Investigation of Lipid Polymer Hybrid Nanocarriers for Oral Felodipine Delivery: Formulation, Method, In-vitro and Ex-vivo Evaluation

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Abstract

The antihypertensive felodipine is a calcium-channel blocking agent. It is practically insoluble in water and shows low oral bioavailability (15%-20%). This investigation aims to formulate and characterize felodipine lipid polymer hybrid nanocarriers (LPHNs) to be given orally by two nanovesicles formulating methods and make comparative analysis through characterization process and in vitro and ex vivo intestinal permeation evaluation. The felodipine LPHNs formulations (HF_1-HF_3) were prepared by the new microwave-based method and that felodipine LPHNs formulations (HF_4-HF_6) were prepared by a single emulsification solvent evaporation technique (SESET). All formulations (HF_1-HF_6) enter the characterization process. The felodipine LPHNs formulations (HF_1-HF_6) were prepared successfully and undergo different characterization processes to make a comparative study between formulations prepared by different methods. It was found that formulas prepare by a microwave-based method are most superior to the SESET. The felodipine LPHNs formulations HF1-HF_3 has lower particle size, lower PDI and higher zeta potential, significantly higher ($p < 0.05$) dissolution rate, and significantly higher ($p < 0.05$) intestinal permeation study than the felodipine LPHNs formulations HF_4-HF_6. The microwave-based method is a very successful technique in preparing felodipine LPHNs formulations (HF_1-HF_3) and prepotent to the SESET. All the felodipine LPHNs formulations (HF_1-HF_6) show extended drug release nanosystem.

Keywords: Lipid-polymer hybrid nanocarriers, Felodipine , Microwave based method, SESET.

التحقيق في تحضيرناقلات نانوية علاجية هجينة من الدهن والبوليمر لتسليم الفلودبين عن طريق الفم:وذلك بمقارنة الطريقة مختبريا خارج الجسم الحي وخلال الأنسجة الحية

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الخلاصة

فيلوديبين الخافض للضغط هو عامل يحجب قنوات الكالسيوم. إنه غير قابل للذوبان عمليا في الماء ويظهر التوافر الحيوي عن طريق الفم (١٥٪ -٢٠٪). الهدف من هذا البحث هو صياغة وتوصيف ناقلات النانو الهجينة الفلوديبين الدهنية (LPHNs) التي يتم إعطاؤها عن طريق الفم من خلال طريقتين لصياغة النواقل النانوية وإجراء تحليل مقارن من خلال عملية التوصيف وتقييم نفاذية الأمعاء في المختبر وخارج الجسم الحي. تم تحضير HF1-HF3 بطريقة جديدة تعتمد على الميكروويف وتم تحضير تركيبات فلوديبين LPHNs HF4-HF6 بتقنية تبخير المذيبات الأحادية الاستحلابSESET . تدخل جميع التركيبات (HF1-HF6) في عملية التوصيف. تم تحضير تركيبات LPHNs الفلوديبين (HF1-HF6) بنجاح وخضعت لعمليات توصيف مختلفة لإجراء دراسة مقارنة بين الصيغ التي تم تحضيرها بطرق مختلفة. وجد أن الصيغ المحضرة بطريقة تعتمد على الميكروويف هي الأكثر تفوقًا على تبخير المذيبات الأحادية الاستحلاب. تحتوي تركيبات فلوديبين LPHNs HF1-HF3 على حجم جزيئات أقل ، وأقل PDI وإمكانات زيتا أعلى ، ومعدل ذوبان أعلى بشكل ملحوظ (p <٠,٠٥) ودراسة نفاذية معوية أعلى بكثير (p <٠,٠٥) من تركيبات LPHNs الفلوديبين HF4-HF6. تعتبر الطريقة المعتمدة على الميكروويف تقنية ناجحة للغاية في تحضير تركيبات فلوديبين LPHNs HF1-HF3 ومتفوقة على SESET. تُظهر جميع تركيبات فلوديبين LPHNs HF1-HF6 نظام نانوي ممتد لإطلاق العقاقير.

الكلمات المفتاحية: ناقلات نانوية هجينة من الدهن و البوليمر ، فيلوديبين ، طريقة تعتمد على الميكروويف ، تبخير المذيبات الأحادية الاستحلاب

Introduction

It became clear that nanotechnology has entered into the development of most areas of life, including the development and manufacture of medicines [1]. Despite the great discoveries of drugs, they remain ineffective until a safe way is found to deliver them to the circulatory system or site targeting. Felodipine is the calcium-channel antagonist. Its use in the treatment of hypertension and angina pectoris.

It is practically insoluble in water and undergoes a hepatic first-pass effect that leads to low oral bioavailability (15%-20%) [2]. The oral route of drug administration is most prevalent where and has many merits such as its safe, easy to administer therapeutic agent, not associated with pain as in the intravascular route. But the oral route has some demerits such as intestinal and hepatic first-pass effect and is associated with low bioavailability

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for drugs that have low solubilities such as felodipine and low permeability[2]. In addition to oral route obstacles, the conventional oral delivery system has many demerits such as poor patient amenability, increased opportunity of dose missing of a drug with a short half-life for which frequent taking is required and presence of typical peak and valley in the blood concentration/time curve lead to fluctuations in therapeutic agent level this will create adverse effects particularly in therapeutic agent with low therapeutic index [3]. Nanoparticles played a major role in carrying therapeutic agents and delivering them to the place of effectiveness. The presence of nanoparticles led to an increase in solubility, an increase in the surface area for drug release, and thus increased absorption and bioavailability [4]. There are many nanoparticles used in drug delivery, but they are associated with some obstacles that interfere with drug efficacy. The most important obstacles are low drug loading, drug expulsion, and drug instability after administration and in long-term storage. The lipid polymer hybrid nanocarriers (LPHNs) system is a nanoparticulate system for drug delivery. It protects the encapsulated drug from obstacles associated with another nanoparticle system[5-7]. The LPHNs consist of lipid content and polymeric ingredient [8]. The lipid content enhances the solubility of hydrophobic drugs, improves membrane permeability leads to increase bioavailability. The presence of polymer within the structure of LPHNs provide more control to release of loaded drug. The hybridization between lipid and polymeric ingredients creates a nanoparticulate system which is LPHNs that characterized mainly by toughness, robust nanoparticles, provide extended-release drug delivery, higher drug payload, and high stability in the human circulatory system and during formulation storage[9]. There are many methods of LPHNs preparation with some limitations such as: the presence of impurities in final products, costly, require high time that delays research field, and low stability. The newly microwave-based method is employed with great success in the formulation of a highly advanced nano system which is LPHNs and characterized by economical, absence of impurities associated with other nanoparticle preparation methods, inexpensive, high stable of final formulation [10-12]. The single emulsification solvent evaporation technique (SESET) is widely used in the preparation of nanocarriers. The SESET has been successfully used to loading a variety of hydrophobic therapeutic agents with good reproducibility, high yield, and ease of scaling up [13].

This study aims to prepare and characterize oral felodipine lipid polymer hybrid nanocarriers (LPHNs) by two nanoparticle preparing methods to make a comparative study through the characterization process and in vitro and ex vivo intestinal permeation evaluation.

Material and Methods
Materials
The Labrasol, PEG laurate, and PEG oleate were purchased from Beijing Yibai Biotechnology Co., Ltd. China. The methanol, ethanol, KCl, HCl, KH_2PO_4, and Na_2HPO_4 Grin land chemical comp. The U.K. The felodipine, lauric acid, polysorbate 80, polysorbate 20, span 80, propylene glycol, and NaOH were purchased from Nanjing Duly Biotech Co., Ltd. China. The aniseed oil, argan oil, and cardamom oil were purchased from Hemani international KEPZ, Karachi, Pakistan. The Olibanum oil was purchased from AI-Emad for plant oil products. Iraq. The fenugreek oil was purchased from BAR-SUR-LOUP GRASSE (A.M).

Methods
The microwave-based method
The hydrophobic blend was prepared under a magnetic stirrer device at 1000 rpm for 5 minutes. It contains felodipine, lauric acid and chitosan that was dissolved in cardamom oil : PEG-laurate. The hydrophilic blend contains distilled water, polysorbate 80 and propylene glycol related to optimized amounts. By the application of microwave device for less than 15 seconds to the mixture of the two blends and under magnetic stirrer device at 1000 rpm for adequate time (seconds to minutes according to a final volume of dosage form), a colloidal dispersion system of felodipine LPHNs will be prepared. The optimized felodipine LPHNs formulations (HF1-HF3) as shown in Table (1), are immediately used for the study or lyophilized to be filled in hard gelatin capsules. In the lyophilization process (freeze-drying), the samples were first frozen at -20 °C for 2 h, then transferred at -80 ºC for 22 h., and then lyophilized at 0.001 mbar at -104 °C for 24 h using lactose 10%(w/w) as a cryoprotectant [10,14].

Single emulsification solvent evaporation technique (SESET)

In this method, the felodipine and the chitosan are dissolved in organic solvent and mix with the lipophilic phase, then it was added into an aqueous phase containing PEG laurate: polysorbate 80: propylene glycol. Under magnetic stirrer device and probe or ultra-sonication process result in the formation oil in water (o/w) emulsion. The organic solvent is removed by a rotary evaporator, yielding the felodipine lipid polymer hybrid nanocarriers. The optimized felodipine LPHNs formulations (HF4-HF6) as shown in Table (1), are immediately used for investigation or pass through freeze drying to fill in hard gelatin capsules. In freeze-drying, the samples were first frozen at -20 °C for 2 h, then transferred at -80 ºC for 22 h., and then lyophilized at 0.001 mbar at -104 °C for 24 h using lactose 10% as a cryoprotectant [15,16].

Table 1. The selected felodipine LPHNs formulations (HF1-HF6) for characterization and optimization.

Formulation code	Method of preparation	Felodipine % (w/w)	Cardamon oil % (w/w)	Lauric acid % (w/w)	Chitosan % (w/w)	PEG-(400) laurate :Polysorbate 80: Propylene glycol % (w/w)	Distilled water % (w/w) up to
HF1	Microwave based method	1	8	2	0.2	17.5:8.75:8.75	100
HF2	Microwave based method	1	8	2	0.25	20:10:10	100
HF3	Microwave based method	1	8	2	0.35	22.5:11.25:11.25	100
HF4	SESET	1	8	2	0.2	17.5:8.75:8.75	100
HF5	SESET	1	8	2	0.25	20:10:10	100
HF6	SESET	1	8	2	0.35	22.5:11.25:11.25	100

Characterization of the felodipine LPHNs formulations(HF1-HF6)
Globule size determination

The globule size is determined by the nanoparticle analyzer model SZ-100 - nanopartica series instruments from Horiba scientific company. The photo correlation spectroscopy (PCS), is a technique that has been used to determine the particle size of felodipine LPHNs formulation. The experiments were performed in triplicate [17].

Polydispersity Index (PDI) determination

The uniformity of the nanosystem of colloidal attributes is determined by the Polydispersity Index (PDI). It is measured by PCS technique. As PDI value increases, the uniformity of nanocarriers of the felodipine LPHNs formulations will decrease. The experiments were performed in triplicate [17].

Zeta Potential (ZP) measurement

The ZP is an index to determine the stability of the colloidal dispersion system that is affected by DLVO forces. It is measured by PCS technique. The ZP determines the surface charge which can develop around nanocarriers in a dispersion medium. The experiments were achieved in triplicate [17].

Entrapment efficiency (EE) and drug loading (DL) determination

The entrapment efficiency of felodipine LPHNs formulation is determined by the indirect method through, 0.1 mL of freshly prepared felodipine LPHNs formulation was taken and add to it 9.9 mL ethanol for dilution. The obtained colloidal dispersion was centrifuged for 15 minutes at 10000 rpm. The supernatant layer was removed and filtered through a 0.45 μm filter. The filtrated liquid was diluted in a sufficient amount of ethanol and analyzed by an ultraviolet (UV) spectrophotometer at 361.5 nm. This study was performed in three trials [18].

The encapsulation efficiency (EE) can be indirectly determined by the following equation (1):

EE (%)= [(Weight of felodipine in LPHNs)/$Total$ felodipine amount)] ×100 …………..… (1)

The drug loading (DL) expressed in percentage (%) is the quantity of active pharmaceutical agents

present in the nanocarriers divided by the total quantity of lipid present in the nanosystem. It is measured by the equation(2):

DL (%)=[(Weight of felodipine in LPHNs)/Weight of LPHNs] × 100 ………..… (2)

The pH determination

It has a great effect on the solubility of the therapeutic agents, the formulation attributes, tolerability, formulation stability, and the therapeutic agent's activity. The pH determination is an important factor in felodipine LPHNs formulations due to its relation to the stability and activity of pharmaceutical nanocarriers. The alteration of pH may be related to chemical interactions that can affect product quality. The digital pH meter employs to measure the pH of the felodipine LPHNs formulations. The study was achieved in triplicate [19].

Percent of light transmittance measurement

It is an important parameter that ascertains colloidal attributes of felodipine LPHNs formulations. The percent of light transmittance was determined by a UV-Visible spectrophotometer to preserve double distilled water as blank at 600 nm. Results were being taken in triplicate [20].

In vitro felodipine release experiment

The experiment was achieved for felodipine LPHNs (HF1-HF3) prepared by microwave-based method and felodipine LPHNs (HF4-HF6) that is prepared by single emulsification solvent evaporation technique and compare it with drug dissolution from pure drug suspension using the combination method of (USP dissolving type I apparatus - dialysis bag technique).Two dissolution media have been used which are HCl buffer pH 1.2 + 0.3 % polysorbate 80 solution and phosphate buffer pH 6.8 + 0.3 % polysorbate 80 solution. The dissolution medium volume is 900 mL for each experiment at $37 \pm 0.5\ ^{\circ}$C with constant stirring at 50 rpm. The drug amount in each of felodipine LPHNs (HF1-HF6) and pure drug suspension was 5mg of felodipine. Samples were withdrawn at predetermined intervals of time (5, 10, 15, 30, 60 minutes, and 2,4,8,12,24,36 hours) and filtered by microfilter paper of 0.45 µm pore size and compensate by equal withdrawn volume. At 361.5 nm, the felodipine concentration determines by spectrophotometrically[21,22]. The study was performed in triplicate and the results were analyzed using ANOVA statistical test at level p< 0.05. The drug liberation kinetics study performed by fit the obtained kinetic data into the various kinetic equations. The value of regression coefficients (R^2) will explain the resultant model. The mechanism of felodipine release was obtained from the slope of the Korsmeyer Peppas equation [23,24].

Ex-vivo intestinal permeation study

The study of ex-vivo intestinal permeation was performed using the non-everted sac technique [25,26]. The fasted male sheep weighing about 16 kg

was slain and anatomized under license university of Baghdad/ College of Pharmacy. The small intestine is a tested region that isolated and mesentery residue was removed and washed with normal saline solution. Several pieces of 5 cm in length and 2 cm diameter of the small intestine was produced after the cutting process. Insert in each piece that ligates from one end one capsule of (felodipine LPHNs HF1-HF6) and (felodipine drug suspension) where all capsules contain 5mg of felodipine and add 4.5 g of phosphate buffer pH 6.8 solution and tied the other end. Insert the tested segments in 900 ml of liquid which is phosphate buffer pH 7.4 solution+0.3% polysorbate 80mg using apparatus 1 rotating basket (Biobase Meihua Trading Co., Ltd.). At predetermined time intervals (5,10,15,30,60,90,120,150,180,210, 240 minutes) samples (5 ml) were taken and filtered by microfilter paper(0.45 µm) then find the felodipine concentration by UV spectrophotometer where the wavelength is 361.5 nm. The replenishment with an equal volume of withdrawn sample by diffusion liquid at once. The study was achieved in three trials and the outcome was analyzed statistically by ANOVA test at p< 0.05.

The effective membrane permeability was achieved by equation (3).

$M = P_{eff}\ S\ C_d\ t_{res}$ …………..… (3)

where,

M = therapeutic agent amount that absorbed

Peff = permeability coefficient (effective membrane permeability).

C_d = drug concentration at donor compartment

tres = residence time of drug in GI lumen.

S = surface area available for absorption

Statistical analysis

The research experimental data documented as mean $\pm$ SD (n=3). A statistical study was performed by analysis of variance (ANOVA) where a p-value less than 0.05 indicates a significant outcome[36].

Results and Discussion

Preparation of felodipine lipid polymer hybrid nanocarriers

The felodipine LPHNs formulations (HF_1-HF_3) were prepared by the new microwave-based method according to specified concentrations of components as shown in Table (1). The microwave's role in the formulation process was according to the following mechanism: when microwave passes through the ingredient of felodipine LPHNs formulation, the hydrogen bond, Vander walls bonds, electrostatic interactions, and hydrophobic forces between the molecules undergo temporary breakage. The microwave causes dipolar rotation and ionic conduction that cause molecular oscillation and molecular collisions that raise the thermal energy of the system lead to weak bonds disruption. The increased thermal energy for the

system create tamed molecules that is more favorable and faster to get self-assembly by the magnetic stirrer agitation to produce felodipine LPHNs formulation[10,14]. The felodipine LPHNs formulations (HF4-HF6) were prepared by single emulsification solvent evaporation technique (SESET) according to specified concentrations of components as shown in Table (1). The mechanism of SESET in felodipine LPHNs formulation depends on the formation of oil/water emulsion that finally produces hybrid nanocarriers through a single formulation step[15,16].

Characterization of the prepared felodipine LPHNs formulations

The following tests were utilized to characterize the prepared felodipine LPHNs systems:

Globule size determination

The results were HF1 (146.2 nm); HF2 (94.2 nm); HF3 (75.1 nm); HF4 (743.2 nm); HF5 (169.7) and HF6 (179.2 nm). The outcomes indicate that all felodipine LPHNs formulations(HF1-HF6) have nanoscale particle size and colloidal dispersion attributes. The analysis of variance indicates that there is a significant ($p<0.05$) decrease in particle size as the increase the concentration of PEG laurate: polysorbate 80: propylene glycol blend at constant lipid content.

Polydispersity Index (PDI) determination

PDI is a homogeneity parameter that determines the size distribution of nanocarriers within a colloidal dispersion system. Its value ranges from 0 to 1. The smaller values near zero indicate a more homogenous globule size distribution while large values that approach 1 indicate a wider globule distribution [27]. The results of felodipine LPHNs formulations (HF1-HF6) are HF1 (0.547); HF2 (0.54); HF3 (0.335); HF4 (0.888); HF5 (0.551) and HF6 (0.411). The outcome explains that there is a significant ($p<0.05$) decrease in PDI as the increase the concentration of PEG laurate: polysorbate 80: propylene glycol blend at constant lipid concentration.

Zeta Potential (ZP) measurement

The zeta potential is a parameter employ to measure the surface charge of felodipine LPHNs. It explains the physical stability of some colloidal dispersion systems. The results of felodipine LPHNs formulations (HF1-HF6) are HF1 (2.3 mV); HF2 (10.3 mV); HF3 (10.2 mV); HF4 (0.6mV); HF5 (-2.8mV) and HF6 (1.3mV). The felodipine LPHNs formulations(HF1-HF6), depend on non DLVO forces which are steric forces and hydration forces to stabilize the nanovesicles. Therefore the low value of zeta potential of LPHNs formulations(HF1-HF6) do not affect the physical stability and remain to withstand the process of nanocarrier aggregation because the stabilization process performed by non DLVO forces [28] The ANOVA explain there is a nonsignificant ($p > 0.05$) correlation between independent variables and zeta potential factor.

Entrapment efficiency (EE) and drug loading (DL) determination

The entrapment efficiency and felodipine loading is an important factor that employs for the evaluation and characterization of felodipine LPHNs formulations. The EE outcome of felodipine LPHNs formulations (HF1-HF6) are HF1 (85.443 % w/w); HF2 (84.81w/w); HF3 (84.177% w/w); HF4 (84.2% w/w); HF5 (83.4% w/w) and HF6 (82.8% w/w). It was found that the preparations (HF1-HF3) are more EE than preparations (HF4-HF6). The ANOVA indicates there is a significant ($p < 0.05$) correlation between independent variables and EE parameter.The DL outcome of felodipine LPHNs formulations (HF1-HF6) are HF1 (8.544% w/w); HF2 (8.481% w/w); HF3 (8.418% w/w); HF4 (7.7% w/w); HF5 (7.5% w/w) and HF6 (7.4% w/w). It was found that the preparations (HF1-HF3) are more DL than preparations (HF4-HF6) due to lower particle size and lower lipid content. The ANOVA indicates there is a significant ($p < 0.05$) correlation between independent variables and the DL factor.

The pH determination

The pH outcome of felodipine LPHNs formulations (HF1-HF6) are HF1 (4.2); HF2 (4.1); HF3 (4.3); HF4 (4.1); HF5 (4.1) and HF6 (4.2). The result shows that the felodipine LPHNs formulations (HF1-HF6) had a suitable acidic pH value in the range of (4.1 – 4.3) that is better for oral administration[29]. Also that the preparations (HF1-HF3) are nearly similar pH values of preparations (HF4-HF6). The analysis of variance shows a significant relationship ($p<0.05$) between independent variables and pH parameter.

Percent of light transmittance measurement

The percent (%) of light transmittance is an attractive parameter to explain physically the colloidal properties of felodipine LPHNs formulations (HF1-HF6).The results of light transmittance percentage of felodipine LPHNs formulations (HF1-HF6) are HF1 (94.3%); HF2 (95.2%); HF3 (92.3%); HF4 (89.2%); HF5 (90.1%) and HF6 (90.8%).The outcomes show that the preparations (HF1-HF3) are more transparent than preparations (HF4-HF6) and all felodipine LPHNs formulations (HF1-HF6) gave features of colloidal dispersion [30]. The analysis of variance indicated a significant relationship between independent variables and light transmittance percentage at a level ($p<0.05$).

In vitro felodipine release experiment

The experiment was done using the combinational method which is (USP type I (Basket) - dialysis bag technique in two dissolution media which are HCl buffer pH 1.2 + 0.3 % polysorbate 80 solution and phosphate buffer pH 6.8 + 0.3 % polysorbate 80 solutions. The outcome ascertains there is no burst release of the therapeutic agent from all felodipine LPHNs formulations (HF1-HF6) and there was an extended-release process over 36 hours from all felodipine LPHNs formulations (HF1-HF6) [46].

In dissolution medium of HCl buffer pH 1.2 + 0.3 % polysorbate 80 solutions as shown in Figures (1,2,3), the felodipine release profile was significantly higher (p-value <0.05) in dissolution rate for HF3 and was significantly lower (p-value < 0.05) in dissolution rate of the pure drug. The comparability profile of felodipine release for formulas have similar concentration with different preparation method was explained as following: HF1> HF4, HF2> HF5 and HF3> HF6 while the comparability profile of the felodipine release from LPHNs formulation (HF1-HF6) and the pure drug suspension explains in the following descending order: HF3 > HF 6 > HF 2 > HF 5 > HF 1 > HF 4 > pure drug suspension. The result indicates that formulas were prepared by microwave-based method to provide a higher dissolution rate in comparison to formulas were prepared by SESET. This is due to the microwave-based method was prepared formulas (HF1-HF3) has lower particle size than formulas (HF4-HF6) which prepared by SESET, which provide higher surface area for exposure to the dissolution medium lead to increase the rate of felodipine dissolution.

In dissolution medium of phosphate buffer pH 6.8 + 0.3 % polysorbate 80 solution as shown in Figures (4,5,6), the felodipine release profile was significantly higher (p-value <0.05) in dissolution rate for HF2 and was significantly lower (p-value < 0.05) in dissolution rate of pure drug suspension. The comparability profile of felodipine release for formulas have similar concentration with a different preparation method was explained as following: HF1> HF4, HF2> HF5 and HF3> HF6 while the comparability profile of the felodipine release from felodipine LPHNs formulations (HF1-HF6) and the pure drug suspension explains in the following descending order: HF2 > HF 3 > HF 1 > HF 6 > HF 5 > HF 4 > pure drug suspension. The result indicates that formulas were prepared by a microwave-based method to provide a higher dissolution rate in comparison to formulas were prepared by SESET. This is due to the microwave-based method was prepared formulas (HF1-HF3) has lower particle size than formulas (HF4-HF6) which prepared by SESET, which provide higher surface area for exposure to the dissolution medium lead to an increased rate of felodipine dissolution.

In the dissolution rate of felodipine profile, it was observed that the pure drug suspension gives a lower release rate of the therapeutic agent in comparison to all optimized LPHNs formulations (HF1-HF6) because that LPHNs formulations (HF1-HF6) have a large surface area that exposes to the dissolution medium and this will allow a higher interaction with the dissolution medium that increases dissolution rate [47,48]. The analysis of variance indicated a significant (p<0.05) relationship between independent variables and felodipine release.
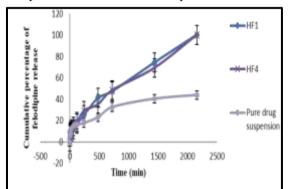

Figure 1. In vitro felodipine release profile from LPHNs formulations HF1,HF4 and the pure drug at HCl buffer pH 1.2 + 0.3 % polysorbate 80 solutions, the values of mean ±SD (n=3).
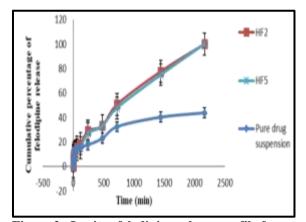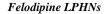

Figure 2. In vitro felodipine release profile from LPHNs formulations HF2,HF5 and the pure drug at HCl buffer pH 1.2 + 0.3 % polysorbate 80 solutions, the values of mean ±SD (n=3).
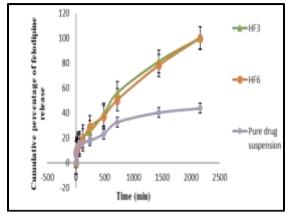
Figure 3. In vitro felodipine release profile from LPHNs formulations HF3,HF6 and the pure drug at HCl buffer pH 1.2 + 0.3 % polysorbate 80 solutions, the values of mean ±SD (n=3).
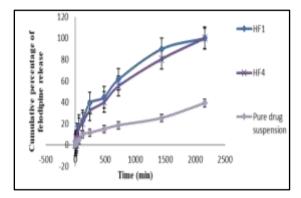

Figure 4. In vitro felodipine release profile from LPHNs formulations HF1,HF4 and the pure drug suspension at phosphate buffer pH 6.8 + 0.3 % polysorbate 80 solutions the values of mean ±SD (n=3).
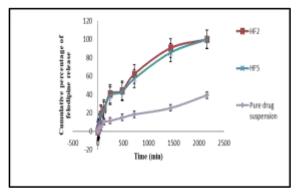

Figure 5. In vitro felodipine release profile from LPHNs formulations HF2,HF5 and the pure drug suspension at phosphate buffer pH 6.8 + 0.3 % polysorbate 80 solutions.
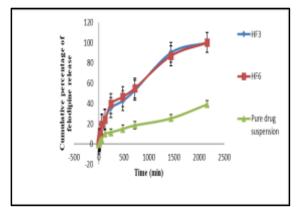

Figure 6. In vitro felodipine release profile from LPHNs formulations HF3,HF6 and the pure drug suspension at phosphate buffer pH 6.8 + 0.3 % polysorbate 80 solutions.

Kinetic analysis of release drug

The kinetic data were summarized in Table (3) and Table (4), were obtained from in vitro release of felodipine LPHNs formulations (HF1-HF6) and the pure drug suspension at HCl buffer pH 1.2 + 0.3 % polysorbate 80 solution and phosphate buffer pH 6.8 + 0.3 % polysorbate 80 solutions. It was fitted to different models that determine the mechanism of therapeutic agent release. The kinetic models that employ in the investigation were zero-order, first-order kinetic, Higuchi model, and Korsmeyer-Peppas model. The outcome indicates Higuchi's model was obtained due to the higher regression coefficient (R^2) is obtained for it. This indicates felodipine was release from the monolithic system. The felodipine liberation from LPHNs formulations (HF1-HF6) following non Fickian/anomalous dissolution (diffusion and erosion) due to the drug liberation exponent were significantly higher (p<0.05) than 0.43[23].

Table (2). Summary of characterization results of felodipine LPHNs formulations (HF1-HF6).

code	Globule size (nm)*	PDI*	Zeta potential (mV)*	Entrapment efficiency % (w/w)*	Drug loading % (w/w)*	pH*	Percent of light transmittance*	Release percent of felodipine in HCl buffer pH 1.2 + 0.3 % polysorbate 80 solutions at 24 hours*	Release percent of felodipine in phosphate buffer pH 6.8 + 0.3 % polysorbate 80 solutions at 24 hours*
HF1	146.2±10.095	0.547± 0.0502	2.3± 0.264	85.443±5.058	8.544±0.478	4.2±0.435	94.3± 0.8	74.21± 0.7	89.882± 1.268
HF2	94.21± 1.587	0.54± 0.078	10.3±0.721	84.81± 4.013	8.481±0.354	4.1±0.264	95.2± 0.964	77.682± 0.532	90.575± 0.238
HF3	75.1±2.816	0.335± 0.005	10.2±0.754	84.177±2.561	8.418±0.256	4.3± 0.36	92.3± 0.888	81.154± 0.872	90.2± 0.435
HF4	743.2±8.861	0.888± 0.007	0.6± 0.264	84.2± 4.529	7.7± 0.608	4.1±0.132	89.2± 0.869	69.21± 0.642	80.6± 1.587
HF5	169.7± 9.553	0.551± 0.005	-2.8±1.081	83.4± 3.984	7.5± 0.529	4.1±0.264	90.1± 1.058	74.98± 1.048	85.3± 0.264
HF6	179.2± 8.827	0.411± 0.011	1.3± 0.2	82.8± 3.862	7.4± 0.435	4.2±0.264	90.8± 0.964	78.1± 1.228	87.1± 0.754

*Values are expressed as mean ± SD (n=3).

Table 3. The correlation coefficient (R2) and release exponent (n) of different kinetic models of felodipine LPHNs (HF1-HF6) and the pure drug released in HCl buffer pH 1.2 + 0.3 % polysorbate 80 solutions.

Formulation code	Zero Order model	First Order model	Higuchi model	Korsemeyer-peppas model	
	R^2	R^2	R^2	R^2	n
HF1	0.971	0.866	0.982	0.948	0.5064
HF2	0.968	0.8821	0.979	0.934	0.496
HF3	0.958	0.906	0.984	0.919	0.4914
HF4	0.969	0.756	0.983	0.960	0.516
HF5	0.972	0.870	0.992	0.942	0.504
HF6	0.968	0.885	0.984	0.929	0.494
Pure drug suspension	0.843	0.89	0.965	0.908	0.4133

Table 4. The correlation coefficient (R^2) and release exponent (n) of different kinetic models of felodipine LPHNs (HF1-HF6) and the pure drug released in phosphate buffer pH 6.8 + 0.3 % polysorbate 80 solutions.

Formulation code	Zero Order model	First Order model	Higuchi model	Korsemeyer-peppas model	
	R^2	R^2	R^2	R^2	n
HF1	0.895	0.965	0.989	0.924	0.7304
HF2	0.891	0.968	0.988	0.964	0.6774
HF3	0.917	0.9563	0.9884	0.9794	0.6356
HF4	0.936	0.909	0.996	0.968	0.649
HF5	0.911	0.937	0.993	0.98	0.645
HF6	0.905	0.944	0.992	0.983	0.622
Pure drug suspension	0.939	0.958	0.9755	0.9318	0.4795

Ex-vivo intestinal permeation study

The permeability coefficient (cm/min) was calculated after obtaining felodipine flux (µg/mL) as shown in Table (5). The comparative study was performed on the felodipine LPHNs formulations (HF1-HF3) that prepare by microwave-based method and felodipine LPHNs formulations (HF4-HF6) that prepare by SESET. It was found that the permeability coefficient (cm/min) has the following descending order HF1 > HF4, HF2 > HF5, HF3> HF6. This indicates that formulas (HF1-HF3) were prepared by microwave-based method provide a higher permeation rate across the intestinal membrane in comparison to formulas (HF4-HF6) were prepared by SESET.
The outcomes of the ex vivo intestinal permeation experiment indicate that the permeability coefficient (cm/min) of felodipine was significantly higher (p-value <0.05) for F3 and was significantly lower (p-value < 0.05) for pure drug suspension. The comparability profile of the felodipine release from LPHNs formulation (HF1-HF6) and the pure drug suspension explains in the following descending order: HF3 > HF 6 > HF 2 > HF 5 > HF 1 > HF 4 > pure drug suspension as shown in Figures (7,8,9).
It was noted that the pure drug suspension gives a lower intestinal flux in comparison to all felodipine LPHNs formulations(HF1-HF6) due to increase solubility and intestinal permeation with felodipine LPHNs as lipid-based technology. In addition, the nanosize of these lipid-based carriers provide a large surface area for drug release and rapid absorption to the systemic circulation [25,26]. The analysis of variance indicated a significant(p-value <0.05) relationship between independent variables and ex vivo intestinal permeation factor.

Table 5. The slope and permeation coefficient for felodipine from LPHNs formulations (F1-F9) and pure drug suspension through non-everted sheep intestine.

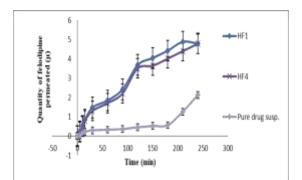| Formulation code	Flux (µg/mL)	Permeability coefficient (cm/min)
HF1	0.0211	0.000559
HF2	0.0213	0.000564
HF3	0.0226	0.000599
HF4	0.0199	0.000527
HF5	0.0211	0.000559
HF6	0.022	0.000583
Pure drug suspension	0.006	0.000159

Figure 7. Permeation of felodipine from LPHNs formulations HF1, HF4 and pure drug suspension through non-everted sheep intestine, the values of mean ±SD (n=3).
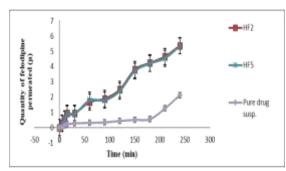

Figure 8. Permeation of felodipine from LPHNs formulations HF2, HF5 and pure drug suspension through non-everted sheep intestine, the values of mean ±SD (n=3).
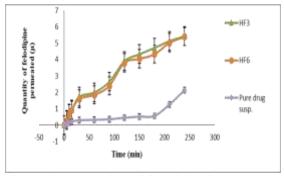

Figure 9. Permeation of felodipine from LPHNs formulations HF3, HF6 and pure drug suspension through non-everted sheep intestine, the values of mean ±SD (n=3).

Conclusion

The microwave-based method is a very effective and reproducible technique in preparing felodipine LPHNs formulations (HF$_1$-HF$_3$) and prepotent to the SESET that prepare felodipine LPHNs formulations (HF$_4$-HF$_6$). All the felodipine LPHNs formulations (HF$_1$-HF$_6$) show colloidal dispersion properties but The (HF$_1$-HF$_3$) superior to that (HF$_4$-HF$_6$) according to the research characterization process. All the felodipine LPHNs formulations (HF$_1$-HF$_6$) show an extended drug release nanosystem that makes it an advanced system for control therapeutic agent delivery to

improve the patient's commitment to taking treatment on time.

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