



Formulation and evaluation of nanosuspension of Lercanidipine HCl

Debashish Borah, Shivanand Kalyanappa and Shantha Kumar GS

Department of Pharmaceutics, Acharya & BM Reddy College of Pharmacy, Soldevanahalli, Hesaraghatta main road, Bangalore 560107, India

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ABSTRACT

The main objective of the present study was to increase the aqueous solubility of poorly soluble Lercanidipine HCl and improve the bioavailability. Lercanidipine is mainly used as antihypertensive drug. The present work was focused on formulation of Lercanidipine HCl loaded nanoparticles using biodegradable polymer chitosan of low molecular weight by ionic gelation method using tripolyphosphate as cross linking agent, tween 80 as stabilizer. The nanoparticles were evaluated for various parameters like particle size, zeta potential, drug entrapment efficiency, surface morphology, *in-vitro* drug diffusion study and stability studies. FT-IR and DSC studies depicted that there was no interaction between drug and polymer. The drug entrapment efficiency was found to be in the range of 74.67 to 84.34%. The developed nanoparticles size was ranging from 106.23-360.17nm. The polydispersity index was found to be in the range of 0.015-0.451. Scanning electron microscopy studies revealed that particles were of irregular shape showing no agglomeration. The zeta potential was found to be in the range of +35.22 to +40.17mV. *In-vitro* drug diffusion study showed that the highest percentage cumulative drug release was found 84.41% for (F3) formulations in 12h. Kinetic modelling revealed that the *in-vitro* drug release follows first order kinetics and non-fickian drug release. Stability study revealed that there is no significant change in physicochemical properties, drug entrapment efficiency and *in-vitro* drug release. Thus, the prepared nanoparticles proved to be a potential candidate as by enhancing the aqueous solubility by reducing the particle size and effective *in-vitro* release and *in-vivo* results.

KEYWORDS: Nanoparticles, Lercanidipine, chitosan, ionic gelation, particle size, zeta potential.



INTRODUCTION

The formulation of poorly water soluble drugs has always been a challenging problem faced by pharmaceutical scientists and it is expected to increase because approximately 40% or more of the new chemical entities being generated through drug discovery programmes are poorly water-soluble. Obviously poorly water-soluble drugs show many problems in formulating them in conventional dosage forms. One of the critical problems associated with poorly soluble drugs is too low bioavailability and/or erratic absorption. Nanosuspension have revealed their potential to tackle the problems associated with the delivery of poorly water-soluble and poorly water-and lipid-soluble drugs, and are unique because of their simplicity and the advantages they confer over other strategies. Solubility is the crucial factor for drug effectiveness, independence of the route of administration. Today the world is really facing a huge problem of poorly water soluble drugs. Many

methods are there for increasing the solubility, but nanotechnology is one of the most prominent and latest technology. It deals with the nanoparticles (having high surface area) which are useful for increasing the solubility of poorly water soluble drugs. Nanoparticles are the end products of a wide variety of physical, chemical and biological processes some of which are novel and radically different, others of which are quite commonplace. A nanometre is 1×10^{-9} m or one millionth of a millimetre. To give a sense of this scale, a human hair is of the order of 10,000 to 50,000 nm, a single red blood cell has a diameter of around 5000 nm, viruses typically have a maximum dimension of 10 to 100 nm and a DNA molecule has a diameter of 2 – 12 nm. The use of the term “nanotechnology” can be misleading since it is not a single technology or scientific discipline. Rather it is a multidisciplinary grouping of physical, chemical, biological, engineering, and electronic, processes, materials, applications and concepts in which the defining characteristic is one of size⁶. The formulation of

*Corresponding Author Address: Debashish Borah, Department of Pharmaceutics, Acharya BM Reddy College of Pharmacy, Soldevanahalli, Hesaraghatta main road, Bangalore 560090, India; E-mail: debashishborah22@gmail.com

poorly water soluble drugs has always been a challenging problem faced by pharmaceutical scientists and it is expected to increase because approximately 40% or more of the new chemical entities being generated through drug discovery programmes are poorly water-soluble¹. Obviously poorly water-soluble drugs show many problems in formulating them in conventional dosage forms. One of the critical problems associated with poorly soluble drugs is too low bioavailability and/or erratic absorption. Nanosuspension have revealed their potential to tackle the problems associated with the delivery of poorly water-soluble and poorly water-and lipid-soluble drugs, and are unique because of their simplicity and the advantages they confer over other strategies. Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including: (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.

Lercanidipine HCl is chemically 2-[(3,3-diphenylpropyl) methylamine]-1, 1-dimethylethylmethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5 pyridine carboxylic ester hydrochloride. Lercanidipine is used in treatment of hypertension, because of its selectivity and specificity on the smooth vascular cells^{5,6}. The drug is administered orally in a dose of 10–20 mg daily as its hydrochloride salt, reducing significantly the diastolic blood pressure⁶. After oral administration, Lercanidipine is completely and erratically absorbed from the gastrointestinal

tract⁷. However, absolute bioavailability is reduced to approximately 10% because of extensive first pass metabolism to inactive metabolites⁶. Half life of lercanidipine is 2.5-4.5 h. Preparation of lercanidipine nanosuspension will increase solubility, thereby, increasing bioavailability.

The main objective of this work is an attempt to overcome the poor solubility and dissolution rate of the model drug (Lercanidipine) by using ionic gelation method. Lercanidipine was used as the model drug, chitosan as polymer and tween 80 as stabilizers were used at different concentrations. The formulations were done by ionic gelation method followed by sonication.

METHODOLOGY

Formulation of Lercanidipine nanoparticles:

Ionic gelation method: The lercanidipine nanoparticles were prepared using different concentrations of drug to chitosan of 1:1, 1:2, 1:3 ratios with different sodium tri poly phosphate concentrations (0.75, 1.0 & 1.25%) with a concentration of 0.25% of tween 80 by ionic gelation method. Specified quantity of tripolyphosphate was added into the above mixture and kept stirring for half an hour at 300rpm in magnetic stirrer. TPP aqueous solution was added drop wise using syringe needle into 100 ml chitosan solution containing 100mg of Lercanidipine HCl. pH was adjusted to 6 by adding 0.1 N NaOH. The stirring was continued for about 30 min. The resultant nanoparticles suspensions were centrifuged at 12000x g for 30 min using C24 centrifuge. The formation of the particles was a result of the interaction between the positive groups of the TPP and the negatively charged amino groups of chitosan. Specified quantity of tween 80 solutions was stirred for 10mins with magnetic stirrer at 300rpm. The Lercanidipine-chitosan solution was poured slowly into the tween 80 solution under stirring.

Table 5: Formulation chart of Lercanidipine nanoparticles (F1-F9)

Formulation Code	Lercanidipine (mg)	Chitosan (mg)	Tween 80(%)	Glacial acetic acid (%)	Sodium TPP (mg)	Distilled Water Q.S. to (ml)
F1	100	100	0.25	1.5	0.75	100
F2	100	100	0.25	1.5	1.0	
F3	100	100	0.25	1.5	1.25	
F4	100	200	0.25	1.5	0.75	
F5	100	200	0.25	1.5	1.0	
F6	100	200	0.25	1.5	1.25	
F7	100	300	0.25	1.5	0.75	
F8	100	300	0.25	1.5	1.0	
F9	100	300	0.25	1.5	1.25	

Evaluation of Lercanidipine nanoparticles:

Particle size analysis: The particle size of nanoparticles was measured by using Brookhaven instruments (Particle size analyzer, S 90 plus). Samples were prepared by diluting nanoparticles with sufficient amount of water in concentration of 0.0001 to 1.0%. The average diameter was determined from the particle size distribution data³⁷.

Scanning Electron Microscopy (SEM): SEM photographs were taken for the prepared nanoparticles using a scanning electron microscope (Carl Zeiss FESEM Model Number: Ultra 55 USA) at specified magnification (50 KX & 100 KX) in room temperature

Zeta Potential: The zeta potential is the electric potential in the interfacial double layer at the location of the slipping plane versus a point in the bulk fluid away from the interface. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle.

Drug entrapment efficiency study: Specified quantity (10 ml) of nanosuspension was transferred to centrifugation tube and placed in the centrifuge. The centrifugation instrument used was operated at 15,000 rpm for 15 mins. After that took the supernatant and diluted it to 10 ml. The percentage of incorporated Lercanidipine was determined by using shimadzu 1700 U.V spectrophotometer at 263 nm.

In-vitro drug diffusion study: The *in-vitro* drug release of Lercanidipine nanoparticles formulations were studied using a dialysis bag. Specified quantity (2 ml) of formulation was taken into a dialysis bag and placed in a beaker containing 100ml of pH 6.8 phosphate buffer. The beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at $37 \pm 0.5^\circ\text{C}$ throughout the study. During the study rpm was maintained at 200 rpm. Samples (1ml) were withdrawn at definite time intervals and replaced with equal amounts of fresh pH 6.8 phosphate buffer. The samples were analyzed for drug concentration by shimadzu 1700 UV-Visible spectrophotometer at 263 nm⁴².

Kinetic modeling: In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with various kinetic equation like zero order (Graph 1), first order (log % drug remaining Vs time) , Higuchi's model (Cumulative % drug

release vs square root of time). The r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots

Stability studies for the most satisfactory formulation: All the nine formulations were placed in plastic bottles and stored for 60 days at:

- $2-8^\circ\text{C}$ in refrigerator.
- $30^\circ\text{C} \pm 2^\circ\text{C}/65\% \text{ RH} \pm 5\% \text{ RH}$ in Thermo lab humidity chamber (GINKYA IM 3500 series).

The optimized formulation was evaluated for % drug entrapment and *in-vitro* drug release at interval of 30 days.

RESULTS AND DISCUSSION

Nanoparticles were prepared by ionic gelation technique. It was found to be discrete through SEM analysis; their mean size distribution was found to be 290 nm. The cumulative drug release of drug-polymer in various ratios of 1:1, 1:2, 1:3 with a different concentrations of Sodium TPP for 9 formulations were found to be 83.45 ± 0.25 , 82.25 ± 0.14 , 84.76 ± 0.53 , 78.34 ± 0.66 , 75.61 ± 0.78 , 72.74 ± 0.52 , 68.76 ± 0.32 , 69.31 ± 0.40 , 65.31 ± 0.31 . that means there is a decrease in drug release by increasing the polymer concentration with increasing concentration of Sodium TPP. Entrapment efficiency was found to be 74.67 ± 0.15 , 78.63 ± 0.05 , 79.41 ± 0.02 , 76.77 ± 0.02 , 75.15 ± 0.04 , 78.59 ± 0.05 . Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The formulation F3 registered highest drug release of ($84.76 \pm 0.53\%$).

Zeta potential for best formulation F3 was found to be +30.08 mV. It was apparent that *in vitro* release of Lercanidipine HCl showed a very rapid initial burst, and then followed by a very slow drug release. An initial, fast release suggests that some drug localized on the surface of the nanoparticles. F3 was showing good sustained release compared to other formulations and it was considered as best formulation. Particle size of formulation F3 shows 106.23 nm. The stability studies showed that maximum entrapment efficiency and *in vitro* drug release was found in F3 formulation which was stored at 4°C and room temperature. Based on drug content, drug entrapment deficiency, particles size morphology, zeta potential and *in vitro* drug release, batch F3 was selected as an optimum formulation. Thus nanoparticles of Lercanidipine HCl with core: coat ration 1:1 was found to be spherical, discrete and free.

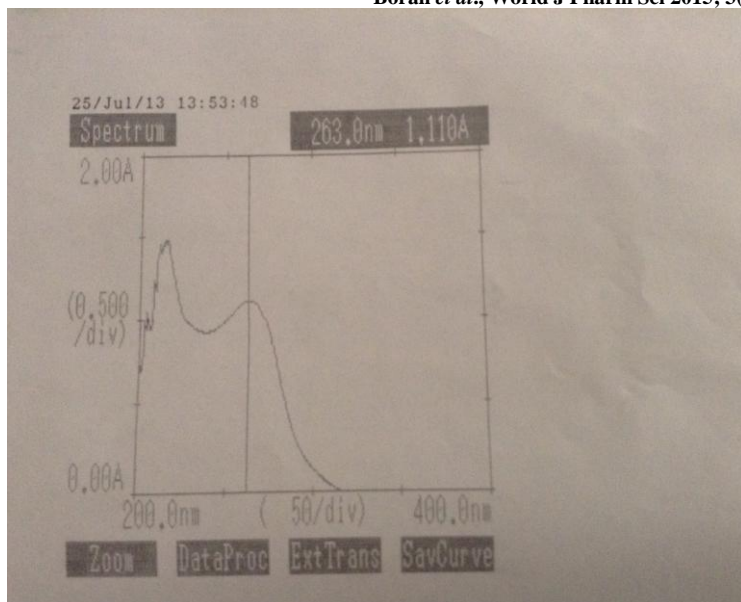
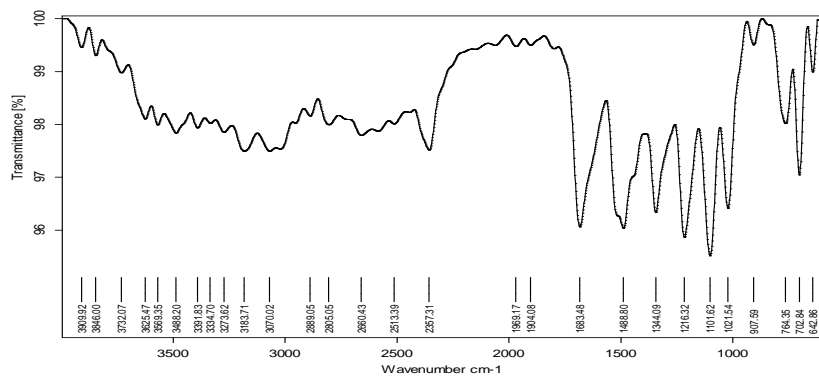


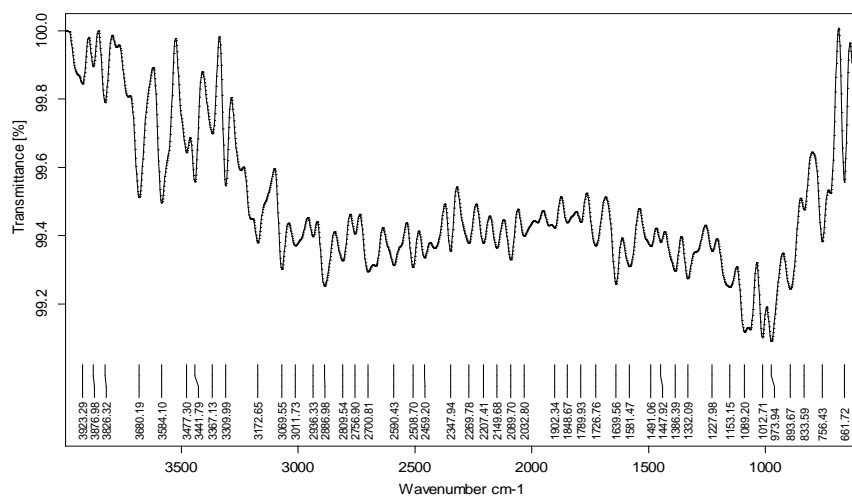
Fig 5: λ_{max} Lercanidipine



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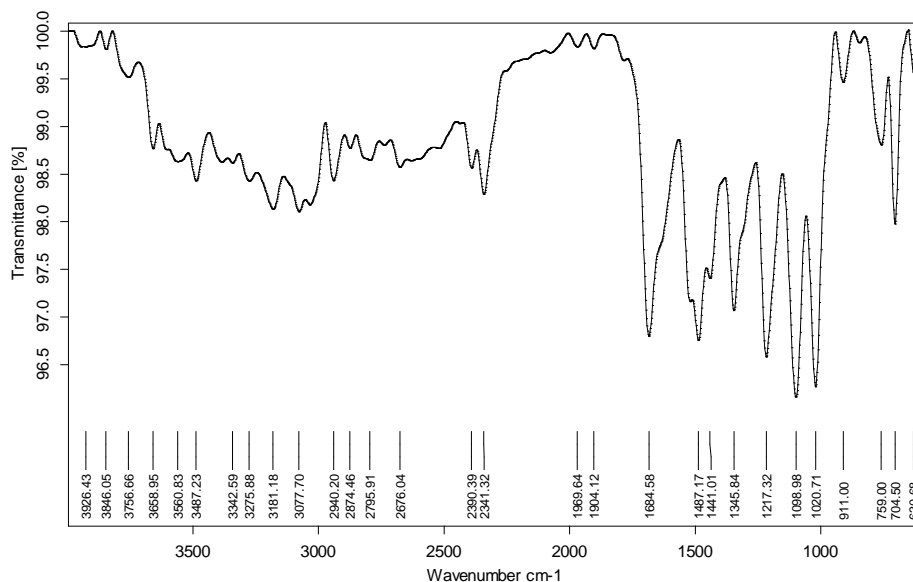
Fig 7: FT-IR spectra of pure drug Lercanidipine:



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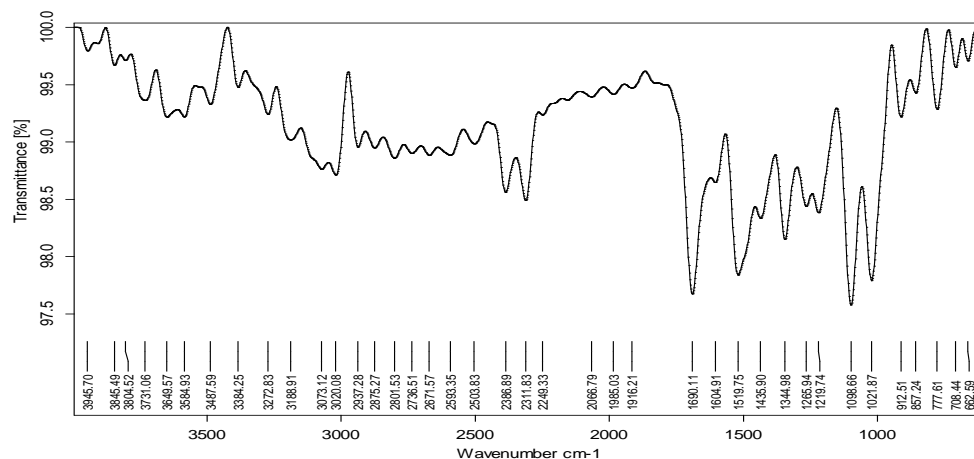
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Fig 8: FT-IR spectra of chitosan polymer



E:\Pharmaceutics\DEBASHISH BORAH\5	LERCANIDIPINE + CHITOSAN	SOLID	23/03/2014
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Fig 9: FT-IR Spectra of Lercanidipine mixture



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Fig 10: FT-IR spectra of lyophilized Lercanidipine nanoparticles (F3)

Differential Scanning Calorimetric Study: The DSC analysis of Lercanidipine alone showed a sharp endothermic peak at 115°C corresponding to its melting point and shown in Fig 8. The DSC analysis of Lercanidipine nanoparticles (F3) demonstrated that negligible change in the melting point of Lercanidipine and shown in Fig 11.

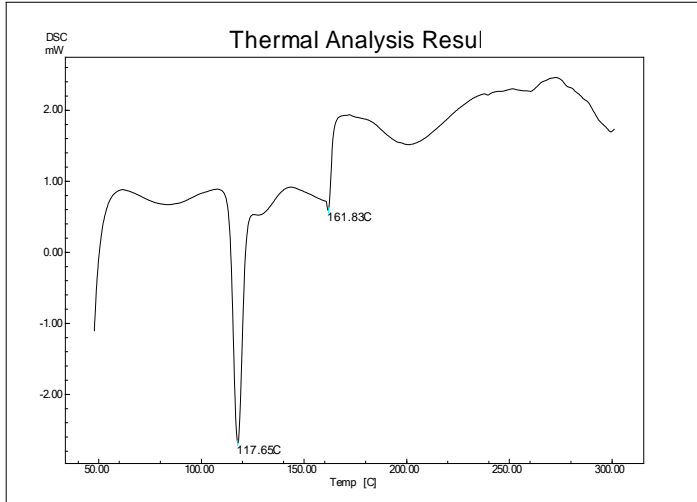


Fig 11: DSC thermo gram of Lercanidipine pure drug

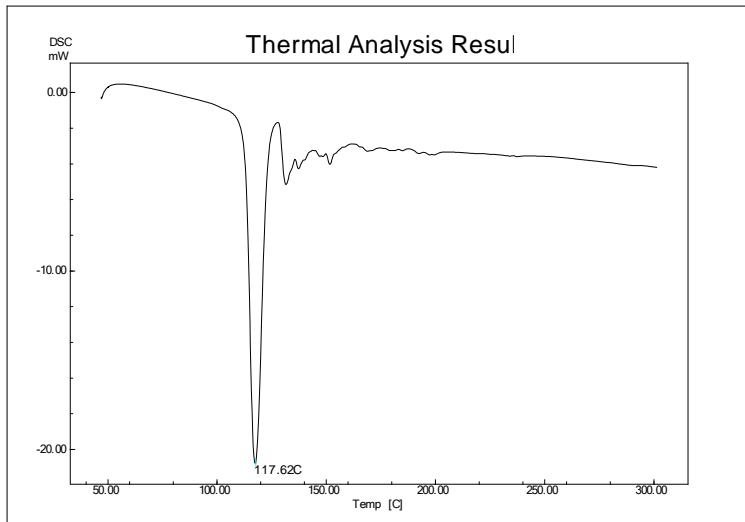


Fig 12: DSC thermo gram of Lercanidipine nanoparticles (F3)

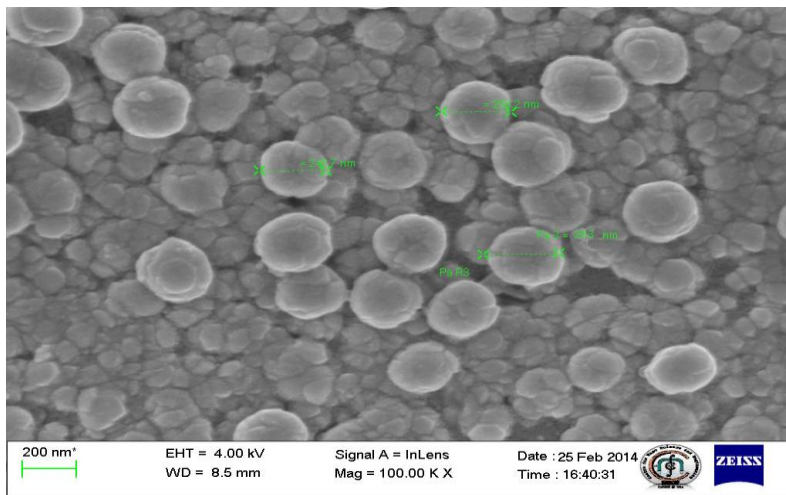
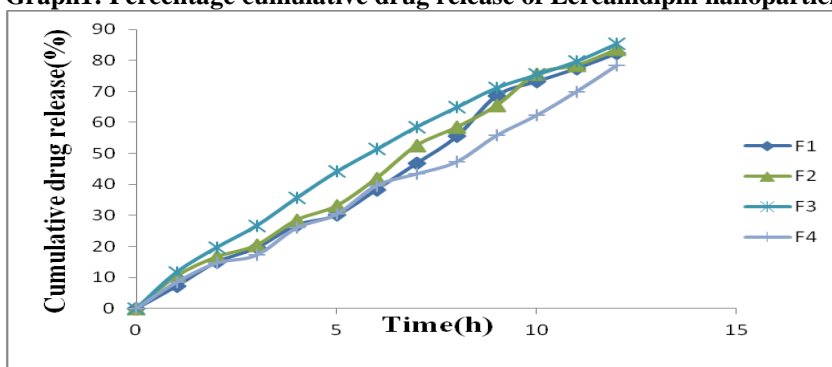
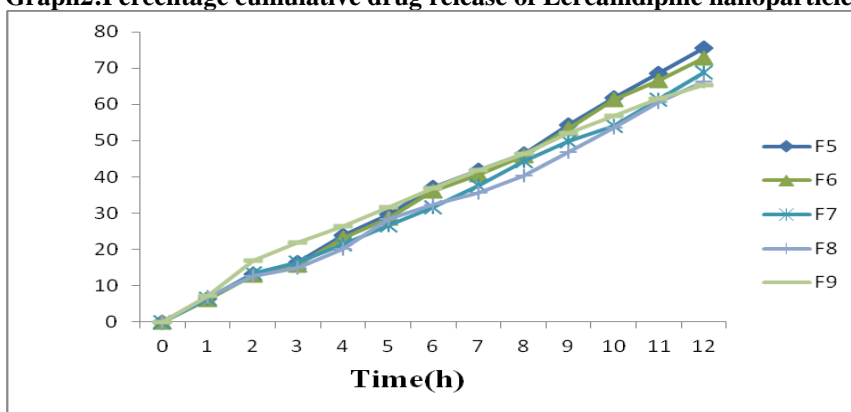


Fig 13: In 50.00 K X magnitude SEM study

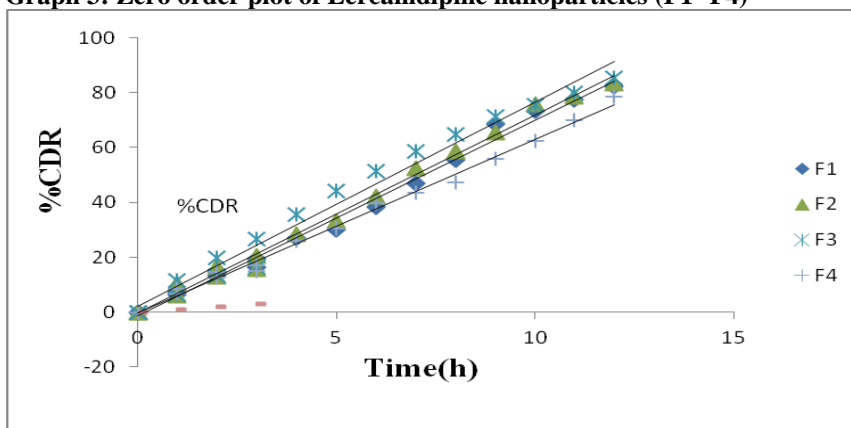
Graph1: Percentage cumulative drug release of Lercanidipin nanoparticles (F1-F4)



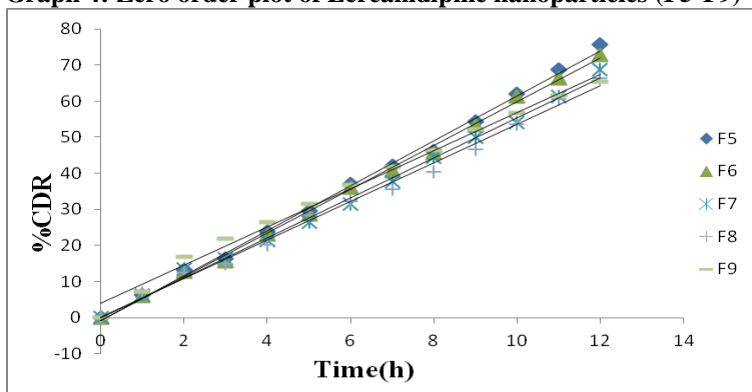
Graph2: Percentage cumulative drug release of Lercanidipine nanoparticles (F5-F9)



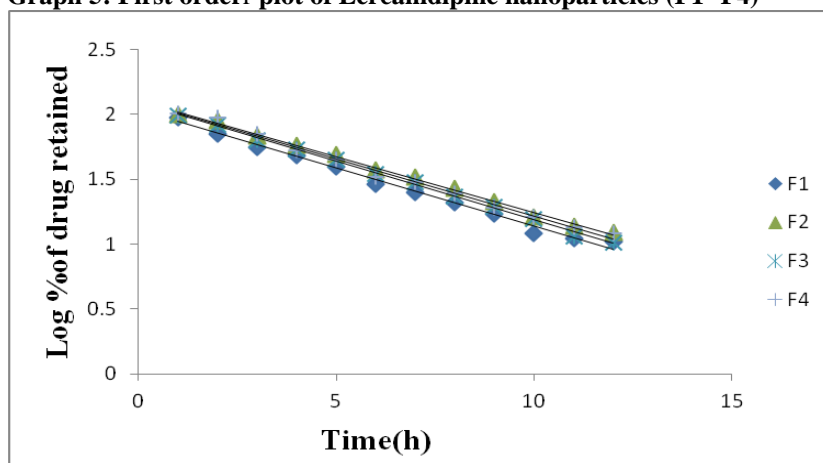
Graph 3: Zero order plot of Lercanidipine nanoparticles (F1- F4)



Graph 4: Zero order plot of Lercanidipine nanoparticles (F5-F9)



Graph 5: First order plot of Lercanidipine nanoparticles (F1- F4)



Graph 6: First order plot of Lercanidipine nanoparticles (F5-F9)

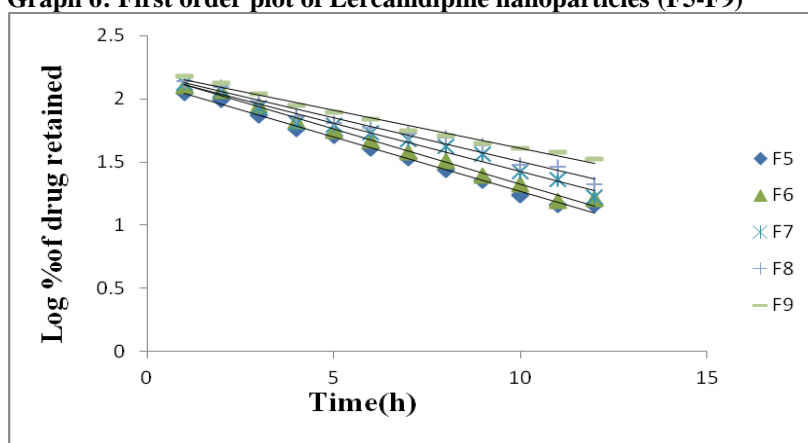
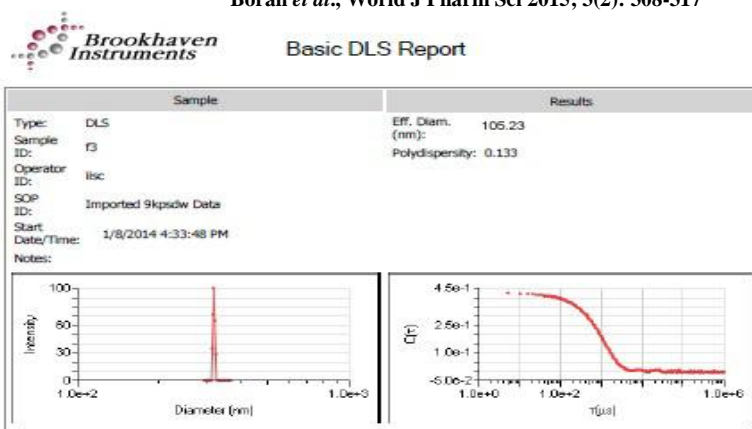


Table 13: Regression co-efficient values of drug release curves for Lercanidipine nanoparticles (F1-F9)

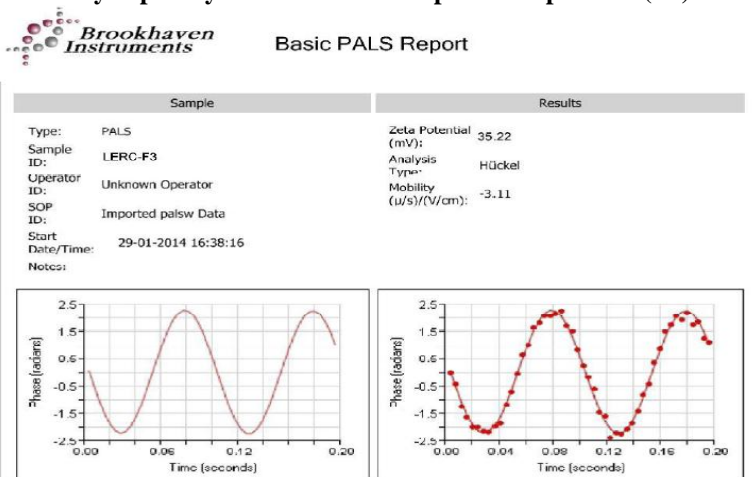
Kinetic model	Parameter	Formulation code								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero order	Regression	0.915	0.922	0.945	0.989	0.988	0.986	0.996	0.998	0.997
First order	Regression	0.995	0.998	0.999	0.991	0.996	0.992	0.991	0.990	0.992
Higuchi model	Regression	0.972	0.970	0.956	0.998	0.989	0.998	0.997	0.993	0.994
Korsmeyer peppas	Regression	0.713	0.704	0.741	0.831	0.836	0.868	0.778	0.823	0.798

Table 14: Particle size and polydispersity index of Lercanidipine nanoparticles (F1-F9)

Formulation code	Particle size(nm)	Polydispersity index(PI)
F1	140.11	0.110
F2	120.21	0.451
F3	106.23	0.133
F4	245.23	0.015
F5	210.43	0.063
F6	220.96	0.018
F7	350.21	0.104
F8	320.34	0.121
F9	360.17	0.024



Particle size and Polydispersity index of Lercanidipine nanoparticles(F3)



Zeta potential of Lercanidipine nanoparticles (F3)

CONCLUSION

The present study confirms that the ionic gelation followed by probe sonication technique is suitable for the preparation of Lercanidipine nanoparticles with high entrapment efficiency. The method of preparation of nanoparticles was found to be simple and reproducible. The slow and constant release of Lercanidipine from nanoparticles maintain constant drug plasma concentration thereby increasing therapeutic efficacy. Finally it was concluded that the developed formulation overcome and alleviates the drawbacks and limitations of other Lercanidipine sustained release formulations. Chitosan nanoparticles with a size of 106 nm can

be obtained when the concentration of chitosan and TPP was set as 1 and 1.25 mg/ml, respectively at pH 4.8. The nanoparticles have no significant influence on the fibroblast viability, demonstrating their good biocompatibility. The applicability of the chitosan nanoparticles as drug carriers is demonstrated by loading and release of a model drug, this work confirms that modified ionic gelation method is useful for the development of chitosan nanoparticles. The concentration of polymer and crosslinking agent as well as sonication time is important factors in the development of Lercanidipine HCl nanosuspension.

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