RESEARCH ARTICLE

Efficacy of Gelling Agents on the *In-vitro* Release and Physical Properties of Loxoprofen Sodium Gel Containing Ultra Elastic Vesicles

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Received: 18th Oct, 19; Revised: 22th Nov, 19, Accepted: 15th Dec, 19; Available Online: 25th Dec, 2019

ABSTRACT

Objective: Preparation of a topical prolong release gel for loxoprofen sodium using optimized nano-sized transfersome dispersion using different gelling agents with in vitro and in vivo evaluation in comparison to the marketed gel prepared by a conventional method.

Method: The optimum nano transfersome dispersion containing 5% egg lecithin (as an oil phase) 1% span 60 (as an emulsifying agent) was converted into a gel using different gelling agents including carbopol 974p, carbopol 940, carbopol 934 and hydroxyl propyl methylcellulose (HPMC K100) at ratio of (1:1). Each prepared gel was then evaluated in vitro to determine the homogenisity, consistency, spreadability, viscosity, and in vitro drug release as well as skin permeation test, human skin irritation test, and in vivo effectiveness.

Result: The selected gel formula (TG3) showed best homogenisity, consistency and spreadability as well as it produced initial burst release of 60.3% (within 1 hour) followed by the prolonged release of 95.4% continued for 6 hours. In addition, the optimum gel formula (TG3) has shown remarkable prolong release, which was significantly higher than the marketed gel (loxonin® gel 1%) without any sign of skin irritation with better effectiveness

Conclusion: This work succeeded in preparing topical gel for loxoprofen sodium using carbopol 974p as gelling agent and utilizing ultra elastic lipid vesicles (transfersomes) with prolong release that reduces dose frequency and improves patient compliance

Keyword: Gel, Loxoprofen sodium, Nanoparticle, Topical application.

International Journal of Drug Delivery Technology (2019); DOI: 10.25258/ijddt.9.4.26

How to cite this article: Maraie, N.K. and Kadhium, H.S. (2019). Efficacy of Gelling Agents on the *In-vitro* Release and Physical Properties of Loxoprofen Sodium Gel Containing Ultra Elastic Vesicles. International Journal of Drug Delivery Technology, 9(4): 671-677.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The topical application of active pharmaceutical ingredients to the skin gave many advantages over the oral and intravenous administration by avoiding the systemic action throughout the localization of drug in one area. Various dermatological products can be applied to the skin, which may vary in their consistency. The most widely used topical preparations are semisolids. They are used for their local effect at the site of application by permeation of a drug into the underlying layer of skin or mucous membrane. Also, topical preparations may be formulated to give sustained local effect with minimum or without any systemic drug absorption.[1] A gel is one of the best dosage forms within the semisolid preparations, which is widely used both in cosmetics and pharmaceutical preparations. Gels preparations, in general, provide faster drug release compared with conventional topical preparations due to high water content, which provide better dissolution of the hydrophilic drug, but the problem is drug penetration through the skin, and this can be overcome through the application of much-advanced technology including ultra elastic lipid vesicles. A gelling agent is classified according to its nature to natural like xanthan gum, semisynthetic, and synthetic like carbopol.[2] The aim of this work, is to study the effect of using different gelling agents to convert the optimized nano transfersome dispersion prepared in our laboratory to a suitable gel with fast onset of action and long duration that can reduce dose frequency and improves patient compliance.

MATERIALS AND METHODS

Materials

Loxoprofen sodium was purchased from A-cathei bureau, carbopol 974p was purchased from Lubrizol Advanced Material, Belgium, triethanolamine was purchased from BDH, England, loxonin® gel, HPMC, potassium dihydrogen

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phosphate, sodium hydroxide, carbopol 943, carbopol 940, were purchased from Himedia, India, egg lecithin was purchased from, span 60 was purchased from Sinopharm Chemical Reagent Co.LTD, China, deionized water.

Preparation of the nano transferosomal gel from nano transferosomal dispersion

The optimized nano transfersomal dispersion prepared in our laboratory containing 5% egg lecithin (as lipid), 1% span 60 (as edge activator) and 1% loxoprofen sodium (as a model drug) by thin-film method[3] was converted into gels using different gelling agents (TG1-TG4) in ratio (1:1) as shown in Table 1 This was accomplished by adding the gel base to the transfersomal dispersion in a ratio (1:1) then mixed together using magnetic stirrer and then by spatula until smooth homogenous gel was obtained.[4,5]

Preparation of the gel base

The preparation of gel bases was done in an accurate and precise way using the following gelling agents:

- *Carpobol 974 p gel, carpobol 940 gel, carpobol 934 gel:* 0.5 gram of each C974 p,C934, C940 powder was dispersed in 100 mL deionized distilled water and continuous stirring at moderate speed, and then pH was changed to (6 to 6.5) by addition of few drops of triethanolamine.
- *HPMC K 100 gel:* It is prepared by dispersing 2.5 g HPMC powder in 100 ml deionized distilled water 100 mL heated to (75°C) with constant mechanical stirring at a moderate speed and then the dispersion was cooled down and left overnight.[5]

Characterization of the prepared nano transfersomal gels

Physical appearance

All the prepared nano transferosomal gel formulations (TG1-TG4) were inspected visually for their homogeneity, color, grittiness, consistency and phase separation.[6]

pH determination

The pH of all nano transfersomal gel formulations (TG1-TG4) was determined using pH-meter and done by positioning the tip of the electrode inside the nano transfersomal gel and the result was recorded after two minutes.[7]

Viscosity Studies

The viscosity of all formulas (TG1-TG4) was carried out with Brookfield digital viscometer, by using spindle number S-64. A specific weight (30 g) of the sample was put in a glass container and then the viscosity measured at different rates (2.5, 5, 10, 20, 30, 50, 60, 100 rpm), the temperature was maintained at 25° C and at 37°C. The viscosity was read directly after 30 seconds.[8]

Table 1: Composition of nano transfersomal gel formulas prepared using the optimized nano transfersomal dispersion formula

Formula code	*Gelling agent*	*Ratio*
TG1	Carbopol 934	1:1
TG2	Carbopol 940	1:1
TG3	Carbopol 974p	1:1
TG4	HPMC K100	1:1

Spreadability studies

A sample of (1 g) of each formula (TG1-TG4) was put between two glass slides then 500 g weight was applied and left for about (five minutes) when no further spreading was expected. Diameters of spread circles were marked and measured in (cm) and compared with the initial circle diameter (diameter of the spread circle that has been made before the application of the weight).[9,10]

Drug content

One gram of nano tranferosomal gel was added in 100ml of ethanol and placed in a water bath sonicator for 2 hours and subjected to centrifugation for 15 minutes at 3000 rpm then filtered by 0.22 µm millipore filter and examined by UV spectroscopically at 223nm.[11]

Extrudability test

The extrudability of loxoprofen sodium of all nano transfersomal gel formulas (TG1-TG4) was determined by measuring the weight that needed to extrude the gel from a syringe and subtracted from weight needed to extruded syringe when it was empty. The syringe had an opening tip of 5 mm.[12]

Extrudability = Weight to extrude gel from the syringe (in gm)–weight to extrude the empty syringe/Area (in cm^2)

In vitro release of the drug from the prepared nano transfersomal gel formula (TG1-TG4)

The release of loxoprofen sodium from the prepared nano transfersomal gel formulas (TG1-TG4) was done using dialysis membrane (MWCO 2000 Da) and rotating paddle dissolution apparatus type II and applying the same procedure explained using phosphate buffer pH 7.4

Selection of the optimum nano transferosomal gel formula

The selection of the optimum formula (TG3) was done according to its best spreading coefficient property, consistency, viscosity, drug content, and drug release profile.

Drug and excipient compatibility study by fourier-transform infrared spectroscopy (FTIR)

The compatibility between pure drug (loxoprofen sodium) and other excipients (surfactant and lecithin) was recorded using the FTIR spectrophotometer. For the pure drug, KBr disk was used while for formula (TG3) liquid cell was applied by dripping several drops of the sample onto NaCl or KBr aperture plate and then sandwiching it under another aperture plate, so that no gas bubbles are trapped. The thickness can be adjusted according to the sample absorbance by appropriately tightening the screws or by inserting spacers between the aperture plates; then it was analyzed by FTIR spectroscopy from 4000 to 400 cm^{-1}.[13]

Human skin irritation test

The irritation test was done on human volunteers, twenty volunteers were selected, and 1.0 g of formulated gel (TG3) was applied on an area of 5 cm^2 to the back of the volunteer's hands and left for 6 hours then the same procedure was repeated by daily application of the formula for a week. The volunteers were examined for any irritation sign.[14]

Skin permeation test of the selected formula TG3

The selected nano transfersomal gel (TG3) was characterized for drug permeation study by using skin mouse and that was done by taking a piece of skin, that was sunken before in formalin and stored in refrigerator at 4°C then put it in paddle dissolution apparatus type II using 500 mL phosphate buffer pH 7.4 and at 37°C temperature, samples were withdrawn every half hour for 6 hours and then analyzed spectroscopically at 223nm.[15]

In vivo evaluation

The optimized gel formula (TG3) was given to six patients suffering from pain in back and arm once daily for 3 days and the follow up of the patients continued two weeks after treatment cut.

Comparison of in vitro release and pH between the selected gel formula (TG3) and the commercially marketed loxonin® gel

The prepared nano transfersomal gel for loxoprofen sodium (TG3) was compared with the marketed loxoprofen sodium gel also containing 1% drug (prepared by conventional method). The comparison included in vitro drug release in phosphate buffer pH 7.4 and phosphate buffer pH 5.5 each one separately. The pH of the selected formula (TG3) and the marketed formula was also recorded

Effect of temperature on the physical properties of the selected formula TG3

The optimum formula was stored at 40 °C, 25 °C and in a refrigerator at 4 °C for 1-month duration according to the International Council for Harmonisation (ICH) guidelines. Samples were then withdrawn and tested for physical appearance, pH, drug content.[16]

RESULT AND DISCUSSION

Characterization of nano transfersomal gel formulas

Physical appearance

All the prepared formulas (TG1-TG4) appeared as a white homogenous creamy gel with no grittiness. The results showed that carbopol containing formulas (TG1-TG3) were thicker than HPMC based formulas (TG4) because HPMC is more hygroscopic, and it is less viscous than carbopol.[17]

pH determination

The pH values of all prepared formulas (TG1= 6.37 ± 0.152, TG2= 6.56 ± 0.11, TG3 = 6.73 ± 0.057 and TG4 =7.35 ± 0.05) and this matches with skin requirements for topical preparations to avoid skin irritation.[18,19]

Viscosity studies

The viscosity of all the nano transfersomal formulas (TG1-TG4) were measured at 37°C and 25°C, respectively (as shown in Table 2). The results showed that the values of viscosity at 37°C is lower than at 25°C because elevated temperature increasing the energy dissipation movement of the molecule, or decreasing the intermolecular interactions, which in turn decrease the interference of the hydrodynamic domain.[20] All formulas possessed a pseudoplastic flow (the apparent viscosity or consistency decreases instantaneously with increase in shear rate), because as the shear stress was increased, the normally disarranged molecules of the gelling material were caused to align their long axes in the direction of flow, such orientation reduced the internal resistance of the material and hence decreased the viscosity. Formula containing carbopol 974p(TG3) had significantly ($p < 0.05$) lower viscosity than formula containing carbopol 940 (TG2)

Table 2: Values of the viscosity of nano transfersomal formulas gel at temperature 37°C and temperature 25°C, values are mean ± SD (n = 3) upon application of different shear stress

At 37 °C				
Speed(rpm)	*TG1 ± SD*	*TG2 ± SD*	*TG3 ± SD*	*TG4 ± SD*
2.5	35800 ± 1.5	19200 ± 7.4	11800 ± 12.5	2400 ± 7.5
5	25700 ± 1.5	14200 ± 9.8	7000 ± 1.2	1200 ± 4.8
10	16500 ± 5.5	9240 ± 5.6	4200 ± 3.2	780 ± 4.5
20	10530 ± 4.8	5490± 4.5	2580 ± 4.2	510 ± 2.3
30	8140 ± 4.8	4040 ± 4.5	1940 ± 7.5	460 ± 2.5
50	5570 ± 5.2	2700 ± 3.2	1390 ± 7.5	400 ± 1.5
60	4809 ± 9.2	2399 ± 1.5	1220 ± 6.3	390 ± 1.4
100	3293 ± 8.2	1600 ± 1.5	900 ± 5.2	348 ± 1.2
At 25 °C				
Speed(rpm)	*TG1 ± SD*	*TG2 ± SD*	*TG3 ± SD*	*TG4 ± SD*
2.5	38600 ± 9.6	20250 ± 0.8	12300 ± 6.8	2608 ± 5.3
5	25890 ± 3.9	14650 ± 9.1	7800 ± 0.2	1306 ± 8.2
10	17100 ± 4.7	9287± 1.8	4920 ± 4.1	850 ± 2.9
20	10740 ± 0.8	5523± 1.1	2960 ± 10.2	620 ± 3.8
30	8350 ± 1.4	4200 ± 2.9	2840 ± 8.5	492 ± 5.1
50	5678 ± 2.4	2985 ± 4.8	1420 ± 4.5	486 ± 4.6
60	4918 ± 6.5	2578 ± 9.6	1555 ± 2.8	430 ± 2.1
100	3874 ± 8.2	1820 ± 1.5	1080 ± 5.2	368 ± 1.2

which showed significantly ($p < 0.05$) lower viscosity than formula containing carbopol 934 (TG1), while the viscosity of carbopol 974p is higher than carbopol 940 which had higher viscosity of carbopol 934,[21] but due to the interpolymer interaction between nonionic surfactant (span 60) and polymer (carbopol) that lead to change in internal viscosity of gel.[22,23] All formulas containing carbopol TG1, TG2, and TG3 showed higher viscosity than HPMC based formula (TG4) due to the higher hygroscopicity of cellulose derivatives in comparison to carbopol.[24]

Spreadability studies

In general, the spreadability is a significant characteristic of topical formulations efficacy. It indicates that the formulas are easy to be spread by a small application of shear and it shows the behavior of gel when it comes out from its tube. In general, there is a relationship between the spreadability of gel and firmness, time of shear, rate produced upon smearing, and the viscosity as well as the temperature of the formulation.[25] The results (TG1 = 1.8 cm ± 0.25, TG2 = 2 cm ± 0.28, TG3 = 2.5 cm ± 0.35, TG4 = 3 cm ± 0.4) showed that the most viscous gel (TG1) had the lowest spreadability and the lowest viscous gel (HPMC based gel TG4) had the highest spreadability. Although all the prepared formulas showed suitable spreadability and agreed with reported data.[26]

Drug content

The drug content of all nano transfersomal gel formulas (TG1= 88.2%± 1.9, TG2 = 86.6% ± 2.8, TG3= 98.13% ± 4.2, TG4 = 102.6% ± 4.5), indicating high adequacy of the preparation method and high content uniformity of the prepared formulas.

Determination of extrudability of nano transfersomal gel formulas

The good extrudability values (TG1= 400 g/cm^2 ± 2.5, TG2 = 380 g/cm^2 ± 7.8, TG3=320 g/cm^2 ± 3.2, TG4 = 300 g/cm^2 ± 5.4) mean its contents can be easily extruded from the tube by simple pressure of the hand's finger. The result showed a significant difference where the more viscous and thicker formula (TG1) needed more weight to extruded, and the less viscous formula (TG4) needed less weight to extruded, this was due to the relationship between viscosity and extrudability as agreed with reported data.[27]

In vitro release of drug from the prepared nano transfersomal gel formulas (TG1-TG4)

The results showed (Figure 1) that the release of drug from formula TG1 and TG2 was significantly ($p < 0.05$) more prolong than TG3 and TG4 because the viscosity of formulas TG3 and TG4 was lower than the viscosity of TG1 and TG2 and in general viscosity of TG4 is less than TG1, TG2 and TG3 due to the hydrophilic nature of cellulose derivatives.[28,29]

The drug release from TG3 and TG4 formulas gave 95.4% and 91% respectively after 360 minutes (6 hours), while the formulas TG1 and TG2 gave prolong drug release 87% and 92.7% respectively after 660 min (11 h), this release pattern showed the significantly effect of viscosity on the % release and the duration.

Selection of optimum formula

Among the four formulas (TG1-TG4) and according to the results obtained such as prolong release of dug 95.4% after 360 minutes (6 hours), drug content (98.13%), good spreadability, consistency, viscosity and other physical properties TG3 formula was selected as the optimum formula for nano transfersomal gel for loxoprofen sodium and subjected for further work.

Drug and excipient study by Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of loxoprofen sodium in formula TG3 is shown in Figure 2 together with the physical mixture of formula content and as well as FTIR of pure loxoprofen sodium in comparison to reported spectrum of loxoprofen sodium. The formula TG3 showed the main characteristic peaks present in

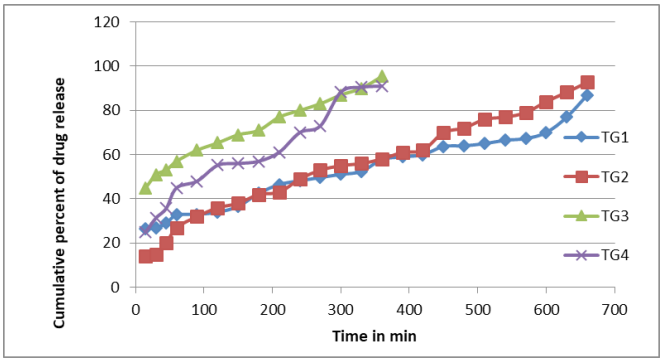

Figure 1: In vitro release profile of loxoprofen sodium from nano transfersomal gel Formulas (TG1, TG2, TG3, and TG4) in phosphate buffer solution (pH 7.4) at 37° C, the values are mean ± SD (n = 3)

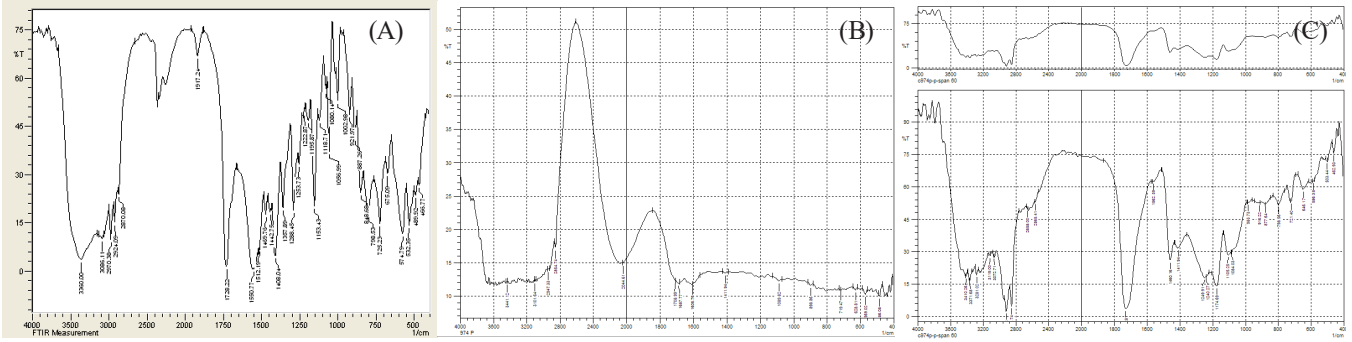

Figure 2: FTIR spectra of (A) pure loxoprofen sodium, (B) selected nano transfersomal gel (TG3), (C) physical mixture

pure drug (Table 3) as well as the physical mixture but with less intensity indicating the uniformity and compatibility of the drug with excipients.

Human skin irritation test for the selected formula TG3

The results showed that there were no signs of irritation on the skin like edema, erythema and ulceration after the application of the optimum formula (TG3) and monitoring of the irritation signs during the 6 hours application and after 24 h of washing away. Also, there were no irritation signs upon daily application for a week, indicating no irritation observed upon single and repeated applications and no sensitivity reactions of the skin, indicating the suitability of the selected formula for skin application.[30]

Skin permeation test of selected formula (TG3)

In vitro permeation of optimum formula TG3 through mouse skin by dissolution apparatus type II showed the maximal permeation of loxoprofen sodium from TG3 was 98.2% after 360 min (6 hours) as shown in figure 3. The high permeation of the drug through the skin may be due to increased association of the drug with the lipid bilayer of transfersomal vesicles, which had ultra flexibility and ultra deformability that permitted the permeation of intact vesicles containing the drug through that may improve drug activity.[31]

In vivo evaluation test

The six patients that were given formula TG3 (containing 1% loxoprofen sodium) once daily showed pain relief within the first day of application and no pain observed during the period of study and no pain observed after 2 weeks of cut off treatment. This proving the fast onset of action of the prepared gel and its prolong duration with reduce dose frequency.

Comparison of in vitro release and pH between the selected gel formula (TG3) and the commercially marketed loxonin® gel

The comparison between TG3 and the commercial loxoprofen sodium (loxonin®) gel (prepared by conventional method) pH measurement and in vitro drug release. The results showed that there was a significant difference ($p < 0.05$) in the pH of TG3 and loxonin gel, as shown in table 4.

The results also showed (figure 4) that loxonin® gel gave 98% of drug released after 120 minutes (2 hours) in both phosphate buffer pH7.4 and pH 5.5 while TG3 formula gave 95.4 % drug release that continue for 360 min (6 hours) in phosphate buffer pH 7.4 and 90.8% drug release for 360 min (6 h) in phosphate buffer pH 5.5 indicating that the ourtransfersomal technology used in the preparation of TG3 formula gave a suitable drug diffusion and permeability that prolong the drug release up to 6 hours that may decrease frequency of doses and improves patient compliance.

Effect of temperature on the physical properties of the selected formula TG3

The effect of temperature on the selected nano transfersomal gel formula (TG3) was studied regarding its physical appearance, drug content, and pH after storage at three different temperature

Table 3: The characteristic absorption bands of pure loxoprofen sodium by FTIR

Characteristic absorption bands groups	*Pure loxoprofen sodium*
C–H bands of the aromatic ring	3086 cm^{-1}
Peak of carbonyl stretching of carboxylic acid	1728 cm^{-1}
CH_2 streching vibration	2870 cm^{-1}
The carbonyl stretching of cyclopentanoe	1730 cm^{-1}
C-H bending	1410 cm^{-1}

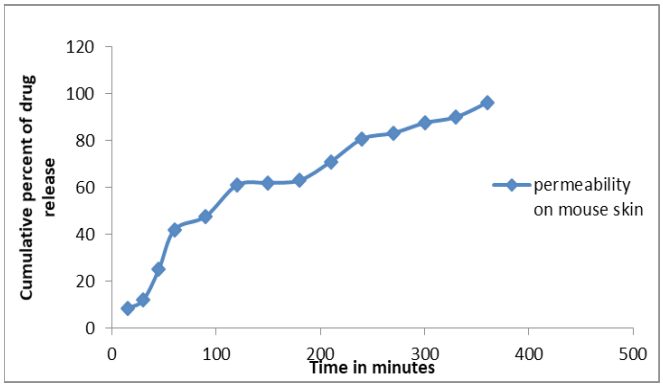

Figure 3: In vitro permeation of loxoprofen sodium from nano transfersomal gel using mouse skin in phosphate buffer solution (pH 7.4) at 37°C, values are mean ± SD (n = 3)

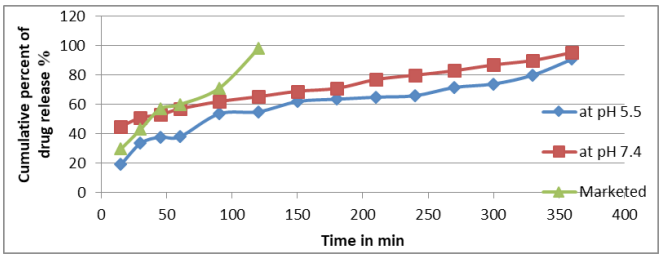

Figure 4: Dissolution profile of the selected nano transfersomal gel (TG3) loxoprofen sodium in phosphate buffer in (pH 7.4 and pH 5.5) and marketed loxoprofen sodium at temperature 37 °C

Table 4: Comparative of pH values between marketed and TG3, values are mean ± SD (n=3)

	TG3 ± SD	*Loxonin® gel ± SD*
pH	6.73 ± 0.057	6.41± 0.03

Table 5: Stability study for the selected nano transfersomal gel Formula (TG3) under accelerated condition (40°C, 25°C and at refrigerator 4°C after 1 month)

Parameters	*25 °C ± SD*	*4 °C ± SD*	*40 °C ± SD*
pH	6.62± 0.05	6.67 ± 0.4	6.58 ± 0.2
Drug content	97.1%	97.8% ± 0.5	96.45% ± 1.2
Physical appearance	+++ good	+++ good	++ good

40°C and in a refrigerator at 4°C for a 1-month period. The results of the stability studies are shown in Table 5. The result showed that no significant difference in the appearance of the prepared TG3 formula, pH and drug content upon storage of the formula at three different temperatures that indicate the suitability of the TG3 formula for storage on a refrigerator, and different room temperature (25°C and 40°C)[32].

CONCLUSION

This study succeeded to prepare topical gel using nano ultra deformable liposomes (transfersomes) utilizing natural lipid (egg lecithin) and carbopl 974p as a gelling agent that gave a sustained release of loxoprofen sodium up to 6 hours with better effectiveness and patient compliance improvement in comparison to the conventionally prepared and marketed loxprofen sodium.

ACKNOWLEDGMENT

The authors would like to thank Mustansiriyah University (www.uomustansiriyh.edu.iq) Baghdad-Iraq for its support in the present work.

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