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Ramipril Loaded Solid Lipid Nanoparticles: Preparation, Characterization, *In Vitro* Evaluations

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Abstract

Objective: This work deals with the design and evaluation of the solid lipid nanoparticles of Ramipril drug using the steric acid and the palmitic acid are as lipid solutions.

Methods: Ramipril solid lipid nanoparticles are formed using modified high shear homogenisation and ultra-sonification techniques. Combination of stearic acid and the palmitic acid were used as lipid solutions. Tween 80 was used as the surfactant. Standard calibration curve has been used to simulate the experimental trend. Scanning electron microscopy (SEM) is used for the analysis of the sample. Parameters measured are entrapment efficiency, in-vitro drug release.

Results: The parameters studied are the percent drug release, entrapment efficiency, particle size of SLN and in-vitro drug release of prepared SLN.

Conclusion: The results obtained in this research work clearly indicated that the formulated solid lipid nanoparticle delivery system for the Ramipril drug, using the stearic acid, palmitic acid as carrier matrices can be used effectively for hypertension.

Keywords: Entrapment efficiency modified high shear homogenisation technique, Hypertension, Ramipril, In-vitro drug release, Stability studies, Scanning electron microscopy (SEM), Solid Lipid Nanoparticles (SLN), Ultra-sonication technique.

1. Introduction

Ramipril is used to treat hypertension. Its poor solubility [1] in water decreases the bioavailability of the drug and it is one of the fundamental drawbacks of several kinds of drugs. Application of solid lipid nanoparticles (SLNs) and the lipid nanoparticles (LNPs) in the drug delivery system improves the performance of the drugs. Studies [2-11] using the SLNs as carriers for the poor soluble drugs has shown the improvements in the efficiency of the drugs. The techniques used for the design of solid lipid nanoparticles (SLNs) and the analysis techniques used like the transmission electron microscopy, differential scanning calorimeter, X-ray diffraction were helped to predict the bioavailability of the drugs and the bioavailability is measured in terms of the parameter the drug entrapment efficiency [12-15]. The reason for the usage of lipid nanoparticles (LNPs) is their biocompatibility of the lipid matrix [16-18]. In the solid lipid nanoparticles surfactant role is to enhance the penetration efficiency and the role of various surfactants in improving penetration efficiency is discussed [19,20].

This research work handles with the preparation of the solid lipid nanoparticle system and also deals with the improvement of the solubility of anti-hypertensive drug of Ramipril. Modified high shear homogenisation followed by ultra-sonification technique is used for the formulation of the Ramipril drug loaded solid lipid nanoparticles. Improvement study for the bioavailability of poor soluble drug is tried. The parameters considered for the improvement study is the drug content, entrapment efficiency of the drug, and the particle size of the prepared SLN. Fourier transform infrared

spectroscopy [21,22] was used for the study of the compatibility of drug and the excipients study. Stability and in-vitro drug release studies are conducted for the prepared SLN.

2. Material And Methods

Materials

Ramipril was purchased from Shreeji Pharma international, Vadodara, Gujarat and stearic acid is purchased form Lobachemi Mumbai, Tween 80 and Palmitic acid were purchased from Sisco research Mumbai. The reagents used in this work all are analytical reagent grade.

The sequence of the steps followed is preparation of standard stock solution, preparation of calibration curve, design of SLNs and the evaluation of Ramipril. The evaluation of Ramipril was carried by using the scanning electron microscopy (SEM), and the Fourier transform infrared studies. After that, the entrapment efficiency, in vitro drug release and the stability studies are conducted.

Standard solution Preparation

Ramipril drug standard solution was prepared by adding 100 mg of Ramipril to 100 ml of methanol using 100 ml standard flask. 1 ml of stock solution was withdrawn from the standard flask and diluted with 10 ml of methanol. It was further diluted by taking 1 ml of stock solution and dilution with the 10 ml of methanol. It is done by similarly for different concentrations of stock solution like 2 ml to 6 ml with 1 ml increment. The samples are kept for absorbance at 214 nm.

Preparation of SLNs

The SLNs were produced using the modified high shear homogenisation and ultra-sonification technique [12]. SLN was prepared by heating different concentration of lipids (stearic acid and palmitic acid) and different concentration of surfactants (10% and 20%). The aqueous phase containing stearic acid is added with the drug Ramipril and heated to a temperature of 70^0C until it dissolves completely. The organic phase consisting of the surfactant tween 80 is added to the aqueous phase after melting at 70^0C. The mixture is kept in magnetic stirrer for 30 minutes to get a cleaner solution. It is then followed by homogenization and sonication for 30 minutes. This similar technique used for preparing SLN with palmitic acid. In table 1 SLNs formulation data was given.

Table 1. Formulation data for the SLNs

Components	S1(mg)	S2(mg)	S3(mg)	S4(mg)	S5(mg)	S6(mg)	S7(mg)	S8(mg)
Drug(Ramipril)	100	100	100	100	100	100	100	100
Stearic Acid	100	100	150	150	-	-	-	-
Palmitic Acid	-	-	-	-	100	100	150	150
Tween 80(ml)	10	20	10	20	10	20	10	20
Distilled water(ml)	100	100	100	100	100	100	100	100

Preparation of calibration curve

The calibration curve was prepared from the absorbance noted from the UV-visible spectrophotometer [23]. A stock solution of concentration 100 µg/ml was used for this study. In series dilutions of from 1 ml to 6 ml with 1 ml increment were used. The absorbance values were recorded at 214 nm. The

prepared solutions were subjected for scanning (200 nm to 400 nm range). From the absorbance values the calibration curve was plotted. The optical characteristics are tabulated and shown in table 2.

Table 2. Absorbance of Ramipril drug

Concentration (ml)	Absorbance
1	0.226
2	0.411
3	0.587
4	0.762
5	0.92
6	1.1

Characterization of Ramipril microspheres

Morphology and particle size measurement

Particle size of solid lipid nanoparticles is very important characteristic. The surface morphology of the solid lipid nanoparticle weremeasured by using the SEM. The photon correlation spectroscopy at 90^0 angle and 25^0C was used to determine the mean diameter of the SLNs. Using the FT-IR studies the interaction between the lipid and the Ramipril drug were identified.

Entrapment efficiency of the Ramipril drug

The centrifugation method was used for measuring the entrapment efficiency of the SLN dispersion. Supernatant liquid was collected by centrifuging the SLN dispersion (containing the 1 mg of Ramipril drug) at 2000 rpm for one hour and then it was filtered to determine the free drug concentration. For the dilution of the sample phosphate buffer saline with pH 7.4 was used. The absorbance was determined at 236 nm with a UV spectrophotometer and the entrapment efficiency was calculated using the equation (1).

$$Entrapment\ efficiency\ of\ the\ drug = \frac{Total\ drug - Entrapped\ drug}{Total\ drug} \times 100 \quad \text{--- (1)}$$

In-vitro dug release

The in-vitro drug release of different dispersions of SLNs were determined using the dialysis bag technique. SLN dispersion containing an equivalent of 5 mg Rampril drug was transferred to the dialysis bag and the bag was sealed tightly. The sealed bag was suspended in the phosphate buffer saline and stirred with the help of a magnetic stirrer. The conditions for the suspended solution were pH 7.4 and the temperature of 37^0C ± 0.5^0C. After that, up to 6 hours, aliquots were withdrawn at different time periods and the quantity of drug released was calculated by using the spectrophotometric studies at 236 nm. The samples with high entrapment efficiencies were identified, for them solubility studies were conducted at two different temperatures of 40^0C and 25 ± 2^0C. The drug content was measured for every 15 days to note the changes in the prepared SLNs.

3. Results And Discussion

Preparing the calibration curve

Ramipril microspheres were successfully prepared by modified high shear homogenisation followed by ultra-sonification technique [12]. Composition of formulations of Ramipril drug is given in table 1.

Calibration curve was drawn to predict the behaviour of the experiment and it is shown in the figure 1. The trend line R^2 value is 0.99944. It shows that the experimental values are reliable.

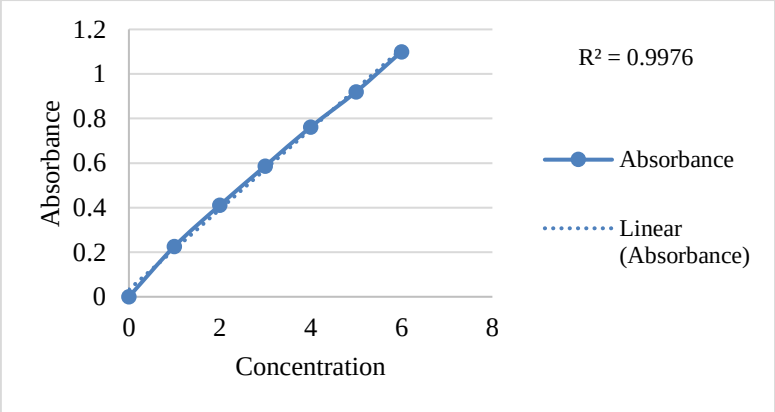

Fig.1. Calibration curve for the Ramipril drug

IR Studies

Sample and total mixture were studied by using IR spectral studies. The FT-IR spectrums of pure drug Ramipril, stearic acid, polysorbate, palmitic acid and of formulation 1 are shown in figures 2,3 4, 5 and 6 respectively. Samples retained their individual absorption characteristics, without undergoing any interaction with one another. From these studies we can conclude that no untoward chemical reactions were observed.

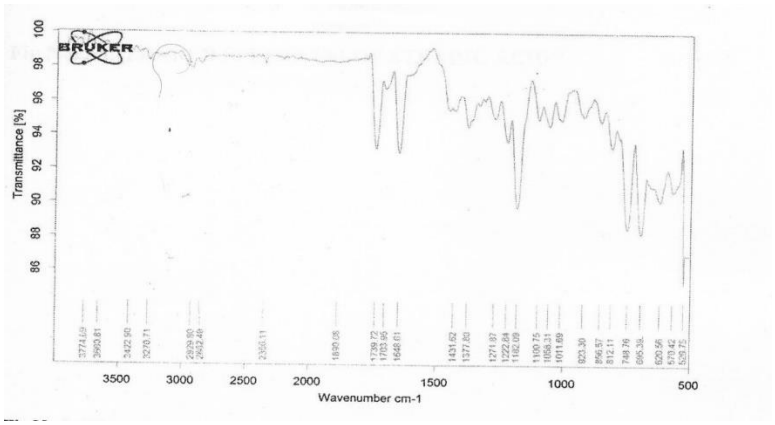

Fig.2 Infrared spectrum of Ramipril

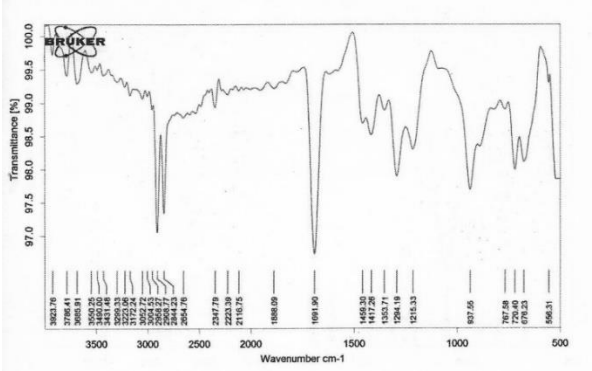

Fig 3. Infrared spectrum of Steric acid

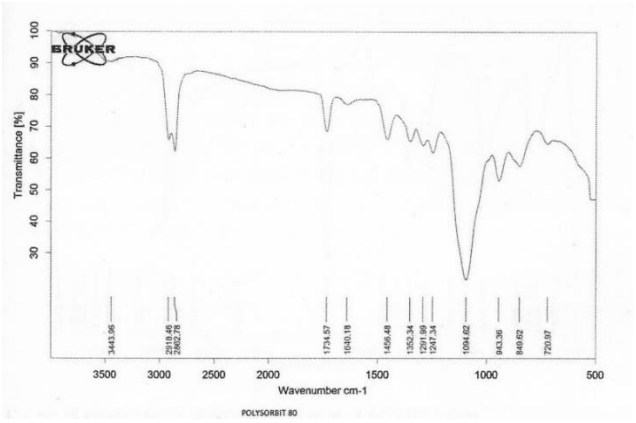

Fig.4 Infrared spectrum of polysorbate 80

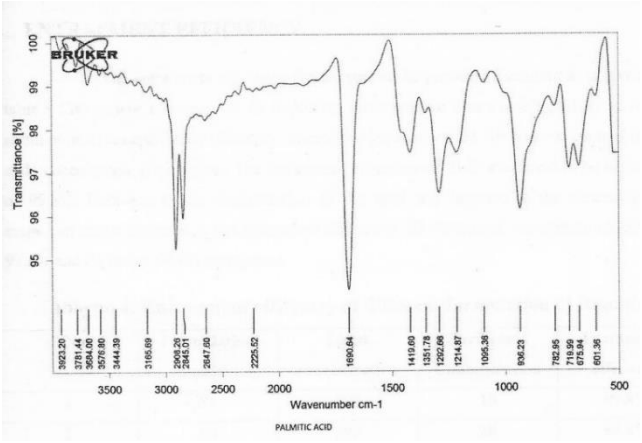

Fig 5. Infrared spectrum of Palmitic acid

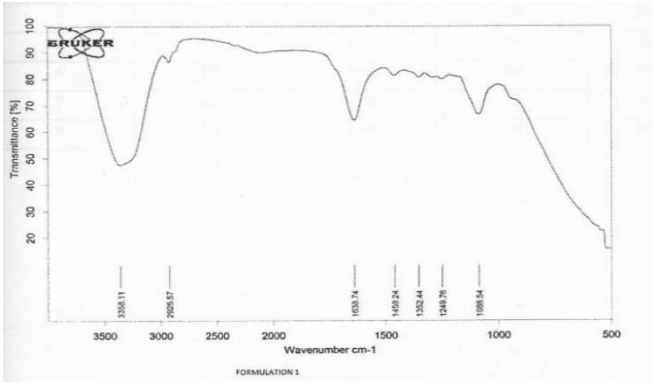

Fig. 6 Infrared spectrum of formulation 1

Entrapment efficiency

In table 3 the entrapment efficiency(responses) of the designed formulations was given. Entrapment efficiency was used to characterize the SLNs. The optimal encapsulation efficiency can decided by several factors like the concentration of the lipids and surfactants. Form the table 3, the entrapment efficiency was in the range of 99.8%. This observation leads the statement that the decrease in the concentration of the lipid and increase in concentration of the surfactant shows increase in entrapment efficiency. S2 shows and entrapment efficiency of 99.8% and P2 shows 99.9% entrapment.

Table 3: Entrapment efficiency of different formulation of Ramipril

S.no	Formulation code	Lipid concentration	Surfactant Concentration	Entrapment efficiency (%)
1	S1	100	10	99.8
2	S2	100	20	99.8
3	S3	150	10	99.8
4	S4	150	20	99.8
5	P1	100	10	99.8
6	P2	100	20	99.8
7	P3	150	10	99.8
8	P4	150	20	99.8

In-vitro drug release

Percentage release of drug with different concentration of lipid stearic acid and the lipid palmitic acid are shown in table 4 and in table 5 respectively. In-vitro release profiles for Ramipril for lipid stearic acid and the lipid palmitic acid wereshown in figure 7 and in figure 8. Figure 7shows that the in-vitro studies of all the batches showed an initial progress of 17% - 30% of drug release for all the batches within 1hour and the 60% was released slowly over a period of 4hours. The batch S1 shows about 80% of the Ramipril drug was released at the end of 6hours. From the figure 8 it is observed that, P2 shows a drug release of 78% at the end of 6hours and it is the highest compared to other lipid palmitic acid batches. In summary, an increase in drug release with increase in surfactants was observed.

Table 4. Percentage release of drug with different concentration of lipid (stearic acid) and surfactant

S.No	Formulation code	Lipid concentration	Surfactant concentration	(%) drug release
1	S1	100	10	86.1
2	S2	100	20	91.5
3	S3	150	10	80
4	S4	150	20	85

Table 5. Percentage release of drug with different concentration of lipid(palmitic acid) and surfactant

S.No	Formulation code	Lipid concentration	Surfactant concentration	(%)drug release
1	P1	100	10	80
2	P2	100	20	86
3	P3	150	10	85
4	P4	150	20	75

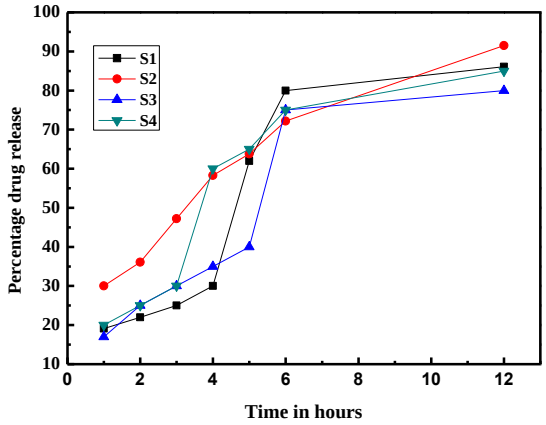

Fig.7 In-Vitro release profile of Ramipril for different batches of drug loaded with Tween 80 and Stearic acid

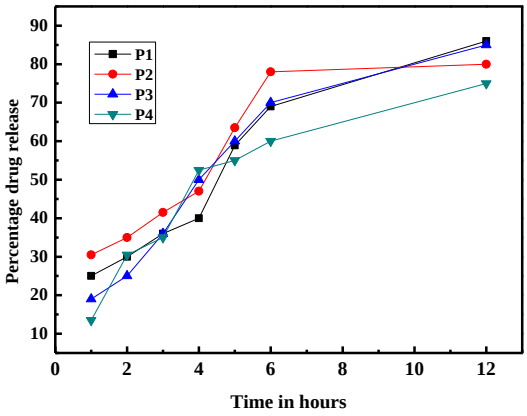

Fig 8. In-Vitro release of Ramipril for different batches of drug loaded with Tween 80 and Palmitic acid

Morphological studies

The SEM images for the SLNs loaded with Ramipril drug were shown in figure 9 and in figure 10. From the morphological studies it is revealed that the SLNs were in spherical shape and the average particle size around 320±5.15 nm (n=200).

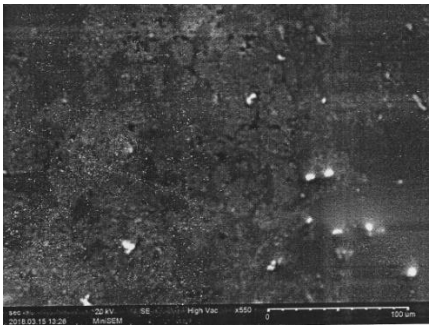

Fig.9 Scanning electron microscopy of Ramipril SLN S2 (Containing Tween 80, Stearic acid and drug concentration – 100 mg)

Fig. 10 Scanning electron microscopy of Ramipril SLN P2 (Containing Tween 80, Palmitic acid and drug concentration – 100)

Stability studies

For the samples with high entrapment efficiencies stability studies were conducted. The temperatures maintained were 4^0C and 25^0C ± 2^0C and the time for the study was 30 days. Stability studies identified that the SLNs were physically stable.

4. Conclusion

In this work the Ramiprilldrug was successfully loaded into SLNs by modified high shear homogenisation and ultra-sonification techniques. The effects of different formulation variables on % entrapment efficiency and physiochemical properties were evaluated. The in-vitro release tests showed that the slow release of drug release. The type and concentration of surfactant as well as the lipid matrix were played a greater role on physiochemical characterisation of SLNs and the in-vitro drug release. SLN formulation S2 and P2 composed of Tween 80 as a surfactant with higher concentration and lower concentration of lipid matrix (100 mg of stearic acid for S2 and 100 mg of palmitic acid for P2) showed the best result in view of the entrapment efficiency as well as in vitro drug release.

Particle size analysis showed that the formed particles were in Nano size. Based on observation it can be concluded that the formulated lipid Nano particulate delivery system of Ramipril could be widely accepted and physiologically safe lipids was capable of exhibiting sustained properties. In conclusion the SLNs designed in this work were shown an efficient drugdelivery systems for poor soluble drugs like the Ramipril drug.

Conflict of Interests

Authors declare no conflict of interest amongst themselves

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