Felodipine oral nanoemulsions

Formulation and Characterization of Felodipine as an Oral Nanoemulsions

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Abstract

Felodipine is a calcium-channel blocker with low aqueous solubility and bioavailability. Lipid dosage forms are attractive delivery systems for such hydrophobic drug molecules. Nanoemulsion (NE) is one of the popular methods that has been used to solve the dispersibility problems of many drugs. Felodipine was formulated as a NE utilizing oleic acid as an oil phase, tween 80 and tween 60 as surfactants and ethanol as a co-surfactant. Eight formulas were prepared, and different tests were performed to ensure the stability of the NEs, such as particle size, polydispersity index, zeta potential, dilution test, drug content, viscosity and in-vitro drug release. Results of characterization showed that felodipine nanoemulsion (F3) with (oleic acid 10%), (Smix 60% of tween80: ethanol in a ratio of 3:1), (DDW 30%) was selected as the best formula, since it has a particle size of (17.01 nm), low PDI (0.392), zeta potential (-22.34 mV), good dilution without drug precipitation, higher percent of drug content (99.098%) with acceptable viscosity, and complete release of the drug after (45 min) with significantly higher (P<0.05) dissolution rate in comparison with the pure drug powder. The selected formula (F3) subjected to further investigations as drug and excipient compatibility study by Fourier transform infrared spectroscopy (FTIR). The outcomes of the (FTIR) explain that the distinctive peaks for felodipine were not affected by other components and displayed the same functional group's band with very slight shifting. This indicates that there was no interaction between felodipine and other NE components. Therefore, these results were deemed to be compatible with felodipine. In conclusion, the NE was found to be an efficient method to enhance the dispersibility and permeation of drugs that have poor water solubility (lipophilic drugs).

Keywords: Nanoemulsion, Felodipine, Lipid dosage forms.

Table 1. Biopharmaceutics classification system (BCS) of the drugs (1)

| Class | Solubility | Permeability | Example |
|-------|------------|--------------|---------|
| Class-I | High       | High         | metoprolol |
| Class-II | Low        | High         | felodipine |
| Class-III | High      | Low          | captopril  |
| Class-IV | Low        | Low          | furosemid. |

Introduction

Biopharmaceutics classification system (depending on drug solubility and permeability) as (BCS) divide the drugs into four categories shown in Table (1).

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Many drugs belong to class-II group display low bioavailability due to poor solubility and insufficient dissolution process, to improve the bioavailability of drugs that belong to class II and obtain a good clinical efficacy, the solubility of these drugs must be increased and the result can be achieved by using several techniques that lead to enhancing the solubility of poorly water-soluble drugs which they are reduction of particle size, pH adjustment, solid dispersion, formation of salt and nanotechnology (1-5).

Nanoemulsion is defined as a novel and advance drug delivery system that has a great devotion to the delivery of drugs. Nanoemulsions, also known as submicron emulsions, are submicron sized colloidal particulate systems deliberated as thermodynamically and kinetically stable isotropic dispersions, which consist of two immiscible liquids like water and oil, stabilized by an interfacial film forming agent consisting of a suitable surfactant and co-surfactant to form a single phase. It leads to improve the solubility of poorly soluble drugs (lipophilic drugs) which results in an improvement of the bioavailability of these drugs (3).

Felodipine (FLD) is a dihydropyridine calcium-channel blocker used in the treatment of elevated blood pressure and angina pectoris (m.w. 384.3 Dalton, m.p. 145°C, pKa 5.39, practically insoluble in aqueous medium, freely soluble in acetone, ethanol, methanol and in methylene chloride). FLD is more selective vasodilator and have fewer cardiac action than non-dihydropyridine calcium-antagonists. But this advantage is absent due to poor bioavailability of this medicament, which (although the drug is absorbed totally from the GIT) is simply 15% of the dose is available in blood circulation when it is administered orally. The low bioavailability of felodipine is attributed to its low aqueous solubility and also due to extreme first-pass metabolism (5). This study aimed to prepare and in vitro evaluate of felodipine nanoemulsion in order to enhance dispersibility and dissolution rate of drug.

**Materials and Methods**

**Materials**

Felodipine powder was purchased from Baoji Guokang Bio- Technology Co.Ltd. Tween 80 was purchased from Riedel-De-Haen, Germany. Tween 60 was provided from Avonchem, England. Dialysis membrane (12000 Da) provided from Schuchardt, Germany. All other chemicals and solvents were of analytical reagent grade.

**Methods**

**Saturation solubility study of felodipine**

Saturated solubility of felodipine was measured in various oils (triacetin, oleic acid, olive oil, corn oil, lavender oil, sunflower oil, lime oil, saessme oil), surfactants (tween 20, tween 60, tween 80), co-surfactants (PEG 400, ethanol and methanol) and dissolution media (0.1 N HCl containing 1% tween80) to ensure sink condition. The measurement of solubility was done as follow: Excess amount of felodipine was added to (5ml) of each selected individual oils, surfactants and co-surfactants contained in stoppered vials separately, then was shaken using a water bath shaker for 72 hrs at 25±1°C for the oils, surfactant and co-surfactants and at 37±1°C for the dissolution media to prepare a saturated solution (5). After reaching equilibrium, the mixtures were centrifuged at 3000rpm for 15min, followed by filtration through a 0.45μm millipore filter, samples were suitably diluted with ethanol and analyzed by UV/Vis spectrophotometer at λmax of felodipine and the measurements were done in triplicate.

**Pseudo-ternary phase diagrams construction**

Construction of the pseudo-ternary phase diagrams was done by using aqueous titration method. Based on the solubility studies, oleic acid was selected as an oil phase, tween 80 and tween 60 were selected as surfactant and ethanol were selected as a co-surfactant, and deionized water (DDW) used as an aqueous phase. The oil: surfactant: co-surfactant (Smix) mixed at different ratio. For each phase diagram, oil and Smix (at a specific ratio) were mixed gradually at different ratios (ranging between 1:9 to 9:1) in different glass vials (6). Smix ratios was 1:3, 1:2, 1:1, 2:1 and 3:1 for Smix (tween 80:ethanol) and 1:2, 2:1 and 3:1 for Smix tween 60:ethanol.

**Preparation of felodipine nanoemulsion**

Different o/w nano emulsion formulations (Table 2) were prepared using the Smix and oil ratios according to pseudo-ternary phase diagrams. The preparation of primary felodipine nanoemulsion occur through dissolving (5 mg) of the drug in the selected oil, then magnetic stirrer was used then the selected Smix added slowly in a fixed proportion until clear solution was gained followed by the addition of deionized distal water dropwise to the clear solution with continuous stirring ((~500 rpm) at room temperature till formation of clear emulsion. The prepared emulsions then were ultrasonicated using a 20 kHz sonicator for 10 min to produce very small droplet size NEs. (7).
Characterization of the prepared nanoemulsion:

**Droplet size and polydispersity index (PDI)**

The droplet size of NE was determined by analyzing the fluctuations in light scattering due to the Brownian motion of the particle using the dynamic light scattering technique (Zetasizer Nano). Nanoemulsion was diluted with distilled water (100-fold) and gently stirred (to increase the homogeneity) before measurement (8). While the measurement of (PDI) gives information about the uniformity of droplet size within the formulated NE. The lower PDI value (near zero) indicates a monodisperse droplet population, whereas a PDI value closer to 1 indicates a wide range of droplet size (9).

**Transmittance percentage (%T)**

The translucence of the prepared nanoemulsions was checked by the turbidity test. By taking 2 ml of each nanoemulsion formula and measuring absorbance at 650 nm (light wavelength) using UV/Vis spectrophotometer and distilled water was used as a blank (10).

**Dilution test**

Aqueous dilution test was done, 1 mL of each nanoemulsion formula from (F1-F8) diluted to 50 mL, 100 mL and 500 mL with distilled water at 37°C with constant stirring and was maintained at 50 rpm. turbidity, clarity and the phase separation for each formula was observed visually (11).

**Drug content estimation**

Accurately, 10 ml of each NE formula which contains (5mg) of felodipine was dissolved in 100 ml ethanol, then filtered using 0.45 μm filter syringe and suitably diluted. The contents of felodipine was determined using UV/Vis spectrophotometer at the selected λ max (12).

**Zeta potential measurement (ζ – potential)**

The droplet charge (zeta potential) of the selected NE formula was determined by using a dynamic light scattering technique (Zetasizer Nano ZS) (13).

**Viscosity measurement**

Viscosity is very important for stability and efficient drug release. Nanoemulsion carrier formulations are basically oil-in-water and so in addition to being less greasy than water-in-oil formulations, often possess lower apparent viscosities. They are therefore expected to exhibit faster release of active ingredients (14).

The low viscosity of systems shows that it is o/w type and high viscosity shows that it is w/o type system. Measurements were performed using viscometer spindle number 2 that was immersed in 100-ml sample of each prepared NE formulas and rotated at different speeds (15).

**In vitro drug dissolution study**

The in vitro release of felodipine loaded NE occur using USP dissolution apparatus type – II (paddle method). Ten ml of each formula which contains (5mg) of felodipine was poured in the dialysis bag (Molecular cut off 12000Da), then this bag immersed in 500 ml of dissolution medium. The dissolution medium was (0.1N HCl with 1% tween 80), the dissolution apparatus set at 37 ± 0.5 °C, and the rotation speed was 50 rpm (16). Nanoemulsion containing felodipine equivalent to one dose(10ml) was placed in a dialysis bag, and five ml of dissolution medium was withdrawn at 5, 10, 15, 30, 45, 60, 90 and 120 min time intervals and the samples then filtered using a 0.45 μm filter syringe and analyzed by UV/Vis spectrophotometer at the λ max of the drug the study was done in triplicate (17).

**Selection of the optimum formula**

The choice of the optimum formula was accomplished, and this achieved according to the droplet size, PDI, transmittance percentage, dilution test, drug content, viscosity, and in vitro release studies.

**Evaluation of the selected felodipine optimum formula**

**Drug and excipient compatibility study by FTIR Fourier transform infrared spectroscopy (FTIR)**

To investigate any possible interaction between the drug and the utilized excipients (oleic acid, tween 80, ethanol) in the selected formula. Pure drug was mixed with potassium bromide and pressed in a form of a disc. Oleic acid, tween 80, ethanol and the selected formula (liquid samples) were analyzed by an FTIR device for liquid samples FTIR spectroscopy analyzed all the samples from 4000-400 cm⁻¹ (18).

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Table 2. Composition of different felodipine nanoemulsion

| NE-F | Felodipine | Oleic acid | surfactant | Co-surfactant | Smix ratio | Smix % w/w | DDW % w/w |
|------|------------|------------|------------|--------------|-------------|-------------|-----------|
| F1   | 0.05 w/w   | 10%        | Tween 80   | Ethanol      | 1:1         | 60          | 30        |
| F2   | 0.05 w/w   | 10%        | Tween 80   | Ethanol      | 2:1         | 60          | 30        |
| F3   | 0.05 w/w   | 10%        | Tween 80   | Ethanol      | 3:1         | 60          | 30        |
| F4   | 0.05 w/w   | 10%        | Tween 80   | Ethanol      | 1:2         | 60          | 30        |
| F5   | 0.05 w/w   | 10%        | Tween 80   | Ethanol      | 1:3         | 60          | 30        |
| F6   | 0.05 w/w   | 10%        | Tween 60   | Ethanol      | 1:2         | 60          | 30        |
| F7   | 0.05 w/w   | 10%        | Tween 60   | Ethanol      | 2:1         | 60          | 30        |
| F8   | 0.05 w/w   | 10%        | Tween 60   | Ethanol      | 3:1         | 60          | 30        |
Results and Discussion

Saturation solubility study of felodipine

The solubility of felodipine as shown in table below was higher in oleic acid so that oleic acid was used in the formulations to keep the drug in solubilized form, and no precipitation of drug will occur\(^{(19,20)}\). Regarding surfactants, tween 80 and tween 60 were selected as a surfactant to obtain a one-phase clear solution. Considering cosurfactants, ethanol was found to have the higher solubilizing capacity for felodipine, it would increase the miscibility of the aqueous and oily phases due to its partitioning between these phases to reduce the interfacial tension also increase the mobility of the hydrocarbon tail and allow greater penetration of the oil into this region\(^{(21)}\).

Table 3. Saturation solubility study of felodipine in different oils, surfactants, co-surfactants and dissolution media.

| Oil          | Solubility (mg/ml) mean ±SD* |
|--------------|------------------------------|
| Sesame oil   | 27.0163±1.8029               |
| Triacetin    | 29.4836±0.6467               |
| Oleic acid   | 49.733±0.6976                |
| Lime oil     | 4.1796±0.1067                |
| Lavender oil | 3.7856±0.0784                |
| Olive oil    | 17.3536±1.9459               |
| Sunflower oil| 4.5463±0.3408                |

| Surfactant   | Solubility (mg/ml) mean ±SD* |
|--------------|------------------------------|
| Tween 60     | 36.89827±0.4072              |
| Tween 80     | 47.0603±0.4776               |
| Tween 20     | 27.119±0.3142                |

| Co surfactant| Solubility (mg/ml) mean ±SD* |
|--------------|------------------------------|
| Ethanol      | 48.8016±0.4834               |
| Methanol     | 32.665±0.23926               |
| PEG 400      | 5.07133±0.0475               |

| Dissolution media | Solubility (mg/ml) mean ±SD* |
|-------------------|------------------------------|
| 0.1 N HCl (with 1% tween 80) | 32.9 ±0.692 |

\*SD standard deviation from the mean, n=3

Construction of pseudo-ternary phase diagrams

Figures 1 and 2 showed the pseudo-ternary phase diagram for the o/w NEs using oleic acid as an oil phase, tween 80 and tween 60 as a surfactant and ethanol as a co-surfactant.

![Figure 1](image1.png)

![Figure 2](image2.png)

Figure (1 and 2). Pseudo-ternary phase diagrams showing the (o/w) nanoemulsion (colored area) regions of oleic acid at different Smix ratios.

Characterization of the prepared nanoemulsions:

Droplet Size and Polydispersity Index (PDI)

Table (4) showed the results of droplet size measurement and polydispersity index. Also, in regard to particle size, the results showed that when the concentration of surfactant increased the particles size reduced, since this high surfactant concentration decreases surface tension and stabilizes newly developed surfaces during homogenization and production of smaller particles, these results may be also due to accumulation of surfactant molecules at the interface provides better stabilization against droplet aggregation and helps in lowering the flocculation rate, as well as greater penetration of the oil phase in the hydrophobic
region of the surfactant, lead to reduction the droplet size \(^{(22)}\). While the PDI refers to the quality of a polydispersity index and it is not stable. The low value of PDI (0.08–0.7) is considered to be desirable for uniform distribution, stability and high of the dispersion \(^{(23)}\). The higher the value of PDI (>0.7) indicate that the sample has a very broad particle size distribution quality and homogeneity of nano-sized droplets within the preparation \(^{(24)}\).

### Table 4. Particle size and polydispersity index of the NE formulas.

| F code | Particle size (nm) | F code | PDI  |
|--------|-------------------|--------|------|
| F1     | 197.6 nm          | F1     | 0.298|
| F2     | 48.88 nm          | F2     | 0.421|
| F3     | 17.01 nm          | F3     | 0.392|
| F4     | 47.40 nm          | F4     | 0.395|
| F5     | 27.44 nm          | F5     | 0.393|
| F6     | 31.74 nm          | F6     | 0.368|
| F7     | 19.70 nm          | F7     | 0.367|
| F8     | 18.75 nm          | F8     | 0.366|

### Figure 3. Particle size distribution of felodipine nanoemulsions.

**Transmittance percentage (%)**

Transmittance percentage of felodipine nanoemulsion formulas demonstrates that all these formulas were translucent, clear and convey the light easily since the values of percentage transmittance closer to 100 % since the reducing droplet sizes to the nanoscale was lead to higher transparency \(^{(25)}\).

**Dilution test**

All nanoemulsion formulas (F1-F8) showed fine bluish to clear nanoemulsion indicating o/w type, proved that they could be diluted in GI fluids and maintaining the nanosized character without drug precipitation. Thus, it is anticipated that absorption will be enhanced \(^{(26)}\).

### Drug content

Results shown in table (6) indicated that all nanoemulsion formulas agreed with the requirements of the British Pharmacopeia range (87.2 % - 109.6 %) indicating that, there was no precipitation of drug in any of prepared formulations...
Table 6. Drug content percentage of the prepared nanoemulsions

| F Code | % Drug content |
|--------|----------------|
| F1     | 93.539         |
| F2     | 94.224         |
| F3     | 99.098         |
| F4     | 96.28          |
| F5     | 95.252         |
| F6     | 93.881         |
| F7     | 95.937         |
| F8     | 95.595         |
| F9     | 98.336         |

Zeta potential measurement

Zeta potential values of merely 30 mV or much lower can supply enough stabilization (27) and the zeta potential value of the selected formula as shown in figure (4) was found to be (-22.34), which would be increase the stability of the nanoemulsion as the individual droplets repels each another in order not to coalescence into larger globules.

Figure 4. Zeta potential of formula (F3)

Viscosity measurements

From the figure (5), it was demonstrated as the concentration of the surfactant increased; the viscosity increased this may be due to entrapping of the water molecules in cross-linking surfactants chains and also highest surfactant concentration would make the dispersion medium more rigid (28). The results also showed that the viscosity decreased as the rotation speed increased (shear rate) indicating the pseudoplastic (shear thinning liquids) flow of the preparation (29).

In vitro drug dissolution study

Higher and faster the absorption, and hence quicker and greater the drug action can be obtained by smaller the particle size of a drug in the dosage forms (30). The release of felodipine from the formula that contain tween 80 as surfactant (F3) was higher than that contain tween 60 (F8) which could be explained by the smaller droplet size of formulas containing tween 80 as compared to that formulas which contain tween 60 leading to greater rate of dissolution. While the release of formulas that contain tween 80 (F2, F4) is greater than formulas contain tween 60 (F7, F6) due to the higher HLB value of tween 80 which is 15 enhanced the continuous distribution and solubilization of the incorporated lipophilic drug within the system. (31) as shown in figures 5, 6.

Figure 5. Viscosities data of prepared felodipine nanoemulsion formulas (F1, F2,F3,F4,F5,F6,F7,F8).
Figure 6. A comparative dissolution profile of felodipine nanoemulsions (F1, F2,F3,F4 and pure felodipine) in 500ml of 0.1 N HCl (containing 1% tween 80) dissolution medium at 37°C.

Figure 7. A comparative dissolution profile of felodipine nanoemulsions (F5, F6,F7,F8 and pure felodipine) in 500ml of 0.1 N HCl (containing 1% tween 80) dissolution medium at 37 °C.

Fourier transform infrared spectroscopy (FTIR)

The spectrum of the selected formulas (F3) represented in figure (9) reveal presence of main peaks of drug which indicates that there is no considerable interaction between drug and excipients during preparation of nanoemulsion.

Figure 8. The FTIR spectrum of pure felodipine

Figure 9. FTIR spectrum of the selected formula (F3)

Conclusion

All the nanoemulsion formulas prepared with oleic acid as an oil phase, tween 80,tween 60 as a surfactant,ethanol as a co-surfactant provided a significant increase (P<0.05) in the dissolution rate compared to pure drug powder. The formula (F3) with oleic acid oil and Smix (tween80: ethanol) in a ratio of (3:1) was selected as an optimum formula. No chemical interaction between felodipine and other components in the preparation of nanoemulsion. The present study proved that nanoemulsion technology is an efficient method for administering aqueous insoluble drugs like felodipine in a liquid dosage form.

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