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# FORMULATION AND EVALUATION OF SEABUCKTHORN OIL ENTRAPPED NLC

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**ABSTRACT**

The Seabuckthorn oil *(hippophae rhamnoides*) is nutraceutical lipophilic drug. The reason for choosing this drug for the study lies in its abundant supply of polyunsaturated fatty acids (PUFA), antioxidants, flavonoids, phytosterols, carotenoids, and vitamins C, E, and K. These valuable components offer considerable benefits for treating a wide range of skin conditions. The literature review suggests that the melt-emulsification technique has been used for Seabuckthorn oil entrapped NLC. However, there have been no reports so far on the application of the microemulsion method for creating Seabuckthorn enriched NLCs. Hence an attempt was made to prepare Seabuckthorn oil entrapped NLC by microemulsion method. The aim of present research work was to formulate, evaluate and optimize the topical gel of Seabuckthorn oil enriched NLC. The preformulation studies were conducted on physical characteriscs (colour, odour, consistency), characterization tests (acid value, saponification value, peroxide value and iodine value), UV visible spectroscopy, FTIR spectroscopy of Seabuckthorn oil. In the current research, the preparation of NLCs involved conducting a screening of excipients, and the composition of these excipients was determined using a pseudoternary phase diagram. The Quality by Design (Qbd) principle was applied during the NLC preparation process, and a $3^2$ factorial design was employed to optimize the formulation.The study aimed to achieve specific characteristics in NLCs, and to do so, the key parameters of total lipid content and Smix concentration were optimized using design expert software and assessed for % Entrapment efficiency, Particle Size, and Polydispersity Index.The optimal combination of 20% total lipid and 25% Smix resulted in NLCs with the minimum size of 308.9 nm, high entrapment efficiency (97.88%) and a narrow polydispersity index (0.320).The obtained optimized NLC formulation was subjected to various evaluations, including physical appearance, % drug loading capacity, viscosity, pH, zeta potential, and Scanning Electron Microscopy. Additionally, a gel version of the selected optimized NLC formulation was developed for topical application. The gel was then assessed for several parameters, including homogeneity, viscosity, pH, percent drug content, spreadability, extrudability and In vitro drug diffusion. From the results it was concluded that the Seabuckthorn oil was successfully loaded in to NLC by microemulsion technique and incorporated in to gel for topical purpose.

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## INTRODUCTION

Seabuckthorn is a deciduous shrub or small tree found in the genus *Hippophae*, belongs to the *Elaeagnaceae* family [1]. It is indigenous to the mountainous areas of Europe and Asia and is notable for its vibrant orange berries and medicinal qualities [2,3]. Numerous studies have reported various pharmacological benefits associated with this plant, including antimicrobial, anti-inflammatory, anti-psoriatic, antioxidant, anticancer, hepatoprotective, immunomodulatory, and anti-platelet activities. Many authors have documented these diverse pharmacological properties. Few authors have reported the Naonostructured lipid carrier by meltemulsification technique [3]. However, the NLC by microemulsion technique have not been reported so far. Therefore, the attempt was made to prepare NLC of Seabuckthorn oil by microemulsion technique. NLCs are prepared by blending liquid lipids and solid lipids, mixture of surfactant and cosurfactant [4]. Literature survey pertains to Seabuckthorn oil enriched lipid carrier formulated by meltemulsification. This drug was selected for the study because it has a good source of polyunsaturated fatty acids (PUFA), antioxidant, flavonoids, phytosterols, carotenoids, and vitamin C, E and K which is beneficial for various skin diseases. The aim of present research work was to formulate, evaluate and optimize the Seabuckthorn oil entrapped NLC and incorporate in to topical gel. In present investigation Seabuckthorn oil enriched NLC was prepared by microemulsion technique which was found to be simple and economic. For the preparation of NLC screening of excipients was done on the basis of saturated solubility method and composition was identified by the construction of pseudoternary phase diagram. For optimization the principle of QbD was applied. $3^2$ full factorial design was adopted and evaluated for %EE, PDI and particle size. In $3^2$ factorial design total lipid content Smix were selected as independent variables with 3 levels (-1, 0, +1), and %EE, PDI and particle size were selected as dependent variables. The obtained optimized batch was further evaluated for its % drug loading capacity, pH, viscosity, zetapotential, FTIR and SEM studies. For the topical purpose the optimized batch was incorporated in to gel and evaluated for its physical appearance, %drug content, pH, viscosity, extrudability, spreadability and In vitro drug diffusion studies.

## MATERIALS AND METHODS

### Materials:

Seabuckthorn oil was purchased from shoprythymstore. Compritol 888 ATO, Labrafil 1994 CS, Labrafil 2125 CS, Labarasol, Transcutol P, Gelucire 50/30 and Capryol 90 were obtained as a gift sample from Gattafose India Pvt. Ltd, Mumbai. The chemical reagents utilised were all of analytical grade, and all additional substances were of pharmaceutical grade.

### Preformulation Studies

**Acid value**

The acid value is a measurement that represents the quantity, in milligrams, of potassium hydroxide required to neutralize the unbound acids found in one gram of the substance.

**Iodine value**

The iodine value is a numerical value that indicates the amount of halogen, specifically iodine, absorbed by 100 grams of the substance under specific conditions.

**Peroxide value**

The peroxide value represents the quantity of active oxygen, measured in milliequivalents, that indicates the peroxide content present in 1000 grams of the substance.

**Saponification value**

The saponification value is a measurement in milligrams of potassium hydroxide required to neutralize the unbound acids and saponify the esters found in 1 gram of the substance.

### Excipients screening and selection

**Screening of solid lipid and selection**

Solid lipids (Compritol 888 ATO, glyceryl monostearate, Gelucire 50/13, Stearic acid, PEG 4000 and Myristic acid) were screened using the principle of phase separation. Fixed amounts of Seabuckthorn oil and solid lipid were combined in a ratio of 1:1 and melted above the melting point of solid lipid. The physical combination was visually inspected every 15 minutes for the first hour, and then after 24 hours to see whether the two lipids were miscible when melted and after a full 24-hour period of recrystallization of the solid lipids [6].

**Screening and selection of liquid lipids**

Liquid lipids (Sunflower oil, Capryol 90, Eucalyptus oil, Castor oil, Isopropyl myristate, Clove oil, Oleic acid, Labrasol, Labrafil 2125CS, Labrafil 1994CS, Olive oil, Groundnut oil and Transcutol P. The screening was done by saturated solubility method. By dissolving an excess amount of the drug in 2 mL oils, the solubility of the drug (Seabuckthorn oil) in various liquid lipids, surfactants and cosurfactants was investigated. This system underwent continuous vortexing after 24 hours at room temperature. Each sample underwent centrifuged after reaching equilibrium solubility, and the supernatant was collected. Using a calibration line equation ($y = 0.8707x - 0.0141$), the solubility of an aliquot of supernatant that had been diluted with hexane was determined [7].

**Screening and selection of surfactants**

Surfactants (Tween 80, Cremophore EL, Cremophore RH 40, Tween 20 and Span 80) are used for screening. The screening was done by saturated solubility method. By dissolving an excess amount of the drug in 2 mL surfactants, the solubility of the drug (Seabuckthorn oil) in various surfactants was investigated. This system underwent continuous vortexing after 24 hours at room temperature. Each sample underwent centrifuged after reaching equilibrium solubility, and the supernatant was collected. Using a calibration line equation (y = 0.8707x - 0.0141), the solubility of an aliquot of supernatant that had been diluted with hexane was determined [7].

**Screening and selection of cosurfactants**

Cosurfactants (Capmul MCM C8, Soylecithin, Polyethylene glycol, Propylene glycol) are used for screening. The screening was done by saturated solubility method. By dissolving an excess amount of the drug in 2 mL pf cosurfactants, the solubility of the drug (Seabuckthorn oil) in various cosurfactants was investigated. This system underwent continuous vortexing after 24 hours at room temperature. Each sample underwent centrifuged after reaching equilibrium solubility, and the supernatant was collected. Using a calibration line equation (y = 0.8707x - 0.0141), the solubility of an aliquot of supernatant that had been diluted with hexane was determined [7].

**Screening of binary lipid combination and selection**

In the compatibility investigation, the binary mixture of solid lipid (Compritol 888 ATO) and liquid lipid (Capryol 90) with the highest solubility for Seabuckthorn oil was used. The solid lipid was heated to their melting point in order to optimize their capacity for solubilization. The liquid lipids were then combined with the solid lipids that had been liquified in a variety of ratios, including 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. Using a magnetic stirrer, these mixtures were swirled for an hour at 75° C (at 200 rpm). The mixture was applied on Whatman filter paper after cooling to test its compatibility with a binary blend. Visible light was used to look for any oil droplets. oil droplets that formed on the filter paper indicated the presence of both solid and liquid lipids were not compatible [8].

**Construction of pseudoternary phase diagram**

By creating pseudo-ternary phase diagrams, the area of the microemulsion existence was identified. Lipids, surfactants, co-surfactants, and aqueous phase were utilised to design the phase diagram based on the solubility studies of the medications. With the same ratio of surfactant to cosurfactant (Smix), i.e, (1:1), different concentrations of oil to Smix were created in different glass vials in the ratios of 1:9 to 9:1 (1:9, 2:8, 3:7, 4:6, 5.0:5.0, 6:4, 7:3, 8:2, and 9:1) which is mentioned Table 1. Additionally, the solid-to-liquid lipid ratio (7:3) for each formulation was maintained. Aqueous titration approach was used to construct a pseudo-ternary phase diagram. Each weight ratio of oil and Smix is slowly titrated with an aqueous phase. Drops of water were added while using a magnetic stirrer (Spectralab, Whirlmatic, India) to continuously agitate the mixture until a uniform dispersion or solution was achieved. After each addition, the system's look and flow characteristics were checked. The point at which the solution became turbid served as the titration's endpoint. It was recorded how much aqueous phase was needed to reach the turbidity point. The physical state of the microemulsion was marked on a pseudo-three-component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and co-surfactant. The pseudoternary phase diagram was constructed in chemix school software [9,10], which is mentioned in Figure 1. Three compositions i.e, (A, B and C) were selected from the enclosed microemulsion region in the pseudo ternary phase diagram which is mentioned in the Table 2 and % entrapment efficiency and % drug loading capacity were determined for each formulation. Thus, point B which is highlighted in pseudoternary showed high % entrapment efficiency (98.97) and % drug loading capacity (51.07) which is mentioned in the Table 3. The formulation B containing highest % entrapment efficiency and % drug loading capacity was selected for further analysis.

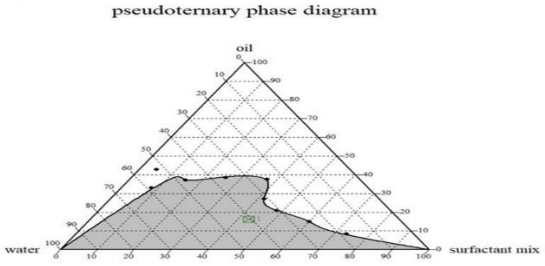



**Figure 1 Pseudoternary phase diagram.**

**Table 1 Formulations containing different ratios of Oil:Smix (1:9 to 1:9)**

| Ratios (oil: Smix) | Oil phase (solid lipid: liquid lipid) (g) | Smix (g) |
|---|---|---|
| 1:9 | 0.45 | 4.05 |
| 2:8 | 0.9 | 3.6 |
| 3:7 | 1.35 | 3.15 |
| 4:6 | 1.8 | 2.7 |
| 5:5 | 2.25 | 2.25 |
| 6:4 | 2.7 | 1.8 |
| 7:3 | 3.15 | 1.35 |
| 8:2 | 3.6 | 0.9 |
| 9:1 | 4.05 | 0.45 |

**Table 2 Compositions of excipients.**

| Excipients | A | B | C |
|---|---|---|---|
| Oil (%) | 16.4 | 21.8 | 23.1 |
| Smix(%) | 43.8 | 25.1 | 34 |
| Wter (%) | 39.8 | 53.1 | 43 |

**Table 3 % Entrapment efficiency and % Drug loading capacity.**

| Formulations | % Entrapement efficiency | % Drug loading capacity |
|---|---|---|
| A | 98.89 | 51.02 |
| B | 98.97 | 51.07 |
| C | 98.96 | 51.06 |

**Development of NLC by microemulsion technique**

A Nanostructure lipid carrier containing Seabuckthorn oil was created using the microemulsion method. The Compritol 888 ATO was melted above its melting temperature, and Seabuckthorn oil was dissolved in Capryol 90. After that, surfactant mix (i.e., Tween 80 and Capmul MCM) was added, and then distilled water was added dropwise into the lipid mix under constant stirring on a magnetic stirrer to form the homogenous mixture at 300 rpm, the endpoint is transparent solution turns turbid. Then the solution was stirred for 20 min on ultra turrax homogenizer at 15000 rpm and sonicated for 30 minutes. The microemulsion is then poured into a cold aqueous solution (2–10°C) under gentle mechanical mixing [11,12]. The composition of excipients used for the preparation of NLC were mentioned in Table 1.

**Full factorial ($3^2$) design for optimization**

Utilising experimental design (full factorial), for $3^2$ factorial design the variables affecting the NLCs' quality aspects were optimised. As input factors, lipid concentration (F1, 15-25%) and surfactant concentration (F2, 20-30%), whereas the response parameters were % entrapment effectiveness (EE, R1), particle size (R2) and PDI (Polydispersity index, R3) [13], which is mentioned in Table 4.

**Table 4 $3^2$ factorial design by design expert software.**

| | | Factor 1 (A:A) | Factor 2 (B:B) | Response | Response 2 | Response 3 |
|---|---|---|---|---|---|---|
| Std | Run | Total Lipid (%) | Smix (%) | % Entrapment efficiency | Particle size (nm) | Poly dispersibility Index |
| 6 | 1 | 25 | 25 | 98.85 | 409.7 | 0.43 |
| 7 | 2 | 15 | 30 | 98.8 | 724.5 | 0.579 |
| 5 | 3 | 25 | 30 | 98.83 | 608.9 | 0.453 |
| 2 | 4 | 20 | 20 | 98.81 | 657.7 | 0.554 |
| 8 | 5 | 20 | 30 | 98.84 | 452.5 | 0.44 |
| 3 | 6 | 25 | 20 | 98.86 | 392.4 | 0.369 |
| 9 | 7 | 20 | 25 | 98.87 | 308.4 | 0.32 |
| 1 | 8 | 15 | 20 | 98.77 | 2175.6 | 0.774 |
| 4 | 9 | 15 | 25 | 98.79 | 1343.9 | 0.6 |

The obtained optimized batch no 7 was selected for evaluation tests and incorporated in to gel. It shows the highest % EE i.e, 98.87%, lowest particle size i.e, 308.4 nm and PDI value of 0.320.

**Preperation of NLC based gel**

In brief, (1% w/v) of gelling agent Carbopol 934 was dispersed in distilled water and allowed to hydrate for 4 to 5 hours, 0.5% of methyl paraben was also added as a preservative. Glycerin (10% w/v) was added stirred for 10 min at 1500 rpm, the 5% of NLC equivalent to 0.25g of Seabuckthorn oil has been then incorporated into the gel and stirred at 1200 rpm for 2 hours. The dispersion was neutralized with few drops of triethanolamine (TEA), sufficient amount of water has been added for constructing final weight of gel up to 100% [14].

**EVALUATION TESTS**

**Evaluation parameters of NLC**

**Determination of λmax and preparation of calibration by UV Spectroscopy**

For the determination of λmax and calibration curve the standard stock solution solution of 1000 µg/mL of Seabuckthorn oil was prepared in hexane. From the stock solution of 1000 µg/mL concentration and then diluting it to different concentrations to prepare 6 calibration standards of 6 series. All standards were prepared in volumetric flasks. Concentrations of the standards were blank, 2, 4, 6, 8, 10 µg/mL and absorbances were measured spectrophotometrically at λmax=233nm using the UV spectrophotometer [15]. The λmax and calibration curve of Seabuckthorn oil were mentioned in Figures 5 and 6.

**FTIR Analysis**

For compatibility study IR spectrum of pure drugand NLC was measured by Fourier Transform Infrared (FTIR) spectrometer using KBr pellets technique at room temperature. In this technique, samples were mixed with KBr and compressed to form a thin pellet that was used for testing. The measurements were recorded in the frequency range of 4000–400 cm−1 [16]. The FTIR spectra of pure Seabuckthorn oil, and the NLC formulation shown in Figures 7 and 8.

**Physical appearance**

The optimized NLC was evaluated for its colour, consistency and homogeneity.

**Drug Entrapment efficiency and drug loading capacity**

The entrapment efficiency (EE, % w/w) was determined by centrifugation method. Briefly, from freshly prepared NLC, 0.5g of NLC was withdrawn and diluted with hexane and it was subjected to centrifugation for 20 min at 4000 rpm, 25°C. After centrifugation, the supernatant was collected and analyzed spectrophotometrically and absorbance were recorded at 233 nm, respectively [17]. The formula for entrapment efficiency and drug loading capacity is given below.

$$\%EE = \frac{\text{(actual weight of drug-weight of unbound drug)}}{\text{(actual weight of drug)}} 100$$

$$\%DL = \frac{\text{(actual weight of drug-weight of unbound drug)}}{\text{(actual weight of drug) + (weight of other excipients)}} 100$$

**Zetapotential, Particle size and Polydispersibility index**

Particle size refers to the dimensions of individual particles in a sample, typically expressed as a diameter. Polydispersity index (PDI) quantifies the degree of variation or heterogeneity in the size distribution of particles within a sample. A PDI value closer to zero indicates a narrow size distribution with minimal variation, while higher values indicate a broader and more heterogeneous distribution. Particle size and PDI is performed using dynamic light scattering (DLS) by diluting the sample. Which is a measure of the magnitude of electrostatic potential or particles' surface charge. The zeta potential is an important parameter in determining the stability of a dispersion system. Particles with zeta potential values higher than (±) 30 mV indicate a stable dispersion system because they have a surface charge that can prevent aggregation [18]. The results of zetapotential, particle size and PDI were mentioned in Figures 9 and 10.

**Viscosity**

The viscosity of freshly prepared NLC was determined as such without dilution using Brookfield Viscometer (model cap 2000+). Spindle no 1 was used for the measurement of viscosity. The speed of the spindle was set at 5 RPM. The holding time and run time was set at 5sec and 33sec respectively at a temperature of 25°C. Viscosity measurement were taken three times in triplicate [18].

**pH**

For the determination of pH, a digital pH meter (model 802, systronics) was used. Before measurements, the pH meter was calibrated by using pH 4.0 and pH 7.0. An accurately weighed quantity of sample was dispersed in 20 ml of purified distilled water in a separate beaker, in which the electrode was dipped in a manner do not touch the bottom of the beaker [19].

**Scanning Electron Microscopy (SEM)**

The Optimized NLC formulation was analyzed by Scanning Electron Microscopy studies (SEM). Analysis was performed at 25$^{o}$C. The NLC dispersion was diluted appropriately. Few drops of the dispersion were placed on the slide, the slide was then attached to aluminium stub using double sided carbon tape and allowed to dry the sample at room temprature. After the samples were dried thoroughly, the sample was coated with gold layer using a sputter coating under a vacuum of 10 Pascle for 10 sec. achieving a thickness of 10 A$^{o}$ and the image was captured at desired magnification [20]. Thes results of SEM studies were mentioned in Figure 11.

**Evaluation parameters of NLC based gel**

**Physical evaluation, viscosity and pH**

The prepared gel were evaluated for its homogeneity, colour, consistency. The same procedure was adopted for viscosity and pH which is mentioned in evaluation parameters of NLC.

**Drug content estimation**

Accurately weighed 1 g of the gel transferred to the 100 ml of volumetric flask containing 20 ml of phosphate buffer pH 7.4. The volumetric flask was shaken for 30 min and the volume was made up to 100 ml with phosphate buffer pH 7.4 solution. After suitable dilution, the sample was filtered through Whatman filter paper. A volume 0.1 ml from the filtrate was pipet out and diluted to 10 ml with phosphate buffer pH 7.4. The concentration of active constituent analyzed at 233 nm by using UV- visible spectrophotometer [19].

**Spreadability**

After 24 hours of gel manufacture, the spreadability of the gels was assessed by measuring the diameter of the spread of 1 gramme of gel that was placed between two horizontal plates and measured after 1 minute (20 20 cm2). The upper plate has always been fastened with a set weight of 220 gm. The equation below has been used to assess the spreadability[14].

Spreadability = M * L/ T

**Extrudability**

In this study, extrudability was evaluated by determining the weight (in grams) required to extrude at least 0.5 cm of gel from a collapsible tube made of lacquered aluminum in 10 seconds. After that, the extrudability was calculated using the formula below [21].

$$\text{Extrudability} = \frac{\text{Applied weight to extrude gel from tube (in gram)}}{\text{Area (in cm}^2\text{)}}$$

**Invitro drug diffusion study**

The in-vitro drug diffusion study for formulation was carried out using a Franz diffusion cell with a cellophane membrane as the diffusion membrane. The glass tube, which serves as the donor compartment, is sealed at one end and covered with a cellophane membrane that has been pre-soaked in the diffusion medium, specifically phosphate buffer at pH 7.4. The entire setup, including the cell, is maintained at a constant temperature of 37$^{o}$C. A magnetic stirrer is used to continuously agitate the solution with a magnetic bead. Over a period of 6 hours, 1 ml samples are extracted from the diffusion medium at precise time intervals and replaced with an equal volume of fresh, pre-warmed diffusion medium. The collected samples are then analyzed spectrophotometrically at a wavelength of 233 nm to determine the cumulative percentage of drug release [19].

**RESULTS AND DISCUSSIONS**

**Excipients screening and selection**

**Solid lipid screening and selection**

After 24 hrs each physical mixture were evaluated on the basis of phase separation. The homogenous mixture was obtained for the drug with Compritol 888 ATO which indicates the drug was soluble in that solid lipid. The other mixture shows the phase separation, which indicates the drug is insoluble in that solid lipid used. The solubility of drug in solid lipid affects the drug entrapment efficiency, drug loading capacity, release of drug and stability. On the basis of phase separation method Compritol 888 ATO was selected as solid lipid for NLC formulation.

**Liquid lipid screening and selection**

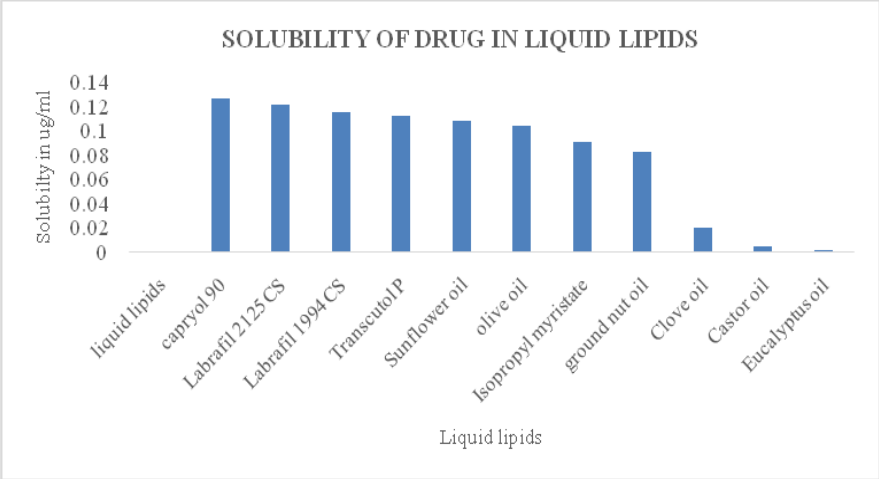



**Figure 2 Screening of liquid lipids.**

The highest solubility was shown by Capryol 90. Hence on the basis of above results Capryol 90 was selected as liquid lipid for NLC formulation.

**Surfactants screening and selection**

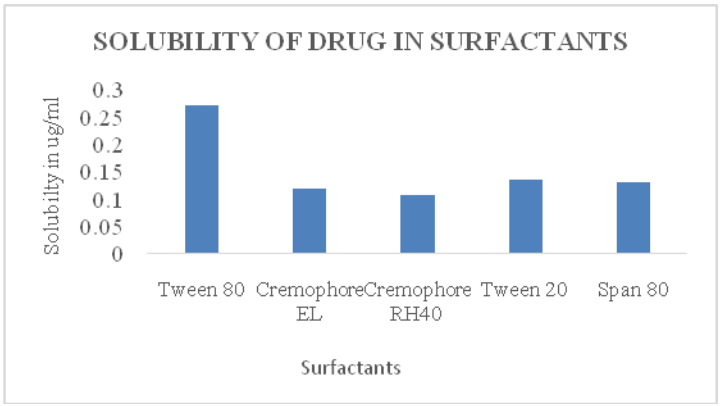



**Figure 3 Screening of surfactants.**

The highest solubility was shown by Tween 80. Hence on the basis of above results Tween 80 was selected as surfactant for NLC formulation.

**Cosurfactants screening and selection**

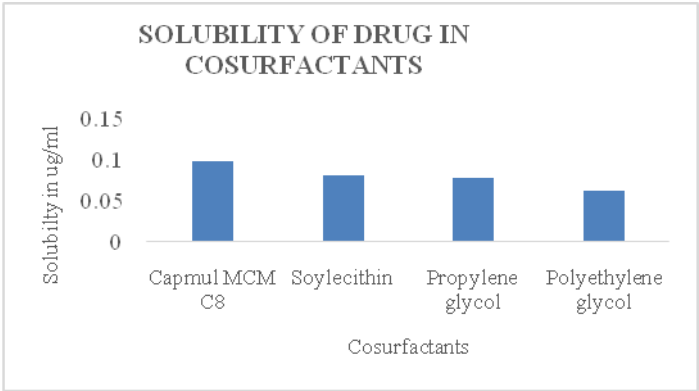



**Figure 4 Screening of cosurfactants.**

The highest solubility was shown by Capmul MCM C8. Hence on the basis of above results Capmul MCM C8 was selected as cosurfactant for NLC formulation.

**Screening of binary lipid combination and selection**

From the different combination of solid lipid: liquid lipid 7:3, 8:2 and 9:1 does not show any oil droplets on Whatman filter paper, which indicated the better compatibility. From these three combination the 7:3 ratio of solid lipid: liquid lipid was selected for NLC formulation.

**Evaluation parameters of NLC**

**Preformulation studies, determination of λmax and preparation of calibration by UV Spectroscopy**

**Table 5 Physical characteristics of Seabuckthorn oil.**

| Sr no | Tests | Results |
|---|---|---|
| 1. | Acid value | 8.976 |
| 2. | lodine Value | 70.701 |
| 3. | Peroxide Value | 16 |
| 4. | Saponification Value | 182.325 |

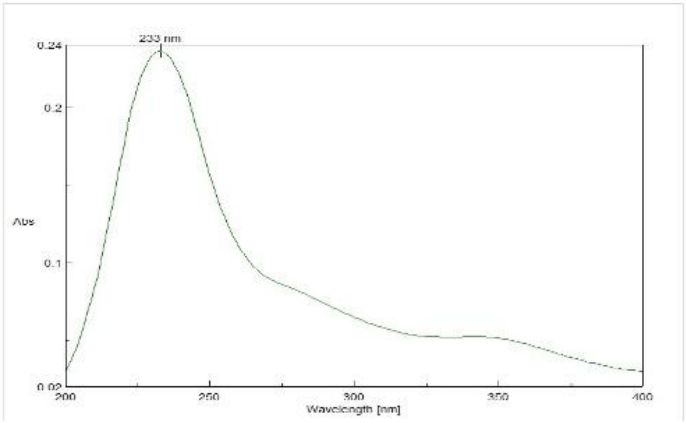



**Figure 5λmax of Seabuckthorn oil.**

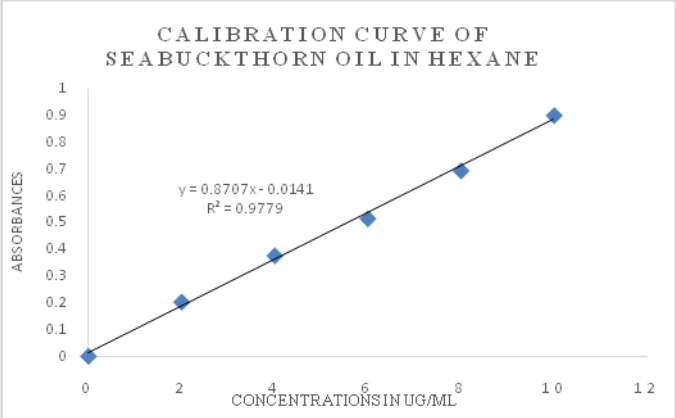



**Figure 6 Calibration curve of Seabuckthorn oil**

**FTIR Analysis**

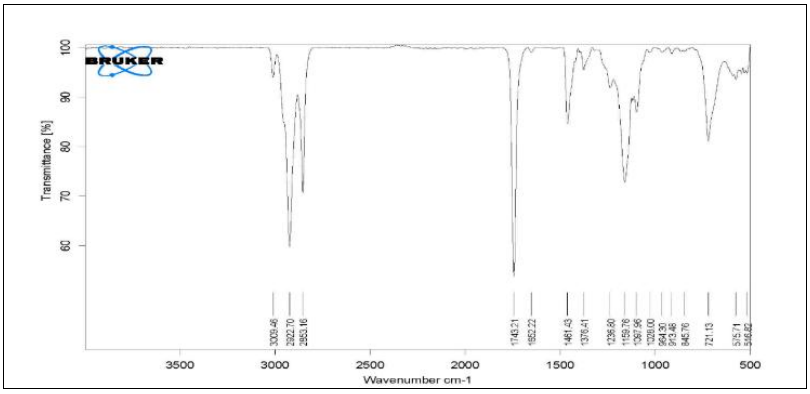



**Figure 7 FTIR spectrum of Seabuckthorn oil.**

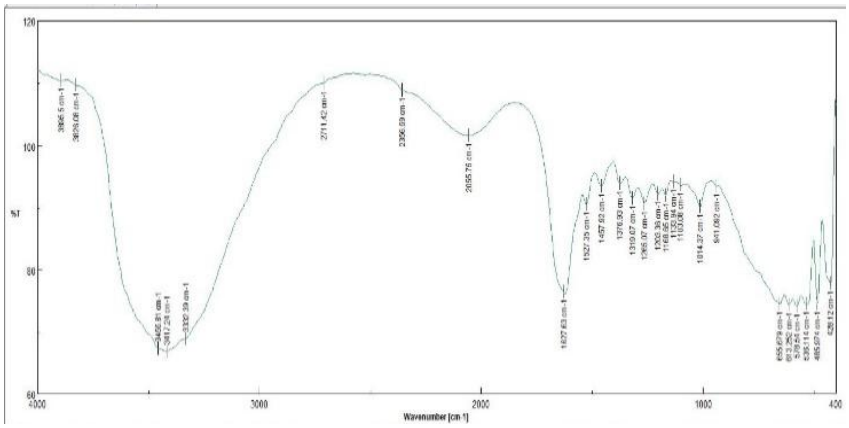



**Figure 8 FTIR spectrum of optimized NLC formulation.**

It was inferred that the preferred functional group frequencies of Seabuckthorn oil were reproducible in NLC but the intensity was changed which may be due to the deep encapsulation of lipid over drug.

**Physical appearance**

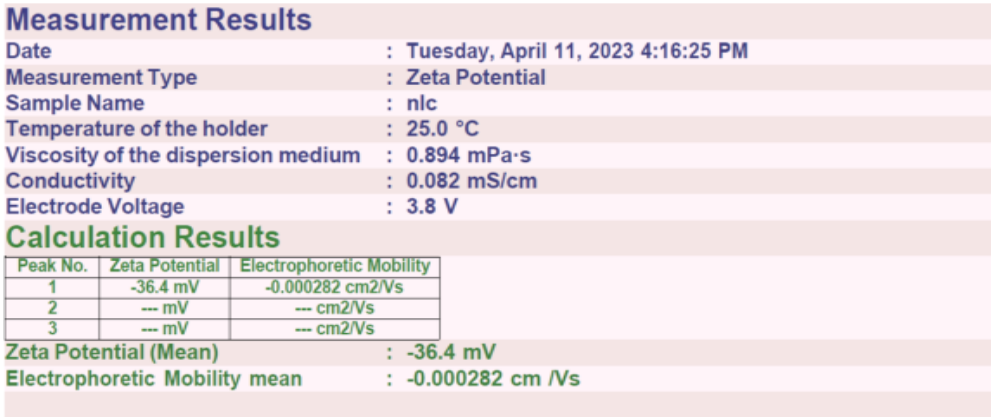
The formulation was found to be white in colour, liquid in consistency and homogenous with no phase separation.

**Entrapment efficiency and drug loading capacity**

As shown in the Table 4 the % Entrapment efficiency of all formulations was found to be in the range of 98.77% to 98.87%.The highest entrapment efficiency was shown by formulation 7 i.e, 98.87% with a drug loading capacity 49.09%. It was concluded study that % EE increases significantly by increasing the total lipid and Smix.

**Zetapotential, particle size and polydispersibility index**

The optimized formulation shows the ZP value at -36.4 mV which indicates the dispersion system is stable, which is mentioned in Figure 9.



**Measurement Results**

| | | |
|---|---|---|
| Date | : | Tuesday, April 11, 2023 4:16:25 PM |
| Measurement Type | : | Zeta Potential |
| Sample Name | : | nlc |
| Temperature of the holder | : | 25.0 °C |
| Viscosity of the dispersion medium | : | 0.894 mPa·s |
| Conductivity | : | 0.082 mS/cm |
| Electrode Voltage | : | 3.8 V |

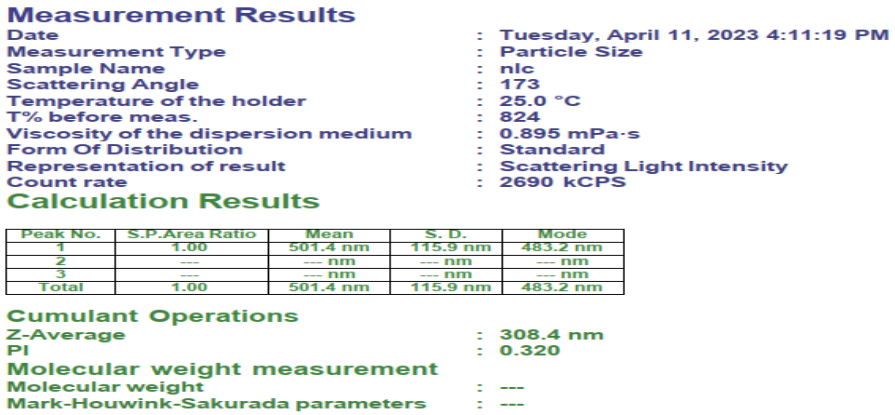
**Calculation Results**

| Peak No. | Zeta Potential | Electrophoretic Mobility |
|---|---|---|
| 1 | -36.4 mV | -0.000282 cm2/Vs |
| 2 | --- mV | --- cm2/Vs |
| 3 | --- mV | --- cm2/Vs |

| | | |
|---|---|---|
| Zeta Potential (Mean) | : | -36.4 mV |
| Electrophoretic Mobility mean | : | -0.000282 cm /Vs |

**Figure 9 ZP of optimized NLC formulation.**

Particle size of all formulations was found to be in the range of 308.4–2175.6 nm, which is due to the varied amount of independent variables or factors in the formulation which is mentioned in Table 3. The particle size statistics shows that, particle size decreased as the amount of total lipid and Smix in the formulation increased. The formulation 7 shows the lowest particle size i.e, 308.4 nm, which is mentioned in Figure 10. Polydispersity values were varied from 0.320 to 0.774, implying as the concentration of Smix increases PDI value decreases, which is mentioned in Table 4. The formulation 7 shows the lowest PDI value i.e, 0.320, which is mentioned in the following Figure 10. A low PDI suggests a more uniform and consistent particle size distribution, which is often desirable in many applications.



**Measurement Results**

| | | |
|---|---|---|
| Date | : | Tuesday, April 11, 2023 4:11:19 PM |
| Measurement Type | : | Particle Size |
| Sample Name | : | nlc |
| Scattering Angle | : | 173 |
| Temperature of the holder | : | 25.0 °C |
| T% before meas. | : | 824 |
| Viscosity of the dispersion medium | : | 0.895 mPa·s |
| Form Of Distribution | : | Standard |
| Representation of result | : | Scattering Light Intensity |
| Count rate | : | 2690 kCPS |

**Calculation Results**

| Peak No. | S.P.Area Ratio | Mean | S. D. | Mode |
|---|---|---|---|---|
| 1 | 1.00 | 501.4 nm | 115.9 nm | 483.2 nm |
| 2 | --- | --- nm | --- nm | --- nm |
| 3 | --- | --- nm | --- nm | --- nm |
| Total | 1.00 | 501.4 nm | 115.9 nm | 483.2 nm |

**Cumulant Operations**

| | | |
|---|---|---|
| Z-Average | : | 308.4 nm |
| PI | : | 0.320 |

**Molecular weight measurement**

| | | |
|---|---|---|
| Molecular weight | : | --- |
| Mark-Houwink-Sakurada parameters | : | --- |

**Figure 10 Particle size and PDI values of optimized NLC formulation.**

**Viscosity**

The viscosity of freshly prepared NLC was found to be in a range of 1012.5cp - 1237.5 cps.

**pH**

The pH of the sample was found to be in the range of 5.04 - 5.37.

**SEM studies**

The Optimized NLC formulation was analyzed by Scanning Electron Microscopy studies (SEM) as shown in Figure11, in which the morphology of NLC was examined as particles are distinct and spherical in shape.

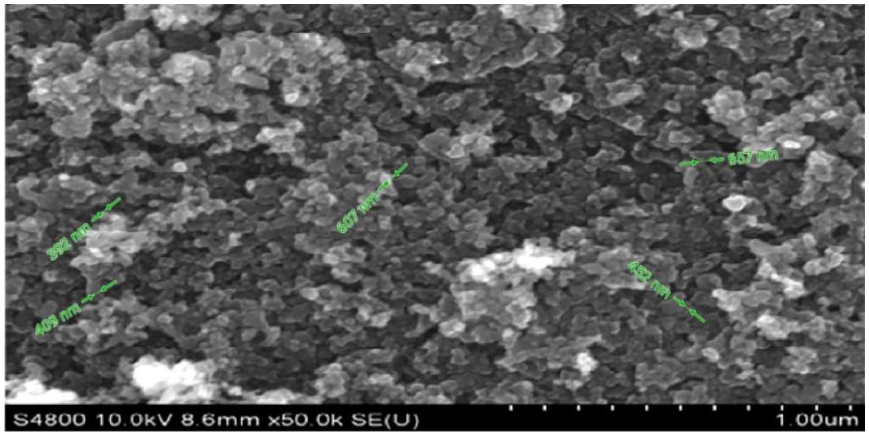



**Figure 11 SEM reults of optimized NLC.**

**Evaluation of NLC based gel**

**Physical evaluation, viscosity and pH, Drug content estimination, spreadability and extrudability**

**Table 6 Physical evaluation, viscosity, pH , Drug content estimination, spreadability and extrudability.**

| Evaluation parameters | Results |
|---|---|
| Physical evaluation | The color of gel formulation were found to be a translucent white appearance and to be smooth on application. The prepared gel was found to be homogenous and well looking with no phase separation. |
| Viscosity | 3312.5 - 4137.5 cps |
| pH | 5.07 |
| Drug content estimation | 90.50%. |
| Spreadability | 4.8 ± 1 to 6.1 ± 0.5 cm |
| Extrudability | 8.9 g/cm$^2$ |

**Invitro drug diffusion study**

**Table 7 % CDR values of Seabuckthorn oil.**

| Time (hrs) | %Cumulative drug release |
|---|---|
| 0 | 0 |
| 0.5 | 10.73 |
| 1 | 12.15 |
| 1.5 | 18.77 |
| 2 | 30.85 |
| 2.5 | 34.15 |
| 3 | 38.2 |
| 3.5 | 51.33 |
| 4 | 67.46 |
| 4.5 | 74.01 |
| 5 | 85.62 |
| 5.5 | 89.22 |
| 6 | 91.51 |

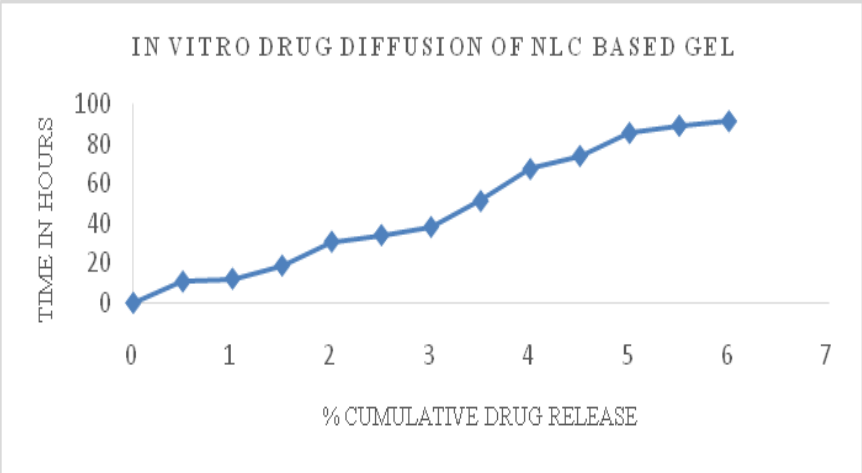



**Figure 12 In vitro frug diffusion study.**

In-vitro release testing gives information about the drug release behaviour from the formulation matrix. The drug release from the formulation affects various pharmacokinetic parameters. A plot of % cumulative release vs time (h) was plotted as shown in Figure 12. NLCs showed controlled release up-to 6 h. About 91.5% of the drug was released in 6 h.

## CONCLUSION

The present paper describes Seabuckthorn oil loaded NLC developed by "microemulsion technique with the construction of pseudoternary phase diagram the composition of excipients was identified by pseudoternary phase diagram which was then optimized using design expert software (2-factor 3-level). The average particle size for the batches was found in the range of 308.4–2175.6 nm with PDI in the range of 0.320 to 0.774 and % entrapment efficiency was found in the range of 98.77-98.87%. The obtained optimized batch had a mean particle size of 308.4 nm with 98.87% entrapment efficiency with a PDI value of 0.320. The obtained optimized batch of NLC was further evaluated for several parameters such as drug loading capacity, viscosity, pH, zetapotential, FTIR and Scanning Electron Microscopy. Zeta potential for optimized batch at -36.4 mV was obtained which stated that the NLCs formed were stable. SEM study revealed that the particles are distinct and spherical in shape which is desirable characteristics of NLCs. FTIR study showed there was no major incompatibility between drug and excipients and drug was encapsulated by the lipids. The gel version was choosen for optimized NLC formulation and assessed for % drug content, pH, spreadability, viscosity, extrudability and In vitro drug diffusion study and incorporated in to gel. The gel was found to show controlled drug release up to 6 h. These research findings suggests that the NLC of Seabuckthorn oil was successfully prepared by microemulsion method, is a feasible method for lab scale production and incorporated in to to gel for topical purpose.

**Conflict of Interest:**

There are no conflicts of interest.

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**ABBREVIATIONS**

| | |
|---|---|
| NLC | Nanostructured Lipid Carriers |
| SLN | Solid Lipid Nanoparticles |
| HPH | High Pressure Homogenization |
| PIT | Phase Inversion Temprature |
| QbD | Quality by Design |
| ZP | Zeta Potential |
| PDI | Polydispersity Index |
| DMSO- | Dimethyl sulfoxide |
| UV- | Ultraviolet |
| FTIR- | Fourier transform infrared |
| SEM | Scanning Electron Microscopy |
| PEG 400 | Polyethylene Glycol 400 |
| PUFA | Polyunsaturated Fatty Acids |
| %EE | %Entrapment efficiency |
| %CDR | %Cumulative Drug Release |
| Smix | Surfactant mixture |
| mV | Millivolt |
| nm | Nanometer |
| mg- | milligram |
| cps | Centipoise |
| ml- | millilitre |
| µl- | microliter |
| µg- | microgram |

**REFERENCES**


1. Wang Z, Zhao F, Wei P, Chai X, Hou G and Meng Q (2022) Phytochemistry, health benefits, and food applications of sea buckthorn (Hippophaerhamnoides L.): A comprehensive review. Front. Nutr. 2022; 9:1036295
2. Li TSC, Schroeder WR. Sea Buckthorn (Hippophaerhamnoides L.): A Multipurpose Plant. Horttechnology. 1996 Oct;6(4):370–80.
3. ChaiyavatChaiyasut , Bhagavathi Sundaram Sivamaruthi, Development and Evaluation of Elaeagnus rhamnoides (L.) A. Nelson Oil-Loaded Nanostructured Lipid Carrier for Improved Skin Hydration, applied sciences, 2022, (12), 8324.17-3
4. Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Res Pharm Sci. 2018;13(4):288.
5. Chauhan I, Yasir M, Verma M, Singh AP. Nanostructured lipid carriers: A groundbreaking approach for transdermal drug delivery., Advanced Pharmaceutical Bulletin. Tabriz University of Medical Sciences; 2020;(10) p. 150–65.
6. Vieira R, Severino P, Nalone LA, Souto SB, Silva AM, Lucarini M, et al. Sucupira Oil-Loaded Nanostructured Lipid Carriers (NLC): Lipid Screening, Factorial Design, Release Profile, and Cytotoxicity. Molecules. 2020 Feb 6;25(3):685.
7. Marwaha TK. Formulation design and evaluation of herbal anti psoriatic emulgel. Journal of Pharmaceutical and Scientific Innovation. 2013 Jun 20;2(3):30–42.
8. Qureshi M, Qadir A, Aqil M, Sultana Y, Warsi MH, Ismail MV, et al. Berberine loaded dermal quality by design adapted chemically engineered lipid nano-constructs-gel formulation for the treatment of skin acne. J Drug Deliv Sci Technol. 2021 Dec;66:102805.
9. Marwaha TK. FORMULATION DESIGN AND EVALUATION OF HERBAL ANTI PSORIATIC EMULGEL. Journal of Pharmaceutical and Scientific Innovation. 2013 Jun 20;2(3):30–42.
10. Joshi M, Pathak S, Sharma S, Patravale V. Design and in vivo pharmacodynamic evaluation of nanostructured lipid carriers for parenteral delivery of artemether: Nanoject. Int J Pharm. 2008 Nov;364(1):119–26.
11. Duong VA, Nguyen TTL, Maeng HJ. Preparation of Solid Lipid Nanoparticles and Nanostructured Lipid Carriers for Drug Delivery and the Effects of Preparation Parameters of Solvent Injection Method. Molecules. 2020 Oct 18;25(20):4781.
12. Suksaeree J, Treelop A, Veeravatanayothin P, Maneewattanapinyo P, Monton C. Stability Test of Nanostructured Lipid Carriers-Loaded Mefenamic Acid prepared by Microemulsion Technique. IOP Conf Ser Mater Sci Eng. 2020 May 1;840(1):012001.
13. Suto B, Weber S, Zimmer A, Farkas G, Kelemen A, Budai-Szűcs M, et al. Optimization and design of an ibuprofen-loaded nanostructured lipid carrier with a 23 full factorial design. Chemical Engineering Research and Design. 2015 Dec;104:488–96.
14. Al-Sarraf MA, Hussein AA, Al-Sarraf ZA.Comparison Between Conventional Gel and Nanostructured Lipid Carrier Gel of Zaltoprofen: Preparation and In-vitro/Ex-vivo Evaluation. International Journal of Drug Delivery Technology. 2021;11(3):988-995.
15. Tavade S, Patil K, Kurangi B, Suryawanshi S. Development and validation of UV-spectrophotometric method for estimation of berberine hydrochloride in marketed formulation and poly lactic co-glycolic acid nanoparticles. Indian J Pharm Educ Res. 2022 Jul 1;56(3):54
16. Elsewedy HS, Shehata TM, Almostafa MM, Soliman WE. Hypolipidemic Activity of Olive Oil-Based Nanostructured Lipid Carrier Containing Atorvastatin. Nanomaterials. 2022 Jun 23;12(13):2160.

17. Qadir A, Aqil M, Ali A, Warsi MH, Mujeeb M, Ahmad FJ, et al. Nanostructured lipidic carriers for dual drug delivery in the management of psoriasis: Systematic optimization, dermatokinetic and preclinical evaluation. J Drug Deliv Sci Technol. 2020 Jun;57:101775.
18. Wulansari A, Jufri M, Budianti A. studies on the formulation, physical stability, and in vitro antibacterial activity of tea tree oil (melaleuca alternifolia) nanoemulsion gel. International Journal of Applied Pharmaceutics. 2017 Oct 30;9:135.
19. Gujjar S, Blr M, Karki R. Formulation and evaluation of topical gel containing nanostructured lipid carriers dispersion of an antifungal drug. ACTA Pharmaceutica Sciencia. 2019;57(4):57.
20. Anthony A. Attama CEU. Formulation Design and Preclinical Evaluations of Surface Modified Lipid Nanoparticles-Coupled Gel Encapsulating Dihydroartemisinin for Treatment of Localized Inflammation. Letters in Applied NanoBioScience. 2021 Sep 11;11(3):3745–69.
21. AmolAmrutkar , Smita Aher , Rishikesh Bachhav, Topical Gels as Drug Delivery System- A Comprehensive Review, International Journal of Trend in Scientific Research and Development, , ISSN: 2456- 6470, 6(2), February 2022, pp.1430- 1436.

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