RESEARCH ARTICLE

Formulation, Characterization, and Optimization of Zaltoprofen Nanostructured Lipid Carriers (NLCs)

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ABSTRACT

This study aimed to prepare nanostructured lipid carriers (NLC) of zaltoprofen to treat rheumatoid arthritis and osteoarthritis. NLCs were prepared by melt-emulsification and ultra-sonication technique in different solid to liquid lipid ratios, different surfactant concentrations, and different total lipid amounts and use different solid and liquid lipid types to study all these effects factors on NLC dispersions. Characterization of the prepared NLC dispersions was done by determining the particle size, polydispersity index, zeta potential, percentages of entrapment efficiency, and drug loading. The optimal formulation was further investigated by X-ray Diffraction Analysis (XRD), Fourier Transform Infra-Red (FT-IR) spectroscopy, visualization by Transmission Electron Microscope (TEM), and also an *in-vitro* release profile study. The results showed that all of the NLCs were within the nanometer size and they were monodispersed system, they showed high percentages of entrapment efficiency ranged from (86.1 ± 2.61 to 96.6 ± 0.37) %, and the drug loading ranged from (4.85 ± 0.74 to 7.57 ± 0.41) %.

The results showed that the optimal formulation (F1) was composed of a 70:30 ratio of solid lipid (stearic acid) to liquid lipid (peppermint oil) in concentrations of surfactant (tween 80) and co-surfactant (PEG400) of 5% and 2.5 % v/v, respectively. Its mean diameter was 78.5 nm, PI was 0.229, and zeta potential equal to -55.52 mV. FT-IR spectroscopy showed no drug-excipient interaction and successful dispersion of the drug within the lipid matrix. XRD demonstrated that the zaltoprofen molecule lost its crystalline nature and produced an amorphous complex within the NLC matrix. TEM analysis showed spherical nanoparticles. The in-vitro experiment proved that zaltoprofen was released gradually over 8 hours from the optimal formulation.

Keywords: Nanostructured lipid carrier, zaltoprofen, nanoparticles, entrapment efficiency, drug loading.

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INTRODUCTION

As well known, the use of large size molecules in drug delivery systems has dramatic challenges like poor absorption in the body, poor solubility, poor bioavailability, problems associated with target-specific delivery, in-vivo instability, and probability of drugs adverse effects. Therefore, using novel drug delivery systems with small particle sizes could be an appropriate option to solve these critical problems.[1]

Hence, nanotechnology is a promising approach for appropriate drug delivery, advanced drug formulations, drug targeting, and controlled drug release. Nanotechnology is the science at the nano-scale size, and it can be used along the entire spectrum of scientific approaches, including healthcare and life sciences.[1,2]

Nanoscale molecules exhibit unique biological, mechanical, structural, electrical, magnetic, and chemical properties, and they can move more quickly in the human body than larger materials. The nanoscale technologies can be classified into lipid-based nanocarriers, polymeric nanocarriers, inorganic nanocarriers, and drug nanoparticles or nanosuspensions.[2,3]

Lipid nanocarriers include lipid core micelles, liposomes, microemulsions and nanoemulsions, solid lipid nanoparticles, and nanostructured lipid carriers. At the same time, polymeric nanocarriers include polymeric micelle, polymeric nanoparticles, nanocapsules, dendrimers, and polymer-drug nanoconjugates.[3]

The inorganic nanocarrier is composed of nanostructures containing various inorganic metals, such as gold nanoparticles, iron oxide (magnetic) nanoparticles, calcium phosphate nanoparticles, and quantum dots, whereas drugs in nanoparticulate form can be used as nanosuspensions. All these colloidal nanocarriers are widely being studied in

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drug delivery researches and have gained marked potential in enhancing drug delivery by different routes of administration.

The successful usage of nanoparticles for drug delivery depends upon their stability in the nano-size, their capability to penetrate several biological barriers, and the sustained release of their components.[3,4]

However, the cytotoxicity of the polymers with their higher cost and lack of suitable large-scale production techniques have minimized the widely spread application of nanoparticles to clinical approach.[5] To overcome the disadvantages of polymeric nanoparticles, lipids have been used as an alternative carrier, particularly for lipophilic drugs. Several types of lipid-based nanocarriers were developed to improve the bioavailability of poorly permeable and poorly soluble drugs, and these include liposomes, microemulsions, nanoemulsions, self-emulsifying systems, lipid nanocapsules, and lipid nanoparticles.[6]

The NLCs are colloidal carriers consisting of a mixture of solid and liquid lipids as a hybrid carrier having an average size of 40–1000 nm. It consists of a lipid matrix with a specialized nanostructure developed by *Müller et al*..[7] This particular type of nanostructure helps to increase drug solubility, drug loading, and bioavailability. Also, NLCs are prepared using that combination of lipids, where less ordered structures are formed, give firmer inclusion of drug molecules within the matrix during storage.[7]

NLC system comprises a biocompatible solid lipid, and liquid lipid with a chemical structure differs. The liquid lipid is better solubilizer of drugs than solid lipid. This carrier comprises biodegradable and physiological lipids exhibiting low cytotoxicity and low systemic toxicity.[7]

Zaltoprofen (ZPF) is an effective non-steroidal anti-inflammatory drug (NSAID) that is come from Japan. ZPF is 2-(10, 11-dihydro-10-oxodibenzo [b, f] thiepin-2-yl) propionic acid.[8] ZPF is used to treat acute and chronic inflammation and is used in arthritic conditions like rheumatoid arthritis, osteoarthritis, and post-operative pain, tooth extraction, injury, and alleviation of inflammation in acute upper respiratory infection.[9]

MATERIALS AND METHODS

Materials

Zaltoprofen was obtained as a gift sample from Shanghai Research Institute of Chemical Industry Testing Co., Ltd, China. Peppermint oil was purchased from Reagent World, Inc., Ontario, USA. Sesame oil and palmitic acid were purchased from HiMedia Lab Pvt. Ltd, Mumbai, India. Aloe, cucumber, shea butter, castor, and black seed oils were supplied by NOW, USA. Stearic acid was purchased from Xinwang, China. Glycerol behenate was supplied by a Gattefossé, France. Tween 20, tween 40, tween 80, mannitol were supplied from Hopkin & Williams LTD. Chandwell Heath. Essex. England. Transcutol P from Provizer Pharma, India. Provizer Pharma, India supplied PEG 200, PEG 400, and PG. Poloxamer 188 was purchased from Sigma-Aldrich, Chemie GMBH, Germany. Hyper-Chem LTD CO, China supplied Cremophor EL and triacetin.

Methods

Screening of Starting Materials:

Screening of Liquid Lipid (Oils)

Solubility Study: The solubility of ZPF in different liquid lipids (peppermint, sesame, aloe, cucumber, shea butter, castor, and black seed) oils was determined by adding excess amounts of drug to 5 mL of oils in small vials. The vials were tightly stoppered and were continuously stirred to reach equilibrium for 48 hours in a water bath shaker. After that, the mixtures were centrifuged using Digital Centrifuge at 6000 rpm for 30 minutes. The supernatant was separated, suitably diluted in methanol, and solubility was quantified by UV visible spectrophotometer (Carry win UV, Varian, Australia) at the λ_{max} of the drug. The solubility studies were done in triplicate, and the results reported as ± SD.[10]

Screening of Solid Lipids

Solubility Study: The solubility determination of ZPF in numerous solid lipids (stearic acid, palmitic acid, and glycerol behenate) was achieved by adding 10 mg of ZPF in a test tube containing the solid lipid, that added in increments of 0.5 g, then the mixture was heated above 5°C of the lipid melting point in a water bath with continuous shaking. The qualitative solubility of ZLP in the molten solid lipid was evaluated visually under normal light. The amount of lipid for complete drug solubilization was calculated, and the experiment was conducted in triplicate.[11]

Screening of Surfactant

Solubility Study: The solubility of ZPF in various surfactants (tween 20, tween 40, tween 80, poloxamer 188, and cremophor EL) was determi ned by adding excess amounts of the drug in 5 mL of the surfactant in small vials. The vials were tightly stoppered and were continuously stirred to reach equilibrium for 48 hours in a water bath shaker. After that, the mixtures were centrifuged at 6000 rpm for 30 min. The supernatant was separated, diluted suitably in methanol, and UV Spectrophotometer quantified solubility at λ_{max} of the drug.[12]

Screening of Co-surfactant

Solubility Study: The solubility of ZPF in various co-surfactants (triacetin, transcutol, PEG 200, PEG 400, and PG) was determined by the same procedure illustrated in the previous section of the surfactant screening.

Preparation of Zaltoprofen Nanostructured Lipid Carrier (ZPF-NLC)

In the present study, ZPF-loaded NLCs were produced by melt-emulsification and ultra-sonication techniques. This method shows superiority over other techniques, as it is considered the most cost-effective and less time-consuming technique for producing NLC formulations. Briefly, the lipid phase composed of the solid lipid and liquid lipid and the drug (ZPF) were melted in 5°C above solid lipid melting point with continuous stirring in a beaker a hot plate magnetic stirrer. The aqueous phase was prepared by dissolving the surfactant and

co-surfactant in D.W. Both phases were heated up to 5°C above solid lipid melting point under magnetic stirring.

Then the hot aqueous phase was added drop by drop to the melted oily phase in hot plate magnetic stirring at 600 rpm for 15 minutes. The primary emulsion was homogenized by probe-ultrasonic (ultrasonic processor UP200Ht, Hielscher, Germany) with an amplitude of 80% pulses were maintained to 10 seconds and the sonication was carried for 5 min. Consequently, the dispersions were cooled down, and the resulting o/w nanoemulsion was cooled at 4 ± 0.5°C for 15-20 minutes by placing the container on an ice bath ZPF-NLC dispersions were formed.[13]

Blank NLC was prepared by using the same previous procedure but without the drug. The NLC dispersions were lyophilized for long-term stability, mannitol as a cryoprotector (5% w/v) was added.

The samples were frozen at -78°C for 10 hours, followed by lyophilization for 36 hours. The lyophilized formulation was used later for further experiments.[14]

CHARACTERIZATION AND OPTIMIZATION OF ZPF-NLCS

1. Determination of Particle size (PS) and Polydispersity Index (PI)

PS determination and PI measurement were done by particle size analyzer, Brookhaven, USA. that works on the principle of photon correlation spectroscopy (PCS), which provides mean particle size of the nano-formulations as well as the PI that measures the width of the distribution. The PS and PI were determined by diluting the formulations (1:50) with double distilled water to obtain suitable scattering intensity and reducing the opalescence followed by membrane (0.45 µm) filtration. The measurements were carried out in triplicate, and standard deviations were calculated at a fixed scattering angle of 90°at 25°C.[15] All measurements were performed in triplicate, and the results were presented as mean ± SD.

2. Determination of Zeta Potential (ZP)

ZP of the prepared ZPF-NLCs was measured by using the Zetasizer potential analyzer (Malvern, UK). The NLCs samples were diluted with double distilled water (1:100) to get a uniform dispersion before the analysis. Zeta potential was measured at 25°C by electrophoretic light scattering using a disposable zeta cuvette.[16] All measurements were performed in triplicate, and the results were presented as mean ± SD.

3. Determination of Entrapment Efficiency (EE) and Drug Loading Capacity (DL) Percentages

EE refers to the amount of encapsulated drug compared with the amount of drug used. While DL is referring to the drug-encapsulated compared with the amount of lipid in the formula.[17] The EE and DL of the prepared ZPF-NLC formulations were measured by calculating the amount of free drug in the dispersion medium using the ultrafiltration method. In this concern, 5 mL of each formula was poured into the Amicon Ultra (an ultrafilter, MWCO 10 kDa, Millipore Company, USA) and centrifuged for 20 minutes at 4000 rpm at 25°C to separate the nanoparticles. Next, this colloidal dispersion was filtered by a 0.45 µm membrane filter, and a UV spectrophotometer analyzed the supernatant containing the free unloaded drug at the drug λ_{max}.[18] The percent of EE was calculated using equation no. (1) and the percent of DL was calculated using equation no. 2.

$$EE\% = (W_{initial\ drug} - W_{free\ drug})/W_{initial\ drug} \times 100 \quad ...eq.\ (1)$$

$$DL\% = (W_{initail\ drug} - W_{free\ drug})/W_{lipid} \times 100 \quad ...eq.\ (2)$$

Here, $W_{initail\ drug}$ is the weight of initial drug used, $W_{free\ drug}$ is the weight of free drug observed in the supernatant after centrifugation of the aqueous dispersion and W_{NLC} is the total weight of lipid in the formula.[19] All measurements were performed in triplicate, and the results were presented as mean ± SD.

4. Examination of ZPF-NLCs

A. X-ray Diffraction Analysis (XRD)

X-ray diffraction analysis (XRD) can be utilized to assess the degree of crystallinity of nanoparticle dispersion. An X-ray diffractometer (XRD-6000 Shimadzu, Japan) equipped with a copper anode for radiation was used to detect the crystallinity of the drug (ZPF), solid lipid (stearic acid), physical mixture of ZPF and stearic acid in ratio 1:1, and lyophilized powder of the optimum ZPF-NLC dispersion. Powdered samples about 10 mm in length were placed on the top of X-ray plates, exposed to a voltage of 45 kV and a 40 mA current at room temperature, with a scanning speed of 5° per minute and a scanning range of 2θ. The X-ray diffractogram patterns were recorded over the range of 20°–80°.[20]

B. Fourier Transform Infra-Red (FT-IR) Spectroscopy

The infrared absorption characteristics of the drug (ZPF), bulk excipient (stearic acid), physical mixture of ZPF and stearic acid in ratio 1:1, and the lyophilized powder of the optimum ZPF-NLC formula were compared by ATR FT-IR (IRAffinity-1, Shimadzu, Japan) in order to investigate the compatibility between the drug and another constituent of the optimum formula.[20]

C. Visualization by Transmission Electron Microscope (TEM)

The examination of the morphology ZPF-NLC dispersion was also carried out by (TEM Morgagni 268-D; FEI Company, Eindhoven, Netherlands). A single drop of the dispersion was placed on a carbon-coated copper grid and kept for 1 minutes to allow the optimum prepared ZPF-NLC to stick on the carbon substrate. The residual dispersion was removed by absorbing the drop with a piece of filter paper.

Then, a drop of 2% phosphotungstic acid was reserved on the grid for 120 s, and this sample was then permitted to dry in the air and then scanned.[20]

5. *In-Vitro* Release Study

The optimum ZPF-NLC formulas according to the result obtained from PS, PI, ZP, percentage of EE, and DL values were investigated in-vitro drug release. The in-vitro release

profiles were done by modified dialysis membrane method using Dissolution apparatus paddle type (Faithful, China). The dialysis membrane (Hi-media, Mumbai, India), with a molecular weight cut off between 12,000-14,000Da, were previously soaked overnight with phosphate buffer 7.4, about 3 mL from the formulations were placed in the dialysis membrane, and both open ends of dialysis membrane were perfectly tied and were fixed to the paddles. The membrane was allowed to immerse in a 250 mL phosphate buffer solution of pH-7.4. The system was stirred continuously at 100 rpm. 2 mL of the samples were collected at an interval of 0, 0.15, 0.30, 1, 2, 3, 4, 5, 6, 7, and 8 hours. A UV spectrophotometer determined ZPF content in the receptor compartment at the λ_{max} of the drug. Sink conditions were conserved in the receptor compartment during all in-vitro release process by replenishment the equivalent volume of fresh dissolution medium to maintain a constant volume. The release profiles were attained by plotting the cumulative drug release percent versus time,[21] the experiment was carried out in triplicate.

Statistical Analysis

To investigate the significance of the difference between the results of studied formulations, the one-way analysis of variance (ANOVA) test was used. The significance level was set at 0.05, and ($p < 0.05$) was considered statistically significant. All the results were illustrated as the mean values ± standard deviation (SD) in three replicates (n = 3).

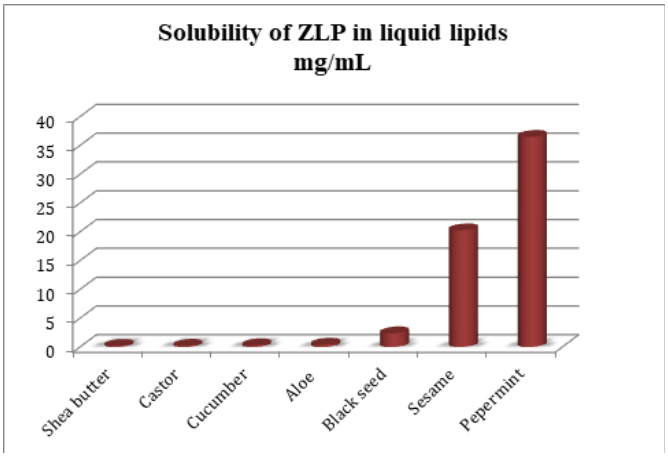

Figure 1: Solubility of Zaltoprofen in different oils

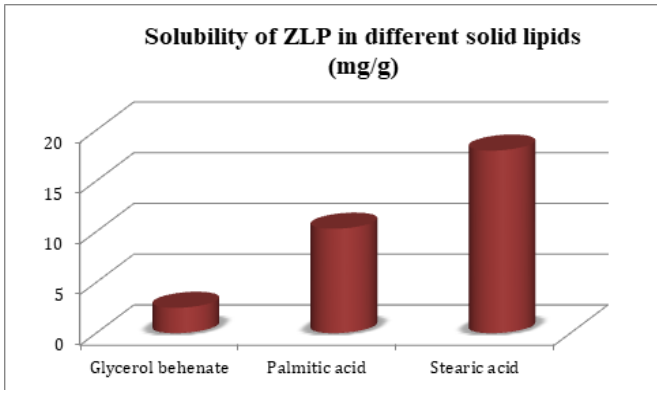

Figure 2: Solubility of Zaltoprofen in different solid lipids

RESULTS AND DISCUSSION

Screening of Starting Materials

Screening of Liquid Lipid (Oils)

Solubility of Liquid Lipid: Solubility of ZPF in different oils was done, and the results were represented in Figure 1. The maximum solubility of ZPF was in peppermint oil 36.4836.48 ± 0.23 mg/mL followed by sesame oil of 20.26 ± 0.11 mg/mL. So, these two oils were selected for NLC formulation and further investigation.

Selection of Solid Lipids

Solubility of ZPF in Solid Lipids

The solubility of ZPF in different solid lipids was investigated, as was represented in Figure 2. It showed maximum solubility in stearic acid about (18 ± 0.012 mg/g), followed by palmitic acid (10.33 ± 0.032 mg/g), and it showed the lowest solubility in glycerol behenate (2.5 ± 0.02).

Screening of Surfactants and Co-Surfactants

Surfactants and co-surfactants screening study were carried out to study the effect of these surfactants on PS, PI, and ZP, %EE, and %DL. The surfactant, which reduces the particle size, minimizes the PI value, and generates more surface charge, is preferred.[22] The solubility of surfactants and co-surfactants were represented in Figure 3.

Preparation and Evaluation of ZPF-NLCs

In the present study, ZPF-loaded NLC formulas were successfully produced by melt-emulsification and ultra-sonication methods. The lipid phase and the aqueous phase were prepared separately. The lipid phase contained 300 mg of solid lipid and liquid lipid in different ratio (70:30, 80:20, and 90:10) and the drug ZPF in a dose of 80 mg. In contrast, the aqueous phase contained surfactant and co-surfactant in a constant ratio of 2:1 in a sufficient quantity (12 mL) of distilled water. A constant temperature was maintained at 75ºC for both phases. The aqueous phase was added to the lipid phase, the rate of addition of aqueous phase to the lipid phase was about 1 mL/min, it was added dropwise, and the subsequent drop is added only when the previous one is homogenously mixed, the obtained mixture was sonicated using a probe sonicator in a manner as mentioned previously. Formulas

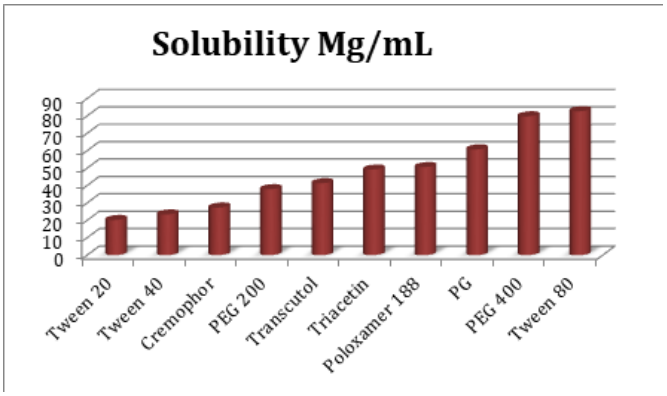

Figure 3: Solubility of ZPF in different surfactants and co-surfactants

F1-F12 were prepared for ZPF-NLCs as illustrated in Table 1, and their properties were explained in Table 2, including mean particle size distribution, polydispersity index, zeta potential, and the entrapment efficiency and drug loading percentages.

CHARACTERIZATION AND OPTIMIZATION OF ZPF-NLCS

1. Determination of Particle size (PS) and Polydispersity Index (PI)

Determination of the average PS and PI were measured for all the prepared ZPF-NLC formulations, and their values were represented in table 2. It was found that all the formulated ZPF-NLCs had particle size in the nanometer range (<1μm), and they were monodispersed systems (their PI was less than 1). So, all the formulations met the characteristics of the NLC system and were identified as an excellent homogenous dispersion.[23]

The type of lipid had an important effect on PS and PI. The F8 utilized sesame oil as liquid lipid. This oil had less ZPF solubility than peppermint oil in F1 (Figure 1), so; it showed a larger particle size and higher PI than F1 (Table 2).

Table 1: Preparation of different formulas of ZPF-loaded NLCs

F No.	*A.of ZPF (mg)*	*Soild lipid (mg)*		*Liquid lipid (mg)*		*Surfactant % v/v*		*Co-surfactant %v/v*	
		Type of oil	*A.*	*Type of oil*	*A.*	*Type*	*%*	*Type*	*%*
F1	80	Stearic Acid	210	Pepp.	90	Tween 80	5	PEG 400	2.5
F2	80	Stearic Acid	240	Pepp.	60	Tween 80	5	PEG 400	2.5
F3	80	Stearic Acid	270	Pepp.	30	Tween 80	5	PEG 400	2.5
F4	80	Stearic Acid	210	Pepp.	90	Tween 80	1	PEG 400	0.5
F5	80	Stearic Acid	210	Pepp.	90	Tween 80	2.5	PEG 400	1.25
F6	80	Stearic Acid	210	Pepp.	90	Poloxa-mer 188	5	PEG 400	2.5
F7	80	Stearic Acid	210	Pepp.	90	Tween 80	5	PG	2.5
F8	80	Stearic Acid	210	Sesame	90	Tween 80	5	PEG 400	2.5
F9	80	Palmitc Acid	210	Pepp.	90	Tween 80	5	PEG 400	2.5
F10	80	Stearic Acid	280	Pepp.	120	Tween 80	5	PEG 400	2.5
F11	80	Stearic Acid	140	Pepp.	60	Tween 80	5	PEG 400	2.5
F12	-	Stearic Acid	210	Pepp.	90	Tween 80	5	PEG 400	2.5

F.:formula, no.:number, ZPF: Zaltoprofen, A.: amount, mg: milligram, pepp.: peppermint.

Notes: the amount of deionized water in all formulas was 12 mL, ratio of surfactant to co-surfactant was kept constant (2:1) in all formulas.

Table 2: Particle Size, Polydispersity Index, Zeta potential, % Entrapment efficiency, and % Drug loading of the prepared ZPF-NLCs Formulas**.**

F. no.	*P.S*	*PI*	*Z-p*	*% EE*	*% DL*
1	78.5 ± 0.82	0.229 ± 0.012	-55.52 ± 0.52	96.12 ± 0.53	7.57 ± 0.41
2	164.5 ± 0.43	0.269 ± 0.005	-26.66 ± 2.6	93.23 ± 0.58	5.9 ± 0.54
3	286.2 ± 0.22	0.291 ± 0.007	-20.76 ± 3.61	90.14 ± 2.36	5.2 ± 0.32
4	104.6 ± 0.63	0.275 ± 0.024	-31.36 ± 2.3	86.6 ± 0.87	6.44 ± 0.61
5	89.7 ± 0.59	0.251 ± 0.006	-22.49 ± 2.4	95.9 ± 0.41	6.62 ± 0.17
6	99.6 ± 0.32	0.212 ± 0.005	-21.12 ± 1.62	92.46 ± 3.2	6.71 ± 0.63
7	163.3 ± 0.32	0.259 ± 0.006	-15.69 ± 0.47	92.85 ± 0.06	6.12 ± 0.34
8	251.5 ± 0.5	0.334 ± 0.025	-20.80 ± 0.26	86.1 ± 2.61	5.22 ± 0.82
9	304.8 ± 0.62	0.404 ± 0.036	-23.98 ± 0.06	87.7 ± 3.52	4.85 ± 0.74
10	221.3 ± 0.62	0.35 ± 0.006	-22.48 ± 1.42	96.6 ± 0.37	6.12 ± 0.44
11	50.1 ± 0.21	0.331 ± 0.003	-27.18 ± 0.67	92.4 ± 1.4	6.36 ± 0.32
12	49.5 ± 0.32	0.235 ± 0.002	-37.62 ± 0.55	-	-

The amount of liquid lipid is another factor that affects the PS and PI because it leads to a decrease in the viscosity and surface tension of the system that offers NLCs of smaller sizes and provides higher surface area.[24] An increase in liquid lipid leads to reducing the particle size. For this reason, F2 had a smaller PS than F3, and F1 showed the smallest PS because of using the highest amount of liquid oil (Table 2), but the effect was insignificant (p-value > 0.05) while the same factor (amount of lipid) showed a significant effect on PI in F1, F2, and F3 (Table 2).

The properties of the NLCs are also influenced by the type of surfactant used, F6 had larger particle than F1 due to the use of poloxamer 188 as a surfactant instead of tween 80 in F1, and this surfactant showed lower solubility of ZPF than that of tween 80 (Figure 3).

The type of stabilizer or co-surfactant affects the average size, charge, and the size distribution of the NLCs, but to a more negligible effect than surfactants, F7 showed larger particle size and higher PI than that of F1 because the co-surfactants utilized was PG, which had lower ZPF solubility than PEG 400 (Figure 3).

F9 utilized palmitic acid, and it showed higher PS and PI (304.8 ± 0.62 and 0.404 ± 0.036 nm respectively) compared to F1 (Table 2); this was because its solid lipid exhibited lower solubility than stearic acid (Figure 2).

When the concentration of surfactant (tween 80) was increased from 1%, 2.5%, and 5% as in F4, F5, and F1, respectively, there was a significant reduction ($p < 0.05$) in particle size from (104.6 ± 0.63 to 89.7 ± 0.59 to 78.5 ± 0.82) nm respectively and also a significant reduction in PI values from 0.275 ± 0.024 to 0.251 ± 0.006 to 0.229 ± 0.012, respectively. This might be attributed to the higher surfactant concentration that covered the lipid phase's surface, resulting in a decrease in the particle size.[25]

From Table 2, it can be noticed that increasing in particle size with increasing of lipid amount, this can be seen in F11, F1, and F10 that contained 200 mg, 300 mg, and 400 mg respectively of the total amount of lipids with maintaining other factors constant, their PS were 50.1 ± 0.21, 78.5 ± 0.82 and 221.3 ± 0.62 nm respectively. However, it was insignificant while the effect of the same factor on PI was significant ($p < 0.05$), their PI values 0.331 ± 0.003, 0.229 ± 0.012, and 0.35 ± 0.008, respectively.

Formula 12 was a blank NLC prepared by the same constituents and same concentrations as F1 but without the drug, and it was observed that a significant decline in PS ($p < 0.05$) in which particle size was 49.5 ± 0.32 nm and also a significant increase in PI (0.235 ± 0.002) compared to drug-containing formula (Table 2).

2. Zeta Potential (ZP)

The surface charge values for the prepared NLC were negative for empty (F12) and drug-loaded NLC samples (F1-F11), represented in table 2. The zeta potential for all ZPF-NLC dispersions was found to be between -15.69 and -55.52 mV (Table 17). NLC with a zeta potential of more than +20 mV or less than -20 mV can be termed as physically stable dispersion. It can be concluded that most of the prepared dispersions had good physical stability, preventing aggregation with aging.[26] The nanoparticle emulsion of a higher zeta potential value in F1 (-55.2) indicates the better stability of the colloidal system of lipid particles.

3. Entrapment Efficiency (EE) and Drug Loading Capacity (DL) Percentages Determination

The percentage of incorporated drug in the lipid matrix was evaluated and represented in Table 2, %EE of the prepared ZPF-NLC formulations varied from 86.1 ± 2.61 to 96.6 ± 0.37%. The relatively high value of % EE of the drug in all the prepared NLCs was mainly because of the lipophilic character of the drug and the binary mixture of liquid and solid lipids, resulting in only a very weak crystallization.[27]

Also, the solubility of many drugs in liquid lipid is greater than in solid lipid, and the addition of liquid lipid to the solid lipid can damage the crystal lattice in the matrix of lipid nanoparticle, and then it can increase the entrapment efficiency of an active ingredient. In the NLC system, the solid lipid matrix encloses a tiny oil section in which drug solubility is considerably higher, which increases their total drug loading capacity.[5] The drug loading for all NLC formulations was good (ranged from 4.85 ± 0.74 to 7.57 ± 0.41) because of the blending of solid and liquid lipids, creating a disordered structure provides higher space for drug loading. Moreover, the drug is more soluble in liquid lipids than solid lipids.[5]

4. Examination of the ZPF-NLC Optimum Formula

A. X-Ray Diffraction Analysis (XRD)

The XRD spectra of ZPF (Figure 4 A) showed distinct and intense peaks at 2θ scale, indicating the high crystalline structure of the drug: 10.5, 15.50°, 23°, 25°, and 28° and several low-intensity peaks. The diffraction curve of stearic

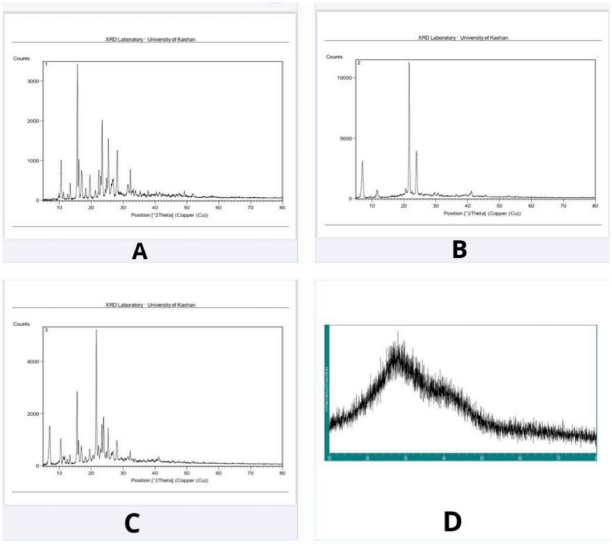

Figure 4: XRD pattern of A) ZPF, B) Stearic acid, C) physical mixture of ZPF: Stearic acid 1:1 and D) ZPF-NLC optimum formula (F1).

acid (Figure 4 B) displayed a sharp peak at 2θ equals 6.5°, 21.5°, and 24°.

The characteristic diffraction peaks of ZPF and the stearic acid were observed in the pattern taken for the physical mixture of ratio 1:1 in the same position (figure 4 C), indicating the compatibility between the drug and the solid lipid.[28] While, the characteristic peaks of ZPF were absent from the XRD pattern of ZPF-NLC optimum formula (F1) (figure 4 D), demonstrating that the ZPF molecule lost its crystalline nature and produced an amorphous complex within the NLC matrix.[29]

B. Fourier Transform Infra-Red (FT-IR) spectroscopy

FTIR spectra of pure ZPF (figure 5 A) showed characteristic peaks such as C-H stretch at 3055 cm^{-1}, aryl C-H stretch at 2978 cm^{-1}, carbonyl C=O stretch of ketone and acid at 1701 cm^{-1} and 1670 cm^{-1} respectively, C-O stretch at 1276 cm^{-1}, 1330 cm^{-1} of CH_3 stretching, C-S stretch at 1419.6 cm^{-1} and C-S-C aromatic stretching peak at 937 cm^{-1} [30,31].

Figure (5-B) showed the FTIR spectrum of stearic acid. The peaks at 2916.37 and 2846.93 cm^{-1} are related to the CH_2 groups. The band at 1697.36 cm^{-1} is assigned to the C=O group. The bands at 1462 and 1188 cm^{-1} are assigned to the CH_2 group, whereas the absorption at 1311.59 cm^{-1} is assigned to the CH_3 group.[32]

The physical mixture spectrum of ZPF: Stearic acid in ratio 1:1 (Figure 5 C) showed most of the characteristic peaks of both the drug and the solid lipid, indicating no drug –excipient interaction.

The FTIR spectrum of ZPF-loaded NLC (figure 5-D) showed the broad peak of O-H stretching at 3100–3500 cm^{-1} detected for water that made the external phase of the NLC.[31] The spectrum did not display all the characteristic ZPF peaks. This was because of the dispersion of the drug within the lipid matrix; the detected peaks were from lipid excipients certain functional groups, the result has confirmed the successful employment of the drug into cavities of the nanoparticle.[20]

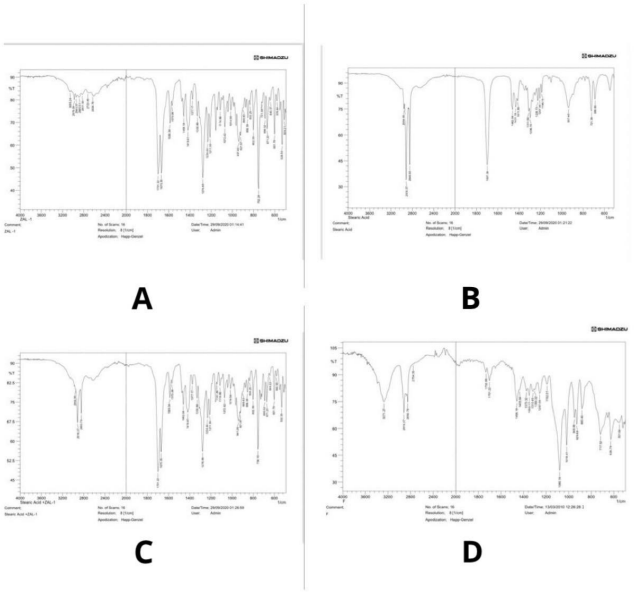

Figure 5: FTIR spectrum of A) ZPF, B) Stearic acid, C) Physical mixture of ZPF: stearic acid 1:1 and D) NLC-Optimum formula (F1).

C. Visualization by Transmission Electron Microscope (TEM)

The TEM images were taken to visualize the morphology of the internal structure of the drug-loaded NLC.[32] The TEM of the optimized ZPF-NLC showed a spherical shape, as shown in Figure 6.

5. *In-vitro* Release Study

The selection of the three following ZAL-NLC formulas for in-vitro release profile was made depending on the lowest particle size, appropriate PI, %EE, and %DL in addition to good Z-potential value. The cumulative %drug release of F1, F5, and F11 was plotted against time for 8 hours in phosphate buffer 7.4 media, as shown in Figure 7.

F11 (of a lower amount of lipid, 200 mg) had a rapid early release profile, but it reached only 70% at the end of the eighth hour due to a lower amount of total lipid 200 mg to 300 mg of lipid in F1.

The amount of the surfactant affected particle's size and hence drug release, as it decreased from 5 to 2.5%, there was a decline in release properties of the NLC formulations as in F1 and F5, respectively, due to reduction in surfactant amount

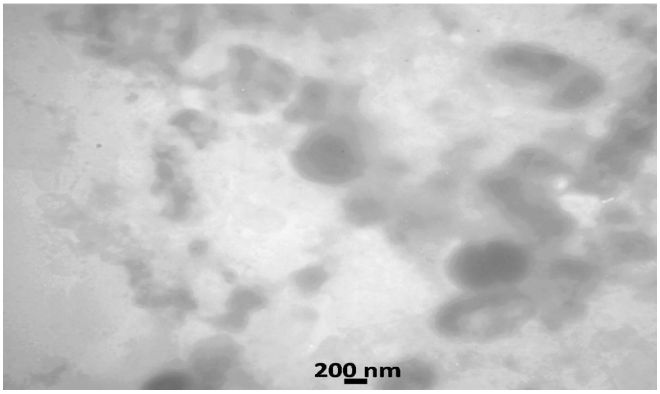

Figure 6: TEM of the optimum formula (F1)

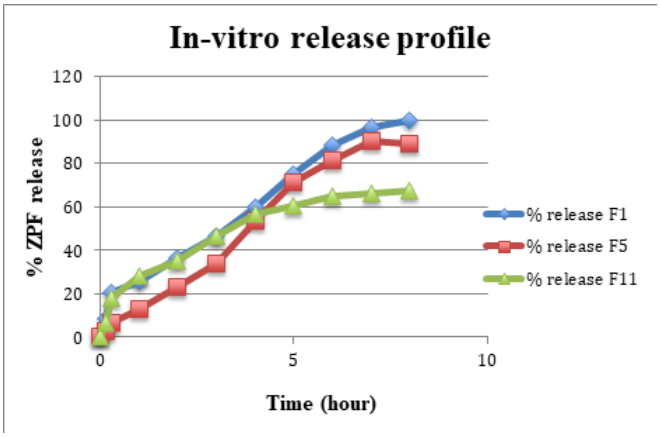

Figure 7: The cumulative release profile for different ZAL-NLCs in pH 7.4 phosphate buffer.

required to cover the nanoparticles surface effectively.[33] It reached about 88% at the end of the eighth hour.

It was also observed that ZPF released from F1 was higher than other formulas; it reached 100 % at the end of the 8th hour; this formula had a small particle size, the highest value of the zeta potential, and good entrapment efficiency.

It is clear from Figure 7 and there was a significant difference for drug release from formulations F1, F5, and F11 ($p > 0.05$). The alterations in the drug release profile from all the above formulas might be related to the effect of lipid matrix composition, and the surfactant concentration, and the drug load.[34]

In conclusion, F1 was chosen as an optimum formula because it had a small PS 0f 78.5 nm with a low PI value of 0.229, that is meant it was homogeneously distributed. It also had an excellent %EE of about 96.12% and the highest %DL of 7.57%; the highest ZP value characterized this formula among all other formulas of about -55.52 mV, this formula excepted to have the maximum stability. It is composed of stearic acid and peppermint oil (as a solid and liquid lipid, respectively) in a ratio of 70:30, tween 80 and PEG 400 (as surfactant and co-surfactant respectively) in ratio of 2:1.

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