Formulation and Characterization of Isradipine Nanoparticle for Dissolution Enhancement

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Abstract

Isradipine belong to dihydropyridine (DHP) class of calcium channel blockers (CCBs). It is used in the treatment of hypertension, angina pectoris, in addition to Parkinson disease. It belongs to the BCS class II drug (low solubility-high permeability). The drug also suffers from extensive first pass metabolism, therefore it had low bioavailability of approximately 15-24%.

The aim of this study was to formulate and optimize a stable nanoparticles of this highly hydrophobic drug by solvent-antisolvent precipitation method to enhance in vitro dissolution rate and improve drug bioavailability.

Ten formulas of isradipine nanoparticles were prepared by antisolvent precipitation method utilizing one of these polymers Poloxamer 188, PVA, and Soluplus at different drugs: polymer ratios. The particle size, and polydispersity index (PDI) were investigated.

Among all the prepared nanoparticles formulas, formula (F9) which contain Soluplus as a stabilizer at polymer: drug ratio of (1:0.75) and solvent: antisolvent ratio of (1:9) was considered as the optimum formula which showed increment in the solubility to about 15-24 times than that of the pure drug. The investigations of the drug-excipients compatibility studies by FTIR and crystalline state by DSC, were done. Moreover, the analysis by DSC of the nanoparticles of the selected formula (F9) indicated a reduction in the crystallinity and amorphization of the drug. In addition, FTIR result indicates that hydrogen bond may be formed between soluplus and the drug, which it may have a contribution to increase affinity between them and resulted in enhanced solubility.

The results of dissolution study showed a significant increase in dissolution rate through particle size reduction. So, it can be concluded that solvent-antisolvent precipitation method was an efficient method for preparation of isradipine nanoparticles for dissolution enhancement.

Keywords: Nanoparticles, Isradipine, Dissolution rate enhancement

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Introduction

Frequent problems are result from a poor solubility of drug candidates in drug research and development. The aqueous solubility of the drug is a critical determinant of its dissolution rate, therefore limited dissolution rate arise from low solubility which results in a low bioavailability of an orally administered drug\(^{(5)}\). The solubility is a significant physicochemical property of a drug substance, mainly the aqueous solubility, so drug must be in solution to enter the systemic circulation and utilizes a therapeutic effect. However, 35-40% of new drugs discovered experienced poor aqueous solubility\(^{(5)}\).

Numerous methods were improved for resolving the drug’s poor aqueous solubility\(^{(5)}\). One of these approach is nanotechnology\(^{(6)}\). Nanoparticles (NP)s are solid particles or particulate dispersions with size in the range of 10–1000 nm. Within nanoparticle matrix the drug is dissolved, entrapped, absorbed, attached or encapsulated. According to the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained with different properties and release characteristics for an encapsulated therapeutic agent \(^{(5)}\).

The advantages of using nanoparticles for drug delivery result from their two main basic properties. First nanoparticles, due to their small size, can pass through smaller capillaries and uptake by cells, which permit an efficient drug accumulation at the target sites. Second, the use of biodegradable materials for nanoparticle preparation allows sustained drug release within the target site over a period of days or even weeks\(^{(5)}\).

Isradipine belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), isradipine binds to calcium channels with high affinity and specificity and inhibits calcium flux into cardiac and arterial Smooth muscle cells\(^{(6)}\). Isradipine is a class II drug according to BSC \(^{(7,8)}\). It is mainly absorbed from gastrointestinal tract after oral administration, undergoes extensive first-pass metabolism; as a result bio-availability is (15 to 24%) \(^{(8,9)}\). The aim of this study is to increase the solubility and enhance the dissolution rate of the poorly water-soluble (class II) isradipine by the Preparation of isradipine nanoparticles in size between 10-1000nm with studying preparation variables that affect the properties of prepared nanoparticles.

Materials and Methods

Materials

Isradipine, polaxamer 188 (PXM 188), poly vinyl alcohol (PVA) were purchased from hyper chem company, china, soluplus supplied by Sigma-Aldrich, Germany. Methanol supplied by Avantor performance materials, Norway. Hydrochloric acid from Grin land chemical comp, United Kingdom. Mannitol were purchased from Jk. Chemical. China.

And filter syringe (0.45μm) supplied by Chem Lab, Spain. Dialysis membrane 12000 Da from Schcuhardt, Germany. Na2HPO4, KH2PO4 and deionized water were supplied by Jancee for chemical and laboratory materials, Baghdad, Iraq.

Methods

Preparation of isradipine nanoparticles by solvent-antisolvent precipitation method:

Isradipine nanoparticles were prepared by using solvent/antisolvent precipitation technique (Nanoprecipitation method). Briefly, 10mg of isradipine was completely dissolved in water miscible organic solvent (methanol), then the obtained drug solution was added drop wise into the water containing one of the stabilizers (PVA, PXM 188, Soluplus) at different ratios of drug to stabilizers which acts as an antisolvent as shown in Table (1). Precipitation of solid drug particles occurred immediately upon mixing. Then, the precipitated nanoparticles stirred at agitation speed of 200 revolution per minute (rpm) on magnetic stirrer for 1 hour to allow the organic solvent to evaporate. And then lyophilized the selected formula using freeze drying system (Labconco, USA) to obtain the nanoparticles powder \(^{(10)}\).

### Table 1. Composition of isradipine nanoparticles formulas

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>Polymer type</th>
<th>Drug:polymer Ratio</th>
<th>Methanol (ml)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>PVA</td>
<td>1:1</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>F2</td>
<td>PVA</td>
<td>1:0.5</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>F3</td>
<td>PVA</td>
<td>1:2</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>F4</td>
<td>PXM 188</td>
<td>1:1</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>F5</td>
<td>PXM 188</td>
<td>1:0.5</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>F6</td>
<td>PXM 188</td>
<td>1:2</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>F7</td>
<td>Soluplus</td>
<td>1:0.5</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>F8</td>
<td>Soluplus</td>
<td>1:0.75</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>F9</td>
<td>Soluplus</td>
<td>1:1.5</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>F10</td>
<td>Soluplus</td>
<td>1:2</td>
<td>3</td>
<td>27</td>
</tr>
</tbody>
</table>
**Characterization of isradipine nanoparticles**

**Particle size and polydispersity index analysis**

Particle size distribution analysis was done by using ABT-9000 nano laser particle size analyzer which is a dynamic light scattering, works by measuring the intensity of light scattered by the molecules in the sample as a function of time, at a constant temperature of 25 °C without dilution the samples. The average particle size was measured for all the prepared formulas. Each sample was measured in triplicate. The polydispersity index (PDI), as measures for the width of the size distribution for each sample was also measured by the instrument (13).

**Selection of optimized isradipine nanoparticle formula**

Depending on the characterization study for the formulations of isradipine such as particle size, and PDI, the optimized formula was selected which should have reliable results in studying characterizations.

The optimized formula was then subjected for further studies such as DSC, drug-excipient compatibility study.

**Characterization of selected isradipine nanoparticles formula**

**Determination of saturation solubility of isradipine nanoparticles**

Saturation solubility of the selected isradipine lyophilized powder was investigated in water, 0.1 N HCl and phosphate buffer pH 6.8. In each case, the excess quantity of sample was added to 10 ml of solvent and agitated at 25°C in a water bath shaker for 72 hrs. After equilibration, the samples were centrifuged at 4000 rpm for 10 min, filtered using 0.45 μm millipore filters, suitably diluted and analyzed by measuring the absorbance using UV-visible spectrophotometer at 327 nm, 328 nm, 329nm for 0.1N HCl, phosphate buffer pH 6.8, and water respectively, for the amount of drug dissolved (12-14).

**Determination of isradipine content in nanoparticles**

The assay was performed using a lyophilized powder, equivalent to 2.5mg isradipine, that dissolved in 100 ml methanol. The samples were stirred and subsequently centrifuged at 6000 rpm for 10 min. The contents of isradipine in a sample were analyzed after suitable dilution using UV spectrophotometer at 326 nm. Drug content was calculated by the equation.

\[
\text{Drug content} = \frac{\text{Analyzed content}}{\text{Theoretical content}} \times 100
\]

**In-vitro dissolution profile of isradipine nanoparticle**

In-vitro dissolution test of isradipine nanoparticles was estimated using (paddle assembly) type II dissolution test apparatus.

Accurately weighed lyophilized nanoparticles of the selected formula equivalent to 2.5 mg of isradipine was mixed with 5ml of the dissolution media and placed in a dialysis bag and rotated by a paddle at 50 rpm at 37 ± 0.5°C in 500ml of dissolution medium, 0.1N HCl with 1% tween 20, to ensure sink condition. An aliquot of 5 ml samples was drawn from receiver compartment at predetermined time intervals and refilled the equal volume of fresh dissolution medium to conserve the constant volume. Then, samples were filtered by 0.45μm filter syringe and assayed spectrophotometrically by UV–spectrophotometer at 327 nm (15). The same procedure was made for the pure drug.

**Crystallinity study**

**Differential Scanning Calorimetry (DSC) analysis**

The DSC was used to assess the crystallinity properties of the various substances and formulations. Pure isradipine powder and lyophilized nanoparticles (3-5mg) were sealed in the flat-bottomed aluminum pan of the differential scanning calorimeter (Shimadzu DSC-60 plus, Japan). Data collection was achieved at a temperature range of 30–300°C and the heating rate was 10°C/min under nitrogen gas at a flow rate of 40 ml/min (16).

**Compatibility study**

**Fourier Transform Infrared Spectroscopy (FTIR)**

The FTIR spectra of pure isradipine, optimized polymer, and lyophilized nanoparticles were performed using FTIR7600, (Australia spectrophotometer). Powders were mixed with potassium bromide (spectroscopic grade) and compressed into disks using hydraulic press before scanning from 4000 to 400 cm⁻¹ (17).

**Statistical analysis**

The experimental results were expressed as a mean triplicate sample ± standard deviation (SD) and were analyzed according to one-way analysis of variance (ANOVA) using SPSS software at which the results would be significant when p < 0.05, and the results would be non-significant if p> 0.05. similarity factors (f2) was also calculated to compare dissolution profiles, where f2 more than 50 consider similar , while less than 50 consider dissimilar using DD Solver program(17, 18).

**Results and Discussion**

**Characterization of isradipine nanoparticles particle size analysis and polydispersity index**

The particle size and PDI results of the prepared isradipine nanoparticles are shown in Table (2). For all the prepared formulas, the average particle size and PDI were analyzed by particle size analyzer .

The size of the particle was in the range of 77.34 – 1582.56 nm. The particle size determines the absorption and bioavailability of the drug; the smaller particle size of nanosize provides a larger
surface area, which leads to faster drug release into aqueous medium \(^{(19)}\).

On the other hand, PDI which is a measure of the size distribution of the nanoparticles; were ranged from (0.005-0.395) depending on formulation variables, (0.005) indicating good uniformity, monodisperse system in the particle size distribution of nanoparticles while (0.395) indicated polydisperse system \(^{(20)}\).

**Table 2. The particle size and PDI of isradipine nanoparticles**

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>stabilizer</th>
<th>Drug:polymer Ratio</th>
<th>Particle size average(nm)</th>
<th>Polydispersity index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>PVA</td>
<td>1:1</td>
<td>554.36± 0.012</td>
<td>0.205</td>
</tr>
<tr>
<td>F2</td>
<td>PVA</td>
<td>1:0.5</td>
<td>913.59±0.004</td>
<td>0.269</td>
</tr>
<tr>
<td>F3</td>
<td>PVA</td>
<td>1:2</td>
<td>769.1±0.143</td>
<td>0.023</td>
</tr>
<tr>
<td>F4</td>
<td>PXM 188</td>
<td>1:1</td>
<td>726.82±0.002</td>
<td>0.395</td>
</tr>
<tr>
<td>F5</td>
<td>PXM 188</td>
<td>1:0.5</td>
<td>1582.5±0.001</td>
<td>0.332</td>
</tr>
<tr>
<td>F6</td>
<td>PXM 188</td>
<td>1:2</td>
<td>1017.8±0.004</td>
<td>0.246</td>
</tr>
<tr>
<td>F7</td>
<td>Soluplus</td>
<td>1:1</td>
<td>120.9±0.120</td>
<td>0.279</td>
</tr>
<tr>
<td>F8</td>
<td>Soluplus</td>
<td>1:0.5</td>
<td>228.35±0.101</td>
<td>0.345</td>
</tr>
<tr>
<td>F9</td>
<td>Soluplus</td>
<td>1:0.75</td>
<td>77.34±0.003</td>
<td>0.005</td>
</tr>
<tr>
<td>F10</td>
<td>Soluplus</td>
<td>1:1.5</td>
<td>219.68±0.005</td>
<td>0.352</td>
</tr>
</tbody>
</table>

**Selection of optimized isradipine nanoparticle formula**

From the overall results of the particle size analysis, PDI of nanoparticle formulations, formula F9 was selected as the best formula that characterized by a low particle size (77.34 nm), with PDI (0.005). Therefore, the selected formula (F9) was subjected to further investigations of drug-excipient compatibility and crystalline state determination.

**Characterization of selected isradipine nanoparticles:**

**Determination of saturation solubility of isradipine nanoparticles:**

The saturated solubility of the lyophilized isradipine powder of F9 was increased significantly (\(p <0.05\)), more than ten times in water, over the pure drug and about six times in HCl and more than ten times in phosphate buffer pH 6.8.

This was due to the decrease in particle size which increased the surface area and due to the presence of surfactant within its composition that enhanced the solubility; as shown in figure (1) and table (3).

**Table 3. Saturated solubility of pure isradipine and selected lyophilized formula F9 in different solvents**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility of pure drug(mg/ml) mean ±SD*</th>
<th>Solubility of lyophilized isradipine nanoparticles (mg/ml) mean ±SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl pH 1.2</td>
<td>0.040± 0.023</td>
<td>0.259± 0.012</td>
</tr>
<tr>
<td>Phosphate buffer pH 6.8</td>
<td>0.010±0.187</td>
<td>0.106± 0.103</td>
</tr>
<tr>
<td>Water</td>
<td>0.014±0.002</td>
<td>0.211± 0.015</td>
</tr>
</tbody>
</table>

*SD Standard deviation from the mean of a triplicate sample

**Determination of isradipine content in nanoparticles**

Drug content of the prepared Isradipine nanoparticles was \(96.89 \pm 0.038\%\), which meet BP (British pharmacopoeia) requirement and were within an acceptable range (95%-110\%).\(^{(21)}\) indicating that, there was no precipitation or lose of drug.

**In-vitro dissolution profile of lyophilized isradipine nanoparticle**

In-vitro drug release studies of isradipine nanoparticles of the selected formula (F9) and pure drug were conducted in 0.1N HCl to simulate in-vivo release in the stomach.\(^{(22)}\)
Comparing the dissolution profiles of the selected formula of isradipine nanoparticle, with the pure drug as a reference. It was observed that the $f_2$ value equal to 23.53 indicating that, the dissolution of the selected formula of isradipine nanoparticles was dissimilar, faster than that of pure drug powder as shown in figure (2).

These results were expected according to Noyes–Whitney equation where the dissolution rate is directly proportionate to its surface area exposed to the dissolution medium and saturation solubility of the prepared drug dosage form (23).

![Figure 2. Dissolution profile for pure drug ,F9 (Isradipine nanoparticles ) in 0.1N HCl at 37°C](image)

**Crystallinity study**

**Differential Scanning Calorimetry (DSC) Analysis**

Isradipine peak was clear in its DSC thermogram as a sharp endothermic peak around its melting point (170.3°C) such sharp endothermic peak indicates that isradipine used is in crystalline and pure state (24). The thermogram of pure isradipine is shown in Figure (3).

![Figure 3. Differential scanning calorimetry of pure Isradipine](image)

**Drug- excipients compatibility study**

**Fourier Transform Infrared Spectroscopy (FTIR)**

The FTIR spectra of pure isradipine and lyophilized isradipine nanoparticle are shown in figures (5 , 6), respectively. Pure Isradipine exhibited characteristic peaks shown in table (4) at 3347 cm$^{-1}$ (N-H stretching), 2948 cm$^{-1}$ (C-H stretching), 1,702.84 cm$^{-1}$ (C=O stretching), and 1,490 cm$^{-1}$ (C=C vibration) (Figure 5). All these peaks had appeared in the spectra of the selected formula (Figure 6)(7).

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of bands</th>
<th>Pure Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C=O vibration</td>
<td>1702.84 cm$^{-1}$</td>
</tr>
<tr>
<td>2</td>
<td>C=C vibration</td>
<td>1490 cm$^{-1}$</td>
</tr>
<tr>
<td>3</td>
<td>C-H stretching</td>
<td>2948 cm$^{-1}$</td>
</tr>
<tr>
<td>4</td>
<td>N-H stretching of amide</td>
<td>3347 cm$^{-1}$</td>
</tr>
</tbody>
</table>

From the FTIR results, it was found there was no changes in the peaks of isradipine spectrum to that of the spectra of lyophilized isradipine nanoparticles F10, and all the functional groups in Isradipine were maintained with low intensity due to dilution with the polymer .In addition hydrogen bond may be formed between soluplus and the drug shown as broad peak at 3363 , which it may have a contribution to increase affinity between them and resulted in enhanced solubility.
Figure 5. FTIR spectrum of pure Isradipine

Figure 6. FTIR of Isradipine nanoparticle (F9)
Conclusions

Based on the results obtained, the study concluded that nanoparticles is a promising approach to improve the solubility, dissolution rate and release of isradipine and solvent-antisolvent precipitation is an efficient method for preparation of isradipine nanoparticles.

Isradipine nanoparticles were successfully prepared using soluplus as stabilizer at ratios (1:0.75 Drug:polymer) that gave a higher in-vitro release profiles compared to pure drug powder. DSC confirm that the crystalline structure of isradipine was lost and transformed to the amorphous state when the drug was converted into nanoparticles. Drug–excipients compatibility studies revealed that hydrogen bond may be formed between soluplus and the drug, which may have a contribution to increase affinity between them and resulted in enhanced solubility.

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References

