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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 (-22.34mV), 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 excipients were found to be compatible with felodipine. In conclusion, the NE was found to be an efficient method to enhance the dispersibility and permeatioins of drugs that have poor water solubility (lipophilic drugs).

Keywords: Nanoemulsion, Felodipine, Lipid dosage forms.

صياغه وتوصيف الفيلودبين كمستحلب نانوي فموي

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الخلاصه

ان العديد من المركبات الصيدلانية الفعالة لديها مشاكل في الذوبان حتى الآن، والتي اصبحت عقبة رئيسية تقيد استخدامها في المستحضرات الصيدلانية. ان عقارالفيلودبين هو مانع لقنوات الكالسيوم له ذوبان مائي وتوافر حيوي منخفض جدا. ان الصيغ الصيدلانية الدهنية هي صيغ دوائية جذابة لمثل هذه الجزيئات القليلة الذوبان والمستحلب النانوي هو واحد من الانظمة الشائعة التي تم استخدامها لحل مشاكل الذوبان في العديد من الأدوية. لقد تم تصييغ الفيلودبين كمستحلب نانوي باستخدام الاوليك اسد ، توين ٨٠ وتوين ٦٠ كخافض للسطح والإيثانول كمضاد للشد السطحي . تم تحضير ثمانيه تركيبات سائلة وأجريت لها اختبارات مختلفة لضمان ثبات المستحلب النانوي مثل قياس حجم القطيرات، مؤشر التشتت، جهد زيتا، اختبار التخفيف، تقدير محتوى العقار، مستوى اللزوجة ومستوى تحررالعقار في المختبر. لقد أظهرت نتائج التوصيف أن التركيبة رقم (٣) لمستحلب الفيلودبين والتي تحتوي (oil: Smix (3:1 DDW بنسب (60:30:10)كأفضل صيغة لها حجم قطيرة (١٧،٠١) نانومتر، مؤشر التشتت (0.392)، جهد زيتا (-22.34 mV)، عدم ترسب الدواء عند اجراء اختبار التخفيف، نسبة مئوية عالية من محتوى الدواء (99.098%)مع لزوجة مقبولة وتحرر العقار الكامل بعد 45 دقيقة واعلى بكثير ($P< ٠،٠٥$) عند المقارنة مع مسحوق الدواء النقي. الصيغة المفضلة (F3) قد خضعت لمزيد من الاختبارات مثل دراسة التوافق مع المكونات الاخرى بواسطة الفحص بالأشعة تحت الحمراء (FTIR) حيث يوضح (FTIR)أن القمم المميزة للـعقار لم تتأثر وهذا يشير إلى وجود توافق بين المستحلب النانوي والمكونات الأخرى. مما يؤكد على عدم وجود تفاعل بين العقار ومكونات الصيغة المفضلة ,لذلك تم التأكد من توافق العقار مع مكونات المستحلب النانوي الاخرى . من النتائج التي تم الحصول عليها تبين ان المحلول النانوي وسيلة فعالة لزيادة قابلية الذوبان لعقار (الفيلودبين) وبهذا يمكن اعتباره طريقة جيدة لتحسين الذوبانية المائية لكثير من الادوية.

الكلمات المفتاحية :مستحلب نانوي ، فيلودبين ، الصيغ الصيدلانية الدهنية .

Introduction

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

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.

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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 improved dissolution process and this 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[2].

Nanoemulsion is defined as a novel and advance drug delivery system that has a great devotion to the delivery of drugs. Nanoemulsions, also is 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. 145C°, 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 [4]. This study aimed to prepare and in vitro evaluate of felodine 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.LtdTween 80 was purchased from Riedel-De-Haen, Germany.Tween 60 was provided from Avonchem, England.Dialysis membrane(12000 Da) provided from Schcuhardt , 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,seassme 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 ratios) 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, 2:1and 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].

Table 2. Composition of different felodipine nanoemulsion

NE-F	Felodipin w/w	Oleic acid %w/w	surfactant	Co-surfactant	Smix ratio	Smix %w/w	DDW% w/w
F1	0.05w/w	10%	Tween80	Ethanol	1:1	60	30
F2	0.05w/w	10%	Tween80	Ethanol	2:1	60	30
F3	0.05w/w	10%	Tween80	Ethanol	3:1	60	30
F4	0.05w/w	10%	Tween80	Ethanol	1:2	60	30
F5	0.05w/w	10%	Tween80	Ethanol	1:3	60	30
F6	0.05w/w	10%	Tween 60	Ethanol	1:2	60	30
F7	0.05w/w	10%	Tween 60	Ethanol	2:1	60	30
F8	0.05w/w	10%	Tween 60	Ethanol	3:1	60	30

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 (ʒ – 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 dropletsize, 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].

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 co-surfactants, 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	27.01633±1.8029
Triacetin	29.4836±0.6467
Oleic acid	49.733±0.6976
Lime oil	4.1796±0.1067
Lavender oil	3.78566±0.0784
Olive oil	17.35367±1.9459
Sun flower oil	4.54633±0.3408
Surfactant	**Solubility(mg/ml) mean ±SD***
Tween 60	36.89827±0.4072
Tween 80	47.06033±0.4776
Tween 20	27.119±0.3142
Co surfactant	**Solubility(mg/ml) mean ±SD***
Ethanol	48.80167±0.4834
Methanol	32.665±0.23926
PEG 400	5.071333±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 1and 2 showed the pseudo-ternary phase diagram for the o/w NEs using oleic acid as an oil phase, tween 80 and tween60 as a surfactant and ethanol as a co-surfactant

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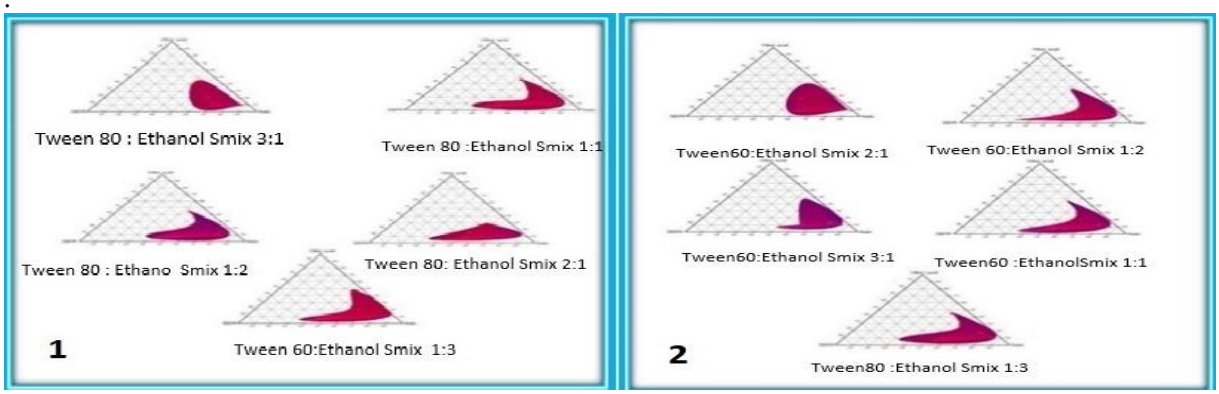

Figure (1and 2). Pseudo-ternary phase diagrams showing the (o/w) nanoemulsion (colored area) regions of oleic acid at different Smix ratios.

Characterization of the prepared nanoemulsions: Droplet Size and Polydispersity Index (PDI)

Table (4) showed the results of droplet size measurement and poly dispersity 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 poly pisprersity 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

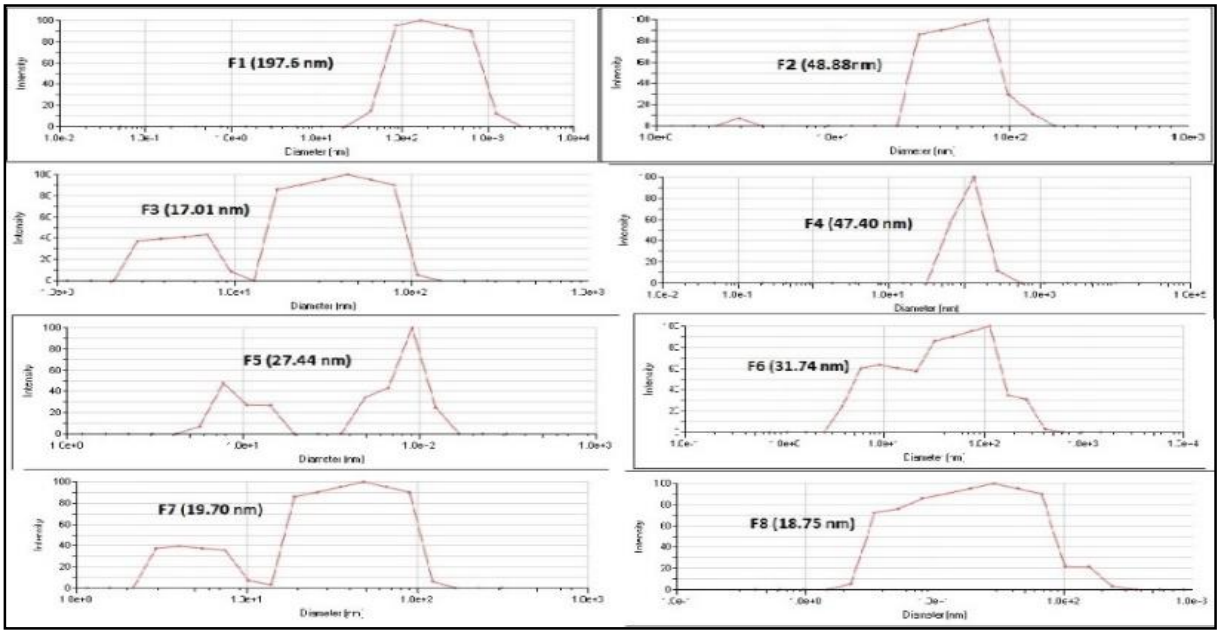

Figure 3.Particle size distribution of felodipine nanoemulsions.

Transmittance percentage (%T)

Transmittance percentage of felodioine 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].

Table 5. Percentage of transmittance (%T) of felodipine nanoemulsion

F code	Absorbance	%Transmittance
F1	0.0031	92.6616
F2	0.0244	94.5366
F3	0.0037	99.15166
F4	0.0211	95.2576
F5	0.0107	97.5663
F6	0.0104	97.6337
F7	0.0042	99.0375
F8	0.0116	97.364

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.

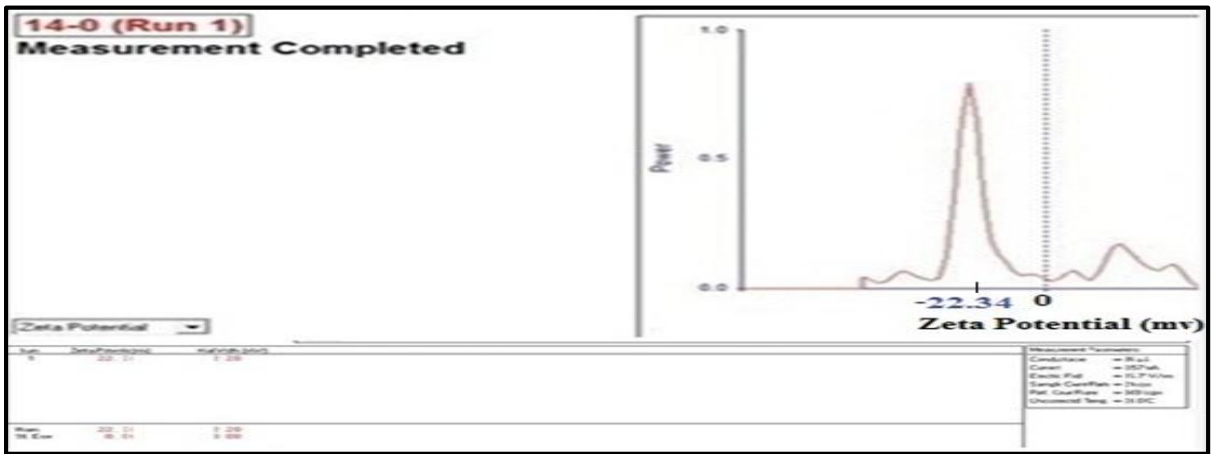

Figure 4. Zeta potential of formula (F3)

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].

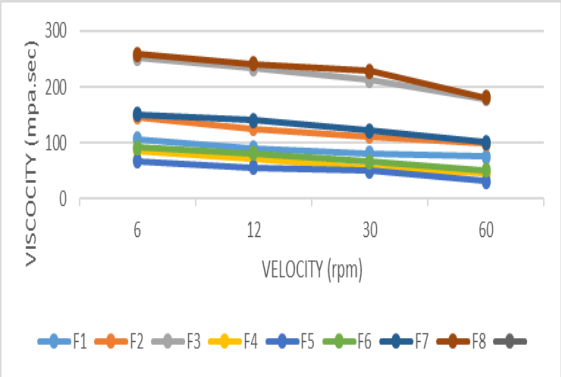

Figure 5. Viscosities data of prepared felodipine nanoemulsion formulas (F1, F2,F3,F4,F5,F6,F7,F8).

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.

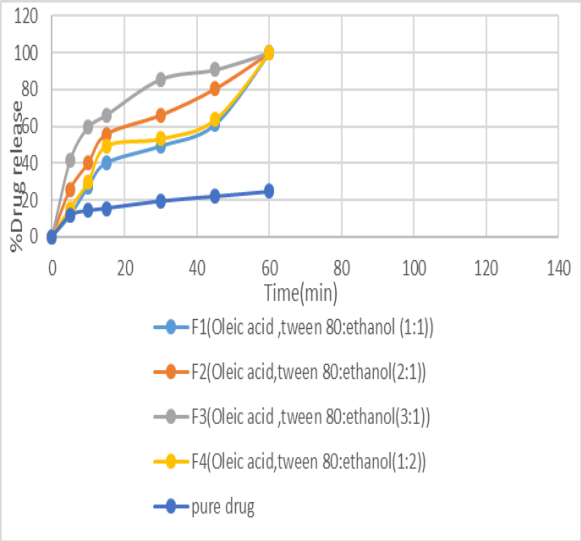

Figure 6. A comparative dissolution profile of felodipine nanoemulsions (F1, F2,F3,F4 and pure felodipine) in 500ml of 0.1 N HCl (containing 1% tween 80) dissolution medium at 37°C.

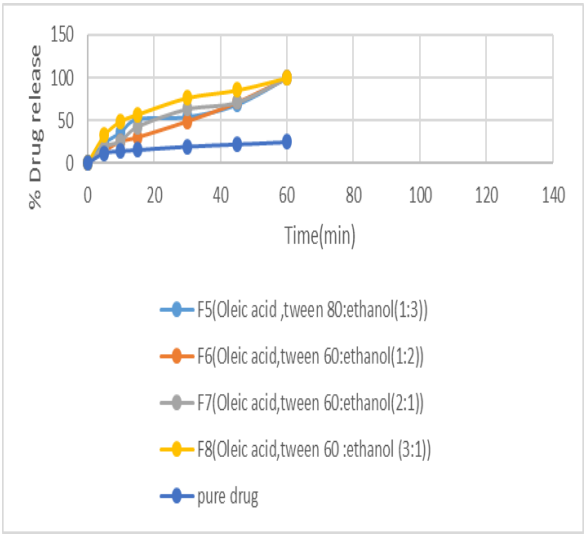

Figure 7. A comparative dissolution profile of felodipine nanoemulsions (F5, F6,F7,F8 and pure felodipine) in 500ml of 0.1 N HCl (containing 1% tween 80) dissolution medium at 37 °C.

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 excepeints during preparation of nanoemulsion.

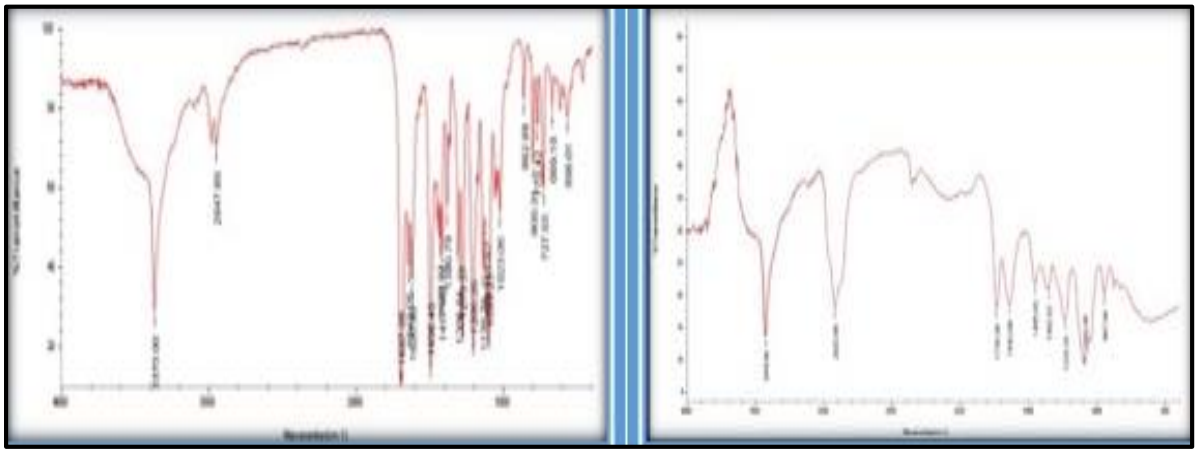

Figure 8. The FTIR spectrum of pure felodipine

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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