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Fabrication and Evaluation of Rimantadine Loaded Niosomes

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Abstract

Influenza virus is a highly contagious zoonotic respiratory disease that causes seasonal outbreaks each year and unpredictable pandemics occasionally with high morbidity and mortality rates, posing a great threat to public health worldwide. Besides the limited effect of vaccines, the problem is exacerbated by the lack of drugs with strong antiviral activity against all flu strains. Rimantadine is one of the effective anti-viral drugs that is used in treating influenza virus. In present study niosomes of Rimantadine were developed by ether injection method. Total 6 formulations were prepared by altering ratios of span 60 and cholesterol. The prepared formulations were evaluated for different parameters. *In vitro* drug release studies were carried out for all the formulations and among five formulations F5 was found to have highest drug release. Prepared niosomes were incorporated in gel and evaluation studies like pH, homogenicity, viscosity and spreadability were carried out. All the parameters were found satisfactory. It was compared with marketed gel. The former has shown drug release till 12 h whereas the latter has shown only till 6 h. Thus, sustained release of drug loaded niosomes was proved.

Keywords: Influenza virus, Rimantadine, Niosomes, Anti-viral drug, Niosomal gel.

Introduction

The basic goal of novel drug delivery system is to achieve a steady state blood or tissue level that is therapeutically effective and nontoxic for an extended period of time. Conventional drug delivery involves the formulation of the drug into a suitable form, such as compressed tablet for oral administration or a solution for IV administration[1]. These dosage forms have been found to have serious limitations in terms of higher doses required lower effectiveness, toxicity and adverse effects. NDDS are being developed rapidly, so as to overcome the limitations of conventional drug delivery. The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all[2]. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a

growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues[3].

From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology[3]. To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name soluble polymers, micro particles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, niosomes and micelles[4].

The carriers can be made slowly degradable, stimuli-reactive (e.g., pH- or temperature-sensitive), and even targeted (e.g., by conjugating them with specific antibodies against certain characteristic components of the area of interest). Targeting is the ability to direct the drug-loaded system to the site of interest[4]. Two major mechanisms can be distinguished for addressing the desired sites for drug release: (i) passive and (ii) active targeting. An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumours as a result of the enhanced vascular permeability of tumor tissues compared with healthy tissue. A strategy that could allow active targeting involves the surface functionalization of drug carriers with ligands that are selectively recognized by receptors on the surface of the cells of interest. Since ligand–receptor interactions can be highly selective, this could allow a more precise targeting of the site of interest[5].

Colloidal drug delivery systems such as liposomes and niosomes have distinct advantages over conventional dosage forms. These systems can act as drug reservoirs and provide controlled release of the active substance. In addition, modification of their composition or surface can allow targeting[6]. Niosomes are non-ionic surfactant based vesicles that had been developed as alternative controlled drug delivery systems to liposomes in order to overcome the problems associated with sterilization, large-scale production and stability. The first niosome formulations were developed and patented by L'Oreal in 1975. They are liposome-like vesicles formed from the hydrated mixtures of cholesterol, charge inducing substance, and nonionic surfactants such as monoalkyl or dialkyl polyoxyethylene ether. The main objective of is to formulate and evaluate Rimantadine niosomes to treat Influenza virus.

Materials & Methods:

Rimantadine was procured from Jigs chemicals, India. Sorbitan Monostearate (Span 60) was procured from Loba Chem. Pvt. Ltd., Mumbai. Cholesterol, Carbopol 934 and Stearic acid were procured from Research lab Fine Chem, Mumbai, India.

Experimental Method:

Preparation of Rim-Niosomes:

The various concentration ranges of span 60, cholesterol and stearic acid were dissolved in ether in a small beaker. In another beaker double distilled water containing drug was taken. Then the dissolved surfactant / lipid were injected slowly at a rate of 0.25 mL / min, via a 24 gauge needle in double distilled water which was magnetically stirred continuously and maintained at 60-65^{0}C for 30 minute and to ensure complete evaporation of the solvent and to get a uniform suspension of niosome. Batches were prepared wherein the content of span 60 % varied since 0.1 to 1% w/v, whereas cholesterol concentration varied from 0.1 to 0.5% w/v. All batches contain 0.05% w/v of Stearic acid. Various batches were prepared using various concentrations of span 60 and Cholesterol. The dispersions were filled in amber coloured glass vial and observed for

appearance, colour, pH, odour and redispersion time[7]. Various Rim-Niosome preparations are given in Table 1.

Table 1: Formulation chart of various Rim-Niosome formulations

Formulation	Drug(mg)	Span 60 (mg)	Cholesterol (mg)	Stearic acid (mg)
F1	100	40	20	10
F2	100	40	40	10
F3	100	50	20	10
F4	100	50	40	10
F5	100	60	20	10
F6	100	60	40	10

Characterization and Evaluation of Rim-Niosomes[8-10]:

1. Entrapment Efficiency (EE):

To calculate the entrapment efficiency, accurately weighed quantity of 100 mg Rim-Niosomes were taken and dissolved in 7.4 pH buffer. It was stirred for 10 min to break the complex. Then the solution was filtered and 2 ml was taken from above solution and diluted up to 10 ml with 7.4 pH buffer[8]. It was kept aside for few minutes and absorbance was measured by UV- spectrophotometer at 270 nm It can be calculated by using the formula.

$$\mathrm{EE} = \frac{\text{Actual drug content in NS}}{\text{Theoretical drug content}} * 100$$

2. *In vitro* drug release studies:

In vitro studies were carried out using cellophane membrane soaked in pH 7.4 buffer overnight. For this studies franze diffusion cell was taken and in donar compartment pH 7.4 buffer was taken. In between donar and receiver compartments cellophane membrane was placed and tightly held using rubber band. On the membrane gel was applied. The buffer which passes from donor to receiver compartments through the membrane was collected in receiver compartment using a syringe tube. Sampling was done at regular intervals of 15, 30 mins, 1 hour, 2 ,3, 4, 5 and 6 hours (each time 2 ml of sample was collected and replaced with similar amount of buffer). The obtained samples were analyzed using UV spectrophotometer. Formulation with highest entrapment efficiency and in vitro drug release was chosen for further studies.

3. Fourier Transform Infrared Spectroscopy (FTIR):

Fourier transform infrared analysis was conducted to verify the interaction between drug and polymer. The sample powder was dispersed in KBr powder and pellets were made by applying 4 Kg/cm^2 pressure. FT-IR spectra were obtained by powder diffuse reflectance on a FT-IR spectrophotometer type 8400S Shimadzu.

4. Differential scanning calorimetry (DSC):

Differential scanning calorimetry was performed on pure drug and its formulations using DSC-60 instrument. Calorimetric measurements were made with empty cell (high purity alpha alumina discs) as the reference. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10 °C min-1. The energy was measured as J/Kcal.

5. Scanning electron microscopy (SEM):

The surface morphology of formulations was determined using a scanning electron microscope. Samples were mounted on aluminium mount, using double-sided adhesive tape and sputtered by gold under vacuum and were scanned at an accelerating voltage of 15 KV before observation.

6. Particle size & Zeta potential analysis:

The average particle size distribution and charge of the resulting nanoparticles was determined by dynamic light scattering using

C:\Microtrac\FLEX 11.0.0.2 Instruments, United Kingdom. The experiment was performed using clear disposable zeta cell, water as a dispersant which has refractive index (RI) - 1.330 and viscosity (cP) - 0.898 and the temperature was kept constant at 25 °C. The optimized SLN formulation was further incorporated into topical nanogel prepared.

Niosomal Gel Preparation:

Appropriate quantity of carbopol 934 was soaked in water (around 5 ml) for a period of 2 hours. Carbopol was then neutralized with triethanolamine (TEA) with stirring. Then specified amount of Rim niosomes were dissolved in appropriate and pre-weighed amount of propylene glycol. Solvent blend was transferred to carbopol container and agitated for additional 20 min. The dispersion was then allowed to hydrate and swell for 60 min; finally, the pH was adjusted with 98% TEA until the desired pH value was approximately reached. During pH adjustment, the mixture was stirred gently with a spatula until homogeneous gel was formed. All the samples were allowed to equilibrate for at least 24 hours at room temperature prior to performing rheological measurements[11]. Formulation chart of niosomal gel was given in **Table 2**.

Table 2: Formulation chart of Nano gel

S No.	Ingredients	F1
1.	Rim-Niosomes (mg)	100
2.	Carbopol 934 (gm)	0.5
3.	Propylene glycol (ml)	0.5
4.	Tri ethanolamine (%)	1
5.	Distilled water (ml)	Q.S

Evaluation studie[12-14]: Evaluation studie[12-14]:

pH determination:

The pH of the gels was determined using digital pH meter by placing the glass electrode completely into the gel system. The readings were taken for average of 3 times.

Homogenicity:

Developed gel was tested for homogenicity by visual inspection after gel has been set in the container. It was tested for the appearance and presence of any aggregates.

Spreadability test:

Spreadability is a mean of measuring the extent at which the semisolid formulations gets readily spread onto the administration site post application of little shear. Place 0.5 gm gel in a premarked circle (1cm diameter) on a glass plate. Another glass plate was then placed over the gel and weight of 500 gm was placed over this upper glass plate for 5 min. Theexperiment was carried out in triplicate and spreadability expressed in gm.cm/sec. Spreadability can be calculated by using the formula.

$$S = M.L/T$$

Where, S =Spreadability
M =Weight tied to upper slide
L=Length of glass slide
T= Time taken to separate the glass slide completely from each other

Rheological studies:

The rheological measurements were performed on the Brook field viscometer. All measurements were carried out by using parallel plates measuring systems having 50 mm diameter and 1 mm gap at 25 ^{o}C. The rheological properties of the formulated gels and nanogel were studied at different shear rates (rpm) and the viscosity was measured in cP.

Comparitive *In vitro* drug diffusion studies:

The niosomal gel and marketed gel were permeated through dialysis bag. 0.5gm of niosomal gel was placed in the bag and is placed in a beaker containing 150 ml of phosphate buffer of pH 7.4 and constantly stirred with a small magnetic bead. During the experiment, temperature was maintained at 37± 0.5^{o}C to simulate the human skin condition. 5ml of samples were withdrawn at 0.5, 1, 2, 6 and 12 h and replaced with fresh receptor solution. The samples withdrawn were analyzed spectrophotometrically at 270 nm. The amount of drug released was calculated and the percentage drug released was plotted against time. Similarly, it was done for marketed gel.

Stability studies:

Stability studies were conducted for 3 months for optimized formulation according to ICH guidelines. The storage requirements were 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH. Changes in physical appearance and drug content of formulation were observed at standard time intervals.

Results and Discussion:

Evaluation of Rim- Niosomes

Entrapment Efficiency:

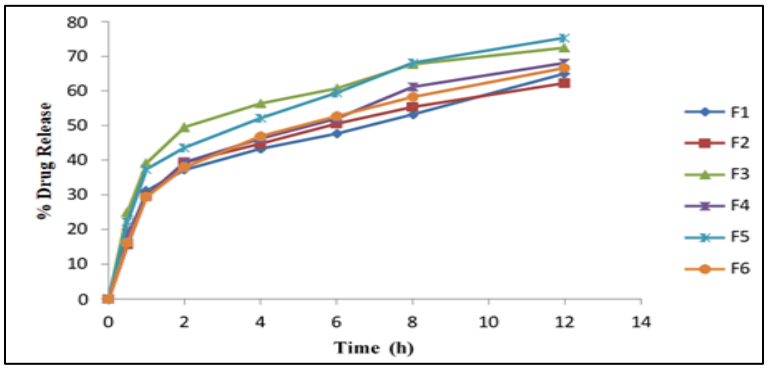
Entrapment efficiency study was carried out for prepared formulation. Percent drug entrapment (PDE) was expressed as fraction of drug incorporated into niosomes relative to total amount of drug used. In the present study the observed percentage entrapment efficiency for all batches were in the range of 69-79%. Percentage entrapment efficiency was significantly affected by concentration of span 60 as well as cholesterol. It was clearly indicated that with increase in concentration of span 60 percentage entrapment efficiency was increased whereas concentration of cholesterol was inversely related to the percentage entrapment efficiency (Table 3).

Table 3: Entrapment efficiencies studies

Sl. No	Formulation	Entrapment efficiency (%)
1.	F1	72.60±1.39
2.	F2	69.05±1.14
3.	F3	73.09±1.94
4.	F4	70.84±1.60
5.	F5	79.60±2.26
6.	F6	74.24±1.50

In vitro drug release studies:

As mentioned in Methodology section, the Rim-Niosomes were subjected to in vitro release studies. At the end of 12th hour 75.32% of drug release was noticed. Obtained results were shown in Figure 1



Fourier Transform Infrared Spectroscopy (FTIR):

Major peaks of Rimantadine, i.e, Methyl C-H asymmetric bond, Carboxylic acid and aliphatic nitro compounds are seen at 2581, 2592 and 2456 respectively. FTIR Spectrum of pure drug is shown in Figure 2. Similar peaks were observed in final formulation along with other characteristics peaks pertaining to drug and excipients. This indicates presence of drug informulation. Some of the major peaks disappeared in final formulation. This shows that though pure drug is present, it is entrapped in the lipid system. This is also confirmed with blunt peaks obtained in FTIR spectrum.

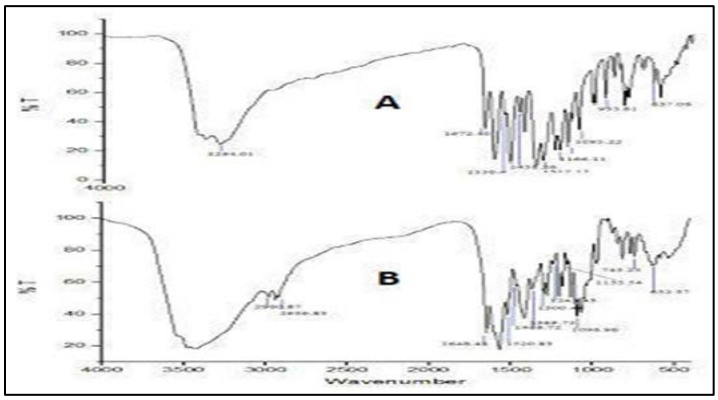


Figure 2: FTIR spectra of A) Rimantadine B) F5 Formulation

Differential Scanning Calorimetry (DSC):

DSC endograms of pure drug and F5 formulations are given in Figure 3. Melting point of pure Rimantadine was observed at 310 °C. In case of F5 formulation , an endothermic peak was seen at 58°C that resembles melting point of stearic acid. Drug peak was observed at around 319 °C but was not sharp. Absence of sharp peak pertaining to drug indicates the presence of drug but embedded in lipid system showing drug-lipid compatibility.

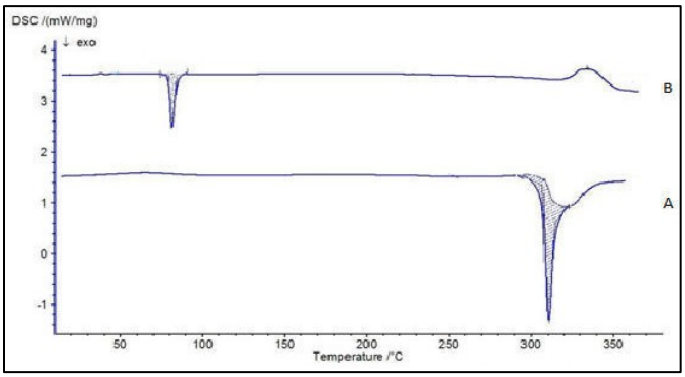


Figure 3: DSC Thermogram of A) Rimantadine B) F5 Formulation

Scanning electron microscopy:

From the results obtained niosomes were roughly spherical. It shows formation of Nanoparticles resembling spheres. SEM image of F5 formulation is given in Figure 4.

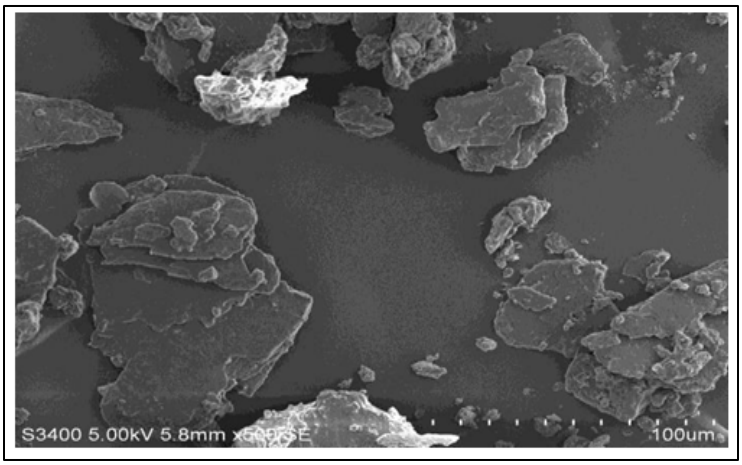


Figure 4: SEM photograph of F5 formulation

Particle size analysis and zeta potential and measurement:

The particle size of niosomes was found between 100-1000 nm (steric acid). The average particle size was 181.5 nm. Obtained results state that prepared Nanoparticles were in Nano size which is one of the objectives of the study. Particle size distribution of both F5 formulation is given in **Figure 5**.

Zeta potential is the major function which determines the interaction of formulation with biological system. It determines the charge type present on the Nano particle surface. Zeta potential of the prepared niosomes was found as -15.2mv (stearic acid). It shows decrease in particle size has lead to increase in surface area that resulted in higher zeta potential.

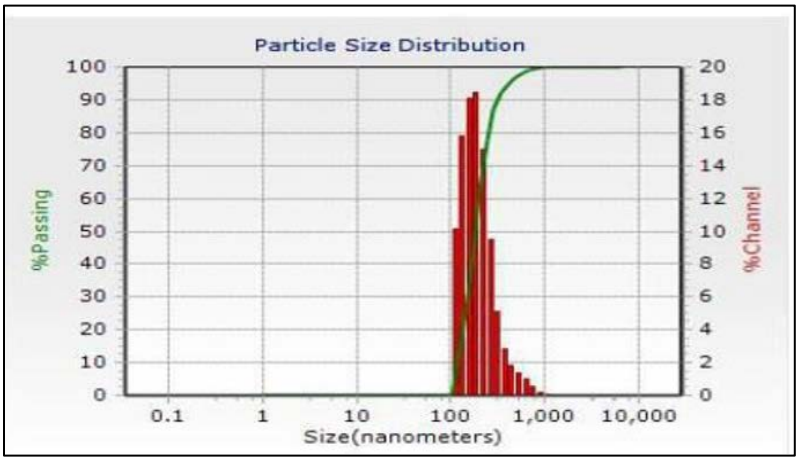


Figure 5: Particle size distribution of formulation F5

Evaluation of Nanogels:

pH determination:

pH of obtained gel was found to be 7.4 which is near to neutral pH. This shows prepared gel does not cause any skin irritation as it is near to skin pH.

Homogenicity:

All the gels prepared clear and transparent. It shows that no aggregates were present and are shown in **Table 4**.

Spreadability:

Prepared gel was spreaded on skin and was found to spread easily. It shows prepared gel has good viscosity.

Rheological studies: Viscosity for prepared gel was found to be good. It shows that obtained results were optimum which helped in good spreadability.

Table 4: Evaluation parameters of F5 niosomal gel

S No	Evaluation parameter	Results
1.	pH	7.2
2.	Homogenicity	Clear & Transparent
3.	Spreadability	Good
4.	Rheological studies (cp)	10583

Comparison with marketed formulation:

***In vitro* drug release studies**

The Niosomal gel and marketed gel were subjected to *in vitro* release studies. The results obtained were plotted in percent cumulative drug release Vs time in Figure 11. It was found that Niosomal gel has shown maximum *in vitro* drug release of 72.49 % and ordinary curcumin gel release was 72.68 %. But sustained release up to 12 h was found in former and it was limited to 6 h in later. This could be due to entrapment and slow release of drug from cniosomes whereas due to availability of free drug, quick release was seen in marketed formulation.

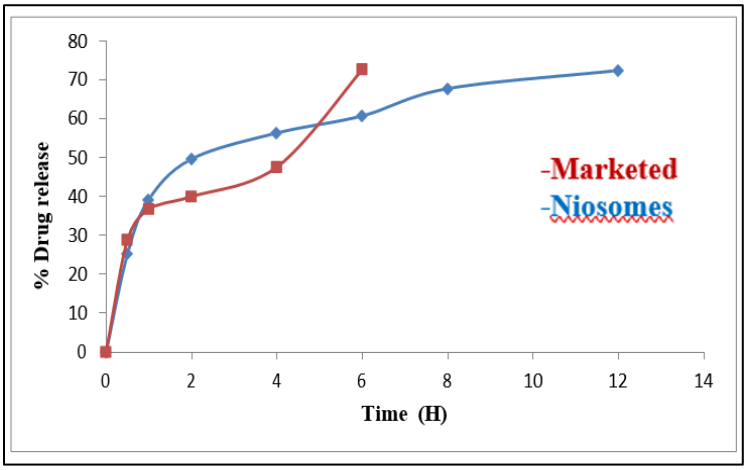


Figure 6: *In vitro* % drug diffusion studies

Stability studies:

As per ICH Guidelines, stability studies were conducted for niosomal gel formulation for a period of 3 months and storage conditions were 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH. The optimized formulation was analyzed for changes in physical appearance and drug content at regular time intervals during the study period. Results obtained are shown in Table 5, which indicated that there was no significant change in appearance and drug content of formulation after subjection to stress testing for the 3 months period.

Table 5: Stability study data of Niosomal gel formulation

Stability testing conditions	Sampling Interval (days)	Physical appearance	% Drug content
25 °C/60% RH	0	No change	92.57 ± 0.37
	15	No change	91.78 ± 0.24
	30	No change	89.92 ± 0.36
30 °C/60% RH	0	No change	91.12 ± 0.35
	15	No change	89.98 ± 0.51
	30	No change	88.23 ± 0.29
40 °C/75% RH	0	No change	91.13 ± 0.37
	15	No change	88.76 ± 0.63
	30	No change	88.56 ± 0.57

*Mean ± SD, n = 3

Conclusion:

The objective of the study was to formulate Rimantadine loaded Niosomes and incorporate the formulation into topical gel. From the UV Visible spectrophometry studies it was found that, the UV spectrum of Rimantadine as shown absorption at 270 nm. Entrapment efficiency study was carried out for prepared formulation. And it was found to be 79.60%. Rim-niosomes were subjected to *in vitro* release studies. At the end of 12^{th} hour 75.32% of drug release was noticed. Prepared Rim-niosomes formulation was incorporated in topical gel. The obtained nanogel was found to be homogeneous which indicates proper distribution of niosomes in gel. pH of niosomal gel was found to be 7.4 which is near to the skin pH and this indicates there is no skin irritation caused by the gel. The spread ability and viscosity studies were performed and the results obtained were within limits. Viscosity for prepared gel was found to be good. It shows that obtained results were optimum which helped in good spreadability. Comparison *In vitro* drug release studies were done for niosomal gel and marketed formulation. It was found that niosomal gel has shown maximum *in vitro* drug release of 72.49 % and marketed gel release was 72.68 %. But sustained release up to 12 h was found in former and it was limited to 6 h in later. This could be due to entrapment and slow release of drug from niosomes whereas due to availability of free drug quick release was seen in marketed formulation. From the stability studies it was confirmed that drug content and color of the formulation did not change even after exposure to various storage conditions. This shows prepared niosomal gel has good stability on exposure to various changes.

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