Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Investigating the influence of formulation variables on the preparation of nimodipine - loaded polymeric nanoparticles

Asmaa Mohammed RASHID[1]*, Mowafaq Mohammed GHAREEB[2]

1 Department of Pharmaceutics, College of Pharmacy, Uruk University, Baghdad, Iraq.
2 Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
* Corresponding Author. E-mail: Mowafaq.abd@copharm.uobaghdad.edu.iq (M.M. G.); Tel. +9647901737638.

Received: 28 January 2024 / Revised: 15 March 2024 / Accepted: 16 March 2024

ABSTRACT: Nimodipine (NID), as a calcium channel blocker used for the treatment of cerebral vasospasm, is classified as class II with poor water solubility, increasing its rate of dissolution by reducing particle size will boost its bioavailability. Nimodipine was formulated as Nimodipine-loaded nanoparticles using a solvent-anitsolvent nanoprecipitation approach to speed up the dissolving process and bring the particle size down to the nanorange. The purpose of the study was to investigate the impact of several variables on particle size, polydispersibility index, entrapment efficiency (%EE), and *in vitro* release behavior, including the type and quantity of polymers and surfactant, stirring speed, and solvent type. It is well established that particle size, polydispersity index, and entrapment efficiency are not only significantly affected by the formulating variables but can also affect drug release. The selected F12 was designated with a (1:4) drug:polymer w/w ratio as well; the (1:9) solvent:anti-solvent ratio was the best ratio to produce particle size (81.86 nm), polydispersibility index (0.053), and 90% entrapment efficiency. A morphology and compatibility study was conducted for the characterization of selected nanoparticles. The *in-vitro* drug release experiments were conducted, which show a marked improvement in the release profile via solubility enhancement.

KEYWORDS: Nanoparticles; Nimodipine; Soluplus; Solvent - antisolvent; Zetapotential.

1. INTRODUCTION

As a baseline management of cerebral vasospasm, nimodipine (NID) has some selectivity for cerebral vasculature and is generally used for the prevention of cerebral vasospasm [1] and resultant ischemia, a complication of subarachnoid haemorrhage (SAH) specifically from ruptured intracranial aneurysms, irrespective of the patient's post-ictus neurological condition [2].Nimodipine is 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid 2-methoxyethyl -1-methylethyl ester (Figure 1) [3]. It belongs to the class of pharmacological agents known as calcium channel blockers (1). After being taken orally, it is almost completely absorbed. and reaches its highest concentration within an hour, but because the liver undergoes considerable first-pass metabolism, its bioavailability is low (13%), and it was reduced when food was consumed [3].

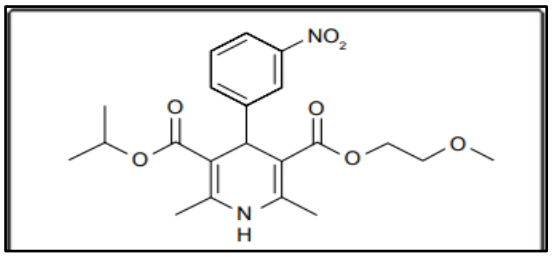

Figure 1. Chemical Structure of NID [3]

How to cite this article: Rashid AM, Ghareeb MM. investigating the influence of formulation variables on the preparation of nimodipine - loaded polymeric nanoparticles. J Res Pharm. 2025; 29(2): 764-775.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1664920

The distinctive characteristics and possible uses of nanoparticles (NPs) have made them a popular approach in recent years, particularly for improving the bioavailability and effectiveness of medications that are poorly soluble by improving their aqueous solubility. NPs have been extensively studied by researchers in the fields of biomedicine and biotechnology, which have poured a lot of time and effort into them, particularly in relation to drug delivery systems. This is due to the fact that NPs have several potential benefits, such as a smaller particle size (PS), better drug stability, a longer therapeutic effect duration, less degradation metabolism, and increased cellular uptake [5, 6]. Since researchers may effectively control many of the physical and chemical properties of NPs by selecting their size and shape, preparing and characterising NPs is today a significant challenge for researchers [7, 8].

Reducing the size of particles from micrometers to nanometers through nanonization techniques improves drug saturation and solubility. This is because the dissolution pressure of small NPs is higher, depending on Ostwald's ripening effect, which states that smaller particles are more soluble than larger ones and that the surface area and adhesiveness of nanomaterials are greater for smaller particles compared to larger ones [9]. However, the small size of NPs makes them prone to aggregation and instability, which can compromise their therapeutic potential. To overcome these challenges, stabilizers are often incorporated into nanoformulations to prevent particle aggregation and improve their stability. The type and concentration of stabilizers (polymer or surfactant), temperature, pH, organic solvent, and stirring speed play a vital role in determining the size, morphology, and distribution of NPs.

There is a wide variety of stabilizers available; choosing the right one for a given drug molecule requires consideration of its hydrophobicity, the stabilizer's molecular weight, the concentration of the stabilizers and stabilizer's nature (hydrophilic or hydrophobic). The formation of agglomerates can be prevented by the use of stabilizers, which include polymers, food proteins, amino acids, and ionic and nonionic surfactants, that prevent particles from sticking together. They act by either electrostatic and/or steric techniques [9]. By using ionic surfactants that can be adsorbed onto particle surfaces, electrostatic stabilization creates a double electric layer around the drug and separates it from the hydrophilic stabilizer. It is possible to prevent nanosized particles from clumping together because of their surface charges by triggering electrostatic repulsion, which occurs when the distance between two drug particles decreases beyond a certain threshold, causing the particles to separate as the two layers of identical charge repel each other [10, 11].

Steric stabilization is achieved by applying polymers or nonionic surfactants to the surfaces of drug-loaded NPs. They preserve the consistency of dispersion over spatial obstacles and function by absorbing hydrophobic molecules on the surfaces of NPs.[12] The extended hydrophilic chains of the polymers that adhere to the surface of the nanocrystals restrict the mobility of drug particles, effectively controlling the spacing between them. [13]. Recently, ionic liquids have gained attention because of their high ion density, moderate toxicity, and relatively high-water solubility. These properties allow for significant electrostatic stabilization and a reduction in surface tension between the drug and the dissolving medium. Hence, it can provide novel uses for serving as a surfactant [14]. Nanoprecipitation is a method used for the preparation of polymeric nanoparticles (PNPs) by employing various polymers, such as polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus®), tween 80, and polyvinyl alcohol.

This study aims to provide an overview of formulation variables such as the types and concentrations of different polymers and surfactants used as stabilizers, as well as the stirring speed and solvent type that influence NID-loaded NPs formulation (NID-NP), dissolution rate, and their impact on the physicochemical properties and stability of NPs [12].

2. RESULTS and DISCUSSION

2.1. Particle Size (PS), Polydispersity Index (PDI), %EE

With the average PS and PDI as an evaluation index, the formulations were screened. The majority of formulas had particles with diameters between 81.53 and 508 nm, while PDI results varied between 0.053 that indicate monodispersion of NP and 0.93 polydispersed NP, and entrapment efficiency (%EE) varied between 68.73% and 99% for prepared formulations, as shown in Table 1. These results were related to the differences in stabilizer and polymer types, ratios, and physicochemical characteristics of the materials.

The %EE is affected by factors as shown in Figure 2. % EE was found to be higher in dispersion made of soluplus and T-80 compared to soluplus and PVA. These results indicated that the %EE was directly proportional to the hydrophobicity and concentration of the polymer, such that the higher the hydrophobicity of the polymer, the greater the resistance to the escape of the encapsulated drug into the outer aqueous phase.

By using soluplus with T-80 F7 (**76.75)** as a less hydrophilic surfactant than PVA, such that increasing amounts of T-80, as in F10, lead to a decrease in %EE (63.14), it could be due to the solubilization effect of NID caused by an excess of surfactants in the aqueous phase that resulted in reducing its adsorption and deposition on the surface of NP [15]. Also, increasing polymer:drug concentration (soluplus) results in increasing the %EE as in F1 and F2, F7 and F9, F10 and F12 [16].

Table 1. The particle size and PDI of different formulations of NID–NP

Formula no.	Particle size (nm)± SD*	PDI± SD*	EE%±SD*
F1	326.9 ±0.11	0.332±0.12	89.04±0.01
F2	300.1±0.14	0.31±0.04	92.78±0.53
F3	228.3±0.43	0.233±0.2	89.23±0.08
F4	508 ±0.53	0.93±0.32	94.71±0.22
F5	264.3 ±0.312	0.375±0.22	76.9±0.54
F6	124.7±0.42	0.113±0.05	71.27±0.12
F7	101.3 ±0.16	0.148±0.54	76.75 ±0.43
F8	91.03 ±0.05	0.244±0.21	68.73±0.02
F9	81.86 ±0.25	0.053±0.11	84.84±0.12
F10	310.5 ±1.2	0.412±0.5	63.14±0.063
F11	118.5±0.5	0.43±0.71	90.15 ±0.17
F12	81.53 ±0.33	0.059±0.23	90.65±0.12
F11a	128 ±0.18	0.116±0.2	99±0.32
F11b	271.1 ±1.51	0.54±0.18	93.7±0.05
F9 a	118.7 ±0. 62	0.43±0.25	97.5±0.19
F9b	94.47 ±0.31	0.22±0.34	98.72±0.1

* **±** SD is the standard deviation, n=3

Regarding the effect of drug to polymer weight ratio, as shown in Figure 2, increasing soluplus amount will cause a significant (p = 0.028) decrease in PS and PDI from 508nm as in F4 (1:1) to 264.3 nm in F5 (1:2) and 124.7 nm in F6 (1:4) due to the presence of soluplus, which is a polymeric solubilizer that has an amphiphilic structure. It is a water-soluble graft copolymer composed of lipophilic and hydrophilic segments (13% of the polymer is made up of PEG residue). Certain medications can be dissolved by micelle production when polyvinyl caprolactam and polyvinyl acetate are used above the critical micelle concentration (CMC). However, below CMC, it is used to hinder drug precipitation via restraining drug nucleation and crystal growth and to provide steric stabilization in a supersaturated state [17]. It acts as a matrix polymer for solids and as a solubilizer via micelle formation in water. The amphiphilic nature of it makes it a good wetting agent because dropping the interfacial tension between the hydrophobic surface of NID particles and the aqueous antisolvent, thus preventing aggregation of the NP due to the effect of steric hindrance, leads to the production of uniform NPs with a narrower size distribution [18]

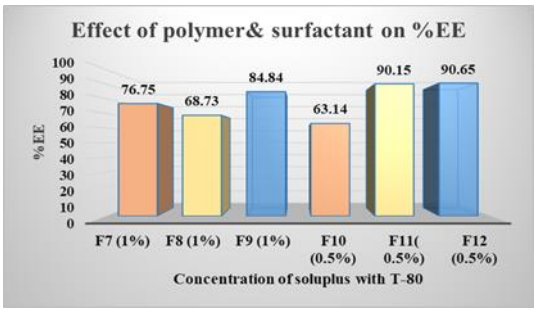

Figure 2. Effect of polymer and surfactant ratio on %EE

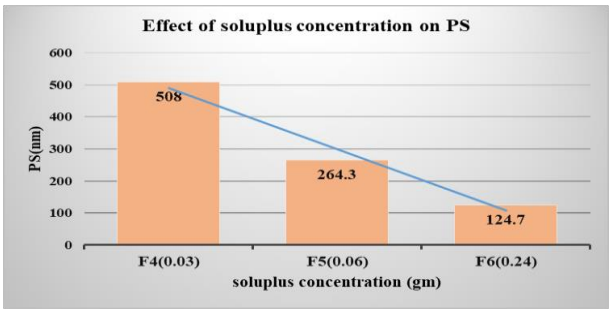

Figure 3. Effect of drug to polymer ratio on PS

2.2. Effect of Stabilizer Type and Concentration

Figure 3 shows that the addition of two stabilizers (T-80 and PVA) in two concentrations (0.5% and 1%) during NP formation organized the size of NID particles to the nanoscale. Regarding T-80, a nonionic surfactant was added to prevent the agglomeration of the NPs and improve physical stability. By comparing the results, the creation of particles of a larger size was seen when using 0.5% T-80 in F10 (310.5nm) in contrast to 1% T-80 in F7 (101.3nm). Similar findings were found in F11 and F12 with F8 and F9, respectively, which resulted from T-80 deposition on the NID-loaded NP surface, which provided a steric repulsion effect that prevented the agglomeration of the particles, while formulas containing lower T-80 content possessed a larger particle size because of the lower amount of T-80, which is not enough to stabilize NID dispersion. In contrast, increasing concentrations of 0.5% PVA (F5 and F6) to 1% (F2 and F3) result in increasing PS significantly ($p<0.05$) which could be due to the increased viscosity and decreased net shear stress, resulting in the production of bigger particles. However, the statistical analysis shows that an increase in PVA and T-80 concentration has a significant effect on increasing the %EE ($p < 0.05$). These results were similar to those of Sukmawati et al. and Ullah F. et al. in studying the effects of T-80 and PVA, respectively [18–20].

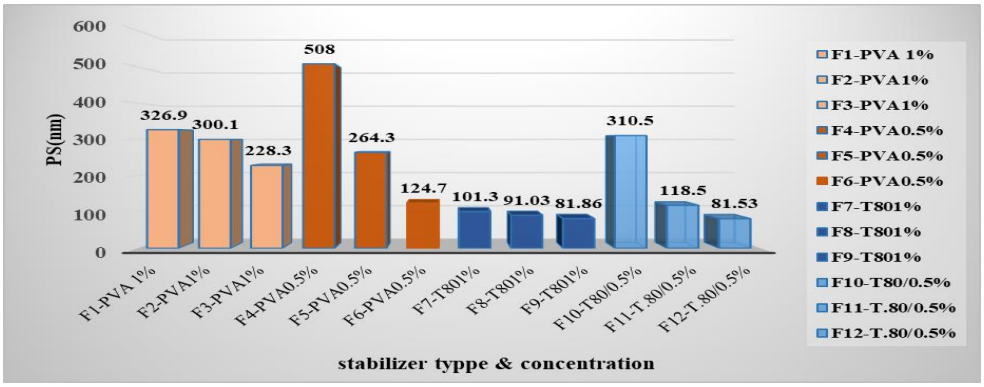

Figure 4. Effect of stabilizer type and concentration on PS

2.3. Effect of Stirring Speed

By matching the PS of prepared formulas, the results show that the PS was decreased when using a stirring speed of 1000 rpm instead of 500 rpm and increased when using a speed of 1500 rpm. while PDI was reduced significantly with increasing stirring speed from 0.54 to 0.116 (Figures 4 and 5). The increase in speed led to a concomitant increase in breaking energy, resulting in smaller PS and thus a narrow PS distribution for NID-NPs [20, 21].

The %EE varies from 93.7% at 500 rpm to a slight increase to 99% at 1500 rpm, as shown in Figure 6. At a lower speed, the PS exhibits a lower %EE on account of the lower surface area and high PDI, which leads to less transport of NID into the external aqueous phase, while at a higher speed, the PS is comparatively lower, the PDI is low, and the surface area is high, resulting in faster passage of the entrapped drug from the organic phase into the aqueous phase. [22] As a result, the optimum speed was 1000 rpm due to low PS (118.5nm) with PDI (0.43) and EE% (90%).

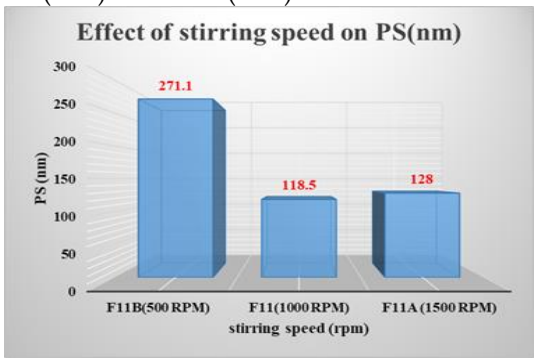

Figure 5. Effect of stirring speed on PS

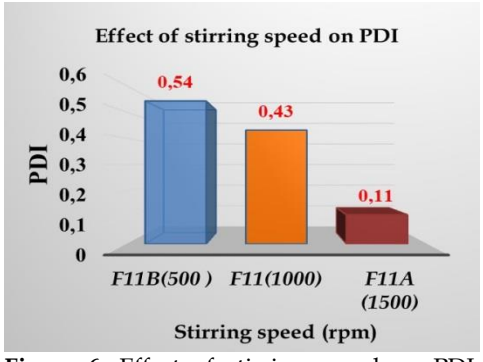

Figure 6. Effect of stirring speed on PDI, Results are expressed as mean, n=3

The study investigated the influence of solvent type on the PS (Figure 7). The findings revealed that using 3 ml of ethanol (F9) resulted in a lower PS of 81.86nm, while using 3 ml of acetone (F9a) resulted in a PS of 94.47nm. Both PDI and EE increased from 0.053 to 0.22 and from 84.84% to 98.72%. The observed outcomes can be attributed to the differences in chemical properties between the organic solvents and the properties of the antisolvent, such as the functional groups, polarity, hydrogen bonding potentials, dielectric constant, dipole moment, and solubility parameters. The efficacy of the antisolvent precipitation depends on the solubility of the solvent in the antisolvent and the insolubility of the drug in the antisolvent. Regarding ethanol, which is a polar protic solvent that possesses O-H bonds and can participate in hydrogen bonding, Meanwhile, acetone is a polar aprotic solvent that possesses C=O bonds and does not participate in hydrogen bonding [23]. In the nanoprecipitation technique used to create polymer-based nanoscale formulations, the polymer is dissolved in an organic solvent that is entirely miscible with water. While introducing the polymer solution into an aqueous phase that is not a solvent for the polymer, the resulting polymer particles are very tiny. Colloidal polymer particles are formed rapidly and without any significant energy input [24].

Nanoprecipitation utilizes a complex hydrodynamic phenomenon (e.g., interfacial turbulence) to compensate for the physicochemical dissimilarities (e.g., viscosity, surface tension) between the utilized solvent and antisolvent phase [25]. The spontaneous blending of the two liquids that are not in equilibrium causes the polymer to separate and settle in the non-solvent. This results in the formation of nanoscale particles when the solvent is replaced [25]. Nanoprecipitation has characteristics similar to the "diffusion-stranding" phenomenon observed in spontaneous emulsification [26]. Solvents with high water affinity and low viscosity improved solvent diffusion into the aqueous phase, forming smaller NPs based on the rapid rate of change in solvent quality [27]. According to polarity, ethanol is more polar with a higher boiling point than acetone. With an increase in the polarity of the solvent, the solvent-interface interactions increase, and fast nucleation occurs, leading to the synthesis of small-size NPs [28]. Thus, choosing the solvent is the primary step in size tuning and encapsulation efficiency for the nanoprecipitation technique, which depends on the solubility of the drug [29, 30].

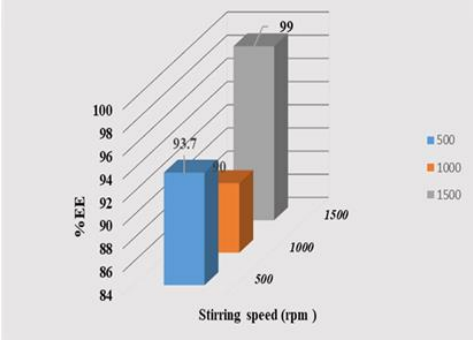

Figure 7. Effect of stirring speed on %EE, Results are expressed as mean, n=3

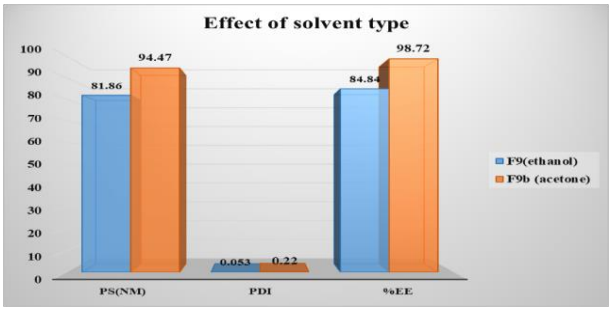

Figure 8. Effect of solvent type on PS, PDI, %EE

Effect of organic /aqueous ratio: Studying the effect of organic to aqueous O/A ratio (Figure 8), by reducing the ratio from 1:9 (F9) to 1:6 (F9a), where a lower volume of water was utilized, an increase value was observed for both the PS from 81.86nm to 118.7 nm, PDI from 0.053 to 0.11, and %EE from 84.84% to 97.5%, respectively, which is a large increase likely due to the poor phase separation. These results were similar to those obtained by Unal Hale in formulating the anticancer agent camptothecin hybrid nanocapsule. Therefore, a 1:9 organic to aqueous phase volume ratio was designated for further studies [31, 32].

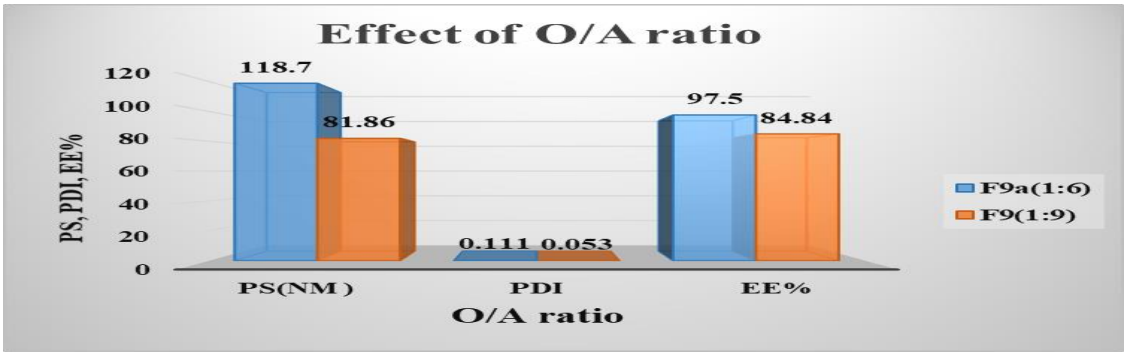

Figure 9. Effect of O/A ratio on PS, PDI, %EE

2.4. The zeta potential (ZP)

The ZP is a measure of a NP's charge with respect to its environment. However, the ZP measures the electric double layer and not the surface charges of individual molecules generated by the ions in the solution's environment. The ZP quantifies the degree of repulsion between neighbouring particles that possess the same charge within a dispersing medium, which is necessary for investigating the stability of colloidal dispersions. ZP was known as a potential at the hydrodynamic shear plane that depends on particle movement under the influence of an electrical field, which is chiefly affected by both the surface charge and the concentration of the electrolyte of the stabilizers used. Figure 9 exhibited the ZP of the selected formula F12 using Soluplus and the nonionic stabilizer T-80, which has a ZP value of -12.33 mV as a result of electrostatic and steric stabilization of the nonionic stabilizer, ZP is mainly depending on steric stabilization due to using nonionic polymers which cause that ZP remain less than 25 mV. A non-ionic surfactant is used as a stabilizer, which provides steric stabilization [33, 34].

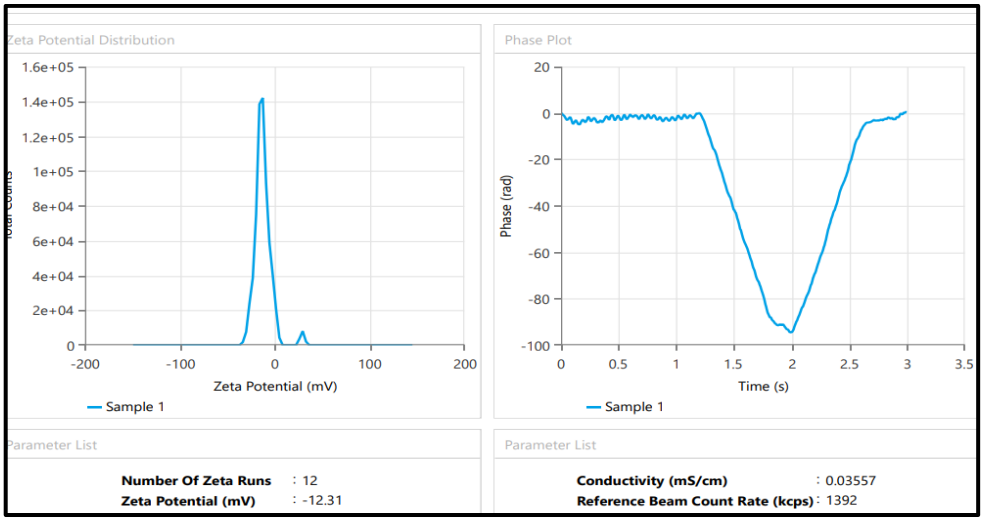

Figure 10. Zeta Potential of Selected NID-PN F12

Morphological Study

Field emission scanning electron microscopy (FE-SEM) revealed a regular rod shape and smooth surface as the morphological properties of nimodipine-polymeric nanoparticles (NID-PNP). As depicted in Figure 10 [34].

2.5. Compatibility Study

Compatibility analysis between the drug and excipient was conducted using Fourier transform infrared spectroscopy (FTIR) for pure NID, Soluplus, T-80, a physical mixture, and the selected F12 formulation, as illustrated in Figure 11. The NID spectrum displays prominent peaks at specific wavenumbers: 3271 cm^{-1} for NH stretching, 3086 cm-1 for C-H aromatic stretching, and 2947 cm-1 for C-H aliphatic stretching. The infrared spectrum reveals the following vibrational frequencies: ester carbonyl stretching at 1701 cm^{-1},

C=N stretching at 1624 cm^{-1}, −C-CH3 at 1381 cm^{-1}, aromatic C=C stretching at 1621 cm^{-1}, pyridine NH at 1648 cm−1, NO2 stretching at 1531 and 1309 cm^{-1}, and C-H bending at 1130 cm^{-1}.

Nevertheless, A broad, prominent peak was observed in the spectra of the formulation at a wavelength of 3275 cm-1, The C=O and NO2 peaks exhibited a decrease in intensity or disappearance of peaks as a result of the establishment of hydrogen bonds with the hydrophilic groups present in soluplus and T-80 with the drug. The analysis indicated the absence of any documented chemical interactions. Therefore, we can assume that the enhanced solubility of the drug is due to the formation of hydrogen bonds [35].

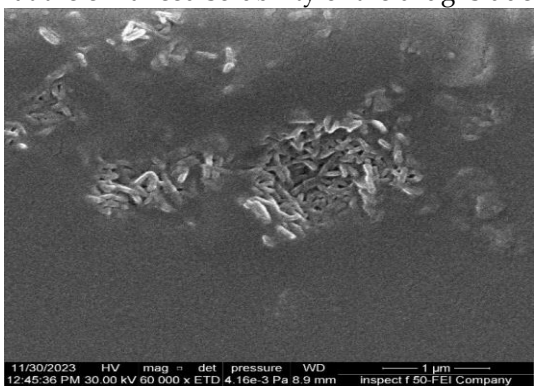

Figure 11. FE-SEM of NID-PNP (F12)

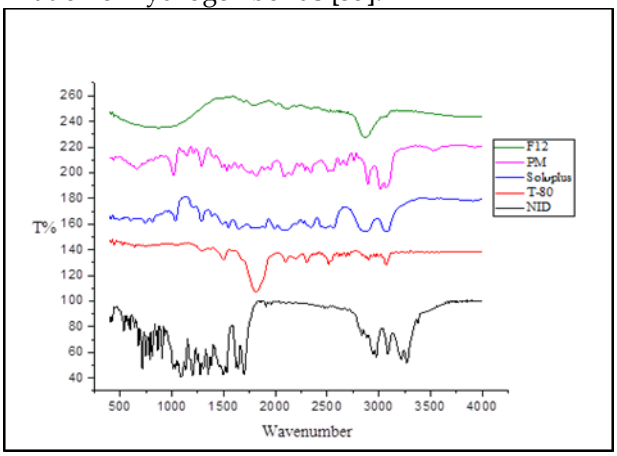

Figure 12. FTIR of pure NID, Soluplus, T-80, physical mixture and NID-PNP (F12)

2.6. *In-vitro* Release of NID- PNP:

In-vitro release profile of NID-PN (F12) compared to pure NID using similarity factor f_2 for comparism revealed a significant improvement in the release rate of NID ($P<0.05$), which was consistent with the Noyes-Whitney equation. Reducing the PS led to an increase in the total surface area and thereby enhanced the solubility and dissolution rate, as shown in figure 12. The bootstrap similarity factor was 11.841, which is lower than 50 for F12, which indicates no similarity appeared between the dissolution profiles of pure NID and prepared NID-PNP.

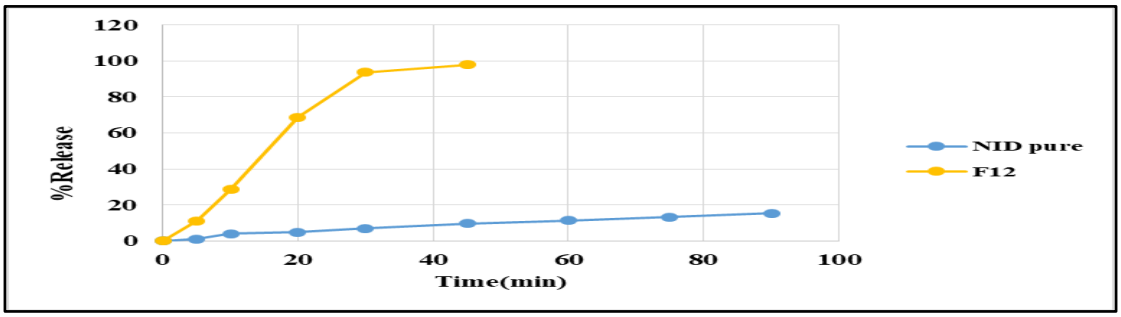

Figure 13. In- vitro release of pure NID, NID-PNP (F12)

3. CONCLUSION

Nimodipine is poorly soluble in water, decreasing its particle size through nanoprecipitation with hydrophilic polymers like Soluplus and T-80. PVA would hasten its dissolving rate. In the preparation of NID-PNPs, the drug-to-polymer (soluplus) ratio was 1:4, and the solvent-to-non-solvent ratio was 1:9 with 0.5% T-80, which resulted in the lowest average PS, PDI, and highest % EE.

4. MATERIALS AND METHODS

4.1. Material

Nimodipine was purchased from Zhejiang Shenzhou pharmaceutical Co., LTD, china. Ethanol was purchased from Honeywell International Inc. USA. Soluplus® was purchased from BASF, Germany. PVA cold from Central drug house, Tween-80 from Himedia Laboratories Pvt. LTD, India. Dialysis bag 8-14 kDa Lab Pvt. Ltd USA. Amicon ultrafilter with a (MWCO 3kDa. Merck) sigma-Aldrich). All other chemicals were of analytical grade.

4.2. Method

The nanoprecipitation technique (solvent-antisolvent precipitation) was employed to synthesize NID polymeric nanoparticles (PNP). In this method, accurately measured quantities (0.03g) of NID and a polymer, specifically polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (Soluplus®), with different concentrations were dissolved together in 3 ml of solvent (ethanol, acetone), serving as the organic phase. The drug-polymer solution, composed of a ratio of 1 part organic to 9 parts aqueous (3 ml to 27 ml), was injected by means of a syringe pump (Hemodiaz. India.) into the aqueous phase. To stabilize the solution, various types and concentrations of stabilizers including polyvinyl alcohol (PVA) and Tween 80 (T-80), were used. The mixture was stirred using a digital magnetic hot plate stirrer, Joanlab, China, at a speed of 1000 rpm at a temperature of 25°C. The solution was mixed for one hour to allow for the evaporation of the organic solvent. The composition of the prepared NID-PNP formulations with different parameters is presented in Table 2 [13].

4.2.1. Characterization of NID-NPs

Particle Size and Polydispersity Index Determination

The Malvern Zetasizer particle size analyzer (Uitra Red, USA) model was used to measure the average PS and polydispersity index (PDI). This instrument detects the variation in light scattering at a room temperature of 25°C and a scattering angle of 90°. PDI values ranging from 0 to 0.05 indicate a monodisperse standard. Values between 0.05 and 0.08 are considered virtually monodisperse; values of 0.08 and 0.7 fall within the moderate polydispersity category; and values over 0.7 are classified as very polydisperse [14].

4.2.2. Entrapment efficiency

The entrapped drug was determined by subjecting 1 ml of the prepared dispersion to centrifugation at 3000 rpm for 15 minutes using an Amicon ultrafilter with a molecular weight cutoff (MWCO) of 10 kDa. The quantity of unbound drug was assessed with organic solvent using spectrophotometry, specifically by determining the absorbance of UV at a wavelength of 237 nm. Organic solvent was used due to the poor water solubility of NID. The amount of drug trapped within the system was then calculated by subtracting the free drug from the total amount of NID and dividing by the total drug content [14].

4.2.3. Effect of process variables on particle size

Process variables such as stirring speed, type of solvent, and type and ratio of polymer and stabilizer used can influence the size. Accordingly, we investigated the effects of these variables on the PS of NID-NPs.

Effect of Drug: Polymer Ratio

Different w/w ratios of drug: polymer (Soluplus®) used in the formulation of NID-PN: 1:1in F1, 1:2 in F2, and 1:8 in F3 were used to investigate the effect of polymer concentration on the NPs properties [8].

Table 2. Constituents of the prepared NID-PNP formulations

Formula no.	NID /gm	Polymer Soluplus amount /gm	Organic solvent/ml	Stabilizer type	Stabilizer concentration (w/v%)	Dw/ml	Speed-rpm
F1	0.03	0.03	Eth.	PVA	1%	27	1000
F2	0.03	0.06	Eth.	PVA	1%	27	1000
F3	0.03	0.24	Eth.	PVA	1%	27	1000
F4	0.03	0.03	Eth.	PVA	0.5%	27	1000
F5	0.03	0.06	Eth.	PVA	0.5%	27	1000
F6	0.03	0.24	Eth.	PVA	0.5%	27	1000
F7	0.03	0.03	Eth.	T-80	1%	27	1000
F8	0.03	0.06	Eth.	T-80	1%	27	1000
F9	0.03	0.24	Eth.	T-80	1%	27	1000
F10	0.03	0.03	Eth.	T-80	0.5%	27	1000
F11	0.03	0.06	Eth.	T-80	0.5%	27	1000
F12	0.03	0.24	Eth.	T-80	0.5%	27	1000
F11a	0.03	0.06	Eth.	T-80	0.5%	27	1500
F11b	0.03	0.06	Eth.	T-80	0.5%	27	500
F9 a	0.03	0.24	Eth.	T-80	1%	18	1000
F9b	0.03	0.24	Acet.	T-80	1%	27	1000

Effect of Stabilizer Type

As an alternative to PVA (F1-F3), T-80 was used as a stabilizer in the formulas (F7-F9), respectively, with the same concentration used previously in PVA to study the effect of changing stabilizer on the resultant NPs fabrication [36].

Effect of Stabilizer Concentration

For both PVA and T-80, two different concentrations (0.5 and 1% w/v) were used in the formulas 1% (F1-F3), 0.5% (F4-F6) for PVA, and 1% (F7-F9) and 0.5% (F10-F12) for T-80, respectively, to show the effect of increasing the stabilizer concentration on the dependent NPs [8, 36].

Effect of Stirring Speed

The effect of speed utilized for achieving nanoprecipitation technique was determined by using three different speeds: 500 (F11b), 1000 (F11), and 1500 (F11a) rpm at a drug-to-stabilizer ratio of 1:2 to study its impact on formulating NPs.

Effect of Solvent Type

The type of organic solvent had been changed using 3 ml (F9) ethanol and 3 ml (F9b) acetone to show its impact on formulating NPs.

Effect of Organic to Aqueous Volume Ratio

The effect of organic to aqueous v/v ratios was determined in F9 (1:9) and F9b (1:6) to show its effect on NPs formulation.

Zeta Potential Measurement of Optimized Formula

Higher zeta potential values, either positive or negative, are necessary to ensure stability, and avoid aggregation of particles. The zeta potential of the selected formula was measured by the Malvern instrument (Malvern zetasizer particle size analyzer (Uitra Red, USA) model), which measures electrophoretic mobility with a voltage of 200V and conductivity of 0.022 ms/cm, then converted into zeta potential, which indicates the degree of stability [37].

In-Vitro Release of Nimodipine from Polymeric Nanoparticles

The release behavior of NID from PNs was measured. 5 mL of PN-dispersion were placed inside a dialysis membrane sac of molecular weight cut off at 8–14 kDa (the cut off was chosen according to macromolecules used in the formulations) that was pre-soaked with dissolution medium for 8 hours., and the open ends of the sac were tied closely to prevent any leakage. The sac was placed in 500 mL of phosphate buffer solution (PBS) pH 7.4 with 0.5% w/v brij-35 in a dissolution medium using a USP Type II dissolution apparatus rotated at 100 rpm and maintaining the temperature at 37 ± 0.1° C, ensuring sink conditions. At regular intervals of 5, 10, 15, 30, 45, 60, and 90 minutes, five millilitres of the sample were collected, filtered through a 0.45 µm syringe filter, and subsequently analyzed by a UV spectrophotometer. To keep the sink in good condition, a fresh buffer solution of the same volume was added right away. The percent release was plotted against time [38, 39].

The statistical analysis of the dissolution investigation for both pure NID and NID-NPs utilized the similarity factor f2 using the DD Solver programme [40]. The pure NID was regarded as the standard, while the NPs were intended to serve as the experimental group. The similarity of release profiles is determined by the range of values for f2, which falls between 50 and 100. The calculation of f2 is derived from equation (1) [35].

$$\{[1 + (1/n) \sum_{t=1}^{n} wt\,(Rt - Tt)\,\hat{}\,2]\,\hat{}\,(-0.5) \times 100\} \ldots\ldots\ldots \text{Eq. (1)}$$

Where Rt represents the percentage of drug dissolved in the reference profile, Tt represents the percentage of drug dissolved in the reference and test profiles at time t, and n denotes the number of sampling instances [35].

4.2.4. Compatibility Study

Fourier Transform Infrared (FTIR) Spectroscopy of NID-NP

The FTIR spectra were acquired using the FTIR (Shimadzu 1800 instrument, Japan). The experiment involved the utilization of pure NID, Soluplus, T-80, and a physical mixture of F12 and the selected formula (F12). These substances were crushed alongside potassium bromide. The acquired spectrum encompassed a range of wavenumbers spanning from 4000 to 400 cm^{-1} [39].

4.2.5. Morphological Study

Morphological examination of the prepared NID-NPs (F12) was performed using field emission scanning electron microscopy (model Inspect 50 FEI, Germany) at a voltage of 10 kV. Briefly, a small quantity of nanodispersion was deposited on a glass slide and permitted to stand at room temperature for 90 seconds to form a thin film on the glass slide. The grid was given enough time to completely dry in the air; to be analyzed Particle 3-dimension figure were obtained at different appropriate magnifications [14, 41]

4.2.6. Statistical analysis

All statistics were calculated using Microsoft Excel 2016 software and the software GraphPad Prism (GraphPad Prism Software, Inc., San Diego, CA, USA). P values < 0.05 were considered significant. Reported averages represent the arithmetic mean of the tested samples.

Acknowledgements: The authors are extremely grateful to the College of Pharmacy/University of Baghdad for their valuable support in providing education and facilities that facilitated this work. .

Author contributions: Concept – M.G.; Design – A.R., M.G.; Supervision – M.G.; Resources – A.R.; Materials – A.R.; Data Collection and/or Processing – A.R.; Analysis and/or Interpretation – A.R., M.G.; Literature Search – A.R.; Writing – A.R.; Critical Reviews – M.G.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

[1] Asasutjarit R, Sorrachaitawatwong C, Tipchuwong N, Pouthai S. Effect of formulation compositions on particle size and zeta potential of diclofenac sodium-loaded chitosan nanoparticles. World Acad Sci Eng Technol. 2013;7(9):568-570.

[2] Jabbarli R, Pierscianek D, Rölz R, Darkwah Oppong M, Kaier K, Shah M, Taschner C, Mönninghoff C, Urbach H, Beck J, Sure U, Forsting M. Endovascular treatment of cerebral vasospasm after subarachnoid hemorrhage: More is more. Neurology. 2019;93(5):e458-e466. https://doi.org/10.1212/wnl.0000000000007862

[3] Teng Z, Yu M, Ding Y, Zhang H, Shen Y, Jiang M, Liu P, Opoku-Damoah Y, Webster TJ, Zhou J. Preparation and characterization of nimodipine-loaded nanostructured lipid systems for enhanced solubility and bioavailability. Int J Nanomedicine. 2018;14:119-133. https://doi.org/10.2147%2FIJN.S186899

[4] Ahad A, Al-Jenoobi FI, Al-Mohizea AM, Aqil M, Kohli K. Transdermal delivery of calcium channel blockers for hypertension. Expert Opin Drug Deliv. 2013;10(8):1137-1153. https://doi.org/10.1517/17425247.2013.783562

[5] Bohrey S, Chourasiya V, Pandey A. Polymeric nanoparticles containing diazepam: preparation, optimization, characterization, in-vitro drug release and release kinetic study. Nano Converg. 2016;3(1):1-7. https://doi.org/10.1186/s40580-016-0061-2

[6] Nugroho AK, Kusumorini N, Pramono S, Martien R. An update on Nanoparticle Formulation Design of Piperine to Improve its Oral bioavailability: A Review. Iraqi J Pharm Sci. 2023;32(1):14-30. https://doi.org/10.31351/vol32iss1pp14-30

[7] Raval JP, Naik DR, Amin KA, Patel PS. Controlled-release and antibacterial studies of doxycycline-loaded poly (ε-caprolactone) microspheres. J Saudi Chem. Soc. 2014;18(5):566-573. http://dx.doi.org/10.1016/j.jscs.2011.11.004

[8] Alzalzalee R, Kassab H. Factors affecting the preparation of Cilnidipine nanoparticles. Iraqi J Pharm Sci. 2023;32(Suppl.):235-243. https://doi.org/10.31351/vol32issSuppl.pp235-243

[9] Hadžiabdić J, Brekalo S, Rahić O, Tucak A, Sirbubalo M, Vranić E. Importance of stabilizers of nanocrystals of poorly soluble drugs. Maced Pharm Bull. 2020;66(03):145-146. http://dx.doi.org/10.33320/maced.pharm.bull.2020.66.03.072

[10] Hong C, Dang Y, Lin G, Yao Y, Li G, Ji G, Shen H, Xie Y. Effects of stabilizing agents on the development of myricetin nanosuspension and its characterization: an in vitro and in vivo evaluation. Int J Pharm. 2014;477(1-2):251-260.https://doi.org/10.1016/j.ijpharm.2014.10.044

[11] Li J, Wang Z, Zhang H, Gao J, Zheng A. Progress in the development of stabilization strategies for nanocrystal preparations. Drug Deliv. 2021;28(1):19-36. https://doi.org/10.1080/10717544.2020.1856224

[12] Demirkurt B, Akdogan Y. Development of an ionic liquid based method for the preparation of albumin nanoparticles. ChemistrySelect. 2018;3(34):9940-9945. http://dx.doi.org/10.1002/slct.201801648

[13] Toma NM, Abdulrasool AA. Preparation and evaluation of microneedles-mediated transdermal delivery of montelukast sodium nanoparticles. Int J Drug Deliv Technol. 2021;11(3):1075-1082. http://doi.org/10.25258/ijddt.11.3.74

[14] Rashid AM, Abd-Alhammid SN. Formulation and characterization of itraconazole as nanosuspension dosage form for enhancement of solubility. Iraqi J Pharm Sci. 2019;28(2):124-133. https://doi.org/10.31351/vol28iss2pp124-133

[15] Hamed HE, Hussein AA. Preparation, in vitro and ex-vivo evaluation of mirtazapine nanosuspension and nanoparticles incorporated in orodispersible tablets. Iraqi J Pharm Sci. 2020;29(1):62-75. https://doi.org/10.31351/vol29iss1pp62-75

[16] Salatin S, Barar J, Barzegar-Jalali M, Adibkia K, Kiafar F, Jelvehgari M. Development of a nanoprecipitation method for the entrapment of a very water soluble drug into Eudragit RL nanoparticles. Res Pharm Sci. 2017;12(1):1. https://doi.org/10.4103%2F1735-5362.199041

[17] Attia MS, Elshahat A, Hamdy A, Fathi AM, Emad-Eldin M, Ghazy F-ES, Chopra H, Ibrahim TM. Soluplus® as a solubilizing excipient for poorly water-soluble drugs: Recent advances in formulation strategies and pharmaceutical product features. J Drug Deliv Sci Technol. 2023:104519. http://dx.doi.org/10.1016/j.jddst.2023.104519

[18] Sukmawati A, Utami W, Yuliani R, Da'i M, Nafarin A. Effect of tween 80 on nanoparticle preparation of modified chitosan for targeted delivery of combination doxorubicin and curcumin analogue. IOP Conf Ser Mater Sci Eng. 2018;311(1):012024. http://dx.doi.org/10.1088/1757-899X/311/1/012024

[19] Attia Shafie MA, Mohammed Fayek H. Formulation and evaluation of betamethasone sodium phosphate loaded nanoparticles for ophthalmic delivery. J Clin Exp Ophthalmol. 2013;4(273):2. http://dx.doi.org/10.4172/2155-9570.1000273

[20] Ullah F, Iqbal Z, Khan A, Khan SA, Ahmad L, Alotaibi A, Ullah R, Shafique M. Formulation Development and Characterization of pH responsive polymeric nano-pharmaceuticals for targeted delivery of anti-cancer drug (Methotrexate). Front Pharmacol. 2022;13:911771. https://doi.org/10.3389/fphar.2022.911771

[21] Jassem N, Rajab N. Formulation and in vitro evaluation of azilsartan medoxomil nanosuspension. Int J Pharm Pharm Sci. 2017;9(7):110. http://dx.doi.org/10.22159/ijpps.2017v9i7.18917

[22] Ray S, Mishra A, Mandal TK, Sa B, Chakraborty J. Optimization of the process parameters for the fabrication of a polymer coated layered double hydroxide-methotrexate nanohybrid for the possible treatment of osteosarcoma. RSC Adv. 2015;5(124):102574-102592. https://doi.org/10.1039/C5RA15859A

[23] Mohammad-Beigi H, Shojaosadati SA, Morshedi D, Mirzazadeh N, Arpanaei A. The effects of organic solvents on the physicochemical properties of human serum albumin nanoparticles. Iran J Biotechnol. 2016;14(1):45. https://doi.org/10.15171%2Fijb.1168

[24] Rao JP, Geckeler KE. Polymer nanoparticles: Preparation techniques and size-control parameters. Prog Polym Sci. 2011;36(7):887-913. http://dx.doi.org/10.1016%2Fj.progpolymsci.2011.01.001

[25] Tan H, Diddens C, Mohammed AA, Li J, Versluis M, Zhang X, Lohse D. Microdroplet nucleation by dissolution of a multicomponent drop in a host liquid. J Fluid Mech. 2019;870:217-246. https://doi.org/10.48550/arXiv.1811.03517
[26] Beck-Broichsitter M. Solvent impact on polymer nanoparticles prepared nanoprecipitation. Colloids Surf. A Physicochem Eng Asp. 2021;625:126928. https://doi.org/10.1016/j.colsurfa.2021.126928
[27] Bovone G, Cousin L, Steiner F, Tibbitt MW. Solvent controls nanoparticle size during nanoprecipitation by limiting block copolymer assembly. Macromolecules. 2022;55(18):8040-8048. https://doi.org/10.1021/acs.macromol.2c00907
[28] Kim M-S, Song H-S, Park HJ, Hwang S-J. Effect of solvent type on the nanoparticle formation of atorvastatin calcium by the supercritical antisolvent process. Chem Pharm Bull. 2012;60(4):543-547. https://doi.org/10.1248/cpb.60.543
[29] Hernández-Giottonini KY, Rodríguez-Córdova RJ, Gutiérrez-Valenzuela CA, Peñuñuri-Miranda O, Zavala-Rivera P, Guerrero-Germán P, Lucero-Acuña A. PLGA nanoparticle preparations by emulsification and nanoprecipitation techniques: Effects of formulation parameters. Rsc Advances. 2020;10(8):4218-4231. https://doi.org/10.1039/C9RA10857B
[30] Alexander K, Thomas EMl. Concepts for the Stabilization of Metal Nanoparticles in Ionic Liquids. In: Scott H. (Eds). Applications of Ionic Liquids in Science and Technology. Rijeka: IntechOpen; 2011. p. Ch. 12. http://dx.doi.org/10.5772/22111
[31] Ünal H, Öztürk N, Bilensoy E. Formulation development, stability and anticancer efficacy of core-shell cyclodextrin nanocapsules for oral chemotherapy with camptothecin. Beilstein J Org Chem. 2015;11(1):204-212. https://doi.org/10.3762/bjoc.11.22
[32] Fayyad MS, Ghareeb MM, Kassab HJ. Study of some variables affecting product properties of felodipine nano precipitation. Int J Pharm Sci Rev Res. 2017;42:265-268.
[33] Dagtepe P, Chikan V. Quantized Ostwald ripening of colloidal nanoparticles. J Phys Chem. C 2010;114(39):16263-16269. http://dx.doi.org/10.1021/jp105071a
[34] Li J, Fu Q, Liu X, Li M, Wang Y. Formulation of nimodipine nanocrystals for oral administration. Arch Pharm Res. 2016 Feb;39(2):202-212. https://doi.org/10.1007/s12272-015-0685-5
[35] Alhagiesa AW, Ghareeb MM. The Formulation and characterization of nimodipine nanoparticles for the enhancement of solubility and dissolution rate. Iraqi J Pharm Sci. 2021;30(2):143-152. https://doi.org/10.31351/vol30iss2pp143-152
[36] Jassim ZE, Hussein AA. Formulation and evaluation of clopidogrel tablet incorporating drug nanoparticles. Int J Pharm Pharm Sci. 2014;6(1):838-851.
[37] Rajab NA, Jassem NA. A Design and in vitro evaluation of azilsartan medoxomil as a self-dispersible dry nanosuspension. Der Pharm Sin. 2018;9(1):12-32. http://dx.doi.org/10.13140/RG.2.2.22215.93606
[38] Zu Y, Li N, Zhao X, Li Y, Ge Y, Wang W, Wang K, Liu Y. In vitro dissolution enhancement of micronized l-nimodipine by antisolvent re-crystallization from its crystal form H. Int J Pharm. 2014;464(1-2):1-9. https://doi.org/10.1016/j.ijpharm.2014.01.020
[39] Alhagiesa AW, Ghareeb MM. Formulation and evaluation of nimodipine nanoparticles incorporated within orodispersible tablets. Int J Drug Deliv Technol. 2020;10(4):547-552. http://doi.org/2010.25258/ijddt.10.4.7
[40] Zeeshan F, Lin PY, Sheshala R. Application of similarity factor (f 2) and time required to drug release (t%) indicators for dissolution profiles comparison of paracetamol tablets. Indian J Pharm Educ Res. 2020;54(3). http://dx.doi.org/10.5530/ijper.54.3.114
[41] Habbal H, Karabet F. Chitosan-(Prunus avium) gum nanocapsules loaded with orange peel extract: Nanocapsules loaded with orange peel extract. Iraqi J Pharm Sci. 2023;32(1):194-201. https://doi.org/10.31351/vol32iss1pp194-201.