FORMULATION,CHARACTERIZATION AND IN-VITRO RELEASE OF ORAL FELODIPINE SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS

BY

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Abstract

Nanotechnology is a cross-disciplinary field, which involves the ability to design and exploit the unique properties that emerge from man-made materials ranging in size from 1 to greater than 100 nm.A self- nanoemulsifying drug delivery system is a fairly similar liquid lipid dosage form designed for oral delivery which composed of oils, surfactants and possibly cosurfactants or cosolvents.Felodipine is a calcium channel blocker (CCB). It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation.

The aim of this paper was to formulate Felodipine self nanoemulsifying system to overcome the poor aqueous solubility of Felodipine (3ug/ml) and hence improving its poor dissolution which is the main cause of its poor oral bioavailability. Evaluation, Characterization and In-Vitro release of orally Felodipine Self-nanoemulsifying Drug Delivery Systems were studied in comparison with the market product.

Formulae B18 (composed of 30% Triacetin: 40% Span 80: 30% Transcutol HP) and C19 (composed of 20% Triacetin: 50% Span 80: 30% Ethanol) were selected for Felodipine SNEDDS.Felodipine SNEDDSs prepared using Transcutol HP (B18) exhibited pseudoplastic flow while Felodipine SNEDDSs prepared using ethanol (C19) exhibited Newtonian flow.Formula prepared with Transcutol HP (B18) has smaller droplet size and PDI than that prepared with ethanol (C19). Felodipine formulae showed negative zeta potential values.The in-vitro release can be arranged in descending order as follows: Felodipine formula B 18 > Felodipine formula C 19 > Market product.

Keywords

Felodipine, In-vitro, Self-nanoemulsifying Drug Delivery Systems, Solubility, oral bioavailability

Introduction

Self-nanoemulsifying drug delivery system (SNEDDS) is isotropic mixtures of oil, surfactant, co-surfactant and drug that form fine oil in water nanoemulsion when introduced into aqueous phase under gentle agitation. SNEDDSs spread readily in the GIT and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification (**Nazzal et al., 2002**)[(1)].

A self-emulsifying / microemulsifying and nanoemulsifying drug delivery system (SEDDS/SMEDDS/SNEDDS), respectively, is a fairly similar liquid lipid dosage form

designed for oral delivery which composed of oils, surfactants and possibly co-solvents. These systems have the ability to form fine oil in water (o/w) emulsions, microemulsions or nanoemulsion upon mild agitation following dilution with an aqueous media. This property makes them good candidates for oral delivery of poorly-water soluble drugs (**Pouton, 1997; Gershanik and benita, 2000**)[2,3].

Solid lipid-based formulations

Solid SEDDS can be used for several dosage forms (dry emulsions, self emulsifying capsules, implants, sustained/controlled release tablets or pellets, beads, microspheres, nanoparticles, suppositories) and can present flexible solution for oral and parenteral administration (**Kumar et al., 2010**)[4].

Hypertension

Hypertension known as high blood pressure (HBP), is a long-term medical condition in which the blood pressure in the arteries is persistently elevated (**Naish, 2014**)[5]. High blood pressure usually does not cause symptoms (**High Blood Pressure Fact Sheet, 2016**)[6]. Long-term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease (**Lackland and Weber, 2015; Mendis et al., 2011**)[7,8].

Calcium channel blockers

Calcium channel blockers, or calcium antagonists, treat a variety of conditions, such as high blood pressure, chest pain and Raynaud's disease.Calcium channel blockers prevent calcium from entering cells of the heart and blood vessel walls, resulting in lower blood pressure. Calcium channel blockers, also called calcium antagonists, relax and widen blood vessels by affecting the muscle cells in the arterial walls.Some calcium channel blockers have the added benefit of slowing heart rate, which can further reduce blood pressure, relieve chest pain (angina) and control an irregular heartbeat.

Examples of calcium channel blockers

Felodipine

Felodipine is a long-acting 1,4- Dihydropyridine calcium channel blocker (CCB) b. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. Felodipine is used to treat mild to moderate essential hypertension.

Pharmacology

Indication: For the treatment of mild to moderate essential hypertension. Associated Conditions: High Blood Pressure (Hypertension)

Absorption:

Felodipine is completely absorbed from the gastrointestinal tract; however, extensive first-pass metabolism through the portal circulation results in a low systemic availability of 15%. Bioavailability is unaffected by food.

Protein binding:

99%, primarily to the albumin fraction.

Metabolism and Interaction:

Felodipine is metabolized by cytochrome P450 3A4, so substances that inhibit or activate CYP3A4 can strongly affects how much felodipine is present.

Half life:

The Half life of Felodipine in hypertensive patients was 17.5 - 31.5 hours; 19.1-35.9 hours in elderly hypertensive patients; 8.5-19.7 in healthy volunteers.Clearance: 0.8 L/min [Young healthy subjects]

Toxicity:

Symptoms of overdose include excessive peripheral vasodilatation with marked hypotension and possibly bradycardia. Oral rat LD_{50} is 1050 mg/kg.

Affected organisms: Humans and other mammals

Solubility:

Felodipine is class II drug, i.e., low solubility and high permeability. Felodipine has poor water solubility and hence poor dissolution and bioavailability after oral administration. Felodipine undergoes extensive first- pass metabolism with a bioavailability of about 15% **(Blychert et al., 1997)**[9].

The major drawback in the therapeutic application and efficacy of Felodipine as oral dosage form is its low aqueous solubility, which is expressed to be approximately 19.17 mg/L at 25°C. Hence, improvement of its water solubility and dissolution is of therapeutic importance **(Budavari S. et al., 1996; Moffat et al., 2002)**[10,11]**.**

Experimental part

Materials

Felodipine kindly supplied from EUG Company, Cairo, Egypt. Felodipine 10 mg tablet kindly supplied from stada Company, Cairo, Egypt. Transcutol® HP (Highly purified diethylene glycol monoethyl ether ER/NF), was kindly supplied from Gattefosse, France. Cremophor EL (Polyoxyl 35 Hydrogenated Castor oil), Span 80, Triacetin, Ethanol HPLC grade and Potassium dihydrogen orthophosphate analytical grade were purchased from Sigma-Aldrich, Germany.

Methodology

HPLC determination of Felodipine

Determination of Felodipine peak and retention time

Felodipine solution of concentration 1 mg/ml in methanol was prepared and injected in the HPLC (Agilent HPLC 1100 PDA, USA) using the following chromatographic conditions: Hypersil ODS 150 mm x 4.6 mm, 5 μm particle size. The mobile phase consisted of 30:70 (v/v) mixture of Sodium acetate: acetonitrile. The column was equilibrated for 30 minutes with the analytical mobile phase before injection; 10 μl of the drug solution was injected after filtration with a 0.2 μ m filter syringe.The mobile phase was pumped isocratically at a flow rate of 2.3 ml/min. The effluent was monitored at 237 nm. Retention time was recorded and sample was analyzed in triplicate.

Formulation of Felodipine self-nanoemulsifying drug delivery systems

Solubility study

The maximum solubility of Felodipine was determined in different oils, surfactants and co-surfactants by adding an excess amount of the drug (Felodipine) in 2 ml of each vehicle in 10 ml screw-capped glass vials, and the mixture was vortexed to facilitate solubilization using a vortex mixer (Vortex mixer, Stuart, UK). Mixtures were equilibrated at 25 ± 2˚C for 3 days in an isothermal shaker (BS-11, shaking water bath) and then centrifuged at 7000 rpm for 30 minutes (Beckman model TJ-6 Centrifuge, Korea) to separate the undissolved drug (**Nielsen et al., 2007**)[12].

The supernatant was diluted with methanol to make suitable dilution and analyzed for Felodipine content by the previously mentioned HPLC method. The solubility of Felodipine was determined in all of these: Triacetin, Cremophor EL, Span 80, Transcutol HP and ethanol.

Construction of ternary phase diagrams

Series of mixtures were prepared with varying ratios of lipids, surfactants and co-surfactants. The components were weighed into 10 mL glass vials and mixed at 50˚C with a stirring at 300 rpm using isothermal shaker, until the components were completely dissolved. The mixtures were cooled to 37 ˚C and 1 g was transferred to a beaker where 250 mL of distilled water at 37 ˚C was added under gentle stirring of 25 rpm.

The dispersions were visually inspected. A mixture was defined to be a suitable SNEDDS and judged good if spontaneous emulsification was obtained after dispersion in 37 ˚C, followed by the formation of a clear transparent nanoemulsion (**El laithy, 2008; Craig et al., 1995; Nazzal et al., 2002**)[13-15]. Ternary phase diagram of surfactant, co-surfactant and oil was plotted; each of them, representing an apex of the triangle (**Sunheer, 2012**)[16] using sigma plot software.

Determination of drug loading capacity of the formulated SNEDDS

An excess of Felodipine powder was placed in 5 ml of the selected SNEDDSs in 10 ml screw-capped glass vials, and the mixtures were vortexed to facilitate solubilization using a vortex mixer, then sonicated for 10 minutes using bath sonicator (Crest ultrasonic Corp., New York, USA). Mixtures were equilibrated at 25 ˚C for 3 days in an isothermal shaker and then centrifuged at 7000 rpm for 30 minutes to separate the undissolved drug. The supernatant was diluted with methanol for the quantification of Felodipine and analyzed by HPLC.

Preparation of Felodipine SNEDDS

Formulae that could solubilize the highest amounts of Felodipine were chosen to be loaded with the drug (in a concentration below its saturation solubility). In 10 ml screw-capped glass vials, weighed amounts of Felodipine were-added to each system (composed of oil, surfactant and co-surfactant)

System No.	Oil	Surfactant	Co-surfactant
Felodipine System A	Triacetin	Cremophor EL	Transcutol HP
Felodipine System B	Triacetin	Span 80	Transcutol HP
Felodipine System C	Triacetin	Span 80	Ethanol
Felodipine System D	Capryol 90	Cremophor EL	Ethanol
Felodipine System E	Capryol 90	Span 80	Transcutol HP
Felodipine System F	Capryol 90	Span 80	Ethanol

sonicated for 30 minutes then kept at 25˚C for further evaluation.

Evaluation of oral Felodipine SNEDDS

Visual inspection

The prepared Felodipine SNEDDSs were inspected for optical transparency and homogeneity by visual observation against strong light. The formulations were also checked for the presence of undissolved drug particles.

Assessment of thermodynamic stability

Felodipine SNEDDSs were subjected to centrifugation for 30 minutes at 3500 rpm. The stable formulations that did not show precipitation of drug were subjected to heating cooling cycles which include six cycles between 45 ˚C and refrigerator temperature 4˚C with storage at each temperature of not less than 48 hours were studied. Furthermore the passed formulae were subjected to freeze thaw cycles (-21 ˚C and +25 ˚C) with storage at each temperature of not less than 48 hours (**Shafiq et al., 2007**)[17].

Conductivity measurements

Conductivity measurements were carried out using digital conductometer (Digital Conductometer, Jennway, UK). The measurements were made at a constant frequency of 1 Hz at constant temperature of 35± 0.1˚C. The cell constant was ascertained by using a standard potassium chloride solution. The different SNEDDSs were titrated with water and their electro conductive behavior was determined.Before each measurement, the cell was prewashed twice with the sample in order to avoid the adherence effect of the surfactant and the cosurfactant upon the cell inner wall and the electrodes.

Assessment of the rheological properties

The rheological properties of the prepared Felodipine SNEDDSs were determined by means of Brookfield rotary viscometer (Brookfield digital Rheometer DVIII, USA). fitted with CP-40 cone and plate spindle. Each formula (0.5 ml) was put in suitable container. The rpm was increased gradually in a suitable range to give torque

values between 10-100 units, at 37 ± 2 ˚C, with 15 seconds between each two successive speeds.The rheological behavior of each system was investigated by plotting the shear stress versus the shear rate and by plotting the viscosity versus the shear rate. The obtained data were determined using an excelcomputer program. The shear rate in sec^{-1} and the viscosity in cp were determined from the instrument readings and fitted to the power law constitutive equation (**Tung, 1994**)[18].

$$\eta = m \gamma^{n-1}$$

The two dimensionless quantities: the consistency index (m) and the flow index (n) characteristic for each formulation were obtained. If n = 1 this indicates Newtonian behavior while if n is less than 1, this corresponds to shear thinning flow. The lower value of n the more shear thinning the formulation (**Copetti et al, 1997; Owen et al, 2000; Chang et al, 2002**)[19-21].

Robustness to dilution and phase separation study

In order to mimic physiological dilution process after oral administration of the prepared Felodipine SNEDDSs, the formulae were diluted 50, 100, 1000 times with different aqueous media including distilled water, 0.1 N HCl and phosphate buffer pH 7.4. The diluted formulae were mixed by vortex mixer. The formed mixtures were set aside for 2 hours, and then they were examined by visual inspection for clarity of the formed emulsions and presence of any precipitate of the drug. Formulae that didn't show significant phase separation or drug precipitation at the end of the 2 hours period were used in the subsequent study (**Kim and ku, 2000; Date and Nagarsenker, 2007; Ofokansi et al., 2009**)[22-24].

Determination of emulsification time, dispersibility and percentage transmittance

The rate of emulsification is an important index for the assessment of the efficacy of emulsification. Evaluation of the self emulsifying properties of SNEDDS formulations was performed by visual assessment. The USP type II dissolution apparatus (Hanson research, USA) was used to evaluate the efficiency of self-emulsification of the selected formulae. One gram of each formula was added drop wise into 500 ml of distilled water maintained at 37˚C with gentle agitation condition provided by rotating paddle at 50 rpm. The time taken for the emulsification (until a clear homogenous system was obtained) formation was assessed visually in triplicates (**Bachynsky et al., 1997**;**Khoo et al., 1998**)[25,26].Transparency of the formed emulsion was determined by measuring % transmittance at 650 nm with purified water as blank through UV Spectrophotometer (Agilent, USA) (**Date and Nagarsenker, 2008a**)[27].

Droplet size analysis

Each formula of the prepared Felodipine SNEDDSs was diluted 100 times using double distilled water (**Wang et al., 2010**)[28]. The mean droplet size and polydispersity index (PDI) of the formed nanoemulsion was determined by using instrument (Nanotrac wave II, Microrack, USA), using the 632 nm line of aHeNe laser as the incident light with angel 90^{o}.

Zeta potential

For measurement, a dilute suspension of the nanoparticles is subjected to a weak electric field, and the mobility of the particles is commonly determined by NICOMP

380 ZLS. This technique is based on the evaluation of a frequency (Doppler) shift that is observed for the light scattered from the particles, motion in the electric field. As a result, the electrophoretic mobility μ (velocity of the particles/electric field strength) of the nanoparticles is obtained.

Investigation of the effect of different surface-active agents on the zeta potential can provide information on the interaction of the particles with surface-active agents. The effect of variations in preparation procedure as well as the potential influence of drug loading or further processing, such as freeze drying or sterilization, on the zeta potential of SNEDDS has also been studied (**Lim et al., 2002**)[29].

Analytical test method of Felodipine by using HPLC technique

HPLC Identification

The retention time of the major peak in the chromatogram of the assay preparation corresponds to that in the chromatogram of the standard preparation, as obtained in the Assay.

Method of assay for Felodipine

Chromatographic conditions used for assaying Felodipine in the selected Felodipine SNEDDSs was illustrated in table (1).

Table (1): Chromatografic conditions for Felodipine HPLC technique

Instrument	Agilent HP1200
System Type	Reverse Phase
Column Type Length & Diameter	Hypersil GOLD C18 (4.6 x 250 mm , 5 μm) or equivalent.
Conditioning	With mobile phase for 45 minutes prior to operating the injection sequence
Column temperature	Ambient
Injection Type Injection volume	Auto Sampler 20 μl
Detector Type Wavelength	UV-DAD 362 nm
Mobile phase Composition	Acetonitrile: Methanol: Buffer solution (40 : 20 : 40) Buffer solution: Dissolve 6.9 g of monobasic sodium phosphate in about 800 mL of water in a 1000-mL volumetric flask. Adjust with 1 M phosphoric acid to a pH of 3.0 ± 0.05, dilute with water to volume, and mix.
Flow rate	1.0 ml/ min

Standard solution: Transfer 20 mg felodipine reference standard to 100 ml volumetric flask.

Assay solution: Transfer amount of formula equivalent to 10 mg Felodipine to 100 ml volumetric flask, then add 70 ml mobile phase, sonicate for 15 min, dilute to volume by mobile phase (soln. 2). Transfer 10 ml from soln. 2 to 50 ml volumetric flask, dilute to volume and mix by mobile phase.

System suitability: After conditioning the column with mobile phase, inject five replicate injections of the standard solution. These injections should be with relative standard deviation of these replicate injections should not exceed 2.0%.

Identification: The retention time of Felodipine peak in assay chromatogram should comply with that of working standard.

Assay: Calculations

$$\text{Assay T \%} = \frac{A_{test} \times P \times St_{wt.} \times D_{fu} \times M \times 100}{A_{standard} \times D_{Fs} \times LC \times test_{wt}}$$

$A_{standard}$	Peak area or height of standard solution (mean of two bracketing standard Injections)
A_{test}	Peak area or height of sample solution (mean of the two injections)
D_{fu}	the dilution factor of the sample solution
D_{fs}	the dilution factor of the standard solution
St_{wt}	the weight of the standard
$test_{wt}$	the weight of the sample
P	the purity of the standard
M	the product average weight
LC	the label claim
MBRF	Mean Bracketing Response Factor

Limit: Felodipine: 90.0 -110.0% of the labeled amount

In-vitro dissolution of Felodipine SNEDDS

Drug dissolution studies of Felodipine SNEDDSs were carried out according to the official method in USP 36-2013. Aliquots of Felodipine SNEDDS each containing 10 mg of Felodipine was installed to the dissolution medium. Also, Plendil 10 mg tablet was tested under the same conditions. Five milliliters of dissolution media was retrieved at timed intervals (10, 30 and 60 minutes) and replaced with fresh dissolution media. The amount of Felodipine was quantified using HPLC method.

Apparatus: USP App II (Paddle)

Medium: pH 6.5 phosphate buffer with 1% sodium lauryl sulfate (Medium is prepared as follows: Transfer 206 ml of 1 M monobasic sodium phosphate monohydrate, 196 ml of 0.5 M dibasic sodium phosphate anhydrous, and 50.0 g of sodium lauryl sulfate to a 5000 ml volumetric flask. Add approximately 4000 ml of

water, and mix well. If necessary, adjust with 1 N sodium hydroxide to a pH of 6.5. Dilute with water to volume, and mix well.)

Volume: 500 ml in each vessel (60 min)

Speed: 50 RPM.

Temperature: 37 ^{0} C + 1 ^{0}C.

Sample: 10 ml and filtered (use Whatman No.5)

Preparation of Felodipine standard solution

Transfer 20 mg felodipine reference standard to 100 ml volumetric flask, add 70 ml mobile phase, Sonicate for 15 min, dilute to volume by mobile phase to give solution No. 1. Transfer 10 ml from the above solution to 100 ml volumetric flask, dilute to volume and mix by dissolution medium to give solution No. 2. Take 10 ml form solution No. 2 then filtere this solution using filter paper (Whatman No.5)

Preparation of Felodipine assay solution

Transfer amount of the selected formula (either B18 or C19) equivalent to 10 mg Felodipine to 100 ml volumetric flask, add 70 ml mobile phase, Sonicate for 15 min, dilute to volume by mobile phase to give solution No. 3. Transfer 10ml from solution No. 3 to 50 ml volumetric flask, dilute to volume and mix by mobile phase then filtere this solution using filter paper (Whatman No.5)

Calculation :

$$\text{In-vitro release \% of Felodipine} = \frac{A_T \times C_{ST} \times P \times 100}{A_{ST} \times C_T}$$

Where;

A_T = Peak area of test solution A_{st} = Peak area of standard solution

C_{ST}= Conc. of standard solution C_t = Conc. of test solution

P_{ST}=Potency of reference standard

Limit: NLT 75% is released in 60 min.

Kinetics for the in-vitro dissolution of Felodipine SNEDDS

Kinetic orders were used to determine the kinetic parameters of the in-vitro dissolution of Felodipine SNEDDS. Zero- and first - order kinetic, as well as, controlled diffusion model were tried to choose the most suitable kinetic order or systems for Felodipine release. Table (2) summarizes all the orders studied.

Table (2): Kinetic treatments and parameters for the in-vitro drug release

Order	X-axis	Y-axis	Slope	intercept	$t_{½}$	Rate equation
Zero	T	X	K	o	a/2k	x=kt
First	T	Log (a-x)	-k/2.3	Log a	0.693/k	Log [a/(a-x)]=kt /2.3
Higuchi	$\sqrt{t}$	X	K	o	$(a/2k)^2$	x= k$\sqrt{t}$

Results and Discussions

HPLC determination of Felodipine

Determination of Felodipine peak and retention time

Although Felodipine can be determined spectrophotometrically at λ_{max} 237nm (**Verma et al., 2017**)[30], but the HPLC method was chosen as the preliminary study revealed that the components of SNEDDS may interfere with Felodipine in the spectrophotometric assay.

The chromatogram of 1 mg/ml Felodipine solution in methanol showed an identified sharp symmetrical peak with good base line and no tailing at a retention time of 4.108 minutes, this is shown in figure (1).

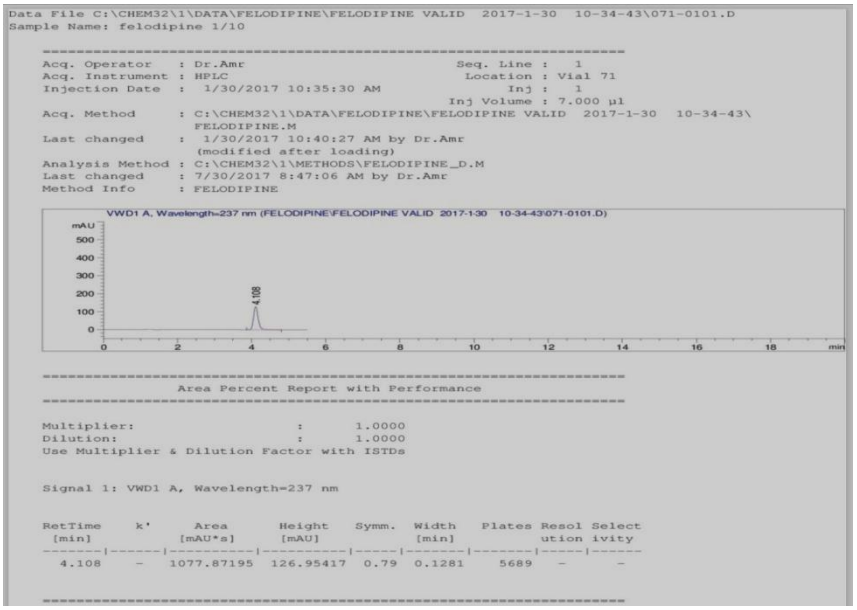

Figure (1): The chromatogram of 1 mg/ml Felodipine solution in methanol

Characterization of self-nanoemulsifying drug delivery systems

Solubility study

Values of the maximum solubility of Felodipine determined in different excipients are recorded in table (3).It was found that the Felodipine solubility values (mg/ml) were in the following descending order in Triacetin>Capryol 90> Lauroglycol 90 > Maisine-35-1 > Labrafil M 2125 >Labrafac PG > Isopropyl myristate. The values obtained were 10.9, 7.9, 3.6, 2.4, 1.18, 0.86 and 0.0514 mg/ml, respectively which are greatly higher than its solubility in water (0.019 mg/ml).

Table (3): Solubility of Felodipine in different oils, surfactants and co-surfactants

Excipients used	**Solubility of Felodipine (mg/ml)**		
	Range	**Mean**	**SD**
Oils			
Triacetin	10.6 – 11.1	10.9	0.0199
Capryol 90	7.8 – 8.1	7.9	0.018
Lauroglycol 90	3.1 – 3.9	3.6	0.099
Maisine-35-1	2.2 – 2.6	2.4	0.068
Labrafil M 2125	1.0 – 1.28	1.18	0.108
Labrafac PG	0.83 – 0.89	0.86	0.028
Isopropyl myristate	0.05 – 0.053	0.0514	0.024
Surfactants			
Span 80	10.55 – 10.84	10.68	0.014
Cremophor EL	2.66 - 2.99	2.79	0.063
Pluronic L64	2.1 - 2.5	2.25	0.097
Labrasol	2.12 - 2.22	2.16	0.024
Tween 20	1.23 - 1.27	1.246	0.017
Tween 80	1.1 - 1.16	1.122	0.032
Co-surfactants/Co-solvent			
Transcutol HP	9.1 – 9.5	9.26	0.022
Ethanol	6.0 – 6.17	6.11	0.077
1-propanol	1.8 – 2.2	2.03	0.016
propylene glycol	1.54 - 1.85	1.72	0.094
PEG 400	1.3 - 1.5	1.39	0.073

Generally, oils can solubilize the lipophilic drug in a specific amount. Their ability to facilitate self-emulsification and increase the fraction of lipophilic drug that is transported via the intestinal lymphatic system, thereby increasing absorption from the GIT, making them the most important excipients (**Kommuru et al., 2001**)[31].

Many researchers choose the oil with highest solubility of the drug for the formulation of self-emulsifying systems to attain successful emulsification and to avoid precipitation of the drug (**Kang et al., 2004; Wei et al., 2005; Franceschinis et al., 2011**)[32-34], consequently Triacetin and Capryol 90 were chosen to be used as oil phases according to the solubility of Felodipine obtained.

Concerning solubility of Felodipine in different surfactants, it could be arranged in the following descending order: Span 80 > Cremophor EL > Pluronic L64 > Labrasol

> Tween 20 > Tween 80. The values observed were 10.68, 2.79, 2.25, 2.16, 1.246, 1.122 mg/ml, respectively.

Among the tested co-surfactants; Transcutol HP (9.26 mg/ml), showed the highest solubility of the drug followed by Ethanol (6.11 mg/ml) and 1-propanol (2.03 mg/ml), propylene glycol (1.72 mg/ml) and PEG 400 (1.39 mg/ml).

Transcutol HP was used in formulation of SEDDSs due to its solving power, good water solubility as well as its absorption and permeability enhancement (**Gao et al., 1998; Lanlan et al., 2005**)[35,36].

Construction of ternary phase diagram

The existence of self nanoemulsified formulations fields that could self nanoemulsify under dilution and gentle agitation was identified from ternary phase diagram of a system containing oil, surfactant and co-surfactant. Six ternary phase diagrams were constructed with the compositions as shown in table (4) and illustrated in figures (2 and 3).

Table (4): Composition of the constructed ternary phase diagrams

System No.	Oil	Surfactant	Co-surfactant
Felodipine System A	Triacetin	Cremophor EL	Transcutol HP
Felodipine System B	Triacetin	Span 80	Transcutol HP
Felodipine System C	Triacetin	Span 80	Ethanol
Felodipine System D	Capryol 90	Cremophor EL	Ethanol
Felodipine System E	Capryol 90	Span 80	Transcutol HP
Felodipine System F	Capryol 90	Span 80	Ethanol

All the components were converted to percent weight per weight before constructing the phase diagram.

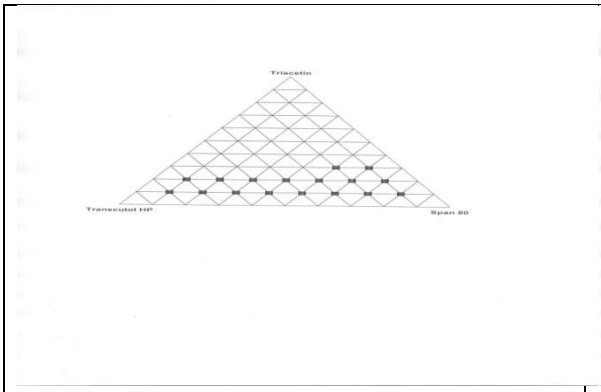

Figure (2): Illustrates the ternary phase diagram of Felodipine System B composed of Triacetin, Span 80 and Transcutol HP, as oil, surfactant and cosurfactant, respectively.

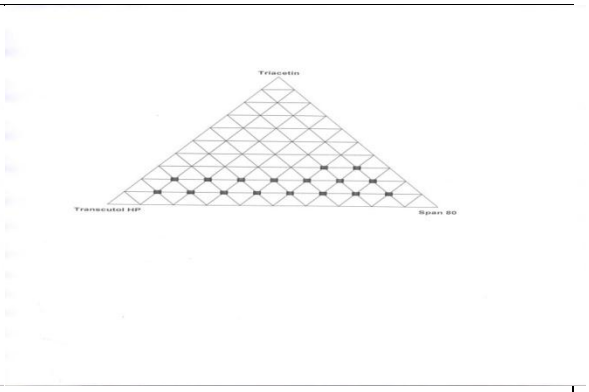

Figure (3): Illustrates the ternary phase diagram of Felodipine System C composed of Triacetin, Span 80 and Ethanol, as oil, surfactant and cosurfactant, respectively.

The marked points represent all formulations that could self- emulsify in seconds and could be infinitely diluted by purified water indicating that the nanoemulsions formed will be capable of keeping Felodipine solubilized. Within this area, the formulations form fine oil in water nanoemulsion with only gentle agitation (Figure 4). This can be attributed to the fact that the surfactant strongly localized to the surface of the nanoemulsion droplets thus decreasing interfacial free energy and provide a mechanical barrier to coalescence forming a thermos mechanically spontaneous dispersion (**Reiss, 1975**)[37].

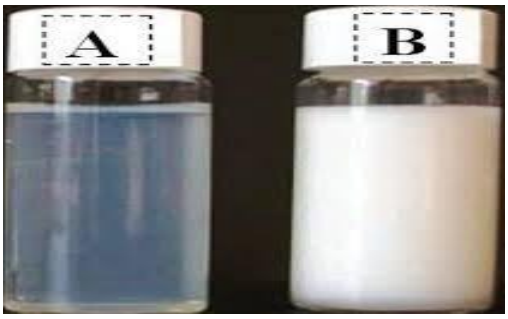

Figure (4): (a) Clear transparent nanoemulsions, (b) White milky emulsion

Moreover, cosurfactant increases interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules (**Constantinides and Sealart, 1997**)[38].

The formed nanoemulsions are clear, isotropic, transparent, and of low viscosity determined by visual inspection.

Determination of drug loading capacity of Felodipine in the formulated self nanoemulsifying drug delivery systems

Since the presence of high surfactant concentrations in the formulation is considered one of the drawbacks of self nanoemulsifying drug delivery system (**Grove and Mullertz, 2007**)[39]. From each phase diagram, the formulae that contained higher amounts of oil and still able to form clear nanoemulsion when infinitely diluted with water, were selected for drug loading as illustrated in table (5).

Table (5): Maximum loading capacity of Felodipine in the selected SNEDDSs

Formula No.	Composition	Loading capacity (mg/ml)	
		Mean	**SD**
A3	Triacetin 20%, Cremophor EL 10%, Transcutol 70%	4.9 ±	0.1
A4	Triacetin 20%, Cremophor EL 20%, Transcutol 60%	5.1 ±	0.02
A8	Triacetin 20%, Cremophor EL 30%, Transcutol 50%	6.3 ±	0.08544
A13	Triacetin 20%, Cremophor EL 40%, Transcutol 40%	5.6 ±	0.055678

A19	Triacetin 20%, Cremophor EL 50%, Transcutol 30%	8.1 ±	0.1
A26	Triacetin 20%, Cremophor EL 60%, Transcutol 20%	7.9 ±	0.06245
A34	Triacetin 20%, Cremophor EL 70%, Transcutol 10%	7.3 ±	0.19
B3	Triacetin 20%, Span 10%, Transcutol 70%	7.9 ±	0.06245
B4	Triacetin 20%, Span 20%, Transcutol 60%	7.5 ±	0.149332
B6	Triacetin 30%, Span 10%, Transcutol 60%	8.2 ±	0.043589
B7	Triacetin 30%, Span 20%, Transcutol 50%	7.9 ±	0.101489
B8	Triacetin 20%, Span 30%, Transcutol 50%	8.4 ±	0.122882
B13	Triacetin 20%, Span 80 40%, Transcutol 40%	8.7 ±	0.045092
B18	Triacetin 30%, Span 80 40%, Transcutol 30%	**9.78 ±**	**0.203162**
B19	Triacetin 20%, Span 80 50%, Transcutol 30%	8.1 ±	0.173494
B26	Triacetin 20%, Span 80 60%, Transcutol 20%	7.3 ±	0.045826
B34	Triacetin 20%, Span 80 70%, Transcutol 10%	6.9 ±	0.879261
C3	Triacetin 20%, Span 80 10%, Ethanol 70%	5.1 ±	0.212838
C4	Triacetin 20%, Span 80 20%, Ethanol 60%	6.5 ±	0.045826
C6	Triacetin 30%, Span 80 10%, Ethanol 60%	6.3 ±	0.362905
C8	Triacetin 20%, Span 80 30%, Ethanol 50%	7.2 ±	0.21
C13	Triacetin 20%, Span 80 40%, Ethanol 40%	7.8 ±	0.459239
C19	Triacetin 20%, Span 80 50%, Ethanol 30%	**9.3 ±**	**0.026458**
C26	Triacetin 20%, Span 80 60%, Ethanol 20%	8.4 ±	0.134536
C33	Triacetin 30%, Span 80 60%, Ethanol 10%	8.1 ±	0.383145
C34	Triacetin 20%, Span 80 70%, Ethanol 10%	5.9 ±	0.07
D19	Capryol 20%, Cremophor EL 50%, Transcutol 30%	6.1 ±	0.42
D26	Capryol 20%, Cremophor EL 60%, Transcutol 20%	5.8 ±	0.445084
D34	Capryol 20%, Cremophor EL 70%, Transcutol 10%	5.4 ±	0.457056

It was found that the systems composed of Capryol 90 and Span 80 produced gel like formulae of high viscosity. So, all formulae from these systems were excluded from this test. The formulae that contained Triacetin, Span 80 and Ethanol in ratio of 20: 50: 30 and the formulae that contained Triacetin, Span 80 and Transcutol HP in ratio of 30: 40: 30, respectively showed the highest loading capacity of Felodipine (9.44 and 9.78 mg/ml, respectively). The type of oil used did not affect the loading capacity of the formulae. SNEDDSs that contain Capryol are able to dissolve amount of Felodipine comparable to those containing Triacetin which has the higher loading capacity of Felodipine.

Preparation of Felodipine self-nanoemulsifying drug delivery systems

Based on loading capacity of the formulae, the best three formulae from each system were chosen to be loaded with Felodipine. Formulae were prepared and loaded with drug and kept at 25 ˚C for further study, the prepared formulae were listed in table (6).

Table (6): The prepared formulae and Felodipine concentration for each selected Felodipine SNEDDSs

Formula No.	Loading Capacity of Felodipine (mg/ml)	
	Value	Ranking order
A19	8.1	6
A26	7.9	8
A34	7.3	9
B8	8.4	4
B13	8.7	3
B18	**9.78**	**1**
C19	**9.3**	**2**
C26	8.4	4
C33	8.1	6
D19	6.1	10
D26	5.8	11
D34	5.4	12

Evaluation of oral Felodipine self-nanoemulsifying drug delivery systems

Visual inspection

Visual inspection of Felodipine SNEDDSs prepared using either Triacetin or Capryol 90 as oily phase showed that all formulae were clear, transparent and homogenous with no recorded signs of phase separation.

Assessment of thermodynamic stability

The physical stability of a lipid-based formulation is important to its performance, which can affect precipitation of the drug in the excipients matrix. In

addition, the poor physical stability of the formulation can lead to phase separation of the excipients, which affects not only formulation performance, but also visual appearance of the formulation. The obtained results are tabulated in tables (7-9).

Table (7): Effect of centrifugation on the thermodynamic stability of Felodipine SNEDDSs

Formula	Drug precipitation	Phase Separation	Centrifugation test result
A19	+	-	Fail
A26	-	-	**Pass**
A34	+	-	Fail
B8	+	-	Fail
B13	-	-	**Pass**
B18	-	-	**Pass**
C19	-	-	**Pass**
C26	-	-	**Pass**
C33	+	-	Fail
D19	+	-	Fail
D26	+	-	Fail
D34	+	-	Fail

Table (8): Effect of heating cooling cycles on the thermodynamic stability of Felodipine SNEDDSs

Formula	Drug precipitation	Phase Separation	Heating cooling result
A26	+	-	Fail
B13	-	-	**Pass**
B18	-	-	**Pass**
C19	-	-	**Pass**
C26	+	-	Fail

Table (9): Effect of freeze thaw cycles on the thermodynamic stability of Felodipine SNEDDSs

Formula	Drug precipitation	Phase Separation	Heating cooling result
B13	+	-	Fail
B18	-	-	**Pass**
C19	-	-	**Pass**

The stability of Felodipine in SNEDDSs was an important issue to be evaluated. Formulae that passed centrifugation test were shown in table (7). Only five formulations showed no precipitation and phase separation. These formulae are the following: A26, B13, B18, C19 and C26. The above selected formulae were subjected to the heating cooling cycles. After heating cooling cycles, only 3 formulae are selected which contain the following formulae B13, B18 and C19 and were able to remain stable as were listed in table (8). Finally the three selected formulae were subjected to freeze thaw cycles and

illustrated in table (9). From the twelve starting tested formulae only B18 and C19 were thermodynamically stable without any precipitation of Felodipine or phase separation of the components.

Conductivity measurements

Conductometry is a useful tool assesses nanoemulsion structure, as there is a consistent correlation between structure type and nanoemulsion electro-conductivity behavior (**Bumajdad and Eastoe, 2004**)[40].

The electrical conductivity measurements can determine the nature of the continuous phase of nanoemulsions, as O/W nanoemulsions are highly conducting because their external phase is water, while W/O are not, since water is the internal or dispersed phase (**Hasse and Keipert, 1997**)[41].

The conductivity of B18 and C19 was checked, as these two formulae passed the thermodynamic stability tests. The observed conductivity curves as a function of water content indicate the use of electroconductimetry to study structural changes in nanoemulsions. Conductivity (O) increased with the increase of the dispersed water volume fraction ($\emptyset$w). The conductivity behavior of system C19 showed a gradual linear increase in conductivity values with the increase of water percent. In contrast, B18 showed an abrupt increase with the increase of water percent.

Results also revealed that the conductivity values of B18 was higher than C19; this may be attributed to the fact that the topological polar surface area of Transcutol that was used in B18 is larger than ethanol that was used in C19, with values of 78.9 and 77.8 A, respectively (**Pubchem. ncbi. nlm. nih. gov, 2015**)[42]. The results are illustrated in Figures (5 and 6).

By plotting the data of the conductivity values for the two selected Felodipine SNEDDSs (B18 and C19), the values obtained were 0.996 and 0.997 for the correlation coefficients, 2.753 and 0.454 for the intercepts and 0.372 and 0.075 for the slopes, respectively.

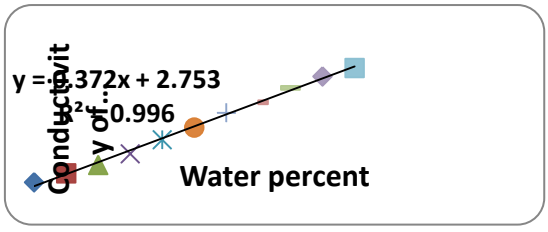

Figure (5): Conductivity values for B 18

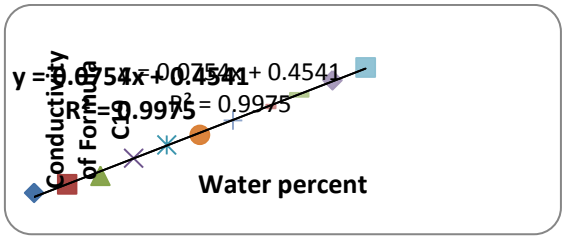

Figure (6): Conductivity values for C 19

Assessment of the rheological properties

The viscosity study of the selected formulae is an important issue. Systems with very high viscosity may create problems in pourability in containers and syringability (**Ram and Ajit, 2011; Attwood D, Florence, 2012**)[43,44].

Assessment of the rheological behavior of Felodipine SNEDDSs prepared using either Transcutol HP (B18) or Ethanol (C19) revealed that the formulae exhibited Pseudoplastic flow and Newtonian flow, respectively. It was noticed that B18 showed higher viscosity values than C19; Viscosity values are listed in table (10).

Formula B18 which show pseudoplastic flow (non-Newtonian flow) indicated by its Farrow's index (N = 2.166), as illustrated in table (11). The N value if greater than 1, it indicates pseudoplastic flow while if N value is less than 1, it indicates dilatant flow.

By applying Tung equation, the values of the consistency index and the flow index for formula B 18 showed the following values, 552.41 and 0.4608, respectively. If n = 1 this indicates Newtonian behavior while if n is less than 1, this corresponds to shear thinning flow. The lower value of n the more shear thinning the formulation.

Table (10) showed that n value for formula C19 is 1.0750 which is approximately near 1 and conformed that this formula exhibited Newtonian flow.

Table (10): Shear rate, Shear Stress and Viscosity Values for Felodipine SNEDDS for the selected formulae (B18 and C19)

Shear rate (S^{-1})	**Shear stress ($dyne/cm^2$)**		**Viscosity (cp)**	
	B18	**C19**	**B18**	**C19**
1100	140	50	12.727	4.545
1200	145	55	12.083	4.583
1300	150	60	11.538	4.615
1400	155	65	11.071	4.643
1500	160	70	10.666	4.667
1600	165	75	10.313	4.687
1700	170	80	10.000	4.706
1800	175	85	9.722	4.722
1900	180	90	9.474	4.737

Table (11): Viscosity parameters for the selected Felodipine SNEDDS

F No.	**Viscosity (cP)**		**N**	**m**	**n**
	High	**Low**			
B 18	12.730	9.474	2.120	552.41	0.4608
C 19	4.737	4.545	---	---	1.0750

N = Farrow's Index m = Consistency index n = Flow Index

Robustness to dilution and phase separation study

It is important to ensure that uniform emulsions are formed from self emulsification of SNEDDSs at different dilution. The prepared Felodipine SNEDDSs formulae were exposed to different folds of dilution in different media in an attempt to mimic the in- vivo conditions to predict whether phase separation or precipitation is likely to occur in the GIT where the formulation would encounter gradual dilution (**Elnaggar et al., 2009; Gupta et al., 2011**)[45,46].

With all tested media, distilled water, 0.1N HCl and phosphate buffer (pH 7.4), visual examination of the formed solutions at 50 and 100 times dilutions showed nanoemulsions with slight bluish appearance and with no precipitation for B18, but those formed of C19 showed insignificant precipitation in the following order 50 fold dilution > 100 fold dilution; However samples of 1000 times dilution showed clear nanoemulsion with no precipitation or phase separation for both B18 and C19.

So, it was clear that formula B18 was robust to all dilutions with different media and did not show any phase separation even after 24 hours of storage. The insignificant precipitation that occurred with C19 may be attributed to the lower solubility of Felodipine in Ethanol than Transcutol that was found in B18. The results are shown in tables (12-14).

Table (12): Effect of 50 times dilution of Felodipine SNEDDSs with different media

Formula No	**0.1 N HCl**		**Distilled water**		**Phosphate buffer pH7.4**	
	Clarity	**Precipitation**	**Clarity**	**Precipitation**	**Clarity**	**Precipitation**
B18	Slightly bluish	-	Slightly bluish	-	Slightly bluish	-
C19	Slightly turbid	±	Slightly turbid	±	Slightly turbid	±

Table (13): Effect of 100 times dilution of Felodipine SNEDDSs with different media

Formula No	**0.1 N HCl**		**Distilled water**		**Phosphate buffer pH7.4**	
	Clarity	**Precipitation**	**Clarity**	**Precipitation**	**Clarity**	**Precipitation**
B18	Slightly bluish	-	Slightly bluish	-	Slightly bluish	-
C19	Slightly turbid	±	Slightly turbid	±	Slightly turbid	±

Table (14): Effect of 1000 times dilution of Felodipine SNEDDSs with different media

Formula No	**0.1 N HCl**		**Distilled water**		**Phosphate buffer pH7.4**	
	Clarity	**Precipitation**	**Clarity**	**Precipitation**	**Clarity**	**Precipitation**
B18	Clear	-	Clear	-	Clear	-
C19	Clear	-	Clear	-	Clear	-

Determination of emulsification time, dispersibility and percentage transmittance

The rate of emulsification is an important index for assessment of the efficiency of emulsification. It was observed that B18 showed less dispersion time when compared

with C19, as it showed emulsification time of 30 ± 1.2 seconds while B18 showed 50 ± 1.1 seconds; The results may be attributed to the higher viscosity value of B18 than that of C 19.

Both formulae B18 and C19 showed clear transparent appearance as a result of forming nanoemulsion of grade A rapidly.

Percentage transmittance was carried out to prove that the emulsions formed from dispersion of SNEDDS are clear and transparent systems. % transmittance of C19 and B18 were 97.5% ± 0.5 and 97.4% ± 0.65, respectively, which is closer to 100%, indicating that clear nanoemulsion was formed when diluted with water. The results are shown in table (15).

Table (15): Self-emulsification time, dispersibility and % transmittance of selected Felodipine SNEDDSs in distilled water

Formula No.	Grade of the formed nanoemulsion	Emulsification time (Sec)		%Transmittance at 238 nm	
		Value	SD	Value	SD
B18	A	50	1.1	97.4%	0.65
C19	A	30	1.2	97.5%	0.5

Droplet size analysis

Droplet size is a crucial factor in self-emulsification performance, because it determines the rate and extent of drug dissolution as well as drug absorption. It has been reported that small size of emulsion droplets may lead to more rapid absorption, thereby improving the bioavailability (**Cui et al., 2009a**)[47]. The smaller the globule size, the larger the surface area provided for drug absorption (**Grshanik and Benita, 2000**)[48]. Furthermore, a decrease in the droplet size reflects the formation of a better packed film of the surfactant at the oil-water interface, thereby stabilizing the oil droplets (**Cui et al., 2009b**)[49].

Depending upon the size of globules, self-microemulsified drug delivery system (SMEDDS) indicated the formulations forming transparent microemulsions with the oil droplet size rang between 100 and 250 nm. Self-nanoemulsified drug delivery system (SNEDDS) is relatively a recent term indicating the globule size less than 100 nm **(Pouton and Porter, 2008)**[50].

The results revealed that the droplet size of Felodipine formula B18 was 64.2 nm (Population % 64.4) and 168.6 nm (Population % 35.6), which is bigger than that of Felodipine formula C19 which was 48.3 nm (Population % 74.2) and 121.4 nm (Population % 25.8). Based on these results both studied Felodipine formulae B 18 and C19 was classified as self nanoemulsified drug delivery system (SNEDDS) as shown in table (16) and illustrated in figures (7 and 8).

The Poly dispersity Index (PDI) is a dimensionless measure of the width of size distribution calculated from the cumulated analysis ranging from 0 to 1. A small value of PDI indicates a broader distribution of droplet size **(Tang et al., 2012)**[51]**.** PDI is the ratio of standard deviation to mean droplet size, which signifies uniformity of droplet size within the formulation. The higher the value of PDI the lower is the uniformity of

droplet size **(Baboota et al., 2007)**[52]. PDI of Felodipine formulae B18 and C19 were found to be 0.314 ± 0.665 and 0.139 ± 0.018, respectively.

Table (16): Droplet size and polydispersity index of Felodipine formulae B 18 and C 19

Formula	**Droplet size (nm)**		**PDI**				
B18	**35.6%**	**64.4%**	**1**	**2**	**3**	**Mean**	**SD**
	168.6	64.2	0.391	0.2791	0.2726	0.314	0.665
C19	**25.8%**	**74.2%**	**1**	**2**	**3**	**Mean**	**SD**
	121.4	48.3	0.135	0.159	0.124	0.139	0.018

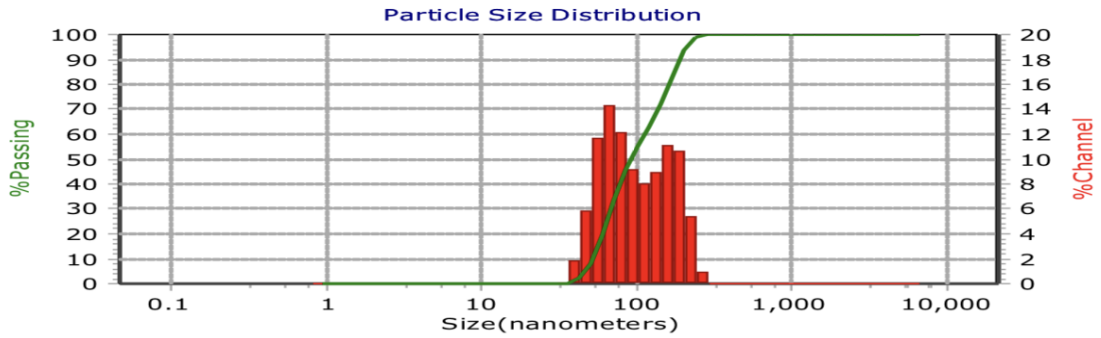

Figure (7): Droplet size measurement of Felodipine formula B18

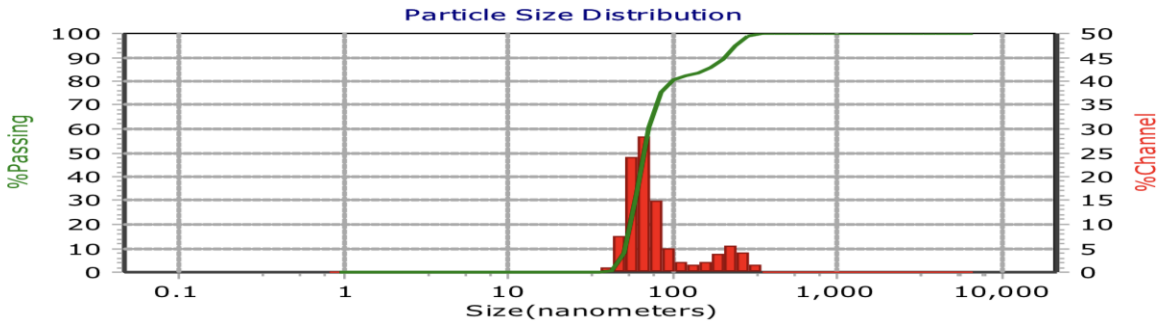

Figure (8): Droplet size measurement of Felodipine formula C19

Zeta Potential Measurement

Zeta potential is a measure for the stability of any formulations based in the nano range. If zeta potential falls in the range of ± 30 mV, this means that the formulations had better stability. The results for the two studied Felodipine formulae B 18 and C 19 showed that zeta potential was 29.133 ± 0.342 mV and 24.057 ± 0.574 mV, respectively. The above values indicated that Felodipine formulae exhibit excellent stability, see table (17).

Table (17): Zeta potential of Felodipine formulae B 18 and C 19

Formula	**Zeta Potential (mV)**				
B18	**1**	**2**	**3**	**Mean**	**SD**
	-29.52	-28.87	-29.01	-29.133	0.342
C19	**1**	**2**	**3**	**Mean**	**SD**
	-24.59	-23.45	-24.13	-24.057	0.574

In-vitro dissolution of Felodipine SNEDDS

Table (18) exhibited the standard calibration data of Felodipine using HPLC technique.

Table (18): Standard Calibration Data of Felodipine

Felodipine Conc.	**Area (cm2)**			**Mean**	**RSD**
	Area 1	**Area 2**	**Area 3**		
10.00	550.29	550.57	550.72	550.53	0.04
15.00	790.59	800.45	820.61	803.88	1.90
20.00	1100.70	1110.01	1110.06	1106.92	0.49
25.00	1370.39	1370.29	1360.42	1367.03	0.42
30.00	1650.03	1650.14	1630.92	1643.69	0.67

The results obtained for the in-vitro release of different Felodipine formulae indicated that the studied formulae can be arranged in descending order, concerning to there in-vitro release as follows: Felodipine formula B 18 > Felodipine formula C 19 > Market product. The data observed are 60.37, 80.80 and 91.98 for Felodipine formula B 18; 42.16, 53.44 and 81.32 for Felodipine formula C 19; 37.86, 46.40 and 75.23 for Felodipine market product after 10, 30 and 60 minutes, respectively.

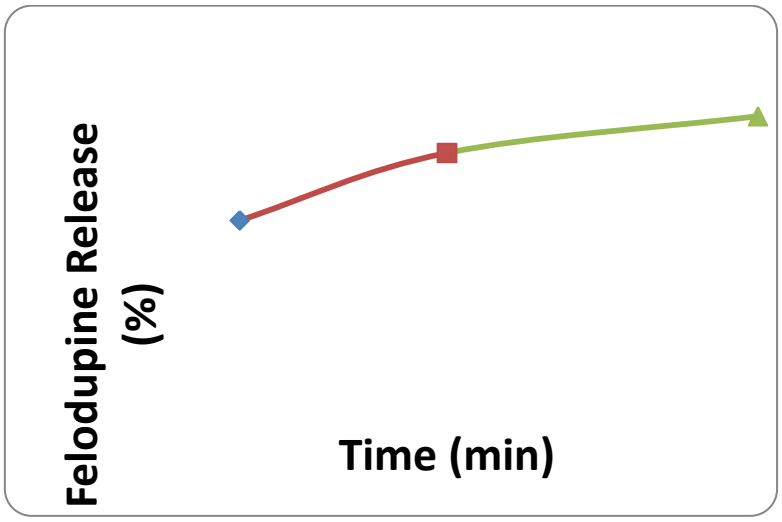

Figure (9): In-Vitro release of Felodipine (%) for System 2(B18)

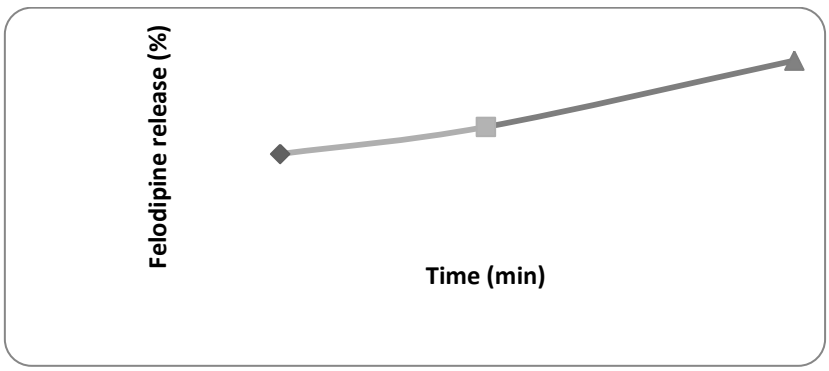

Figure (10): In-Vitro release of Felodipine (%) for System 1(C19)

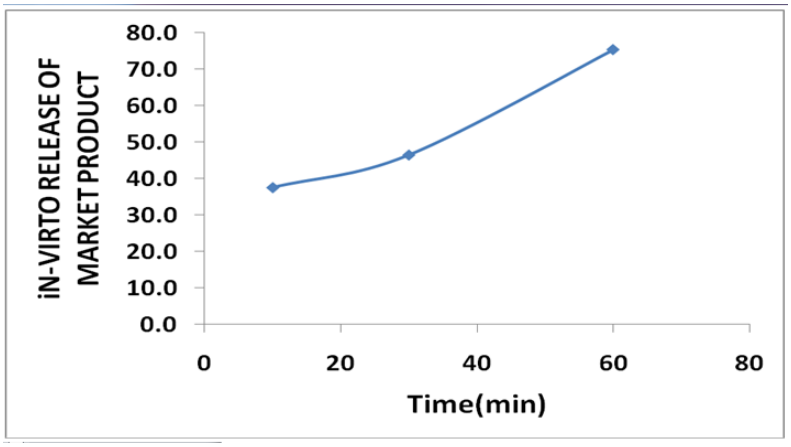

Figure (11): In-Vitro release of Felodipine (%) for Market product

Table (19): In-Vitro release of Felodipine (%) for System 2(B18)

Time (min)	In-vitro release of Felodipine (%)						Mean	RSD
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	60.41	60.32	60.40	60.37	60.38	60.34	60.37	0.0003
30	80.74	80.90	80.90	80.71	80.81	80.71	80.80	0.0009
60	92.27	91.61	91.42	92.04	92.30	92.23	91.98	0.0037

Figure (9): Chromatogram showing In-Vitro dissolution of (System 2 B18) at 10 min.

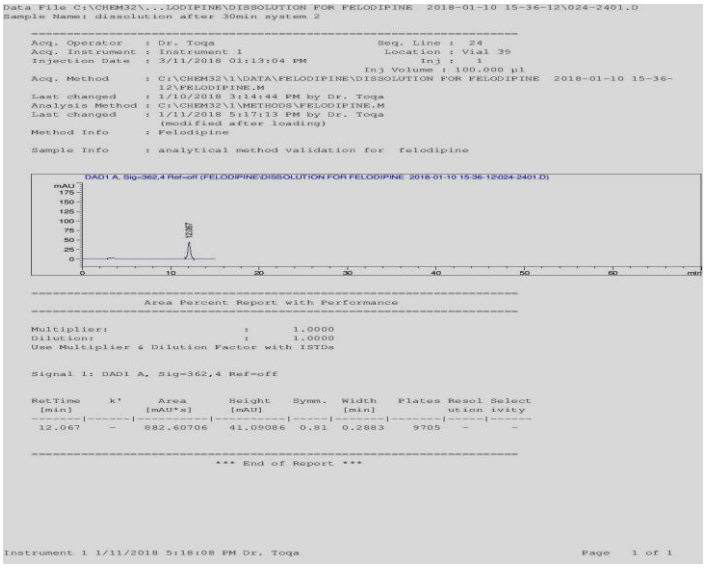

Figure (10): Chromatogram showing In-Vitro dissolution of (System 2 B18) at 30 min.

Figure (11): Chromatogram showing In-Vitro dissolution of (System 2 B18) at 60 min.

The obtained areas for Felodipine SNEDDS were converted to in-vitro release percent as shown in table (20). The in-vitro release percent of formula C 19 (system 1) was found to reach 42.16, 53.44 and 81.32% after 10, 30 and 60 minutes of dissolution.

Figures (12-14) showed the three chromatograms for formula C 19 corresponding to the three release percent of Felodipine. The above figures represented the chromatographs for Felodipine formula C 19 after in-vitro release for 10, 30 and 60 minutes.

Table (20): In-Vitro release of Felodipine (%) for System 1(C19)

Time (min)	In-vitro release of Felodipine (%)						Mean	RSD
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	42.23	42.14	42.21	42.18	42.19	42.16	42.16	0.0003
30	53.46	53.62	53.62	53.43	53.54	53.44	53.44	0.0009
60	81.36	81.61	81.42	81.13	81.39	81.32	81.32	0.0016

Figure (12): Chromatogram showing In-Vitro dissolution of (System 1 C19) at 10 min.

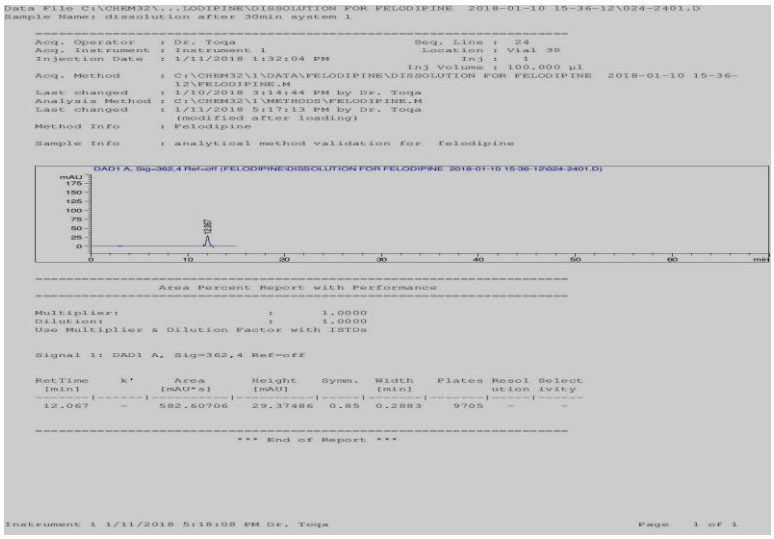

Figure (13): Chromatogram showing In-Vitro dissolution of (System 1 C19) at 30 min.

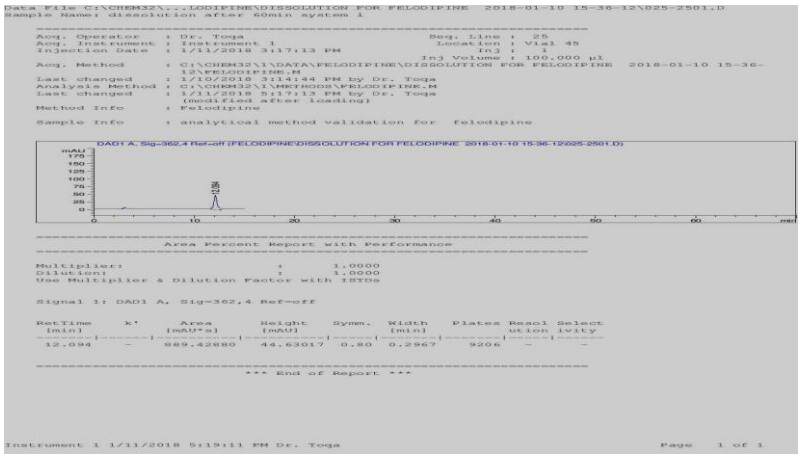

Figure (14): Chromatogram showing In-Vitro dissolution of (System 1 C19) at 60 min.

The obtained areas for Felodipine market product were converted to in-vitro release percent as shown in table (21). The in-vitro release percent of the market product was found to reach 37.86, 465.40 and 75.23% after 10, 30 and 60 minutes of dissolution.

Figures (15-17) showed the three chromatograms for the market product corresponding to the three release percent of Felodipine. The above figures represented the chromatographs for Felodipine market product after in-vitro release for 10, 30 and 60 minutes.

Table (21): In-Vitro release of Felodipine (%) for Felodipine 10 mg marketed tablet

Time (min)	In-vitro release of Felodipine (%)						Mean	RSD
	1	2	3	4	5	6		
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	37.10	37.76	36.69	38.29	38.60	37.04	37.86	2.02
30	47.00	45.93	46.39	46.51	45.81	47.36	46.40	1.29
60	75.43	75.19	74.63	74.29	76.46	76.46	75.23	1.02

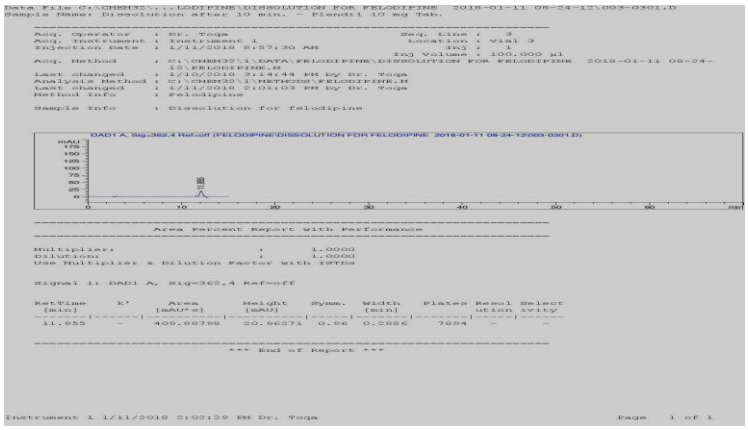

Figure (15): Chromatogram showing In-Vitro dissolution of Felodipine 10 mg marketed tablet at 10 min.

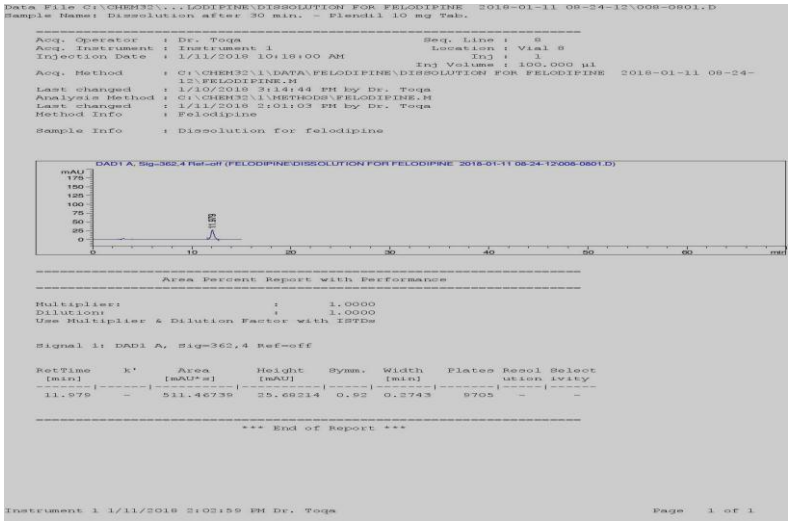

Figure (16): Chromatogram showing In-Vitro dissolution of Felodipine 10 mg marketed tablet at 30 min.

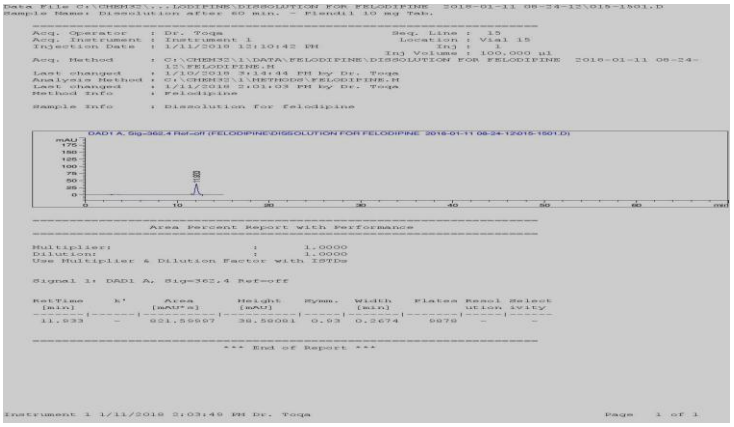

Figure (17): Chromatogram showing In-Vitro dissolution of Felodipine 10 mg marketed tablet at 60 min.

The results obtained for the in-vitro release of different Felodipine formulae indicated that the studied formulae can be arranged in descending order, concerning to there in-vitro release as follows: Felodipine formula B 18 > Felodipine formula C 19 > Market product. The data observed as illustrated in tables (36, 38 and 40) are 60.37, 80.80 and 91.98 for Felodipine formula B 18; 42.16, 53.44 and 81.32 for Felodipine formula C 19; 37.86, 46.40 and 75.23 for Felodipine market product after 10, 30 and 60 minutes, respectively.

Kinetic study of Felodipine in-vitro release

According to the results obtained from the in-vitro release, kinetic behaviors of all Felodipine formulae were studied. Zero order, First order and Higuchi diffusion model were tried in this study to investigate the kinetics of the in-vitro release of Felodipine formulations (formula B 18, formula C 19 and Market product).

Table (22) represents the kinetic treatment while table (23) represents the kinetic parameters for the in-vitro release of Felodipine formulations (formula B 18, formula C 19 and Market product).

From the obtained data, it is found that in-vitro release of Felodipine formulae follows:

Felodipine formulae (B 18 and C 19) obey Higuchi diffusion model while Felodipine market product obey zero-order kinetic.

The previous kinetic data showed that the in-vitro release of Felodipine formulae follows different kinetic orders and no single kinetic order can be used to express the drug release from specific type of these formulations.

Table (22): Kinetic treatments of Felodipine in-vitro release for formula B 18, Formula C 19 and Market product

Time (min)	Zero- order	First- order	Higuchi model
	Percent released versus	Log percent retained	Percent released versus square root of
B 18			
	% released	Log % Retained	Square root of time
10	60.37	1.598024	3.1623
30	80.80	1.283301	5.4772
60	91.98	0.904174	7.7460
C 19			
	% released	Log % Retained	Square root of time
10	42.16	1.7622	3.1623
30	53.44	1.6680	5.4772
60	81.32	1.2713	7.7460
Market product			
	% released	Log % Retained	Square root of time
10	37.86	1.7934	3.1623
30	46.40	1.7292	5.4772
60	75.23	1.3939	7.7460

Table (23): Kinetic Parameters of Felodipine in-vitro release for formula B 18, Formula C 19 and Market product

F No.	a	B	r	k	t½
	Zero – Order				
B 18	14.6943	1.1814	0.9256	1.1814	42.32
	First – Order				
	0.8611	0.0125	0.4097	0.0289	23.93
	Higuchi Diffusion Model				
	2.9665	10.0731	**0.9875**	10.0731	24.64
C 19	**Zero – Order**				
	25.9333	1.2942	0.8351	1.2942	38.63
	First – Order				
	0.7786	0.0067	0.2566	0.0154	44.84
	Higuchi Diffusion Model				
	9.5892	11.8881	**0.9598**	11.8881	17.69
Market product	**Zero – Order**				
	27.6879	0.7643	**0.9822**	0.7643	65.42
	First – Order				
	1.9135	- 0.0082	- 0.9668	- 0.0189	- 36.52
	Higuchi Diffusion Model				
	8.7168	8.1377	0.9525	8.1377	37.75

a = Intercept b = Slope r = Correlation Coefficient

k = Reaction rate constant t½ = Half life

Conclusions

Based on the solubility results of Felodipine, Triacetin and Capryol 90 were selected as oil phase, Cremophor EL and Span 80 as surfactant; Transcutol HP as cosurfactant and Ethanol as cosolvent to construct ternary phase diagram.

Different SNEDDS were prepared and loaded with Felodipine, visually inspected showing that the formulae were clear, transparent and homogenous without phase separation.

Formulae B18 (composed of 30% Triacetin: 40% Span 80: 30% Transcutol HP) and C19 (composed of 20% Triacetin: 50% Span 80: 30% Ethanol) were thermodynamically stable with no precipitation of Felodipine.

Thus, Formulae B18 and C19 were selected for further studies in the following chapters.

Conductivity results showed that, its values increases with increasing the ratio of water added to the prepared formulae. Thus the obtained data indicated the formation of O/W nanoemulsion when water was added to Felodipine SNEDDSs.
Felodipine SNEDDSs prepared using Transcutol HP (B18) exhibited pseudoplastic flow while Felodipine SNEDDSs prepared using ethanol (C19) exhibited Newtonian flow.
Robustness to dilution and phase separation study showed that B18 was robust to all dilution with the three used media (water, 0.1 N HCl and Phosphate buffer pH 7.4), while C19 Showed slightly precipitation in case of 50 and 100 folds dilution and was robust to 1000 fold dilution with three tested media studied.
The passed formulae B18 and C19 were able to form grade A emulsions with rapid emulsification time (30 and 50 seconds) and high % transmittance (97.4 % and 97.5 %), respectively.
Formula prepared with Transcutol HP (B18) has larger droplet size and PDI than that prepared with Ethanol (C19). But both formulae, B18 and C 19 were classified as SNEDDS.

Both SNEDDS Felodipine formulae showed negative zeta potential values which indicated the stable nature of nanoparticles owing to electrostatic repulsion.

The results obtained for the in-vitro release of different Felodipine formulae indicated that the studied formulae can be arranged in descending order, concerning to there in-vitro release as follows: Felodipine formula B 18 > Felodipine formula C 19 > Market product, also it was showed that the presence of transcutol in B18 affect positively on the dissolution rather than ethanol in C19.

It was considered that the in-vitro release of C19 was 81% which is higher than the market felodipine 75%.

It was found that in-vitro release of Felodipine formulae follows: Felodipine formulae (B 18 and C 19) obey Higuchi diffusion model while Felodipine market product obey zero-order kinetic.

The previous kinetic data showed that the in-vitro release of Felodipine formulae follows different kinetic orders and no single kinetic order can be used to express the drug release from specific type of these formulations.

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الملخص العربى

تحضير وتقييم والاتاحة المعملية لعقار فيلودبين في صورة نظام دوائي ذاتي الإستحلاب في حجم النانوميتر

للسادة الدكاترة

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مـــــــن

قسم الصيدلانيات والصيدلة الصناعية بصيدلة الازهر بنين القاهرة

تعد تقنية النانو مجالًا متعدد التخصصات، يشتمل على القدرة على تصميم واستغلال الخصائص الفريدة التي تنشأ من المواد المحضرة التي يتراوح حجمها من ١ إلى أكبر من ١٠٠نانوميتر. إن نظام توصيل الأدوية ذاتية الاستحلاب/ الاستحلاب في حجم الميكرو او الاستحلاب في حجم النانو هو شكل جرعة دهنية سائلة مشابهة إلى حد ما، مصممة للاعطاء عن طريق الفم وتتكون من الزيوت، والمواد الفعالة السطحية ، وربما عوامل فعالة مساعدة أو سوائل مذيبة مساعدة.

فيلوديبين من ضمن مجموعة حاصرات قنوات الكالسيوم طويلة الأمد. وهو يعمل بشكل أساسي على خلايا العضلات الملساء الوعائية عن طريق تثبيت قنوات الكالسيوم من النوع ل الموصلة بالجهد الكهربي في شكلها الخامل.

يتناول هذا البحث تحضير وصياغة المادة الدوائية في صورة نظام دوائي ذاتي الإستحلاب في حجم النانوميتر واجراء كافة الاختبارات المميزة لهذا النظام ودراسة انطلاق المادة الدوائية معمليا لاختيار أحسنها من ناحية المعدل وكمية الإتاحة

صيغ فيلوديبين ذاتية الاستحلاب في حجم النانوميتر المحضرة باستخدام ترانسكيوتول اتش بي (بي ١٨) عرضت تدفق بلاستيكي زائف بينما صيغ فيلوديبين ذاتية الاستحلاب في حجم النانوميتر المحضرة باستخدام الإيثانول (سي ١٩) عرضت التدفق النيوتوني.

الصيغة التي تم تحضيرها باستخدام ترانسكيوتول اتش بي (بي ١٨) لها حجم قطري ومؤشر تشتت متعدد أكبر من تلك التي تم تحضيرها مع الإيثانول (سي ١٩). لكن كلا الصيغتين بي ١٨، سي ١٩ صنفتا على أنهما صيغ ذاتية الاستحلاب في حجم النانوميتر.

أظهرت كلتا الصيغتين بي ١٨، سي ١٩ ذاتية الاستحلاب في حجم النانوميتر قيم سالبة لـ جهد زيتا التي أشارت إلى طبيعة مستقرة من الجسيمات النانوية بسبب التنافر الالكتروستاتيكي.

النتائج التي تم الحصول عليها من الانطلاق المعملي (الإتاحة المعملية) لصيغ الفيلوديبين ذاتية الاستحلاب في حجم النانوميتر أشارت إلى أنه يمكن ترتيب الصيغ المختارة بترتيب تنازلي ، فيما يتعلق بالإتاحة المعملية كما يلي: صيغة فيلوديبين بي ١٨> صيغة فيلوديبين سي ١٩> مستحضر السوق.