Preparation and in Vitro Evaluation of Soya Lecithin Based Nano Transfersomal Dispersion for Loxoprofen Sodium

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Abstract:

This work involves investigation and evaluation of the factors that affect the preparation and release of the model class I drug (loxoprofen sodium) to optimize the efficiency of its prepared nano transfersomal dispersion to give rapid onset of action (burst effect) within the first 2 hours that can continue for further four hours. The work involved preparation of twenty formulas of transfersomal dispersion by thin film method which were nano-sized by probe sonication and characterized morphologically by Transmission electronic microscopic (TEM), zeta potential, particle size, polydispersity index (pdi), pH, physical appearance, entrapment efficiency, viscosity, transmittance and in vitro drug release. The selected nano transfersomal formula (SF16) showed pH (7.2), particle size (393 nm), pdI (0.259), zeta potential (-25), entrapment efficiency (91%) and gave a 73% drug release within the first 2 hours of the in vitro test and continued until it gave 86.35% drug release after 4 h. This work succeeded in preparing optimized transfersomal dispersion for loxoprofen sodium using different soya lecithin / edge activator percent and different types of edge activator. The optimum formula gave an immediate release of the model drug (loxoprofen sodium) and it was ready to be incorporated in any suitable dosage form to give continuous drug release.

Key words: Transfersomes, loxoprofen sodium, soya lecithin, edge activators, nanotechnology

**الخلاصة:**

هذا العمل يتضمن تحضير وتقييم الوسائط التي تؤثر على تحضير وتحرير دواء من نوع الصنف الأول لوكسوبروفين صوديوم لاختيار الكفاءة من الهاام الترانсинسيرمومي المحضر ليعطي تأثير سريع خلال ساعتين الأولى ويستمر لاربع ساعات. هذا العمل يحتوي على تحضير عشرة فورملا من الهاام الترانسينسيرموي بواسطة طريقة فلم الريق ثم استخدام البروب سونيكشير لتضييق الجسيمات إلى حجم النانو. تم العمل بها تقسيم المخثثات التي يحتوي الميكرروسركب الإلكتروني، مقترح جيداً للحصول على الحدود النانوية، العامل المحمولة، كفاءة التقليل وتحرير الدواء في المختبر لفوريلا الترانسينسيرموي الفورملا المختارة ذات الحجم النانو لها عامل حموضة (2.73) حجم جزيئي (263±20 نانومتر)، وزيتا (79.6±2 مللي فولت). معدل انتشار (69.0±10)، لزوية، عامل مرتبة (20.3±1)، تحرير الدواء خلال ساعتين الأولي 3.2%، مما يسمح إلى 86.3% لاربع ساعات. هذا العدد نجح في تحقيق هلام ترانسينسيرمو من خلال

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Introduction
Lipid nanocarriers refer to a large panel of drug delivery systems. Lipid vesicles are the most conventional, having the ability to carry both lipophilic and hydrophilic active ingredients because it has a head group (hydrophilic) and tail group (hydrophobic). Vesicular approach that included liposomes, ethosomes, niosomes, spherosomes and transfersomes[1]. Transfersomes are considered the first generation of highly elastic or deformable vesicles, it is a liposome with modification, it can cross into deeper skin since they can be squeezed through the pores in stratum corneum. Transfersomes compose of several phospholipid bilayers with an additional component that named as the edge activator (EA) [2]. Loxoprofen sodium (LXP) is a recently developed novel NSAID (non-steroidal anti-inflammatory drug), a prodrug of propionic acid derivatives and is chemically as sodium-2-[4-(2-oxocyclopentyl-1-methyl)phenyl] propionate, it has antipyretic and anti-inflammatory properties. LXP acts by inhibiting isoforms of cyclo-oxygenase 1 and cyclo-oxygenase 2. After oral administration, LXP is absorbed as the free acid more than the sodium salt from the gastrointestinal tract, which causes only weak irritation of the gastric mucosa. it is converted to the trans- OH form which is the active metabolite by reduction of the ketone carbonyl which is the enzyme that is found in GIT and skin , and used for the management of pain and inflammation associated with musculo-skeletal, joint disorders and operative procedures[3], the half-life of loxoprofen sodium is short (75 min)(4). The aim of this study is to prepare and in vitro evaluate loxoprofen sodium nano transfersomal dispersion using nano transfersomal technology

Material and methods
Materials:
Loxoprofen sodium was purchased from Al-Catheil bureau, sodium deoxycholate, tween 80 were purchased from CDH, India , span 80, span 60 were purchased Sinopharm, China, soya lecithin was purchased from M/S Provisor pharma, India, methanol was purchased from Chem-lab, Belgium, sodium hydroxide , potassium dihydrogen phosphate, chloroform were purchased from (Himedia, India), deionized water from Al-Basheer bureau.

Methods
Melting point determination:
The melting point of loxoprofen sodium was determined using capillary tube method according to the USP. The glass tube is sealed from one side and the other opened side was dipped in a small quantity of loxoprofen sodium powder then positioned inside the electrical melting point apparatus and complete drug powder temperature melting was reached after gradually increasing the temperature[5].The melting point of loxoprofen sodium was further approved by using Differential Scanning Calorimetry (DSC) that is a basic method used to provide information about the thermal behaviour and structure changes,and it represents more accurate method than method mentioned above to know the melting point [6].

Fourier Transform Infrared Spectroscopy (FTIR)
FTIR spectra of drug, was determined by grounded solid sample with potassium bromide and compressed in special disc then measured under infrared spectroscopy[7]

UV absorption maxima (λ. max) determination:
Loxoprofen sodium (10 mg) was dissolved in 100 mL of each phosphate buffer pH 7.4
as well as methanol respectively to prepare the stock solution of loxoprofen sodium (0.1 mg/mL) and then a suitable working solution in each medium was scanned using UV visible spectrophotometer \(^{(8)}\), over a wavelength range of 200 to 400 nm.

**Preparation of calibration curve:**
Calibration curve of loxoprofen sodium in phosphate buffer pH 7.4 was obtained by preparing serial dilutions of the drug through transferring (1, 1.5, 2, 3, 3.5, 4, 4.5, 5 and 5.5 mL) from the stock solution (5mg/100 mL = 0.05 mg/mL) to 10 mL volumetric flasks and diluted up to 10mL. The absorbance of these diluted solutions was determined spectrophotometrically at the previously estimated \(\lambda\) max and plotted against concentration to get the calibration curve. The \(R^2\) value and calibration curve equation was obtained. Calibration curve of loxoprofen sodium in methanol was also prepared \(^{(9)}\).

**Preparation of transfersomes dispersion formulas:**
The required quantities of soya lecithin and surfactant (keeping the concentration of dispersion phase 6%), were taken into a round bottom flask and dissolved in 9 mL mixture of chloroform and methanol (2:1) by shaking. Then mixture was evaporated using rotary evaporator for 15 minutes at 55 °C and a thin film was obtained (as shown in figure 1). Vacuum was applied for 30 minutes to dry the film. Loxoprofen sodium (100 mg) was dissolved in 10 ml phosphate buffer pH 7.4 which was heated to 55 °C. Then, the film was hydrated with the heated buffer by rotary evaporation for 2 h and then the transfersomal dispersion sonicated by probe sonication for 5 minutes at 300 W to produce small transfersomes vesicle \(^{(10)}\). Twenty formulas were prepared using soya lecithin as shown in table 1.
Characterization of the prepared nano transfersomal dispersion formulas:

**Physical appearance:**
Evaluation of the physical properties (color and homogeneity) for all the prepared nano transfersomal dispersion formulas (SF1-SF20) was done visually.

**pH determination:**
The pH of the formulations (SF1-SF20) was determined using a digital pH meter by immersing the electrode of the device in a beaker containing 10 mL of each of the nano transfersomal dispersion formulas and the results were recorded after two minutes.

**Separation test:**
This test is considered very important to check the physical stability of the dispersions. Cooling centrifugation of each nano transfersomal dispersion formula (SF1-SF20) for 30 min at (10,000 rpm) was applied to check the nano transfersomal dispersions resistance for separation.

**Viscosity test for transfersomal dispersion:**
The viscosity of transfersomal formulas (SF1-SF20) were measured by Brookfield viscometer using spindle number S-62 at 37 °C, the viscosity was read directly after 30 seconds. This test was repeated three times for each formula and average viscosity for each formula was determined.\(^{11}\)

**Entrapment efficiency:**
For the determination of entrapment efficiency, 1 mL of each of the transfersomal dispersions (SF1-SF20) was

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Table (1): Composition of the prepared nano transfersomal dispersion formulas using soya lecithin:

<table>
<thead>
<tr>
<th>Formulas</th>
<th>Soya lecithin</th>
<th>SDC</th>
<th>Tween 80</th>
<th>Span 80</th>
<th>Span 60</th>
<th>Drug</th>
<th>Buffer 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>5.5%</td>
<td>0.5%</td>
<td></td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF2</td>
<td>5%</td>
<td>1%</td>
<td></td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF3</td>
<td>4%</td>
<td>2%</td>
<td></td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF4</td>
<td>3%</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF5</td>
<td>2%</td>
<td>4%</td>
<td></td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF6</td>
<td>5.5%</td>
<td>_</td>
<td>0.5%</td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF7</td>
<td>5%</td>
<td>_</td>
<td>1%</td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF8</td>
<td>4%</td>
<td>_</td>
<td>2%</td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF9</td>
<td>3%</td>
<td>_</td>
<td>3%</td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF10</td>
<td>2%</td>
<td>_</td>
<td>4%</td>
<td></td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF11</td>
<td>5.5%</td>
<td>_</td>
<td>_</td>
<td>0.5%</td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF12</td>
<td>5%</td>
<td>_</td>
<td>_</td>
<td>1%</td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF13</td>
<td>4%</td>
<td>_</td>
<td>_</td>
<td>2%</td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF14</td>
<td>3%</td>
<td>_</td>
<td>_</td>
<td>3%</td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF15</td>
<td>2%</td>
<td>_</td>
<td>_</td>
<td>4%</td>
<td></td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF16</td>
<td>5.5%</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>0.5%</td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF17</td>
<td>5%</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>1%</td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF18</td>
<td>4%</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>4%</td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF19</td>
<td>3%</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>3%</td>
<td>1%</td>
<td>q.s</td>
</tr>
<tr>
<td>SF20</td>
<td>2%</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>2%</td>
<td>1%</td>
<td>q.s</td>
</tr>
</tbody>
</table>
diluted with phosphate buffer pH 7.4 up to 10 mL and then subjected for cooling centrifugation at 16000 rpm for a period of 45 minutes, and the supernatant liquid was collected, diluted appropriately with the phosphate buffer and estimated using UV visible spectrophotometer at λ max 223 nm. The percent of entrapment efficiency (%EE) was calculated using the following equation:

\[
\text{%EE} = \frac{(\text{Total drug} - \text{Free drug})}{\text{Total drug}} \times 100
\]

Effect of different variables on the entrapment efficiency of the prepared transfersomal dispersion formulas:

Effect of types of edge activator (EA) and lipid: edge activator ratio on entrapment efficiency:
Formulas (SF1-EF5, SF6-SF10, SF11-SF15, SF16-SF20) were used to study the effect of types of edge activators (SDC, tween 80, span 80, span 60) respectively and the effect of lipid: edge activator ratios (5.5:0.5, 5:1, 4:2, 3:3, 2:4% ) for each type of edge activator on entrapment efficiency.

Effect of (lipid /surfactant) total percentage on entrapment efficiency:
Formula (SF17) was prepared using 6% total lipid /surfactant dispersion and was re-prepared using 3% total lipid /surfactant dispersion to study the effect of lipid / surfactant total percentage on entrapment efficiency.

Effect of method of preparation of transfersomal dispersion:
Formula (SF17) was prepared by thin film method as well as it was re-prepared using hot melt method where soya lecithin and span 60 are melt at 70 °C, then this mixture was added drop by drop to pre-heated phosphate buffer pH 7.4 containing loxoprofen sodium (100mg) was heated at 70 °C under mechanical stirring at 1000 rpm for 3 h to produce homogeneous dispersion and then sonicated by probe sonicator for 5 min at 300 W. This study was done to investigate the effect of different methods on entrapment efficiency of transfersomal dispersion.

According to the results obtained upon application of different tests as well as studying of the previously mentioned variables, two formulas (SF16, SF17) were selected according to their homogenous appearance, suitable pH, separation test, suitable viscosity and entrapment efficiency. Therefore, the two formulas were subjected to further investigations.

Drug content:
Accurately, one mL of each nano transfersomal dispersion formulas (SF16, SF17) was transferred to volumetric flask (100 mL) and 70 mL methanol was appended, and clear solution was achieved after sonication for 2 h. The solution volume was completed with methanol to 100 mL and subjected to centrifugation for 15 minutes at 3000 rpm then filtered using millipore filter 0.22 μm. Content of loxoprofen sodium was determined spectrophotometrically using UV-Visible spectrophotometer at λ max 223 nm.

Zeta potential, particle size and polydispersity index determination:
Measurement of the mean particle size (mean diameter), zeta potential (particle surface charge) and polydispersity index (size range of particles) of formulas (SF16, SF17) was done by using dynamic light scattering method, by this technique the light scattering fluctuations were examined, this fluctuation is due to the Brownian motion of transfersomal dispersion particles. It measures the size accurately in the range of 0.3 nm to 10 µm. One mL of the diluted transfersomal dispersion was injected into folded capillary zeta cell and monitor the light scattering at 25°C (190° angle).
Study the shape of transfersomes formulas (SF16, SF17) by Transmission Electron Microscopy (TEM):
The nano transfersomal dispersion formulas (SF16, SF17) was further characterized by TEM operating at 30 KV. A drop of diluted formula was allowed to be deposited on the circular copper film grid of 300 mesh previously coated with 1nm of carbon to increase conductivity and the drop was left for about 3 minutes and then stained with formvar (uranyl acetate) and left for 24 h for complete drying. The size and shape of the transfersomes were measured after complete drying of the slide by observing the slide under TEM (Transmission Electron Microscopy)\cite{18}.

Transmittance test (turbidity test):
The transmittance percent of the formulas (SF16, SF17) were measured by taking 1 mL of nano transfersomal dispersion and diluted with phosphate buffer pH 7.4 and then the transmittance was recorded at 650 nm by UV spectroscopically \cite{19}.

In vitro drug release:
The release of loxoprofen sodium from the nano transfersomal dispersion formulas (SF16, SF17) was done by using dialysis membrane (MWCO 2000 Da) and rotating paddle dissolution apparatus type II. The sealed dialysis bag containing (1 mL) of the nano transfersomal dispersion formula (equivalent to 10 mg loxoprofen sodium) was sunken in 500 mL phosphate buffer (pH 7.4 dissolution medium) with a speed of 50 rpm. The temperature of the medium was maintained at 37 °C. Five mL aliquots were outgoing for suitable time periods and immediately making the replacement with fresh phosphate buffer\cite{20}.

Results and discussion
Melting point determination:
Loxoprofen sodium melting point was found to be 204°C, which is consistent with the reported data and proved the high purity of loxoprofen sodium powder\cite{21}.

Further work was applied, where the DSC spectrum of the drug (figure 2) showed sharp peak at 204°C which corresponding to melting point of the drug.

![DSC spectrum of loxoprofen sodium](image)

**Figure (2): DSC spectrum of loxoprofen sodium**

Fourier Transform Infrared Spectroscopy (FTIR)
The results of the of FTIR tests transmittance bands of pure loxoprofen sodium powder were demonstrated in figure 3, where C–H bands of aromatic ring is 3086 cm\(^{-1}\), Peak of carbonyl stretching of carboxylic acid is 1728 cm\(^{-1}\).
**Figure 3: FTIR spectrum of pure loxorofen sodium**

**UV absorption maxima (λ max) determination**
Loxoprofen sodium solution was scanned in phosphate buffer pH 7.4 as well as in methanol by UV- spectrophotometer and showed to be identical with the reported data of loxoprofen sodium, (as shown in figure 4) and the selected λ max was at 223 nm.

![Figure 4](image)

**Figure (4): λ max of loxoprofen sodium (A) in phosphate buffer pH 7.4, (B): in methanol**

**Preparation of calibration curve:**
The calibration curve of loxoprofen sodium in phosphate buffer pH 7.4 as well as in methanol at λ max 223 nm was shown in (figure 5), where the absorbance was plotted against the concentration and gave a straight line with high regression coefficient (R²) and it follows Beer’s law within the range of concentrations used.

![Figure 5](image)

**Figure (5): Calibration curve of loxoprofen sodium (A) in phosphate buffer pH 7.4, (B) in methanol**
Preparation and characterization of the prepared nano transfersomal dispersions:
All formulas (SF1-SF20) of nano transfersomal dispersion of loxoprofen sodium were prepared by thin film method using soya lecithin as phospholipids together with different types and ratios of edge activator. The dispersion formulas were characterized according to their physical appearance, pH, phase separation, viscosity and entrapment efficiency.

Physical appearance:
The formulas (SF1-SF20) appeared as homogeneous brown (milk tea) dispersion.

pH determination:
The pH of the nano transfersomal formulations (SF1-SF20) was measured using pH meter. The pH values were ranged (7.08-7.45), and this matches the skin requirements for topical preparations to avoid skin irritation[23].

Separation test:
No phase separation, sedimentation or creaming was observed for all the prepared nano transfersomal dispersion formulas (SF1-SF20) indicating the resistance and stability of the prepared formulas[24].

Viscosity determination of nano transfersomal dispersion:
The viscosity of all formulas of nano transfersomal dispersion were measured using viscometer at 37 °C for 3 times and the results is shown in table 2. The results showed that in formulas SF1-SF5 (containing SDC as edge activator) as the ratio of SDC increased the viscosity was decreased because it is anionic surfactant and hydrophilic in nature with low molecular weight[25]. While other formulas containing other types of edge activators including SF6-SF10 (containing different ratios of tween 80), SF11-SF15 (containing span 80) and SF16-SF20 (containing span 60) showed increasing in the viscosity as the ratio of edge activator was increased because tween 80, span 80 and span 60 are nonionic surfactants that have no charge on the hydrophilic head and increasing their concentration lead to increasing their viscosity due to increase in flow resistance in the batch emulsification process[26].

<table>
<thead>
<tr>
<th>Formulas</th>
<th>Viscosity(cp) ± SD</th>
<th>Formulas</th>
<th>Viscosity(cp) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>787 ± 6.2</td>
<td>SF11</td>
<td>442 ± 1.9</td>
</tr>
<tr>
<td>SF2</td>
<td>729 ± 4.5</td>
<td>SF12</td>
<td>585 ± 4.5</td>
</tr>
<tr>
<td>SF3</td>
<td>558 ± 3.5</td>
<td>SF13</td>
<td>657 ± 7.1</td>
</tr>
<tr>
<td>SF4</td>
<td>446 ± 2.4</td>
<td>SF14</td>
<td>777 ± 4.6</td>
</tr>
<tr>
<td>SF5</td>
<td>336 ± 6.3</td>
<td>SF15</td>
<td>1125 ± 6.5</td>
</tr>
<tr>
<td>SF6</td>
<td>206 ± 2.5</td>
<td>SF16</td>
<td>1010 ± 7.2</td>
</tr>
<tr>
<td>SF7</td>
<td>396 ± 3.2</td>
<td>SF17</td>
<td>1145 ± 4.6</td>
</tr>
<tr>
<td>SF8</td>
<td>833 ± 1.3</td>
<td>SF18</td>
<td>1180 ± 7.3</td>
</tr>
<tr>
<td>SF9</td>
<td>929 ± 2.5</td>
<td>SF19</td>
<td>1255 ± 4.5</td>
</tr>
<tr>
<td>SF10</td>
<td>1017 ± 2.2</td>
<td>SF20</td>
<td>1363 ± 5</td>
</tr>
</tbody>
</table>

Entrapment efficiency
The entrapment efficiency of loxoprofen sodium nano transfersomal dispersion formulas is shown in table 3. Most of the formulas showed good entrapment efficiency indicating that the thin film method used for their preparation is efficient and reliable. The results agreed with reported data where this method was reported to be applicable for different lipid based vesicles[27, 28]. The effect of different
variables on entrapment efficiency was further studied.

### Table 3: Percentage of entrapment efficiency of twenty formulas of nano transfersomal dispersion, values are mean ± SD (n=3):

<table>
<thead>
<tr>
<th>Formulas</th>
<th>Entrapment efficiency ± SD</th>
<th>Formulas</th>
<th>Entrapment efficiency ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>35% ± 2.5</td>
<td>SF11</td>
<td>79% ± 2.3</td>
</tr>
<tr>
<td>SF2</td>
<td>41% ± 2.1</td>
<td>SF12</td>
<td>89% ± 2.5</td>
</tr>
<tr>
<td>SF3</td>
<td>60% ± 2.2</td>
<td>SF13</td>
<td>90% ± 1.2</td>
</tr>
<tr>
<td>SF4</td>
<td>87% ± 2.8</td>
<td>SF14</td>
<td>85% ± 1.5</td>
</tr>
<tr>
<td>SF5</td>
<td>89% ± 1.5</td>
<td>SF15</td>
<td>79% ± 2.3</td>
</tr>
<tr>
<td>SF6</td>
<td>73% ± 1.5</td>
<td>SF16</td>
<td>91% ± 1.2</td>
</tr>
<tr>
<td>SF7</td>
<td>83% ± 2.3</td>
<td>SF17</td>
<td>89% ± 1.8</td>
</tr>
<tr>
<td>SF8</td>
<td>87% ± 1.4</td>
<td>SF18</td>
<td>87% ± 1.5</td>
</tr>
<tr>
<td>SF9</td>
<td>90% ± 1.4</td>
<td>SF19</td>
<td>86% ± 1.5</td>
</tr>
<tr>
<td>SF10</td>
<td>87% ± 1.3</td>
<td>SF20</td>
<td>81% ± 1.3</td>
</tr>
</tbody>
</table>

**Effect of different variables on the entrapment efficiency of the prepared transfersomal dispersion formulas:**

**Effect of types of edge activators (EA) and lipid: edge activator ratio on entrapment efficiency**

From the obtained results; it was found that span 60 at lowest ratio of lipid : EA ratio (5.5:0.5) in formula SF16 gave significantly highest entrapment efficiency than other edge activators at the same ratio (in formulas SF1, SF6 and SF11), the same results was observed for span 60 at ratio 5:1 (in formula SF17) in comparison to formulas containing other surfactants at the same ratios (SF2, SF7 and SF12), because span 60 having long alkyl chain length and saturated acyl chain (stearyl C-18) compared to other spans, as well as span 60 having high phase transition temperature and low HLB value 4.7 while SDC having high HLB value 16 and tween 80 with HLB value 15 which make span 60 as better emulsifying agent. The same results were obtained with diclofenac sodium and bupivacaine lipid based vesicles [29, 30].

The results in table 9 showed that as the ratio of edge activators was increased, the entrapment efficiency increased (or no significant difference) since the emulsification ability increased and the vesicles number increased leading to encapsulating the drug inside the vesicles [31].

**Effect of (lipid /surfactant) total percentage on entrapment efficiency:**

When the formula (SF17) was re-prepared using 3% total lipid /surfactant dispersion, the entrapment efficiency was decreased significantly (p < 0.05) to 53% in comparison to that prepared using 6% total lipid /surfactant which was 89%. this indicates that as the total content of the transfersomes (lipid and edge activators) increased the vesicles number was increased leading to an increase in entrapment efficiency [28, 32].

**Effect of method of preparation of transfersomal dispersion:**

The entrapment efficiency obtained from using hot melt method to prepare formula (SF 17) was 86.5% which is not significantly different (p> 0.05) than entrapment efficiency (89%) obtained from using thin film method to prepare the same formula [33]. But thin film method is more preferable because it is a simple, most commonly used method and can be applied for different types of lipid
mixtures as well as heat sensitive materials \cite{34, 35}.

According to the results obtained upon application of different tests as well as studying of the previously mentioned variables, two formulas (SF16 and SF17) were selected according to their homogenous appearance, suitable pH, separation test, suitable viscosity and entrapment efficiency. Therefore, the two formulas were subjected for further investigations.

**Drug content**
The drug content of nano transfersomal dispersion formulas (SF16 = 90.5% ± 1.2 and SF17 = 97.7% ± 0.6) results are consistent with the requirements of the USP, indicating high adequacy of the preparation method and high content uniformity of the prepared formulas \cite{36}.

**Zeta potential, particle size and PDI determination:**
Particle size measurement was showed that all particles of the dispersion (SF16, SF17) are in nanometer size range (SF16 = 393 nm ± 3.2 and SF17 = 975 nm ± 4.1), with a polydispersity index of < 1. Low value of polydispersity index (SF16 = 0.289 ± 0.02 and SF17 = 0.363 ± 0.03) is considered to be desirable for uniform distribution and homogeneity of nano-sized particles within the preparation, while PDI value > 0.7 to less than 1 is considered to have broad distribution of particle size \cite{37}. The zeta potential of all formulas were comparably low (SF16 = -25 mV ± 0.02 mV and SF17 = -25 mV ± 0.3 mV) indicating the stability of the prepared formulas, which could be due to the absence of charge in transfersomal dispersion ingredients, this is mainly because of the use of non-ionic surfactant (span 60) which act by steric stabilization \cite{38}. The values of zeta potential in the range (≤ -30 mV) to (≥ +30 mV) refer to good stability and values in the range (≤ -60 mV) to (≥ +60 mV) refer to an excellent stability in the formulation \cite{39}.

**Study of the shape and size of the nano transfersomes in formulas (SF16, SF17)**
The shape and size of the nano transfersomal in formulas (SF16 and SF17) were characterized by the use of transmission electron microscopy (TEM) as in figure (6). The TEM images showed the outlines and the core of the well identified sealed spherical structure \cite{40}. No disruption of vesicular structure even after mechanical stress (sonication). \cite{41}.

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{image1.png}
\caption{(A) SF16, (B) SF17}
\end{figure}
Transmittance test for formulas (SF16, SF17)
The turbidity of the transfersomal dispersion was measured using UV absorbance at 650 nm. The results (SF16 = 98.4% ± 3.1 and SF17 = 98.52% ± 1.4) showed that there is no significant difference between formulas in transmittance percentage, indicating suitable transparency and homogeneity [42, 43].

In-vitro release test:
Loxoprofen sodium based nano transfersomal dispersions (SF16- SF17) were prepared with a different proportion of soya lecithin using span 60 and they were subjected to in vitro drug release studies at phosphate buffer (pH 7.4). The cumulative release percentage of loxoprofen sodium at different time intervals for each nano transfersomal dispersion is shown in figure (7). The results showed that SF17 gave the maximum amount 87.24% after 270 min which is significantly higher than others formulas whereas SF16 gave 86.54%, within the first 2 h there is no significant difference in the release of drug from the prepared formulas although SF17 gave slightly higher release, since it contains higher amount of EA (span 60) which leads to decrease the ordered lipid membrane and made it more leaky and thus gave more drug release [44].

![Figure 7: In vitro release profile of loxoprofen sodium from nano transfersomal dispersion formulas (SF16, SF17) in phosphate buffer solution (pH 7.4) at 37°C, value SD (n=3)](image)

According to the results obtained upon application of different tests as well as studying the effect of different variables, SF16 formula was selected since it showed homogenous appearance, suitable pH, no phase separation, suitable viscosity, high drug content (90.5%), entrapment efficiency (91%), smaller particle size (393nm), spherical shape under TEM, high transmittance (99.02%), and high percentage of drug release 86.54% after 270 minutes.

Conclusion:
Depending on the results obtained in this study, this work revealed that using soya lecithin with different lipid: surfactant ratio as well as type of edge active used and using probe sonication led to improving entrapment efficiency and gave immediate release of the drug from the prepared dispersion as well as the unique structure of the prepared transfersomes that may improve drug permeability, improve drug effectiveness leading to reduce dose amount and frequency that improves patient compliance.
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