glass slide and average size of 300 LP microspheres was determined in each batch. In order to be able to define a size distribution or compare the characteristics of particles with many different diameters, the size distribution can be broken down into different size ranges, which can be presented in the form of a histogram. Histogram presents an interpretation of the particles size distribution and enables the percentage of particles having a given equivalent diameter to be determined.

❖ **Percentage yield**:

Percentage practical yield is calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of LP microspheres recovered from each batch in relation to the sum of starting material.

The percentage yield of prepared LP microspheres was determined by using the formula:

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

❖ **Determination of percentage drug entrapment efficiency (PDE) 8, 9**

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula:

$$\text{PDE} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

❖ **Theoretical drug content** was determined by calculation assuming that the entire LP present in the polymer solution used gets entrapped in LP microspheres, and no loss occurs at any stage of preparation of LP microspheres.

❖ Practical drug content was analyzed by using the following procedure,

Weighed amount of LP microspheres equivalent to 100 mg of LP was dissolved in 100 ml of water. This solution was kept overnight for the complete dissolution of the LP in water. This solution was filtered and further diluted to make a conc. of 10 µg/ml solution. The absorbance of the solutions was measured at 206 nm using double beam UV-Visible spectrophotometer against distilled water as blank and calculated for the percentage of drug present in the sample.

❖ **Calibration curve of Losartan potassium**

• Scanning of Losartan potassium by UV-spectrophotometer in water

Standard stock solution of Losartan potassium was prepared by dissolving accurately weighed 10 mg of Losartan in water in 100 ml volumetric flask. The volume was then made up mark by using water, so as to get the solution of 100 µg/ml.

• **Procedure for Calibration curve of Losartan potassium in water λmax 206 nm**

From the Losartan potassium standard stock solution (100µg/ml). From this solution, aliquots of 0.5, 1, 1.5,2,2.5,3,3.5 and 1.0 ml were transferred to the series of 10 ml volumetric flasks and final volume is made with water, so as to get drug concentrations of 0.5 to 10.0 µg/ml respectively. The absorbences of these drug solutions were estimated at λmax 206 nm. This procedure was performed in triplicate to validate the calibration curve.

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In vitro dissolution studies.

❖ **Procedure for In-vitro dissolution study**

 The release rate of LP microspheres was determined by employing USP XXIII apparatus by rotating basket method. The dissolution test was performed using 900 ml PH 7.4, in 37 ± 0.5°C at 50 rpm. LP microspheres equivalent to 50 mg were placed in a basket to avoid floating of microspheres. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus hourly for 12 hrs, and the samples were replaced with fresh dissolution medium. The samples were passed through Whatman filter paper and the absorbance of these solutions was measured at 206 nm. Dissolution profiles of the formulations were analyzed by plotting drug release versus time plot. Data obtained was also subjected to kinetic treatment to understand release mechanism.

Kinetics of drug release

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q_0-Q) v/s t], Higuchi's square root of time (Q v/s $t^{1/2}$) and Korsemeyer Peppas double log plot (log Q v/s log t) respectively, 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.

In short, the results obtained from *in vitro* release studies were plotted in four kinetics models of data treatment as follows.

➤ Cumulative percentage drug release Vs. Time (zero order rate kinetics)

➤ Log cumulative percentage drug retained Vs. Time (first order rate kinetics)

➤ Cumulative percentage drug release Vs. √T (Higuchi's classical diffusion equation)

➤ Log of cumulative percentage drug release Vs. log Time(Peppas exponential equation)

Differential Scanning Calorimetery (DSC)

The physical state of drug in the LP microspheres was analyzed by DSC. The thermograms of

LP, LP microspheres with different polymers were obtained at a scanning rate of 10°C/min conducted over a temperature range of 25–350ºC, respectively.

X-Ray power Diffractometry (XRD) study [10]

X-ray diffractometry of the LP and LP microspheres were performed by a diffractometer using model (Joel JDX-8030, Japan) equipped with a graphite crystal monochromator (Cu-Kα) radiations to observe the physical state of LP in the microspheres.

RESULTS AND DISCUSSION

Drug polymer interaction (FTIR) study

From the spectra of LP, physical mixture of LP and individual polymer, LP loaded microspheres it was observed that all characteristic peaks of LP were present in the combination spectrum, thus indicating compatibility of the LP and polymer.

Surface morphology of Losartan potassium microspheres (SEM)

The surface morphology of the LP microspheres was studied by SEM. SEM photographs of the various formulations were shown in the Figure. 1. Surface smoothness of the LP microspheres was increased by increasing the polymer concentration, which was confirmed by SEM. At lower polymer conc. (1:1) rough and wrinkled surface of LP microspheres was obtained. [Figure. 1 (LP1)] and at higher polymer conc. (1:6) the LP microspheres with smooth surface was obtained [Figure. 1 (LP6)].
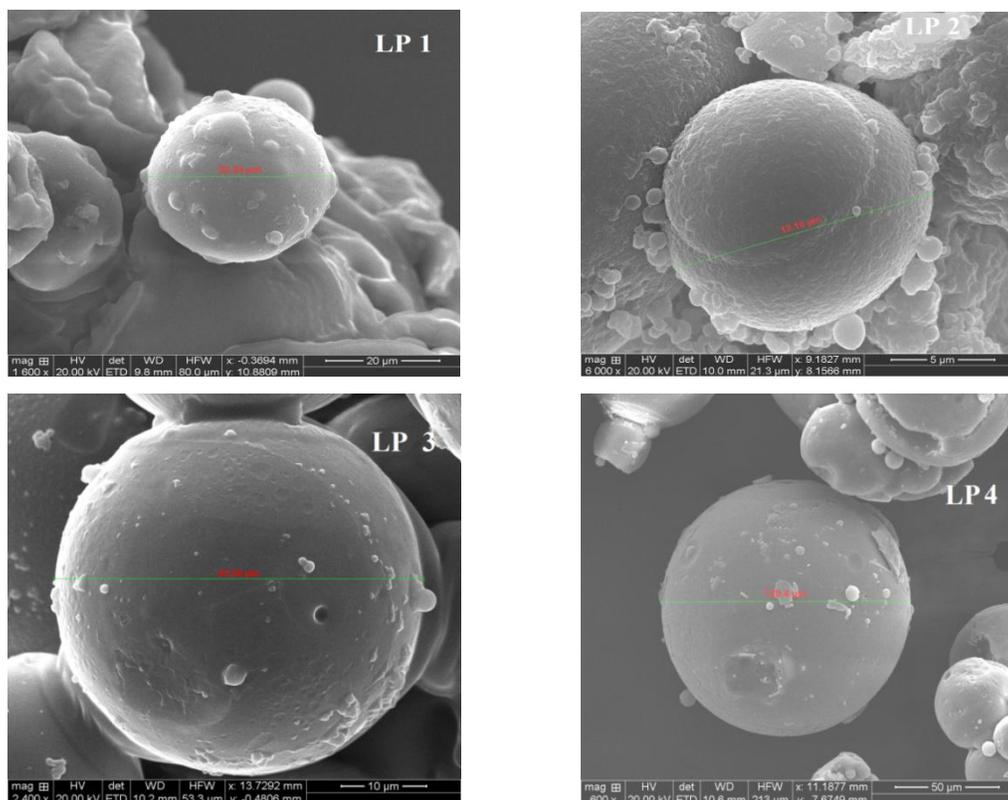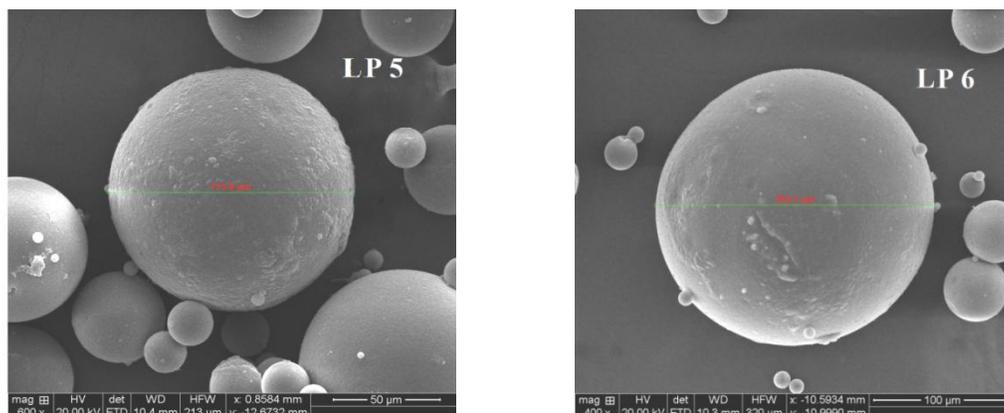
Figure1: SEM photographs of LP microspheres

LP1, LP2, LP3, LP4, LP5 and LP6 refers to LP microspheres prepared by using sodium alginate with drug: polymer ratio 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6

❖ **Determination of Average particle size**

As the LP to polymer ratio was increased, the mean particle size of LP microspheres was also increased. The significant increase may be because of the increase in the viscosity of the droplets (may be due to the increase in concentration of polymer solution). LP microspheres with sodium alginate was shown in table 2.

Table 2: Average size, percentage and Entrapment efficiency of Losartan potassium

Formulation	Average size(μm)	%Entrapment efficiency
LP1	47.6	53.78
LP2	76.00	59.71
LP3	99.8	62.35
LP4	107.2	70.80
LP5	113.17	78.88
LP6	150.3	89.60

❖ **Percentage drug entrapment efficiency**

Entrapment efficiency increases with increase in the polymer conc. From the results it can be inferred that there is a proper distribution of LP in the microspheres and the deviation is within the acceptable limits.

The percent of drug content in the formulations was found to be in the range of 26.45% to 13.57%. The percentage entrapment efficiency was found to be 53.78% to 89.50%. The results obtained are given in Table. 2 and their histograms shown . A maximum of 89.50% drug entrapment efficiency was obtained in the LP microspheres which were prepared by using NA. It was further observed that the drug entrapment was proportional to the LP:

polymer ratio and size of the LP microspheres. By increasing the polymer conc., the encapsulation efficiency was increased.

In vitro dissolution studies

The *in vitro* performance of LP microspheres showed prolonged and sustained release of LP. The results of the *in vitro* dissolution studies of formulations LP1 to LP6 are shown in Table 3 and Figure 2. The study indicated that the amount of drug release decreases with an increase in the polymer concentration. The formulations LP1 showed a maximum of 94.64% and LP6 showed a minimum of 59.50% cumulative drug release.

Table 3: *In vitro* release data of Losartan potassium microspheres with Sodium alginate

Time (h)	% Cum. drug release($\pm$SD)					
	LP1	LP2	LP3	LP4	LP5	LP6
O	0	0	0	0	0	0
1	24.815 $\pm$0.62	20.97 $\pm$ 0.1.	12.23 $\pm$ 0.60	17.47 $\pm$ 0.09	7.68 $\pm$ 0.50	7.33 $\pm$ 0.59
2	31.64 $\pm$ 0.49	27.44 $\pm$ 0.20	18.35 $\pm$ 0.62	22.52 $\pm$ 0.14	13.98 $\pm$ 0.54	7.86 $\pm$ 0.55
3	38.65 $\pm$ 0.69	32.53 $\pm$ 0.10	24.30 $\pm$ 0.60	27.45 $\pm$ 0.06	17.48 $\pm$ 0.60	10.66 $\pm$ 0.66
4	44.44 $\pm$ 0.68	38.49 $\pm$ 0.04	31.48 $\pm$ 0.61	33.24 $\pm$ 0.12	23.61 $\pm$ 0.68	15.39 $\pm$ 0.57
5	56.17 $\pm$ 0.67	44.62 $\pm$ 0.26	36.74 $\pm$ 0.56	36.75 $\pm$ 0.10	28.34 $\pm$ 0.60	17.49 $\pm$ 0.66
6	64.59 $\pm$ 0.60	52.51 $\pm$ 0.41	42.01 $\pm$ 0.59	42.01 $\pm$ 0.24	34.30 $\pm$ 0.58	21.00 $\pm$ 0.58
7	71.96 $\pm$ 0.67	59.18 $\pm$ 0.16	49.02 $\pm$ 0.57	47.28 $\pm$ 0.09	38.16 $\pm$ 0.60	26.25 $\pm$ 0.57
8	78.65 $\pm$ 0.52	66.38 $\pm$ 0.07	54.29 $\pm$ 0.61	52.55 $\pm$ 0.27	44.12 $\pm$ 0.69	28.02 $\pm$ 0.66
9	83.76 $\pm$ 0.68	74.63 $\pm$ 0.16	61.31 $\pm$ 0.63	57.82 $\pm$ 0.14	49.04 $\pm$ 0.73	31.53 $\pm$ 0.58
10	86.42 $\pm$ 0.56	79.39 $\pm$ 0.16	66.41 $\pm$ 0.61	63.09 $\pm$ 0.14	54.84 $\pm$ 0.66	45.52 $\pm$ 0.66
11	91.08 $\pm$ 0.59	83.41 $\pm$ 0.36	72.35 $\pm$ 0.62	67.46 $\pm$ 0.06	59.56 $\pm$ 0.56	52.51 $\pm$ 0.69
12	94.64 $\pm$ 0.53	87.43 $\pm$ 0.15	78.47 $\pm$ 0.59	72.53 $\pm$ 0.09	64.35 $\pm$ 0.66	59.50 $\pm$ 0.63
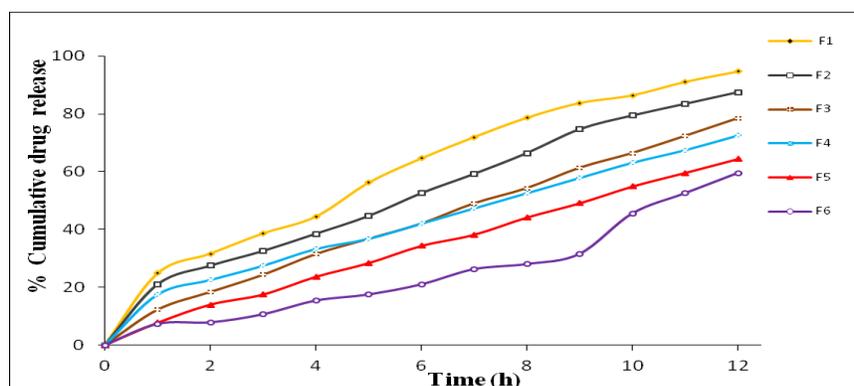
Figure2: Comparative *in vitro* release profile of Losartan potassium microspheres

Release kinetics of Losartan potassium microspheres

The slopes and the regression co-efficient of determinations (r^2) were listed in Table 4. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics.

Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model was found to be in the range of 0.5 to 1 for the LP microspheres prepared with NA indicating Non-Fickian of drug through LP microspheres.

Table 4- Pharmacokinetic release of Losartan potassium microspheres with Sodium alginate

Formulation	Zero order	First order	Higuchi Matrix	Peppas plot r^2 value	'n' value
LP1	0.9664	0.9537	0.9842	0.9807	0.5874
LP2	0.9950	0.9645	0.9745	0.9746	0.6150
LP3	0.9953	0.9687	0.9517	0.9940	0.7656
LP4	0.9893	0.9716	0.9727	0.9788	0.5937
LP5	0.9983	0.9838	0.9370	0.9966	0.8601
LP6	0.9414	0.8786	0.8066	0.9144	0.8920
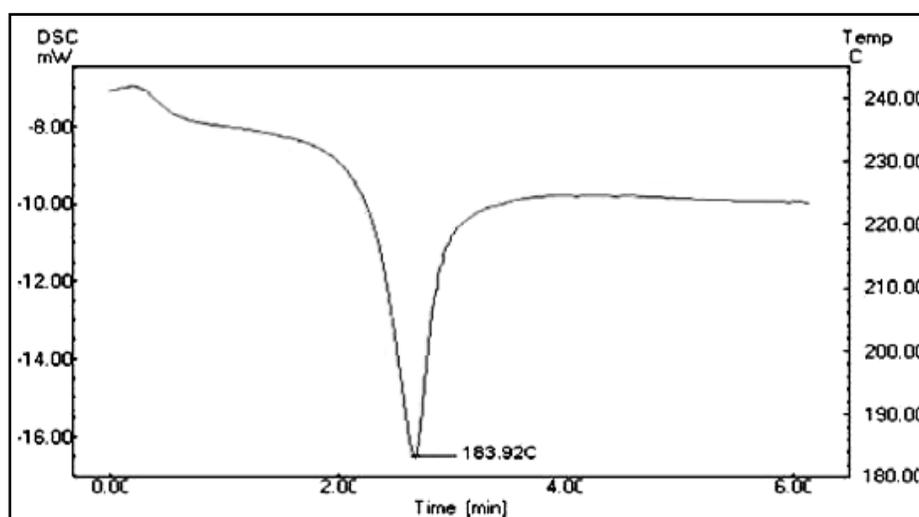
Figure 4. DSC Thermogram of Losartan potassium
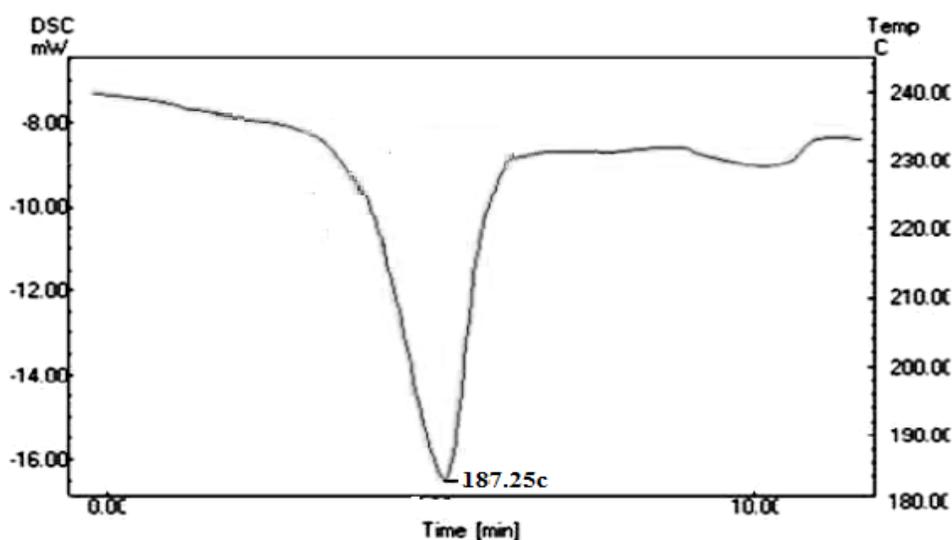
Figure.5 DSC Thermogram of formulation of Losartan potassium microspheres

Differential scanning calorimetry (DSC)

In order to confirm the physical state of LP in the microspheres, DSC of the LP, LP microspheres were carried out and were shown in Figure 4-5. The DSC trace of LP showed a sharp endothermic peak at 183.92, its melting point. The melting point range of LP is between 183-184.5°C, thus indicating there is no change of LP in pure state, formulation of microspheres. The endothermic peak of the LP at 187.25°C in the DSC of the LP microspheres suggests that the LP existed in an amorphous as a molecular dispersion in polymeric matrix.

CONCLUSION

The concept of formulating microspheres containing LP offers a suitable, practical approach to achieve a prolonged therapeutic effect by continuously releasing the medication over extended period of time. microspheres of LP were prepared successfully by solvent evaporation method using the different concentration of individual polymers like Sodium alginate. by means of improving the oral bioavailability of the drug. It would be faster and more economical to alter beneficially the properties of the existing drugs than developing new drug entities hence this formulation will be boon to novel drug dosage forms.

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