Formulation and Characterization of Lercanidipine Loaded Nanoparticles

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Abstract-

Aim of the present study was to investigate the potential of polymeric nanoparticles in improving the biopharmaceutical properties of lercanidipine. Lercanidipine loaded nanoparticles were prepared by modified emulsion-diffusion-solvent evaporation technique. The resulting nanoparticles were freeze dried and then characterized for particle size, zeta potential, encapsulation efficiency, DSC, XRD, AFM, TEM and *in vitro* drug release. Amongst all the screened batches, optimized batch (B. No. PN-05) exhibited a particle size of 200.2 nm, PDI of 0.130, zeta potential, -20.6 mV and encapsulation efficiency of 77.41%. The AFM analysis illustrated spherical, non-aggregated, smooth particles whereas TEM images showed smooth, regularly spherical homogeneous mass of the particles. DSC studies suggested amorphization of the drug in nanoparticles and same was also observed in XRD diffractograms as reflected by the amorphous humps. The optimized batch exhibited a significantly different drug release profile from that of lercanidipine. It showed an initial rapid release of 17.96% in 1 h and followed an extended release profile releasing 82.74% in 24 h. The novel polymeric lercanidipine nanoparticles demonstrated 2.12-fold increase in absolute bioavailability as compared to free lercanidipine. In conclusion, encapsulation of lercanidipine in nanoparticles forms a sound basis for improving its bioavailability and for better management of hypertension.

1.0 INTRODUCTION

The pharmaceutical industry is one of the fastest growing sectors in terms of its size and revenue and has continued to exercise its tremendous sphere of influence for as long as mankind continue to exist. In spite of this apparent certainty, the industry is facing unprecedented level of challenges like, waning pipeline, looming patent expiries, increased generic competition, stringent regulations, amplified development costs, slashed public-health care budgets etc. which are threatening to crumble even the largest pharmaceutical industries.

To accommodate these dynamic challenges the pharmaceutical industry is continuously retooling their research efforts by adopting new delivery systems to rescue failed compounds due to their poor biopharmaceutical properties, and to extend patent lives through innovative repositioning and reformulation Colloidal drug delivery systems in the last few decades have provided the pharmaceutical industry with alternative formulation approaches for exigent molecules. Various colloids including nanoparticles, polymeric micelles, lipid based deliveries (liposomes, solid lipid nanoparticles, proliposomes and microemulsions) have been developed as carriers for encapsulation of both hydrophilic and hydrophobic

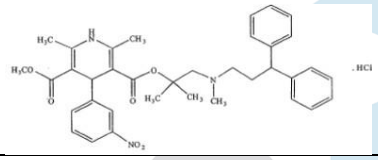
drugs Nanoparticles are exciting class of colloidal carriers shown to offer great potential for peroral drug administration. By definition, nanoparticles are solid, colloidal particles ranging in the size between 10-1000 nano-meters. The therapeutic agent of interest can be dissolved, encapsulated, absorbed or conjugated onto the surface of nanoparticles The nanoparticles also offer multifaceted advantages such as, 1) provides high stability by protecting drug against pH and enzymatic degradation, 2) improves oral bioavailability of the poorly water soluble drugs, 3) reduction in particle size and increased surface area enhances the solubility of encapsulated drugs, 4) high carrier capacity, 5) feasibility of incorporation of both hydrophilic and hydrophobic drugs, 6) control/sustain the drug release from the matrix and, 7) avoids first-pass metabolism through specialized uptake mechanisms. Overall, these properties of the nanoparticles increase therapeutic efficacy, reduce toxicity and thereby improves the patient compliance and convenience. Importantly nanoparticulate systems are utilized to overcome the limitations associated with poorly soluble compounds. These compounds have reasonable membrane permeability and possess dissolution as the rate limiting step for their absorption, resulting in poor bioavailability. The nanoparticles because of their nano-meter size range exhibits high surface area to mass ratio which increases the dissolution velocity according to Noye's-Whitney equation. The uptake process of orally delivered nanoparticles is discussed first from a recent historical perspective, emphasising on size and nature of the particles . Predominantly, GI absorption of nanoparticles delivered through oral route occurs with three possible mechanisms, 1) transcellular uptake, 2) paracellular uptake, and mainly 3) uptake via membranous epithelial cells (M-cells) of Payer's patches in the gut associated lymphoid tissue. Transcellular transport of nanoparticles occurs by transcytosis, in which particles are taken up by cells. Transport of nanoparticles by this pathway depends upon the physicochemical properties like, size, charge on particles and hydrophobicity of surface of the particles as well as the GI physiology . Paracellular transport involves crossing of particles between the adjacent intestinal cells. Many of the surfactants enhance the absorption of poorly soluble drugs through. Most of the literatures reiterate that nanoparticles uptake occurs by intestinal lymphatic tissues, i.e. membranous epithelial cells (M-cells) of Payer's patches. Here, particles localize through apical surfaces of M-cells and then gets internalized through these cells . The nanoparticle uptake through this mechanism is also governed by influencing factors such as, particle size and balance between hydrophobicity and hydrophilicity of the particles. It is generally adopted that nanoparticles anywhere less than one micron size are taken up by the M- cells of payer's patches.

Lercanidipine (LER) is a dihydropyridine calcium antagonist which selectively inhibits influx of calcium ions through L-type calcium channels present in the cardiac and vascular smooth muscle cells. Orally administered lercanidipine is erratically absorbed with peak plasma concentration occurring in 1.5 to 3 h after administration with a plasma half-life of about 2 to 5 h. Lercanidipine exhibits absolute bioavailability of only 10% due to its extensive and saturable first-pass metabolism. Further administration of lercanidipine along with food increases the absorption and hence the bioavailability. The food dependence dosing of lercanidipine is highly undesirable and can result in dose fluctuations, ineffectiveness, larger inter-patient

variability and consequent patient compliance problems.

2.0 Drug Profile

Lercanidipine Hydrochloride

Name	Lercanidipine Hydrochloride
CAS Registry Number	132866-11-6
Appearance	Lercanidipine HCl (crystalline form) is a yellow powder soluble in methanol and practically insoluble in water.
Description	Lercanidipine is a dihydropyridine derivative. It is a racemate due to the presence of a chiral carbon atom at position 4 of the 1, 4-dihydropyridine ring.
Structural Formula	
Chemical Name	3, 5-pyridinedicarboxylic acid, 1, 4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-2-[(3,3-diphenylpropyl)methylamino]-1,1-dimethylethyl methyl ester hydrochloride
Molecular Formula	C36H41N3O6 .HCl
Molecular Weight	648.19 g/mol
Log P Value	6.42
Indications	Lercanidpine HCl is indicated for the treatment of mild to moderate essential hypertension.
Mechanism of Action	It is a calcium antagonist of the dihydropyridine group and inhibits the transmembrane influx of calcium into cardiac and smooth muscles. The mechanism of its antihypertensive action is due to a direct relaxant effect on vascular smooth muscle thus lowering total peripheral resistance.
Pharmacokinetics	Lercanidipine is completely absorbed from the gastrointestinal tract after oral doses. Due to the high first- pass metabolism, the absolute bioavailability of orally administered drug under fed conditions is around 10%. Oral availability of lercanidipine increases 4-fold when it is ingested up to 2 h after a high fat meal. Its distribution is from plasma to tissues and organs is rapid and extensive. The degree of serum protein binding of lercanidipine exceeds 98%. Lercanidipine HCl is extensively metabolised by CYP3A4. A mean terminal elimination half life of Lercanidipine HCl is 8-10 h. Oral administration of Lercanidipine HCl leads to plasma levels of lercanidipine not directly proportional to dosage (non-linear kinetics).
Posology and Method of Administration	The recommended dosage is 10 mg orally once a day at least 15 minutes before meals; the dose may be increased to 20 mg depending on the individual patient's response.
Commercially Available Dosage Form	Film coated tablets
Common Strengths	10 nd 20 mg

3.0 EXPERIMENTAL WORK

3.1 Chemicals and Reagents

Lercanidipine Hydrochloride, 3, 5-pyridinedicarboxylic acid, 1, 4-dihydro-2, 6-dimethyl- 4-(3-nitrophenyl)-2-[(3,3-diphenylpropyl)methylamino]-1,1-dimethylethyl methyl ester hydrochloride was a generous gift from Cipla Ltd., Mumbai, India. Poly (D, L-lactide- co-glycolide), acid terminated, (PLGA 50:50), (molecular weight 24,000-38,000), was purchased from Sigma-Aldrich, Co, St. Louis, MO, USA. Poly (ethyl acrylate-co-methyl methacrylate-co-trimethylammonioethyl methacrylate chloride) (Eudragit® RL PO) was procured from Evonik Degussa India Pvt. Ltd., Mumbai, India. Methyl vinyl ether- maleic anhydride copolymer (Gantrez® AN-119BF) was a kind gift provided by ISP (India) Pvt. Ltd., Ashland Specialty Ingredients, Mumbai, India. Poly (vinyl alcohol), 87- 90% hydrolyzed (average molecular weight 30000-70000) was purchased from Sigma- Aldrich, Inc., St. Louis, MO, USA and D-Mannitol was purchased from HiMedia Laboratories Ltd., Mumbai, India. Ethyl Methyl Ketone was procured from Nice Chemicals Pvt. Ltd., Cochin, India. HPLC grade solvents such as Dichloromethane, Acetonitrile and Methanol were obtained from Merck Specialties Pvt. Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade. HPLC grade water was obtained from Merck Specialties Pvt. Ltd., Mumbai, India.

3.2 HPLC Analysis of Lercanidipine

A specific, sensitive and validated reverse phase high performance liquid chromatography (RP-HPLC) method was used for quantification of lercanidipine in Nanoparticles. HPLC system, LC-2010CHT (Shimadzu Corporation, Kyoto, Japan) equipped with low pressure quaternary gradient pump along with dual wavelength ultra violet (UV)/ visible detector, column oven and auto sampler was used for the analysis. Chromatographic data was processed using LC solution, version 1.24 SP1 software. Phenomenex® Gemini C_{18} (250 × 4.6 mm, 5µ) column was used for the estimation of lercanidipine. The mobile phase consisted of acetonitrile and 25mM potassium dihydrogen phosphate (KH_2PO_4) buffer (pH 3.5 ± 0.2 adjusted with ortho phosphoric acid) with a volumetric ratio of 70:30 at a flow rate of 1.0 mL/min. The column temperature was maintained at 25 °C and the samples were loaded at 4 ± 2 °C. The injection volume was 25 µ L. Effluent was monitored by UV/visible detector at 242 nm. The calibration curve was generated for concentrations ranging from 0.5 to 25.0 µg/mL.

3.3 Preformulation Studies

Preformulation is a primary tool in the formulation development stage which is helpful in designing quality pharmaceutical products. Methodically planned preformulation studies are essential to predict the deficiencies of the drug product as well as to forecast the compatibility with different excipients that are used during the formulation development. Preformulation studies significantly reduce the failures that may arise in the advanced stages of formulation development (Wen and Park, 2010).

3.4 Drug-excipient compatibility studies

The choice of excipients used in the lercanidipine nanoparticles formulation was based on drug-excipient compatibility studies. Lercanidipine was triturated in a mortar with individual excipients based on their customized ratio and then they were transferred into USP Type-1 flint glass vials hermetically sealed with bromobutyl rubber stopper and crimped with an aluminium crimper. The closed vials were charged into stability chamber at 25 ± 2 °C/60 ± 5% RH for 4 weeks time period. The control samples were kept at 5 ± 3 °C. After the study period, observation was done for the change in physical attributes. The selected sets of samples in combination with the drug were also screened for solid-state analysis using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) studies as shown in Table 6.1.

S.No.	Sample
1	Lercanidipine
2	Lercanidipine + PLGA 50:50 + PVA + Mannitol (LPPM)
3	Lercanidipine + Eudragit RLPO + PVA + Mannitol (LEPM)
4	Lercanidipine + Gantrez AN-119BF + PVA + Mannitol (LGPM)

Table 3.1 Samples analyzed for drug-excipient compatibility studies by FTIR and DSC

3.5 Determination of solubility profile

Solubility of lercanidipine was evaluated in water and in different buffers of pH ranging from 1.2 to 7.4 by the shake flask method (Pathak et al., 2010). An excess amount of lercanidipine was added to 20 mL vial containing 5 mL of respective aqueous medium. These vials were incubated in Orbital Shaking Incubator (Labtop Instruments Pvt. Ltd., Thane, India) at 37 ± 2 °C at 150 rpm. The samples were withdrawn after 24 h and centrifuged at 10,000 rpm for 10 min to separate the undissolved solid mass from the saturated solution. The obtained supernatant was filtered through 0.22 µm membrane filter (Durapore® PVDF Membrane Filter, Millipore, Billerica, MA, USA) and analyzed by HPLC after appropriate dilution. The study was performed in triplicate.

3.6 FORMULATION DEVELOPMENT

Polymeric nanoparticles have been widely explored in recent years for their application in drug delivery. These show capabilities to accommodate all type of molecules and delivering them through a sustained and/or controlled fashion thereby enhancing the patient compliance (Pinto et al., 2006). Several investigations have been reported previously pertaining to utility of polymeric nanoparticles for oral drug delivery. In the present study, lercanidipine was encapsulated into polymeric nanoparticles with an aim to improve its delivery efficacy. Different polymers were screened for providing appropriate encapsulation of lercanidipine in the formulation process. The nanoparticles were also added with suitable stabilizer to avoid

aggregation on standing. Finally, lercanidipine nanoparticles were freeze dried to improve their long term stability as well as their elegance in achieving oral drug delivery.

3.6.1Preparation of Lercanidipine Nanoparticles

- **Modified emulsion-diffusion-solvent evaporation method**

PLGA, Eudragit and Gantrez loaded nanoparticles were produced by modified emulsion- diffusion-solvent evaporation method (Sahana et al., 2008; G´omez-Gaete et al., 2007). In brief, lercanidipine and polymer were separately dissolved in organic solvent and were homogenized at 15000 rpm using high speed homogenizer (Polytron® PT 3100, Kinematica AG, Lucerne, Switzerland) for 15 min. The obtained organic phase containing drug and polymer were subjected to probe sonication (Sonics-Vibra-Cell™, Sonics and Materials Inc., CT., USA) at 60 amplitude for 6 min with 2 sec pulsar intermittently under cooling conditions. The sonicated pre-emulsion was then added to 30 mL of an aqueous phase containing 1% w/v stabilizer and subsequently homogenized at 15000 rpm for 20 min. The resulting o/w emulsion was placed on ice bath and again probe sonicated at 80 amplitude for 8 min with 2 sec pulsar intermittently. The organic phase of the preparation was evaporated with moderate magnetic stirring at room temperature and the resultant nanoparticles were further subjected to centrifugation step.

- **Separation of nanoparticles by centrifugation step**

The nanoparticles were separated by two-step centrifugation process by refrigerated centrifuge unit (Sigma® 3K30, Laboratory Centrifuges, Germany). Micron sized particles and unentrapped drug was initially separated at a low-spin of 5000 rpm for 10 min, followed by recentrifugation of obtained supernatant at 20000 rpm for 30 min. The supernatant was decanted from the pellet and subjected to freeze drying.

- **6.5.4 Freeze drying of nanoparticles**

The obtained nanoparticulate pellet was freeze dried with the aid of cryoprotective agent (10% w/v) prepared in MilliQ water. The solution was filtered through 0.20 µm single use non-pyrogenic filter unit and combined with nanoparticle pellet under constant vortexing. The sample was filled into glass vials, bath sonicated for 15 min and kept for deep freezing (Sanyo Ultra Low Temperature Freezer LV, Sanyo Electric Biomedical Co., Ltd., Japan) at -80 ˚C for about 6 h and then subjected to freeze drying. For freeze drying process, Labtech® Freeze Dryer, LFD-5508, Daihan Labtech Co. Ltd., Korea, was used. The temperature of the freeze dryer was maintained in the range of -40 to -50 ºC and vacuum below 50 mTorr. The deep freezed vials containing sample were fitted inside the 600 mL wide neck filter bottles (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) stoppered with rubber caps. The process of freeze drying was carried out for atleast 48 h to get a free-flowing nanoparticle powder. The product was stored in a refrigerator in airtight glass container sealed with parafilm around the container rims to ensure a tight seal until further experiments.

In the process of preparation, dichloromethane was used as organic phase for PLGA and Eudragit nanoparticles whereas ethyl methyl ketone was used for Gantrez nanoparticles.

3.5 FORMULATION OPTIMIZATION

In order to achieve desired properties in the final form of the LER nanoparticles, a different set of formulation optimization experiments were carried out. Initially, nanoparticles were prepared by using different type of polymers such as PLGA 50:50, Eudragit RLPO and Gantrez AN-119BF. The polymers were varied for different concentration levels with respect to the drug in the composition. Table 5.2 indicates Drug: Polymer weight ratio details used in the development of lercanidipine nanoparticles. Several stabilizers like PVA, Pluronic F-68, Pluronic F-127 and Sodium Cholate were screened for their control on physicochemical properties of the nanoparticles. The influence of homogenization speed and sonication parameters on the size reduction of the formulation was also investigated and final homogenization speed and sonication conditions were optimized. Various cryoprotectants *viz.* sucrose, fructose, dextrose, mannitol and trehalose along with suitable concentration was tested for their effect on freeze drying process of lercanidipine nanoparticles. Finally, based on the ideal physicochemical properties and drug release profiles best possible lercanidipine nanoparticles was selected and further characterized.

Drug:Polymer weight ratio				
S.no.	**B. No.**	**LER:PLGA 50:50**	**LER:Eudragit RLPO**	**LER:Gantrez AN-119BF**
1	PN-01	1:05	-	-
2	PN-02	1:10	-	-
3	PN-03	1:20	-	-
4	PN-04	1:30	-	-
5	PN-05	1:40	-	-
6	PN-06	1:50	-	-
7	PN-07	1:60	-	-
8	PN-08	-	1:05	-
9	PN-09	-	1:10	-
10	PN-10	-	1:20	-
11	PN-11	-	1:30	-
12	PN-12	-	1:40	-
13	PN-13	-	1:50	-

14	PN-14	-	1:60	-
15	PN-15	-	-	1:05
16	PN-16	-	-	1:10
17	PN-17	-	-	1:20
18	PN-18	-	-	1:30
19	PN-19	-	-	1:40
20	PN-20	-	-	1:50
21	PN-21	-	-	1:60

Lercanidipine amount used – 10.0 mg; Stabilizer amount used – 30.0 mL of 1.0% w/v solution; Cryoprotectant amount used – 10.0 mL of 10.0% w/v solution.

3.6 FORMULATION CHARACTERIZATION

3.6.1 Measurement of Size, Polydispersity Index and Zeta Potential

Particle size, polydispersity index (PDI) and zeta potential of the lercanidipine loaded nanoparticles were analyzed using Zetasizer Nano ZS, ZEN 3601 (Malvern Instruments Ltd., UK) instrument as described in section 4.5.1. The particle size was measured using Dynamic Light Scattering (DLS) technique and zeta potential using Laser Doppler Micro-electrophoresis principles. All the measurements were carried out in triplicate by dissolving nanoparticles in appropriate volume of MilliQ water (Refractive Index-1.330 and Viscosity-0.887). The sample was filled into disposable folded capillary cell and measured in manual mode at 25 ˚C.

✓ **7.1.2 Determination of Drug Encapsulation Efficiency**

The percentage encapsulation efficiency of lercanidipine in nanoparticles was estimated using developed HPLC method. Based on the polymers employed in the preparation of nanoparticles, different solvent systems were used to extract the drug from nanoparticles as given below:

- ✓ **PLGA nanoparticles:** 1 mg of freeze dried nanoparticles was dissolved in 1 mL of solvent mixture of acetonitrile: methanol (1:1 ratio) and vortexed for 10 min. The mixture was centrifuged at 10000 rpm for 10 min at 4 ˚C and supernatant was carefully separated and analyzed by HPLC method.
- ✓ **Eudragit and Gantrez nanoparticles:** 1 mg of freeze dried nanoparticles was dissolved in 1 mL of dichloromethane for Eudragit nanoparticles whereas for Gantrez nanoparticles 1 mg of formulation was dissolved in 1 mL in ethyl methyl ketone. The mixture was vigorously vortexed for 10 min and centrifuged at 10000 rpm for 10 min at 4 ˚C. The supernatant solvent obtained was evaporated using stream of nitrogen in a

Turbo-Vap evaporator (Zymark, Hopkinton, MA, USA) for 10 min at 50 °C to obtain thin solid film at the bottom of a nitrogen evaporator tube. The solid film was reconstituted with 1 mL of mobile phase mixture and vortexed for 5 min to ensure its complete dissolution in mobile phase. Subsequently, the reconstituted solution was pipetted into 1.5 mL of eppendorf centrifuge tube and centrifuged at 10000 rpm for 10 min at 4 °C. The supernatant was carefully separated and injected into the HPLC system. The percent encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation Efficiency}\,(\%) = \frac{\text{Actual mass of lercanidipine determined (mg)}}{\text{Theoretical mass of lercanidipine loaded (mg)}} \text{x}100$$

✓ ***In vitro* Drug Release Studies**

The release of lercanidipine from nanoparticles was determined using USP type-I dissolution test apparatus (TDT-08L, Electrolab, Mumbai, India) at a stirring speed of 75 rpm and the dissolution medium used was 900 mL of 0.01 N HCl. The temperature of the medium was thermostated at 37 ± 0.5 °C. Freeze dried lercanidipine nanoparticulate powder equivalent to 10 mg of lercanidipine (calculated based on % EE) was added to each basket. At selected time intervals, 5 mL of the sample was withdrawn and same amount was replaced with fresh 0.01 N HCl medium. The test samples were filtered through 0.22 µm membrane filter and the amount of lercanidipine released was determined using HPLC method as stated in the section 4.2.2. The drug release profile of lercanidipine nanoparticles was compared with that of pure drug results already reported in section 4.6.4. The experiment was carried out in triplicate and the mean of three samples was used for the data analysis.

✓ **Transmission Electron Microscopy Analysis**

The size, shape and relevant surface properties of the prepared lercanidipine nanoparticles was evaluated by Transmission Electron Microscopy (TEM) H-7650 (Hitachi High-Technologies Instrumentation, Tokyo, Japan). The procedure followed for sample mounting was similar to that described in section 4.5.5. The images were recorded digitally using high sensitivity integrated computer controlled digital camera.

4.0 RESULTS AND DISCUSSION

4.1 Drug-Excipient Compatibility Studies

The purpose of the drug-excipient compatibility studies was to understand any possible interaction between lercanidipine and other formulation components *viz.* polymer, stabilizer and cryoprotectant used in the development of lercanidipine nanoparticles. In the process, lercanidipine was triturated in a mortar with individual excipients based on their customized ratio and assessed for their physical observation as well as their interaction using FTIR and DSC studies.

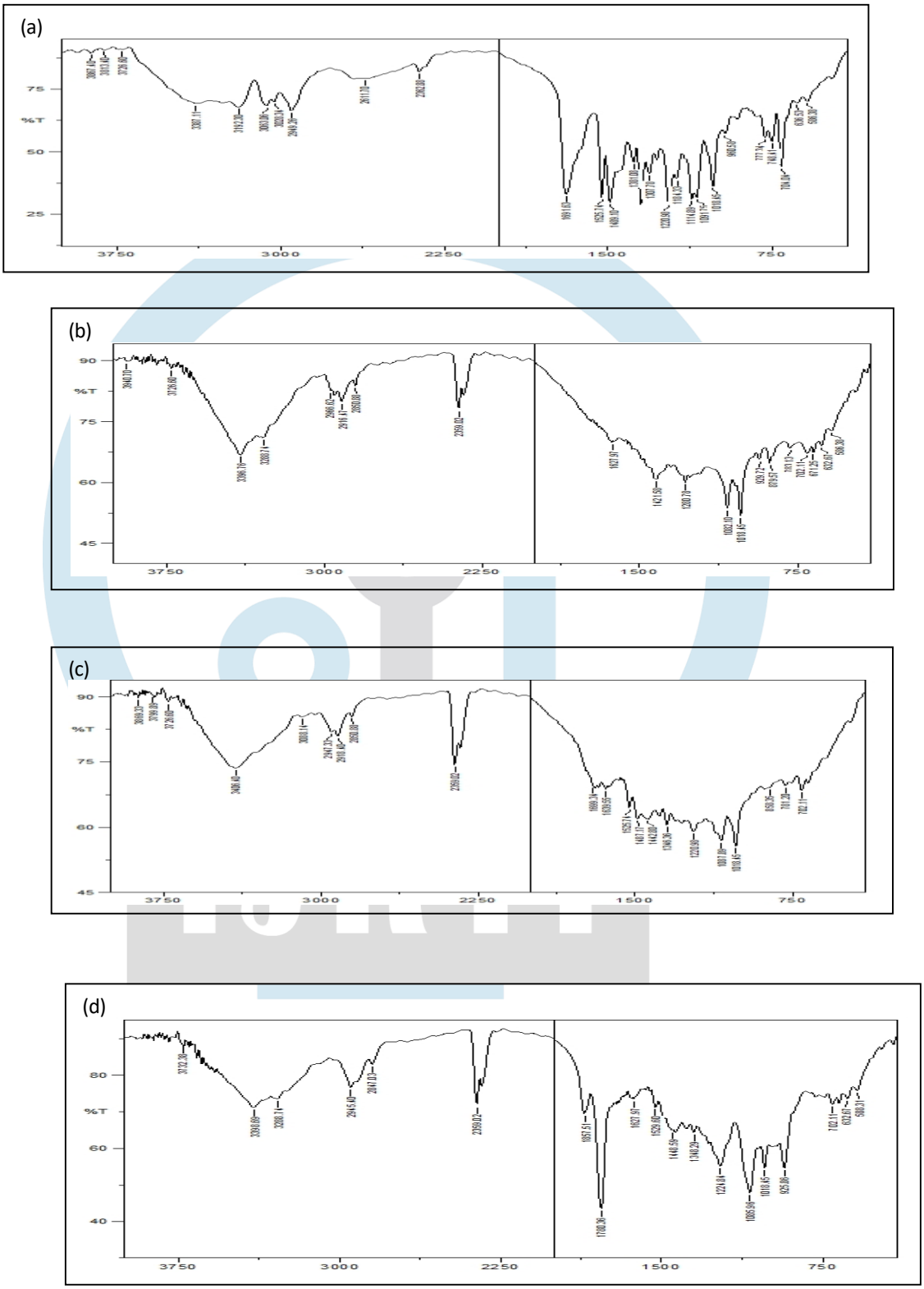


Fig. 4.1. FTIR spectra of pure drug, Lercanidipine (a), physical mixture of Lercanidipine + PLGA 50:50 + PVA + Mannitol (LPPM) (b), Lercanidipine + Eudragit RLPO + PVA+ Mannitol (LEPM) (c), Lercanidipine + Gantrez AN-119BF + PVA + Mannitol (LGPM) (d).

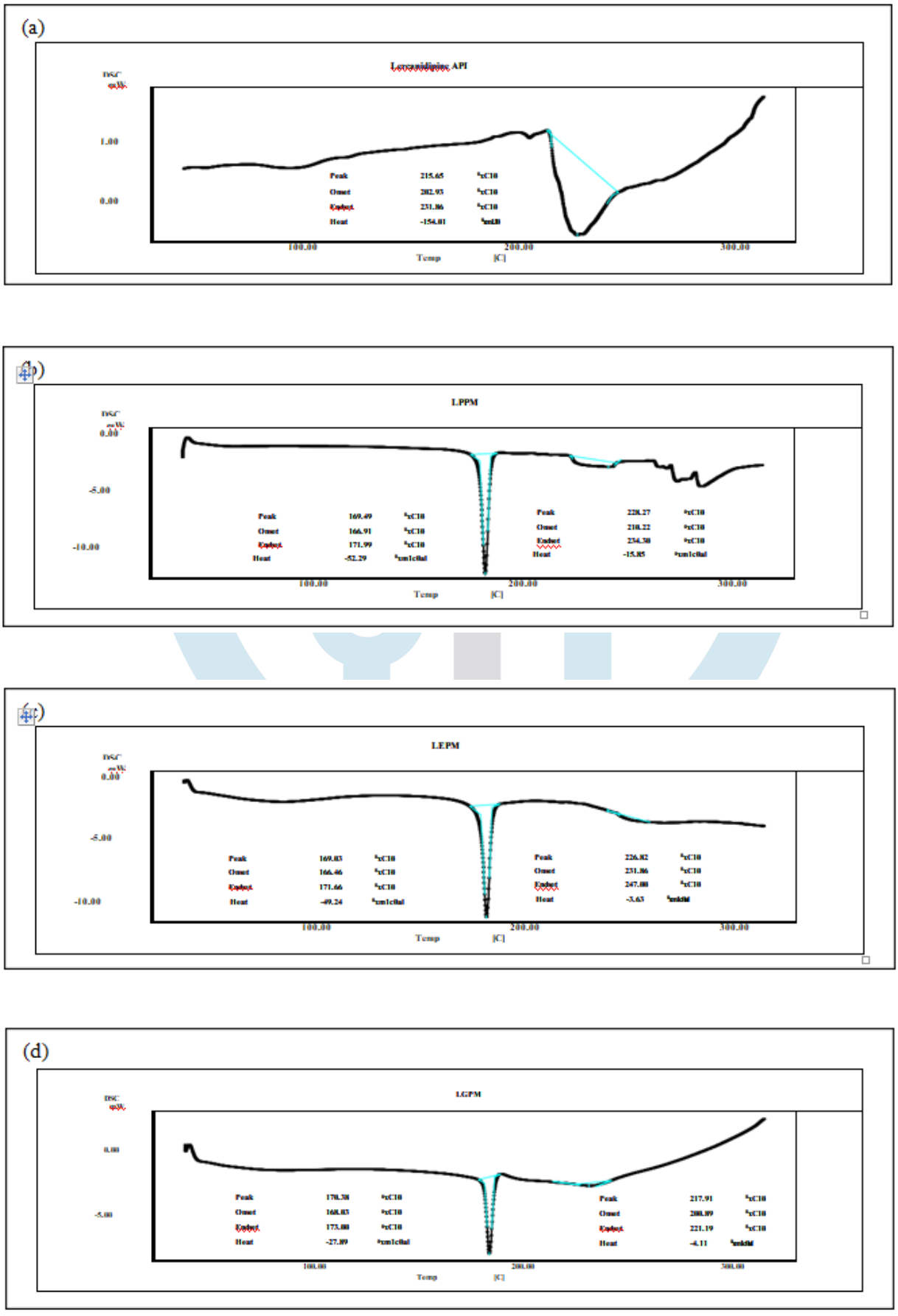


Fig. 4.2. DSC thermograms of pure drug, Lercanidipine (a), physical mixture of Lercanidipine + PLGA 50:50 + PVA + Mannitol (LPPM) (b), Lercanidipine + Eudragit RLPO + PVA + Mannitol (LEPM) (c), Lercanidipine + Gantrez AN-119BF + PVA + Mannitol (LGPM) (d).

4.2 Formulation, Optimization and Determination of Particle Size, PDI, Zeta Potential and % Encapsulation Efficiency of Lercanidipine Nanoparticles

S.No.	B. No.	Particle size (nm; Mean ± SD)	PDI	Zeta potential (mV)	%EE
1	PN-01	722.2 ± 4.31	0.571 ± 0.52	-27.3 ± 4.06	16.79 ± 1.19
2	PN-02	338.6 ± 1.04	0.388 ± 0.04	-16.4 ± 3.98	14.95 ± 1.61
3	PN-03	194.4 ± 2.16	0.085 ± 0.08	-20.5 ± 2.13	38.35 ± 3.48
4	PN-04	185.7 ± 0.80	0.100 ± 0.53	-25.7 ± 1.61	52.42 ± 0.66
5	PN-05	200.2 ± 2.73	0.130 ± 0.07	-20.6 ± 2.07	77.41 ± 1.54
6	PN-06	325.8 ± 4.47	0.265 ± 0.15	-13.6 ± 3.89	73.57 ± 3.89
7	PN-07	729.6 ± 1.08	0.649 ± 0.09	-23.2 ± 5.42	82.62 ± 1.12
8	PN-08	122.8 ± 2.51	0.157 ± 0.02	48.0 ± 2.96	14.48 ± 5.04
9	PN-09	212.6 ± 1.55	0.246 ± 0.36	52.9 ± 1.89	17.55 ± 3.80
10	PN-10	195.9 ± 4.18	0.190 ± 0.74	58.9 ± 2.67	21.51 ± 3.97
11	PN-11	218.8 ± 2.00	0.234 ± 0.65	66.4 ± 1.67	28.06 ± 1.28
12	PN-12	223.6 ± 4.04	0.185 ± 0.58	64.4 ± 3.83	51.37 ± 2.79
13	PN-13	186.6 ±2.23	0.219 ± 0.19	52.4 ± 6.20	54.36 ± 3.17
14	PN-14	247.8 ± 2.82	0.479 ± 0.21	56.3 ± 3.33	57.90 ± 2.19
15	PN-15	1474 ± 16.09	0.828 ± 0.08	-52.1 ± 2.08	18.48 ± 6.08
16	PN-16	390.3 ± 10.98	0.695 ± 0.54	-30.1 ± 2.92	20.10 ± 9.05
17	PN-17	319.9 ± 3.42	0.249 ± 0.09	-54.3 ± 4.38	23.17 ± 6.45
18	PN-18	347.6 ± 7.65	0.673 ± 0.31	-49.1 ± 1.15	35.28 ± 2.23
19	PN-19	305.6 ± 4.59	0.407 ± 0.30	-48.5 ± 5.93	57.18 ± 1.67
20	PN-20	891.0 ± 14.13	0.348 ± 0.06	-51.3 ± 3.16	66.93 ± 2.38
21	PN-21	1132.0 ± 9.23	0.778 ± 0.23	-39.0 ± 4.54	78.34 ± 3.84

All values reported are mean ± S.D. (*n* = 3).

Table 4.4 Particle size, PDI, zeta potential and % encapsulation efficiency details of lercanidipine loaded nanoparticles.

***4.5 In Vitro* Drug Release Studies**

Polymeric nanoparticles have been widely used to encapsulate hydrophobic drugs and used to deliver them for a prolonged period of time to reduce fluctuations in plasma concentrations, to improve therapeutic benefit and overall patient compliance (Swami et al., 2012). In this facet testing of drug release by *in vitro* methods is an excellent tool which provides predictive estimation of the drug performance *in vivo* (Nainar et al., 2012). Therefore, in the present study, drug release behaviour of lercanidipine and lercanidipine loaded nanoparticles was evaluated using USP type-I basket method and monitored over a period of 24 h.

As illustrated in Fig. 7.11, batches were selected from all the three polymers based on their physicochemical properties and compared with pure drug. As shown in Fig. 7.11a, pure drug was rapidly released (71.16%) in 1 h and later it began to plateau and showed a staggered release up to 24 h. In case of PLGA based nanoparticles, B. No. PN-04 exhibited faster release profile releasing almost 29% in 1 h and 79% within in 12 h. Further, as the polymer concentration was increased, B. No. PN- 05 to PN-07 showed slower release profile compared to B. No. PN-04. The release rate of B. No. PN-05 was significantly different ($p < 0.05$) from that of lercanidipine. It showed an initial rapid release of 17.96% in 1 h and then followed an extended release profile releasing 82.74% in 24 h as shown in Fig. 5.11b. However, B. No. PN-06 and PN-07 exhibited incomplete release of 68% and 41.39% indicating the role of higher concentration of polymer. Out of selected batches of Eudragit based nanoparticles (Fig. 7.11c), B. No. PN-12 showed a rapid release of 44.13% within 1 h whereas B. No. PN- 13 showed sustained fashion of % CDR of 98.73% in 24 h. B. No. PN-14 on the other hand exhibited sustained but incomplete % CDR of 77.38 in 24 h. The batches prepared with Gantrez nanoparticles (Fig. 7.11d) exhibited almost slower release profile compared to other two polymers. B. No. PN-18 showed 71.31% in 24 h whereas, B. No. PN-19 showed similar drug release profile as that of B. No. PN-18 up to 2 h and showed more prolonged release profile releasing 73.58% in 24 h.

Amongst the above mentioned batches, B. No's. PN-05, PN-13 and PN-19 illustrated initial rapid release which may be due to non-encapsulated or weakly bound drug to the large surface area of the nanoparticles. These formulations also gave an incremental drug release values up to 24 h without reaching plateau conditions as observed in case of pure drug. Here, the fact is that, nano-meter particle size must have played a pronounced role in improving the solubility of lercanidipine and hence the drug release was almost complete when it was incorporated in nanoparticles. Apart from this, extended release of the drug from nanoparticles may be explained by several mechanisms such as, drug diffusion through polymer matrix, nanoparticle-polymer matrix erosion and/or degradation and/or combination of these processes. The polymer matrix degradation must have played a significant role in the release of the encapsulated drug from nanoparticles (Mohanraj and Chen, 2006; Italia et al., 2007; Shavi et al., 2011). Further, amongst all the polymers used, nanoparticles prepared with PLGA displayed better release properties as compared to Eudragit and Gantrez based nanoparticles. PLGA nanoparticles after initial burst release of 17.96% within 1 h showed a slow and incremental order of drug release whereas Eudragit nanoparticles released 78.48% within

12 h. Gantrez based nanoparticles exhibited a slower release profile releasing only 73.58% even after 24 h as compared to PLGA and Eudragit nanoparticles. Therefore, based on these results, PLGA based lercanidipine nanoparticles demonstrated better extended release profile compared to Eudragit and Gantrez based nanoparticles. Similar to the present study, Italia et al. (2007) discussed the PLGA polymer degradation and its release mechanism for the entrapped drugs within it. They observed that, PLGA degradation occurs due to autocatalytic hydrolysis of ester bonds. The formation of acidic monomers and

oligomers further catalyze the degradation and hence play a key role in release mechanism of encapsulated drug.

Thus based on suitable physicochemical and *in vitro* drug release properties, B. No's. PN-05, PN-13 and PN-19 (one batch from each polymer) were selected for further characterization.

Thus based on suitable physicochemical and *in vitro* drug release properties, B. No's. PN-05, PN-13 and PN-19 (one batch from each polymer) were selected for further characterization.

4.6 Transmission Electron Microscopy Analysis

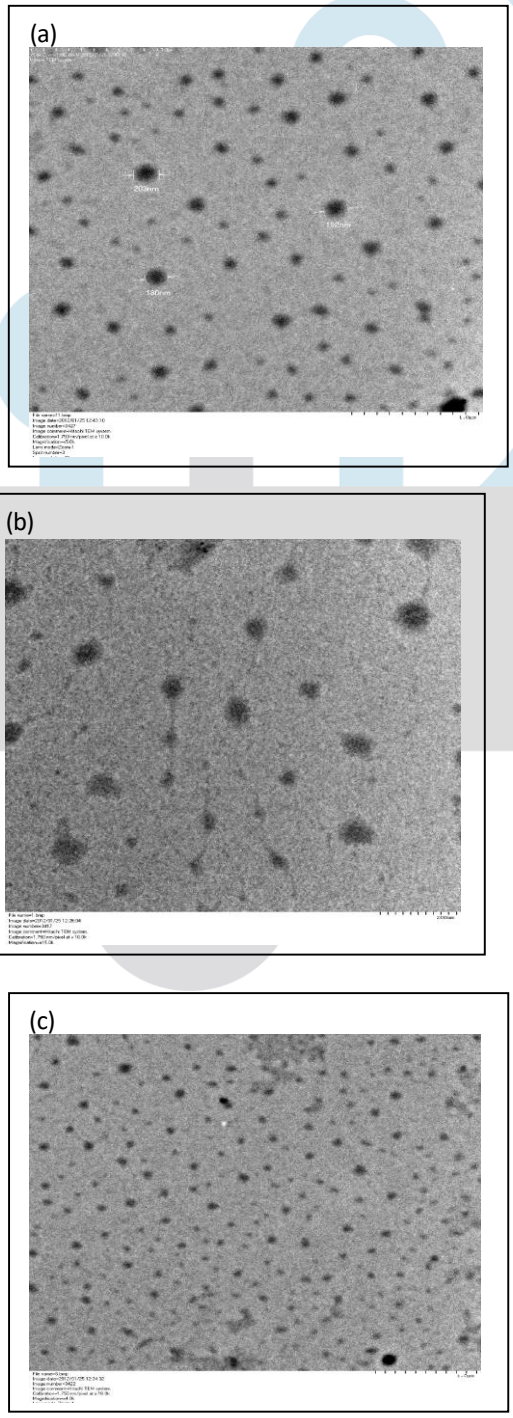


Fig. Transmission electron microscopy image of freeze dried lercanidipine loaded nanoparticles (magnification range of 4 k -15.0 k ×) (a) PN-05, (b) PN-13 and (c) PN-19

5.0 SUMMARY

Present study demonstrates the ample role of polymeric nanoparticles in oral delivery of lercanidipine, to the best of our knowledge, is the first of its kind. Among the different polymers used, PLGA based lercanidipine nanoparticles produced encouraging results. The novel freeze dried formulation presented desirable particle size and excellent encapsulation efficiency. The pronounced increase in absorption and bioavailability of lercanidipine may be due to its loading in polymeric nanoparticles which facilitated the drug to get internalized through M-cells of intestinal payer's patches present in the small intestinal segment thus bypassing the first pass metabolism. increase in intestinal absorption, enhanced bioavailability and prolonged control of antihypertensive activity of the developed lercanidipine nanoparticles may concomitantly improve the therapeutic outcome and can help in patient compliance. However, further detailed investigations are warranted to understand exact mechanism of nanoparticle uptake.

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