ORIGINAL ARTICLE

Formulation and Assessment of Stability Parameters for Acitretin-Loaded NLC Gel

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Abstract

Purpose: The present investigation intended to develop a lipid-based nanocarrier for topical delivery of acitretin an analog of Vitamin A used in the management of psoriasis by mixing suitable lipids and blends of surfactants and to assess stability parameters for various formulations as per regulatory guidelines. **Methods:** Nanostructured lipid carriers of acitretin were prepared by the hot homogenization method. Formulations were prepared by varying the concentrations of surfactants (Tween 80 and sodium lauryl sulfate). Prepared formulations were subjected to accelerated stability testing for 6 months the stability samples were evaluated for color, clarity, homogeneity, viscosity, pH, and zeta potential. **Results:** Higher stability was observed in the F3G formulation. **Conclusion:** Higher stability in F3G formulation might be attributed to the presence of surfactants in optimum concentration.

Key words: Clarity, homogeneity, stability, surfactants

INTRODUCTION

New generation nanocarriers have been developed that are prepared using blends of different lipids (solid and liquid lipids). The combination of lipids with different properties provides a nanostructure that has several advantages such as improved drug loading and prevention of drug leakage while storage.[1-3] The topical route of drug administration implies that the formulation is applied on the surface of the skin, and both the application location and pharmacodynamic effects are thereof observed locally. NLC offers vivid advantages such as increased skin hydration, enhancement of skin penetration of drug, increased skin occlusion, and enhanced ultraviolet protection through topical route.[4] Stability testing of the formulation helps determine the shelf life of the product and helps in estimating the expiration date. The test for stability includes a set of complicated procedures that involve both time and cost but provides considerable scientific support for the safety and efficacy of drug products.[5] With the pace of time, the pharmaceutical preparations change pH, viscosity, consistency, color, particle size distribution, etc., that may arise as a result of temperature fluctuations, stress, abrasion, and vibrations. Alterations in the physical, chemical, and microbiological properties of formulation end in the degradation of the product, loss of potency of the active ingredient, and diminished activity of excipients such as preservatives and antioxidants.[6] The importance of stability testing lies in the fact that the majority of drugs degrade into non-efficacious by-products and ultimately may result in up to 80% efficacy of formulation which can be of major concern for well-being of the patient. The studies involve testing of those characters of formulation that changes over the pace and can influence the quality, purity, identity, efficacy, potency, and safety of the drug.[7] Surfactants such as Tween-80 and sodium lauryl sulfate are used in pharmaceutical formulations to impart stability and to prevent the phenomena of coalescence. At optimum concentration, surfactants result in decreasing the tension that exists between the two phases and make the system stable.[8]

MATERIALS AND METHODS

Materials

ACT was obtained as a gift sample from Sun Pharmaceutical Industries Limited, Vadodara, India. Oleic acid and stearic

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acid were purchased from SD Fines Chemicals Ltd. (Mumbai, India). Tween 80 and sodium lauryl sulfate (SLS) were procured from CDH Pvt. Ltd. (Delhi, India).

Methods

Fabrication of NLC loaded with ACT

Figure 1 presents the schematic preparation of NLC formulation. The hot homogenization method was employed for the formulation of ACT-loaded NLC. Stearic acid and oleic acid as solid lipid and liquid lipid were used in 3:7 ratios along with Tween 80 and SLS as surfactants as per Table 1. Stearic acid was heated at temperatures 5–10°C ahead of its melting point. To the molten solid lipid phase, liquid lipid was added with continuous stirring. The blend of surfactants was added with continuous stirring over a magnetic stirrer. The drug was added into the same and hot pre-emulsion obtained was subjected to homogenization at 85°C. The process of homogenization was repeated to obtain nanoemulsion of a satisfactory size range. Obtained nanoemulsion was cooled down. During the cooling process; lipid droplets of the nanoemulsion recrystallized and formed NLC.[9] The obtained NLC was permuted to gel using Carbopol in a suitable concentration.

Stability studies

Stability studies were performed in a stability chamber (Remi CHM 6 Plus, India) capable of maintaining the temperature and humidity in the range of ±1°C and ± 3%, respectively. The chamber was set at a temperature of 40°C and 75% RH over 6 months.

Method for stability studies

About 1 g of each formulation was placed in five transparent vials separately marked as F1G, F2G, F3G, F4G, and F5G, respectively. The vials were tightly screwed and placed in a stability chamber at 40°C and 75% RH over 6 months.[10] Samples were withdrawn from respective vials and evaluated for different stability parameters.

Macroscopic analysis of formulations

The prepared formulations were investigated visually for color, clarity, and homogeneity. All macroscopic evaluations were performed using natural light as a source of illumination.[11]

Viscosity measurement

Viscosity measurement of all formulations was performed at 40°C ± 2°C at different sampling intervals as per the guidelines for accelerated stability testing.[12,13] Viscosity was ascertained by utilizing Brookfield viscometer (Brookfield DV-II + Pro) using spindle number RV 6 at the different shear rate at 40°C ± 2°C.[14]

pH measurement

The pH of ACT incorporated NLC gel was determined using a digital pH meter (Mettler Toledo).

Zeta potential

The electrokinetic potential was determined at regular sampling intervals as per guidelines for stability testing. Zeta potential of prepared NLC gel was adjudicated using Malvern Zetasizer (Malvern Zetasizer, Worcestershire, UK). The study was performed after diluting the sample with deionized water. Dilution of the samples was performed to avoid multiple scattering phenomena.[15]

RESULTS

ACT-loaded NLC gels were appraised for their macroscopic characters such as clarity and homogeneity. Viscosity measurement, pH estimation, and determination of zeta potential were employed to ascertain the stability of formulated gels overtime a period of accelerated stability studies (ICH guidelines: 40 ± 22°C and 75 ± 5% RH). Formulation F3G was found to be persistent; the characteristics of the formulation remained congruous throughout the conduct of studies.

Macroscopic analysis of formulations

The prepared formulations were assessed for diverse parameters such as color, clarity, and homogeneity at the 0, 3rd, and 6th months. Table 2 exhibits the results of varied evaluation parameters for ACT-loaded NLC gel. Formulation

Table 1: Formulation chart for ACT-loaded NLC

Formulation code	Drug (mg)	Solid lipid (parts)	Liquid lipid (parts)	Tween 80 (parts)	SLS (parts)	Carbopol 940p (%)
F1G	20	3	7	1	1	1
F2G	20	3	7	2	1	1
F3G	20	3	7	3	1	1
F4G	20	3	7	1	2	1
F5G	20	3	7	1	3	1

SLS: Sodium lauryl sulfate

F3G manifested the desirable characters vital for stable formulation during the tenure of stability studies.

Viscosity measurement

The viscosity of gel reflects the consistency of formulation.[16] Viscosity is measured for quality control and pharmacopoeial regulations during the development of the product and also controls the rate of diffusion of the drug from the formulation. Viscosity was determined at minimum and maximum shear rates. Minimum rate of shear is used to reflect viscosity at rest, for example, on shelf while viscosity at maximum shear rate reflects viscosity during the manufacturing process.[15] Viscosities of formulations at a minimum and maximum shear rate are tabulated in Table 3. Formulation F3G proclaims minimum undulation in viscosity during the extent of the stability study. The commensurate viscosity of F3G formulation at various time intervals at the different shear rates is depicted in Figure 2.

pH measurement

The pH of all prepared formulations is expressed in Table 4. pH of F3G was found to be in the range of 5.5 ± 0.1. It is stipulated that the formulation F3G retains its pH and is stable over the period and worth for topical application.

Zeta potential

Zeta potential is an abbreviation of the electrokinetic potential of a colloidal system.[17] The value of zeta potential is related to the short-term and long-term stability of the system. Table 5 shows the changes in the zeta potential of formulations during storage at different time intervals. Figure 3 depicts the zeta potential of F3G formulation during different periods of the stability study. Figure 4 demonstrates the graphical presentation of various formulations at different times of stability study.

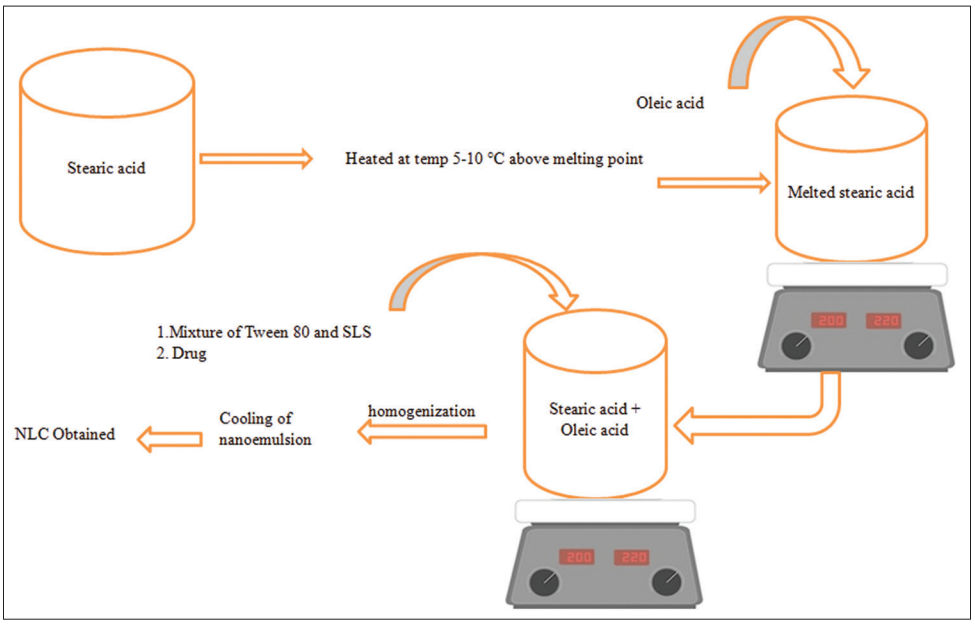

Figure 1: Schematic presentation of NLC formulation

Table 2: Macroscopic analysis of gels at the 0, 3rd, and 6th month

Code	Clarity/color			Homogeneity		
	0 month	3rd month	6th month	0 month	3rd month	6th month
F1G	Transparent/ light yellow	Transparent/ light yellow	Transparent/ light yellow	Homogenous	Separation of phases	Separation of phases
F2G	Transparent/ light yellow	Transparent/ light yellow	Transparent/ light yellow	Homogenous	Homogenous	Separation of phases
F3G	Transparent/ light yellow	Transparent/ light yellow	Transparent/ light yellow	Homogenous	Homogenous	Homogenous
F4G	Transparent/ light yellow	Transparent/ yellow	Opaque/dark yellow	Homogenous	Separation of phases	Separation of phases
F5G	Transparent/ light yellow	Opaque/dark yellow	Opaque/dark yellow	Homogenous	Separation of phases	Separation of phases

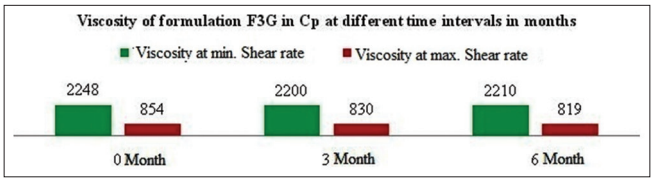

Figure 2: Viscosity of formulation F3G

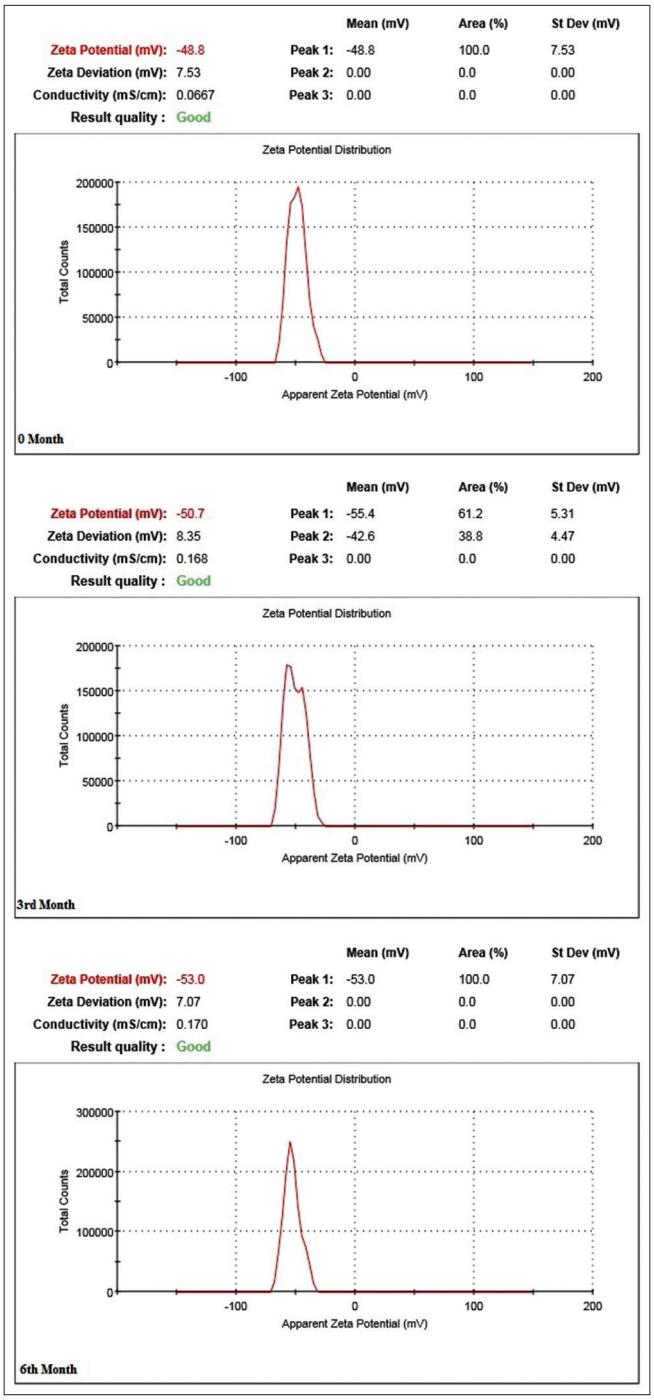

Figure 3: Zeta potentials of F3G formulation at the 0, 3rd, and 6th month

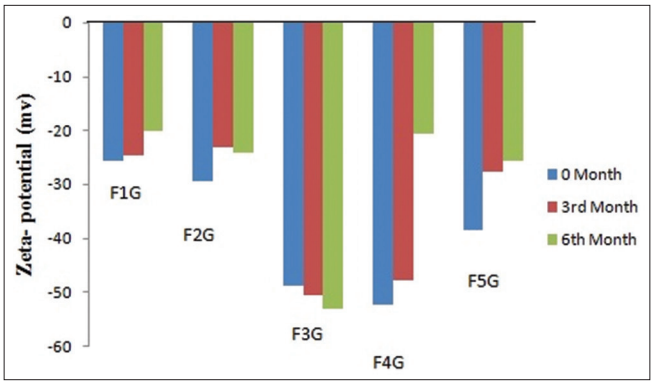

Figure 4: Zeta potential of various formulations at different period

DISCUSSION

The ACT-loaded NLC gel formulations were assessed for stability as per the ICH guidelines to assure quality, safety, and efficacy. Characters such as clarity, homogeneity, viscosity, pH, and zeta potential were used as parameters to evaluate the stability of gel formulations.

Formulation F3G exhibited better quality characteristics. F3G formulation was found to be clear transparent yellow color preparation with homogenous nature throughout the conduct of the study [Table 2]. Consistency of formulation is one of the important features of topical formulation as it is to be applied on the surface of the skin. Viscosity is related to the retention ability of the gel formulation at the skin surface and also has an important role in controlling drug permeation.[18]

The pH values of the F3G formulation were found to be in the range of 5.1 ± 0.1–5.5 ± 0.0. The pH value showed that the formulation would not irritate the skin, as the same is preferable pH for topical preparations. The pH value also supports the formation of gel with desired viscosity as a change in pH to acidic (below 5) and basic (above 9) results in a decrease in viscosity of Carbopol gel that is caused due by the presence of excessive electrolytes which result in electrostatic repulsion of ionized carboxylic groups.[19]

No significant change in the zeta potential of F3G was observed which indicates that the formulation retains its stability. Change in zeta potential of other formulations leads to the interpretation that changes in nature of particles has occurred that has resulted in alteration of electrophoretic mobility of suspended particles.[20] All NLCs had a negative charge even though the formulations were prepared using a combination of Tween 80 and SLS. This negative charge can be assumed due to the preferential adsorption of OH^- ions from water by lipid particles.[21] The change in zeta potential from −48.8 to −53.3 mv suggests the further adsorption of SLS to form a single layer of adsorbed ions. The presence of

Table 3: Viscosity of different formulations at the minimum and maximum shear rate

Code	0 month		3rd month		6th month	
	Viscosity at min. shear rate (Cp)	Viscosity at max. shear rate (Cp)	Viscosity at min. shear rate (Cp)	Viscosity at max. shear rate (Cp)	Viscosity at min. shear rate (Cp)	Viscosity at max. shear rate (Cp)
F1G	1800	750	1575	642	1484	642
F2G	2100	1000	1900	856	1750	732
F3G	2248	854	2200	830	2210	819
F4G	2570	1050	2312	987	1945	721
F5G	2305	912	2165	830	1730	687

Table 4: pH of formulations at different time period

Code	pH (mean±SD, n=3)		
	0 month	3rd month	6th month
F1G	5.5±0.2	3.8±0.3	2.1±0.2
F2G	5.7±0.1	6.5±0.2	4.2±0.2
F3G	5.5±0.0	5.7±0.1	5.1±0.1
F4G	6.5±0.2	5.2±0.3	4.8±0.3
F5G	7.6±0.1	5.7±0.1	4.6±0.3

Table 5: Changes in the zeta potential of formulations during storage at different time intervals

Code	Zeta potential		
	0 month	3rd month	6th month
F1G	−25.65	−24.80	−20.06
F2G	−29.56	−23.21	−24.19
F3G	−48.8	−50.7	−53.08
F4G	−52.5	−48.0	−20.6
F5G	−38.51	−27.80	−25.65

similar charges supports electrostatic repulsion that results in the stability of F3G throughout the stability study.

CONCLUSION

From the results obtained from this study, it was concluded that the ACT-loaded NLCs were successfully incorporated in Carbopol to obtain topical gel formulation. Formulation F3G showed minimum changes in parameters evaluated for the conduct of stability studies. Therefore, it was concluded that formulation F3G could be a stable product and a promising alternative for topical application. In the future, the production can be taken up to pilot plant scale-up.

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