

Formulation and characterization of niosomes for controlled delivery of tolmetin

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Abstract

The main objective of the present study is to formulate of tolmetin niosomes as vesicular carrier in which the tolmetin incorporated inside the vesicle for prolonged duration of action and select the optimum formula after characterization. Tolmetin is a nonsteroidal anti-inflammatory drug commonly used for the treatment of rheumatoid arthritis,

Niosomes are formed by thin film hydration method by hydrating mixture of different amount of cholesterol and non-ionic surfactants like span20, 40, 80 and tween 20,40, 80. Effect of type and amount of non-ionic surfactants, amount of cholesterol, and the effect of ultrasonication time were studied.

The prepared niosomal dispersion were evaluated for their entrapment efficiency, physical stability, in vitro dissolution study, particle size and zeta potential, the niosomes morphology were also tested using optical microscope and TEM.

Among the formulations, Tolmetin niosomal dispersion prepared by using span80 with cholesterol in molar ratio of 4:1 and sonicated for 3 min, showed spherical vesicles with entrapment efficiency of $87.4 \pm 0.8\%$, mean vesicular diameter of 286 nm, zeta potential of -33.5 mV, and in-vitro drug release of $92 \pm 1.2\%$ in 24 hr.

The results indicated niosomal formulation could be one of the promising delivery systems for tolmetin with improved delivery and controlled drug release profile.

Keywords: niosomes, tolmetin, Non-ionic surfactants, span80, vesicles, thin film

INTRODUCTION

Tolmetin is a poorly water soluble, non-steroidal anti-inflammatory drug, which acts specifically on inflammatory sites and thereby decreases the inflammation. It is highly effective as an anti-inflammatory drug for various inflammatory conditions like rheumatoid arthritis, osteoarthritis and ankylosing spondylitis by inhibits Cox activity with a reduction in the tissue production of prostaglandins.

Although tolmetin has a strong therapeutic effect, it is associated with several gastrointestinal side effects, when taken orally for prolonged period. Its usefulness is limited due to its short plasma half-life of 30–60 min following oral dosing, which necessitates frequent administration of the drug in order to maintain the desired steady state levels, Hence, the aim of the present study was to formulate and optimize the tolmetin niosomes for extended period of time (1).

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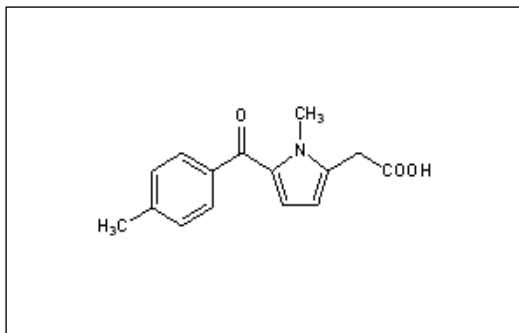


Fig.1. Chemical structure of Tolmetin (2)

Niosomes are colloidal carriers which are formed by self-assembly of nonionic surfactants forming lipid bilayers surrounding an aqueous core, thus they could accommodate drugs with different solubilities. Their composition as well as their characteristics such as size, lamellarity and surface charge, could be varied to fulfill a desired goal⁽³⁾.

MATERIALS AND METHODS

Chemicals and Drugs

Tolmetin (TM) obtained from DAYANG CHEM (HANGZHOU) CO, Chin. Chloroform obtained from Millipore Sigma. Cholesterol from Direvo industrial biotechnology, Germany. Span80, Span40, Span20, Tween 80, Tween40, and Tween20 were purchased from Himedica laboratories, India. All other chemicals were of analytical grade and used as supplied.

CHARACTERIZATION OF TM POWDER

Determination of melting point

The melting point of TM was measured using capillary tube method. Enough quantity of TM introduced to the closed side capillary tube. The tube was then placed inside the melting point apparatus, and the temperature was raised gradually. The temperature at which the powder passes into the liquid phase was recorded as the melting point⁽⁴⁾

Spectrophotometric analysis

Ten milligrams of TM powder were dissolved in 100 ml of Phosphate buffer PBS (pH 7.4), and Methanol in volumetric flasks to prepare (100µg/ml) stock solution. From these stock solutions suitable dilutions were prepared then scanned by UV-visible spectrophotometer at the range of 200-400nm in order to determine the wavelength of maximum absorbance (λ max) of TM in those media. Serial dilutions 2, 4, 6, 8, 9, 10 µg/ml of TM in PBS (pH

7.4) and in methanol were prepared from 100µg/ml stock solution. The prepared samples were analyzed spectrophotometrically for TM at its λ max. The absorbance values were plotted against concentrations to obtain a calibration curve.

Preparation of TM niosomes

In this work, thin film hydration method was employed in the preparation TM niosomes as reported in literature with some modifications. The drug, surfactant, and cholesterol were dissolved in an organic solvent (chloroform, 10 mL) in round Bottom Flask; subsequently, the chloroform was evaporated by a rotary evaporator (100 rpm, 60 °C) for 25 min or until thin film formed. Afterward, the dried thin films were hydrated by 15 ml PBS (pH 7.4) for 30 min at the same temperature and speed but without vacuum. Lastly, the resulting niosomal suspension was mixed by vortex mixing for 5 min and sonicated in prob sonicator for measured time with 4sec rest every 30 sec intervals. Then niosomal suspension was transferred into container and stored in refrigerator at 4°C overnight for further study^(5,6,7). The composition of TM niosomes formulations are illustrated in Table 1

CHARACTERIZATION AND EVALUATION OF TOLMETIN-LOADED NIOSOMES

Visual evaluation

All the prepared formulas were visually observed for turbidity and flocculation in transparent containers⁽⁸⁾.

Determination of entrapment efficiency

Free TM was removed from niosomal suspension by filtration through Ultra-15- Centrifugal Filter Units (Amicon tube) with 3 kDa molecular weight cut off to achieve both high sample recovery and fast sample processing.

Accurately, 4 ml of TM niosomal suspension was poured in the Amicon tube and then was centrifuged at 5,000 rpm for 30 minutes. the drug-carrying niosomes resided in the top chamber, and free drugs passed through the filter membrane. The filter solution at the bottom was collected to determine the free amount of TM by UV-visible spectrophotometer.

The total amount of TM was determined in the niosomal suspension prior filtration. The entrapment efficiency (EE%) was calculated by the following equation:

$$EE (\%) = \frac{(A-A_1)}{A} \times 100$$

where A is the total amount of TM and A1 is the free un-entrapped amount of TM transmitted through the membrane⁽⁵⁾⁽⁷⁾⁽⁹⁾.

Table 1. Composition of different TM niosomal drug delivery systems formulas (mg)

<i>F</i> <i>.</i> <i>N</i> <i>o</i>	<i>Sur:chol</i>	<i>Span</i> <i>20</i>	<i>Span</i> <i>40</i>	<i>Span</i> <i>80</i>	<i>Tween2</i> <i>0</i>	<i>Tween</i> <i>40</i>	<i>Tween</i> <i>80</i>	<i>Choles</i>	<i>Soni.</i> <i>time</i> <i>(min)</i>
1	1:1	138.5	-	-	-	-	-	154.4	3
2	1:1	-	161	-	-	-	-	154.4	3
3	1:1	-	-	171	-	-	-	154.4	3
4	1:1	-	-	-	491	-	-	154.4	3
5	1:1	-	-	-	-	513	-	154.4	3
6	1:1	-	-	-	-	-	524	154.4	3
7	1:2	-	-	171	-	-	-	308.8	3
8	1:4	-	-	171	-	-	-	617.6	3
9	2:1	-	-	343	-	-	-	154.4	3
10	4:1	-	-	686	-	-	-	154.4	3
11	1:2	-	-	-	-	-	524	308.8	3
12	1:4	-	-	-	-	-	524	617.6	3
13	2:1	-	-	-	-	-	1048	154.4	3
14	4:1	-	-	-	-	-	2096	154.4	3
15	4:1	-	-	686	-	-	-	154.4	0
16	4:1	-	-	686	-	-	-	154.4	20

Drug Content Determination

Each quantity was sonicated with 10mL methanol for 30 min, then filtered and the drug concentration was measured at its λ_{\max} by ultraviolet/visible [UV/VIS] spectrophotometer. The drug content was calculated from equation and the experiment was carried out in triplicate) ⁽¹⁰⁾.

$$\% \text{ Drug content} = \frac{\text{Actual amount}}{\text{Theoretical amount}} \times 100$$

Measurement of particle size and distribution of niosomes

For size and size distribution of niosomes were determined by particle size analyzer. Sample was filled in cuvette carefully and there should be no bubble formation in cuvette. Then, the cuvette was inserted into the instrument and sample was analyzed.

particle size distribution (PDI) was determined by averaging of 10 measurements at an angle of 90° in 10 mm diameter cells at 25 °C. and monitored the light scattering. The average particle size and polydispersity index values were recorded.

The real refractive index and the imaginary refractive index were set at 1.456 and 0.01, respectively. PDI value of less than 0.1 was considered as a homogeneous distribution of

vesicles, while large values that approach 1 indicate a wider globule distribution (11,12).

Separation test of TM niosomal dispersion

To checks the physical stability of the dispersions, centrifugation (10,000 rpm) of selected samples at of niosomal dispersion for 30 min in order to show the resistance niosomal dispersions for the separation ⁽¹³⁾.

Dilution test of TM niosomal dispersion

For more checking the physical stability of niosomal dispersion formulas, aqueous dilution test was carried out by diluting 1ml of each niosomal dispersion formulas to 50 mL, 100 mL and 500 mL with deionized water at 32±0.5°C with 50 rpm stirring speed and observed the turbidity, cracking and phase separation ⁽¹⁴⁾.

pH Determination of TM niosomal dispersions

The pH of TM niosomal dispersion formulas with high %EE and physical stability was determined by using a beaker containing 10 mL of each formula and immersing the electrode of a digital pH meter device in it for two minutes then the results were recorded. this test was carried out to identify the pH of good stable formula.

In Vitro Release of TM Niosomes

For drug release comparison purposes, two milliliter of the formulation was accurately placed inside dialysis bag (MWCO = 12 kDa), which was sealed with a closure clip to prevent leakage. This bag which acts as a donor compartment was positioned in receptor compartment beaker of 100 mL PBS solution pH 7.4 accompanied by gradual stirring (50 rpm) at 37 ± 0.5 °C in order to mimic the in vivo condition. Aliquots were taken at particular time intervals (1, 2, 4, 6, 8, 10, 12, 14, 16, 20, 24 hr) and replaced with fresh buffer to maintain sink conditions. The cumulative amount release of TM (%) was measured by UV/VIS spectrophotometer⁽⁵⁾.

Microscopic examination of TM niosomes

To identify the morphology of the TM niosomes, one drop of niosomal dispersion was taken on the glass slide and observed under projection microscope with 40× magnification and 100× if possible⁽¹⁵⁾.

CHARACTERIZATION OF THE SELECTED FORMULA:

1. Morphology by TEM

One drop of selected niosomal suspension placed on a carbon-coated grid and allowed to dry. and examined with an electron microscope. and photomicrograph was taken at acceleration voltage of 20 KV in different manifestation and different location (100–1000 nm) to get best photo for vesicles⁽¹⁶⁾.

2. Zeta Potential Determination

The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system. The optimized formulation was analyzed for zeta potential value which is related to the stability of vesicular formulations. the test was carried out by using Zetasizer Nano ZS-90 (Malvern Instruments Ltd., UK) at 25°C⁽⁸⁾ (15,16).

3. Compatibility study by ATR-FTIR study

FTIR study with a diamond ATR (attenuated total reflectance) was performed in order to investigate possible interaction of TM with the excipients of niosomes formulation at the molecular level. The pure drug (TM), cholesterol, surfactant, and the optimized formula were scanned by pouring a small amount of each homogeneous sample on the window and scanned over a wave number range of 4000–500 cm⁽¹⁷⁾.

Statistical analysis

The research experimental data documented as mean \pm SD (n=3). A statistical study was performed by analysis of variance (ANOVA) where a p-value less than 0.05 indicates a significant outcome

RESULTS AND DISCUSSION

Determination of melting point

The measured melting point of TM powder was 158-160°C which is in agreement with the documented result which indicates the purity of drug powder.

DSC thermogram. shown in Fig. with the endothermic peak was observed at about 160° that is appeared as sharp characteristic peak. These results were also reported by other published studies corresponds to the melting peak indicating the high purity of the compound (18).

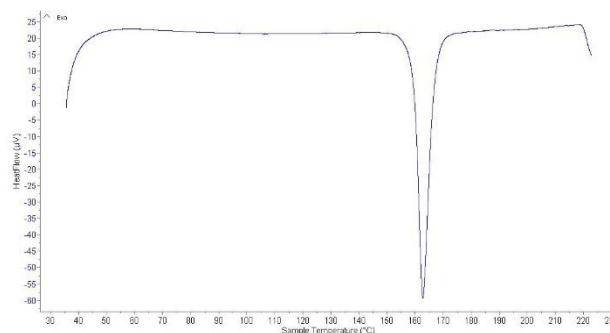


Fig.2. DSC Thermogram of TM

Spectrophotometric analysis

The UV spectrum of TM was obtained with a wavelength of maximum absorption (λ_{max}) at 323 nm in phosphate buffer (pH 7.4) and 312nm in Methanol. Figures (3,4) showed the calibration curves in the involved media. Straight lines were obtained indicating that the calibration curves obey Beer-Lambert law within the range of concentrations used

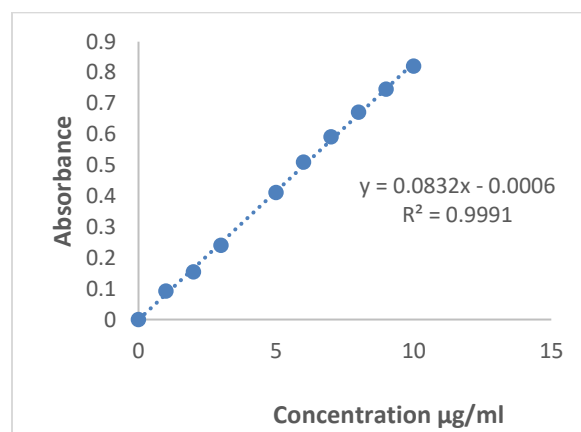


Fig.3: The calibration curve of TM in phosphate buffer pH 7.4

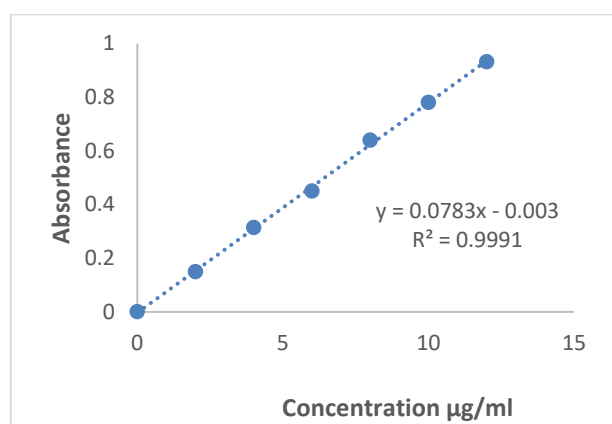


Fig.4: The calibration curve of TM in Methanol

CHARACTERIZATION OF THE PREPARED TM-LOADED NIOSOMES FORMULAS

Visual evaluation

All the samples prepared with span and tween are pale yellowish color because of the color of cholesterol and tolmetin. In addition, the formulas with tween are more milky.

Entrapment efficiency, Drug Content, particle size and distribution of TM niosomes

Table 2: Characterization of niosomal formulations

<i>F.No</i>	<i>%EE</i>	<i>%DC</i>	<i>Mean P.S</i>	<i>PDI</i>
1	80.20± 0.4	96± 0.5	211	0.47
2	81.9± 0.9	97± 0.7	400.6	0.27
3	83.18 ± 1.4	97.7± 0.3	308.6	0.2
4	72.6± 1	98± 0.7	1225	0.83
5	74.7± 0.5	98.6± 0.65	1001	0.24
6	79.16 ± 0.7	96.2± 0.6	326.7	0.26
7	79.4± 1	98.7± 0.5	152.2	0.34
8	78.90± 0.6	98.3± 0.7	161	0.27
9	85.2± 0.3	98.9± 0.3	262	0.6
10	87.4± 0.8	96.9± 0.6	286	0.096
11	75.22± 1.3	98.7± 0.5	111.2	0.107
12	73.80± 1.4	97± 0.5	366.7	0.23
13	82± 0.5	98.2± 0.6	285.5	0.32
14	84.76± 0.6	97± 0.4	356	0.26
15	87.2± 1.2	96.3± 0.9	340	0.4
16	56.4± 1	97.2± 0.7	210	0.1

Separation and dilution tests

All the prepared samples show no sedimentation, creaming or phase separation upon centrifugation, which indicates their high stability⁽¹⁹⁾. And by adding water to niosomal dispersions, formulas remained clear in less than 1 minute with no cracking or separation, further indication that they are all o/w type.

pH Determination of TM niosomal dispersions

all prepared formula was within pH range of 6.2 – 6.7 and this contributed to the drug and excipients used in dispersion of TM niosomes.

Factors affecting niosomal formulations:

1. Type of surfactant

The results in the table (2) showed increasing the entrapped drug in span formulation compared with tween may contributed to increasing the hydrophobicity nature of the span than tween. In addition, the vesicular size of tween20

(F4) was larger than tween40 (F5) and tween80 (F6), and this suggests that when the hydrophilicity of the surfactant increases, the vesicle size increases. Similar results were observed by Ruckmani (20). In addition, the increasing the surfactant HLB value results in the formation of large vesicles diameter of niosomes due to the increased surface free energy according to previous literature (21), and decreasing the hydrophobicity of the surfactant resulted in an increase of the vesicles size, these finding is agreement with previous study (16).

P.D.I of different surfactant formulation were between 0.2-0.8, where F3 and F6 showed PDI value 0.2 and 0.26 respectively and highest %EE so these non-ionic surfactants were selected for other factors experimental studies.

Microscopic examination of TM niosomes

The influence of surfactant types on shape was clearly demonstrated the niosomes of F10 appeared on spherical while F14 appeared as large vesicles with tubular shape as shown in (figure.5).

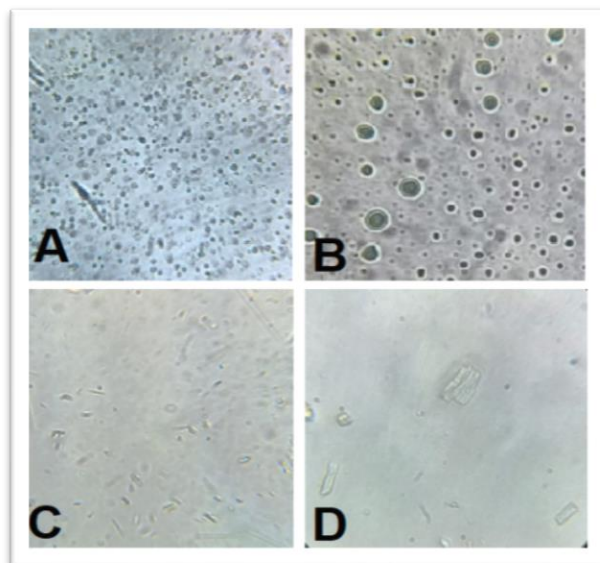


Fig.5: Optical images of niosomal formulation.

A: F10 X40
 B: F10 X100
 C: F14 X40
 D: F14 X100

2. Amount of surfactant

There is clearly noted the effect of surfactant concentration on entrapment efficiency between F3, F9, and F10 in (fig.6) as increasing surfactant concentration will increase the %EE in span80 formulations, and the same finding in comparison between F6,F13 and F14 in tween80 formulations that results may be contributed to increasing

amount of surfactant lead to increase the number of vesicles formed, which consequently leads to a higher volume of the hydrophobic bilayer domain available to house a hydrophobic drug. The impact of surfactant concentration was similar when a low concentration was used to prepare niosomes, a small number of niosomes was obtained, and it was recommended that a higher surfactant concentration may improve drug entrapment ^(22, 23).

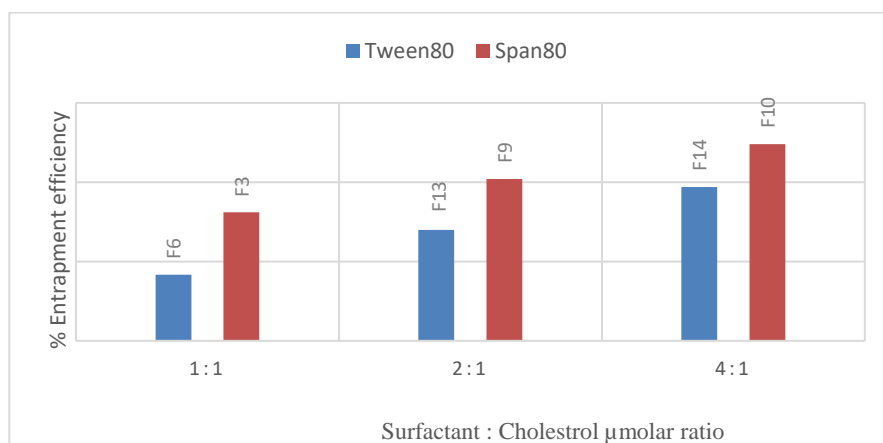


Fig.6: Effect of surfactant amount on percent entrapment of TM

In vitro TM release profile of F3, F9, and F10 in fig (7) shows the effect of increasing span80 in TM release from niosomes, F10 observed best sustained gradually release of

TM. And F6, F13, and F14 TM release profile in fig. (8) shows the effect of increasing amount of tween80 promoting TM releasing rate as a carrier for the drug.

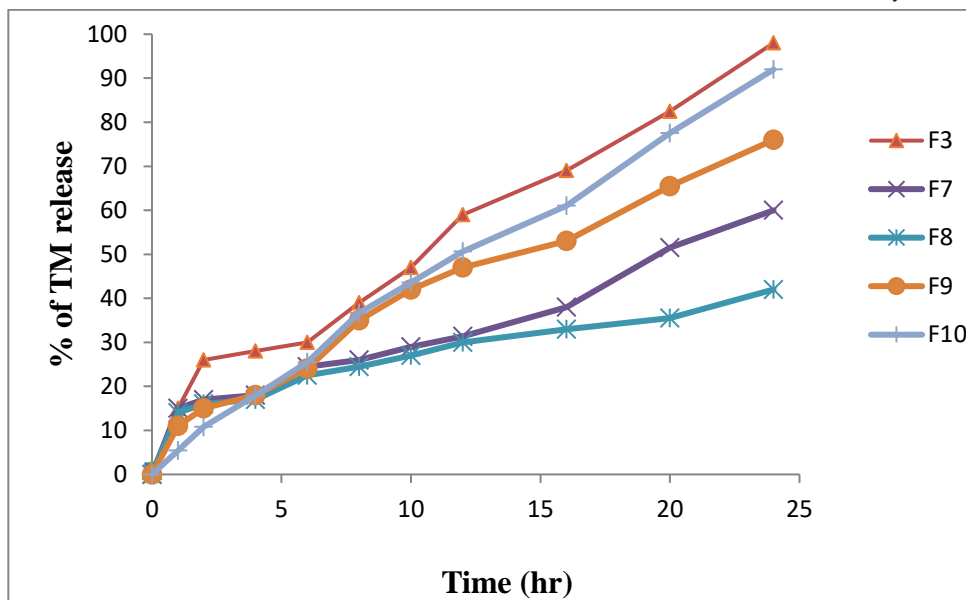


Fig.7: In-vitro release profile of TM niosomal dispersion from different formulations containing span80.

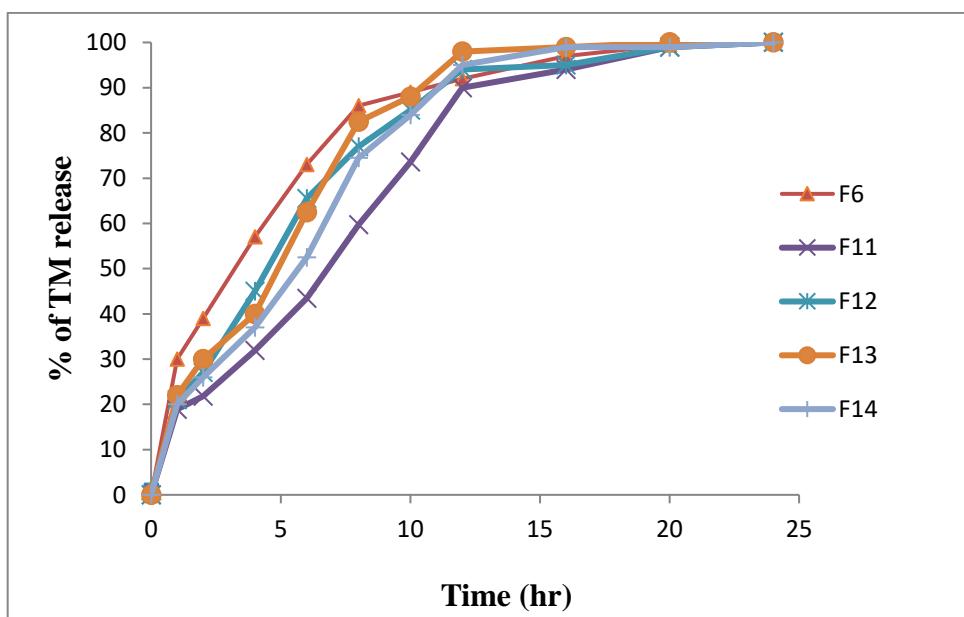


Fig.8: In-vitro release profile of TM niosomal dispersion of different formulations containing Tween80.

3. Effect of cholesterol amount

In the study the effect of cholesterol amounts the results in table (2) of (F3, F7, and F8) and (F6, F11, and F12) shows decrease in the %EE of TM observed when the cholesterol amount was increased during the formulation of TM niosomes as shown in (Fig. 9). This means that the amount of cholesterol taking part in the bilayer niosomal shell so the

encapsulation decreased as the cholesterol amount increased. These results were the same with ⁽¹⁶⁾⁽²⁴⁾. Another study suggested that decreasing the entrapment efficiency with increasing cholesterol ratio above a certain limit may be due to the fact that increasing cholesterol beyond a certain concentration can disrupt the regular linear structure of vesicular membranes ⁽²⁵⁾.

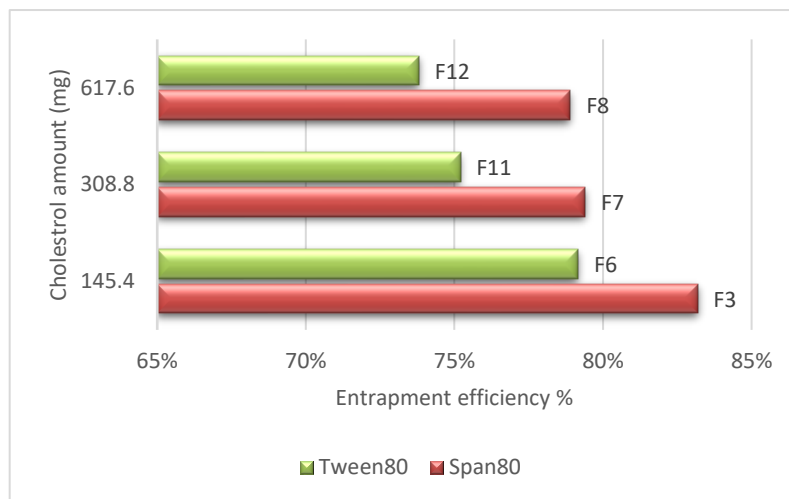


Fig.8: Effect of cholesterol amount on percentage entrapment of TM niosomal formulations

As the cholesterol content increases, also increased the hydrophobicity of bilayer membrane thus increasing vesicles radius in a way to establish more thermodynamic stable form. Rigid structure of bilayer membrane due to cholesterol content also provides resistance to reduce size due to sonication and results in vesicles with bigger size (26).

In vitro release profile of TM niosomes in F3, F9, F10 to study the effect of increasing the amount of cholesterol shows in fig. (4) which significantly decreasing in releasing rate with increasing cholesterol and that because of increasing the rigidity of the niosomes structure that slowing down the TM release from the niosomes. Effect of

increasing the cholesterol in formulation F6, F11, F12 observed the same rigidity effect of cholesterol that may slowing down the TM release by increasing hydrophobicity and stability of bilayers vesicles fig (5).

4. Effect of sonication time

Table (2) shows significantly decreasing in PDI value with increasing sonication time.

But upon sonication 20min the decreasing in %EE observed from 86.4% to 56.4% in F10 and F16 respectively may be contributed to breaking the vesicles structure of niosomes and leakage out the TM.

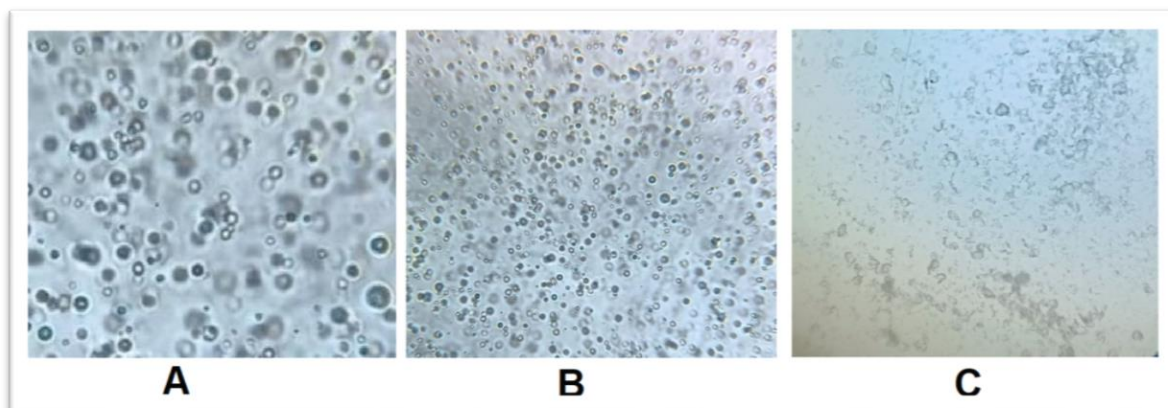


Fig.(9) Optical microscopy of niosomal formulation 40X A: F15, B: F10, and C: F16

Effect of sonication time on F10 microscopic shape also observed in (fig.9) with ruptured non specific shape after 20min sonication in F16 and lost the vesicles structure and

this agreement with the result of in vitro releasing profile in fig (10) which observed fast releasing of F16 and loss the aim of controlling release.

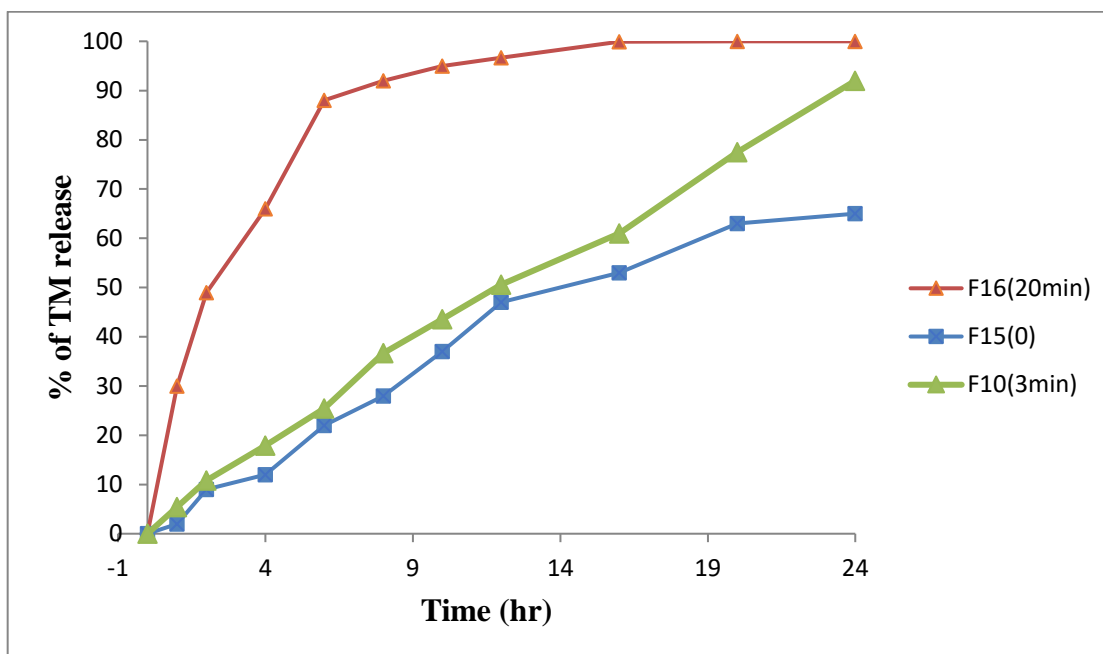


Fig.10 In-vitro release profile of F10, F15, F16

Optimization of TM-Loaded niosomes

According to the obtained results, the optimum in vitro retardation of drug release with homogenous spherical shaped and size distribution and good TM loading efficiency was obtained from F10 prepared using span80 as non-ionic surfactant at surfactant to cholesterol molar ratio of 4:1 and sonication of only 3min

CHARACTERIZATION OF THE SELECTED FORMULA:

Morphology by TEM

It was found that the conventional spherical vesicles were obtained in case of niosomes prepared from Span80 in fig (11). And well separated from each other and in the nano-size, which confirms the results of optical microscopic picture and particle size measurement.

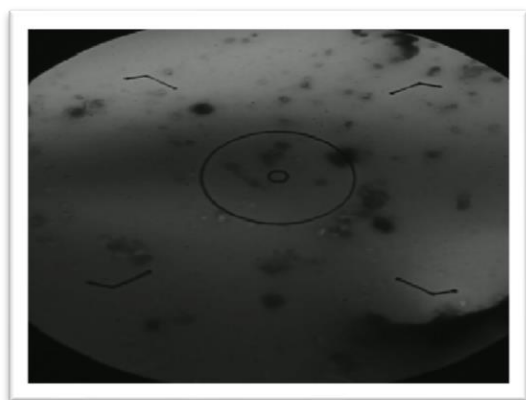


Fig.11: Transmission electron micrographs of F12 dispersions

Zeta Potential

Measurements of zeta potential is another essential index for the stability of niosomes. A high zeta potential absolute value indicates a high electric charge on the surface of niosomes which can prevent aggregation by repulsion force. (Fig.12) show that F10 niosomes possess negative charge (-33.06 mV), This might be attribute to the TM itself and presence of cholesterol⁽²⁷⁾.

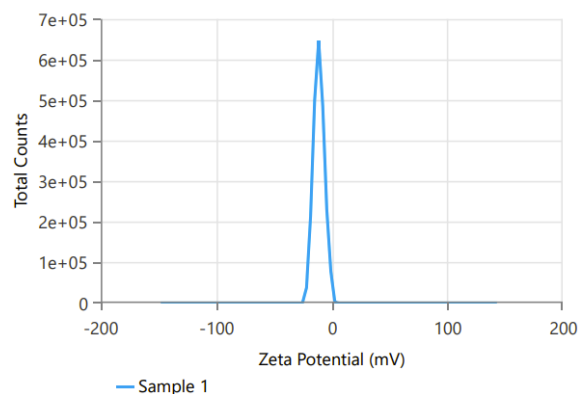


Fig.12: Zeta potential values of F10 niosomal dispersion

ATR-FTIR study

FTIR is for evaluating any possible chemical interaction between TM and any excipient niosomal dispersion preparation. (Figure. 13) showed the ATR- FTIR spectra of the TM, Cholesterol, Span80, and F12 respectively.

The spectrum of untreated TLM showed an intense characteristic peak at 1698.98 cm^{-1} indicates the carbonyl (C=O) group of carboxylic acid. 1267 (C-O), 1616 (C=C), an intense characteristic peak at 1696 cm^{-1} that represents

the ketone carbonyl (C=O) group. and presence of a peak at 3104.03 cm⁻¹ indicates sp² C–H stretching vibrations.

All obtained mean peaks of span80 and cholesterol were also observed in the ATR spectrum of the final formulas, with small decreasing in TM intensity peaks suggesting to small amount of drug and high content of excipients and no new peaks suggesting to no interaction with drug and excipients, and all bands were compared with the reference (28,29).

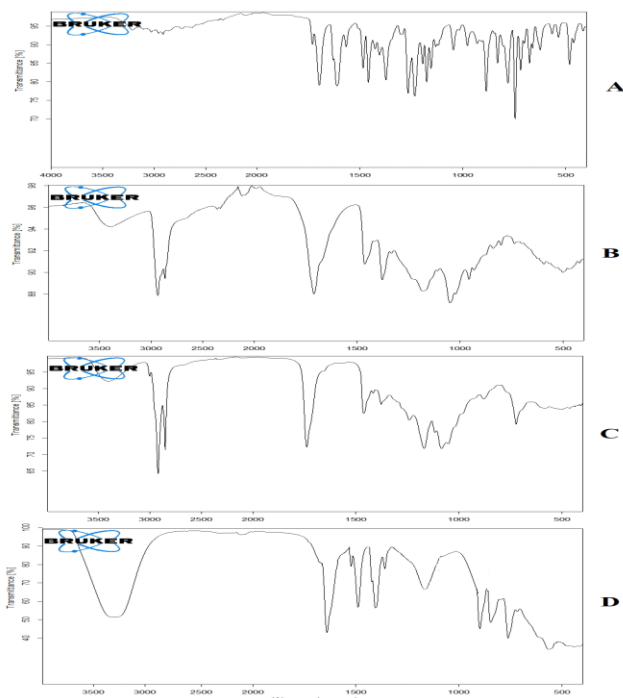


Fig.13 ATR spectrum of A: TM, B: Cholesterol, C: Span80, and D: F12

CONCLUSION

The results from this study indicated that the type and amount of surfactants and amount of cholesterol as well as sonication altered the entrapment efficiency and in vitro release of TM-loaded niosomes. The spherical shape with high entrapment efficiency and accepted sustained release were obtained from the niosomes prepared with Span80 at 1:4 surfactant to cholesterol molar ratio and 3min sonication time. From the present study, it was concluded that niosomes can be used efficiently for enhancing absorption and for sustained delivery of tolmetin.

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