RESEARCH PAPER

Preparation and Evaluation of L-Leucine Loaded Polymeric Nanoparticles for the Management of Liver Disease

Pushpendra Kumar[1], Suman Shrivastava[2]

[1]Shri Shankaracharya Professional University, Bhilai
[2]Shri Shankaracharya College of Pharmaceutical Sciences Junwani Bhilai

ABSTRACT

Polymeric nanoparticles (PNs) are the first generation of lipid-based nanocarriers that are formulated from lipids, which are solid in the body temperature and stabilized by emulsifiers. PNs are composed of well-tolerated and biodegradable solid lipids such as mono-, di-, and triglycerides, fatty acids, waxes, and steroids, as well as lipophilic and hydrophilic emulsifying agents. This composition of biocompatible molecules makes PNs one of the most successful options for the administration of drugs with different routes of administration. PNs have been sought as a means to improve the solubility and bioavailability of many drugs, both hydrophilic and lipophilic, especially drugs belonging to class two (high permeability and low solubility drugs) and four (low permeability and low solubility drugs) of the biopharmaceutical classification system (BCS). PNs provide several indirect ways to address resistance problems, such as achieving a sustained release profile of a drug, maintaining concentrations within its therapeutic range, and thus avoiding potential adverse effects.

Keywords: L-Leucine, Polymeric Nanoparticles, Colloidal carriers, Liver Disease, Sustained release

How to cite this article: Kumar P, Shrivastava S, Preparation and Evaluation of L-Leucine Loaded Polymeric Nanoparticles for the Management of Liver Disease. Int J Drug Deliv Technol. 2026;16(10s): 731-740; DOI: 10.25258/ijddt.16.10s.86.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Polymeric nanoparticles (PNs) are sub-micron colloidal carriers used in drug delivery, made of solid lipids like triglycerides and emulsifiers. They are a versatile system for delivering drugs through various routes like oral, parenteral, and transdermal, offering advantages such as improved bioavailability, drug protection from degradation, and potential for targeted delivery [1]. However, they can have drawbacks like low drug loading capacity and potential for drug expulsion due to changes in lipid crystallinity. Polymeric nanoparticles (PNs) have emerged as a remarkable nanocolloidal system for drug delivery [2]. Nanotechnology-based drug delivery systems have increasingly been developed in the last decades and have significantly impacted the pharmaceutical sciences. They play a critical role in delivering hydrophobic drugs, which comprise more than 40% of approved drugs [3]. The poor water solubility of these hydrophobic drugs is one of the major limiting steps that considerably affect drug release and bioavailability, which can be resolved using nanotechnology-based drug delivery systems. In addition, the incorporation of drugs in nanoparticles (NPs) increases drug stability, reduces enzyme degradation, prolongs circulation time, and improves the uptake of target cells, which, thereby, enhances the overall effectiveness and safety [4].The information regarding various aspects about PNs, i.e., PN morphology, structural features, preparatory methods, and their characterizations. This carrier system allows advancing the therapeutic efficacy of drugs belonging to several categories. The present uses of PNs include cancer therapy, infectious conditions, diabetes, central nervous system disorders, cardiovascular disorders, cosmeceuticals, and others [5]. PNs facilitate improved the pharmacokinetics and modify the drug releases. The prospect of surface modification, enhanced permeation against various biological barriers, the ability to resist chemical degradation, and the possibility of encapsulation of two or more therapeutic agents simultaneously has gained attention for PNs universally [6]. Simultaneously, this review emphasizes on recent research trends pertaining to this carrier system. Polymeric nanoparticles (PNs) offer an amalgamating effect of several carrier systems such as liposomes and niosomes. Similar to other carrier systems, PNs are also constituted by biocompatible excipients that are physiologically accepted and homology to polymeric nanoparticles [7]. The solid matrix in PNs also proposes to protect the loaded therapeutic molecules against the rough biological environment and also shield the other chemical degradations with maximum feasibility to alter the release profiles of the therapeutic molecule [8].In a recent study funded by a significantly large degree of

resources, researchers investigated with therapeutic potentials, which have been used for centuries to treat liver disease animal models. Treatment of liver disease continues to be difficult in modern medicine, with several hurdles yet to be taken. Currently, there are no pharmaceuticals in our health care system that can stimulate liver activity, prevent injury to hepatocellular components or promote regeneration of hepatic cells. The drug encapsulating particles are more compact and therefore have more total surface area resulting in the ability to dissolve at the site of the circulation. The application of the approach is to develop nanoparticles that can modulate and control the release rate of the drug in the selected area of the body. The nanoparticles will improve drug circulation and bioavailability, and attenuate side effects. There are multiple administration routes to introduce nanoparticles to defined controlled release of nanoparticles, oral or nasal, parenteral, and intra-visually. Nanoparticle medicated drugs also have a larger surface area, meaning they will dissolve into the blood stream much more at a time, this decreases identification toxicity as well as have therapeutic implications and enhance membrane permeability.

MATERIAL AND METHODS

Determination of maximum wavelength of l-leucine: Phosphate buffer saline (PBS) (pH 7.4) dispersing mediums are utilized for making determination of maximum wavelength of drug l-leucine. Initially one stock solution was prepared by dissolving 10 mg of drug in 10 mL of particular solvent and then dilutions were made in concentration from 10µg/ mL by adding phosphate buffer saline (PBS) (pH 7.4).

Calibration curve of l-leucine: A number of different aqueous dispersing mediums are utilized for making calibration curves of drug l-leucine. Initially one stock solution was prepared by dissolving 10 mg of drug in 10 mL of particular solvent and then dilutions were made in different concentration starting from 1µg/ mL to 10µg/ mL by adding phosphate buffer saline (PBS) (pH 7.4).

FTIR study: The compatibility i.e. drug-excipients interaction studies are helpful for dosage form design. For compatibility studies drug / excipients ratio are selected and investigated based on the reasonable drug / excipient ratio in the final product.The compatibility i.e. drug-excipient interaction studies are useful for dosage form design. In general, solid-state reactions are slower and more difficult to interpret than reaction in solution, because of a reduced number of molecular constants between drug substances and excipients molecules and the occurrence of multiple-phase reactions. For compatibility studies drug / excipients ratio are chosen and investigated based on the reasonable drug / excipient ratio in the final product.

Formulation of l-leucine loaded polymeric nanoparticles:

Screening of lipids: The solubility analysis of lipids, commonly use lipids reported for the preparation of polymeric nanoparticles were taken into consideration. To select the most appropriate lipid for the development of polymeric nanoparticles, these solubility studies were investigated.For this study, the organic solvents in 2 ml quantity were taken into small vials of 5 ml capacity and lipid in the excess quantity was dissolved in these solvents taken in vials. The solvents utilized in the study include ethyl alcohol. The quantity of lipid varies from 10 mg to 300 mg in all vials of a particular solvent system. The lipid selected for the screening were lecithin, phosphatidyl choline, chitosan. The vials were kept in sonicator at normal room temperature for 30 seconds. The lipid is supposed to be soluble when it disappears in the particular solvent on visual observation.

Preparation of polymeric nanoparticles: The polymeric nanoparticles in the current research utilized solvent emulsification evaporation method for preparation. The drug was intended to be encapsulated in the lipid matrix with the help of surfactant. The use of stabilizer in the formulation was for improvement in the stability of developed polymeric nanoparticles. The method optimization for polymeric nanoparticles was on the quality and size of lipid nanoparticles, polydispersity index, entrapment efficiency and drug loading. The lipid was first melted above its melting point and drug separately l-leucine (10 mg) was dissolved into the melted lipid. The drug-lipid mixture was dissolved completely in an organic solvent by using sonication technique. The surfactant was dissolved in purified water to make an aqueous phase and the water phase was heated to the same temperature as that of lipid phase. The lipid phase was added slowly into hot aqueous phase using high speed mechanical stirring. As the high-speed stirring takes place, the temperature was increased due to the heat generated. Because of increased temperature, the volatile organic solvent gets evaporated and lipid nanoparticles start to precipitate due to low concentration of dispersion medium. The resultant colloidal dispersion was mixed with a 5 mL chitosan solution (0.5% *w*/*v*) and stirred for 1 h. To remove unreacted chitosan, the nanoparticle suspension was centrifuged and the pellet was resuspended in distilled water. This process was repeated thrice. These lipid nanoparticles were solidified through cooling at room temperature and filtered through membrane filter. Washed nanoparticles were lyophilized for stable formulation.

Table 1: Response surface regression in different batches prepared for l-leucine polymeric nanoparticles using 3^3 factorial designs by solvent evaporation technique

Formulation Code	Lipid content (X_1)		Polymer	Amount of surfactant (X_2) (%) (Tween 20)	Addition of sonication time (X_3) (Min.)
	Sodium alginate (SA) (mg) X_a	Xanthan gum (XG) (mg) X_b	Chitosan (CH) (mg) X_c		
LL-SE-PN1	150	0	150	10	10
LL-SE-PN2	0	150	150	10	10
LL-SE-PN3	150	50	100	10	10
LL-SE-PN4	50	150	100	10	10
LL-SE-PN5	150	100	50	10	10
LL-SE-PN6	50	100	150	10	10
LL-SE-PN7	100	150	50	10	10
LL-SE-PN8	100	50	150	10	10

Method optimization for fabrication of PNs: The polymeric nanoparticles in the current research employed three techniques for the fabrication of polymeric nanoparticles. The technique was optimized based on the particles size of the resultant nano lipid particles with poly dispersity index (PDI). The technique through which lower particle size with high entrapment efficiency derived was selected for further progress in formulation development.

Optimization of formulation variables: The optimization of variables employed in the formulation was performed using 3^3 factorial designs. In the present study three independent variables were taken into consideration for the designing of formulation. The dependent variables for the selection of optimized process and batch were considered the particle size (X) and polydispersity index (Y). These were the parameters of importance for selection of appropriate combination of variables. Total 3^2 possible combinations were made and studies for designing of polymeric nanoparticles by various methods. The data obtained for the experimental design were fit to generate polynomial equation. The individual effect of these variables was investigated in development of polymeric nanoparticles.

Selection and optimization of process variables: The variables utilized in the formulation plays an important role in quality of desired formulation. The dependent variable for the present research was speed of homogenization, time of stirring, temperature and the effect of filtration method for getting polymeric nanoparticles. The individual effect of these variables was investigated in development of polymeric nanoparticles. The response was recorded for particles size in nanometres and PDI.

Scanning electron microscopy (SEM): The surface morphology is useful for study 3D structure of a particles. The study was performed using scanning electron microscopy. The sample was fixed and dehydrated with acetone. After drying, the sample was coated with gold for imaging. The SEM was performed at 25°C.

Transmission electron microscopy (TEM): Transmission electron microscopy (TEM) was utilized to evaluate surface characteristics of developed solid lipid nanoparticles. The prepared polymeric nanoparticles dispersion was first diluted with purified water in the ratio of 1:100. The diluted dispersion was filtered through membrane filter of 0.45 µm. The filtered sample was kept on a copper grid coated with carbon. The sample was put on the dried grid mixed with diluted solution of phospho-tungstic acid. The slide was fixed by a cover slip and observed under light microscope.

in-Vitro Dissolution: In-vitro dissolution studies were performed using membrane dialysis method. The dialysis membrane was made of cellulose. It was treated with specific treatment before performing dissolution studies. The in-vitro release of drug from L-theanine and L-leucine loaded PNs was performed using treated dialysis membrane. The PNs suspension in 1 mL quantity was poured to dialysis tube and sealed. The tube was transferred to a vial having 10 mL of phosphate buffer solution pH 7.4 mixed with 2% tween 20. The sample was subjected to a shaker apparatus maintained at 37±1°C. The speed of strokes was fixed at 5o min-1. The samples in 2 mL quantity from the vial were taken out at time hour of 0, 0.5, 1, 2, 4, 8, upto 12h. The sink conditions were maintained by replacing

the amount of sample with fresh media. The samples were analysed by UV spectroscopy method L-Leucineat 240 nm. The release of drug from PNs was compared with the release of drug from pure drug suspension.

RESULTS AND DISCUSSION

The absorption maxima (λ-max) of L-leucine (10 µg / ml) in phosphate buffer saline (PBS) (pH 7.4) solution were found to be at 240 nm.L-leucine drug was estimated in-vitro by reported UV spectrophotometric methods. The reported UV spectrophotometric methods were slightly modified and optimized according to the existing laboratory conditions.

The calibration curves show excellent linearity of data as evidenced by the values of correlation coefficients that were found to be greater than 0.99.

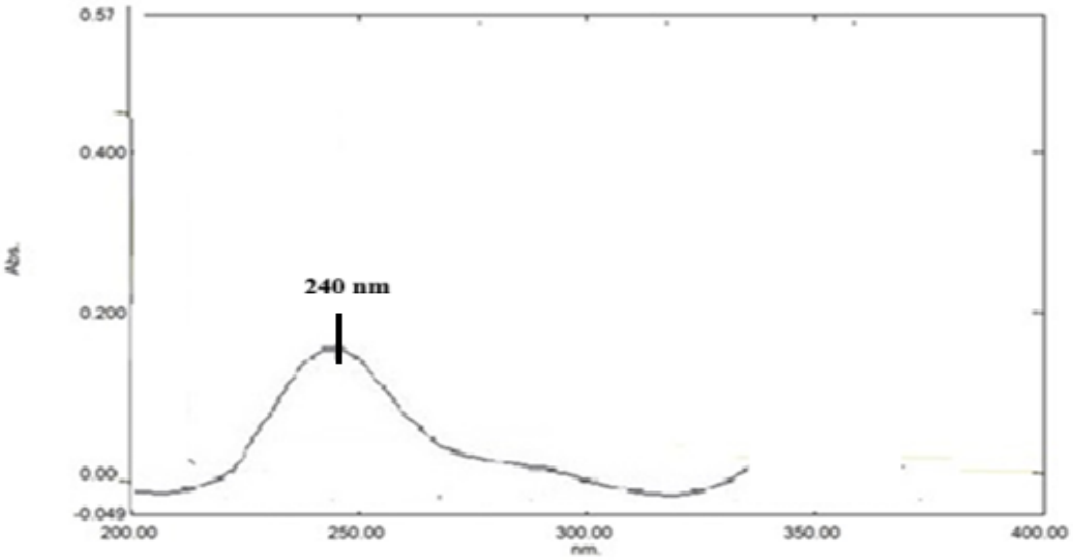

Figure 1: Absorption maxima (λ-max) of L-leucine in phosphate buffer saline (PBS) (pH 7.4) solution (10 µg/ml)

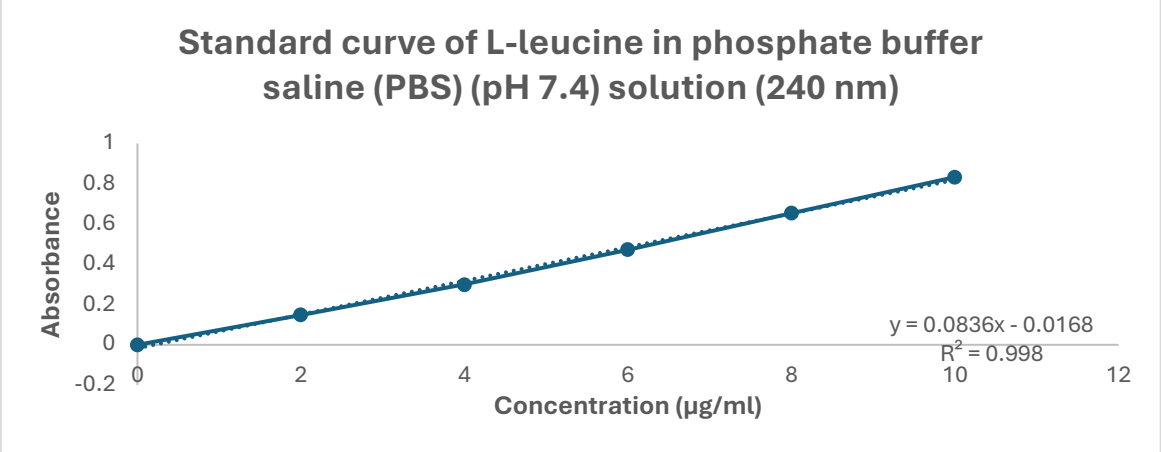

Figure 2: Standard curve of L-leucine in phosphate buffer saline (PBS) (pH 7.4) (240 nm)

The characteristic peaks of sample measured in the range of 400 to 4000 cm-1. Interpretation of FTIR spectra of sample showed almost identical to the reference spectrum of drug. Functional groups leading to characteristic IR spectra of L-leucine include (a) methyl group, (b) amino group, (c) carboxyl group (Figure 3). OH, C=O and C-O stretching can exhibit strong IR absorption in the regions around 3000, 1700, 1300 cm-1, respectively [2]. The methyl group characterized by aliphatic CH3 bending can be accounted for strong IR absorption bands in a region around 2800-3200 cm-1. A strong IR band cluster often occurs in regions of 1020-1250 cm-1 and 1590-1627 cm-1, being attributed to C-N skeletal vibraton and RNH2 bending, respectively.There are no significantly changes between the drug excipient spectra so drug and

excipient both are compatible. Functional groups and bond vibrations are responsible for IR spectra.

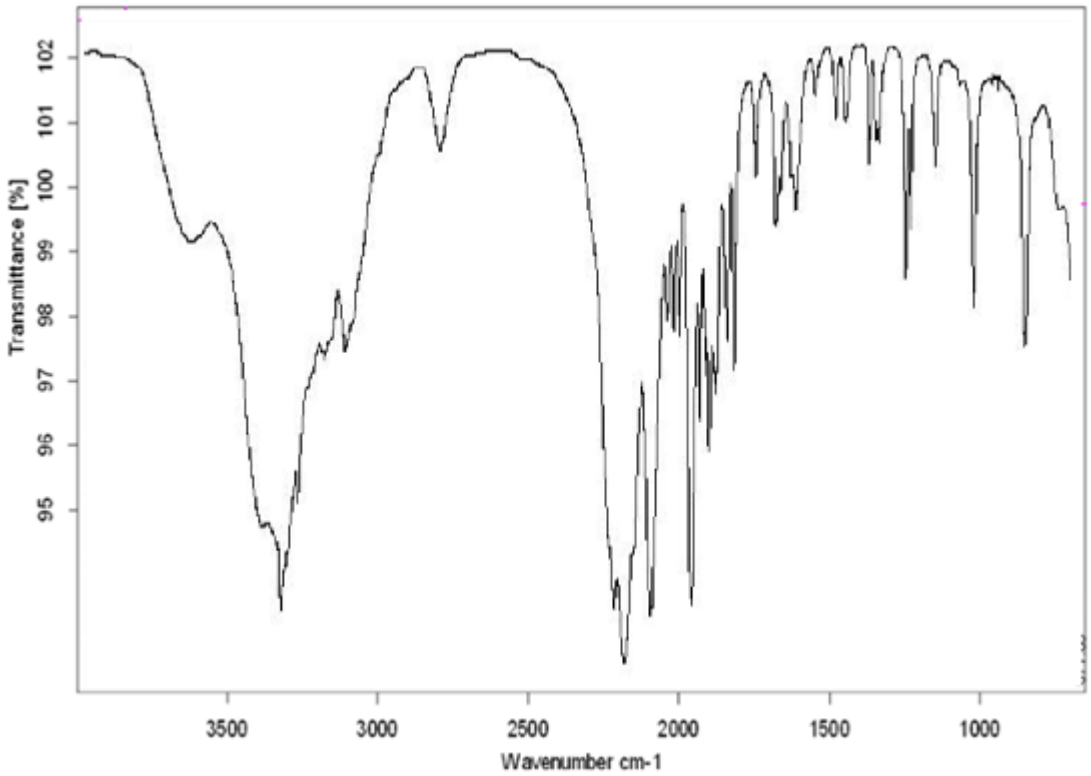

Figure 3: The I. R. Spectrum of sample of pure L-leucine

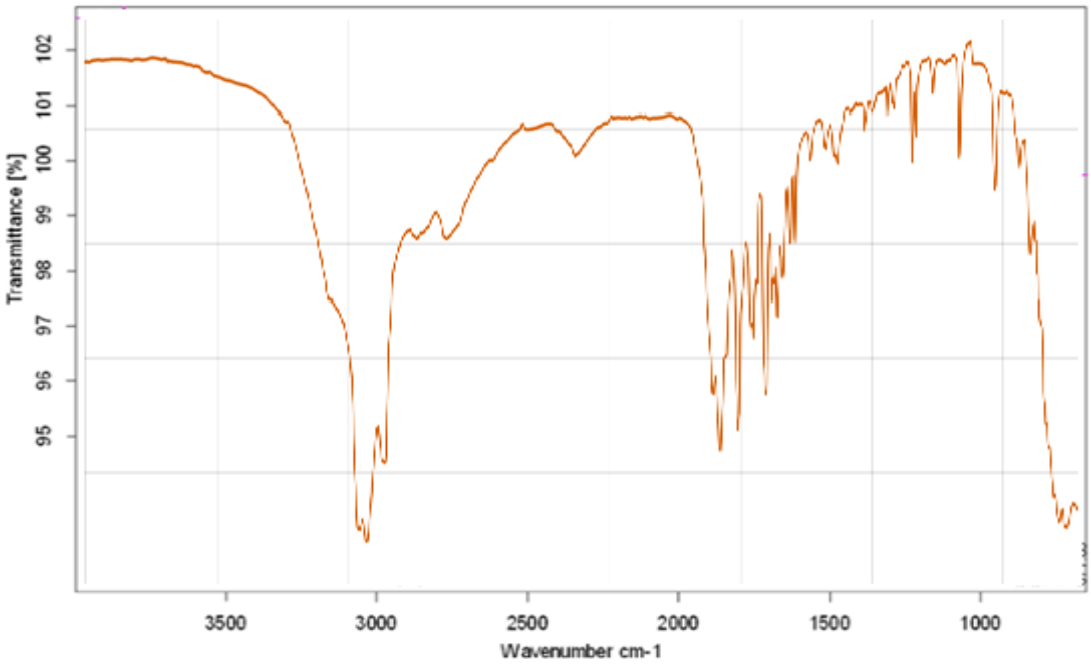

Figure 4: The I. R. Spectrum of sample of L-leucine and all excipients

Lipid selection: Polymeric nanoparticles consist of lipid matrix that facilitates the solubilisation and encapsulation of lipophilic drug molecule. The core material l-leucine in lipid matrix is stabilized by addition of a surfactant. The solvent or dispersing

medium was considered if it dissolved the lipid completely on visual observations.

Table2: Solubility of chosen lipids in ethanol

Lipid content	10 mg	30 mg	50 mg	100mg	150mg	200mg	250mg	300mg
LC	Y	Y	Y	Y	Y	Y	N	N
PCH	Y	Y	Y	Y	Y	Y	Y	Y
CH	Y	Y	Y	Y	Y	Y	Y	Y
Glu	Y	Y	Y	Y	Y	Y	Y	Y

(LC - Lecithin, PCH- Phosphatidylcholine, CH-Chitosan, Glu-Gelucerire)

Y Yes N No)

The lipid solubility of lecithin, phosphatidylcholine, chitosan, Gelucerine were investigated in ethyl alcohol and observed that all lipids in their excess quantities were soluble in ethyl alcohol except stearic acid which showed low solubility in higher amount.

Characterization of L-leucine Loaded PNs: Entrapment efficiency is the study of drug amount that is encapsulated in the lipid matrix and quantity of drug present in supernatant layer received after. The entrapment efficiency is the ratio of actual amount of drug loaded and theoretical amount of drug loaded in lipid nanoparticles. The loading of drug can be measured by subtracting the free drug amount from the total quantity of drug used in the formulation. Drug entrapment efficiency of polymeric nanoparticles were dependent of drug lipid matrix and physico-chemical properties of drug and lipid used in the formulation. The entrapment efficiency (%), percentage yield and % drug loading of optimized formulation of drug loaded PNs mentioned.Yield of the formulation indicates the quantity of polymeric nanoparticles achieved after the preparation. The yield is expressed as the ratio of lipid present after drying and used initially.

The study of particle size and its distribution in the developed formulation is an important tool to give information of existing size range of the particles formed in the formulation. A no. of techniques was employed in the determination of particle size analysis. The size and size distribution of PNs are crucial properties affecting the stability and efficacy of the drug, and PNs with a small size and narrow size distribution have better stability and efficacy than larger PNs and broad size distribution.

Table 3: Physical characterization of prepared L-leucine PNs

Formulation Code	Particle size (nm)	Layers	Zeta potential (mV)	PDI	Drug Entrapment (%)
LL-SE-PN1	127.21±1.11	Single	-24.21±1.09	0.216±0.95	76.53±1.3
LL-SE-PN2	129.01±0.91	Single	-22.91±1.03	0.219±0.05	79.78±1.1
LL-SE-PN3	129.99±0.65	Single	-22.16±1.05	0.221±0.27	71.86±1.1
LL-SE-PN4	126.11±1.09	Single	-24.81±1.19	0.215±0.02	81.37±0.8
LL-SE-PN5	128.11±1.03	Single	-23.01±1.03	0.217±0.35	68.32±1.2
LL-SE-PN6	131.78±0.88	Single	-21.91±0.95	0.229±0.07	76.53±0.9
LL-SE-PN7	130.19±1.11	Single	-22.02±1.04	0.228±0.05	63.05±0.8
LL-SE-PN8	128.01±0.91	Single	-23.01±0.96	0.226±0.95	65.12±1.2

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 126.11	**Peak 1:**	**126**	121.21	109
PdI: 0.215	**Peak 2:**	0.00	0.0	0.00
Intercept: 0.313	**Peak 3:**	0.00	0.0	0.00

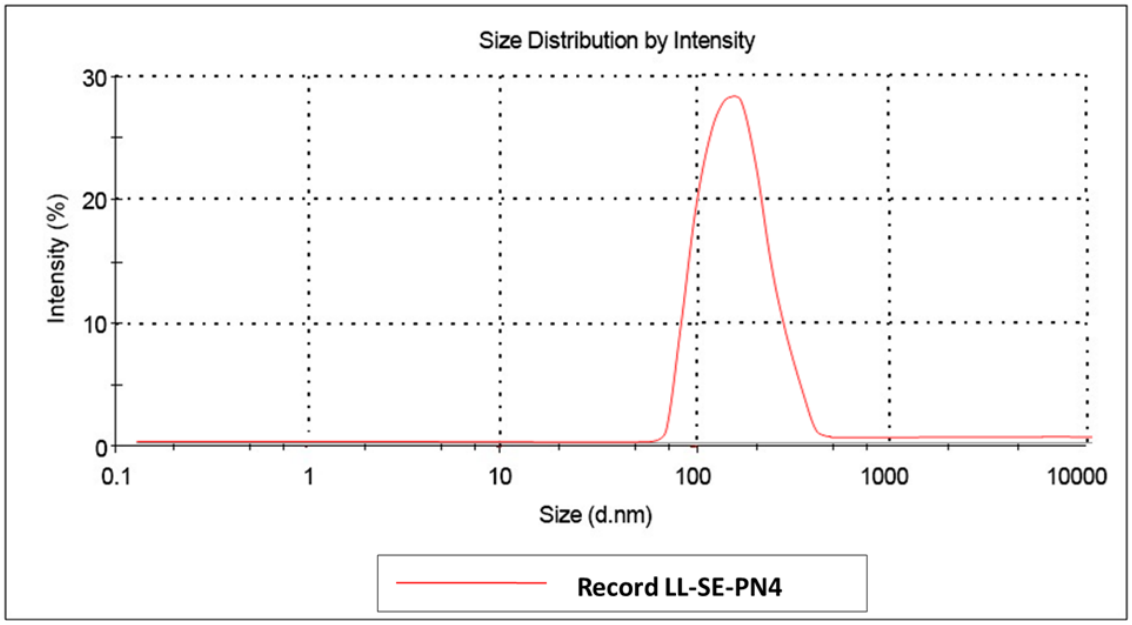

Figure 5: Particle size distribution & Polydispersity Index (PDI) of prepared L-leucine PNs (LL-SE-PN4)

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -24.81	**Peak 1:** -24.81		100	5.79
Zeta Deviation (mV): 78.29	**Peak 2:** 0.00		0.0	0.00
Conductivity (mS/cm): 0.331	**Peak 3:** 0.00		0.0	0.00

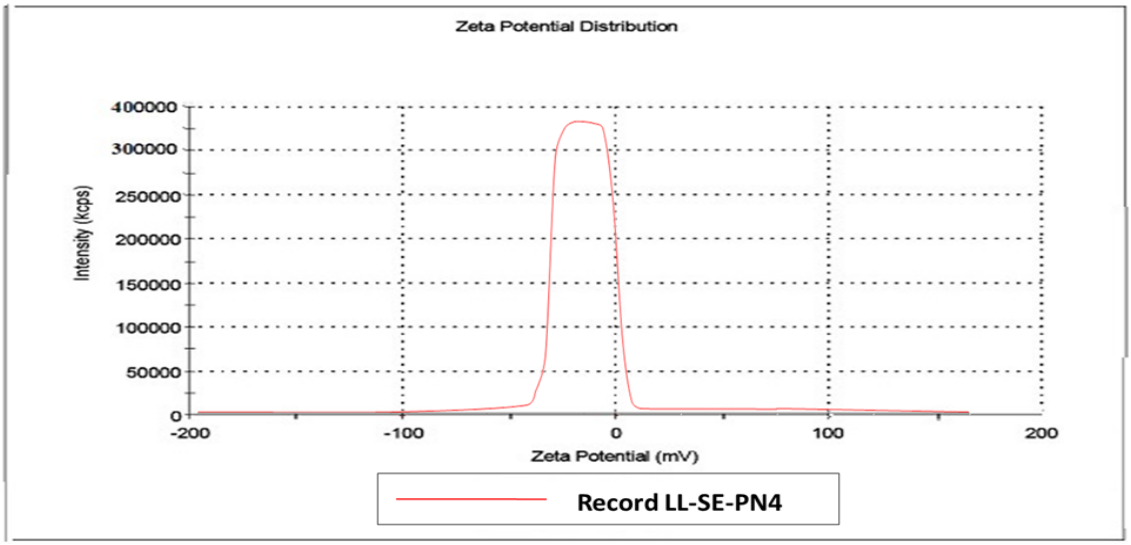

Figure 6: Zeta potential (mV) of prepared L-leucine PNs (LL-SE-PN4)

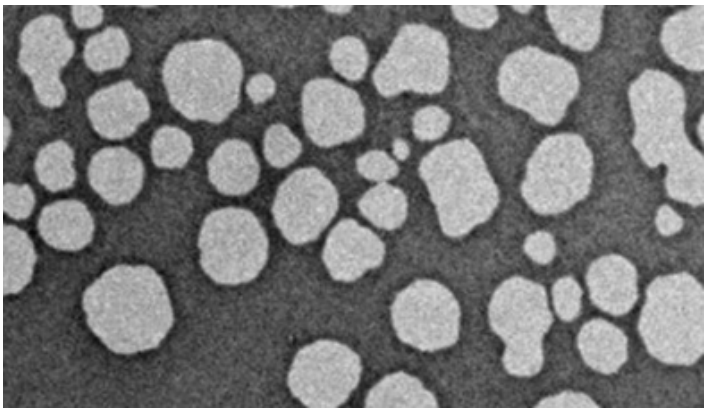

Figure 7: Scanning Electron Microscopy (SEM) image of LL-SE-PN4

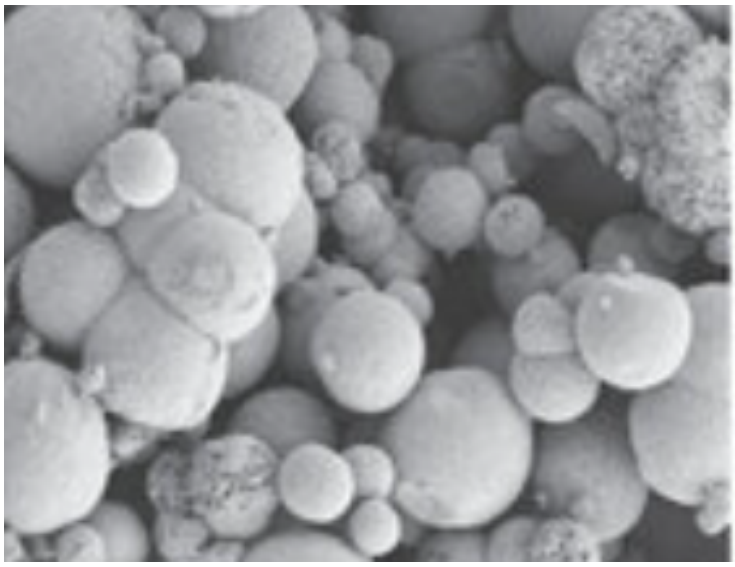

Figure 8: Transmission Electron Microscopy (TEM) image of LL-SE-PN4

The solid lipid nanoparticles **LL-SE-PN4**were examined for their surface morphological studies. Surface morphology was studied using Transmission electron microscopy (TEM). In this technique, electronic rays were passed through the sample and image of the sample was formed.

in-vitro dissolution study: The in-vitro release of drug from L-theanine and L-leucine loaded PNs was performed using treated dialysis membrane. The in-vitro dissolution studies were investigated using phosphate buffer pH 7.4 mixed 2% tween 20 as surfactant. The release of drug from PNs was compared with the release of drug from pure drug suspension. The concentration of drug was calculated by extrapolating of curve and by making a graph between timesversus % cumulative release.

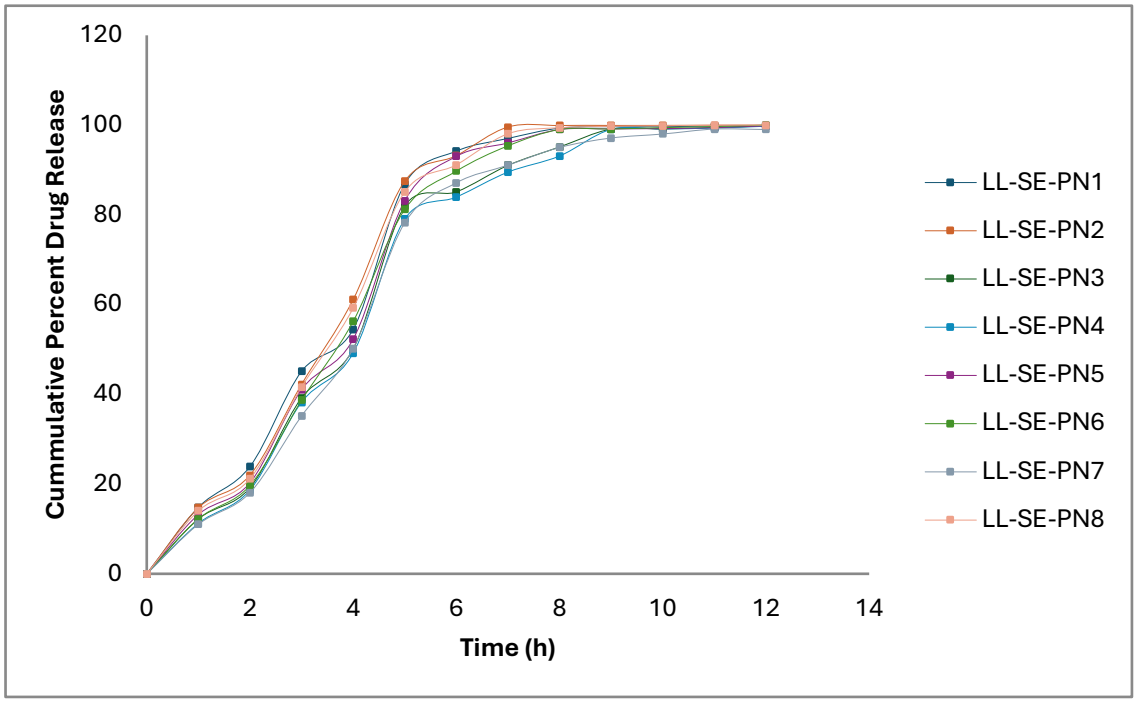

Figure 9: Zero-order plot in-vitro drug diffusion analysis of prepared L-leucine PNs by solvent evaporation technique (LL-SE-PN1- LL-SE-PN8)

SUMMARY AND CONCLUSION

The solvent emulsification-evaporation method includes two steps: (i) preparation of oil/water nanoemulsions and (ii) solvent evaporation. In this method, lipids and a drug are dissolved in a solvent or a solvent mixture to form the oil phase, which is, subsequently, emulsified in an aqueous phase. The solvent evaporation is, usually, carried out using a rotary evaporator or mechanical stirring. During solvent evaporation, the concentration of lipids in droplets increases gradually, resulting in lipid precipitation and the formation of PNs. The present study has shown that the PNs being a versatile technology have the potential to improve the biopharmaceutics properties of poorly water-soluble drugL-leucine and open up new perspectives for the formulation of drugs having low aqueous solubility and high permeability. The PN approach was used in an attempt to increase its oral bioavailability.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

No competing interests to declare.

ACKNOWLEDGEMENTS

The authors thank Shri Shankaracharya College of Pharmaceutical Sciences, Shri Shankaracharya Professional University, Bhilai, Chhattisgarh, India, for providing facilities for research work.

REFERENCES

1. Tariq, F., Zaman, M., Waqar, M.A., Saeed, M.A. and Sarfraz, R.M., 2024. Design, optimization & characterization of niosomal & polymeric nanoparticles. International Journal of Polymeric Materials and Polymeric Biomaterials, 73(15), pp.1353-1366.
2. Hnamte, M. and Pulikkal, A.K., 2024. Biocompatible polymeric nanoparticles as carriers for anticancer phytochemicals. *European Polymer Journal*, *202*, p.112637.
3. Choodet, C., Toomjeen, P., Chuaephon, A., Phanchai, W., Srikulwong, U., Wiwasuku, T. and Puangmali, T., 2024. Polymeric nanoparticles engineered for optimal drug delivery using atomistic computational simulation. *ACS Applied Nano Materials*, *7*(16), pp.19698-19708.
4. Hu, T., Shen, C., Wang, X., Wu, F. and He, Z., 2024. Tumor microenvironment-sensitive polymeric nanoparticles for synergetic chemo-photo therapy. *Chinese Chemical Letters*, *35*(11), p.109562.
5. Rostami, N., Ghebleh, A., Noei, H., Rizi, Z.S., Moeinzadeh, A., Nikzad, A., Gomari, M.M., Uversky, V.N. and Tarighi, P., 2024. Peptide-functionalized polymeric nanoparticles for delivery of curcumin to cancer cells. *Journal of Drug Delivery Science and Technology*, *102*, p.106337.

6. Malhotra, M., Chotaliya, D., Debnath, M., Patel, R. and Kulkarni, A., 2024. Varying the hydrophobic core composition of polymeric nanoparticles affects NLRP3 inflammasome activation. Biomaterials Science, 12(18), pp.4790-4805.
7. Bazzazan, M.A., Fathollazadeh, P., Shahbaz, S.K. and Rezaei, N., 2024. Polymeric nanoparticles as a promising platform for treating triple-negative breast cancer: Current status and future perspectives. *International Journal of Pharmaceutics*, *664*, p.124639.
8. Elmowafy, M., Shalaby, K., Elkomy, M.H., Alsaidan, O.A., Gomaa, H.A., Abdelgawad, M.A. and Mostafa, E.M., 2023. Polymeric nanoparticles for delivery of natural bioactive agents: recent advances and challenges. *Polymers*, *15*(5), p.1123.
9. Tehrani SF, Bharadwaj P, Chain JL, Roullin VG. Purification processes of polymeric nanoparticles: How to improve their clinical translation?. Journal of Controlled Release. 2023 Aug 1;360:591-612.
10. Hosseini, S.M., Mohammadnejad, J., Salamat, S., Zadeh, Z.B., Tanhaei, M. and Ramakrishna, S., 2023. Theranostic polymeric nanoparticles as a new approach in cancer therapy and diagnosis: a review. Materials Today Chemistry, 29, p.101400.
11. Yu Z, Shen X, Yu H, Tu H, Chittasupho C, Zhao Y. Smart polymeric nanoparticles in cancer immunotherapy. Pharmaceutics. 2023 Feb 26;15(3):775.
12. Castro, K.C.D., Costa, J.M. and Campos, M.G.N., 2022. Drug-loaded polymeric nanoparticles: a review. International Journal of Polymeric Materials and Polymeric Biomaterials, 71(1), pp.1-13.
13. Pontes, A.P., Welting, T.J., Rip, J. and Creemers, L.B., 2022. Polymeric nanoparticles for drug delivery in osteoarthritis. *Pharmaceutics*, *14*(12), p.2639.
14. Karam, M., Fahs, D., Maatouk, B., Safi, B., Jaffa, A.A. and Mhanna, R., 2022. Polymeric nanoparticles in the diagnosis and treatment of myocardial infarction: Challenges and future prospects. Materials Today Bio, 14, p.100249.
15. Caraway, C.A., Gaitsch, H., Wicks, E.E., Kalluri, A., Kunadi, N. and Tyler, B.M., 2022. Polymeric nanoparticles in brain cancer therapy: A review of current approaches. Polymers, 14(14), p.2963.
16. Wewers, M., Finke, J.H., Czyz, S., Van Eerdenbrugh, B., John, E., Büch, G., Juhnke, M., Bunjes, H. and Kwade, A., 2022. Evaluation of the formulation parameter-dependent redispersibility of API nanoparticles from fluid bed granules. Pharmaceutics, 14(8), p.1688.
17. Yassin, A.Y., Abdelghany, A.M., Shaban, M.M. and Abdallah, Y.M., 2022. Synthesis, characterization and electrochemical behavior for API 5L X70 carbon steel in 5% sulfamic acid medium using PVVH/PEMA blend filled with gold nanoparticles. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 635, p.128115.