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# Preparation and Characterization of Topical Letrozole Nanoemulsion for Breast Cancer

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## Abstract

Letrozole (LZL) is a non-steroidal competitive aromatase enzyme system inhibitor. The aim of this study is to improve the permeation of LZL through the skin by preparing as nanoemulsion using various numbers of oils, surfactants and co-surfactant with deionized water. Based on solubility studies, mixtures of oleic acid oil and tween 80/ transcutol p as surfactant/co-surfactant (Smix) in different percentages were used to prepare nanoemulsions (NS). Therefore, 9 formulae of (o/w) LZL NS were formulated, then pseudo-ternary phase diagram was used as a useful tool to evaluate the NS domain at Smix ratios: 1:1, 2:1 and 3:1. All the prepared formulae were characterized for thermodynamic stability studies, zeta potential, droplet size, polydispersity index (PDI), % transmittance estimation, pH, % drug content, electro-conductivity and *in vitro* drug release. NS-7 (compose of 5% oleic acid, 45% Smix of 1:9 ratio and 50% water) was chosen as an optimum prepared formula for many reasons. Initially, it has a lower PDI (0.054), optimum droplet size (75.9 nm), highest transmittance percent (99.89±0.015%), high drug content (99.88%±0.03%), acceptable pH (5.96±0.025), highest electro-conductivity (230±1 µS/cm) and optimum drug release% (99.58±1.92) after 75 min in phosphate buffer (pH 6.8) compared to other NS formulations.One can conclude that preparation of LZL NS is an effective method for improving the permeation of LZL throught the skin.

**Keywords: Letrozole, Nanoemulaion, Permeation and *in vitro* release.**

# تحضير ودراسة خواص الليترزول على هيئة مستحلب نانوي دقيق

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## الخلاصة

ليتروزول (LZL) هو مثبط نظام إنزيم أروماتيز تنافسي غير الستيرويد.الهدف من هذه الدراسة هو تحسين نفوذية الLZL من خلال الجلد. بناءً على دراسات الذوبان ، تم استخدام مخاليط من زيت حمض الأوليك وتوين ٨٠ / ترانسكيوتول بي كخافض للشد السطحي / مساعد خافض للشد السطحي (Smix) بنسب مئوية مختلفة لإعداد المستحلبات النانوية (NS). لذلك ، تمت صياغة ٩ صيغ (o / w) لـ LZL NS ، ثم تم استخدام مخطط الطور الكاذب كأداة مفيدة لتقييم مجال NS عند نسب Smix ١:١ ، ١:٢ و ٣: ١.
كل الصيغ المحضرة كانت قد خضعت لدراسات الثبات الديناميكي الحراري ، جهد زيتا ، حجم القطرة ، مؤشر تعدد الانتشار (PDI) ، وتقدير النفاذية٪ ، درجة الحموضة pH ، محتوى الدواء٪ ، التوصيل الكهربائي وتحرير الدواء في المختبر.تم اختيار NS-7 كصيغة معدة مثالية لعدة أسباب. في البداية ، يحتوي على اقل PDI (0.054) ، وحجم القطرة الأمثل (٧٥,٩ نانومتر) ، أعلى نسبة نفاذية (٩٩,٨٩٣٣٣ ± ٠,٠١٥٢٧٥٪) ، نسبة عالية من الدواء (٩٩,٨٨٣٣٣٪ ± ٠,٠٢٥١٦٦٪) ، درجة الحموضة pH مقبولة (٥,٩٥٦٦٦٧ ± ٠,٠٢٥١٦٦) ، اعلى توصيل كهربائي (٢٣٠ ± ١ S / cm/) وأسرع تحرير للدواء ٪ (99.58±1.92) بعد 75 دقيقة في المخزن المؤقت للفوسفات (درجة الحموضة ٦,٨) مقارنة مع تركيبات NS الأخرى.
يمكن أن نستنتج أن تحضير LZL NS هو وسيلة فعالة لتحسين نفوذية الLZL من خلال الجلد.

**الكلمات الرئيسية: ليتروزول ، مستحلب نانوي ، النفوذيه وتحرير الدواء في المختبر.**

## Introduction

LZL is one of the most effective aromatase inhibitors present nowadays for management of breast cancer. LZL has gained attention since it has demonstrated high safety and effectiveness profile in comparison to tamoxifen. Its chemical structure (4, 40-[(1H-1, 2, 4-triazol-1-yl) methylene] bis-benzonitrile) as seen in figure 1[1, 2].

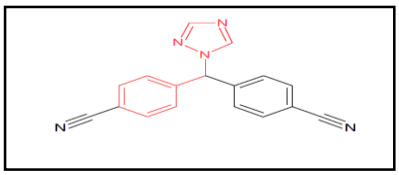

**Figure 1. Chemical structure of LZL[3]**

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LZL practically insoluble in water [3]. To improve the solubility either crystal modification, self-emulsification, particle size reduction, amorphization, or pH modification, are considered to be effective. Moreover, NSs are emulsion of submicron sized that are under extensive investigation act as carriers of drug for enhancing the therapeutic agent delivery. Also NS can be defined as "oil-in-water (o/w) emulsions with mean droplet diameters ranging from 50 to 1000 nm". Depending on the droplet size, it can be divided into groups of milky (up to 500 nm) and the transparent or translucent (50–200 nm) [4].They can exist either as oil in water (o/w) or water in oil (w/o) emulsion forms, where the core of the internal particle is either oil or water, respectively.

The aim of this study was to formulate LZL as NS in order to improve its permeation through the skin.

## Materials and Methods

### *Materials*

Letrozole powder was obtained from Baoji Guokang Bio-Technology Co., Limited, China. Cremophore EL 200 (polyoxyl 35 castor oil), Transcutol®P (Highly purified diethylene glycol monoethyl ether), PEG 200 (Polyethylene Glycol 200) and Span 20(Poly sorbate 20) were bought from Shanghai Ruizheng chemical Tech Co., Ltd, China. Methanol was bought from Sigma-Aldrich, Germany. Oleic acid oil, tween 80(Poly sorbate 80) and Olive oil were supplied from Central Drug House (P) Ltd. (CDH®), New Delhi, India. PEG 400 (Polyethylene Glycol 400) was from SCRC, China. All other solvents and chemicals were of analytical grade.

### *Experimental methods*

### *Solubility screening of excipients*

Excess quantity of LZL was dissolved in 2 ml of different oils, surfactants and co-surfactants individually[4]. The oils that were selected for solubility testing of LZL were: oleic acid, sesame oil, olive oil, peppermint oil, sunflower oil, glycerol mono-oleate (GMO) and corn oil. The surfactants and co-surfactants that were selected for solubility testing were Tween 40, Tween 20, Span 20, Tween 80, Tween 60, Span 80, PEG 200, PEG 300, PEG 400, PEG 600, and transcutol P. Besides, vortex mixer was used for mixing in order to get uniform disperse system [5]. Shaking mixtures in a water bath at 25 ± 0.5 ºC for 72 hrs was then performed. This was followed by 20 min centrifugation at 5000 rpm. A filter syringe of 0.45 µm was used to filter the supernatant. The concentration of drug was determined spectrophotometrically at its $\lambda_{max}$ (240 nm) after dilution of the supernatant with methanol. The unknown concentration of LZL dissolved in certain surfactant or oil was determined by formerly established calibration curve[4].

### *Phase diagrams*

Depending on the LZL solubility and phase diagram result, various components including oil, surfactant and co-surfactant were scanned and NS was formulated with the aim of construct the diagrams. The titration method was employs as follows: mixed the surfactant and co-surfactant completely in ratios of fixed quantity (1:1, 2:1, 3:1) and then Smix were mixed with the oil at ambient temperature. For each ratio of Smix, phase diagram, the oil ratio to the Smix were differed as follow, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. During titration, water had added drop by drop to the oil–Smix combination that was under stirring vigorously, then kept aside for visual assessment for clarity or turbidity[6]. Chemix school software v 7.00 (Arne Standnes , Bergen, Norway) was used to draw these diagrams.

### *Drug loaded NS preparation*

Letrozole required amount was first dissolved in the selected oil, and then followed by the Smix addition. Both of them (the oil and the Smix) were properly mixed by vortex and then eventually titrated with deionized water to get NS. The adding excipients order is extremely crucial to formulate the NS[7].

### *Evaluation of the produced LZL NS*

### *Thermodynamic stability studies*

### *Heating/cooling cycles/studies*

Six cycles between two temperature of 4°C (refrigerator temperature) and temperature 45°C with storage and at each high temperature not less than two days were conducted [8].

### *Centrifugation test*

The NS system was centrifuged for ten minutes at 5000 rpm to determine the signs of phase separation or creaming by examine the formula visually for its appearance [5, 9].

### *Freezing/thawing cycles test*

Deep freezing at −21 ◦C in a freezer (Vest frost, India) and at 25±2 ◦C (room temperature) for 48 h were performed (three cycles each).

### *Measurement of globule size and polydispersity Index*

Scattering of light had been observed at room temperature and at a scattering angle of 90°. The NS (1–1.5 mL) was placed into a cuvette of disposable polystyrene with the micropipette help. The mean droplet size was measured in triplicate. The NS dilution has no effect on their size of globule. The mean polydispersity index and particle size were measured at room temperature by using a Malvern Nanosizer (Malvern Instruments, USA)[10, 11].

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***Measurement of zeta potential***

Zeta potential study was often used as an indicator of the droplet stability in NS. NSs were put into clear and disposable zeta cells and zeta potential that points out the developed NSs surface charge. It was measured by Zetasizer at 25°C [12].

***pH measurement***

The pH of the formulated NS was measured by dipping directly the pH meter electrode into a sample of 10 ml at ambient temperature[12]. All measurements were obtained in triplicates and mean calculated.

***Electro-conductivity measurement***

The NS conductance is determined by a conductometer by immersing its probe in 10 ml of the formula (at 25 ºC) then the instrument measure the conductivity in µS/cm. The apparatus probe composes from two metal electrodes 1 cm apart one from the other. These two electrodes immersed in the NS sample and then a constant voltage was appertained across these electrodes. An electrical current flowed via the aqueous sample only.

***Drug content uniformity***

It was measured as LZL percent found in each formula in comparison with a theoretical amount of the formulation. One ml of the NS was diluted in suitable volume of methanol then mixed and sonicated for complete extraction the drug. Finally centrifuged for 15 min at 3000 rpm to separate and exclude the undissolved excipients. The supernatant was taken and filtered via 0.45µm syringe filter[13]. Then diluted to be analyzed by UV-VIS spectrophotometer at its $\lambda_{max}$ (240 nm).

**Drug content = (measured content / theoretical content) ×100**

***Measurement of Transmittance***

Percent transmittance was made to obtain information regarding the internal phase globule size as well as developed NS stability. The formulated NS percent of this test was calculated at 650 nm wave length using UV–visible spectrophotometer and D.W was used as a blank[14].

***In vitro drug release study***

Using dissolution apparatus type II, a 0.9 L of freshly prepared dissolution medium. The *in vitro* drug release for all the produced NS formulas was performed using dialysis membrane and has pore size of 2.4 nm with approximately 8000-14000 kDa molecular weight (it was treated previously by soaking it into the medium of dissolution for 24 hours only prior to beginning of the test experiment).

The phosphate buffer of 6.8 pH was used as release medium [15].The study was done at 37±1 °C and 50 rpm stirred for 2 hr. The NS involving 2.5mg of LZL was put in the bag. The bag should be closed properly at both end of bag to avoid leakage. A sample of 5 ml of release medium should be drawn at a time interval of 5, 10, 15, 30, 45, 60, 75, 90, 105, 120 min for the NS and 5 ml of fresh medium was added in sake to ensure the sink condition maintenance. These samples were then filtered via syringe filter of 0.45 µm and LZL quantitative estimation at its $\lambda_{max}$ of 240 nm [16, 17].

***Selection of optimum LZL nanoemulsion formula***

Choosing the optimum formula had depended on the results and data of transmittance percent, electro-conductivity, pH, Smix concentration, drug content, and *in vitro* drug dissolution. Basically, the optimum nanoemulsion formula is preferred to have particle size, PDI and zeta potential as <100 nm, <0.3 and more than ±30 nV, respectively[7].

***Morphology evaluations of optimum LZL nanoemulsion***

***Field emission scanning electron microscopy (*FE-SEM*)***

The shape, size and morphology of the surface can be examined by using FE-SEM. A small quantity of NS without dilution was spread above a piece of slab and left to dry. Using platinum to coat the sample, then in all fields the results obtained of particle size of the particles were calculated by using SEM software operated as 5.0kV [18].

***Fourier-transform infrared spectroscopy (FTIR)***

It is important to explore the purity and compatibility of LZL with components of nanoemulsion. Using potassium bromide substance to be packed with the pure drug as a disc (using special cuvette to measure the liquid sample) then setting the range of scanned wavelength to be (400 to 4000 $cm^{-1}$) then registered the shown spectrum data to be analyzed and record if there any incompatibility between the samples or not [19].

***Statistics***

The one way ANOVA test was used to compare results gained from data of our study with 95% confidence interval set for the variation in the mean of samples [20].

## Results

***Solubility screening***

The solubilization of LZL in the oils was with a maximum concentration in oleic oil (76.57 mg/ml) as illustrated in figure 2. Additionally, the value of HLB (hydrophilic/lipophilic balance) is required to be higher than 10 to obtain o/w NS since the greater value leads to faster formation of the oil in water droplets which meaning fast dispersion

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production. According to the solubility study in figure 3, Tween 80 which is a water soluble non-ionic surfactant has a superior solubilizing concentration (132.5577±0.00295mg/ml). It has a good HLB =15 which is essential consideration for o/w NS preparations[21, 22].

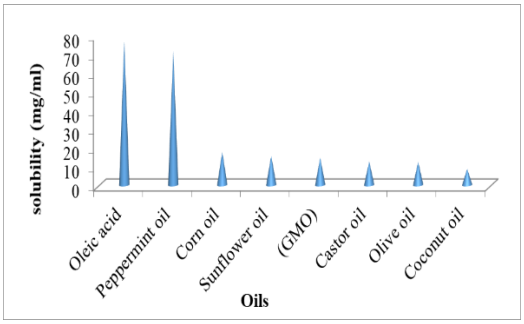


**Figure 2. Chart of solubility results of letrozole in various oils**

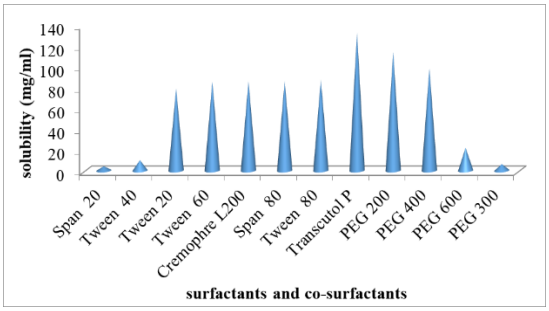


**Figure 3. Letrozole solubility in different surfactants and co-surfactants**

***Phase diagrams construction***

They were created from a mixture of different ratios of oleic acid as oil, tween 80 and transcutol p as surfactant and co-surfactant, respectively. Figure 4 involves the obtained diagrams which were prepared using various Smix; R1: Smix (1:1), R2: Smix (2:1), R3: Smix (3:1) with an existence field of NS (specific coloured area). In general, changing Smix ratios lead to obtain different NS areas. Additionally, when the size of NS field is large that is meaning obtaining the more efficiency of NS system. The NS region has been increased when the concentration of tween 80 increased. Therefore, an increasing the amount of tween 80 which employed as surfactant relative to the amount of transcutol p that used as co-surfactant resulted in improvement the NS region. It seems that the largest size of NS region was reached as Smix of 3:1. There are many studies and debates have presented that the region of NS are basically affected by the Smix ratio as well as the type and concentration of the oil used. Accordingly, Smix 3:1 has associated with high emulsification efficacy which was resulted in diminishing the interfacial tension to the small level and optimum tween 80/transcutol p with forming high flexible coherent film at oleic acid oil/deionized water interface.

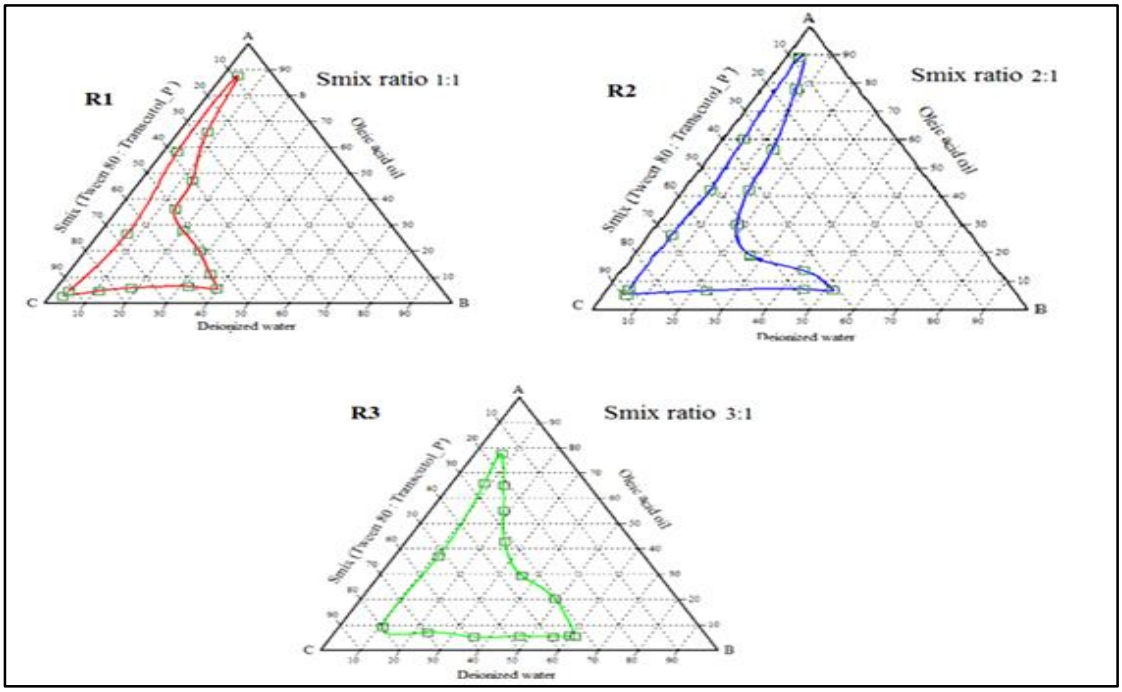


**Figure 1. Pseudo-ternary phase diagrams of the quaternary containing oleic acid / tween 80/ transcutol p® / water as oil/surfactant/co-surfactant/aqueous phase, respectively at different Smix ratios; R1: Smix (1:1), R2: Smix (2:1), R3: Smix (3:1)**

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***Preparation of LZL loading in nanoemulsion according to pseudo-ternary results***

A titration method by low energy process was used to prepare different formulations of LZL loaded NS. Firstly, added 2.5 mg of drug to a volumetric flask and poured a specific amount of oleic acid oil then placed in sonicator to be mixed and dissolved at room temperature for 10 minutes. Secondly, the supposed amount of Smix was added to the prepared mixture and moved the volumetric flask to the vortex mixer. Finally, deionized water was added drop to form the formula. Continues the dropping till obtain clear and transparent liquid formulas. Then, the formulas were moved in tightly closed containers in ambient temperature till apply for further characterization, details of the formulas in table 1[23].

Using Smix (surfactant and co-surfactant) more than 50% would decrease the drug release because high amount of mixture could prevent the dissolution media from getting in contact with the internal phase of the NS as it acts as barrier.

**Table 1**. **Composition of LZL loaded nanoemulsion**

| NS | Oleic acid W/W % | Smix Tween 80: Transcutol P W/W % | Oil: Smix ratio | Smix ratio | Deionized water W/W % |
|---|---|---|---|---|---|
| **NS-1** | 5 | 45 | 1:9 | 1:1 | 50 |
| **NS-2** | 10 | 50 | 2:8 | 1:1 | 40 |
| **NS-3** | 10 | 45 | 3:7 | 1:1 | 45 |
| **NS-4** | 5 | 50 | 1:9 | 2:1 | 45 |
| **NS-5** | 10 | 55 | 2:8 | 2:1 | 35 |
| **NS-6** | 10 | 60 | 3:7 | 2:1 | 30 |
| **NS-7** | 5 | 45 | 1:9 | 3:1 | 50 |
| **NS-8** | 10 | 55 | 2:8 | 3:1 | 35 |
| **NS-9** | 10 | 60 | 3:7 | 3:1 | 30 |

***Evaluation of prepared LZL-NS formulations***

***Thermodynamic stability studies of LZL-NS formulations***

There was no cracking or creaming or even phase separation appeared in tested samples[24, 25]. Therefore, the formulae were examined at different conditions using heating-cooling, centrifugation and freeze-thawing then the samples were taken to be studied for dispersion test as described in table 2.

**Table 2. Thermodynamic Stability Studies and Dispersibility Grades Results of Prepared LZL NSs.**

| NS | H-C/C | CF/T | FT/T | DSG | ET (Sec.) | FR |
|---|---|---|---|---|---|---|
| **NS-1** | √ | √ | √ | A | 33 | Passed |
| **NS-2** | √ | √ | √ | A | 45 | Passed |
| **NS-3** | √ | √ | √ | A | 41 | Passed |
| **NS-4** | √ | √ | √ | A | 20 | Passed |
| **NS-5** | √ | √ | √ | A | 41 | Passed |
| **NS-6** | √ | √ | √ | A | 32 | Passed |
| **NS-7** | √ | √ | √ | A | 20 | Passed |
| **NS-8** | √ | √ | √ | A | 29 | Passed |
| **NS-9** | √ | √ | √ | A | 37 | Passed |

H-C/C= heating-cooling cycles, CF/T= Centrifugation test, FT/T=Freezing-thawing cycles, DTG= dispersibility test grade, ET= emulsification time, FR= final result.

***Particle size distribution and polydispersity index determination fo LZL-NS formulations***

The most valuable consideration to distinguish the microemulsion from NS is to determine its droplet size that should be in the nano range [6]. For that reason, all prepared formulation ought to be examined and should have nano size particle to pass this test. As shown in table 3, it was observed that formula (NS-6) showed the maximum average droplet size (396.8) nm. On the other hand, formula NS-7 has the smallest particle size (75.9 nm). Generally, using tween 80 as a surfactant can extremely reduce the particle size of formulations better than others because of its

low molecular weight (1,310 g/mol). On another hand, to depict the degree of non-uniformity of particle size distribution the term polydispersity. It is a measurement of the distribution of particle size within a prepared formulation. The acceptable value of dispersity ranges from (zero to 1.0); the first range is for a uniform sample while the second is for multiple particle size populations with highly polydispersed sample [26-28]. Generally, equal to 0.2 or below is deemed most acceptable value for nanoparticle formulations since it refers to narrow droplet size distribution. The small polydispersity values lead to better stability against Ostwald ripening (destabilization phenomena)[29, 30]. As shown in table 3, values of polydispersity for LZL-NS formulations are small and within excellent rang (0.054-0.305) and indicates uniformity of droplet size within each formulation.

**Table 3.Results of particle size distribution and PDI for LZL nanoemulsion formulationm.**

| NS | Particle size (nm) | PDI | NS | Particle size (nm) | PDI |
|---|---|---|---|---|---|
| **NS-1** | 105.1 | 0.095 | **NS-6** | 396.8 | 0.305 |
| **NS-2** | 334.5 | 0.301 | **NS-7** | 75.9 | 0.054 |
| **NS-3** | 370 | 0.262 | **NS-8** | 98.3 | 0.085 |
| **NS-4** | 145.7 | 0.204 | **NS-9** | 198.1 | 0.123 |
| **NS-5** | 214.2 | 0.121 | | | |

***Measuring of zeta potential (ζ-potential) of prepared nanoemulsion formulations***

According to the rule of thumb, ζ-potential values more than 30 mV is a good indication of ensuring physical stability of prepared formulation of NS. Table 4 includes the main values of zeta potential scale and the their indications for nanoparticles [31].

**Table 4. Zeta potential (ZP) distribution results of LZL nanoemulsion**

| NS | ZP | NS | ZP |
|---|---|---|---|
| NS-1 | **-39.09** | **NS-6** | **-109.46** |
| NS-2 | **-83.45** | **NS-7** | **-113.76** |
| NS-3 | **-87.47** | **NS-8** | **-70.65** |
| NS-4 | **-90.43** | **NS-9** | **-102.55** |
| NS-5 | **-111.82** | | |

***Measurement of pH of LZL-NS formulations***

The results obtained after dipping the pH meter inside the solution that the formulation are suitable to be used topically since they are between 4.97±0.092 and 5.99±0.091 as presented in table 5. Additionally, there is no significance difference in the pH measurement ($p>0.05$).

**Table 5. Measurement of pH of LZL Nanoemulsions; (mean ± SD);n=3**

| NS | pH | NS | pH |
|---|---|---|---|
| NS-1 | 4.93±0.005 | **NS-6** | 5.19±0.074 |
| NS-2 | 5.28±0.028 | **NS-7** | 5.99±0.091 |
| NS-3 | 4.97±0.092 | **NS-8** | 5.95±0.391 |
| NS-4 | 5.11±0.098 | **NS-9** | 5.83±0.037 |
| NS-5 | 4.91±0.122 | | |

***Measurement of electro-conductivity (σ) of LZL-NS formulations***

The target of this test is to explore the nature of the external phase whether it is aqueous (o/w NSs) or oily (w/o NSs) since the first has highly conducted (more than 10 µS/cm) because of there are more freedom for moving of ions in compared to the latter which has the water in internal or dispersed phase. The data in table 6 involves the results of LZL-NS formulations were measured by conduct meter pen, they have a range from 77 to 229 µS/cm. There is no significant variation ($p>0.05$) in conductivity among LZL-NS formulations.

**Table 6. Electro-conductivity prepared LZL NS; (mean±SD); n=3**

| NS | σ (µS/cm) | NS | σ (µS/cm) |
|---|---|---|---|
| NS-1 | 200±2.081 | **NS-6** | 77±1.008 |
| NS-2 | 130±2.093 | **NS-7** | 229±0.569 |
| NS-3 | 160±3.009 | **NS-8** | 101±2.881 |
| NS-4 | 177±3.921 | **NS-9** | 79±2.987 |
| NS-5 | 121±0.931 | | |

***Drug content measurement***

According to the requirements of USP, the acceptable range of drug content is between (85-%115%)[32]. Table 7 presents the result of this test for LZ-NS formulation and all of them are within the acceptable range. That means the high content uniformity and no signs of precipitation too. Also, there is no significance variation ($p>0.05$) among the samples.

**Table 7. Drug Content Percent Measurement of LZL-NS; (Mean±SD); n=3**

| NS | Drug content %±SD | NS | Drug content %±SD |
|---|---|---|---|
| NS-1 | 98.11±0.019 | **NS-6** | 98.83±3.009 |
| NS-2 | 99.19±0.82 | **NS-7** | 99.89±0.987 |
| NS-3 | 96.81±1.903 | **NS-8** | 99.71±1.009 |
| NS-4 | 97.68±3.491 | **NS-9** | 98.69±0.944 |
| NS-5 | 99.74±3.334 | | |

***Transmittance percent measurement***

The samples are considered as transmit light easily, clear and transparent in nature when the percent transmittance is more than 99% [31].The highest percent value was equal to 99.98% for NS-7 and the lowest transmittance value was found to be 97% for NS-6. It seems from table 8, that the results of transmittance % increase with lessen the globule size of the formulations of LZ-NS. Nevertheless, there is no significant difference among the results of this test ($p>0.05$).

**Table 8. Transmittance percent measurement of LZL-NS formulations; (Mean±SD); n=3.**

| NS | Transmittance % | NS | Transmittance % |
|---|---|---|---|
| NS-1 | 99.64±0.30 | **NS-6** | 98.89±0.09 |
| NS-2 | 98.96±0.04 | **NS-7** | 99.89±0.02 |
| NS-3 | 99.52±0.21 | **NS-8** | 99.74±0.03 |
| NS-4 | 99.30±0.22 | **NS-9** | 99.35±0.30 |
| NS-5 | 99.70±0.12 | | |

***In vitro* drug release study**

The dissolution of LZL from the prepared NS samples is shown in figures 5, 6 and 7 in 6.8 pH media. The shortest dissolution time was 30 min for NS-4 which was 100.42±2.16%. It is clear that might be due to improved solubility of LZL in the oleic acid, existence of tween 80 and in particular, small particle size of molecules of NS. Generally, the small droplet size with a large surface area promotes rapid release of the drug from the formulations [9].

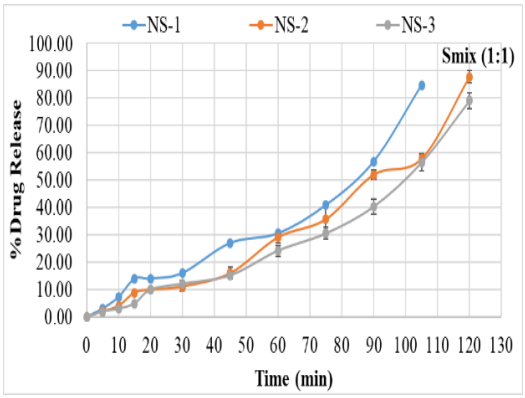


**Figure 5. A dissolution profile of LZL NSs (NS-1, NS-2 and NS-3) in 900 ml of 6.8 pH dissolution medium at 37 °C, all the results represent mean ± SD, where n=3**

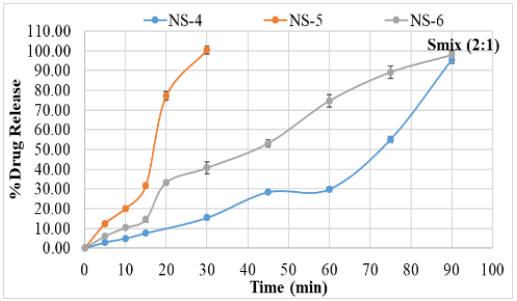


**Figure 6. A dissolution profile of LZL NSs (NS-4, NS-5 and NS-6) in 6.8 phosphate buffer pH at 37 °C, all the results represent mean ± SD, where n=3.**

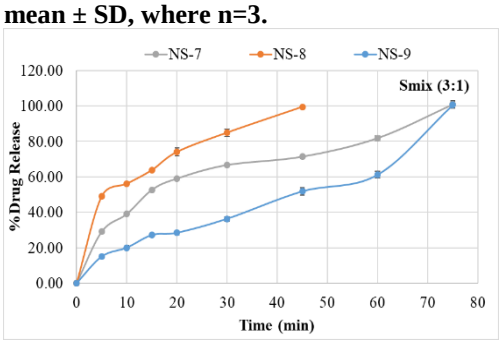


**Figure 7. A dissolution profile of LZL NSs (NS-7, NS-8 and NS-9) in 900 ml of 6.8 pH dissolution medium at 37 °C, all the results represent mean ± SD, where n=3.**

***Selection of optimum LZL nanoemulsion formula***

Many factors should be considered to select the optimum formula and NS-7 was chosen as an optimum prepared NS formulation for many reasons. Initially, it has lower PDI (0.054) and optimum droplet size (75.9 nm) as seen in figure 8 and zeta potential (-113.76) as seen figure 9, highest transmittance percent (99.89±0.02%), high drug content (99.88±0.03), pH (5.96±0.03) and optimum drug release (99.58±1.92) after 75 min compared to other NS samples.


**Figure 8. Globule size distribution and PDI of NS-7**

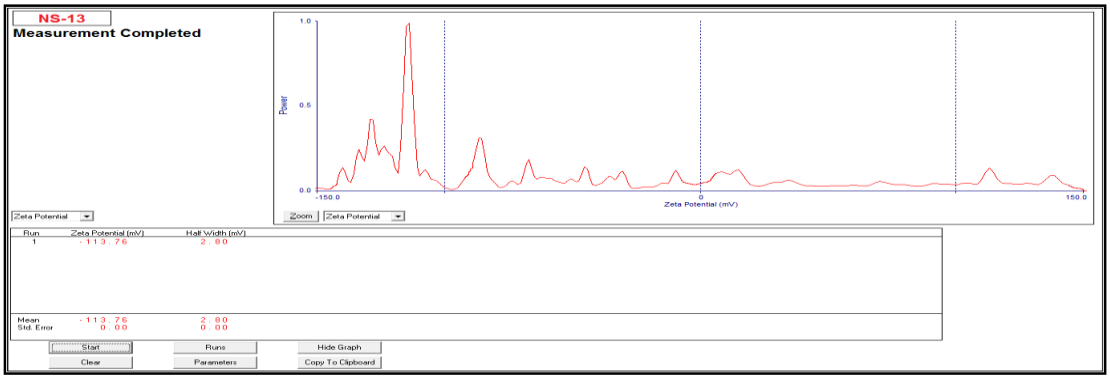


**Figure 9. Zeta potential of optimum NS-7**

***Morphology examination of optimum LZL NS-7 nanoemulsion formula***

Microscopic examinations are fundamental method to obtain actual range of data regarding the morphology of optimum NS formulation.

***Fourier transforms infrared spectroscopy (FTIR)***

There was no significant variation on position and shape of the obtained peaks between the pure drug and optimum formula diagrams (figure 10 and 11). LZL pure powder in figure 10 illustrated major peaks at 3010 $cm^{-1}$ for $sp^2$ CH stretching 2250 $cm^{-1}$ for C≡N stretching and 690-900 $cm^{-1}$ for out-of-plane CH bending (as shown in table 9). No significant difference in shape and position of the absorption peaks of drug has been observed between the spectra.

**Table 1. The FT-IR characteristic Functional Groups of Materials**

| Materials | Functional group | Reference peaks ($cm^{-1}$) | Result peaks ($cm^{-1}$) |
|---|---|---|---|
| **Letrozole** | C≡N stretching | 2250 | 2229 |
| | sp2 CH stretching | 3010 | 3055 |
| | out-of-plane CH bending | 690 - 900 | 790 |
| **Oleic acid oil** | O-H bond | 2674 - 3006 | 2524 |
| | (-CH2-) asymmetric and the symmetric stretching | 2925 - 2856 | 2854 |
| | C=O stretching | 1714 | 1706 |
| | C-O elongation | 1284 | 1284 |
| | C-O-H bond | 1412 | 1415 |
| | plan of O-H bond | 939 | 949 |
| **Tween 80** | (–CH3) | 2920 | 2904 |
| | –CH2-stretching | 2864 | 2858 |
| | C=O | 1735 | 1735 |
| | stretching of C–O–C | 1095 | 1090 |

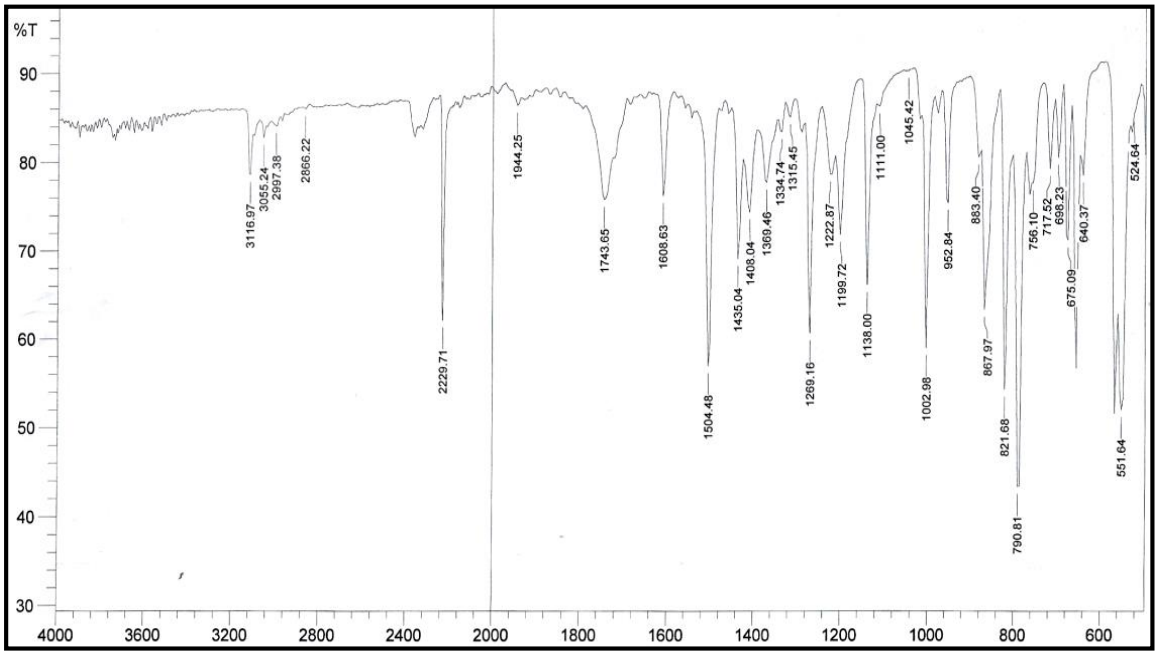


**Figure 10. the FT-IR spectrum of pure LZL powder.**

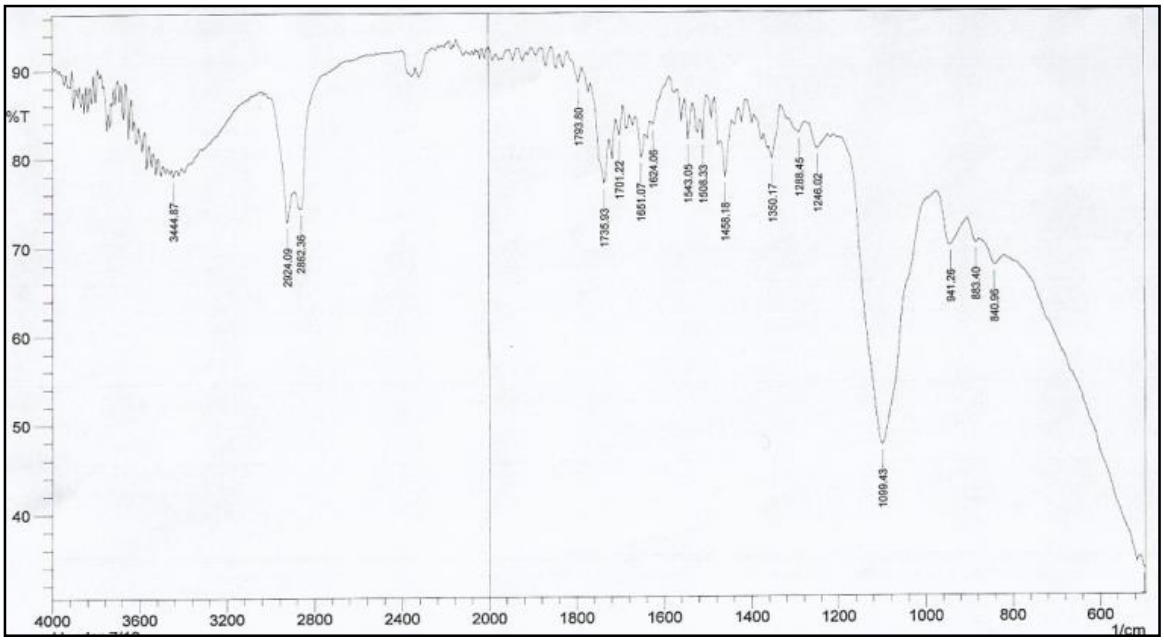


**Figure 11.FT-IR spectrum of optimum formula NS-7**

***Field emission scanning electron microscopy (***FE-SEM***)***

FE-SEM is microscopy examined which can approve the particle size of optimum formula NS-7. As it clear from figure 12 the microscopy could investigate the nano sized particles of NS-7 formulation. The average range is 43.7-79.23 nm with spherical and not adherent shape.

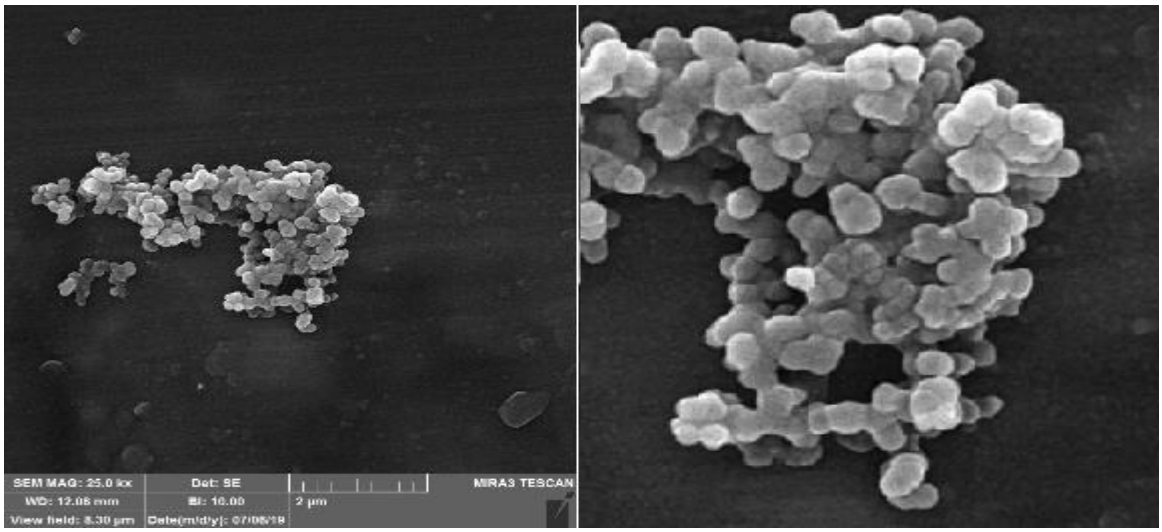


**Figure 12. FE-SEM of optimum NS-7 formulation**

## Conclusion

NSs is a promising new system that can improve the permeation of drugs through skin by using low energy of emulsification method. Oleic acid, tween 80 and transcutol p were chosen as oil, surfactant, and co-surfactant, respectively. One can conclude that preparation of LZL NS is an effective method for improving the permeation.

In future, choose a suitable gelling agent to increase the viscosity of NS to be more adhesive and studying their properties and evaluations. Also, a deeper understanding and studying is necessary in order to develop the topical LZL NS in pharmaceutical applications by undergoing to *in vivo* test to assess the clinical performance of the formulated dosage form

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