International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Design and Characterization of Lercanidipine Microcrystals for Enhancement of Dissolution Rate

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Abstract: ***Improved bioavailability is an added advantage for most of the poorly water soluble drugs. In recent years research work is concentrated on various methods to improve the solubility characteristics of poorly soluble drugs and crystallization phenomenon is one amongst them. The solubility problem can be solved by changing the crystal habit of drug, which improves the solubility and dissolution. So, in the present investigation an attempt has been made to improve the solubility characteristics of Lercanidipine HCl an anti - hypertensive drug, using solvent change method. In this method drug was dissolved in organic solvent (methanol) to form organic phase. Aqueous phase was prepared by dissolving stabilizing agents in water. Poloxamer 407 (1%) and Polyvinyl pyrollidone K30 (1.1%) are used as stabilizing agents. The formulated crystals of LercanidipineHCl were subjected to various physico - chemical parameters like size distribution, shape and surface characteristics using SEM, solubility studies, in - vitro dissolution studies, drug content, solvent interactions with DSC, FT - IR, and crystallographic studies by PXRD. The microcrystals produced with Poloxamer 407 showed better dissolution as compared to pure drug and microcrystals formulated using PVP K30. FTIR and DSC results showed that there was no interaction between the drug, solvent and the stabilizer. PXRD of micro - crystals showed higher peak height than pure drug indicating that crystal habit modification occurred in the microcrystals without any polymorphic changes and were found to be smaller in size than pure drug and free from any interactions.***

Keywords: Microcrystals, LercanidipineHCl, Poloxamer 407, PVP K 30, methanol

1. Introduction

A common observation throughout the pharmaceutical industry is that as the potency and specificity of new drug candidates are improving, the poor aqueous solubility is often becoming a problem. This may result in poor bioavailability of the active pharmaceutical ingredients. Given the increasing number of compounds emerging from discovery programs having poor aqueous solubility and/or dissolution, pharmaceutical scientists are constantly seeking new formulation approaches in order to obtain an adequate oral bioavailability[1].

Several techniques are commonly used to improve dissolution and bioavailability of poorly water - soluble drugs, such as size reduction[2], the use of surfactants[3], the formulation of solid dispersions[4], complexation with cyclodextrins, and the transformation of crystalline drug to amorphous state[5]. In addition to the general solubility enhancement techniques described above, drug particle size reduction has often been used, in regards to the Noyes–Whitney and Ostwald– Freundlich equations, to enhance dissolution of poorly water soluble compounds[6].

Particle size reduction is achieved because adsorption of excipients onto the particle surface that inhibits particle growth[7]. Particle size can be reduced and formulated into micro - crystals. Crystal morphology may be altered by preferential adsorption of stabilizing agent onto specific faces of the growing crystal[8]. Crystallization is a phenomenon in which solid particles formed by solidification under favorable conditions of a chemical element or a compound, whose boundary surfaces are planes symmetrically arranged at definite angles to one another in a definite geometric form. The polymorphic changes will have a definite influence on the solubility and thereby bioavailability of a particular compound due to structural differences resulting from different arrangements of molecules in the solid state. Lercanidipine Hydrochloride is a long - acting dihydropyridine CCB[9] with high vascular selectivity, and thus has many of the characteristics that are desirable in an antihypertensive agent. LercanidipineHCl microcrystals were developed by solvent change precipitation method. The microcrystals were evaluated by various studies like powder X - ray diffraction, DSC, FTIR and scanning electron microscopy for micromeritic properties and finally for solubility.

2. Materials and Methods

LercanidipineHCl (LER) was supplied as a gift sample by Apotexpvt research Ltd, (Bengaluru, India). Poloxamer 407 and polyvinyl pyrollidone K30 was supplied by Apotexpvt research Ltd, (Bengaluru, India). Methanol of analytical grade was procured from S. D. Fine (Mumbai, India).

Preparation of LER Microcrystals:
Microcrystals of LER (LM - I and LM - II) were prepared by solvent change method[10] using Poloxamer 407 and PVP K30 respectively. Briefly, a fixed amount of LER (7.5 g) was dissolved in 30 ml of methanol. This organic phase was added at room temperature, under constant mechanical stirring (600 rpm) to 100 ml of 1 % and 1.1% w/v aqueous solution of poloxamer 407 and PVP K30 respectively. Stirring was continued for 30 min. Microcrystals were collected after filtration, washed with deionized water and dried at room temperature.

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Table 1: Formulation chart to prepare LER Microcrystals

Formulation code	Amt of drug (gm)	Stabilizing agent	Amt of stabilizing agent (gm)	Organic solvent (ml)	Stirring speed (rpm)	Temp (°C)
- I	7.5	Poloxamer 407	5	30	600	25
LM - II	7.5	PVP K30	5.5	30	600	25

Percentage yield:

The practical percentage yield[11] was calculated from the weight of dried microcrystals (Practical mass) to the initial weight (Theoretical mass) & the results are reported in the percentage yield was calculated by using the following formula:

$$\% \text{ Yield} = \frac{\text{Practical Mass}}{\text{Theoretical Mass}} \text{ X } 100$$

Drug content:

A weighed quantity of the microcrystals were dispersed in 100 ml of 0.1 M HCl.1ml of resultant solution was withdrawn and diluted to 10 ml. The above solution was analyzed by UV - Visible Spectrophotometer (Shimadzu UV - 1700, Japan) at 241.5nm. It was carried out in triplicate.

Solubility studies:

The solubility[12] of Lercanidipine microcrystals in water was determined by taking excess quantity of microcrystals and adding to 100ml volumetric flask filled with water and sonicated. The solution was filtered through whatmann filter paper and the drug concentration was determined spectrophotometrically at 241.5nm.

Particle size determination:

Particle size[13] of the prepared microcrystals was determined by optical microscopy. The optical microscope was fitted with an ocular micrometer and a stage micrometer. The eyepiece micrometer was calibrated. The particle diameters of more than 200 microcrystals were measured randomly by optical microscope. The average particle size was determined by using the Edmondson's equation:

$$\mathbf{D_{mean}} = \frac{\sum \mathbf{nd}}{\sum \mathbf{n}}$$

Where,
n – Number of microcrystals observed
d – Mid point range.

***In - vitro* dissolution studies:**

The *in vitro* dissolution studies were carried out using USP type II dissolution apparatus (Rotating Basket type). The dissolution study was carried out in 0.1M HCl solution of pH 1.2. The dissolution medium was kept in a thermostatically controlled water bath, maintained at 37 ± 0.5^{0}C. The rotation of basket was set to 75 rpm. At predetermined time intervals between 0 and 80 min, 5ml of dissolution medium was withdrawn and analyzed for the drug release at 241.5 nm. At each time of withdrawal, 5ml of fresh corresponding dissolution medium was replaced into the dissolution flask. The samples withdrawn were analyzed by UV method at 241.5nm against blank.

Powder x - ray diffraction:

X - ray powder diffraction patterns were used to detect possible polymorphic transition during the crystallization process. X - ray powder diffraction[14] were obtained at room temperature (25ºC) using Bruker $_a$XS D8 Advance diffractometer (Cu Kά source λ = 1.5418 Å) with tube copper anode over the interval 5 to 60^0 of 2θ. The operation data were as follows: generator tension (voltage) 40 kV, generator current 30mA and scanning speed 0.02^0/min.

Shape and surface morphology:

The shape and surface characteristics of the prepared microcrystals were evaluated by means of Scanning electron microscopy[14] **(SEM)** (Using Ultra - 55 Carl Zeiss Field emission scanning electron microscope). The samples for scanning electron microscopy were prepared by gently sprinkling the microcrystals on a double adhesive tape, which is stuck to an aluminium stub. The stubs were then coated with gold using a sputter coater under high vacuum and high voltage to achieve a film thickness of 30nm. The samples were then imaged using a 3 kV electron beam.

Fourier transform infrared radiation (FT - IR)

In order to check the integrity (Compatibility) of drug in the formulation, FT - IR spectra of the microcrystal formulations with that of pure drug were compared (using Shimadzu FT - IR 8400 spectrophotometer) using potassium bromide (KBr). The samples were thoroughly blended with dry powdered potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the spectrophotometer and the spectrum was recorded. The FT - IR spectra of the formulations were compared with the FT - IR spectrum of the pure drug

Differential scanning calorimeter: Differential scanning Calorimetry (Mettler - 7, Germany) was performed to study the thermal behaviours[14] of drug alone and mixture of drug and polymer. Samples of about 1 - 3 mg were weighed and placed in aluminium pans and the lids were crimped using a shimadzu crimper. An empty pan sealed in the same way as for the sample was used as a reference. Thermal behaviour of the samples was investigated under nitrogen gas at scanning rate of 20°C/min, covering a temperature range of 30 - 300°C.

Stability studies: The selected formulations were packed in the containers and are tightly closed with the cap. They were stored at the stated conditions for one month[15]. Samples were analyzed after 30 days and they were evaluated for drug content.

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3. Results and Discussions

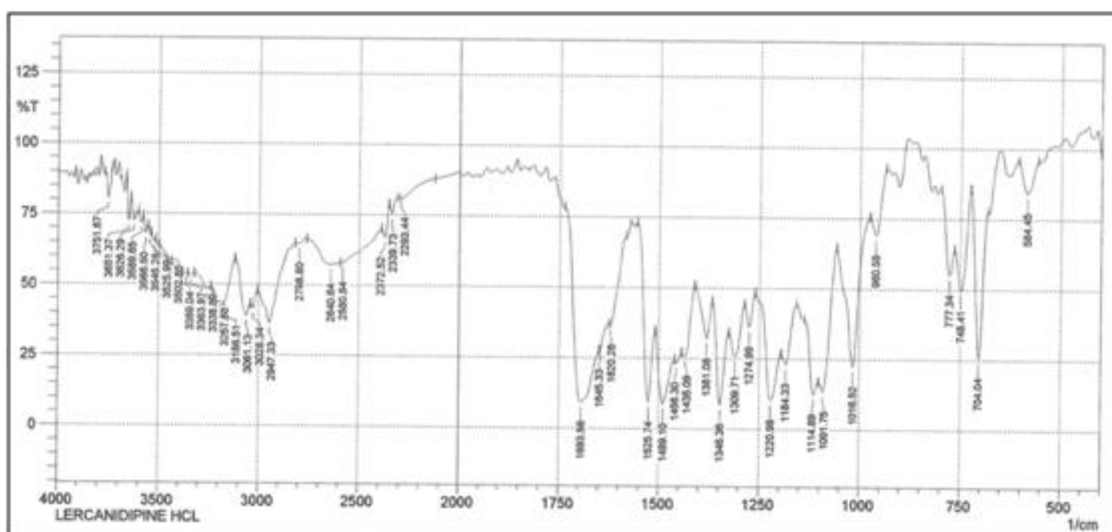

Figure 1: FT - IR Spectrum of Lercanidipine HCl

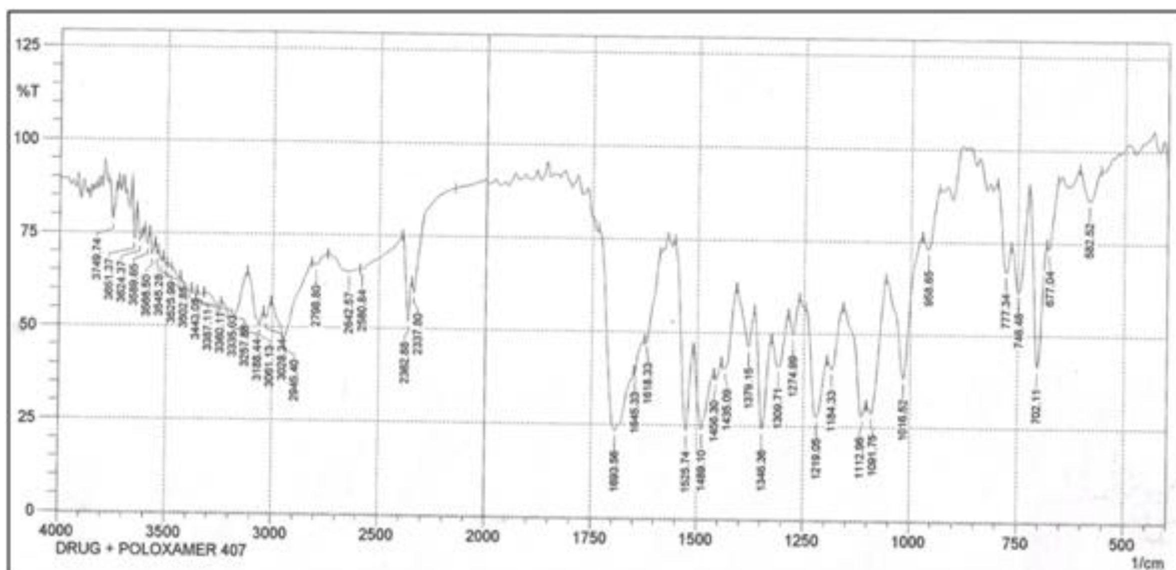

Figure 2: FT - IR Spectrum of formulation LM - I:

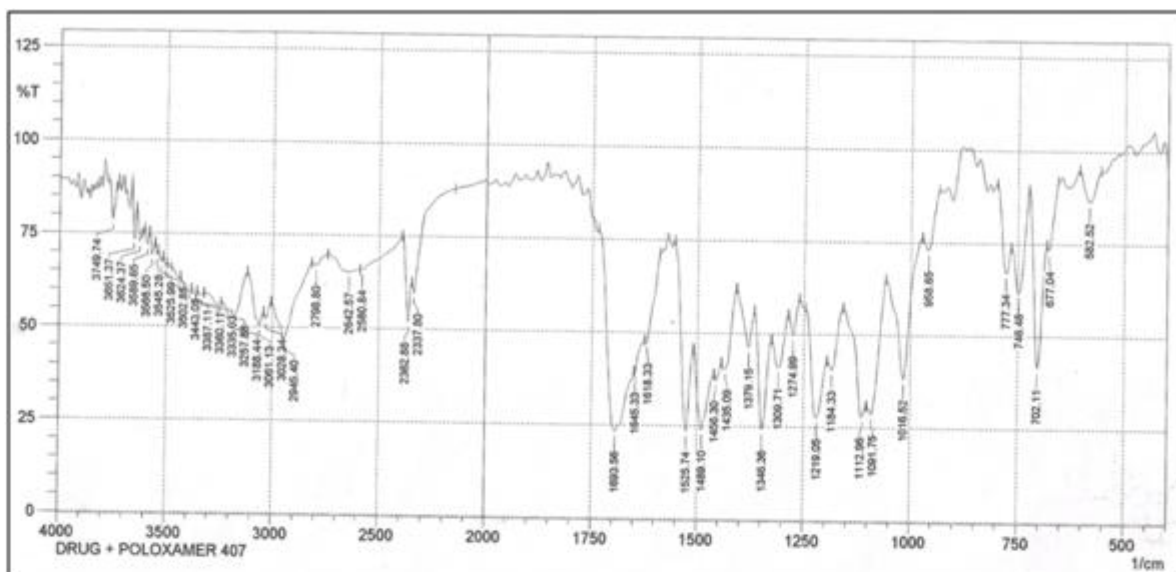

Figure 3: FT - IR Spectrum of formulation LM - II

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Table 2: Comparison of FT - IR spectra of Lercanidipine HCl, Microcrystals LM - I and LM - II:

Sl. no	Drug / polymer	OH stretch (cm^{-1})	N – H stretch (cm^{-1})	CH Stretch (Aromatic) (cm^{-1})	CH3 stretch (cm^{-1})	C=O (amide) (cm^{-1})	C=C stretch (cm^{-1})	C - N (cm^{-1})
1	Lercanidipine Hcl	3186.51	3389.04	3061.13	1435.09	1489.10	1525.74	1346.36
2	LM - I	3188.44	3387.11	3061.13	2875.96	1489.10	1525.74	1346.36
3	LM - II	3190.37	3390.37	3063.06	2845.10	1489.10	1525.74	1346.36

Table 3: Percentage Yield and Drug Content:

Formulations	Drug content (%)	Percentage yield (%)
LM - I	99.09	97.50
LM - II	97.77	96.66

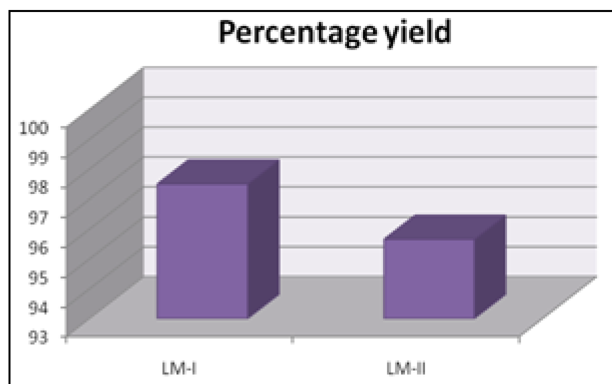

Figure 4: Graphical representation of % yield values for Microcrystals

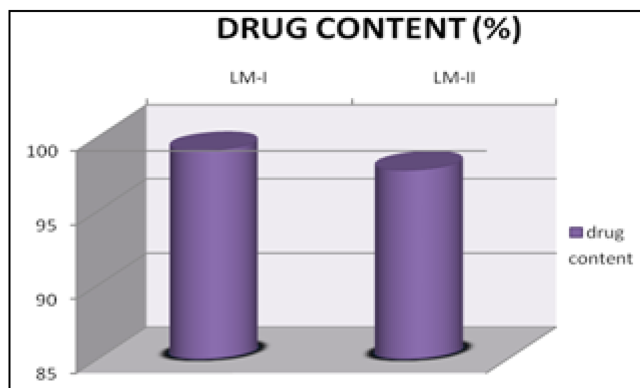

Figure 5: Graphical representation of drug content values for Microcrystals

Table 4: Solubility results of formulations and pure drug in water at 25 ^{0}C.

Sl. No	Formulation	Solubility (µg/ml) ±SD
01	Lercanidipine HCl	51.89 ± 0.002
02	LM - I	130.22 ± 0.001
03	LM - II	118.01 ± 0.002

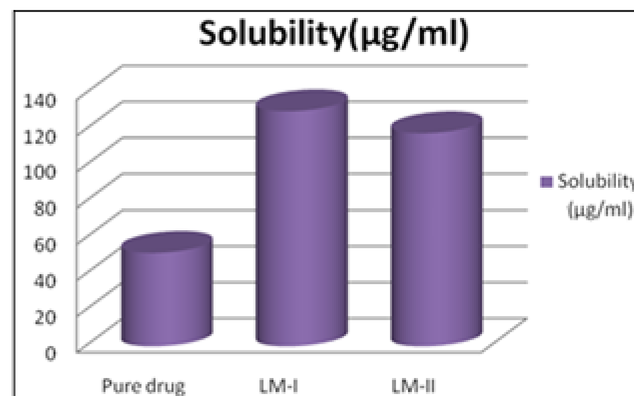

Figure 6: Graphical representation of solubility of formulations and pure drug.

Table 5: Particle size analysis for the prepared microcrystals

Frequency of Distribution		
Particle Size (µm)	Micro Crystals	
	LM - I	LM - II
0 – 10	20	09
10 – 20	23	14
20 – 30	27	23
30 – 40	61	67
40 – 50	49	39
50 – 60	16	30
60 – 70	04	18
Avg. Particle Size (µm)	**34.50**	**38.75**

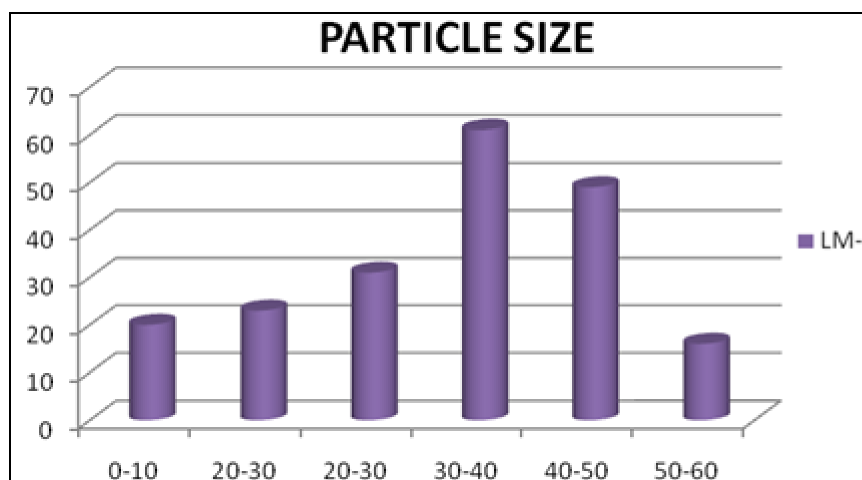

Figure 7: Particle size frequency distribution of formulation LM – I

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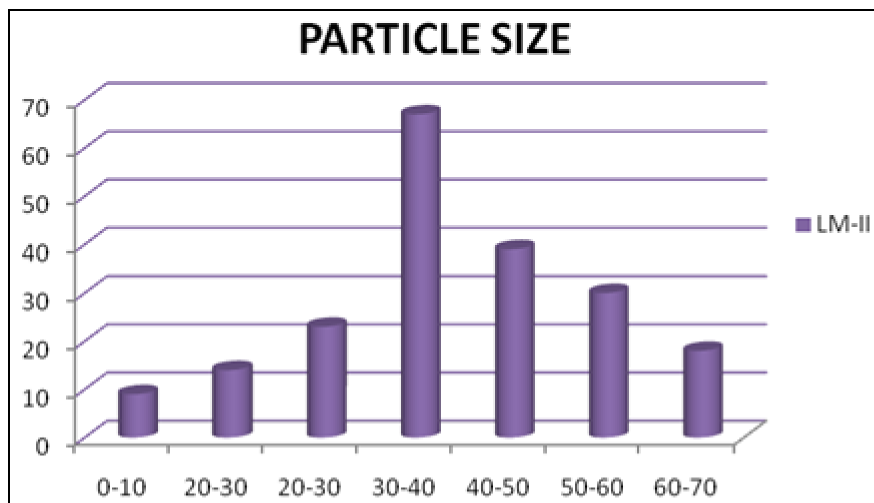

Figure 8: Particle size frequency distribution of formulation LM - II

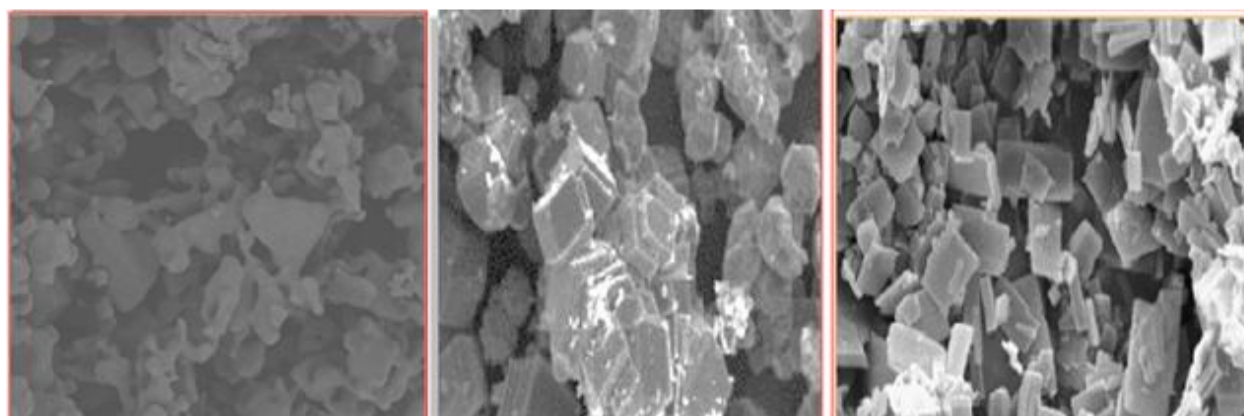

Figure 9: SEM photographs of, A= Lercanidipine HCl, B= LM - I, C=LM - II.

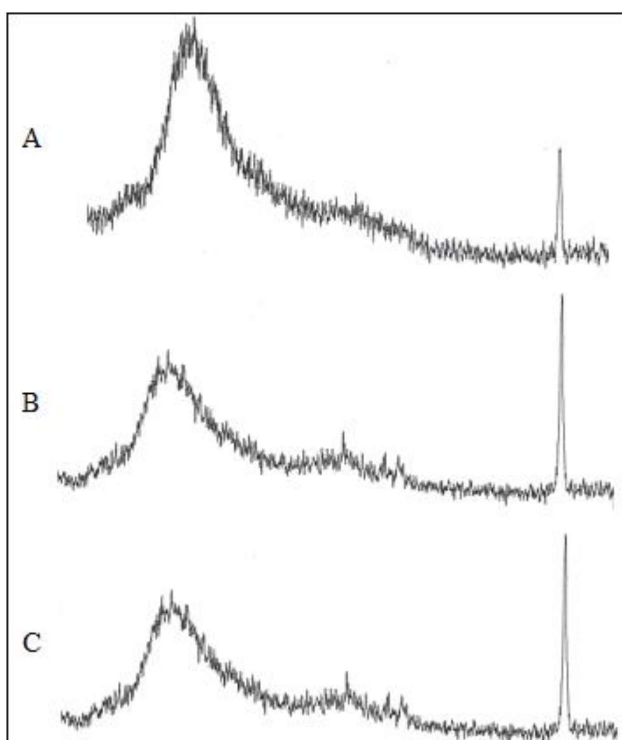

Figure 10: Powder X - Ray Diffractometry graphs of A= pure drug, B= LM - I, C= LM - II.

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ISSN: 2319-7064
SJIF (2022): 7.942

Table 6: *In - vitro* drug release data of pure drug & prepared microcrystals

SI. No	Time (min)	% Cumulative Drug Release		
		Pure Drug	LM - I	LM - II
1	0	0	0	0
2	10	4.98	12.86	11.60
3	20	9.63	25.56	23.46
4	30	16.86	39.79	36.17
5	40	23.16	57.12	52.95
6	50	28.64	71.10	63.28
7	60	38.16	83.28	72.70
8	70	47.91	93.34	83.25
9	80	59.21	99.02	94.34

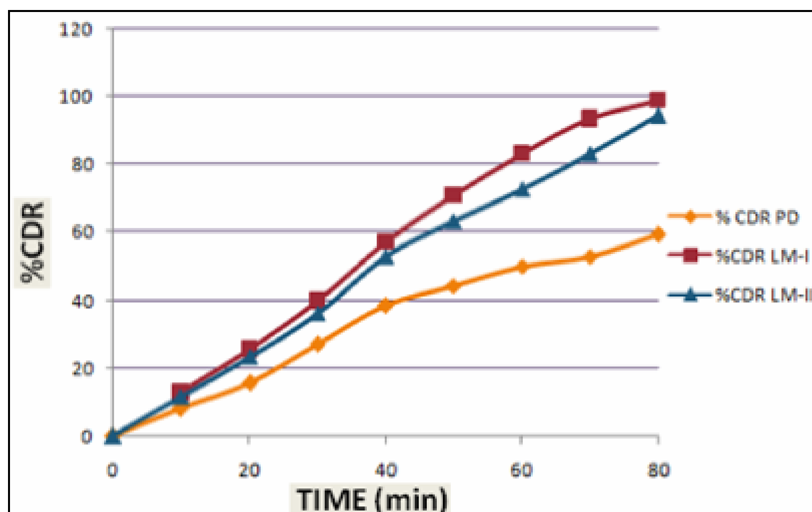

Figure 11: Comparative *In - vitro* drug release profile of Microcrystals to that of pure drug (PD) at the end of 80min:

Differential scanning calorimeter studies:

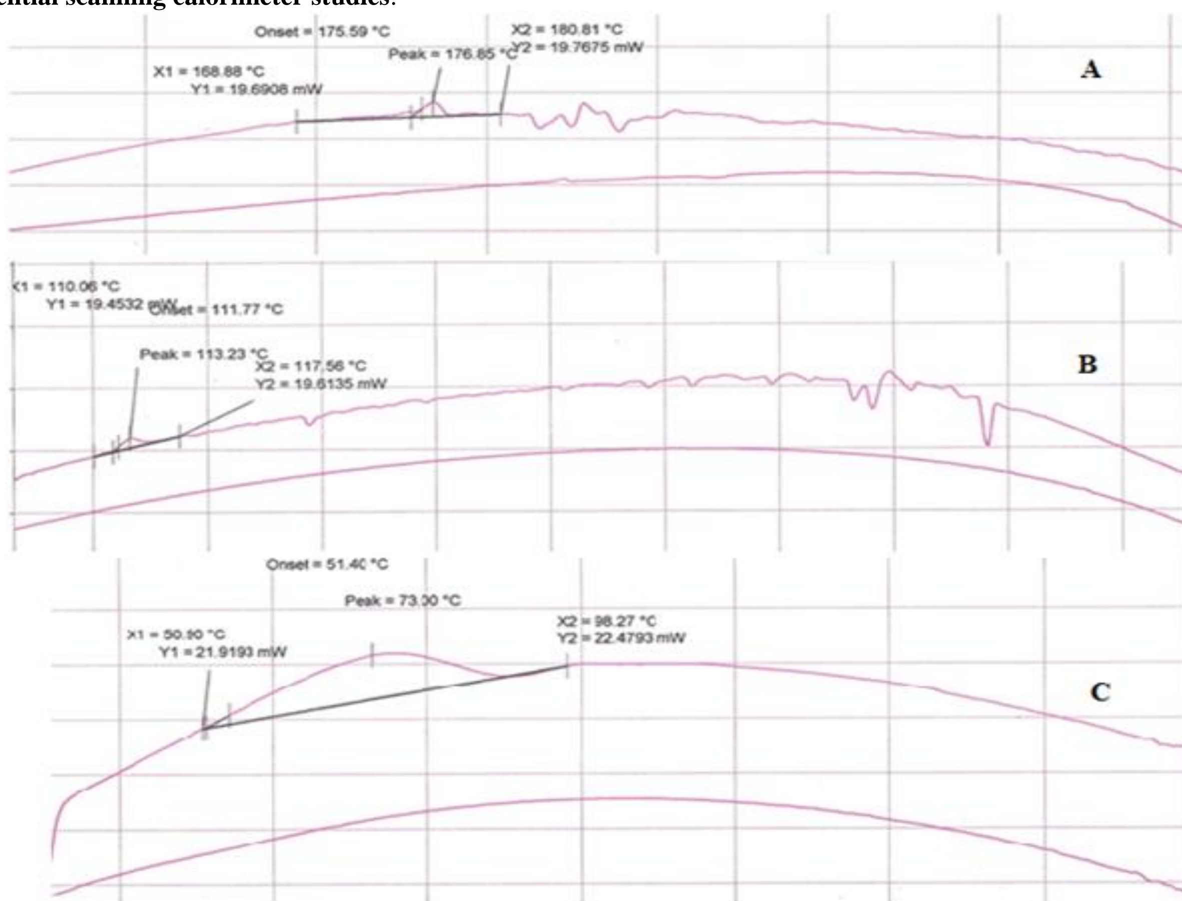

Figure 12: DSC studies of A= Pure drug, B= LM - I, C= LM - II.

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Table 7: Stability Studies

Stability condition	Formulations	Physical stability			Drug content (%)		
		no. of days			no. of days		
		0	15	30	0	15	30
5°Ambient	LM - I	No Change In Appearance			99.09	99.05	99.01
	LM - II				99.77	99.73	99.71
25°C/60%RH	LM - I	No Change In Appearance			99.09	99.09	99.06
	LM - II				99.77	99.77	99.76
40°C/75%RH	LM – I	No Change In Appearance			99.09	99.09	99.05
	LM - II				99.77	99.77	99.75

4. Discussions

Preparation of LER Microcrystals:
Microcrystals of Lercanidipine HCl were prepared by solvent change method using hydrophilic binders as stabilizing agent. The selection of a good solvent depends on the miscibility with water and the solubility of drug in that solvent. Different proportion of stabilizing agent: solvent was selected and microcrystals were prepared at 600rpm (table no.1). A first requirement for a stabilizing system is that it provides wetting of the hydrophobic surfaces of the drug particles. Surfactants used in the preparation of microcrystals stabilized these particles and avoided its growth. The solvent change method for preparation of microcrystals was found to be efficient.

Drug Content, Solubility and Dissolution Studies:
The drug content was found to be good and uniform among the different formulations of the prepared samples and was found to be 99.09 to 97.77 % (table no.3, fig no 5). As water is a universal solvent, apparent solubility studies were carried out in deionized water. In solubility studies of the samples, the crystals prepared using poloxamer 407 have showed highest solubility of the drug in water (130.22 ± 0.0012 μg/ml) as compared with the untreated drug (51.89 ± 0.002 μg/ml) shown in table no.4 and fig no.6.

The dissolution profiles of the prepared crystals and the control (raw drug material) are illustrated in table no 6 (fig no 11). Those samples prepared with surfactants showed the faster dissolution rate, with approximately more than 50% of the drug being released within 40 min compared to 23 % for the control. At the end of 80 min, more than 90 % of the drug was released from all crystals except for the control. This effect can be explained by an increased specific surface area which is hydrophilized due to the adsorbed hydrophilic polymers. Increase in solubility may be due to optimum amount of stabilizing agent was used and because of reduction in size, hydrophilicity of stabilizing agent, better solubility and wettability of microcrystals.

SEM, PXRD and DSC Studies:
The characteristic peak of the Lercanidipine in the PXRD 2θ ranges from 10° to 80°. The relative intensities of prepared microcrystals were increased to nearly two fold than that of pure Lercanidipine (fig no 10). This could be attributed to the markedly different crystal habits of the samples. The X - ray diffraction pattern of the microcrystals showed that Lercanidipine peak intensity was much higher than the pure drug. This could be due to the increase in crystalline nature of prepared microcrystals. Morphology and appearance of the drug and microcrystals were examined by Ultra - 55 Carl Zeiss Field Emission Scanning Electron Microscope. As seen from the photograph (fig no 9), the crystals were rectangular and square in appearance. The DSC curve showed that Lercanidipine HCl appeared an endothermic peak at about 176.85^{0}C corresponding to its melting (fig no 12). However, the crystals prepared with poloxamer 407 (LM - I) and PVP K30 (LM - II) shows shift of endothermic peak towards lower temperature at 113.23^{0}C and 73.00^{0}C respectively. Shift of the endothermic peak towards lower temperature dictates decreased melting point of the drug in the formed crystals. This decreased melting point accounts for increased solubility of the drugs.

FT - IR and Stability studies:
The FTIR studies indicated that there is no strong interaction at molecular level when compared formulations to pure drug as shown in table no 2 (spectrum shown in fig no 1, 2, and 3). Stability studies were performed as per ICH guidelines. The results indicated that there was no evident change in the physical appearance of formulations at the end of the 1 month storage period at 5°C / ambient, 25°C / 60% RH & 40°C / 75% RH conditions. The drug content of LM - I and LM - II at the end of 30 days for 50 ambient was 99.01% and 99.71% respectively, for 25° C/ 60% RH was 99.06 and 99.76 respectively, for 40° C/ 75% RH was 99.05 and 99.75% respectively (table no 7).

5. Conclusion

Microcrystals of Lercanidipine HCl were prepared by solvent change technique. From the above discussion it has been concluded that the prepared Microcrystals of Lercanidipine HCl exhibited good solubility, dissolution rate and physicochemical properties in comparison to that of the pure drug. The FTIR studies indicated that there is no strong interaction at molecular level. PXRD studies revealed changes in the crystalline structure of formulated microcrystals leading to enhanced solubility of the drug sample. DSC results clearly showed that changes in melting characteristics of formulated crystals helps in the solubilization of drugs. Hence microcrystals of the drug can be filled in capsules or formulated as tablets of Lercanidipine by direct compression in order to achieve enhanced solubility and improved bioavailability of the drug.

Acknowledgements:
I am thankful to Apotex research Pvt Ltd, Bengaluru for providing gift sample of Lercanidipine HCl, PVP K30, and Poloxamer 407. I am thankful to our principal, Karavali College of Pharmacy for permitting to avail the research facilities in the college.

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References

[1] Lipinski C., Poor aqueous solubility – an industry wide problem in drug discovery, Americ. Pharm. Rev., 2002, 5, 82 - 85.
[2] E. L. Parrott, Comminution, in: J. Swarbrick, J. C. Boylan (Eds.), Encyclopedia of Pharmaceutical Technology, vol.3, Marcel Decker, New York, 1990, pp.101–121.
[3] Raghavan, S. L., Trividic, A., Davis, A. F., Hadgraft, J., 2001. Crystallization of hydrocortisone acetate: influence of polymers. Int. J. Pharm.212, 213–221.
[4] Leuner, C., Dressman, J., 2000. Improving drug solubility for oral delivery using solid dispersions. Eur. J. Pharm. Biopharm.50, 47–60.
[5] Goddeeris C., Willems T., Mooter G. V. D., 2008. Formulation of fast disintegrating tablets of ternary solid dispersions consisting of TPGS 1000 and HPMC 2910 or PVPVA 64 to improve the dissolution of the anti - HIV drug UC 871. Eur. J. Pharm. Sci.34, 293–302.
[6] Mosharraf, M., Nyatrom, C., 1995. The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. Int. J. Pharm.122, 35– 47.
[7] Lechuga - Ballesteros, N. Rodriguez - Hornedo, 1995. The influence of additives on the growth kinetics and mechanism of L - alanine crystals. Int. J. Pharm.115, 139–149.
[8] Lechuga - Ballesteros, N. Rodriguez - Hornedo, 1995. Effects of molecular structure and growth kinetics on the morphology of L - alanine crystals. Int. J. Pharm.115, 151–160.
[9] Gasser R, Klein W, Köppel H. Lercanidipine: A new third generation Ca - antagonist in the treatment of hypertension. Journal of Clinical and Basic Cardiology.1999; 2 (2): 169 - 174
[10] J. Varshosaza, R. Talaria, SA. Mostafavia, A. Nokhodchic. Dissolution enhancement of gliclazide using in situ micronization by solvent change method. Science direst. http: //dx. doi. org/10.1016/j. powtec.2008.02.018
[11] Yadav V, Yadav A. Directly Compressible RoxithromycinRecrystalized Agglomerates by Solvent Change Technique. Der Pharmacia letter 2010; 2 (1): 25 - 40.
[12] Nighute AB, Bhise SB. Enhancement of Dissolution rate of Rifabutin by preparation of Microcrystals using Solvent Change Method. Int J PharmTech Res.2009; 1 (2); 142 - 148
[13] NagobaShivappa N, Rao KP, Nagoba BS and Kasture RM. Studies on Piroxicam Microcrystals for Improved Drug Therapy. Int J Res Pharma and Bio Sci.2011; 2 (3).
[14] Flavia PiresMaximiano, Livia Maria de Paula, Vivian PaulinoFigueiredo, Isabel Mayer de Andrade, Andre Talvani, Livia. Sa - Barreto, Maria Terezinha Bahia, Marcilio S. S. CunhaFilho. Benznidazole microcrystal preparation by solvent change precipitation and in vivo evaluation in the treatment of Chagas disease. Eur J Pharma Biopharma.2011; 78: 377–384.
[15] ICH Harmonized Tripartite Guidelines, 2003. Stability testing of New Drug Substances and Products. Q1A (R2).

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