Original article

Nanoemulsion and Solid Nanoemulsion for Improving Oral Delivery of a Breast Cancer Drug: Formulation, Evaluation, and a Comparison Study

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ABSTRACT

Letrozole (LZ) is an aromatase inhibitor, which inhibits the formation of estrogens from androgens. Nanoemulsion is a liquid emulsion formulation utilized to increase solubility, bioavailability, and drug delivery to cancer cells. This study aims to improve LZ oral delivery through formulating solid nanoemulsion (SNE). Peppermint oil, tween 80, and transcutol P were used as an oil, surfactant, and co-surfactant, respectively. The optimized nanoemulsion (NE-3) was then incorporated into solid polyethylene glycol (PEG) to formulate (SNE). The optimized (NE-3), SNE-2, and the available marketed tablet have been compared. The optimized (NE-3) was selected according to specific parameters of optimum small nano-size 80 nm, PDI of 0.181, the zeta potential of-98.2, high transmittance (99.78%), optimum pH (5.6), a high percent of LZ content (99.03 ± 1.90), the relatively low viscosity of 60.2 mPa.s, and a rapid release of LZ within 30 min. NE-3 was selected to be formulated as SNE. LZ's best release rate was 80% in 5 min with a content homogeneity of 99.85 ± 0.04 for SNE-2. Zero-order kinetics is determined to have the greatest R^2 values. Field emission scanning electron microscopy (FE-SEM) detected that SNE-2 was (36.75–96.64 nm) with a spherical form and no adhesion or aggregation. FT-IR showed no significant variations in position and shape of the absorption peaks between the pure drug and optimal formulation diagrams. This novel nanoemulsion technology aids in improving the solubility of poorly water-soluble drugs, particularly the SNE delivery method, which has a higher in-vitro release rate and expiration date of LZ than others.

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1. Introduction

Oral administration is the most popular and preferred method of administration since it is an easy-to-administer and non-invasive method that increases patient compliance. However, oral administration of the drugs has the disadvantage of poor bioavailability because of variable absorption affecting food and drug efflux through GIT lumen P-glycoprotein transporters (Mei et al., 2013). As an example, cancer chemotherapy is preferred to be given orally but the main obstacle is the poor bioavailability. For this reason, Letrozole 'LZ' was studied in this research as it is one of the most effective aromatase inhibitors present nowadays for the management of breast cancer. Besides, it has gained attention since it has demonstrated high safety and effectiveness profile in comparison to tamoxifen (Keshaviah et al., 2005). LZ is a non-steroidal competitive aromatase enzyme system inhibitor; it inhibits the conversion of androgen to estrogens. Moreover, it inhibits the enzyme by binding to the cytochrome P450 heme subunit, leading to estrogens biosynthesis reduction in all tissues (Yue et al., 1998, Cuenca et al., 2006). LZ was also well known for its poor water solubility which will influence its bioavailability and according to BCS (Biopharmaceutical Classification System), it is classified as class II (Wempe et al., 2007, Klein et al., 2009, Sodeifian and Sajadian 2018, Alemrayat et al., 2019). Thereupon, many strategies have been used to improve the bioavailability of the poorly water-soluble drug including pH modification, complexation, amorphization, crystal modification, self-emulsification, and particle size reduction (micronization or nano-crystal formation) (Junyaprasert and Morakul 2015, Alaayedi et al., 2020, Kadhim et al., 2020, Mahmood et al., 2020).

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Nanoemulsions are a group of dispersed particles used for biomedical, pharmaceutical aids, and vehicles that show great promise for the future of diagnostics, cosmetics, drug therapies, and biotechnologies (Shah et al., 2010). Many of the nanoemulsion features were established, including stability, ease of manufacture, and high loading capacity; make them all well-suited for drug delivery (Sainsbury et al., 2014). Nanoemulsions are emulsions of submicron-sized that are under extensive investigation act as carriers of the drug for enhancing the therapeutic agent delivery (Shah et al., 2010). It can be defined as "oil-in-water (o/w) or water-in-oil (w/o) emulsions with mean droplet diameters ranging from 50 to 1000 nm" (Yukuyama et al., 2016, Singh et al., 2017). The average droplet size is usually 100–500 nm. Depending on the droplet size, it can be divided into groups of milky (up to 500 nm) and transparent or translucent (50–200 nm) (Yukuyama et al., 2016). Formulations of oral nanoemulsion were developed to improve the bioavailability of the poorly water-soluble drugs especially for chemotherapeutic treatments such as paclitaxel (Shenoy and Tiwari 2006, Shah et al., 2010).

However, liquid dosage forms may have many disadvantages orally such as no masking for the unpleasant taste of the formulation without flavoring or sweetening agent, inaccurate dose adjustment, and others. Consequently, the solid dosage form could be preferred in general cases. A solid nanoemulsion (SNE) is a highly recommended dosage form due to its robustness and scalability, as well as its ability to gain all the benefits of a liquid system. Thus, this nanoparticulate system is anticipated to improve the bioavailability and therapeutic profile of poorly water-soluble drugs (Ahmad et al., 2014). In a short, this research study aims to formulate and evaluate letrozole as SNE to improve its dissolution, solubility behavior, and stability by formulation and characterization of different formulations and compare the SNE with LZ nanoemulsion and the available marketed drug tablets.

2. Materials and methods

2.1. Materials

LZ was bought from Baoji Guokang Bio-Technology Co., Limited, China. Methanol was purchased from Sigma-Aldrich, Germany. Oleic acid, olive oil, sesame oil, and tween 80 were bought from Central Drug House (P) Ltd., New Delhi, India. Sunflower oil, propylene glycol, peppermint oil, and castor oil were bought from Wuhan Senwayer Century Chemical Co., Ltd, China. Corn oil was purchased from Shaanxi Guanjie Technology CO, China. Tween 20 was bought from SCRC, China, while tween 40 was procured from Avondale Lab, England. Tween 60 was purchased from CP, China. Span (20, 60, and 80), PEG (200, 400, and 600), and transcutol P were all bought from Shanghai Ruizheng Chemical Tech Co., Ltd, China.

2.2. Methods

2.2.1. LZ solubility studies

An excess amount of the drug was dissolved in different oils, surfactants, and co-surfactants. The oils that were used for this study were oleic acid, olive oil, sesame oil, corn oil, sunflower oil, and peppermint oil. The surfactants used were tween (20, 40, 60, and 80), and span (20, 40, 60, and 80). While the co-surfactants used were PEG (200, 400, and 600), and transcutol P. The mixtures were shaken vigorously in a vortex mixer (IKA vortex, GENIUS 3, Germany), then the resulted solutions were placed in a water bath shaker (Vision scientific, VS-1205SW1, Korea) for 72 h at 25 °C. Afterward, the equilibrated samples were removed from the water bath shaker and centrifuged at 3000 rpm for 15 min. Using a UV spectrophotometer (PHARMA TEST DFC-820SP, Germany), the LZ concentration was obtained for each of the supernatant solutions after filtrating these solutions by a syringe filter of 0.45 μm. Correspondingly, the drug amount was determined for each solution at a lambda max of 240 nm (Parveen et al., 2011, Ahmad et al., 2013, Hosny and Banjar 2013, Patel et al., 2013, Ahmad et al., 2014).

2.2.2. Phase diagram construction

Following the choice of the optimum solubilizing oil, surfactant, and co-surfactant (without involving the drug). A pseudo-ternary diagram was drawn to the nanoemulsion main components' behavior over a concentration range. The surfactant: co-surfactant (Smix) each ratio of 1:1, 2:1, 3:1 and 4:1 was mixed with different volumes of oil in a ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, then each mixture of oil and Smix titrated against deionized water under stirring condition. The clarity and turbidity were observed (Parveen et al., 2011, Hosny and Banjar 2013). This method was performed to evaluate various surfactant/cosurfactant mixtures (Smix) depending on the formed area of the emulsion and the different Smix ratios were used to achieve the required HLB value for o/w emulsion of 8–18. The Smix ratio with a larger area of monophasic solution points was selected to prepare the nanoemulsion.

2.2.3. Preparation of LZ nanoemulsion formulations

A simple titration method was used to prepare the LZ nanoemulsion formulas. The drug, oil, and Smix were mixed using a vortex mixer, then titrated against water. The used components of the formulas were listed in (Table 1).

2.2.4. Evaluation of LZ nanoemulsion formulations
2.2.4.1. **Studies of LZ nanoemulsion thermodynamic stability** (Altamimi et al., 2021). **Heating/Cooling cycles:** All the coming formulations from the former test were gone into 6 cycles of 4 and 45 °C for 2 d each.

Centrifugation test: All the LZ nanoemulsion formulations were subjected to this test. This test was performed by centrifuga-

Table 1
LZ nanoemulsion formulations.

Formulation Code	LZ mg/5mL nanoemulsion	Peppermint oil (%)	Smix (Tween 80/Transcutol P) %		Water %
			Ratio	Total %	
NE-1	2.5	5	3:1	45	50
NE-2	2.5	8	3:1	48	44
NE-3	2.5	5	3:1	50	45
NE-4	2.5	5	4:1	54	41
NE-5	2.5	8	4:1	58	34
NE-6	2.5	5	4:1	60	35

tion at 3500 rpm for half an hour. The formulation that was still homogenous and pure without any turbidity was subjected to the next test.

Freezing/Thawing cycles: Three cycles of freeze temperature of −21 °C and room temperature were passed the formulations through for 2 d each cycle.

2.2.4.2. Determination of particle size and polydispersity index (PDI). The particle size analyzer instrument (particle size analyzer device - Brookhaven Corp 90 Plus, NY, USA) was used to determine these two parameters of the nanoemulsion formulations. These tests were performed to ensure the stability and uniformity of the prepared formulations. This instrument uses a scattered light of 90° angle at room temperature. The light was directed to the specific volume of the nanoemulsion in a cuvette (Baboota et al., 2007, Araujo et al., 2011, Sood et al., 2014, Mahtab et al., 2016). The analysis tests were implemented six times to set the mean values.

2.2.4.3. Zeta potential determination. This test was performed using Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK). The zeta potential unit is in micrometer per second since it depends on the measurement of electrophoretic mobility. Any particle with a zeta potential of more than + 30 mV or less than −30 mV is stable (Tiwari and Amiji 2006, Dalmolin and Lopez 2018).

2.2.4.4. Measurement of formulations viscosity, electroconductivity, filter paper test, and miscibility. These four tests were used to determine the type of produced nanoemulsions if they were o/w or w/o. A Brookfield digital viscometer (LVDV-E, USA) with spindle no. 62 was used to determine the formulation viscosity and their rheological characteristics at room temperature. The spindle was inserted into the formulation for 3 min at 10 rpm. This test was performed in triplicate and the results were obtained as mean ± SD (Srilatha et al., 2013).

Electro conductometer (Electro conductivity meter pen, TDS&EC meter, GHB, China) was used to measure the electrical conductivity of the nanoemulsions. The instrument electrode was inserted in nanoemulsion formulations and the results were obtained at room temperature (Xu et al., 2011). The analysis tests were performed three times to establish the mean values.

The nanoemulsion samples were placed on paper in the filter paper test. O/W emulsion type spread out rapidly while w/o spread very slowly. In the last test, hydrophilic amaranth red color dye was added to each formula. The o/w type nanoemulsion colors homogenously whereas w/o is not (Ali and Hussein 2017).

2.2.4.5. pH determination. A calibrated pH meter (WTW- INO LAB, Switzerland) was used to measure the pH of all prepared formulations by immersing the instrument bulb into 30 mL of each formulation (Mahtab et al., 2016, Ren et al., 2021).

2.2.4.6. Percentage of transmittance measurement. The clarity of the formulated nanoemulsions was determined through the percent transmittance study. This study was made using a UV–Vis spectrophotometer (Shimadzu 1800, Japan) at the drug Lambda max of 240 nm and deionized water was the blank (Jaiswal et al., 2015).

2.2.4.7. Drug content estimation. In this evaluation test, the percent of LZ in each formulation was calculated in comparison to the theoretical amount. The volume of 1 mL of each formulation was diluted in a suitable volume of methanol to dissolve all the loaded amount of drug in the oil internal phase of the o/w emulsion and measure its accurate content value. The supernatant dissolved layer after centrifugation of the sample at 3000 rpm was taken and filtered through syringe filter 0.45 μm, then analyzed in UV–

Vis spectrophotometer at lambda max of 240 nm (Beg et al., 2013). This test was done in triplicate and the results were earned as mean ± SD.

2.2.4.8. In vitro LZ release study. A drug release study was done using a dissolution apparatus type II (PHARMA TEST DFC-820SP, Germany). The dissolution media was 900 mL of simulated gastric fluid of pH 1.2. All the nanoemulsion formulations were subjected to this study in different pH by being placed in a bag of dialysis membrane. A sample of 5 mL was drawn at a specific time interval and replenished with a fresh medium. Each sample was filtered with a syringe filter of 0.45 μm before being analyzed with a UV–Vis spectrophotometer at lambda max of 240 nm (Miryala and Kurakula 2013, Ahmed et al., 2018). Each experiment was performed six times to determine the results as mean ± SD.

2.2.4.9. Release kinetics. In this study, the data obtained from the release study to determine the kinetic of LZ release. The kinetic could be fitted to a different model of zero order, first order, Korsmeyer's, or Higuch's models (Kawish et al., 2017).

2.2.4.10. Selection of optimum LZ nanoemulsion formulation. The election of the optimum formulation among the produced LZ nanoemulsion formulations depends on the droplet size, PDI, zeta potential, pH, electroconductivity, percent transmittance, viscosity, and drug release (Khames 2019).

2.2.4.11. Examination of the optimum formulation morphology. Many tests were done to examine the morphology of the optimum LZ nanoemulsion formulation including field emission scanning electron microscopy technique (FE-SEM; using SEM software work as 5 kV) using (TESCAN - VEGA 3, Czech Republic) (Araujo et al., 2011, Parveen et al., 2011, Thadkala et al., 2015, Thakkar et al., 2015, Mahtab et al., 2016, Robertson et al., 2016).

2.2.5. Preparation of LZ solid nanoemulsion formulations

The solid inert carrier for the nanoemulsion was polyethylene glycol (PEG) which solidified the nanoemulsion to produce solid nanoemulsion (SNE). PEG with different grades was used including PEG 4000 and 6000, separately. The heat fusion method was used to prepare SNE with a temperature range of 60–70 °C. In this method, the optimum nanoemulsion formulation was poured into melted PEG with stirring to produce a homogenous mixture, then left to solidify after cooling at room temperature. Six SNE formulations were prepared using different ratios of SNE to each PEG (4000 and 6000) 0.5:1, 1:1; 1:0.5 (Ahmad et al., 2014).

2.2.6. Evaluation of solid formulations
2.2.6.1. Drug content estimation. A similar procedure used in Section 2.2.4.7 was used for the determination of SNE drug content.

2.2.6.2. In vitro LZ release study. Dissolution apparatus type II was used in this study using different media for each formulation including an acidic medium of pH 1.2 and a phosphate buffer of pH 6.8 at 37 °C. Both SNE formulations and the marketed tablet of the drug were subjected to this study under the same conditions and procedure mentioned in Section 2.2.4.8.

2.2.6.3. Release kinetic. The kinetic study that was applied for the nanoemulsion formulations using their release data, applied for the SNE release data as mentioned before.

2.2.6.4. Selection of the solid nanoemulsion optimum formulation. According to the SNE evaluation tests, the optimum SNE formulation was chosen.

2.2.6.5. Advance assessments of the optimum drug solid nanoemulsion formulation. These assessments were including FE-SEM and Fourier-transform infrared (FTIR) (Shimadzu 8400S, Japan). FTIR is one of the important assessing tests for the pure drug and other ingredients in the formulas that explain if there was any interaction between the drug and the rest of the used ingredient (Vyas et al., 2009, Thadkala et al., 2015).

2.2.7. Stability studies for optimum LZ nanoemulsion and solid nanoemulsion formulations

Three batches of both nanoemulsion and SNE were taken separately and subjected to the different temperatures of 30, 40, 50, and 60 °C for 90 d at constant humidity. At specific time intervals, samples of each patch were taken to assess the LZ content using a UV spectrophotometer study at a lambda max of 240 nm. The amount of drug that remains and that decomposed through time was calculated. The LZ degradation order was determined graphically and for each temperature, degradation rate constant 'K' was obtained. To determine the shelf life of the optimum nanoemulsion and SNE formulations, an Arrhenius plot was drawn between K and 1/T. From this plot, the rate constant at room temperature 'K′$_{25}$ was obtained. The shelf life of each optimum formulation was calculated according to the following equation (Alam et al., 2012, Krishna et al., 2013, Mohima et al., 2015, Ali and Hussein 2017a, 2017b):

$$Shelf-life = \frac{0.1052}{K}$$

2.2.8. Statistics

One-way ANOVA was used for the statistic studies to explain if there are any significant differences ($P < 0.05$) among data.

3. Results and discussion

3.1. LZ solubility study

The best solubilizing liquids had been chosen through this study. LZ showed the best solubility in peppermint oil (as main oil), Tween 80 (as a surfactant), and transcutol p (as co-surfactant). Tween 80 is a nonionic surfactant that is non-toxic and has no interaction with proteins and mucosa. Moreover, tween 80 has an HLB value of more than 10 that is required to prepare o/w emulsion. For this purpose, the surfactant molecules were added, which may make adsorption at the oil–water interface and screening the thermodynamically unfavorable molecular interactions between the oil and water phases. Moreover, tween 80 may suggest decreasing the interfacial tension and the decrease in oil droplet disruption (Wadhwa et al., 2012, Gupta et al., 2013, Sullivan et al., 2014, Yuan et al., 2014, Moghimipour et al., 2017, Charoo et al., 2019, Zhu et al., 2019). This study data was illustrated in Figs. 1, 2, and 3, for oils, surfactants, and co-surfactants, respectively.

3.2. Pseudoternary phase diagram

Different ratios of oil and Smix (surfactant and co-surfactant) were mixed and titrated against water. The results were constructed as diagrams, as shown in Fig. 4. The diagrams of both 3:1 showed a larger area of nano-emulsification than other ratios. Therefore, this ratio of Smix was used to prepare the nanoemulsion formulations in different ratios with oil to produce stable nanoemulsions. This Smix ratio may result in a further reduction of the interfacial tension, which can increase the dispersion entropy, increase the interfacial area, increasing the fluidity of
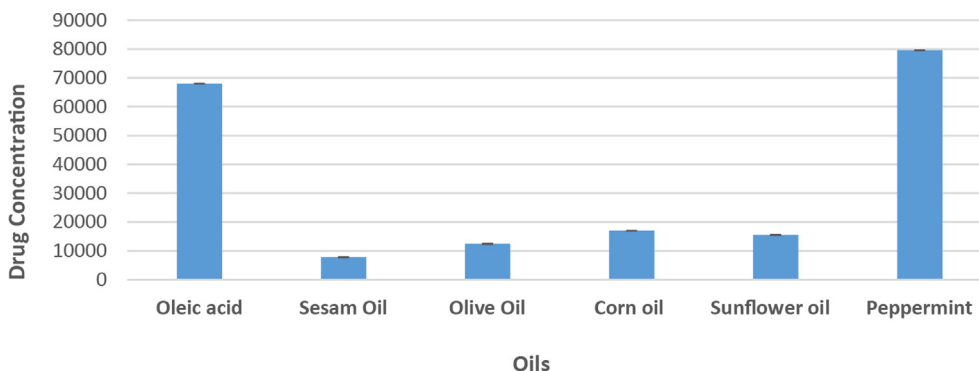

Fig. 1. LZ solubility study in a group of oils separately, all the results represent mean LZ concentration (μg/ml) ± SD.
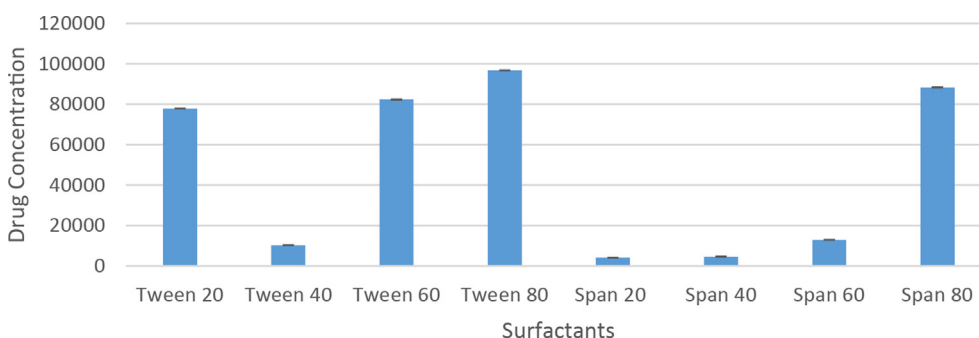

Fig. 2. LZ solubility study in a group of surfactants separately, all the results represent mean LZ concentration (μg/ml) ± SD.
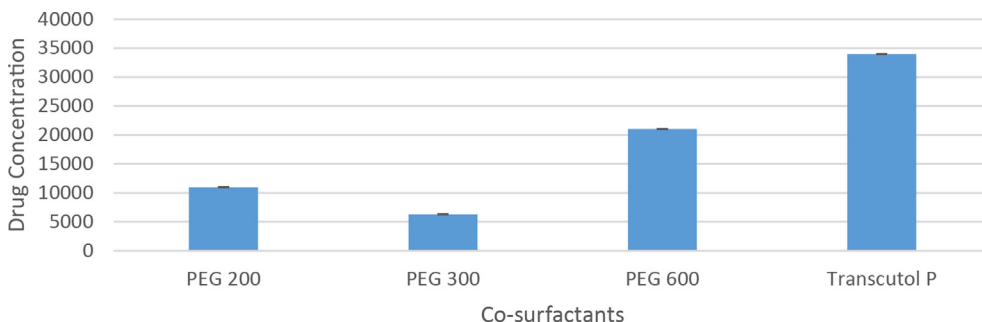
Fig. 3. LZ solubility study in a group of co-surfactants separately, all the results represent mean LZ concentration (μg/ml) ± SD.
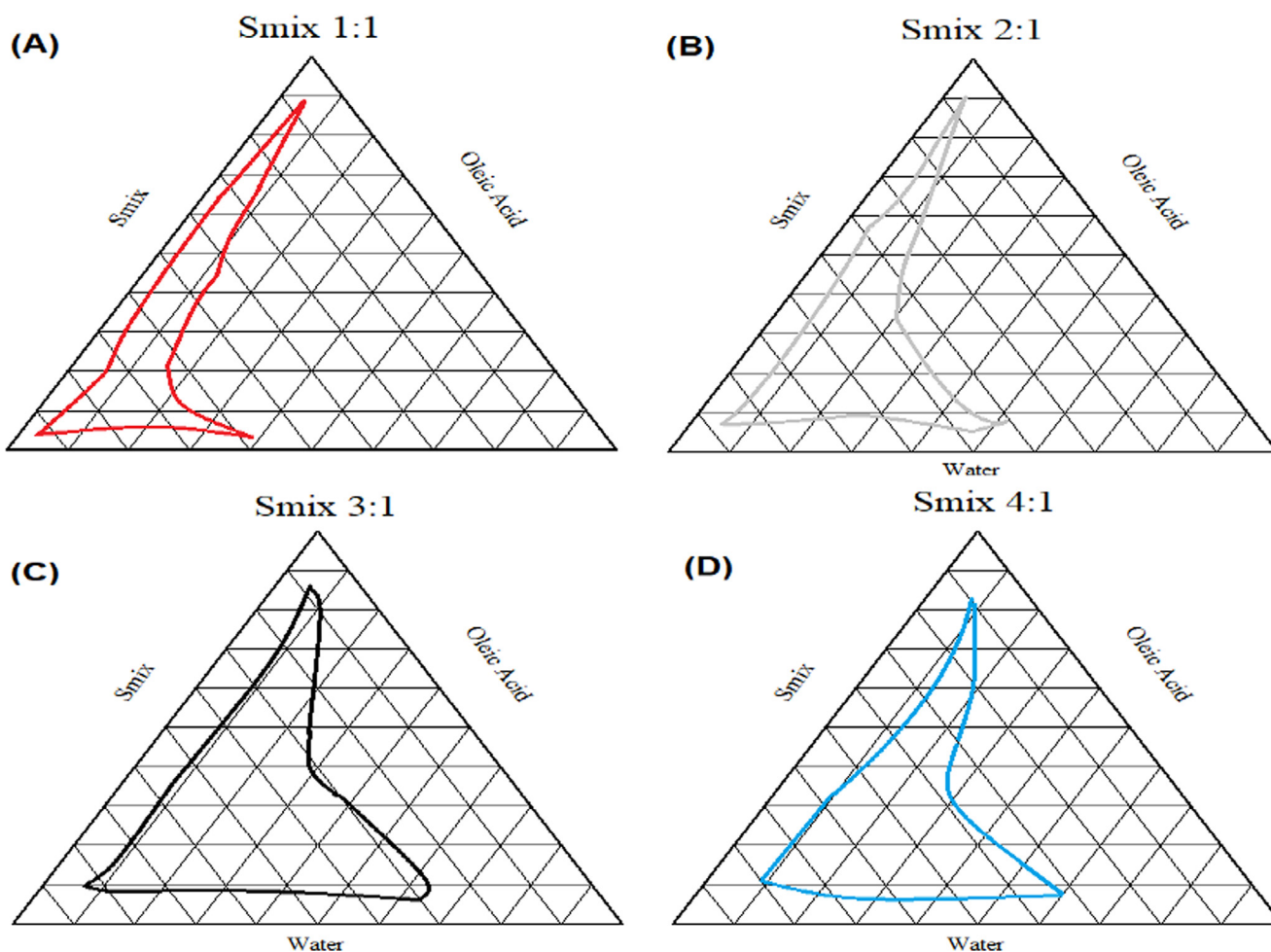

Fig. 4. Pseudoternary phase diagrams of peppermint oil (oil), Smix (tween 80[surfactant]:transcutol p[co-surfactant]) and water at different Smix ratios of 1:1 'A', 1:2 'B', 1:3 'C' and 1:4 'D'.

the interface, and thus lower the free energy of the system to a very low value with the minimum concentration which is thermodynamically stable (Souto et al., 2011).

3.3. Evaluation of LZ nanoemulsion

3.3.1. Thermodynamic stability tests

All the nanoemulsion formulations were subjected to these tests to ensure that they were stable without any separation or precipitation. All of them were successfully passed these tests (Liu et al., 2012, Aziz et al., 2019).

Table 2
Particle size distribution, PDI, and zeta potential of the formulated nanoemulsions. The results represent mean ± SD (n = 6).

Formulations	Particles size (nm)	PDI	Zeta Potential (mV)
NE-1	99 ± 0.43	0.198	−76.5
NE-2	98 ± 0.64	0.201	−98.1
NE-3	76 ± 0.26	0.181	−98.2
NE-4	102 ± 0.71	0.217	−108.4
NE-5	112 ± 0.55	0.274	−99.2
NE-6	107 ± 0.36	0.267	−102.4

Table 3
Viscosity and electroconductivity, filter paper test, and miscibility results of the produced LZ nanoemulsion formulations. All the results represent mean ± SD (n = 3).

Formulations	Viscosity (mPa.s)	Filter paper test	Dye test	Electrical conductivity (μs/cm)	Nanoemulsion Type
NE-1	50.1 ± 1.33	Highly spreadable	Miscible	201.34 ± 1.45	o/w
NE-2	56.3 ± 1.24	Highly spreadable	Miscible	200.34 ± 1.56	o/w
NE-3	60.2 ± 1.16	Highly spreadable	Miscible	193.01 ± 2.80	o/w
NE-4	61.9 ± 1.65	Highly spreadable	Miscible	187.09 ± 1.02	o/w
NE-5	87.2 ± 1.23	Highly spreadable	Miscible	198.33 ± 2.97	o/w
NE-6	90.7 ± 1.46	Highly spreadable	Miscible	189.91 ± 1.73	o/w

3.3.2. Measuring size distribution and PDI

These two characteristics of the nanoemulsion formulations were related to the concentration of both peppermint oil and tween 80. All the six prepared formulations were within the required nano-scale, as explained in (Table 2). There were differences in size among formulations and this may relate to the concentration of the oil with surfactant. The size of the formulations is inversely related to the amount of the surfactant and co-surfactant in them. NE-3 formulation had the smallest size of nm. PDI of all the formulations was less than 0.4 and that indicated the homogeneity and uniformity of the formulations (Baboota et al., 2007, Chen et al., 2011, Acharjya et al., 2012, Danaei et al., 2018).

3.3.3. Zeta potential measurement

The zeta potential is an indication of the repulsion force among the particles. It has been demonstrated that the zeta potential of more than 30 mV indicates the good stability of the formulated nanoemulsion (Lowry et al., 2016, Gurpreet and Singh 2018). The zeta potential of the prepared formulations was shown in (Table 2). The negative charge of the droplet that was recorded is due to the presence of the anionic group in the oil and glycol in the cosurfactant (Transcutol-P: diethylene glycol monoethyl ether).

3.3.4. Measurement of formulations viscosity, electroconductivity, filter paper test, and miscibility

The viscosity of the produced formulation of LZ nanoemulsion was optimized using the Smix to produce stable formulations. The viscosity of the formulations was within the range of (50.1–90.7 mPa.s). Formulations that contained a higher amount of co-surfactant, had the lowest viscosity. The formulations with a higher amount of tween 80, were more viscous (Ahmad et al., 2014). The rapid spreadability for all formulations over the filter paper, the homogenous coloring of them with hydrophilic dye, and been conductor to electricity indicated that they were o/w type emulsion (Hassan 2015). The data of the four mentioned tests are explained in (Table 3).

3.3.5. pH and percent transmittance of the nanoemulsions

All the produced nanoemulsions were had pH within the normal range of the mouth pH of 5–7. The results of the percent transmittance were close to 100% indicating that the formulations were transparent, clear, and able to transmit light. The results of these two tests mentioned above in this section were shown in (Table 4).

3.3.6. Drug content

The results of this study were within the accepted range (85–115) %, according to USP. This indicated that there was no precipitation or loss in the drug during formulation or storage. The results of drug content were shown in (Table 4).

3.3.7. In vitro release study

The release study results show that most nanoemulsion formulations (NE-1 - NE-4) release most of the drug within the first 60 min. Whereas, formulations (NE-5 and NE-6) takes more time to release their content. The release data pattern indicates the effect of nanoemulsion particle size effect, where the formulations with the smallest size had the rapid onset of release. NE-3 has the smallest size with the most rapid release of LZ. Additionally, the formulations containing a higher amount of surfactant had slow
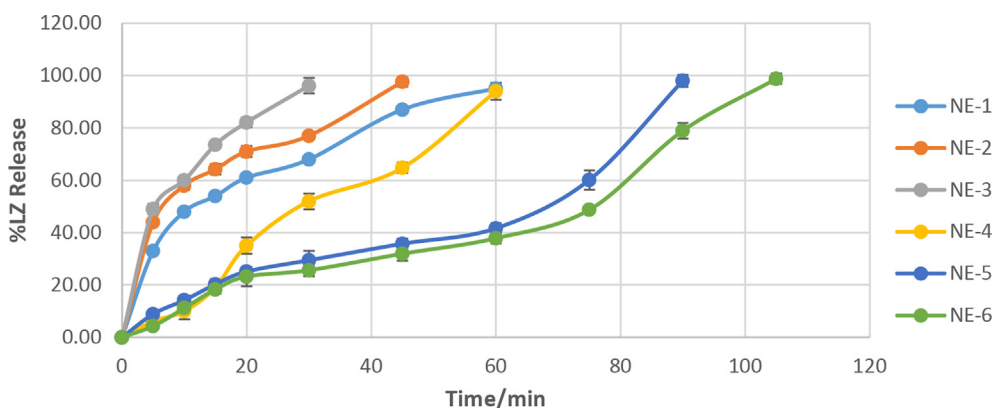
Table 4
pH and percent transmittance of the LZ nanoemulsions. The results represent mean ± SD (n = 3).

Formulations	pH	Transmittance%	Drug content
NE-1	5.4	99.12	96.92 ± 1.01
NE-2	5.2	99.01	97.12 ± 2.11
NE-3	5.6	99.78	99.03 ± 1.90
NE-4	5.6	99.43	99.30 ± 1.49
NE-5	5.9	98.38	98.00 ± 2.09
NE-6	6.1	98.42	97.35 ± 2.14

Fig. 5. Percent of LZ release in pH 1.2 medium, the results represent mean drug amount ± SD, n = 6.

Table 5
LZ releases kinetic models.

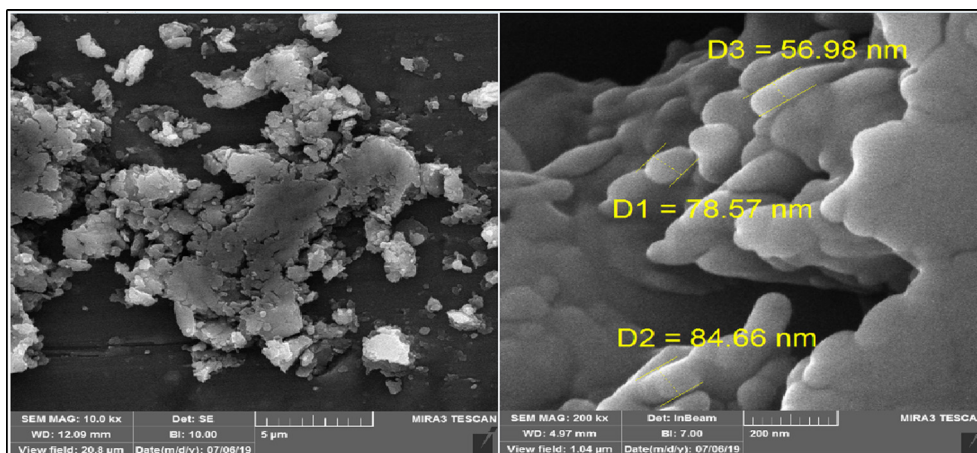| Formulations	Zero-order model R^2	First-order model R^2	Higuchi model R^2	Koresmeyer Peppas model	
				R^2	n
NE-1	0.9817	0.8534	0.9527	0.9635	0.724
NE-2	0.9751	0.8966	0.9696	0.962	0.6892
NE-3	0.9711	0.8921	0.9389	0.9857	0.3857
NE-4	0.9421	0.8391	0.9396	0.8952	0.8821
NE-5	0.8719	0.6142	0.9218	0.999	0.4482
NE-6	0.8001	0.6512	0.9696	0.9838	0.7102

Fig. 6. Morphology of the optimized NE-3 formulation of the LZ nanoemulsion using SEM.

release due to the effect of tween 80 on LZ escape and being available in dissolution medium (Thassu et al., 2007, Sinko 2011, Lokhandwala et al., 2013, Ali and Hussein 2017a, 2017b). The *in vitro* release pattern of LZ was shown in Fig. 5.

3.3.8. Kinetics of LZ nanoemulsion release

As mentioned in the method part, this study investigated the kinetic of LZ release from the nanoemulsion using the *in vitro* release results to determine if the release follow zero or first-order kinetics, Higuchi model, or Korsmeyer-Peppas model according to their equation bellow;

$Mt = M_0 + K_0t$ (Zero-order model equation)

$lnMt = lnM_0 + K_1t$ (First order model equation)

$M_t = M_0 + kH.t1/2$ (Higuchi model equation)

$\frac{Mt}{M\infty} = k \times tn$ (Korsmeyer – Peppas model equation)

Where 't' is time, 'M_t' is the amount released, 'k' is Korsmeyer model constant, 'k'_0 is zero-order constant, 'k'_1 is first-order kinetic constant, 'k_H' is Higuchi model constant, 'M'_0' is the loaded drug in the formulations, 'n' is release exponent. The highest R^2 (correlation coefficient) is the best-fitted model, as shown in (Table 5). The highest diffusion exponent of the Koresmeyer equation determines the release mechanism of LZ from the prepared formulations that were Non-Fickian because all the released exponents were between 0.43 and 0.89 (Małgorzata et al., 2016; Rajabi-Siahboomi et al., 2013; Siepmanna and Peppas, 2001).

3.4. Optimum LZ nanoemulsion selection

The optimum LZ nanoemulsion formulation (NE-3) was selected according to specific parameters of optimum small nano-size of 80 nm, PDI of 0.181, the zeta potential of −98.2, high transmittance (99.78%), optimum pH (5.6), a high percent of LZ content

(99.03 ± 1.90), of relatively low viscosity of 60.2 mPa.s, rapid release of LZ within 30 min.

3.4.1. Optimum formulation morphology examination

FE-SEM is a microscopic test that can approve the particle size of the optimum formulation (Anuar et al., 2020), which is NE-3. As clear from Fig. 6 the microscopy could investigate the nanosized particles of NE-3 formulation. The average range is (56.98–84.66) nm with a spherical non-adherent shape.

3.5. Preparation of solid nanoemulsion (SNE)

The optimum nanoemulsion formulation 'NE-3' was selected to be formulated as SNE by dispersing the nanoemulsion into PEG 4000 and 6000 in a different ratio, as shown in (Table 6).

3.5.1. Evaluation of the prepared SNE
3.5.1.1. LZ content.
As shown in (Table 7) below, the LZ content of the formulated SNE formulations was within the accepted range (Ali and Hussein 2017, Committees 2018).

Table 6
The SNE formulations of the optimum LZ nanoemulsion.

Formulations	Nanoemulsion: PEG 4000 ratio	Nanoemulsion: PEG 6000 ratio
SNE-1	0.5:1	–
SNE-2	1:1	–
SNE-3	1.5:1	–
SNE-4	–	0.5:1
SNE-5	–	1:1
SNE-6	–	1.5:1

3.5.1.2. In vitro release study of the produced SNE. The release of LZ from the six formulations was shown in Fig. 7. The results demonstrated that SNE-2 is the best formulation and it was the only formulation that releases 80% of LZ within 5 min. The same results were obtained as comparing the release of the drug from optimum

nanoemulsion, SNE, and the marketed drug were all compared as shown in Fig. 8. The results demonstrated that solid nanoemulsion release is better than nanoemulsion and marketed products.

3.5.1.3. In vitro release kinetics study. According to that mentioned information in the nanoemulsion release kinetic section, the release kinetic of LZ from the solid formulations was performed and the results data is illustrated in (Table 8). The results followed zero-order kinetic since it has the highest values of R^2. As well as this and according to the Korsmeyer-Peppas model, the SNEs formulations mode of diffusion follows Fickian (case I) diffusion.

3.5.1.4. Examination of SNE formulations morphology. It seems from Fig. 9 that the FE-SEM can detect the spherical shape of nanosized SNE-2 formulation as well as it is not adherent or aggregate to each other. The average particle size was (36.75–96.64 nm). Therefore,
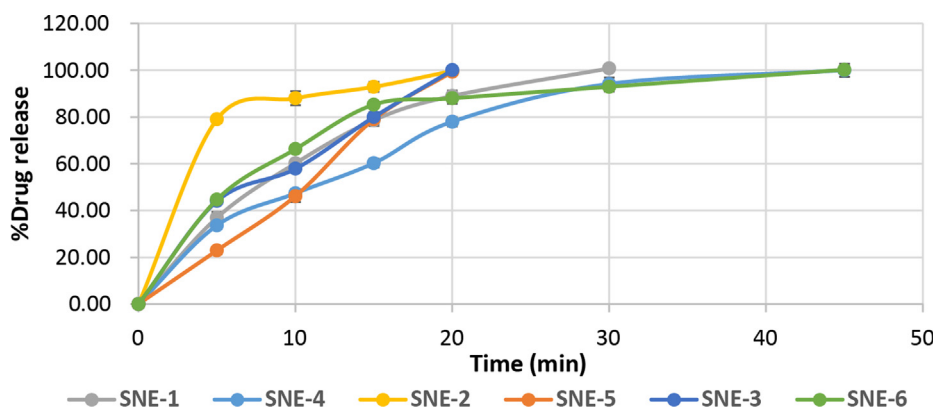
Table 7
The LZ content of the formulated SNE. Each result represents mean ± SD (n = 3).

Formulations	%Drug content
SNE-1	99.03 ± 0.02
SNE-2	98.65 ± 0.03
SNE-3	99.85 ± 0.04
SNE-4	98.63 ± 0.02
SNE-5	98.15 ± 0.04
SNE-6	98.98 ± 0.01

Fig. 7. The dissolution profile of LZ release from SNE formulas in pH 1.2 medium, data were given in mean ± SD, n = 6.
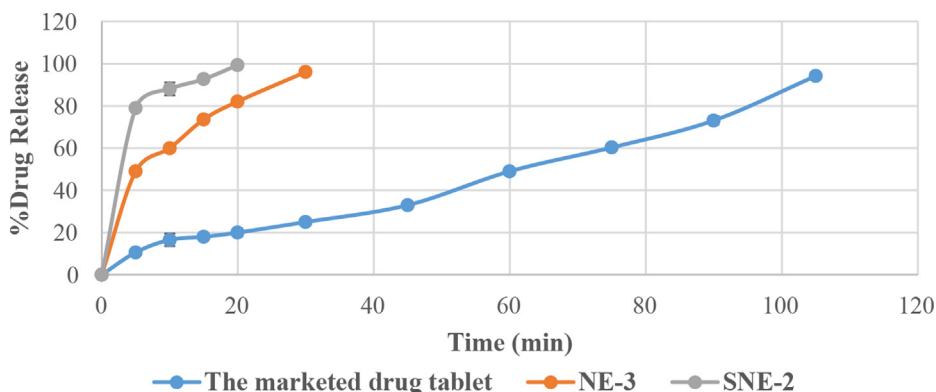

Fig. 8. The dissolution profile for comparing of LZ release from nanoemulsion, SNE, and the marketed available formulation, data were given in mean ± SD, n = 6.

Table 8
The coefficient of correlation (R^2) and the exponent of release (n) of various kinetic models of SNE formulations release in acidic buffer (pH 1.2).

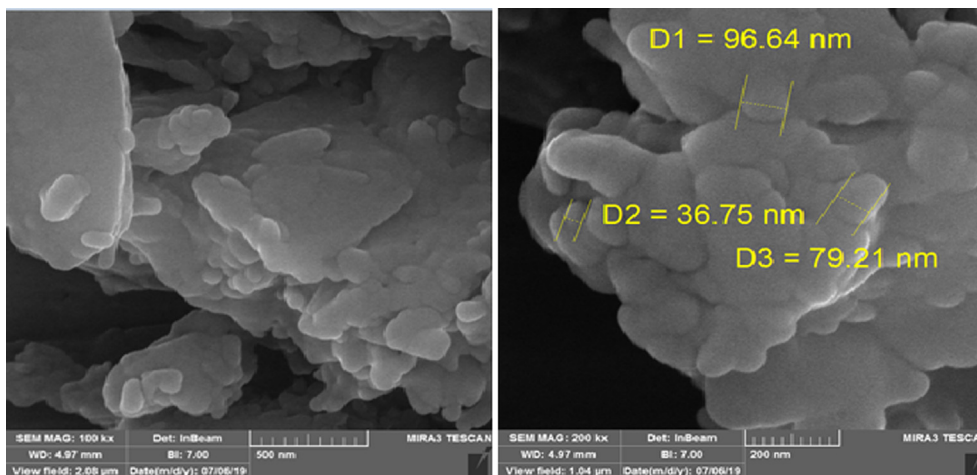| SNE	Zero-order model R^2	First-order model R^2	Higuchi Model R^2	Korsmeyer peppas model	
				R^2	n
SNE-1	0.8586	0.6959	0.9898	0.9879	0.3845
SNE-2	0.6249	0.7793	0.8623	0.8569	0.1602
SNE-3	0.8286	0.5177	0.9767	0.9395	0.430
SNE-4	0.9999	0.5899	0.999	0.9999	0.3998
SNE-5	0.9185	0.6599	0.997	0.99	0.3992
SNE-6	0.9999	0.6999	0.9986	0.999	0.389

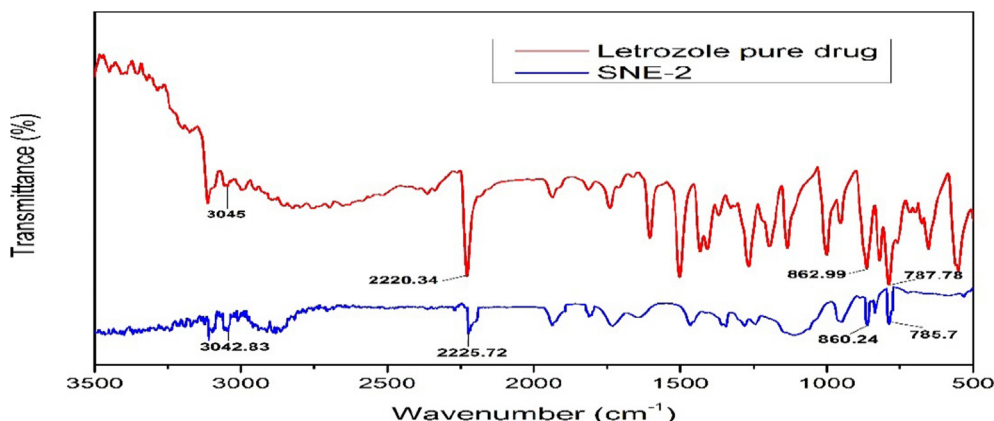**Fig. 9.** FE-SEM of optimum solid nanoemulsion (SNE-2).

Fig. 10. The combined FTIR spectrum of optimum solid nanoemulsion (SNE-2) in comparison with pure LZ drug.

the SNE-2 formulation was still successfully being within the theoretical nanosized.

3.5.1.5. Fourier transform infrared spectroscopy (FT-IR). It showed that no significant differences in shape and position of the absorption peaks could be observed clearly between the pure drug and optimum formulation diagrams. LZ pure powder showed major peaks at 3045 cm^{-1} for sp^2 CH stretching hybridized, 2220 cm^{-1} for C≡N stretching, 690 900 cm^{-1} for out-of-plane CH deformation modes of vibration. It can be concluded that there was a negligible variation as compared between the peaks and no strong chemical interaction occurred between drug and other formulation excipients as illustrated in Fig. 10. No significant difference in shape and position of the absorption peaks of the drug has been observed among the spectra (Dey et al., 2009, Gomathi et al., 2017).

3.6. Stability studies of LZ in optimum nanoemulsion and SNE formulations

The percent of remaining drug in NE-3 at different temperatures during the period of storage was not less than 95%. The order of drug degradation was graphically determined at each temperature; it was first-order since the degradation rate is directly related to the single reactant concentration first power. The first and zero-order degradation correlation coefficients of LZ were determined at each temperature. The rate of degradation constant was deter-

mined from the slope of the graph line at all selected temperature using the following equation:

$$Slope = -\frac{K}{2.303}$$

The NE-3 degradation rate constant for each time is explained in (Table 9). The drug remaining percent log was drawn against time and the slope of the lines was determined then K according to the equation above. K plotting against 1/T was studied the effect of temperature on the degradation (Shafiq et al., 2007, Lovelyn and Attama 2011). The degradation rate constant at room temperature (K_{25} = - 2.44904) was determined by the plot extrapolation then shelf-life was calculated which was 2.6 years.

The optimized drug nanoemulsion formulations must be stable during the intended period of shelf-life; therefore, the formulation was subjected to accelerated temperature for 3 months. Overall, the degradation study showed that there was no significant change

Table 9
K of LZ in NE-3 and SNE-2 at different temperatures during storage.

K (month^{-1})	NE-3	SNE-2
K_{30}	0.0066787	0.005297
K_{40}	0.0112847	0.011285
K_{50}	0.0179634	0.017273
K_{60}	0.0202664	0.020497

in LZ content at all temperatures through the study interval. Thus NE-3 was stable (Glass and Haywood 2006). The stability study mentioned above was applied to the optimum solid nanoemulsion, the degradation rate constant at room temperature was (K_{25} = -2.61978) and the shelf-life was 3.66 years. The optimized SNE-2 has a considerably higher expiration date than NE-3 since the solid demonstrated more stability of solid than liquid preparations (Rajabi-Siahboomi et al., 2013, Małgorzata et al., 2016).

4. Conclusion

The planned formulations were prepared successfully and the aim of this study was reached. The formulated nanoemulsion has a promising result in improving the oral LZ release and then it could be shown in future bioavailability studies. Optimized nanoemulsion (NS-3) and SNE (SNE-2) have been chosen. Besides, they exhibited a required *in vitro* pharmaceutical result with a significant increase in the LZ solubility (P < 0.05) in comparison with the marketed conventional tablet dosage form.

Human and animal rights

No Humans and animals were used for studies that are based on this work.

Consent for publication

Applicable.

Availability of data and materials

Applicable

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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