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Formulation, Optimization, and *Invitro* Characterization of Lipid-Based Nanoparticles for Effective Delivery to the Liver

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Abstract - Chronic liver disorders are the major causes of illness and mortality worldwide. Patients with chronic liver diseases have a greater chance of developing cirrhosis, hepatocellular carcinoma, progressive liver fibrosis, and subsequently liver failure. Currently there are no effective treatments available for patients with the various kinds of liver diseases. The use of nanotechnology is considered a rapidly growing field of interest for the safe and targeted delivery of insufficiently water-insoluble hepatoprotective drugs. Therefore, the nanoparticle combination improves bioavailability and plasma stability of drugs with poor aqueous solubility. Thus, this study aims at developing chemically and physically stable Fenretinide loaded solid lipid nanoparticles (FEN-SLNs) for successful delivery to the liver. The nanoencapsulation of FEN in Gelucire-based, surfactant-free SLNs was developed. SLNs were characterized in terms of physicochemical properties, surface morphology, drug loading, release behavior as well as *in vivo* biodistribution study. The results showed that adopting hot homogenization method for preparation of FEN loaded solid lipid nanoparticles using Gelucire 50/13 and Precirol provided chemically and physically stable FEN-SLNs. Further, the optimized FEN-SLNs has particle size 298.3 ± 2.54 and PDI 0.3 with negative zeta potential -15.2 ± 3.61 mV, and Entrapment efficiency exceeding 92%. Furthermore, *in vitro* release experiment ensured sustained release of FEN over > 24 h with no signs of degradation. In addition, TEM photomicrographs showed spherical particles. Noteworthy, the *in vivo* biodistribution results showed that fluorescently labeled SLNs retained in the liver for 8h with diminished migration to the other organs unlike the free dye. In conclusion the study highlights the effective encapsulation of FEN and effective delivery to the liver.

Keywords**:** Solid lipid nanoparticles, Gelucire, tissue distribution, liver

1. Introduction

Despite the availability of different medications and/or vaccinations, hepatic fibrosis, cirrhosis, and hepatocellular carcinoma are the main consequences of liver diseases that obviously contribute to mortality globally. In addition, the failure to deliver sufficient drug concentration to the liver disorder resulted in unfavourable results which is considered the main disadvantages of traditional therapy [1]. According to the centre of disease control and prevention (CDC), liver diseases and cirrhosis (end stage for hepatic fibrosis) are ranked among the top leading cause of death in 2020 with a rate of 15.7 per 100,000 [2]. Liver fibrosis is an excessive deposition of extracellular matrix (ECM) produced by the activated hepatic stellate cells (HSC), which leads to severe pathophysiological disturbances in the liver. Several factors could induce the activation of HSC, such as: viral infection (e.g., hepatitis B or C virus), excessive use of drugs or alcohol, autoimmune injury, cholestasis, and genetic diseases [3]. Since, the liver fibrosis is a crucial reversible intermediate phase for the liver cirrhosis and/ or liver cancer. There are different treatment protocols to stop the progression of this disease. Lately antifibrotics have been introduced to halt the progression of the fibrosis and help it to regenerate new healthy cells [4, 5].

Fenretinide (FEN), a synthetic derivative of retinoic acid, is reported to be used as a chemo preventive for different type of cancers such as Breast, Prostate, Pancreas and skin cancers [6]. In addition, it has been recently repurposed to be used as anti-fibrotic drug for both cystic fibrosis and liver fibrosis [7, 8]. However, working with this drug is challenging on various levels due to its poor physicochemical properties, for instance it is highly hydrophobic, low bioavailability, as well as its sensitivity towards light and heat. In addition, this drug is not readily available in the market [9]. Notably, drug delivery can be made more precise using nanotechnologies made of biocompatible materials, by improving the physicochemical

characteristics of the drug nanocarriers (such as their size and surface properties) that enable the drug targeting to the diseased side with minimum side effects [10].

Solid lipid nanoparticles (SLNs) consist of solid lipid, emulsifier, and water/solvent. They offer several advantages for instance, they have excellent biocompatibility, can easily prepared by conventional emulsion preparation techniques, provide excellent encapsulation for both hydrophilic and hydrophobic drugs, control drug release over long period of time and improve drug stability over time [11] . SLNs are usually prepared with different lipids such as, glyceryl behenate (Compritol) fatty acids (stearic acid and palmitic acids), etc. Gelucires® are multifunctional lipid excipients made of mono-, di-, and triglycerides as well as mono- and di-fatty acid esters of polyethylene glycol. They are usually regarded as safe. Moreover, they have distinctive compositions that operate as a lipid matrix in drug delivery systems by using surfactants, cosurfactants, and lipid phases. Two values are typically used to characterise Gelucires®; the first one is the lipid melting point and the second one is the HLB value. As a result, Gelucires® has intriguing qualities such as emulsification, improved medication solubility, and granule formation [12] . Nevertheless, it was discovered that using them in the lipid nanocarrier system improved drug loading and stabilised the lipid nano system. Therefore, in the current study, the main aim is to encapsulate FEN in Gelucire-based SLNs that maintain drug stability both during preparation and storage and guarantee its efficient delivery to the site of Liver.

2. Material and Methods

Materials

Fenretinide (high purity, >98%) was obtained from Sigma-Aldrich, Canada. Lipids such as (Gelucire 43/01, Gelucire 50/13, Compritol 888 ATO and Precirol) were kindly donated samples from Gattefosse, Lyon, France. Poloxamer 407 (Kolliphore 407) was supplied from BASF, Ludwigshafen, Germany. Polysorbate 80 and potassium dihydrogen phosphate were purchased from El-Nasr Pharmaceutical Co, Cairo, Egypt. Coumarin-6 was provided from Polysciences, Europe GmbH, Germany. HPLC grade of Acetonitrile was purchased from Fischer scientific, Pittsburgh, United states of America.

Methods

Solubility Study of Fenretinide (FEN) in Lipids (Qualitative Screening)

As previously Published by Nafea *et al.* [13] with little modifications, 100 mg of each lipid (Gelucire 43/01, Gelucire 50/13, Compritol 888 ATO, Precirol ATO5 and Precirol) were melted at 5°C above the lipid melting point. Then FEN was added gradually to the molten lipids, in ratio 1:10 with simple stirring till no more drug was dissolved. After that, the drug solubility was observed visually and under optical microscope to see if there are any drug crystals. The total weight of FEN increments showing no crystals under microscope in the melted lipid is considered as solubility of FEN in the lipid.

Preparation of Fenretinide loaded solid lipid nanoparticles (FEN-SLNs) and optimization of process parameters for stable formulation.

FEN-SLNs were prepared by hot homogenization technique followed by ultra-sonication [13]. First, lipids (Gelucire 50/13 and Precirol) were melted in water bath at 80 °C and then FEN (0.05 %*w/v*) was dissolved in the melted lipids. After that, the aqueous medium was added dropwise at the same temperature under stirring rate at 800 rpm. Hereafter, the dispersion was homogenized at 15,000 rpm for 10 minutes at the same temperature followed by sonication at 40 °C for 10 minutes. Finally, nanoparticle dispersion was allowed to stabilize by stirring at room temperature at 600 rpm for 30 minutes.

Various factors were studied to optimize and stabilize the loaded solid lipid nanoparticles, different lipids were tried in different concentrations with/ without different emulsifier such as Poloxamer 407 or Tween 80. The composition of these SLNs formulations is shown in Table 1. On the chosen formulation (FEN-SLNs 4), to study the impact of both homogenization speed and time on colloidal stability of SLNs. Three different formulations were prepared at different homogenization speed (10,000, 15,000 and 20,000 RPM) for 10 minutes. In addition, effect of homogenization time was evaluated by preparing another 4 formulations at different homogenization time (5, 10, 15, 20 minutes) and maintaining homogenization speed at 15,000.

Table 1: Composition of FEN-SLNs formulations

Formula code	Concentration (% *w/v*)					
	Drug	Lipids			Emulsifier	
	Fenretinide	Gelucire 50/13	Compritol	Precirol	Poloxamer 407	Tween 80
Blank 1A	—	—	—	0.5	0.1	—
Blank 1B	—	—	—	0.5	0.2	—
Blank 1C	—	—	—	0.5	0.5	—
FEN-SLNs-1	0.05	—	—	0.5	0.5	—
Blank 2A	—	0.1	0.5	—	—	—
Blank 2B	—	0.2	0.5	—	—	—
Blank 2C		0.5	0.5			
FEN-SLNs-2	0.05	0.5	0.5	—	—	—
Blank 3A	—	0.5	—	0.5	—	0.125
Blank 3B	—	0.5	—	0.5	—	0.25
Blank 3C	—	0.5	—	0.5	—	0.5
Blank 3D	—	0.5	—	0.5	—	1
FEN-SLNs-3	0.05	0.5	—	0.5	—	0.125
Blank 4A	—	0.5	—	0.5	—	—
FEN-SLNs-4*	0.05	0.5	—	0.5	—	—
FEN-SLNs-5	0.05	0.7	—	0.3	—	—
FEN-SLNs-6	0.05	0.85	—	0.15	—	—

**Formula FEN-SLNs-4 will be used for further investigations*

Preparation of fluorescently labeled solid lipid nanoparticles (Cou6-SLNs) for in vivo biodistribution.

Similarly, fluorescently labeled SLNs loaded with Coumarin-6 to allow their visualization using fluorescent microscope (λ_{ex} 450 nm, λ_{em} 505 nm). SLNs were prepared as mentioned in the previous section while replacing FEN with coumarin-6 (10 µg/ml of SLNs dispersion). The Cou6-SLNs contain Gelucire 50/13 and Precirol in concentration 0.5% *w/v* each, per SLNs dispersion.

HPLC analysis of Fenretinide

Standard solutions and calibration curve

A standard stock solution for FEN was prepared in acetonitrile in concentration 100 mg% *w/v*. After that, the working solutions for calibration curve were prepared by dilution of the stock solutions. Different volumes corresponding to concentrations in the range of 0.1–1.0 mg% *w/v* were withdrawn from FEN stock solution and diluted with mobile phase (Acetonitrile: Water 90:10) in 10 ml volumetric flasks.

Chromatographic conditions and construction of calibration curve

The HPLC analysis was performed using a system equipped with a reverse phase C18 column. The isocratic mobile phase, consisting of a mixture of acetonitrile and water (90:10 *v/v*), was eluted at a flow rate 1 ml/min. The injection volume was 20 µl. The eluent was monitored by the diode array detector from 190 to 400 nm, and chromatograms were extracted at the wavelength of 360 nm. All determinations were performed at 26 °C. Triplicate injections were made for each concentration and chromatographed as under the previously described LC conditions. Under these conditions, a calibration curve was constructed by plotting measured peak area versus corresponding FEN concentration and the best straight line was drawn.

Physicochemical characterization of FEN-SLNs

Measurement of colloidal stability

The particle size, polydispersity index (PDI) and zeta potential of blank and drug loaded SLNs were measured by Zeta-Sizer Nano-ZS (Malvern Instruments, Malvern, UK). The samples were properly diluted with Milli Q water in ratio 1:10 followed by sonication for 5 minutes prior to measurements. All samples were measured in triplicates and the results were calculated as average of three samples and standard deviation.

Morphological examination

The particles morphology for both blank and FEN-SLNs was observed by Transmission electron microscopy (TEM, JOEL, 100 CX, Japan). Samples were diluted with Milli Q water and then added on grid and then left for drying and then stained with Uranyl acetate as a negative staining.

Calculation of Entrapment efficiency (EE)

The drug entrapment efficiency was measured directly by using centrifugal ultrafiltration using Centrisart-I® MWCO 10kDa, Sartorius AG, Goettingen, Germany as previously reported by *Gaber et al.* [14]. One ml of FEN-SLNs was added in Centrisart and centrifuged at 5000 rpm for 10 minutes. Consequently, the encapsulated FEN was measured directly by extracting the entrapped drug in SLNs with acetonitrile and melt it in water bath at 80°C followed by sonication and then filtered with 0.45 µm syringe filter and measured with above mentioned validated HPLC method. The % EE was calculated using the following equation:

$$\% \text{ EE} = (\text{Amount of entrapped drug in SLNs} / \text{ Total drug content}) * 100 \quad (1)$$

In vitro release study

The release profile of FEN from SLNs was investigated using dialysis method. The release medium was 100 ml phosphate buffer (pH 7.4): ethanol (1:1 *v/v*) in amber glass bottles. Three ml of SLNs containing 0.05 %*w/v* FEN were instilled in dialysis bag in comparison to same concentration of FEN suspension in water: ethanol (2:1). The dialysis bags used are (12–14 KDa molecular weight cut off, VISKING dialysis tubing, SERVA, electrophoresis, Germany). The dissolution set was transferred to a horizontal shaking incubator at 100 rounds/min, the temperature adjusted at 37 ± 0.5°C. One ml sample was withdrawn at certain time intervals (1, 4, 6, 8 and 24 h) and replaced with an equal volume of a pre-warmed fresh release medium. The amount of drug released was detected by the previously mentioned HPLC method after dilution sample with acetonitrile in ratio 1:1 and filter with 0.45 µm syringe filter.

In vivo Pharmacodynamics (Tissue deposition and Organ distribution) of fluorescent labelled SLNs

Experimental animal study protocol

The *in vivo* biodistribution study was performed to prove the impact of nanosytem to effectively deliver the drug to the liver in comparison to free dye. This study was carried out on male Wistar albino rats 170-250g.

The rats were kept at room temperature (25 ℃) and 50% relative humidity and then housed in stainless-steel cord mesh cages. Rats were randomly categorized into 2 groups (4 rats/ group) and fasted overnight with no access restriction to water before treatment. Group (1) - rats were injected intraperitoneal (IP) with normal saline containing 500 ng free Coumarin 6, group (2) – rats were IP injected with NP suspension (Cou-SLNs) equivalent to 500 ng Cou 6. The experimental protocol was approved via the Animal Care and Use Committee of the Faculty of Pharmacy, Alexandria University.

Biodistribution study

After 1 and 8 h after the administration, two rats from each category were sacrificed, and neutral formalin in 10% saline was perfused through the heart for dye fixation [15]. After that, organs (Liver, kidney, spleen and brain) were collected and kept in 10% neutral formalin for preservation. Further, tissues of 2–3 mm thickness from every organ had been trimmed with a scalpel and located in a tissue cassette that processed into paraffin and embedded in a paraffin block, sectioned on a microtome to a thickness of 2 µm, placed on a microscope slide, following standard histology techniques.

Fluorescence microscopy

To detect coumarin 6 in the selected tissue samples, fluorescence microscopy was used. Slices of thickness (±2 mm) of various tissues had been fixed in sample holder and covered with a glass cover slip for examination through fluorescent microscope (Olympus BX 41, Olympus America Inc., Mellville, NY, USA) supplied with planachromat N 20X Objective Lens. The NPs were depicted as green colour.

3. Results and discussion

Solubility of FEN in Lipids (Qualitative)

One of the most important parameters to formulate SLNs is the selection of the most suitable solid lipids, considering their ability to dissolve the required drug and improve its incorporation in the melted lipids. Four different lipids (Gelucire 50/13, Gelucire 43/01, Precirol and Compritol) were tried and assessed qualitatively. Fig.1 showed the results where, the maximum solubility was denoted for Gelucire 50/13 in comparison to the other lipids as there are no drug crystals observed under the microscope (Fig. 1C). These results were in accordance with our previous findings where, this enhancement in drug solubility was attributed to the amphiphilic characters of Gelucire 50/13 as it contains both polar heads and non-polar

tail. On the other, hand drug crystals were recorded in case of Compritol and Gelucire 43/01 (Fig. 1A-B). in Contrast, FEN showed good affinity to Precirol where few crystals were observed (Fig. 1D). accordingly, both Gelucire 50/13 and Precirol were selected for preparation of nanoparticles.

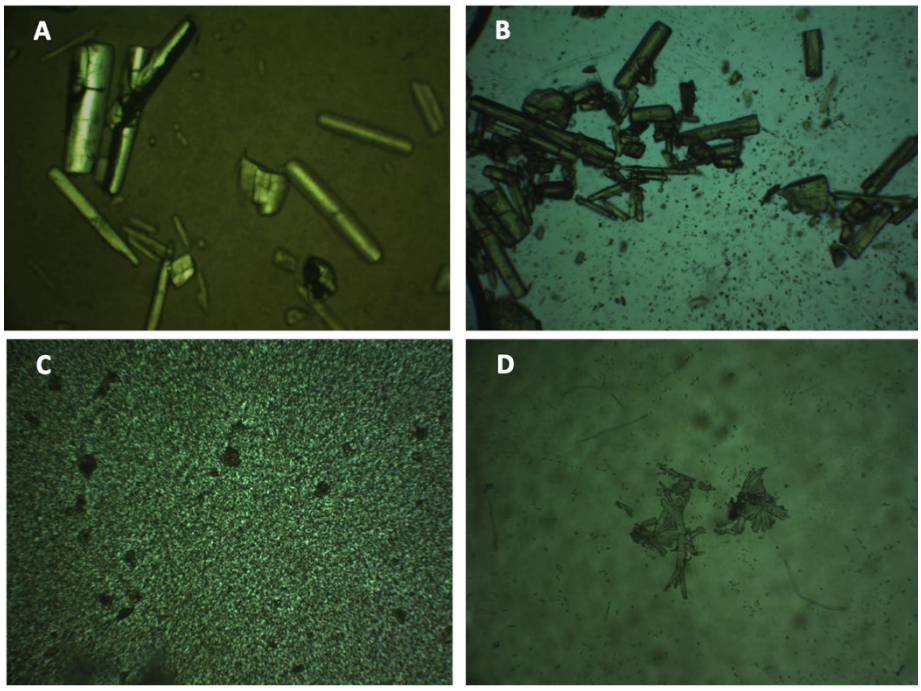

Fig. 1: Solubility of Fenretinde in different lipids; (A) Compritol 888 ATO, (B) Gelucire 43/01, (C) Gelucire 50/13, and (D) Precirol.

HPLC analysis of FEN

Linearity and regression equation

A validate HPLC method was used to quantify the amount of FEN. Fig 2A showed the chromatogram of FEN solution in concentration 0.5 mg% *w/v* diluted with mobile phase (Acetonitrile: Water 90:10). The chromatogram showed a symmetric and sharp peak with retention time 6.073 minutes. The linearity of the HPLC method was demonstrated by analysing a series of different concentrations for FEN. A calibration curve was drawn between the measured peak area at 360 nm versus the proportional drug concentration. The standard calibration curve was linearly correlated (R^2=1) over different FEN concentration ranging from (0.1-1 mg% *w/v*) (Fig. 2B).

Limit of detection and Limit of quantification

The concentration of the analyte with a signal-to-noise ratio of 3:1 is known as the limit of detection (LOD). While considering the ratio is 10:1 is the limit of quantitation (LOQ). Using the signal-to-noise ratio calculation, the LOD and LOQ values were determined to be 0.297 and 0.901 mg % *w/v*, respectively. The suggested approach demonstrated low noise levels and high drug responses, which allow for the quantification and detection of low concentrations, according to both LOD and LOQ values.

Inter- and intra-day precision

To examine the method precision, five different concentrations of FEN were measured (3 replicate each) for each concentration during the same day and the following day resembling, intra-day and inter-day precision, respectively. The findings demonstrated that this HPLC method used for the calculation of FEN concentration had a high repeatability and accuracy, with relative standard deviation (%RSD) < 2%.

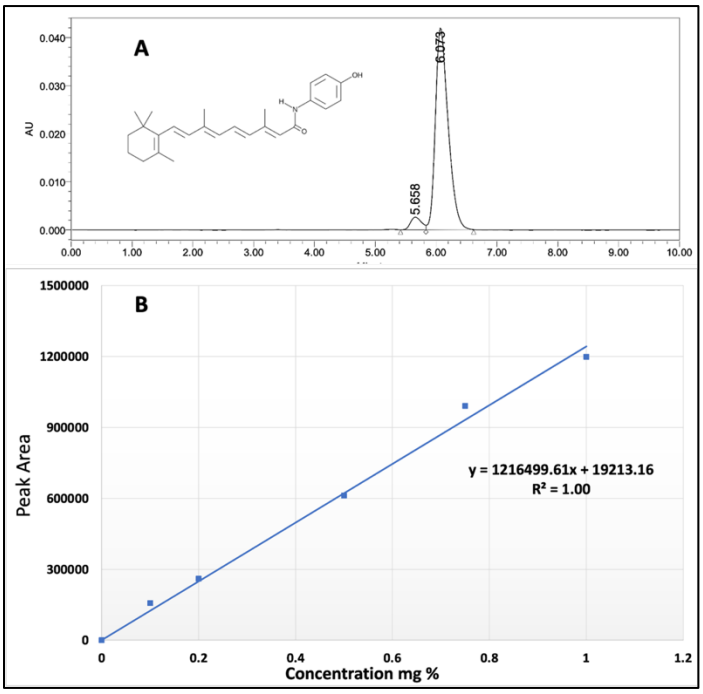

Fig. 2: (A) HPLC chromatogram of FEN, insert represents the chemical structure of FEN, (B) Calibration curve of FEN in acetonitrile: water (90:10).

Formulation variables affecting nanoparticles characteristics

In the present study, FEN was encapsulated in solid lipid nanoparticles. The optimum formulation (FEN-SLNS-4) was prepared and stabilized by using Gelucire 50/13 and Precirol in concentration 0.5 % *w/v* each. Measurements of entrapment efficiency revealed high entrapment efficiency 95.4 ± 1.98%, particle size 298.3 ± 2.54 and PDI 0.3 with negative zeta potential -15.2 ± 3.61 mV (Fig. 3).

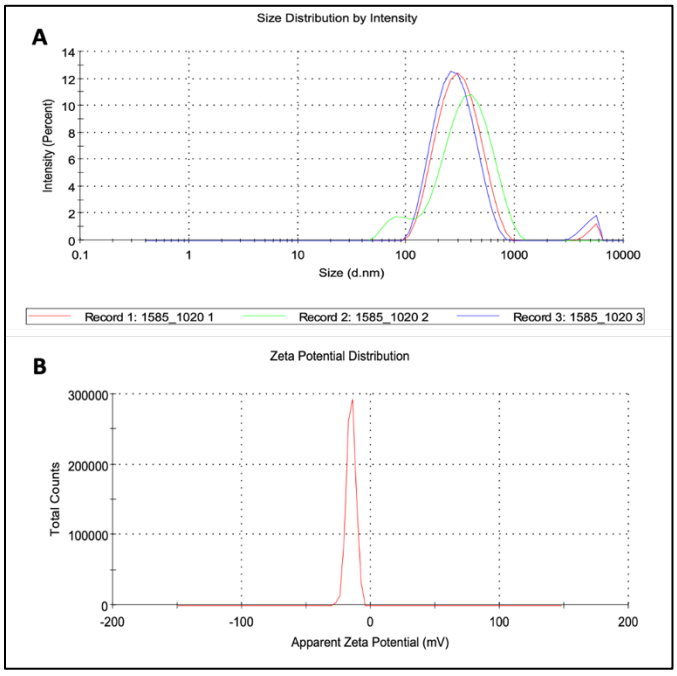

Fig. 3: (A) Particle size distribution of FEN-SLNs 4, (B) Zeta potential distribution

In case of Precirol SLNs (Blank 1A-1C), it was found that by increasing Poloxamer concentration (conc.) from 0.1 to 0.5% resulted in

decreasing particle size from 201.2 ± 1.44 nm to 134.4 ± 2.06 nm (Fig. 4A). These results were in accordance with our previous findings [13] and this decrease in particle size associated with increase in surfactant concentration could be attributed to decrease in surface tension and better particles portioning during homogenization. However, after encapsulating FEN in conc. 0.05% *w/v* (FEN-SLNs-1) was unstable after 48h from preparation and drug partitioned out of SLNs and precipitated. Similar results were obtained by replacing Poloxamer with Tween 80 in conc. range from 0.125-1% *w/v* (Blank 4A-D). Where, Particle size decreased with increasing surfactant conc. from 0.125 to 0.25% (Fig. 4B). Further increase in Tween 80 conc. resulted in aggregation and increase in particle size. This might be due to excessive amounts of emulsifier could dramatically increase the size via particle bridging and or multilayer formation as was the case in formula (Blank 4C-D).

Preparation of Compritol based SLNs, in this case Gelucire 50/13 was used in conc. (0.1-0.5% *w/v*) (Blank 2A-C) as an emulsifying agent and lipid. Blank 2A was unstable and aggregation occurred due to insufficient surfactant conc. and this was confirmed by visual inspection and presence of lumps whereby, a sort of very viscous liquid or most probably liquid crystals were developed. However, by increasing concentration of Gelucire 55/13 to 0.2 and 0.5 % *w/v* (Blank 2B-C) resulted in colloidal stable SLNs with particle size and 301.3 ± 1.19 nm, and 255.7 ± 2.06 nm. FEN-SLNs-2 prepared with Compritol and Gelucire 50/13 (0.5% *w/v* each) showed low value of entrapment efficiency (62.1 ± 3.05%) this might be due to poor FEN solubility in Compritol as previously expressed in the above section. Accordingly, the use of Compritol will be omitted and replaced with Precirol.

Therefore, to maximize drug encapsulation, three formulations namely (FEN-SLNs 4-6) were prepared with different Gelucire 50/13: Precirol ratio as described in table 1. It was found that by increasing Gelucire 50/13 concentration from 0.5 to 0.85% *w/v* resulted in increase in particle size and aggregation (Fig. 4C) (FEN-SLNs 5-6). This is due to increase of drug migration from SLNs core to the surface and drug precipitation, and that was confirmed with EE% measurements. Where, % of drug encapsulated decreased from 95.4 ± 1.98% to 83.4 ± 1.15% and 75.2 ± 2.11% for FEN-SLNs 4-6, respectively, with raising Gelucire conc.

To study effect of homogenization time and speed on colloidal stability of SLNs, it was found that by elevating homogenization speed from 10,000 to 15,000 rpm for 10 minutes, the particle size diminished from 392.3 ± 0.354 nm to 229.2 ± 0.28 nm. While, further increasing in speed, aggregation in SLNs occurred (Fig.4D). Similar results were obtained by increasing homogenization time at speed 15,000 rpm.

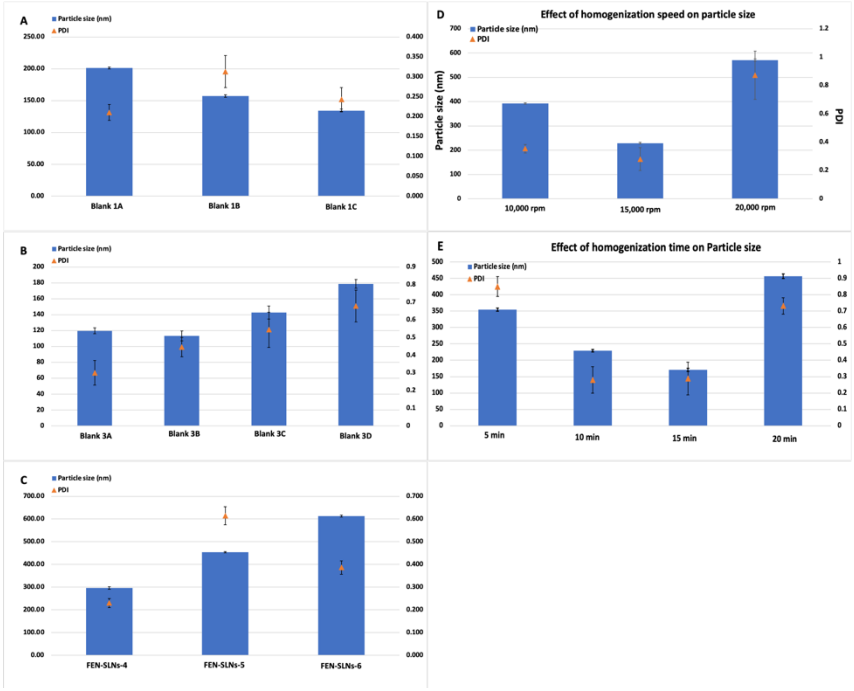

Fig. 4: Effect of different factors on colloidal stability of SLNs formulations (A) Poloxamer concentration, (B) Tween 80 concentration, (C) ratio of Gelucire 50/13: Precirol, (E) Homogenization speed for10 min, (F) Homogenization time at 15,000 rpm.

Morphological examination of SLNs

The morphology of both blank and drug loaded SLNs was detected by TEM (Fig.5). Similarly, to particle size measurements by DLS, the blank particles showed smaller particle size and looked more homogeneous than FEN-SLNs (Fig. 5A). This might be due to drug incorporation in SLNs resulted in adsorption of FEN on surface od SLNs and resulted in increase in particle size and that was confirmed by observing different phase contrast which reveals drug incorporation (Fig.5B).

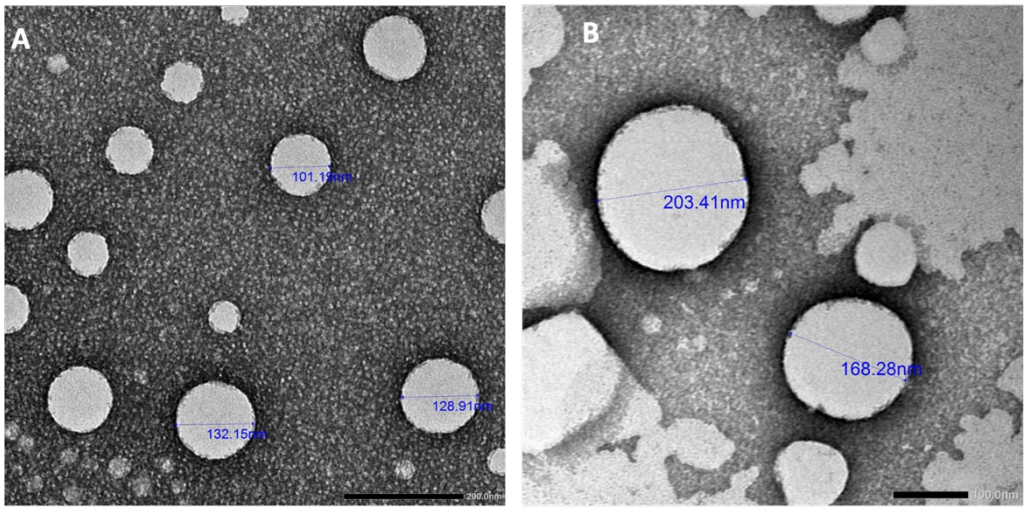

Fig. 5: TEM microphraphs of SLNs formulations; (A) Blank SLNs and (B) FEN-SLNs

In vitro release of FEN from SLNs

The amount of drug released was plotted as a function of time. Owing to the poor solubility of FEN, the free drug suspension in water exhibited a time-dependent release pattern over 8 h (Fig. 6). On the other hand, inclusion of the drug in SLNs obviously provided sustained drug release over 24 h (n.m.t. 45% FEN released within 24) h.

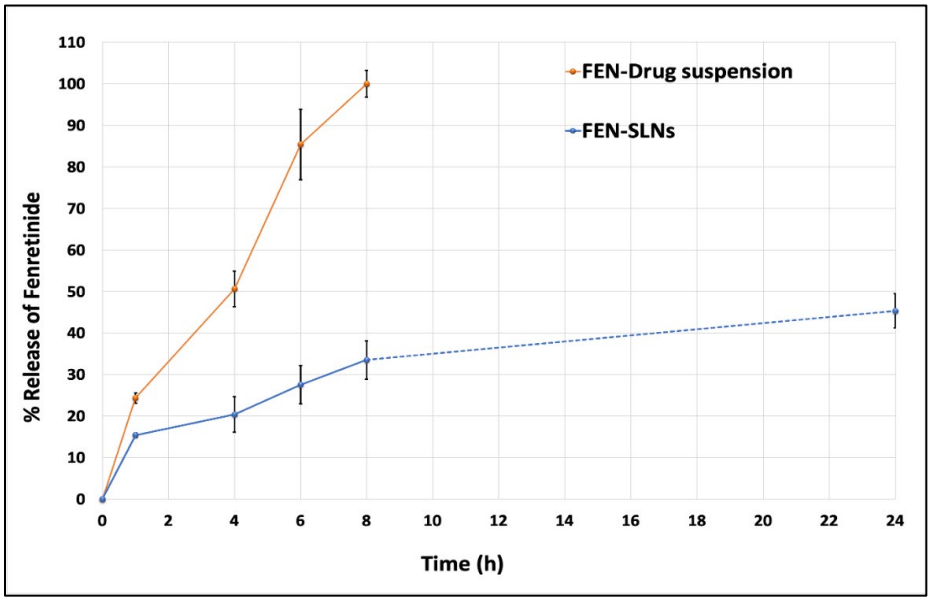

Fig. 6: *In vitro* release of FEN from optimized formulae of FEN-SLNs and free drug suspension in 100 ml water/ ethanol (1:1), 100 strokes per min at 37°C. Each point represents the mean ±S.D (n=3).

In vivo biodistribution

As a point of interest to explore the effect of drug deposition and organ biodistribution after intraperitoneal administration of both fluorescently labelled SLNs compared to free dye as control. Therefore, in this part we will evaluate the role of nano system for efficient delivery of drug to the liver in comparison to free dye as control. Fig. 7 represent the fluorescent photomicrographs of sections from different organs (Liver, kidney, spleen and brain) 1- and 8h- following IP administration of the forementioned samples. Noteworthy, after 1h it was found that both Cou6-SLNs and Free dye showed

distinct localization in the liver, this might be due to the lipid structure of SLNs besides the lipophilic nature of Cou 6 (log P 55.43) (Fig. 7A). However, after 8 h revealed faster migration of the free dye more than Cou6-SLNs (Fig. 7B). This was revealed by the remarkable increase of fluorescent particles in kidney and spleen and brain, while diminished in liver. Noticeable, encapsulation of Cou 6 in lipid based nano system showed superior retention in the liver and protected them from clearance to other organs, which highlights the role of SLNs in efficient delivery and retention.

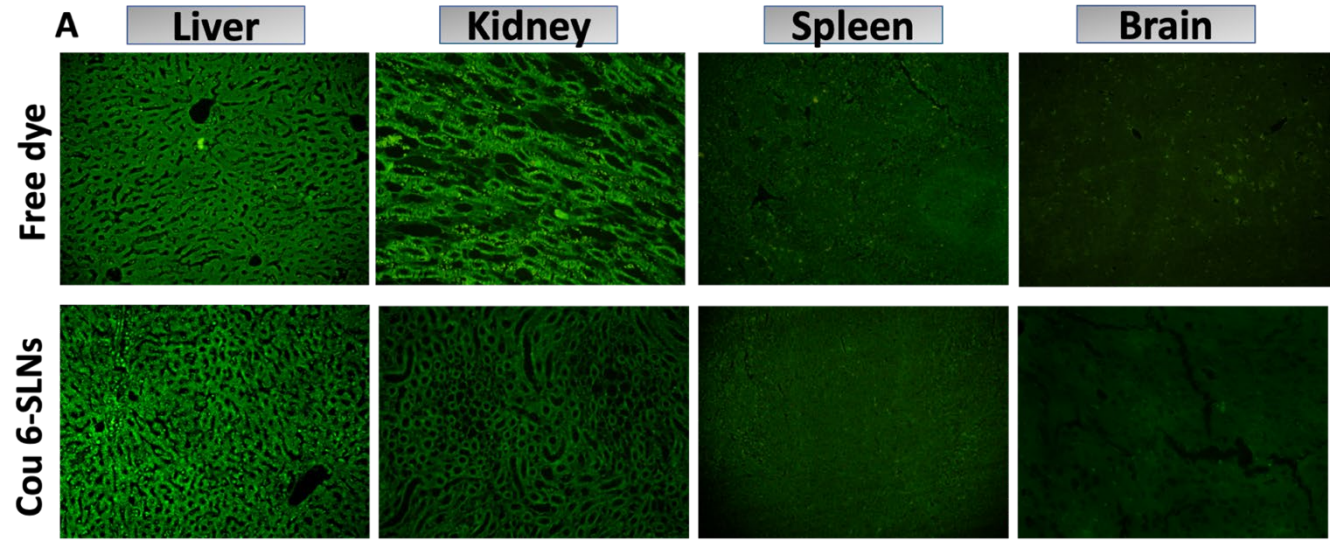

Fig. 7A: Fluorescence photomicrographs of different organs tissues 1h following the intraperitoneal administration of; Free dye solution, and Cou6-SLNs (Magnification power 20X).

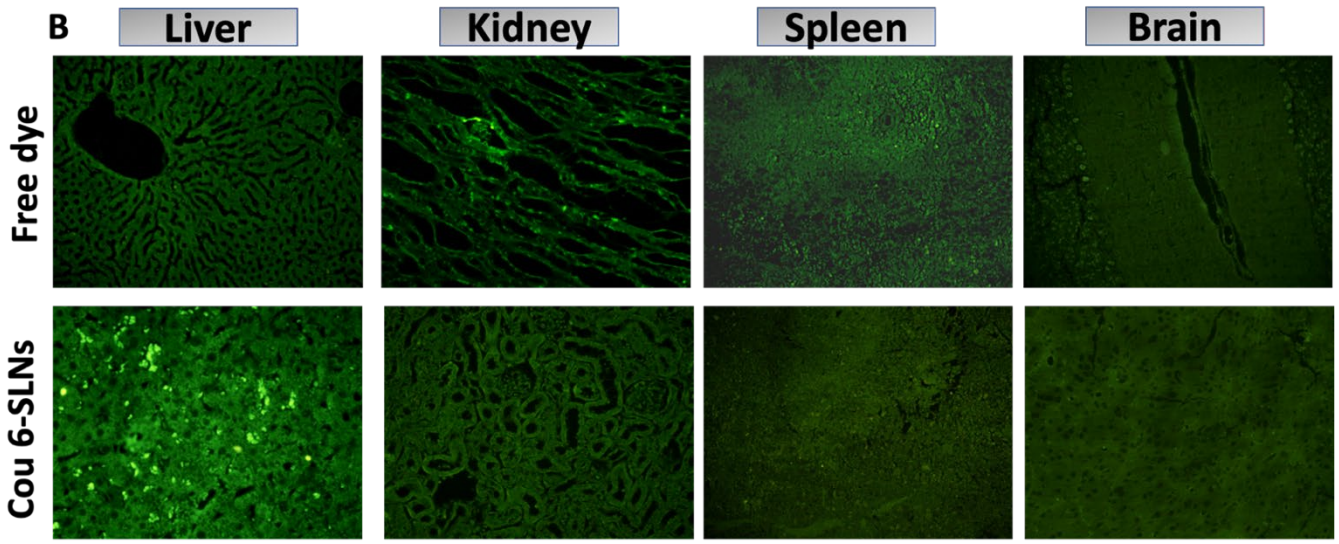

Fig. 7B: Fluorescence photomicrographs of different organs tissues 8h following the intraperitoneal administration of; Free dye solution, and Cou6-SLNs (Magnification power 20X).

4. Conclusion

The use of Gelucire 50/13 in preparing SLNs offers good benefits owing to its amphiphilic characters. It provides SLNs with good lipid core and surfactant free particles. Further, the inclusion of Precirol in the core in concentration 0.5% *w/v* produced SLNs with rigid core with particle size <300 nm and narrow distribution besides high drug encapsulation efficiency and provided slow FEN release over 24 h. In brief, this FEN-SLNs give promises for efficient delivery of FEN to the liver and diminished rate of clearance to other organs. Noteworthy, this is an opportunity to pave a way for developing a system with good retention and controlled drug release to ameliorate liver fibrosis. Yet, there are still a lot of obstacles to overcome before these technologies may be turned into effective treatments. The recently developed nano-system solves additional problems with respect to meeting carrier toxicological and biocompatibility international criteria.

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