Archives of Pharmaceutical Sciences Ain Shams University 2023; Vol. 7(1): 41-59

OPEN ACCESS

Pharmaceutics and Industrial Pharmacy

Original Article

Preparation and characterization of mangiferin-loaded lipid nanocapsules

Sara S. Barakat[a*], Maha N.Sayed[b], Sabry S.Badawy[a], Nahed D.Mortada[b]

[a]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Misr International University, Egypt*
[b]*Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt*

ABSTRACT

Mangiferin (Mgf), a xanthone glucoside of natural origin, is well known to possess different pharmacological actions such as analgesic, antiviral, antidiabetic, anti-inflammatory, hepatoprotective, and antitumor. However, concerning water solubility it is sparingly soluble; in addition, it is classified as a BCS class IV compound which limits its bioavailability. Lipid nanocapsules (LNCs) are a promising carrier for oral delivery as it provides better solubility of the drug and enhances the drug absorption after oral administration owing to their small size. In this study, mangiferin-loaded lipid nanocapsules (Mgf-LNCs) were prepared using phase inversion temperature using solutol and cremophor RH40. This was followed by the characterization of the formed Mgf-LNCs by measuring particle size (PS), zeta potential (ZP), polydispersity index (PDI), *in vitro* release, viscosity, and stability after storage was also evaluated. The obtained results showed that PS for the prepared LNCs ranged from 22.88 ± 3.10 to 436.35 ± 69.37 nm. The ZP range of prepared LNCs was from -6.48 ± 2.13 to -1.90 ± 0.08 mV and showed a PDI ranging from 0.05 ± 0.01 to 0.72 ± 0.28 and viscosity ranging from 6.21 ± 2.90 to 1361.20 ± 92.21 mPa*s. The cumulative % release ranged from 48.89 ± 2.54 to 94.33 ± 9.63% after 6 hours period. After storage for 3 months in the refrigerator it is clear that the prepared formulae showed significant stability. Finally, it is concluded that LNCs are promising nanocarriers for the delivery of Mgf via the oral route.

Keywords: *Mangiferin; nanocapsules; solutol; cremophor RH40; phase inversion temperature; xanthone; natural compounds.*

***Correspondence** | **Sara S. Barakat**; Department of *Pharmaceutical Technology, Faculty of Pharmacy, Misr International University, Egypt*. Email: Sara.barakat@miuegypt.edu.eg

Citation | Barakat SS, Sayed MN, Badawy S S, Mortada N, 2023. Preparation and characterization of mangiferin-loaded lipid nanocapsules. Arch Pharm Sci ASU 7(1): 41-59

DOI: 10.21608/aps.2023.194792.1109

Print ISSN: 2356-8380. **Online ISSN**: 2356-8399.

Received 12 March 2023. **Accepted** 28 March 2023.

Copyright: ©2023 Barakat *et al.* This is an open-access article licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Published by: Ain Shams University, Faculty of Pharmacy

1. INTRODUCTION

The attractiveness of the oral route lies in its superiority in being non-invasive offering good patient compliance, ease of drug administration and handling, low cost, lack of the urge for sterility procedures, and ease of scaling up manufacturing of oral dosage forms **[1]**. Nevertheless, the oral route is characterized by having a large surface area (>300 m^2), with many enterocytes distributed in different intestinal parts **[2]**. Despite the numerous advantages of the oral route, it suffers many challenges **[1]**. Besides, many factors influence oral drug absorption, including factors related to the body's physiology and factors related to the properties of the drug **[3]**. It is well-known that the release of the drug from the dosage form is a must before its solubility in GIT fluids to be available for absorption **[1]**, that's why solubility and intestinal

permeability of the drug are major factors affecting the rate and extent of oral absorption **[3]**.

Mangiferin (Mgf). Mgf is a neutraceutical compound of polyphenolic C-glucosyl xanthone (1,3,6,7-tetrahydroxyxanthone-C2-β-Dglucoside) structure, $C_{19}H_{18}O_{11}$ **[4]** that has gained great attention recently. Mgf is found in many plant species, however, its major plant source is found in the leaves, stem barks, and fruits of the mango tree (*Mangifera indica*), belonging to the family *Anacardiaceae* **[5, 6]**. From a pharmacological perspective, MgF possesses many actions against different pathological conditions **[7]**. It was found to act as antidiabetic **[8],** anti-inflammatory **[9]**, analgesic **[10]**, **immunomodulatory [11]**, antiviral **[12]**, nephroprotective **[13]**, neuroprotective **[14]**, cardioprotective **[15]**, hepatoprotective **[16]**, and antitumor **[17]**. Despite the pharmacological merits of MgF, its pharmaceutical challenges lead to poor bioavailability (around 1.2%), which restricts its clinical development and application **[6, 18]**. Therefore, an increasing number of studies have been concerned with enhancing the bioavailability of Mgf by formulating it in nanocarriers, to increase its solubility and membrane permeability.

Nanocapsules are among the most promising types of nanoparticles. Generally, nanocapsules are vesicular delivery systems in the nanometer range, characterized by having an oil core with a rigid shell surrounding it. The inner oily core is for hosting the hydrophobic drugs while the surrounding shell is for protection and providing stealth properties to the nanocapsules owing to PEG moieties **[19]**.

Nanocapsules can be classified according to the type of oils constituting the core into lipid nanocapsules (LNCs) and lipid core nanocapsules (LCNCs). The term lipid nanocapsules (LNCs) indicate that all the oily ingredients in the capsule core are in the liquid state. LNCs can be further classified according to the nature of the surrounding shell; which can either be polymeric in nature forming polymer-shelled nanocapsules or surfactant in nature forming surfactant-shelled nanocapsules **[20]**. The advantages of LNCs include their preparation using generally recognized as safe (GRAS) ingredients through solvent-free and soft-energy techniques, their very small size, and their ability to encapsulate lipophilic and hydrophilic active substances **[21]**. Moreover, LNCs show a higher drug-loading capacity and longer physical stability compared to liposomes and solid lipid nanoparticles. Additionally, LNCs were reported to possess a sustained release of the drug, with a low initial burst effect unlike PLGA nanoparticles, which show a noticeable burst effect **[22]**. Besides, the advantages of LNCs include delivery by different routes, ease of manufacture and scale-up, dispersion stability over a long period, and small particle size (adjustable between 20 and 150 nm) **[23]**.

It was suggested that the use of LNCs can lead to enhancement in the pharmacological effect of the orally administrated drugs, owing to their ability to prevent GIT degradation of drugs by protecting them through encapsulation. Moreover, LNCs can increase the interaction with the mucosal surface lining the intestine, maintain the time of drug residence, and enhance drug absorption by improving the drug permeability across the mucosal epithelium, consequently leading to enhanced oral bioavailability of drugs **[24]**. In addition, the external polyethylene glycol (PEG) casing of the LNCs effectively avoids recognition by the phagocyte system and so prolonging blood circulation, and they also show an outstanding ability in transient suppression of P-gp function that decreases the bioavailability of many drugs orally, thus, cancer treatments are good candidates for LNC-based therapy **[25]**.

Nevertheless, LNCs were shown to interact with efflux pumps in glioma cells leading to avoiding multidrug resistance **[26].** Concomitantly, LNCs' stealth properties have prevented the opsonization and macrophage uptake, and their shells are suitable for grafting with ligands for efficient targeted drug delivery **[27]**.

Despite their anticipated merits in improving the drug's oral bioavailability, a small number of studies on the oral drug delivery of LNCs were conducted. Paclitaxel (PTX); an anticancer class IV drug showing poor solubility and permeability that results in low bioavailability through the oral route, was encapsulated in nanocapsules composed of captex® 8000, lipoid® S75-3 and solutol® HS15 **[28]**. Results demonstrated the mucus stability of LNCs, and that they enhanced PTX diffusion and membrane permeability. The pharmacokinetic results after oral administration of the PTX–loaded LNCs showed a significant increase in the AUC and C_{max} compared to the commercial PTX formula, proving oral bioavailability enhancement **[28]**.

Therefore, owing to the merits of LNCs for drug delivery through the oral route, the present work aimed to prepare LNCs for oral delivery of Mgf. Preliminary experiments were conducted to test the optimum conditions for the preparation of the lipidic nanocapsules. Characterization of Mgf LNCs was performed regarding their particle size, polydispersity, zeta potential, viscosity, *in vitro* drug release, and storage stability.

2. MATERIALS AND METHODS

2.1. Materials

Cremophor RH40 and isopropyl myristate were purchased from Alpha Chemika, India. Dialysis membrane (Spectra/ Por) 12,000–14,000 molecular weight cut-off was purchased from Spectrum Laboratories Inc, USA. Kolliphor® HS 15 (Solutol), ethyl oleate, and oleic acid were purchased from Sigma Aldrich, Germany. Mangiferin was purchased from Skin Actives Scientific L.L.C., USA. Epikuron® 200 (Soyabean lecithin) consisting of 92 phosphatidylcholines and 8% of accompanying phospholipids was kindly provided by Cargill Texturizing Solutions, Germany. Sodium chloride (NaCl), potassium dihydrogen orthophosphate, and disodium hydrogen orthophosphate anhydrous were purchased from Adwic, El-Nasr Chemical Co, Egypt. Tween® 20 was purchased from SDFCL Sd Fine Chem Limited, India. Labrafac®, Labrafac™ Lipophile, Labrafil®, and Maisine® were kindly provided as a gift from Gattefosse's company, France.

2.2. Preparation of the Mgf-loaded lipidic nanocapsules (Mgf-LNCs)

Before the preparation of Mgf-LNCs, Mgf solubility in various oils, (Labrafac®, Labrafac™, Lipophile, Labrafil®, Maisine®, ethyl oleate, oleic acid, and isopropyl myristate IPM) was determined by dissolving fixed amount of Mgf (10 mg) in increasing amount of each oil (0.1 mL until 3 mL). The mixtures were vigorously agitated by hand and then kept at 37 °C for 48 h in a thermostatically controlled shaking water bath (Thermo Haake shaking water bath, model SWB25, USA), and assessed by visual inspection. Since IPM provided complete solubilization for Mgf in contrast to the other oils which displayed Mgf precipitate, IPM was the selected oil for preparing Mgf-LNCs.

The phase inversion method (PIT) was used in preparing Mgf lipid nanocapsules **[29, 30]**. Briefly, 10 mg drug (Mgf) was mixed with (IPM) oil, solutol® HS15 or cremophor RH40 surfactant, and Epikuron® 200 (100 mg). In addition, sodium chloride (100 mg) and distilled water were added. Weighing of the chemicals was done using an electric balance (Radwag Analytical balance model: AS 220/C/2, Poland). The magnetic stirrer (Jenway, model 1000, England) was used for stirring the emulsion while

increasing the temperature to 85 °C to be higher than the phase inversion temperature forming w/o microemulsion; that was then cooled under magnetic stirring to 55 °C to ensure its inversion to an o/w microemulsion. This process was redone for three cycles followed by the sudden addition of distilled water at 0 °C resulting in the formation of capsules under the shock effect done by cooling. The formed dispersion was then subjected to further stirring at room temperature for 10 min.

2.3. Experimental design and data analysis

A 2^3 full factorial experimental design was done for evaluating the main factors and interactions of three chosen independent variables namely; the type of surfactant used (X_A), the surfactant concentration (X_B), and the oil concentration (X_C) on LNCs size as shown in **Table 1**. Eight formulations of Mgf-LNCs were separately prepared by using various concentrations (10%w/w) and (25%w/w) of IPM and numerous concentrations (10%w/w) and (40%w/w) of solutol® HS15 or cremophor RH40 surfactant**s**. The composition of Mgf LNCs is shown in **Table 2**. The design was then statistically evaluated using ANOVA, correlation coefficient (R^2), adjusted (R^2), predicted (R^2), and adequate precision. The complete 2^3-factorial design setup is illustrated in **Table 2**. All the statistical and factorial analysis was done using Design-Expert® version 11.

Table 1. Factors and levels employed for the 2^3 full factorial model

Factors (independent variables)		Minimum level	Maximum level
X_A	Type of surfactant	Solutol®	Cremophor RH40®
X_B	Concentration of surfactant (%)	10	40
X_C	Concentration of IPM oil (%)	10	25

Table 2. Composition of the Mgf-loaded lipidic nanocapsules

Formulae code*	Composition of formulations: Type of surfactant X_A	Percentage of Surfactant X_B (%w/w)	Percentage of Oil X_C (%w/w)
F1	Solutol®HS15	10%	10%
F2			25%
F3		40%	10%
F4			25%
F5	Cremophor® RH40	10%	10%
F6			25%
F7		40%	10%
F8			25%

*All formulations were prepared using 10 mg Mgf, 100 mg sodium chloride, and 100 mg epikuron®200. The volumes of the added distilled water either at the beginning or at the end were calculated to ensure a total formula weight of 10 g.

2.4. Characterization of the prepared Mgf- LNCs

2.4.1. Determination of the particle size, polydispersity index (PDI), and zeta potential of Mgf-LNCs

After diluting the formulae 1:100 (v/v) in deionized water, the zeta size device (Nano ZS 3600, Malvern Instruments Ltd., UK) was used in measuring the mean particle size, PDI, and zeta potential of the formed Mgf-LNCs **[30-32]**.

2.4.2. Measurement of the viscosity of the Mgf-LNCs

The prepared Mgf-LNCs viscosity was measured at a temperature of 25 °C using Anton Paar Physica MCR 51 rheometer (model Anton Paar Physica MCR 51, Austria). Measurements were performed in triplicate using the shear rate of 50 s^{-1} **[33-35]**.

2.4.3. *In vitro* release of Mgf from the prepared lipid nanocapsules

Determining the release of Mgf from the prepared LNCs was done using a modified rotating basket method at 37 °C and 100 rpm **[36]**. Briefly, the method involves the addition of one ml of the formulation to a glass cylinder (2.5 cm diameter). The cylinder showed a cellulose membrane 0.45 μm (Whatman® membrane filters, USA) tied to one end and the other end hooked to the metallic shaft of the dissolution apparatus (Varian VK7000, USA) (in the place of the basket). The shaft was then lowered till it reaches the dissolution medium surface. The dissolution medium was 100 mL of PBS containing 1% v/v tween 20 and at pH 7.4 **[37]** using the pH meter (Jenway, model 3510, UK) ensuring sink conditions for Mgf. At different time intervals (0.25, 0.5, 0.75, 1, 2, 3, 4, 5, and 6 hours), a 3 mL aliquot was withdrawn with replacement by fresh medium with equivalent volume. The amount of Mgf released was analyzed spectrophotometrically by the ultraviolet spectrophotometer (Evisa-Shimadzu model: UV-1650PCUV-1650PC, Germany) at the predetermined maximum wavelength of maximum absorbance **[38]**.

2.4.4. Assessment of storage stability on Mgf-LNCs

After storing the prepared Mgf-LNCs in the refrigerator (4 °C) for three months, their physical stability was assessed by re-measuring the particle size, PDI, and zeta potential **[31, 35]**.

2.5. Statistical analysis

The obtained data from the performed experiments were statistically analyzed using the Graph pad Instat program. Data were represented as the mean ± standard deviation (S. D). One-way analysis of variance (ANOVA) was used in comparing the mean values followed by Tukey – Kramer Multiple Comparisons Test. In addition, Design-Expert® version 11 (Stat-Ease, USA) was utilized in carrying out the factorial design and the relevant statistical analysis. Statistical significance was set at p-value $\leq$ 0.05. All measurements were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Preparation of the Mgf-loaded lipidic nanocapsules (Mgf-LNCs)

The PIT was used in preparing Mgf lipid nanocapsules successfully as it allows preparing nanocapsules in the nano range by manipulating the oil/water system thermally **[29, 30]**. The PIT method is a low-energy method that does not involve the use of solvents; it depends on changing the temperature with subsequent variation in polyoxyethylene (POE)-type nonionic surfactants solubility **[39]**. The increase in temperature breakdown the hydrogen bonds between the surfactant and water molecules resulting in the dehydration of polyoxyethylene chains turning the surfactant lipophilic. This leads to inverting the oil-in-water (o/w) emulsion to water-in-oil (w/o) emulsion and vice versa in case of low temperature **[40]**.

Regarding Mgf-LNCs, they were prepared using either the nonionic solutol HS 15 or cremophor RH40. The lowest and highest concentrations (10% and 40%) were based on the allowable limit that was reported in the literature for the surfactants used for LNCs preparation (between 10-40%) **[40-42]**. Solutol HS 15 is a hydrophilic surfactant that is made of a blend of free polyethylene glycol 660 and polyethylene glycol 660 hydroxy stearate **[21]**. It has been widely used as an important component in many drug delivery carriers owing to its potential in enhancing the dissolution and bioavailability of hydrophobic molecules **[43]**. Solutol HS 15 is mainly responsible for LNCs formation and stability due to the PEG portions and is responsible for the nanocapsule's stealth properties **[30]**. The other nonionic surfactant used in preparing the nanocapsules was cremophor RH40 which is polyoxyl 40 hydrogenated castor oil **[44]**. Cremphor has also been widely used as a nonionic surfactant for preparing LNCs **[29, 45]**.

IPM is polar low-density pharmaceutical fatty acid ester oil that was also used for the preparation of LNCs **[46]**. Since the allowable oil concentration for use in nanocapsules preparation lies in the range between 10-25%, so the lowest and highest concentrations selected for preparation of Mgf-LNCs were 10% and 25% respectively **[40-42]**.

For stabilizing the LNCs rigid shell epicurean 200 was used; in addition to its ability in enhancing biocompatibility with biological membranes. The water was supplemented with sodium chloride for lowering the phase inversion temperature of the nonionic surfactant to more reachable levels **[30]**.

3.2. Experimental design and data analysis

Factorial research designs aim at studying the interactions between different experimental factors, by the use of more than one independent variable and changing their levels to set the ideal experimental conditions **[47]**. The 2^3 full factorial design studied particle size (P.S.) as a dependent variable, and the model was obtained by varying the three selected independent variables, namely; the type of surfactant (X_A), the concentration of the used surfactant (X_B) and concentration of IPM oil (X_C).

It is well known that PS is an important NPs characteristic affecting the drug's physical stability, release, and oral bioavailability **[42, 48]**. The oral absorption and biodistribution of NPs are significantly affected by their particle size, which in turn determines the therapeutic efficacy **[49]**. Reducing the size of the encapsulated lipophilic compounds to the nanosize range has maximized their oral bioavailability **[50]**. Also, PS affects the drug mucus penetration, showing high spreadability of smaller particles (40 -100 nm) over the mucosa reaching the deep intestinal epithelium so prolonging their residence time, compared to large particles (200-500 nm) that spread only over the surface **[51]**. Moreover, it was reported that Caco-2 and Caco-2/HT-29 cellular NP uptake was higher for smaller particles (50 nm) than that for larger particles (1000 nm) indicating the inverse relation between cellular uptake and the NP size **[52]**. In addition, NPs of size 100 nm have shown a dramatic increase in uptake by the GIT in comparison to 1000 nm NPs **[49]**.

The significant influence of solutol surfactant concentration on the particle size of the developed LNCs was obvious in formulations F1-F4, in which the increase in the concentration of solutol from 10% w/w in formulations (F1, F2) to 40% w/w in formulations (F3, F4) significantly decreased the particle size from 50.16±5.52 and 126.47±4.67 nm respectively to 22.8±3.10 and 37.07±3.79 nm respectively

(P<0.05) (**Fig. 1**).

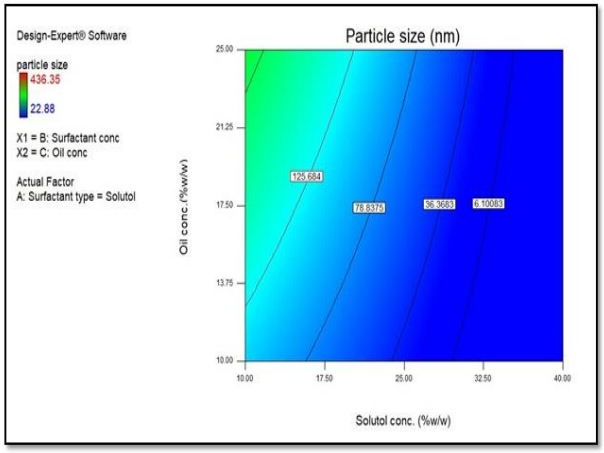

Fig. 1. Contour plot of particle size for formulations prepared using solutol surfactant.

As explained by Lamprecht et al. **[53]**, increasing the concentration of solutol increases the number of solutol molecules adsorbed at the interface between oil and water, which decreases the interfacial tension thus preventing the aggregation of the emulsion droplets, and consequently reducing the size of LNCs **[35, 45]**. This result is similar to the outcome of earlier studies showing a decrease in particle size with the use of higher solutol HS15 content **[21, 42, 54]**. Similar results were obtained with cremophor RH40 surfactant, which increases its concentration from 10% w/w in formulae (F5, F6) to 40% w/w in (F7, F8) formulae significantly causing the decrease in the size of LNCs from 317.35±84.5 and 436.35±69.37 nm respectively to 30.31± 1.49 and 53.885±5.38 nm in F7 and F8 respectively (P<0.05) (**Fig. 2**). This can be interpreted similarly to solutol HS15, and was found to concur with the results of other authors **[55]**.

Comparing the particle size of solutol LNCs to cremophor LNCs, it was obvious that at the same surfactant and oil concentrations, the particle size of cremophor LNCs (F5, F6, F7, F8) was significantly higher than solutol LNCs (F1, F2, F3, F4) (P<0.05), similar to what was shown by others who attempted formulating cisplatin loaded LNCs using solutol and cremophor surfactants **[45]**.

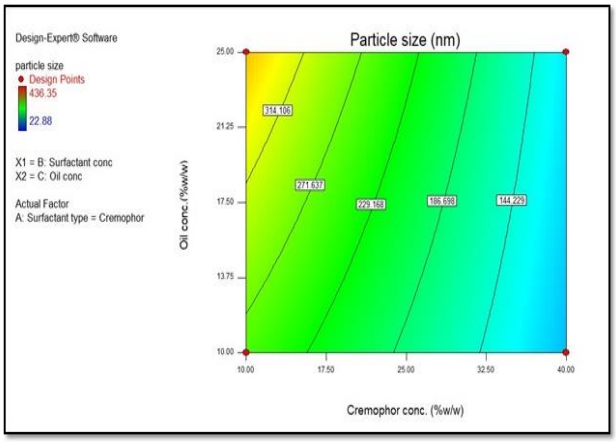

Fig. 2. Contour plot of particle size for formulations prepared using Cremophor RH40 surfactant.

As also shown in **Table 3**, the increase in oil percentage caused the increase in the size of LNCs, as similarly reported by Huynh et al.**[40]**. At the same solutol concentration (10% w/w) in F1 and F2, it was found that increasing the amount of IPM from 10% w/w to 25% w/w significantly increased LNCs size from 50.16±5.52 to 126.47±4.67 nm respectively (P<0.05). Similarly, at solutol concentration (40% w/w) in F3 and F4, it was found that increasing the amount of IPM from 10% w/w to 25% w/w significantly increased the particle size from 22.88±3.10 to 37.07±3.79 nm respectively (P<0.05). The same effect was obtained in formulations F5-F8 prepared using cremophor RH40. At 10%w/w cremophor RH40 (F5 and F6) it was found that the increase in IPM from 10% w/w to 25% w/w leads to a significant increase in the particle size from 317.35±84.5 to 436.35±69.37 nm respectively (P<0.05). Similarly, at 40%w/w cremophor RH40 (F7 and F8) it was found that by increasing IPM from 10%w/w to 25% w/w the particle size significantly (P<0.05) increased from 30.31±1.49

to 53.885±5.38 nm respectively. This could be owing to the increase in the LNCs core size with the use of higher oil content in the center of the particle **[42]**. Also, increasing the oil amount could lead to an increase in the viscosity of the organic phase, producing particles with larger sizes **[56]**. Similar results were obtained showing the increase in the size of nanoemulsions particles formed by IPM oil with increasing its concentration **[57]**.

Table 3. 2^3 Full factorial design setup and particle size values of Mgf-LNCs

Formula code	Type of surfactant (X_A) *	Concentration of surfactant (X_B) **	Concentration of IPM oil (X_C) ***	Mean particle size (nm) ± S.D.
F1	Solutol	10	10	50.16 ± 5.52
F2	Solutol	10	25	126.47 ± 4.67
F3	Solutol	40	10	22.88 ± 3.10
F4	Solutol	40	25	37.07 ± 3.79
F5	Cremophor RH40	10	10	317.35 ± 84.50
F6	Cremophor RH40	10	25	436.35 ± 69.37
F7	Cremophor RH40	40	10	30.31 ± 1.49
F8	Cremophor RH40	40	25	53.89 ± 5.38

-X_A *: Type of surfactant (Solutol HS15 or Cremophor RH40)
-X_B **: Concentration of surfactant used (% w/w) at the minimum level (10%) and maximum level (40%).
-X_C ***: Concentration of IPM oil (% w/w) at the minimum level (10%) and maximum level (25%).

The contour plots illustrated in **Fig. 1 and 2** display the effect of different parameters of preparation on the particle size of Mgf-loaded LNCs and figure out the relationship between different factors X_B and X_C at both levels of X_A (Solutol and Cremophor RH40 surfactants).

The Design Expert® 11 software was used to analyze the achieved particle size results by performing ANOVA tests. The proposed model was linear with significance (P<0.0082). The experimental design that studied the main effects and interactions illustrated that the particle size of Mgf-LNCs was significantly affected by the type of surfactant (X_A), the concentration of surfactant (X_B), and concentration of oil (X_C) (P < 0.0001). The following **Equation** is used in representing the model of the linear regression obtained from the full factorial study:

1.0/(particle size) = +0.019 -5.192E-003* X_A +0.011* X_B -5.507E-003* X_C **(Equation)**

All aforementioned factors were proven statistically significant (P<0.0001), as obvious in **Table 4**. The proposed model was inverse and showed R^2 a value of 0.9778, adjusted R^2 0.9481, and a predicted R^2 of 0.8420; which were considered to be acceptable high. The

adequacy of the model in predicting the size of the nanoparticles was clarified by the closeness in the values between the adjusted and predicted R^2 **[58]**. Moreover, since the adequate precision value was more than 4 (16.433) This indicates that this model can be useful in the navigation of the design space **[59]**. Plotting the power of response transformation (symbolized mathematically by the Greek letter lambda) ranging against the natural log of the sum of squares of the residuals (ln residual SS) generated the Box-Cox Plot for power transformations, which indicates if measuring the response on a different scale could provide an improvement on the fit of the model or not **[60]**, and also for obtaining the best fitting models; it suggests the best power (lambda) that all the response data should be raised to. Accordingly, the current suggested powers are -1 for the particle size data (inverse recommended transformation) as clear in **Fig. 3.**

Table 4. Summary of ANOVA analysis of the particle size model derived from the factorial study determining the significance of the independent variables

	Sum of squares	Degrees of freedom (d.f.)	Mean square	F value	P value
Model	1.489E-003	4	3.722E-004	33.00	0.0082
X_A	2.156E-004	1	2.156E-004	19.12	0.0221
X_B	9.889E-004	1	9.889E-004	87.67	0.0026
X_C	2.426E-004	1	2.426E-004	21.51	0.0189
Residual	3.384E-005	3	1.128E-005		
Cor. Total	1.523E-003	7			

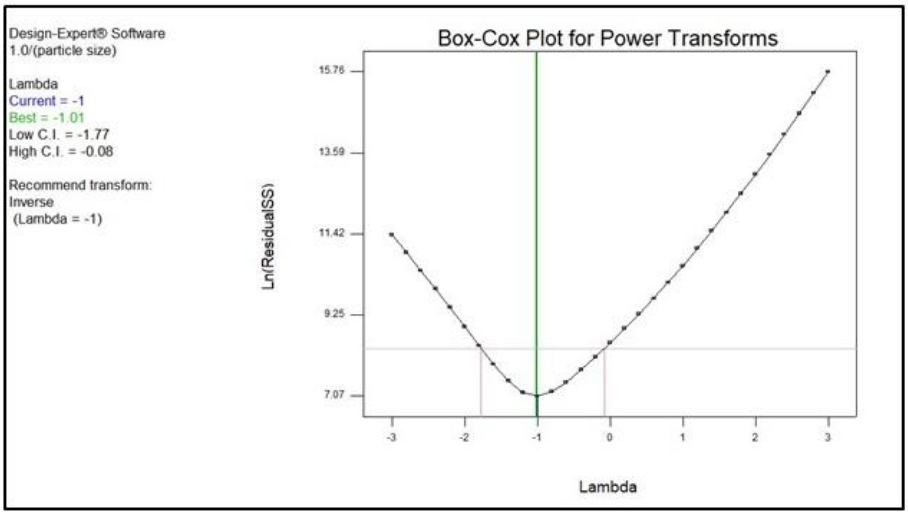

Fig. 3. Box-Cox plot for power transformations for the particle size model.

As obvious from the results, the Mgf-LNCs formulation (F3) composed of 10% IPM and 40% solutol surfactant showed the smallest particle size (22.88±3.10 nm) while the Mgf-LNCs

formulation (F6) composed of 25% IPM and 10% cremophor surfactant showed the largest particle size (436.35±69.37 nm).

3.3. Determination of PDI and zeta potential of Mgf-LNCs

Results in **Table 5** show that all prepared Mgf-LNCs formulae show generally low PDI values (0.05-0.20) indicating their homogeneity, except for formulations F5 and F6. Solutol is a surfactant; therefore it is mainly arranged at the interface between the oil and water resulting in a reduction in the interfacial tension. Thus, producing particles with small and homogenous size values **[53]**.

As also shown in **Table 5** the prepared Mgf-LNCs are all negatively charged, with a zeta potential range of (-1.9 to -6.48 mV). The negatively charged phospholipids present at the interface of LNCs **[61]**, in addition to the presence of solutol with PEG dipoles **[31]** are the cause of the negative charge of the LNCs. These values concurred with the results of other authors formulating LNCs **[31, 35]**.

Table 5. PDI and zeta potential of the prepared Mgf-LNCs

Formula code	Type of surfactant	Concentration of Surfactant (%w/w)	Concentration of IPM oil (%w/w)	PDI Mean ± S.D.	Zeta Potential (mV) Mean ± S.D.
F1	Solutol	10	10	0.20 ± 0.09	-3.14 ± 2.92
F2	Solutol	10	25	0.07 ± 0.03	-2.42 ± 1.37
F3	Solutol	40	10	0.07 ± 0.03	-4.36 ± 0.19
F4	Solutol	40	25	0.05 ± 0.01	-6.48 ± 2.13
F5	Cremophor RH40	10	10	0.72 ± 0.28	-1.90 ± 0.08
F6	Cremophor RH40	10	25	0.43 ± 0.11	-3.01 ± 1.33
F7	Cremophor RH40	40	10	0.19 ± 0.13	-3.67 ± 0.64
F8	Cremophor RH40	40	25	0.09 ± 0.01	-2.81 ± 1.29

3.4. Viscosity of Mgf-LNCs

The high viscosity of formulations was reported to retard the absorption of drugs from the GIT **[62]**, therefore, the viscosity of Mgf-LNCS was measured accordingly. As shown in **Table 6**, the viscosity of the prepared Mgf-LNCs was found to be significantly influenced by the surfactant concentration, in which increasing the surfactant's concentration (Solutol or Cremophor RH40) led to a significant viscosity increase ($P<0.05$), which is similar to the results reported by Bseiso et al. **[35]**, and can be explained by the inherent viscous nature of the two surfactants. On the other hand, the influence of increasing oil concentration or changing the surfactant type on Mgf-LNCs was not conclusive, suggesting that the main determinant factor for the viscosity of

Mgf-LNCs was the concentration of surfactant.

As clear from the results, Mgf-LNCs formulation (F3) composed of 10% IPM and 40% solutol surfactant possessed the highest viscosity value (1361.20 ± 92.21 mPa*s), while the Mgf-LNCs formulation (F1) composed of 10% IPM and 10% solutol surfactant possessed the lowest viscosity value (6.21 ± 2.90 mPa*s).

Table 6. Viscosity values of Mgf-LNCs

Formula code	Type of surfactant	Concentration of Surfactant (%w/w)	Concentration of IPM (%w/w)	Mean Viscosity (mPa*s) ± S.D.
F1	Solutol	10	10	6.21 ± 2.90
F2	Solutol	10	25	36.55 ± 5.21
F3	Solutol	40	10	1361.20 ± 92.21
F4	Solutol	40	25	229.89 ± 3.39
F5	Cremophor RH40	10	10	8.94 ± 1.82
F6	Cremophor RH40	10	25	30.10 ± 8.04
F7	Cremophor RH40	40	10	698.10 ± 26.79
F8	Cremophor RH40	40	25	246.64 ± 34.32

3.5. *In vitro* release of Mgf from LNCs

The release of Mgf from LNCs formulae was carried out at 37 °C±0.5 °C in a dissolution medium of 100 mL (PBS with 1% v/v tween 20 at pH 7.4) to ensure Mgf sink condition for a duration of 6 h as commonly reported by Zhang et al. **[37]**. The reported results in **Tables 7 and 8** and **Fig. 4 and 5** show the satisfactory sustained drug release rates of Mgf-loaded LNCs within 6 h, in the range of 48.89% to 94.33%; similar to what was obtained by Zhang et al. who formulated efavirenz in LNCs **[42]**.

By observing the results, it is delineated that Mgf-LNCs with high surfactant (solutol, cremophor RH40) concentration of 40%w/w namely (F3, F4, F7, F8) generally exhibited significant ($P<0.05$) lower release of Mgf than their counterparts prepared using lower surfactant concentration of 10%w/w (excluding F2/F4 that exhibited non-significant change ($P>0.05$)). This may be due to the viscosity values which were higher for F3, F7, and F8 formulated with higher surfactant concentration (40%w/w). This increase in viscosity is expected to decrease the rate of release by resisting the diffusion of the drug **[42]**. Moreover, increasing the density of surfactant at the oil/water interface provides steric restrictions and makes the membrane behaves as a barrier, leading to a decrease in the drug release from LNCs formulated with higher surfactant concentrations, as similarly reported by Lamprecht et al., and Safwat et al. **[30, 53]**. Also, the increase in the oil concentration from

10% to 25% w/w decreased the cumulative percent released of Mgf from LNCs, but in an insignificant manner (P>0.05). This could be explained by the increase in the lipophilic nature of the LNCs upon increasing the concentration of oil, causing Mgf to favorably reside in the oil core, and to diffuse at a slower rate.

Table 7. Cumulative percent released of Mgf from the LNCs prepared using Solutol surfactant over a period of 6 h

Time (h)	Cumulative drug released % from different formulations (Mean± S.D.) F1	F2	F3	F4
0.25	11.11 ± 0.73	9.90 ± 1.01	10.40 ± 0.79	11.20 ± 0.65
0.5	18.56 ± 0.58	13.70 ± 1.54	12.42 ± 0.94	13.28 ± 1.30
0.75	21.90 ± 0.72	16.89 ± 1.50	15.12 ± 0.73	15.71 ± 0.52
1	26.44 ± 0.87	18.87 ± 1.20	17.26 ± 0.36	17.70 ± 2.13
2	41.18 ± 1.28	24.98 ± 3.57	25.57 ± 1.07	26.56 ± 1.73
3	52.26 ± 3.12	32.55 ± 3.01	34.36 ± 1.16	32.22 ± 0.61
4	64.54 ± 4.45	39.82 ± 3.70	44.32± 1.75	40.27 ± 1.29
5	75.62 ± 5.63	47.87 ± 4.82	55.12 ± 2.52	47.88 ± 1.16
6	86.64± 6.45	54.55 ± 4.39	65.51 ± 4.07	55.13 ± 1.95

Table 8. Cumulative percent released of Mgf from the LNCs prepared using Cremophor RH40 surfactant over a period of 6 h

Time (h)	Cumulative drug released % from different formulae (Mean± S.D.) F5	F6	F7	F8
0.25	10.82 ± 1.32	11.53 ± 2.14	7.99 ± 1.06	7.78 ± 0.69
0.5	14.64 ± 0.47	17.20 ± 4.17	9.73 ± 1.03	9.31 ± 0.95
0.75	18.98 ± 0.65	19.75 ± 5.33	11.10 ± 0.93	11.70 ± 0.63
1	23.40 ± 0.76	23.73 ± 5.99	12.70 ± 1.22	13.58 ± 0.67
2	41.43 ± 4.60	39.14 ± 11.68	18.68 ± 2.21	19.12 ± 0.48
3	59.30 ± 8.43	52.69 ± 13.95	24.99 ± 3.72	24.24 ± 0.71
4	72.19 ± 4.45	65.88 ± 14.85	31.64 ± 2.56	31.41 ± 2.53
5	85.16 ± 9.46	80.29 ± 15.86	38.76 ± 1.25	37.02 ± 0.50
6	94.33 ± 9.64	92.82 ± 15.77	51.73 ± 2.11	48.89 ± 2.54

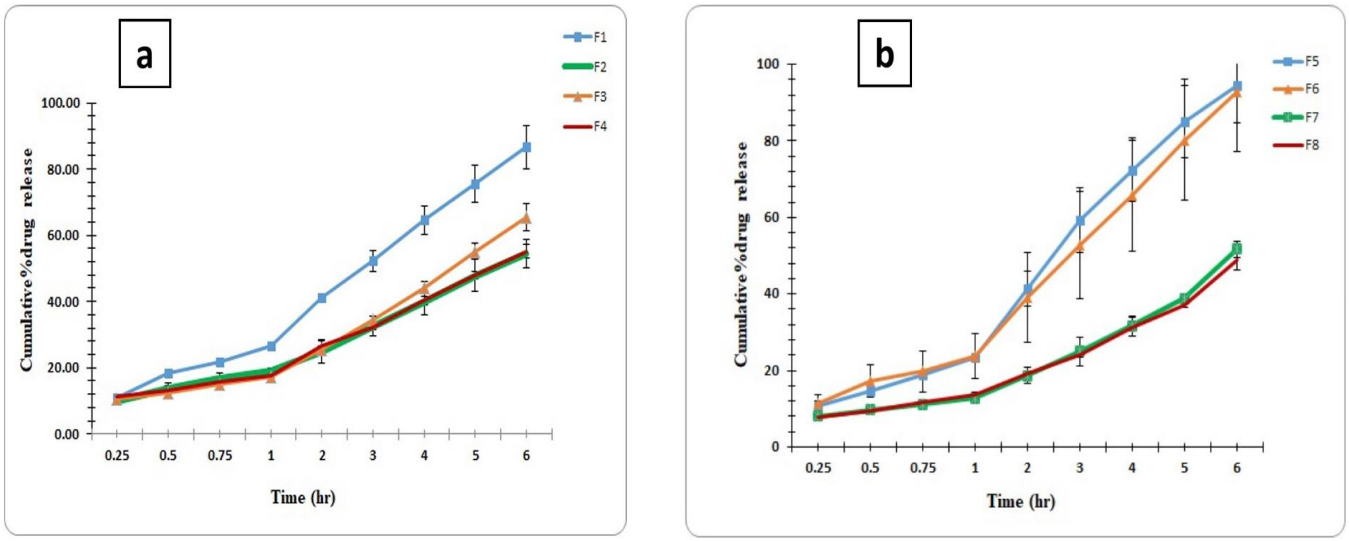

Fig. 4. *In vitro* release profiles of Mgf from formulations prepared with (a) Solutol surfactant and (b) Cremophor RH40 surfactant in PBS containing 1% v/v tween 20 at pH 7.4.

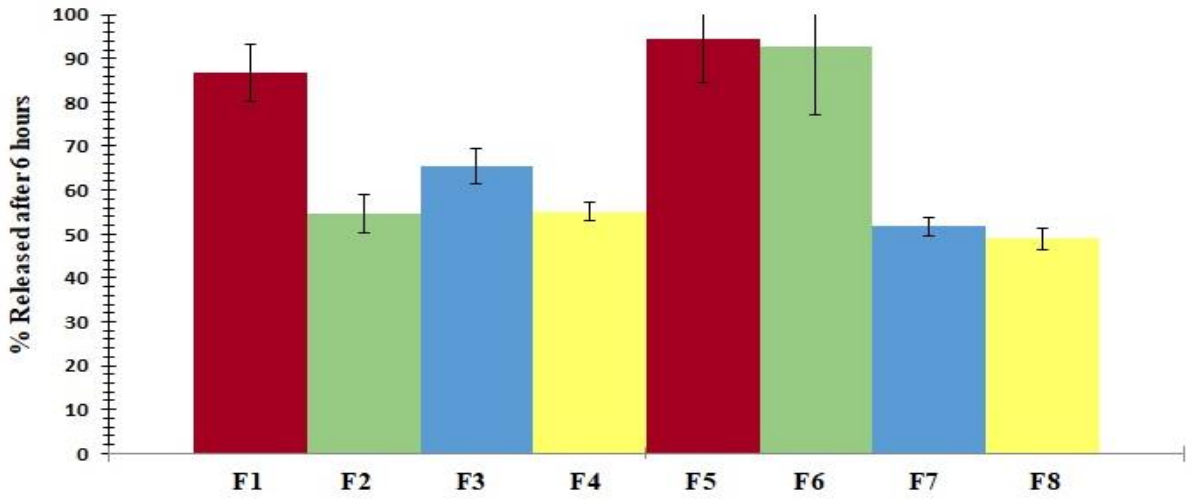

Fig. 5. Cumulative percent release of Mgf from LNCs PBS containing 1% v/v tween 20 at pH 7.4.

3.6. Storage stability study on Mgf-LNCs

Results shown in **Table 9** and **Fig. 6** represent the influence of the storage at 4 °C in refrigeration for a period of 3 months on the particle size, zeta potential, and PDI of the prepared Mgf-LNCs. Mgf-LNCs displayed changes that were generally insignificant after the storage period ($P>0.05$), which delineates the stability of the prepared LNCs. Although the values of zeta potential were below -30 mv, the steric stabilization caused by solutol surfactant was the major cause in maintaining LNCs stability, as previously reported by Heurtault et al. **[63]**.

Table 9. Influence of three months storage on the physical characteristics of Mgf-LNCs

Formulae Code	Particle size (nm) ± S.D.		Zeta potential (mV) ± S.D.		PDI ± S.D.	
	Freshly prepared nanocapsules	After 3 months storage	Freshly prepared nanocapsules	After 3 months storage	Freshly prepared nanocapsules	After 3 months storage
F1	50.16±5.52	56.47±8.10	-3.14±2.92	-5.18±1.99	0.20±0.09	0.24±0.06
F2	126.47±4.67	122.35±3.61	-2.42±1.37	-3.49±1.78	0.07±0.03	0.12±0.11
F3	22.88±3.10	25.18±2.13	-4.36±0.19	-3.62±0.66	0.07±0.03	0.16±0.01
F4	37.07±3.79	43.73±0.62	-6.48±2.13	-4.25±1.58	0.05±0.01	0.14±0.01
F5	317.35±84.50	483.45±85.35	-1.90±0.08	-1.46±0.22	0.72±0.28	1.00±0.20
F6	436.35±69.37	346.1±27.01	-3.01±1.33	-1.39±0.43	0.43±0.11	0.86±0.18
F7	30.31±1.49	32.35±0.93	-3.67±0.64	-4.77±1.34	0.19±0.13	0.21±0.01
F8	53.89±5.38	65.85±0.88	-2.81±1.29	-4.23±0.30	0.09±0.01	0.16±0.03

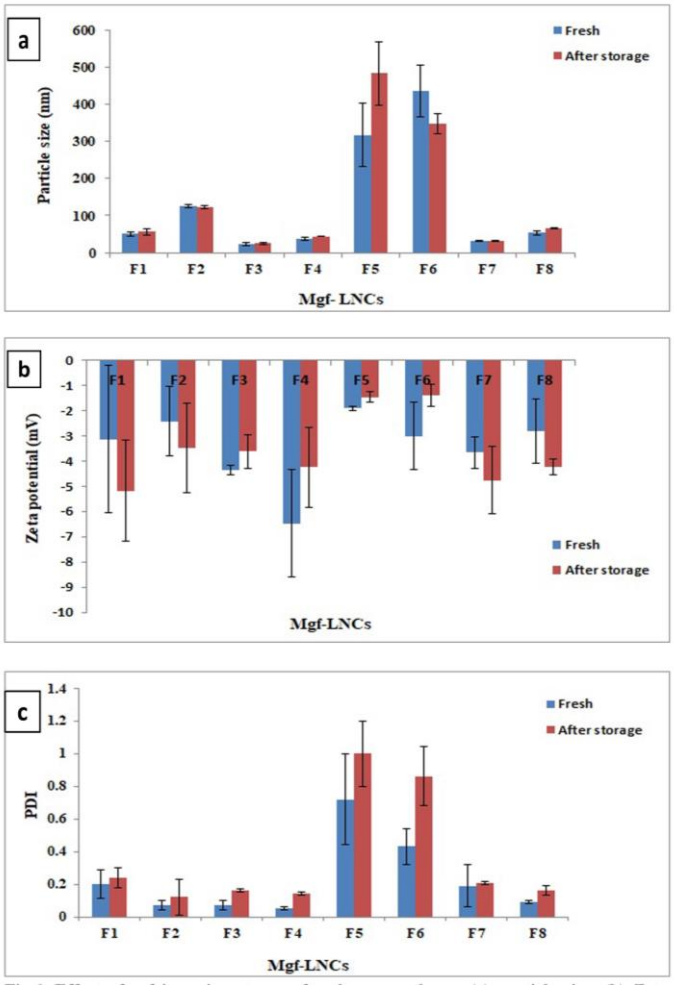

Fig. 6. Effect of refrigeration storage for three months on (a) particle size, (b) zeta potential, and (c) PDI of Mgf-LNCs.

CONCLUSION

Mgf- LNCs were successfully formed using the PIT method. Results of the full factorial design study revealed that the surfactant type (X_A) was proven to affect the PS of the formulae significantly, in which the use of cremophor RH40 produced LNCs of larger size than those prepared using solutol surfactant. Increasing the surfactant concentration (X_B) significantly decreased the PS of Mgf-LNCs. Moreover, increasing the IPM oil concentration (XC) increased the PS of Mgf-LNCs significantly. It was also concluded that both the surfactant type and the IPM oil concentration were found to exhibit an insignificant effect on the viscosity of the formulae. All LNCs formulations were shown to have a mono-modal particle size distribution with low PDI values (0.05-0.72). Negatively charged Mgf-LNCS, with zeta potential ranging from -1.9 to -6.48 mV were obtained, indicating relatively good stability and high dispersion quality. All Mgf-LNC formulations showed sustained release of Mgf over a 6 h period. Moreover, the increase in surfactant concentration generally decreased the release significantly at constant IPM concentration. However, the increase in IPM concentration decreased the release generally in an insignificant way at constant surfactant concentration. The release of Mgf is not affected by surfactant type. Finally, Mgf-LNCs displayed reliable storage properties as manifested by the general insignificant changes in PS, PDI, and zeta potential values.

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

Data analyzed during this study are all included in the main manuscript.

Competing interests

No competing interests were declared by the authors.

Funding statement

No funding source was received

Authors' contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by [Sara Sherif Barakat] and [Maha Nasr Sayed]. The first draft of the manuscript was written by [Sara Sherif Barakat] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

5. References

1. Alqahtani MS, Kazi M, Alsenaidy MA and Ahmad MZ. Advances in oral drug delivery. Frontiers in Pharmacology 2021;12(618411. DOI: org/10.3389/fphar.2021.618411.
2. Homayun B, Lin X and Choi HJ. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. Pharmaceutics 2019;11(3):DOI: 10.3390/pharmaceutics11030129.
3. Song N-N, Zhang S-Y, and Liu C-X. Overview of factors affecting oral drug absorption. Asian J Drug Metab Pharmacokinet 2004;4(3):167-76. DOI:
4. Khurana RK, Rao S, Beg S, Katare O and Singh B. Systematic development and validation of a thin-layer densitometric bioanalytical method for estimation of mangiferin employing analytical quality by design (AQbD) approach. Journal of Chromatographic Science 2016;54(5):829-41. DOI: 10.1093/chromsci/bmw001.
5. Núñez Selles AJ, Daglia M and Rastrelli L. The

potential role of mangiferin in cancer treatment through its immunomodulatory, anti-angiogenic, apoptosis, and gene regulatory effects. Biofactors 2016;42(5):475-91. DOI: 10.1002/biof.1299.

6. Khurana RK, Bansal AK, Beg S, Burrow AJ, Katare OP, Singh KK, and Singh B. Enhancing biopharmaceutical attributes of phospholipid complex-loaded nanostructured lipidic carriers of mangiferin: Systematic development, characterization, and evaluation. Int J Pharm 2017;518(1-2):289-306. DOI: 10.1016/j.ijpharm.2016.12.044.
7. Telang M, Dhulap S, Mandhare A and Hirwani R. Therapeutic and cosmetic applications of mangiferin: A patent review. Expert Opinion on Therapeutic Patents 2013;23(12):1561-80. DOI: 10.1517/13543776.2013.836182.
8. Muruganandan S, Srinivasan K, Gupta S, Gupta PK and Lal J. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin-diabetic rats. J Ethnopharmacol 2005;97(3):497-501. DOI: 10.1016/j.jep.2004.12.010.
9. García-Rivera D, Delgado R, Bougarne N, Haegeman G and Berghe WV. Gallic acid indanone and mangiferin xanthone are strong determinants of immunosuppressive anti-tumour effects of Mangifera indica L. bark in MDA-MB231 breast cancer cells. Cancer Lett 2011;305(1):21-31. DOI: 10.1016/j.canlet.2011.02.011.
10. Ojewole JA. Antiinflammatory, analgesic, and hypoglycemic effects of Mangifera indica Linn. (Anacardiaceae) stem-bark aqueous extract. Methods Find Exp Clin Pharmacol 2005;27(8):547-54. DOI: 10.1358/mf.2005.27.8.928308.
11. Leiro J, Arranz JA, Yáñez M, Ubeira FM, Sanmartın ML and Orallo FJIi. Expression profiles of genes involved in the mouse nuclear factor-kappa B signal transduction pathway are modulated by mangiferin. 2004;4(6):763-78. DOI: 10.1016/j.intimp.2004.03.002.
12. Wang RR, Gao YD, Ma CH, Zhang XJ, Huang CG, Huang JF, and Zheng YT. Mangiferin is an anti-HIV-1 agent targeting protease and is effective against resistant strains. Molecules 2011;16(5):4264-77. DOI: 10.3390/molecules16054264.
13. Pal PB, Sinha K and Sil PC. Mangiferin attenuates diabetic nephropathy by inhibiting oxidative stress-mediated signaling cascade, TNFalpha-related and mitochondrial-dependent apoptotic pathways in streptozotocin-induced diabetic rats. PLoS One 2014;9(9):e107220. DOI: 10.1371/journal.pone.0107220.
14. Andreu GLP, Maurmann N, Reolon GK, de Farias CB, Schwartsmann G, Delgado R, and Roesler RJEjop. Mangiferin, a naturally occurring glucoxilxanthone improves long-term object recognition memory in rats. 2010;635(1-3):124-8. DOI: 10.1016/j.ejphar.2010.03.011.
15. Prabhu S, Narayan S, and Devi CS. Mechanism of protective action of mangiferin on suppression of inflammatory response and lysosomal instability in a rat model of myocardial infarction. Phytother Res 2009;23(6):756-60. DOI: 10.1002/ptr.2549.
16. Pan C-w, Pan Z-z, Hu J-j, Chen W-l, Zhou G-y, Lin W, Jin L-x, and Xu C-lJEjop. Mangiferin alleviates lipopolysaccharide and D-galactosamine-induced acute liver injury by activating the Nrf2 pathway and inhibiting NLRP3 inflammasome activation. 2016;770(85-91. DOI: 10.1016/j.ejphar.2015.12.006.
17. Du S, Liu H, Lei T, Xie X, Wang H, He X, Tong R, and Wang Y. Mangiferin: An effective therapeutic agent against several disorders (Review). Mol Med Rep 2018;18(6):4775-86. DOI: 10.3892/mmr.2018.9529.
18. Tian X, Gao Y, Xu Z, Lian S, Ma Y, Guo X, Hu P, Li Z, and Huang C. Pharmacokinetics of mangiferin and its metabolite-Norathyriol, Part 1: Systemic evaluation of hepatic first-pass effect in vitro and in vivo. Biofactors 2016;42(5):533-44. DOI: 10.1002/biof.1291.
19. Nasr M and Abdel-Hamid S. Lipid-based nanocapsules: a multitude of biomedical

applications. Current pharmaceutical biotechnology 2015;16(4):322-32. DOI: 10.2174/1389201016041502181035555.

20. Feng L and Mumper RJ. A critical review of lipid-based nanoparticles for taxane delivery. Cancer Lett 2013;334(2):157-75. DOI: 10.1016/j.canlet.2012.07.006.

21. Kiani A, Fathi M, and Ghasemi SM. Production of novel vitamin D3-loaded lipid nanocapsules for milk fortification. International Journal of Food Properties 2017;20(11):2466-76. DOI: org/10.1080/10942912.2016.1240690.

22. Aparicio-Blanco J and Torres-Suarez A-I. Glioblastoma multiforme and lipid nanocapsules: a review. Journal of biomedical nanotechnology 2015;11(8):1283-311. DOI: 10.1166/jbn.2015.2084.

23. Tran P, Jang J-H, Jeong S-H and Lee Y-B. Oral and Lymphatic Delivery of Paclitaxel via Lipid Nanocapsules. Yakhak Hoeji 2021;65(5):375-85. DOI: org/10.17480/psk.2021.65.5.375.

24. Erdoğar N, Akkın S and Bilensoy E. Nanocapsules for Drug Delivery: An Updated Review of the Last Decade. Recent Pat Drug Deliv Formul 2018;12(4):252-66. DOI: 10.2174/1872211313666190123153711.

25. Moura RP, Pacheco C, Pêgo AP, des Rieux A, and Sarmento B. Lipid nanocapsules to enhance drug bioavailability to the central nervous system. Journal of Controlled Release 2020;322(390-400. DOI: 10.1016/j.jconrel.2020.03.042.

26. Garcion E, Lamprecht A, Heurtault B, Paillard A, Aubert-Pouessel A, Denizot B, Menei P, and Benoît JP. A new generation of anticancer, drug-loaded, colloidal vectors reverses multidrug resistance in glioma and reduces tumor progression in rats. Mol Cancer Ther 2006;5(7):1710-22. DOI: 10.1158/1535-7163.mct-06-0289.

27. Hirsjärvi S, Dufort S, Bastiat G, Saulnier P, Passirani C, Coll J-L, and Benoît J-P. Surface modification of lipid nanocapsules with polysaccharides: from physicochemical characteristics to in vivo aspects. Acta Biomaterialia 2013;9(5):6686-93. DOI: 10.1016/j.actbio.2013.01.038.

28. Groo AC, Saulnier P, Gimel JC, Gravier J, Ailhas C, Benoit JP, and Lagarce F. Fate of paclitaxel lipid nanocapsules in intestinal mucus given their oral delivery. Int J Nanomedicine 2013;8(4291-302. DOI: 10.2147/ijn.s51837.

29. Abdel-Mottaleb MM, Neumann D and Lamprecht A. Lipid nanocapsules for dermal application: a comparative study of lipid-based versus polymer-based nanocarriers. Eur J Pharm Biopharm 2011;79(1):36-42. DOI: 10.1016/j.ejpb.2011.04.009.

30. Safwat S, Hathout RM, Ishak RA and Mortada ND. Augmented simvastatin cytotoxicity using optimized lipid nanocapsules: a potential for breast cancer treatment. Journal of liposome research 2017;27(1):1-10. DOI: 10.3109/08982104.2015.1137313.

31. Mohsen K, Azzazy HM, Allam NK, and Basalious EB. Intranasal lipid nanocapsules for systemic delivery of nimodipine into the brain: In vitro optimization and in vivo pharmacokinetic study. Materials Science and Engineering: C 2020;116(111236. DOI: 10.1016/j.msec.2020.111236.

32. Paillard A, Hindré F, Vignes-Colombeix C, Benoit J-P and Garcion E. The importance of endo-lysosomal escape with lipid nanocapsules for drug subcellular bioavailability. Biomaterials 2010;31(29):7542-54. DOI: 10.1016/j.biomaterials.2010.06.024.

33. Pramod K, Shanavas S and Baby JN. Rheology profiling of a hydrogel drug delivery vehicle. J. Chem. Pharm. Res 2015;7(818-25. DOI:

34. dos Santos PP, Paese K, Guterres SS, Pohlmann AR, Costa TH, Jablonski A, Flôres SH, and Rios AdO. Development of lycopene-loaded lipid-core nanocapsules: physicochemical characterization and stability study. Journal of Nanoparticle Research 2015;17(2):DOI: 10.1007/s11051-015-2917-5.

35. Bseiso EA, AbdEl-Aal SA, Nasr M, Sammour OA and El Gawad NAA. Nose-to-brain delivery

of melatonin lipidic nanocapsules as a promising post-ischemic neuroprotective therapeutic modality. Drug Deliv 2022;29(1):2469-80. DOI 10.1080/10717544.2022.2104405.

36. Barakat SS, Nasr M, Ahmed RF, Badawy SS, and Mansour S. Intranasally administered in situ gelling nanocomposite system of dimenhydrinate: preparation, characterization and pharmacodynamic applicability in chemotherapy-induced emesis model. Scientific Reports 2017;7(1):1-13. DOI: 10.1038/s41598-017-10032-7.

37. Zhang Y, Wu X, Meng L, Zhang Y, Ai R, Qi N, He H, Xu H, and Tang X. Thiolated Eudragit nanoparticles for oral insulin delivery: preparation, characterization and in vivo evaluation. International journal of pharmaceutics 2012;436(1-2):341-50. DOI: 10.1002/mabi.201300515.

38. Liu R, Liu Z, Zhang C, and Zhang B. Nanostructured lipid carriers as a novel ophthalmic delivery system for mangiferin: improving in vivo ocular bioavailability. Journal of pharmaceutical sciences 2012;101(10):3833-44. DOI: 10.1002/jps.23251.

39. Anton N, Gayet P, Benoit J-P and Saulnier P. Nano-emulsions and nanocapsules by the PIT method: an investigation on the role of the temperature cycling on the emulsion phase inversion. International Journal of Pharmaceutics 2007;344(1-2):44-52. DOI: 10.1016/j.ijpharm.2007.04.027.

40. Huynh NT, Passirani C, Saulnier P and Benoît J-P. Lipid nanocapsules: a new platform for nanomedicine. International Journal of Pharmaceutics 2009;379(2):201-9. DOI: 10.1016/j.ijpharm.2009.04.026.

41. Heurtault B, Saulnier P, Pech B, Proust J-E and Benoit J-P. A novel phase inversion-based process for the preparation of lipid nanocarriers. Pharmaceutical research 2002;19(6):875-80. DOI: 10.1023/a:1016121319668.

42. Varshosaz J, Taymouri S, Jahanian-Najafabadi A and Alizadeh A. Efavirenz oral delivery via lipid nanocapsules: formulation, optimization, and ex-vivo gut permeation study. IET nanobiotechnology 2018;12(6):795-806. DOI: 10.1049/it-not.2018.0006.

43. Chauvet S, Barras A, Boukherroub R and Bouron A. Lipid nanocapsules containing the non-ionic surfactant Solutol HS15 inhibit the transport of calcium through hyperforin-activated channels in neuronal cells. Neuropharmacology 2015;99(726-34. DOI: 10.1016/j.neuropharm.2015.08.043.

44. Thakkar PH. Influence of excipients on drug absorption via modulation of intestinal transporters activity. Asian Journal of Pharmaceutics (AJP) 2015 69-82. DOI: 10.22377/AJP.V9I2.435.

45. Zhai Q, Li H, Song Y, Wu R, Tang C, Ma X, Liu Z, Peng J, Zhang J, and Tang Z. Preparation and optimization lipid nanocapsules to enhance the antitumor efficacy of cisplatin in hepatocellular carcinoma HepG2 cells. AAPS PharmSciTech 2018;19(5):2048-57. DOI: 10.1208/s12249-018-1011-6.

46. Piotrowski M, Szczepanowicz K, Jantas D, Leśkiewicz M, Lasoń W and Warszyński P. Emulsion-core and polyelectrolyte-shell nanocapsules: biocompatibility and neuroprotection against SH-SY5Y cells. Journal of Nanoparticle Research 2013;15(2035. DOI: 10.1007/s11051-013-2035-1.

47. Collins LM, Dziak JJ and Li R. Design of experiments with multiple independent variables: a resource management perspective on complete and reduced factorial designs. Psychological methods 2009;14(3):202. DOI: 10.1037/a0015826.

48. Emami J, Boushehri MS and Varshosaz J. Preparation, characterization, and optimization of glipizide controlled release nanoparticles. Res Pharm Sci 2014;9(5):301-14. DOI:

49. He C, Yin L, Tang C, and Yin C. Size-dependent absorption mechanism of polymeric nanoparticles for oral delivery of protein drugs. Biomaterials 2012;33(33):8569-78. DOI: 10.1016/j.biomaterials.2012.07.063.

50. Zhou H, Liu G, Zhang J, Sun N, Duan M, Yan Z, and Xia Q. Novel lipid-free nanoformulation for improving oral bioavailability of coenzyme Q10. BioMed research international 2014;2014(DOI: 10.1155/2014/793879.

51. Wang Y, Pi C, Feng X, Hou Y, Zhao L, and Wei Y. The influence of nanoparticle properties on the oral bioavailability of drugs. International Journal of Nanomedicine 2020;15(6295. DOI: 10.2147/IJN.S257269.

52. Banerjee A, Qi J, Gogoi R, Wong J and Mitragotri S. Role of nanoparticle size, shape and surface chemistry in oral drug delivery. Journal of Controlled Release 2016;238(176-85. DOI: 10.1016/j.jconrel.2016.07.051.

53. Lamprecht A, Bouligand Y and Benoit J-P. New lipid nanocapsules exhibit sustained release properties for amiodarone. Journal of controlled release 2002;84(1-2):59-68. DOI: 10.1016/s0168-3659(02)00258-4.

54. Barras A, Mezzetti A, Richard A, Lazzaroni S, Roux S, Melnyk P, Betbeder D, and Monfilliette-Dupont N. Formulation and characterization of polyphenol-loaded lipid nanocapsules. International Journal of Pharmaceutics 2009;379(2):270-7. DOI: 10.1016/j.ijpharm.2009.05.054.

55. Khalil RM, Abd El-Bary A, Kassem MA, Ghorab MM and Basha M. Influence of formulation parameters on the physicochemical properties of meloxicam-loaded solid lipid nanoparticles. Egyptian Pharmaceutical Journal 2013;12(1):63. DOI: 10.7123/01.EPJ.0000428643.74323.d9.

56. Esmaeili A and Ebrahimzadeh M. Preparation of polyamide nanocapsules of Aloe vera L. delivery with in vivo studies. AAPS PharmSciTech 2015;16(2):242-9. DOI: 10.1208/s12249-014-0203-y.

57. Zheng WW, Zhao L, Wei YM, Ye Y, and Xiao SH. Preparation and the in vitro evaluation of nanoemulsion system for the transdermal delivery of granisetron hydrochloride. Chem Pharm Bull (Tokyo) 2010;58(8):1015-9. DOI: 10.1248/cpb.58.1015.

58. Karimi M, Avci P, Ahi M, Gazori T, Hamblin MR and Naderi-Manesh H. Evaluation of chitosan-tripolyphosphate nanoparticles as a p-shRNA delivery vector: formulation, optimization, and cellular uptake study. Journal of nanopharmaceuticals and drug delivery 2013;1(3):266-78. DOI: 10.1166/jnd.2013.1027.

59. Abd-Allah H, Abdel-Aziz RTA and Nasr M. Chitosan nanoparticles making their way to clinical practice: A feasibility study on their topical use for acne treatment. Int J Biol Macromol 2020;156(262-70. DOI: 10.1016/j.ijbiomac.2020.04.040.

60. Habib BA, Sayed S and Elsayed GM. Enhanced transdermal delivery of ondansetron using nano vesicular systems: fabrication, characterization, optimization and ex-vivo permeation study-Box-Cox transformation practical example. European Journal of pharmaceutical sciences 2018;115(352-61. DOI: 10.1016/j.ejps.2018.01.044.

61. Hussein A, Abdel-Mottaleb MM, El-assail M and Sammour O. Novel biocompatible essential oil-based lipid nanocapsules with antifungal properties. Journal of Drug Delivery Science and Technology 2020;56(101605. DOI: org/10.1016/j.jddst.2020.101605.

62. Tanaka Y, Matsubara R, Furukawa K, Satonaka S, and Kasaoka S. The influence of viscosity-enhancing agents on oral absorption of drugs. Pharmazie 2019;74(11):661-4. DOI: 10.1691/ph.2019.9097.

63. Heurtault B, Saulnier P, Pech B, Proust J-E and Benoit J-P. Physico-chemical stability of colloidal lipid particles. Biomaterials 2003;24(23):4283-300. DOI: 10.1016/s0142-9612(03)00331-4.