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Solid Lipid Nanoparticle: A Novel Lipid Based System of Coenzyme Q10 for Skin Enrichment

Pallavi K. Urade*, Mitali M. Bodhankar

Department of Pharmaceutics, Gurunanak College of Pharmacy, Nagpur – 440026, Maharashtra, India.

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ABSTRACT: Background: Coenzyme Q10 is a fat soluble vitamin-like substance. It has antioxidant properties. The antioxidant role of CoQ10 as a free radical scavenger was widely studied. **Aim:** The study was aimed to develop Solid Lipid Nanoparticle **(**SLNs), a novel lipid based system of coenzyme Q10 for skin enrichment. **Method:** Coenzyme Q10 SLNs were prepared from solvent evaporation method containing Glyceryl monostearate as lipid carrier, Span 80 as surfactant, Tween 80 as co-surfactant and acetone, chloroform and dichloromethane as organic solvents. The drug and excipient compatibility was confirmed by FTIR study. The developed SLN formulations were evaluated for particle size, zeta potential, DSC, total drug content, entrapment efficiency, *in vitro* dissolution and stability studies. **Result:** SLN formulation batch F5 was considered as the overall best formulation as it showed highest *in vitro* drug release and also shows good anti-wrinkle property. Short term stability studies revealed that the optimized formulation was stable as there was no significant change occurring in drug content. **Conclusion:** Solid lipid nanoparticle F5 containing the drug and carrier in the ratio of 1:5, is the best optimized formulation as it possesses maximum encapsulation efficiency and shows good anti-aging property as compared to other formulations.

Corresponding author*
Ms. Pallavi K. Urade
Research Scholar
Gurunanak College of Pharmacy,
Nagpur, 440026.Maharashtra, India.
Tel: +91-9420197440
Mail ID: pallaviurade@gmail.com

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INTRODUCTION:

Coenzyme Q10, a vitamin-like substance with a yellow-coloured crystalline powder form and the melting point of 49 ºC, is widely biosynthesized in living organisms such as plants and animals [1]. It has been found in virtually all cells of the human body, including the heart, liver and skeletal muscles [2]. Initially, it became a popular supplement due to participation in two major physiological activities: as a mitochondrial electron-transporter in the high-energy metabolic pathways of liver cells and other cells of the body and as an antioxidant against free radicals and lipid peroxidation [3-5]. Recently, Co Q10, as a cutaneous antioxidant and

energizer, had been demonstrated to prevent photo aging in topical application. It not only penetrates into the viable epidermis and reduces the level of oxidation and wrinkle depth but also reduces the detrimental effects of ultraviolet A (UVA) on dermal fibroblasts, which maintain the dermal matrix. To be able to act as a cutaneous antioxidant and energizer, Co Q10 needs to penetrate into the above living layers [6-8].

Skin drug delivery system have been widely used now a days for several purposes, e.g. to provide surface effects (e.g. sunscreens, cosmetics and anti-infectives), dermal effects (e.g. corticosteroids), and systemic effects (e.g. nicotine patches) as well as deeper tissues (e.g. non-steroidal anti-inflammatory drugs). However, several problems have been reported when the drug is applied via topical/dermal route by the conventional formulations, for instance, low uptake rates due to the barrier functions of stratum corneum and absorption to systemic circulation leading to unwanted systemic side effects. This can be avoided by the use of special approaches that are able to enhance the drug absorption and delivery to the target site. Those are the colloidal drug delivery systems as Niosomes, Ethosomes and SLN. SLN have been introduced as an alternative colloidal carrier systems to traditional carriers (e.g. NE, liposomes and polymeric nanoparticles), for both pharmaceutical and cosmetic applications.

Solid lipid nanoparticles have been introduced as a potent carrier system for various pharmaceutical and cosmetic active compounds. In the second half of the nineties there was an increasing interest in investigating solid lipid nanoparticles for dermal application. They are capable of protecting unstable active compounds against degradation [8], releasing the active ingredients in controlled way [9] and prolonging residence time of some actives such as sunscreens in the stratum corneum [10] as well as targeting drug to the upper layer of skin [11]. They also show occlusive properties in vitro and decrease water evaporation from the skin to the atmosphere [12,13]. Occlusion promotes the penetration of actives into the skin. Due to the reduced water loss caused by occlusion, skin hydration is increased after dermal application of SLN or SLN-containing formulations [14].

SLN also possesses a number of advantages for the topical route of administration. Due to small particle size, SLN ensures close contact to stratum corneum and thereby increases penetration of encapsulated drugs into the viable skin. Sustained release of the drug from SLN supplies the drug to the skin over a prolonged period and thereby reduces systemic absorption. For topical SLN, all excipients used in current topical cosmetic and dermal pharmaceutical products can be used. SLN shows occlusive properties as a result of film formation on the skin, which reduces dermal water loss. Increase of water content in the skin reduces the symptoms of atopic eczema and also improves the appearances of healthy human skin [15].

Since they are made from solid lipids, chemically unstable lipid soluble compounds that are sensitive to oxidation can be successfully encapsulated in the SLNs [10]. Several molecules were incorporated into lipid nanoparticles, e.g. ascorbyl palmitate,

Resveratrol, retinol and retinyl palmitate, vitamin E and vitamin E acetate (8,-11-14). It has been shown that systemic uptake of active substances (e.g. isotretinoin) through skins could be avoided and SLN could increase the accumulative uptake of the active material in the skin [16]. SLNs are produced as liquid dispersions and their incorporation into Hydrogels or emulsions helps enhance the retention time in the applied area and patient compliance [17,18].

Hydrogels are generally formed from carbomers that have the ability to absorb water, get hydrated and swell by neutralization with base. Carbomers have been mainly used in liquid or semi- solid pharmaceutical formulations in order to modify their flow characteristics [19,20].

The objective of this work was to carry an efficient amount of Q10 to epidermis via SLNs without being oxidized. This study was therefore designed to prepare Q10 loaded SLNs and incorporate them into Carbomer based hydrogel.

MATERIALS AND METHODS:

Coenzyme Q10 was received as a gift sample from Inventia HealthCare Pvt. Ltd, Mumbai. Glycerol monostearate, Tween 80, Span 80, Chloroform, acetone, dichloromethane and dialysis membrane-70 were procured from Himedia Laboratories Pvt. Ltd, Mumbai. The Carbopol 947P was purchased from Merck, India.

Preparation of coenzyme Q10-SLNs:

Coenzyme Q10 SLNs were prepared from a solvent evaporation method containing GMS as lipid carrier, Span 80 as surfactant, Tween 80 as co-surfactant and Acetone, Chloroform and Dichloromethane as organic solvent. Coenzyme Q10 was dissolved in organic solvent. GMS and Span 80 were melted and dissolved in organic solvent containing drugs.

Tween 80 was dispersed in water which was used as aqueous phase. The aqueous phase was also heated to the temperature above 10 ^{0}C that of temperature required to melt the lipid. Aqueous phase was stirred at 3000 rpm for 20 min, and then organic phase was added slowly to aqueous phase and stirred at 10,000 rpm for 30 min. The details of formulation design of Coenzyme Q10 loaded SLNs by solvent evaporation method [19] are tabulated in Table 1.

Table 1. Composition of different batches of Solid lipid nanoparticles.

Composition (mg, ml)	F1	F2	F3	F4	F5
Drug	200	200	200	200	200
GMS	200	400	600	800	1000
Span 80	200	200	200	200	200
DCM	-	5	5	5	-
Chloroform	-	-	-	-	10
Acetone	10	-	-	-	-
Tween 80	40	80	120	160	200
Water	100	100	100	100	100

DCM – Dichloromethane.

Characterization of coenzyme Q10- SLNS:

***Particle size analysis*:**

The particle size of a pharmaceutical substance is strictly maintained in order to get optimal biological activity. Optical microscopy is one of lab scale techniques to study size and surface morphology of prepared SLNs. The size of the prepared Solid lipid nanoparticles was measured by the optical microscopy method using a pre calibrated stage micrometer [21].

***Shape and surface morphology*:**

Shape and surface morphology of nanoparticles was done by scanning electron microscopy (SEM). SEM is the most commonly used method for characterizing drug delivery systems because of its simplicity in sample handling and ease of operation. SEM has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured surfaces.

Small volume of Nanoparticulate suspension was placed on an electron microscope brass stub. The stubs were placed briefly in a drier and then coated with gold in an ion sputter. Pictures of nanoparticles were taken by random scanning of the stub. The shape and surface morphology of the nanoparticles was determined from the photomicrographs of each batch [22].

***Zeta potential determination*:**

Zeta potential is highly useful for assessment of the physical stability of colloidal dispersions. Zeta potential can be measured by determination of the movement velocity of the particles in an electric field (electrophoresis measurements). Zeta limits ranged from -200mV to +200 mV. In the present work, the SLN dispersion was diluted 10 times and placed in a small disposable zeta cell and zeta potential was measured in triplicate manner [23].

***Differential Scanning Calorimetry*:**

Differential scanning calorimetry (DSC) studies were performed using a Mettler DSC821. The DSC measurements were carried out on the following samples: unloaded SLN, Co Q10, CoQ10 loaded SLN and Glycerol monostearate as a lipid phase in SLN formulation. Each sample (1 to 3 mg) was transferred into a 40 µL aluminium container, and an empty standard aluminium pan was used as reference. The samples were heated from 25 to 80 ºC and subsequently cooled down to 0 ºC at a rate of 5 ºC/min while flushing with nitrogen (80 ml/min) [23].

***Total drug content*:**

Coenzyme Q10 loaded SLNs (1 ml) were diluted to 100 ml with ethanol, and total drug content was determined by using UV-Spectrophotometer (Shimadzu 1800, Japan) at 275 nm by taking Ethanol as blank [14,23].

***Entrapment efficiency*:**

The entrapment efficiency of CoQ10 loaded SLNs was determined by collecting the filtered supernatant after centrifugation and the amount of incorporated drug was determined by measuring its absorbance at 275 nm using UV-Spectrophotometer by taking ethanol as blank [22]. Encapsulation efficiency was calculated by subtracting the amount of drug in the filtrate from the amount of drug originally added to the formulation [23].

***In vitro dissolution study*:**

For the *in vitro* release study, 1 ml of Co Q10 loaded SLN dispersion was added to a dialysis bag, which was previously soaked in medium overnight. The dialysis bag was tied from both the sides and added into a 500 ml conical flask containing 375 ml of a phosphate buffer solution (pH 7.4) with 0.8 % v/v Tween 20 as a medium. The flask was kept at 37 ºC in the shaker incubator (REMI, India). At predetermined time intervals, 5 ml aliquots were taken and replaced with the same amount

of fresh medium. The amount of Co Q10 released from the SLN was measured by UV Spectrophotometer [20,23].

***Stability study*:**
The stability studies were conducted according to ICH guidelines. The optimized formulation was divided into 3 samples sets and stored at 4 ± 1 ºC in the refrigerator, 25 ±2 ºC / 65 % RH ± 5 % in stability chamber and 40 ± 2 ºC / 75 % RH ± 5 % in stability chamber. After one month interval drug content of sample was determined by the method discussed previously [21].

Preparation of SLNs based Hydrogel:
The Carbopol 974P was added into SLNs dispersion under continuous stirring (1500 rpm) at room temperature for 3 to 5 h. After 24 h, the gels were neutralized with sodium hydroxide (C= 2 $molL^{-1}$) to give a gel matrix with a pH value of 5.4. The amounts of SLN were added into gel such that the prepared gel will have 1 % w/w of Coenzyme Q10 concentration. Control gel (1 % w/w) was made under the same conditions. In that, free drugs were incorporated instead of CoQ10 loaded SLNs [17].

Characterization of COQ10 loaded SLNs based Hydrogel:
The prepared plain and SLNs based hydrogel were evaluated for the following parameters.

***Physical examination*:**
The formulations were tested for their color, odour and homogeneity by visual appearance [17].

***pH*:**
The pH of SLN based gel was determined by using digital pH meter (Model no. NIG333, Naina solaris Ltd, New Delhi). About 1 g of gel was dissolved in 20 ml distilled water. The measurement of pH of SLN based gel was performed in triplicate and average values were calculated [17].

***Viscosity measurement*:**
Brookfield viscometer DV-II model (REMI, India) was used for determination of viscosity. Gel was filled in a beaker of suitable size and the spindle was lowered perpendicularly taking care in such a way that the spindle does not touch the bottom of the beaker. The spindle was rotated at such a speed so as to generate the torque >30 %. The viscosity of gel was then obtained by multiplying the viscometer reading with the multiplication factor given in Brookfield viscometer catalogue [17].

Spreadability test:
Spreadability of the formulation was determined by an apparatus [6], which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. A rectangular ground glass was fixed on this block. An excess of gel (About 3 g) under study was placed on this ground plate. The gel was then sandwiched between this plate and another glass plate the dimension of a fixed ground plate and provided with the hook. A 1 kg weight was placed on the top of the plates for 5 min to provide a uniform film of the gel between the plates. Excess of the gel was scraped off from the edges. The top plate was then subjected to a pull of 80 g. With the help of a string attached to the hook and the time (in seconds) required by the top plate to cover a distance of 10 cm be noted.
A shorter interval indicates better Spreadability [17]. Spreadability of the formulation may be determined by the following formulation,
$S = (M \times L)/ T$ …… (1)
Where, L is the length moved by glass slide, T is the time in seconds, M is the weight in the pan and S is the Spreadability.

***In vitro drug release study*:**
In vitro drug release studies were performed in a vertical Franz diffusion cell. A phosphate buffer solution (pH 7.4) with 0.8 % v/v Tween 20 (25 ml) was placed in the receiver compartment. About 0.2 g of gel (Q10 loaded SLN gel, simple Q10 gels) was placed in the donor compartment. A dialysis membrane was used to separate the donor and receiver compartments. The diffusion cells were maintained at (37± 0.5 ºC) with stirring at 600 rpm throughout the experiment. At a fixed time interval, 1 ml of the sample was withdrawn from the receiver compartment through the side tube and the drug was analysed by UV - visible spectrophotometer at 275 nm.

***Protection against UV light*:**
Female hairless mice with an initial weight of 20 ± 2 g are used. Groups of 20 animals are treated topically with formulations. One another group is not treated topically, but irradiated. A further group is neither treated nor exposed to UV-B. A special light source (Osram Ultra-Vitalux No. 2) was used. This light source was almost free from UV-C. For UV exposure groups of 5, mice were immobilized under a fine wire net of 14 × 14 cm at a distance of from the lamp, resulting in an irradiation energy of 14mW /cm^2 for UV-B and 33 mW/cm^2 for

UV-A. Irradiation was performed once a day except Saturday and Sunday for a duration of 4 weeks. Exposure time was 45, 60, 90 and 120 s for the 1st, 2nd, 3rd and 4th week respectively. The dorsal skin of the hairless mice was lifted up by pinching gently. Wrinkle formation was evaluated visually by 3 trained graders according to the scaling grade [19] for 4 weeks.

***Stability study*:**

Accelerated stability of the SLN based gel was carried out as per ICH guidelines. All the formulated SLN based were kept in glass containers and kept undisturbed in the environmental chamber (Remi, programmable environmental test chamber) for a period of three months [19]. The analytical condition was 40 ± 2 ºC temperature or 75 ± 5 % RH and 25 ± 2 ºC temperature or 60 ± 5 % RH. Then after one month interval, the samples were withdrawn and studied for diffusion study.

RESULTS:

Five formulations of Coenzyme Q10 were formulated using Glycerol monostearate as lipid which is shown in Table 1. The formulations were subjected to evaluation parameters like particle size, surface morphology, drug entrapment efficiency, drug content and *in vitro* drug release and stability studies.

Particle size:

Solid lipid nanoparticles were spherical lipid particles in the nanometer size range. Thus particle size is a critical parameter for evaluation during and after formulation of solid lipid nanoparticles. The particle size of Coenzyme Q10 loaded SLN dispersion was shown in Fig 1. From these figure, it was found that the particle size was 2 µm.

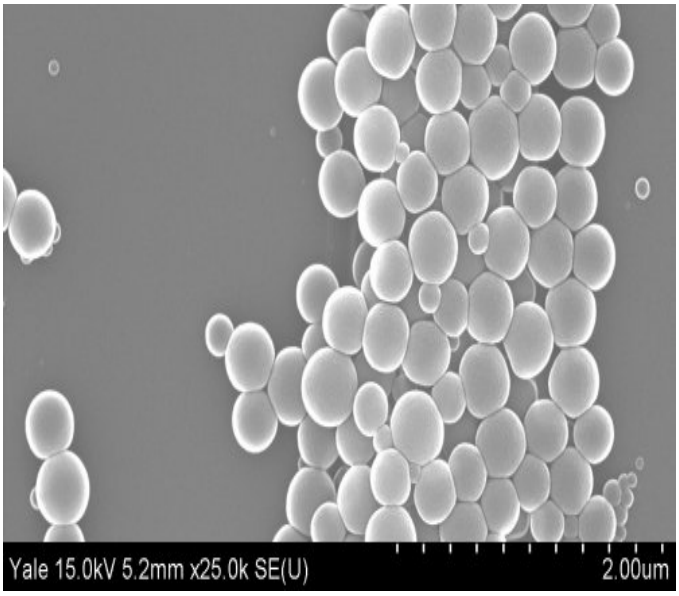


Fig 2. The SEM images of SLN Formulation F5.

Shape and surface morphology:

The shape and surface morphology of Coenzyme Q10 loaded SLN dispersion prepared via solvent evaporation method were studied using Scanning Electron Microscopy. In the SEM study, the size of the lipid nanoparticles was found to be in agreement with the dynamic light scattering data for all the samples (Fig 2). All the particles were found to be roughly spherical in shape with a well-defined periphery. SEM image of Coenzyme Q10 loaded SLN also provided the structural information of the SLN. The lipid nanoparticles appear to be less dense in the core with a well-defined shell. No obvious aggregation of the lipid nanoparticle was observed in the SEM images.

Zeta potential:

The magnitude of the zeta potential is an important factor that determines the stability of SLN dispersions. The zeta potential value for SLN loaded with drug was -44.4mV. Glycerol monostearate is a fatty acid ester that imparts a negative surface charge on the lipid particles and thus the SLN formulation possesses a negative zeta potential. The drug molecule covering and coating the surface of the drug incorporated SLN is likely contributing to the reversal of surface charge.

DSC study:

DSC is used for detecting potential drug excipient incompatibilities as well as melting and recrystallization behavior of crystalline materials like SLNs. As depicted in Fig 3, the DSC profiles of GMS as a solid lipid component of SLN formulation showed appearent endothermic peaks at about 58 ºC. The melting point of Coenzyme Q10 is 49 ºC. The profile of the powder of Co Q10-loaded SLN showed a single peak around 51 ºC, whereas the SLN without CoQ10 had an endothermic peak at 52 ºC. The height of the endotherm in the thermogram of SLN-containing Q10 was less than in SLN without Q10, suggesting loss of crystallinity of the lipids and Q10 after incorporation into SLN. During the production procedure, Q10 was in melted lipid phase. Glycerol monotearate showed one peak at 58 ºC, indicating no polymorphic forms. The peak of GMS in the formulation had a slight shift to the lower temperature side. This could be due to a reduction in particle size and an increase in surface area, leading to a decrease in melting enthalpy.

The results indicate that Q10 incorporated in SLN was not in a crystalline state but in an amorphous form. When the materials were formulated as SLN, the

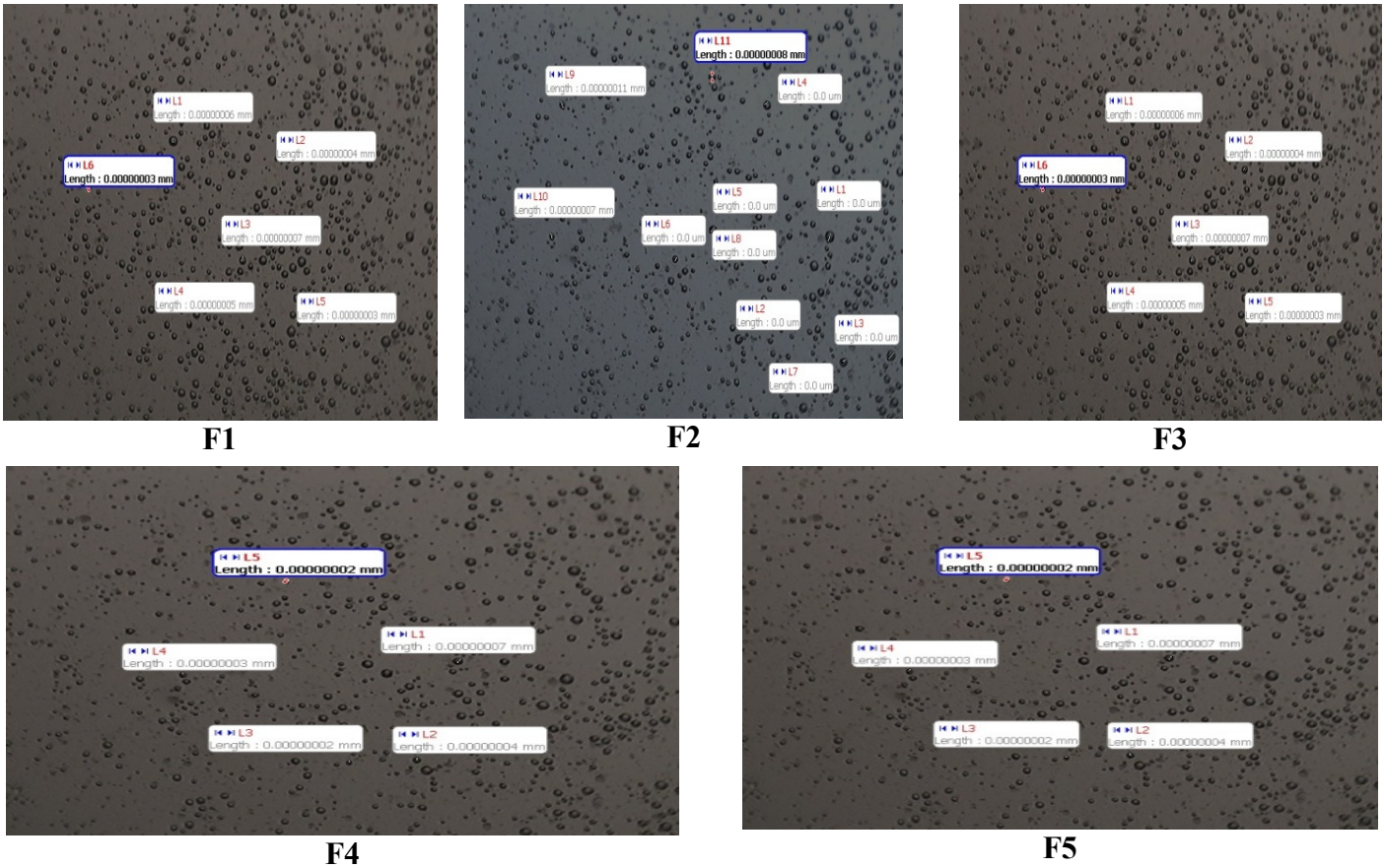


Fig 1. Particle size of different SLN formulations.

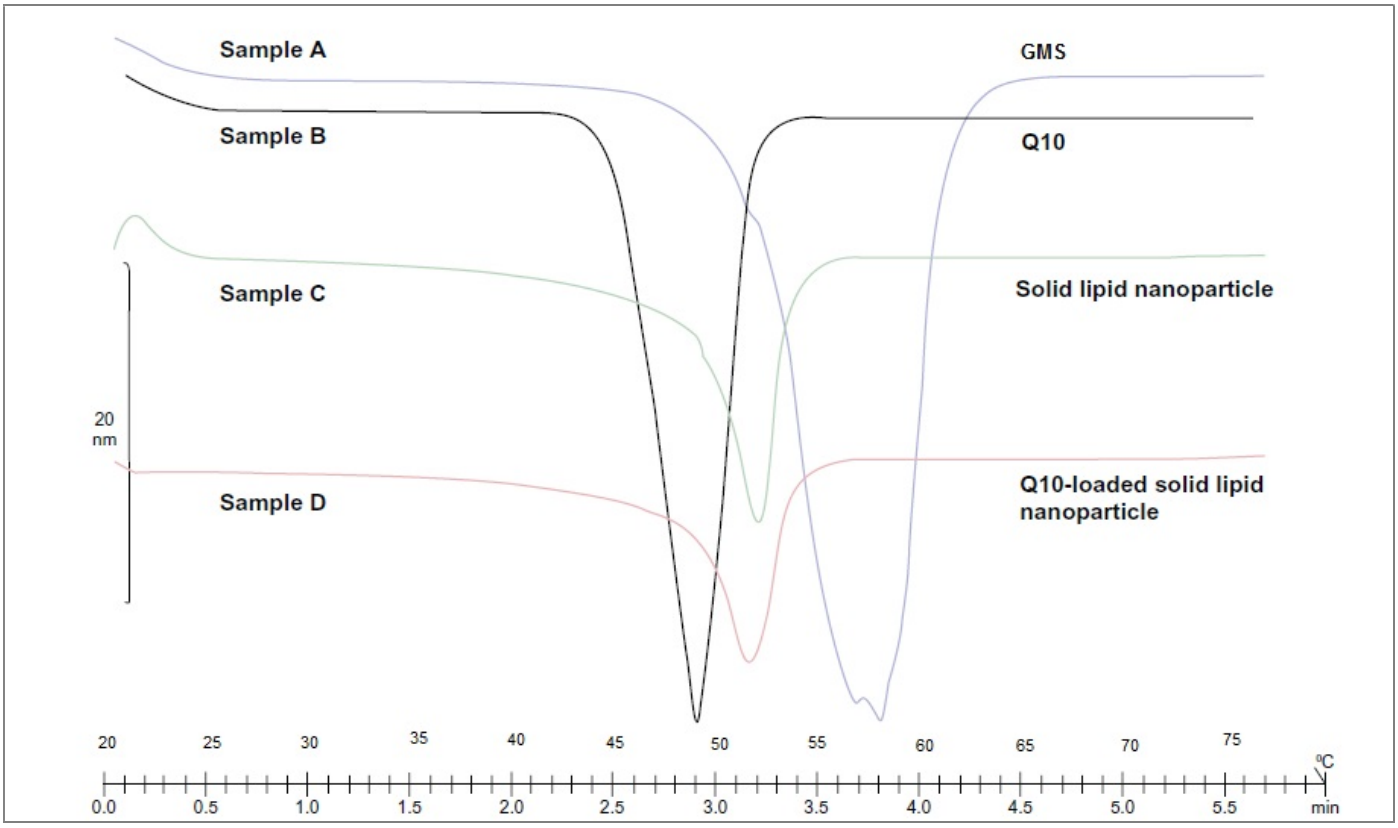


Fig 3. Differential scanning thermogram of Sample A (GMS), Sample B (Coenzyme Q10), Sample C (Solid lipid nanoparticle), and Sample D (Q10 loaded solid lipid nanoparticles).

endothermic temperature was lower. Along with a certain effect of surfactants, the observed depressed endothermic peak for SLN may be due to the nanometric size of the particles with a huge surface area.

Drug content:

The drug content in SLN dispersion was found in the range of 88.58 to 89.62 % which was presented in Table 2.

Drug entrapment efficiency (%):

The % entrapment efficiency of Coenzyme Q10 from the batch of F1 to F5 in SLN was found in range 58.13, 60.88, 65.48, 73.47, and 74.72 % respectively (Table 2). The entrapment efficiency in five batch of Coenzyme Q10 was studied. As the lipid concentration was increase from 200 mg to 1 g, the entrapment efficiency was increase in every lipid, highest encapsulation efficiency was found in the batch F5.

Table 2. Drug content and entrapment efficiency of SLN formulations.

Formu-lation	Drug Content (%)	EE (%)
F1	88.58±0.05	58.13
F2	88.85±0.07	60.88
F3	89.14±0.10	65.48
F4	89.36±0.02	73.47
F5	89.62±0.07	74.72

All data are presented as mean ± Standard deviation (n = 3).

***In vitro* drug release studies:**

In vitro drug release from SLN was performed in phosphate buffer pH 7.4 with 0.8 % v/v Tween 20 by using a dialysis bag. The calibration curve of Coenzyme Q10 was constructed by measuring the absorbance at 275 nm of solutions of five different concentrations of the drug. *In vitro* drug release profile obtained from the dialysis bag experiment is shown in Table 3. Drug release was found to be 37.97 % after 2 h (Fig 4). The release profile was characterized by an initial and variable rapid release followed by a continuous release. The initial burst release may be due to the drug located on or near the surface of the SLNs and the large surface to volume ratio of the nanoparticles geometry owing to their size. After the initial burst release, the release profile displayed a plateau, resulting from the diffusion of the drug dispersed in the lipid matrix. Water soluble drugs exhibit the tendency to migrate to the aqueous phase, hence concentrating at the surface of the particles and exhibiting the burst effect. The low drug release from the SLNs could be due to the structural integrity of the SLN matrix which may impede drug diffusion.

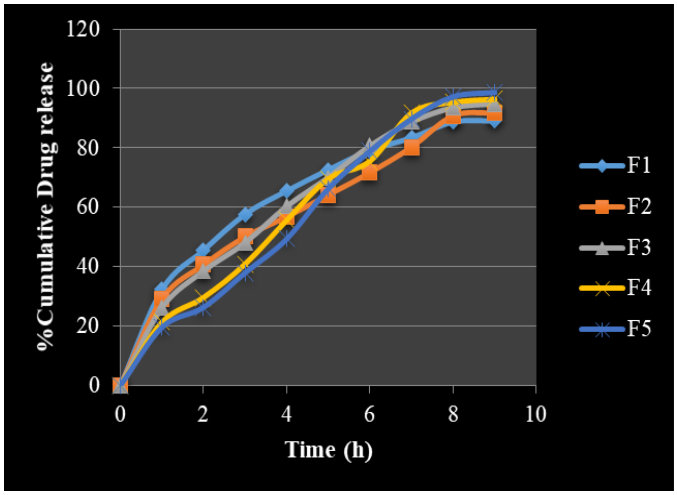


Fig 4. Dissolution Profiles of SLNs formulations F1-F5.

Stability studies:

The stability study of optimized formulation F5 was found to be stable. There is no deviation occur in the drug content of the three month stability study. The drug content of F5 formulation was found to be 88.58 ± 0.05, 88.85 ± 0.07, 89.14 ± 0.10, 89.36 ± 0.02 and 89.62 ± 0.07 % respectively (Fig 5).

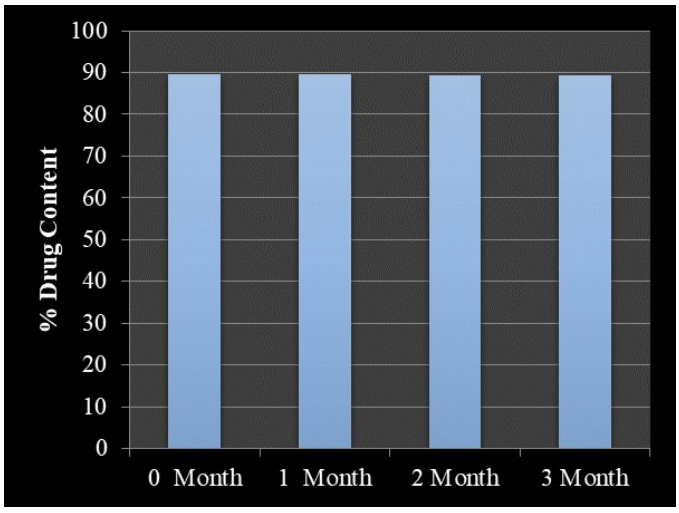


Fig 5. Drug content of formulation F5 kept for stability study.

Evaluation of SLN based Hydrogel:

***Physical examination*:**

The prepared Coenzyme Q10 loaded SLN based gel and plain gel formulations were yellow, odorless preparations with a smooth and homogeneous appearance.

***pH*:**

The pH values of prepared SLN based gel and plain gel were 5.68 ± 0.49 and 5.81±0.15 respectively.

Table 3. *In vitro* drug release data of solid lipid nanoparticles.

Time (h)	F1	F2	F3	F4	F5
0.5	32.29±0.13	29.16±0.26	26.23±0.40	21.30±0.40	19.60±0.13
1	45.54±0.66	40.62±0.13	38.54±0.13	29.64±0.13	26.32±0.26
2	57.67±0.40	50.09±0.40	48.10±0.26	40.90±0.53	37.97±0.13
3	65.53±0.53	56.81±0.53	60.32±0.13	55.68±0.26	49.33±0.40
4	72.63±0.13	64.20±0.26	70.07±0.53	69.60±0.13	66.28±0.53
5	79.16±0.26	71.68±0.13	80.68±0.26	75.56±0.26	78.88±0.13
6	83.42±0.13	80.30±0.26	88.73±0.13	91.76±0.40	89.77±0.26
7	88.73±0.66	90.81±0.13	93.56±0.26	95.35±0.13	97.25±0.13
8	89.10±0.13	91.85±0.53	94.88±0.80	96.40±0.26	98.67±0.53

All data are presented as mean ± Standard deviation (n = 3).

***Viscosity*:**

The measured viscosity of prepared SLN based gel and plain gel was found to be 12359± 14 and 10095±80 cPs respectively.

***Spreadability test*:**

Spreadability was found to be 25.4 ± 0.2 and 24.1 ± 0.5 (g.cm/s) for SLN based gel and plain gel respectively. These values were indicative of good spreadability of SLN based gel.

***Diffusion study*:**

As shown in Table 5, the release of Co Q10 from SLN based gel was faster than plain gel. Due to the solid matrix of the nanoparticles and the subsequent drug immobilization, the release of encapsulated Q10 appeared different from the Q10 gel. The percentage of released Q10 from simple plain gel was more than SLN based gel at the first hour time point, but after 6 hours it released only about 41 % of Q10 contents (Fig 6).

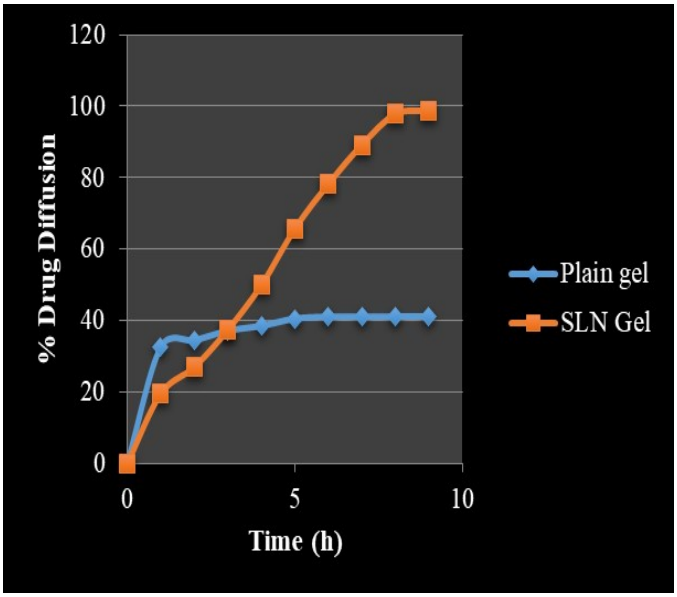


Fig 6. Diffusion profile of hydrogel and plain gel.

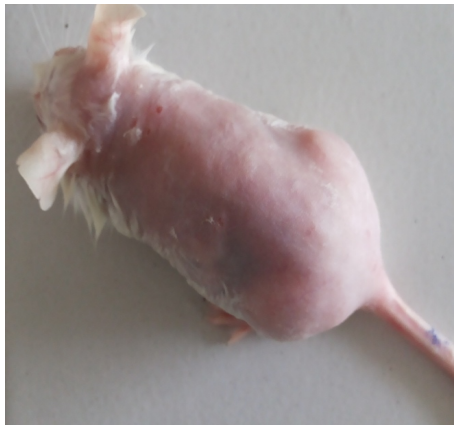
The initial release within the first 5 h was faster for the SLN formulation than for the simple plain gel, which can be explained by enrichment of Q10 in the outer shell of the particles. The prolonged release in the second phase can be explained by slower diffusion of Q10 from the solid lipid of SLN. The solid lipid matrix creates a slow release pattern of Q10. The Q10 release from the gel formulation appeared to be slower than release from SLN based gel, although it did not reach a significant level. It has been reported that chemical stability of Q10 increases after incorporation into lipid nanoparticles. The SLN gel has a higher viscosity compared with plain gel, which slows down the release. In the simple plain gel, Q10 appeared to mix homogenously with the gel base, leading to an almost constant Q10 release.

Animal study:

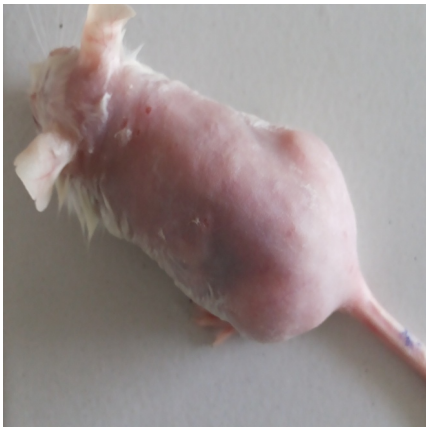
Protection against UV light

Topical application of CoQ10 loaded SLN based gel inhibits UV-irradiation induced skin wrinkle formation in female hairless mice (Table 4). Estimation of increase in wrinkle formation was done by using the visual grading method. In order to evaluate the anti-photo aging activity of Co Q10loaded SLN based gel, 1 % gel was topically applied to female hairless mice. In control group repetitive exposure to UV radiation increases the wrinkle grade in the mice. However, topical treatment of CoQ10 loaded SLN based gel significantly suppressed the increase in the wrinkle grade. Result did not show any notable phenotypic and behavioral adverse side effects in mice by topical treatment with the Co Q10 loaded SLN based gel during the experiment. From the observations shown in Fig 7 and 8, it is confirmed that Co Q10 loaded SLN based gel could significantly suppress the signs of Photo aging including wrinkle formation.

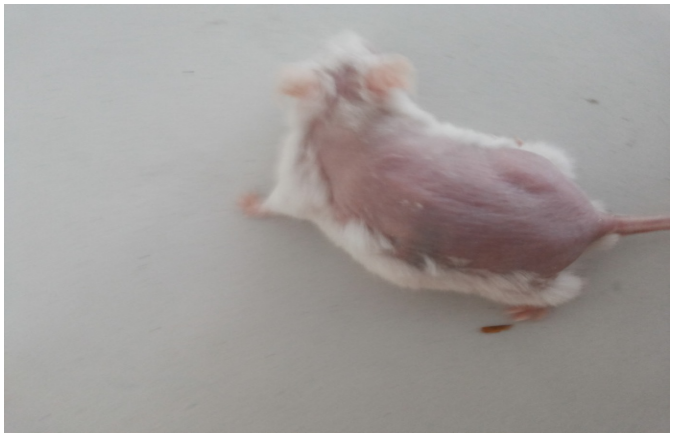
Initial



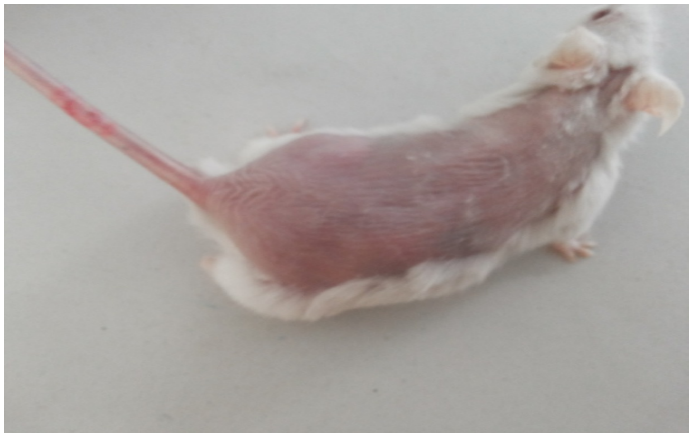
After 1 week



After 2 weeks

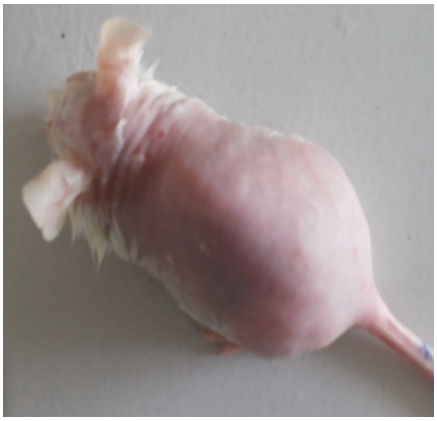


After 3 weeks



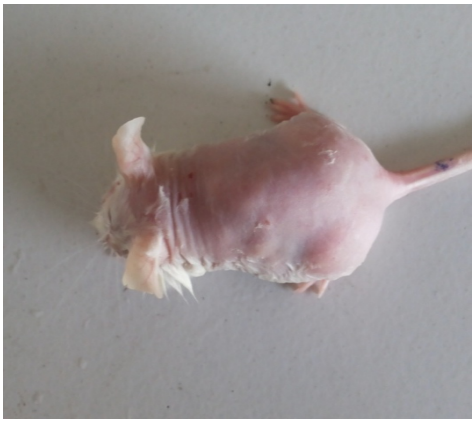
After 4 weeks

Fig 7. Animal study result for control group.

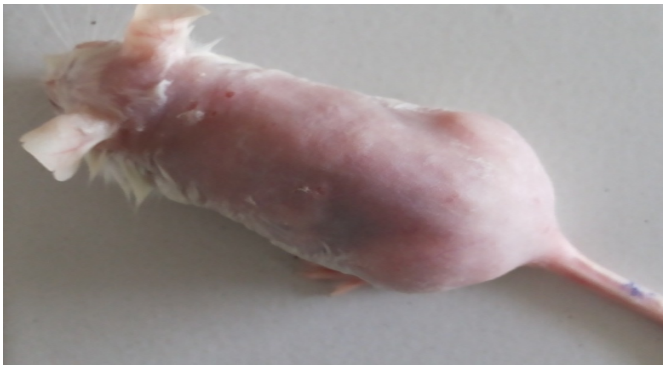


Initial

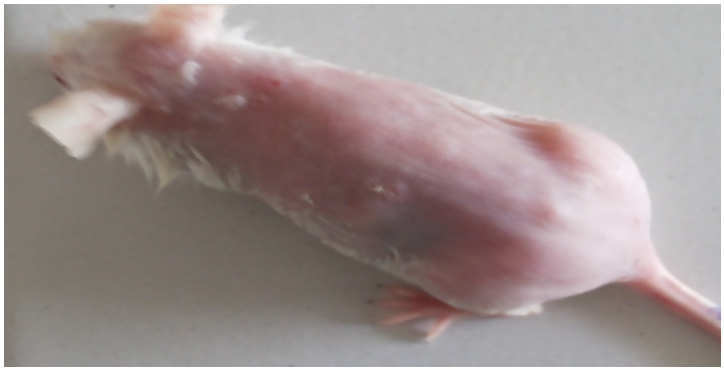
After 1 week



After 2 weeks



After 3 weeks



After 4 weeks

Fig 8. Animal study result for treated group.

***Stability study*:**

The stability study of Co Q10 loaded SLN based gel was found to be stable. There is no deviation occur in the diffusion study of the three month stability study. The diffusion study results are shown in Fig 9. From the observations it could be concluded that the SLN based gel was stable.

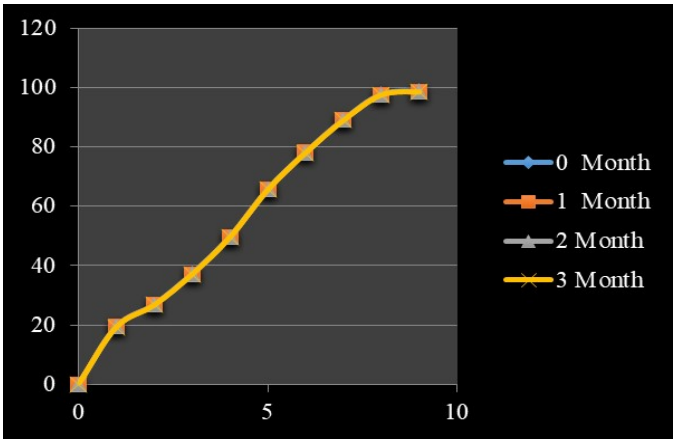


Fig 9. Stability study profile of SLN based hydrogel.

DISCUSSIONS:

From motic images and analysis it is observed that the particle size is around 2 µm. The SEM image shows a well-defined intact shell. They are perfectly circular and having less particle size. Zeta potential is well within the range and shows fairly good stability. The DSC results indicate that Q10 incorporated in SLN was not in a crystalline state but in an amorphous form. When the materials were formulated as SLN, the endothermic temperature was lower. Along with a certain effect of surfactants, the observed depressed endothermic peak for SLN may be due to the nanometric size of the particles with a huge surface area. The drug content and % entrapment efficiency of SLN formulation was found to be good in all formulation and was highest in F5 formulation. The stability study shows fairly good stability which was confirmed by physical examination and drug content analysis after stability studies.

The diffusion studies of gel formulation containing SLN shows 98.79 % of drug diffuses in 8 h which is very good for enrichment of skin because it penetrate into deeper layer of skin. Solid lipid nanoparticles containing Glycerol monostearate shows better stability than Liposomes, Ethosomes, Transferosomes and other lipid based system. The animal study shows promising result as the UV- irradiation by specially design equipment having Osram Ultra- Vitalux No. 2 as light source shows significant reduction in the wrinkle development. At the same time there was no irritation or adverse reaction observed during the study. Coenzyme Q10 a powerful antioxidant can be used internally and externally.

The aim was to prepare external gel formulation which should penetrate into deeper tissues so that; the long lasing antioxidant effect is obtained. Even though to some extent it may show some systemic absorption as the penetration is good, but it will be harmless and no side effects or adverse reaction are observed in animals throughout the study during 4 weeks and two weeks even after that. All the animals survive and treated animals were recovered from wrinkle formation. This unique lipid based drug delivery system can efficiently deliver Coenzyme Q10 as antioxidant and anti-aging preparation and can be successfully used for skin enrichment.

CONCLUSION:

It could be concluded that Solid lipid nanoparticle F5 is the best optimized formulation as it possess maximum encapsulation efficiency and shows good anti-aging property as compared to other formulations.

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