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Preparation and Characterization of Alpha Tocopherol Loaded Solid Lipid Nanoparticles By Hot Homogenization Method

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Shylaja P.A.*, Dr Manju Maria Mathew

Department of Pharmaceutics, Nirmala College of pharmacy, Muvattupuzha, Kerala, India.

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ABSTRACT

The aim of present work was to formulate and evaluate the Solid lipid nanoparticle (SLN) containing Alpha Tocopherol. Twelve formulations of Tocopherol loaded SLN were prepared. Prepared solid lipid nanoparticles were evaluated for the drug content, percentage yield, encapsulation efficiency, surface morphology and *in vitro* drug release studies. All the formulations showed better results i.e. there is no any major difference between the formulations. But considering the *in vitro* release and entrapment efficiency, it concludes that F6 and F11 are the best formulations among all the formulations. Based on *in vitro* release data F6 and F11 formulations were taken as optimized formulation as it shows 72.81% and 74.33% of drug release respectively at the end of 8^{th} hour.

INTRODUCTION

Solid lipid nanoparticles (SLN) are sub-micron colloidal carriers ranging from 50 to 1000 nm, which are composed of physiological lipid, dispersed in water or in aqueous surfactant solution. SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve Solid lipid nanoparticles (SLNs) have been considered as an alternative drug carrier system with several advantages, including enhanced physical stability, dual loading ability for lipophilic and hydrophilic drugs, improved physical stability, low cost, and high feasibility for large scale manufacturing. The prepared SLN can be administered orally, intravenously or topically [1,2].

Tocopherol is a chain-breaking antioxidant which prevents the propagation of free-radical reactions [3]. It is a primary and reference antioxidant that interrupts auto-oxidation by reacting with lipid radicals as an electron donor and converts free radicals into more stable species [4]. Tocopherol is known to have a skin anti-aging effect due to its antioxidant property [5, 6]. A well-known phenomenon in skin aging (photoaging) is the sun exposure (ultraviolet-UV and infrared-IR radiation) leading to the formation of free radicals [7]. But it degrades in the presence of air and high temperature. Because of stability problems, use of Tocopherol has been limited in cosmetics. Therefore incorporation of Tocopherol into SLN will have a

- Improved stability and
- Sustained release

Hence the objective of the study was to formulate SLN dispersion of Alpha –Tocopherol in order to enhance stability of Tocopherol and to attain sustained release.

MATERIALS AND METHODS

Materials

Alpha Tocopherol was purchased from HiMedia Laboratories Pvt ltd., Mumbai. Stearic acid, and Tween 80 were provided by Nice Chemicals Pvt ltd., Kochi. All other chemicals and solvents were of analytical reagent grade.

FTIR spectroscopy

FTIR spectral analysis of pure drug was carried out individually using FTIR instrument (Jasco 4100). Observation was made whether changes in the chemical constitution of drug after combining it with the excipients occurred.

Preparation of Solid Lipid Nanoparticles

SLN formulations were prepared using hot homogenization technique with high shear homogenizer (REMI, Vasai). Stearic acid was heated over 5-10ºC of its melting point (70ºC) and Alpha Tocopherol was dissolved in the molten lipid. The lipid phase containing Alpha Tocopherol was dispersed in hot surfactant (78 ºC) (Tween 80) solution of distilled water using a mechanical stirrer (KEMI, Kerala) for 3 minutes at 78°C. This pre-emulsion was then homogenized by high shear homogenization at 9500 rpm 10 minutes, with 30 seconds intervals every two minutes. The dispersion thus obtained was allowed to cool to room temperature.

Evaluation of Solid Lipid Nanoparticulate Dispersion

Scanning Electron Microscopy (SEM):

The SEM analysis of prepared SLN was performed for morphological studies. The formulations are poured into circular aluminum stubs using double adhesive tape, and coated with gold in HUS -5GB vacuum evaporator, and observed in Hitachi S-3000N SEM at an acceleration voltage of 20 Kv and a magnification of 1000X.

Total drug content:

From the prepared SLN 1 ml of dispersion was dissolved in the 9 ml of methanol. The amount of Alpha- Tocopherol was determined using UV spectrophotometer (Shimadzu UV 1800) at 292nm. The total Alpha- Tocopherol content was calculated.[8]

Percentage Drug Entrapment:

The prepared dispersion was centrifuged at 15000 rpm for 30min at 4°C using refrigerator centrifuge (KEMI, Kerala). Then the supernatant was made up to a desired volume with methanol to measure the absorbance at 292 nm to estimate the unentrapped Alpha-Tocopherol for the calculation of Entrapment Efficiency.

Equat gbion 1:

$$EE(\%) = \frac{total\ drug\ content - free\ drug\ content}{total\ drug\ content} x100$$

***In-vitro* release studies**

The *in vitro* release study was carried out by using a modified Franz diffusion cell at 37°C which is fitted with a pre-hydrated cellophane membrane. 1 ml dispersion is placed into the donor compartment and the 25 ml of methanolic Phosphate Buffer Solution (PBS) is used to fill receptor compartment. The solution on the receptor side was stirred by externally driven Teflon coated magnetic beads. At predetermined time intervals of 0,1,2,3,4,5,6,7 and 8 h, 1 ml of the aliquot was withdrawn from receptor compartment through the sample port. Fresh methanolic phosphate buffer pH 7.4 was replaced to maintain constant volume. Samples were suitably diluted and analyzed by UV spectrophotometrically at 292 nm.[9]

Stability study

All solid lipid nanoparticulate lotions were kept for stability studies. Formulations were sealed tightly and studies were carried out for 60 days by keeping at 4°C, 25^{o}C and 40^{o}C/75%RH.Samples were withdrawn on 30th, 60th and 90th day and were analyzed for physical appearance, drug content and entrapment efficiency.

Kinetic study

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q_0-Q) v/s t], Higuchi's square root of time (Q v/s $t_{1/2}$) and Korsmeyer Peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q_0-Q) is the cumulative percentage of drug remaining after time t.

In short, the results obtained from *in vitro* release studies were plotted in four kinetics models of data treatment as follows:-

Mathematical models:

- **Zero Order Kinetics**

To study the zero order release rate kinetics the release rate data were fitted to the following equation

$$Qt = Qo + Ko\ t$$

Qt = Amount of drug dissolved in time t,

Qo = Initial amount of drug in the solution and

Ko = Zero order release constant.

The plot made: cumulative% drug release vs time (zero order kinetic model).

- **First Order Kinetics**

To study the first order release rate kinetics the release rate data were fitted to the following equation.

$$\log Q_t = \log Qo + K_1 t / 2.303$$

Q_t = Amount of drug released in time t,

Qo = Initial amount of drug in the solution and

K_1 = First order release constant.

The plot made: log cumulative of % drug remaining vs time (first order kinetic model).

- **Higuchi Model**

Higuchi developed several theoretical models to study the release of water soluble and low-soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media.

$$Qt = KH\ x\ t^{1/2}$$

Qt = Amount of drug released in time t and

KH = Higuchi dissolution constant

Determination of Diffusion exponent

- **Korsmeyer-Peppas Release Model**

To study this model the release rate data is fitted to the following equation

$$\mathbf{Mt / M\infty = K. tn}$$

Mt / M∞ = Fraction of drug release,

K= Release constant,

t = Drug release time and

n = Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

The plot made: log cumulative % drug release vs log time[10,11]

RESULTS AND DISCUSSION

Determination of compatibility of Alpha Tocopherol with excipients by FTIR

Fourier Transform Infrared spectroscopy studies were carried out for pure drug (Alpha Tocopherol) and for the Alpha Tocopherol and various excipients physical mixtures. The results are summarized in the Figure 1 and 2 and there were no changes in the major peaks of Alpha Tocopherol when mixed with excipients. This revealed that the Alpha Tocopherol and the excipients are compatible with each other.

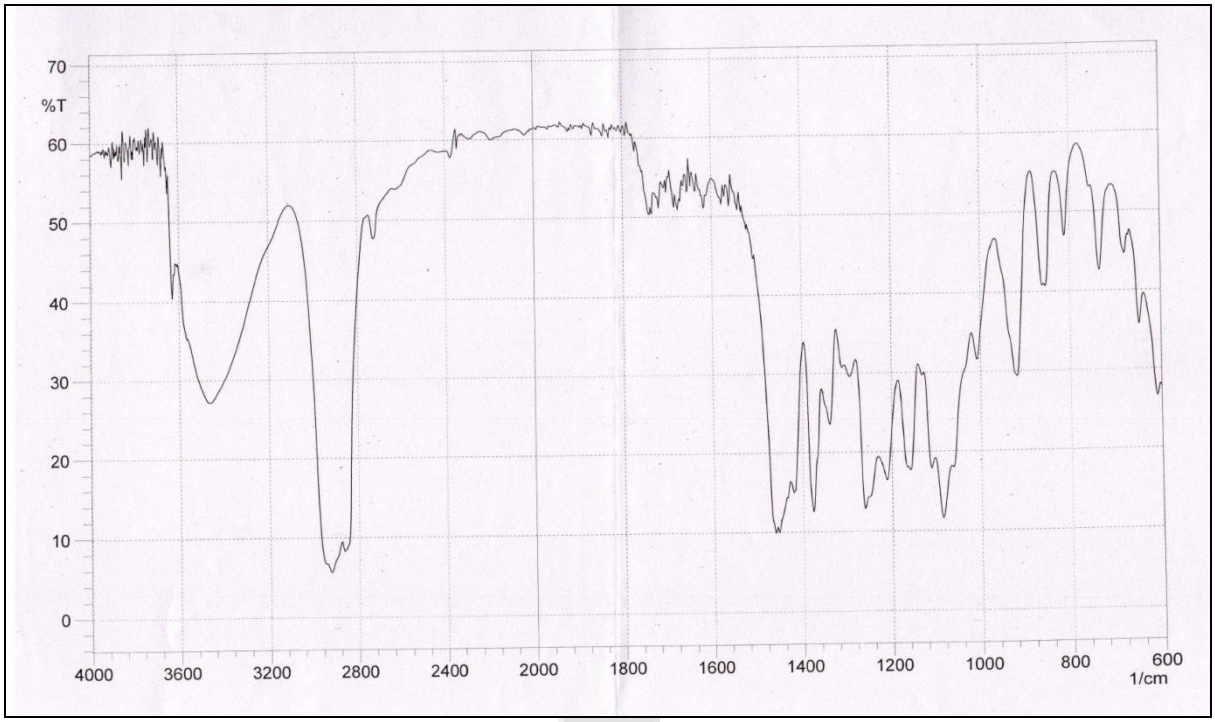

Fig 1: FTIR Spectra of Alpha Tocopherol

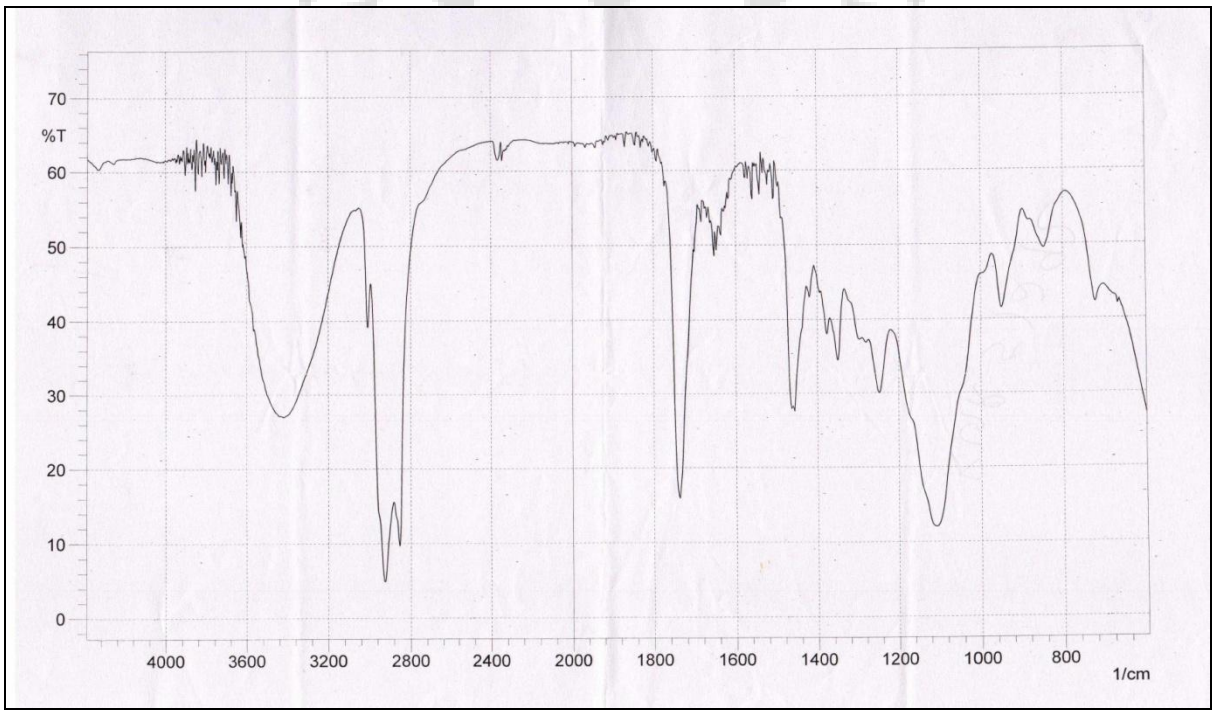

Figure 2: FTIR spectrum of Tocopherol, stearic acid and tween 80 physical mixture

Morphological Study using SEM

The SEM photograph of solid lipid nanoparticulate dispersion is shown in figure 3. The SEM photograph illustrates the spherical shape of nanoparticles entrapping the drug. The SLN dispersion showed the particle size was found to be less than 1000 nm in size with the spherical shape, almost smooth surface and they are well separated on the surface.

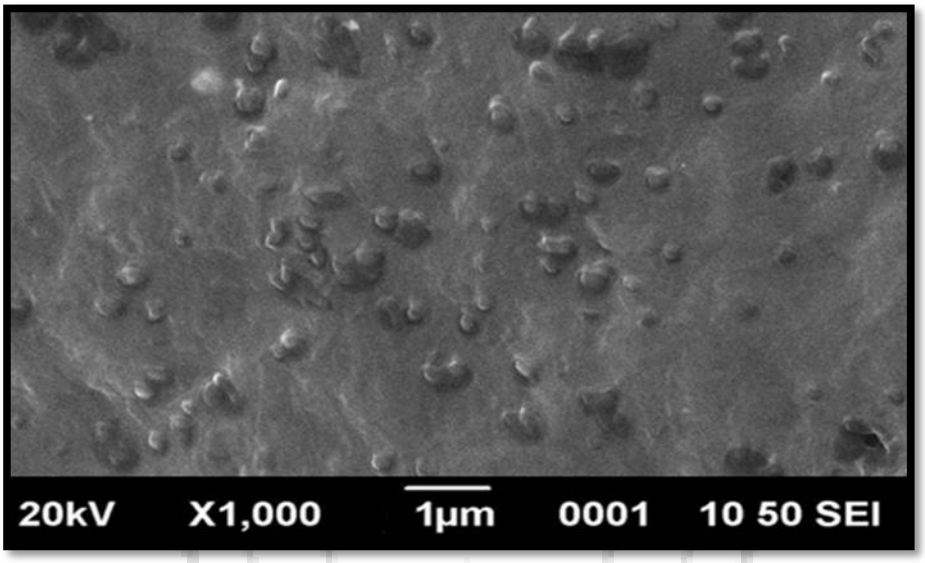

Figure 3: SEM image of Solid Lipid Nanoparticles

Total Alpha Tocopherol Content (%)

Total Alpha Tocopherol Content (%) in different formulations F1 to F12 was calculated and the Alpha Tocopherol content was found to be almost similar in all formulations. The minimum content of Alpha Tocopherol was found in formulation F1 (97.02%). This indicates that amount of Alpha Tocopherol lost during the preparation is negligible or almost all the amount of added Alpha Tocopherol is present in the formulations. Such high incorporations were possible because Alpha Tocopherol is lipid soluble.

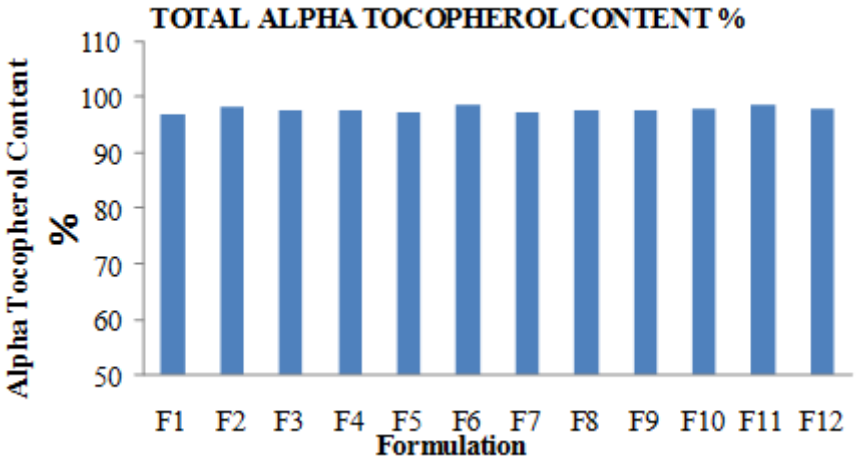

Figure 4: Total Alpha Tocopherol Content (%) of formulations F1-F12

Entrapment Efficiency (%)

Percentage Alpha Tocopherol entrapment of different formulations F1 to F12 were calculated and the drug entrapment was found to be in the order:-
F12>F8>F4>F11>F6>F10>F7>F3>F2>F1>F5>F9

The entrapment efficiencies were found to range between 75.77 % and 98.67 %.

Table 1: Percentage entrapment of Total Alpha Tocopherol

Formulation	Entrapment Efficiency (%)
F1	81.30
F2	86.08
F3	90.11
F4	98.21
F5	80.53
F6	94.13
F7	90.68
F8	98.44
F9	75.77
F10	93.84
F11	94.25
F12	98.67

The entrapment efficiency (EE) of the formulations were found to be increasing with increase in lipid concentration. This may be due to the higher concentration of lipid which would have provided more space to Alpha Tocopherol content and also reduces the escaping of drug into the external phase thus ensuring highest % EE.

***In vitro* release studies**

The release of the drugs from SLN formulations ranked in the order F11>F6>F10>F9>F5>F1>F2>F7>F3>F12>F8>F4.The *in vitro* release of Alpha Tocopherol from nanoparticulate dispersions in the initial 2 hours was less, probably because of the slow diffusion of drug from the lipid. This is followed by a prolonged release. Formulations F12, F8 and F4 were found to have slower release. This may be due to the high entrapment of Tocopherol within the lipid. The amount of Alpha Tocopherol released after 8 hours was higher for F11and F6 (74.33% and 72.81% respectively).

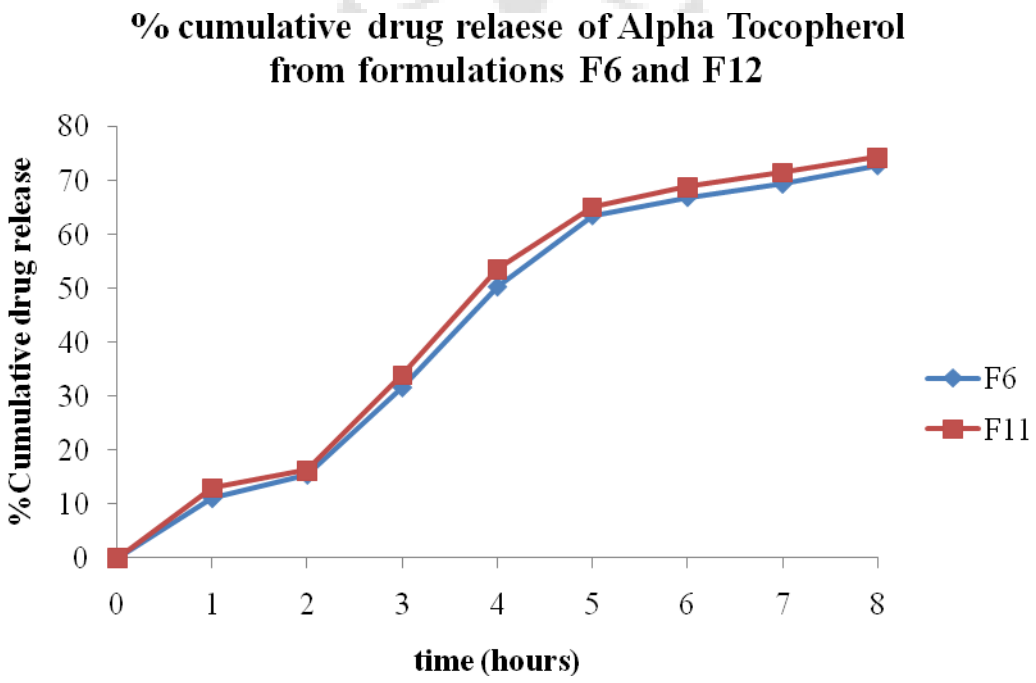

Figure 5: % Cumulative Release Alpha Tocopherol from optimized formulations F6 and F11

Stability study

All the solid lipid nanoparticulate dispersions were stored at different temperatures for the course of 60 days and evaluated at different days of storage. From the stability study data

obtained, no significant changes in physical appearance, Alpha Tocopherol content, and Entrapment Efficiency were seen. This indicates the stability of Solid Lipid Nanoparticulate dispersions.

KINETIC DATA ANALYSIS

In order to understand the release kinetics, the results obtained from *in-vitro* release studies of formulation F11 was fit to various kinetic equations such as zero order (cumulative % release vs. time), first order (log % drug remaining vs. time), and Higuchi's model (cumulative % drug release vs. square root of time).

The release kinetics data indicated that the release of Alpha Tocopherol from formulation F11 fits to first order release model. That means the release happens at different rate and time to achieve prolonged action.

Table 2: Regression coefficient (R^2) values of kinetic models for F11L

Zero order	First order	Higuchi model
0.976	0.987	0.965

The exact mechanism of drug release was determined by the Korsemeyer–Peppas model (log drug release vs. log time).

Table 3: Mechanism of drug release from F11L

0.908	Super case II transport

The value of diffusion exponent, (n) for F11L was found to be 0.908 indicates super case II Transport mechanism of release. i.e., drug release by both diffusion and relaxation of the system.

CONCLUSION

From the above discussion it was concluded that solvent hot homogenization technique shows better control of particles, drug content and *in vitro* drug release data. So it was concluded that the hot homogenization was an efficient method to formulate Solid Lipid Nanoparticle. Tocopherol release from SLN occurs slowly in a super case II transport

mechanism. Tocopherol appears to be distributed molecularly in the lipid carrier with probable physical interaction with the lipids leading to slow release. Stability studies indicated little or no changes in the physical appearance, drug content and entrapment efficiency. Thus, SLN appears as a promising sustained delivery system for Tocopherol to overcome its stability problems.

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