RESEARCH ARTICLE

Rheological and physicochemical characterization of *alpha*-tocopherol loaded lipid nanoparticles in thermoresponsive gel for topical application

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Abstract

Features of a thermoresponsive gel are advantageous for topical application. Lipid nanoparticles is incorporated as a carrier for a hydrophobic active ingredient which is not stable in the aqueous gel system. In this work, a thermoresponsive gel mixture consists of carboxymethyl cellulose (CMC) and *iota*-carrageenan (*ı*-C) was prepared. Nanostructured lipid carriers (NLC) made of fatty acids and lecithin was incorporated into the gel formulation as a carrier for *alpha*-tocopherol. Temperature effect on the rheological behavior of the prepared gel mixture was investigated. The NLC was evaluated for its mean particle size, zeta potential, morphology, encapsulation efficiency and *in vitro* drug release. Physicochemical characterization showed that *alpha*-tocopherol loaded NLC being stored at 4˚C was stable for 1 month and encapsulation efficiency of *alpha*-tocopherol was more than 90% for all formulations due to its hydrophobicity to stay in the lipid matrix. *In vitro* release studies proved that NLC was able to provide slow release of loaded *alpha*-tocopherol. On the other hand, the phase transition from gel-like to liquid-like of the formulated gel mixture was proven to be activated by temperature changes. The rheological gelling point of the gel mixture was represented by the crossover point of storage modulus, *G'* and loss modulus, *G''* which was obtained in the vicinity of body temperature 37˚C. This property is useful in topical application for its better spreadability on skin upon usage and hence better penetration for the *alpha*-tocopherol loaded NLC across the skin barrier. Prolong release of *alpha*-tocopherol with enhanced stability is favored as *alpha*-tocopherol is commonly known for its potent antioxidant activities. These results suggested that gel mixture of CMC and *ı*-C is a good candidate to be developed as a thermoresponsive gel while lipid nanoparticles is a promising carrier system for *alpha*-tocopherol in topical use.

Keywords: Rheology, thermoresponsive gel, lipid nanoparticles, topical application

INTRODUCTION

Topical drug delivery represents a promising route mainly for treatment of localized skin diseases and cosmetic use. A gel is commonly defined as a phase dispersed in another continuous phase forming a three-dimensional network by chemical covalent bond or physical interactions (Nishinari, 2009). It is one of the most general semisolid formulations used in topical application for its good stability against separation and lack of greasiness upon usage. However, active compounds tend to degrade in the dispersing aqueous medium of the gel during storage, thus greatly reduces the effectiveness of the product. Moreover, active compounds are difficult to penetrate across our skin barrier. In fact, skin being the organ most exposed to the environment prevents most external substances penetrating into our body (Friberg, 1990). In order to enhance the penetration and stability of active compounds, a carrier system is incorporated into the gel formulation to provide a more efficient drug delivery.

Colloidal carrier systems had been widely employed to overcome the limitations of conventional dermal formulations in pharmaceutical and cosmetics industries. As an alternative to emulsions, liposomes and polymeric nanoparticles, lipid nanoparticles such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) were developed not only to provide improved features but also to meet the

industrial needs including low cost and easiness for large scale production. Those features found in lipid nanoparticles are advantageous for dermal products for instances occlusive properties, modified release profile and avoidance of systemic uptake (Beck *et al.,* 2011). Lipid nanoparticles are mainly utilized to load hydrophobic drug in its lipid core or lipid outer shell depending on the production method (Üner *et al.,* 2007). The encapsulated drug is therefore protected against chemical degradation caused by the surrounding environment and dispersing medium such as light, oxidation and hydrolysis by water. In addition, the nanoparticles provide prolong release of drug to enhance its effectiveness and avoid irritation to the skin (Dingler *et al.,* 1999; Grana *et al.,* 2013).

Alpha-tocopherol is a form of lipid-soluble vitamin E with potent antioxidant properties essential for health. Oxidation is always related to numerous diseases especially cancer and aging, therefore daily intake of vitamin E is necessary (Podda *et al.,* 2001; Rizvi *et al.,* 2014). Besides dietary intake either from food or supplements, vitamin E can be applied topically to nourish our skin with the main purpose to prevent aging. Shylaja *et al.* (2016) had shown the enhanced stability and slow release property of vitamin E loaded in SLN.

In this study, *alpha*-tocopherol was loaded in lecithin-fatty acid nanoparticles which were then incorporated into a thermoresponsive gel mixture. The aim of employing a thermoresponsive gel instead of a

conventional gel system is to enhance its spreadability upon usage by manipulating the transition temperature of gel from solid-like to liquid-like in the vicinity of body temperature. The physicochemical characteristics and release profile of *alpha*-tocopherol loaded NLC was investigated while the rheological behavior of the gel was evaluated.

EXPERIMENTAL

Materials

Fatty acids (stearic acid and oleic acid), Tween 80, *iota*-carrageenan and phosphate buffered saline tablets were purchased from Sigma Aldrich (St. Louis, USA). Calcium chloride anhydrous was obtained from Merck while carboxymethyl cellulose was bought from Dai-Ichi Kogyo Seiyaku (Kyoto, Japan), respectively. Lecithin was purchased from Alfa Aesar (Massachusetts, USA) and *alpha*-tocopherol was obtained from Spectrum (New Jersey, USA). All solutions and samples were prepared by using deionized water with resistivity of 18.2 Ωcm^{-1}, which was supplied from a Barnstead Diamond Nanopure Water Purification unit coupled with a Barnstead Diamond RO unit (Barnstead International, USA).

Preparation of nanostructured lipid carrier (NLC)

NLC was prepared by hot homogenization method using a high shear homogenizer (Heidolph Silent Crusher M, Germany). Stearic acid, oleic acid and lecithin were heated at 80 °C until the mixture became clear liquid and *alpha*-tocopherol was added into the molten lipid. Surfactant solution (Tween 80) was pre-heated at the same temperature and dispersed into the lipid phase under strong agitation at 18,000 rpm for 10 minutes. The hot emulsion was then poured into cold water at approximately 2°C under magnetic stirring where solidification of lipid occurred. The NLC dispersions were stored at 4°C for further investigation.

Preparation of thermoresponsive gel and NLC-gel

Carboxymethyl cellulose (CMC) and *iota*-carrageenan (*ι*-C) were weighed and added into 0.06 % (w/w) Ca^{2+} solution. The mixture was then heated in a water bath up to 80°C until a clear gel was obtained and kept at room temperature for 24 hours before incorporation of NLC dispersion. 30% of NLC dispersion (w/w) was incorporated into the gel system and mixed homogeneously at 10,000 rpm for 1 minute. The NLC-gel was stored at 4°C for 24 hours for further characterization.

Mean particle size and zeta potential measurement of NLC

The mean particle size and zeta potential of NLC were measured with Zetasizer Nano ZS (Malvern Instruments, U.K.) using Dynamic Light Scattering. NLC dispersions were diluted to an appropriate concentration before being placed into a 1 cm path length quartz cuvette for particle size measurement. A disposable folded capillary cell (DTS1070) was used to perform zeta potential measurement.

Morphology of NLC

The morphology of the *alpha*-tocopherol loaded NLC was observed by an Energy Filtered Transmission Electron Microscopy (TEM) model LIBRA 120 (Zeiss, Germany) with accelerating voltage of 120 kV. A drop of NLC was placed on a 400 mesh copper-coated carbon grid and stained with 2% phosphotungstic acid solution. The excess solution was removed by filter paper. After drying in a desiccator for 24 hours, the grid was ready to be examined by TEM.

Encapsulation efficiency of *alpha*-tocopherol

Encapsulation efficiency of *alpha*-tocopherol loaded NLC can be determined by utilizing centrifugal ultrafiltration method. The dispersion was filled into the upper chamber of a centrifugal filter tube with 50 kDa molecular weight cut-off (Vivaspin 6, Sartorius Stedim Biotech, Germany) and centrifuged at 10,000 rpm for 1 hour. After centrifugation of the dispersion, the supernatant in bottom chamber was dissolved in methanol and vortex homogeneously. *Alpha*-tocopherol was detected spectrophotometrically (Cary 50 UV-Vis Spectrometer, Agilent Technologies, USA) at wavelength 290 nm. The concentration of *alpha*-tocopherol in each sample was determined from a standard calibration curve. Encapsulation efficiency was calculated with the following equation:

$$EE = \left(\frac{W_T - W_F}{W_T} \right) \times 100 \% \qquad (1)$$

where *EE* is encapsulation efficiency of *alpha*-tocopherol in NLC, W_T is the weight of *alpha*-tocopherol added during preparation and W_F is the weight of unloaded *alpha*-tocopherol detected in filtered aqueous phase, respectively.

In vitro release

The release of *alpha*-tocopherol from *alpha*-tocopherol solution, *alpha*-tocopherol gel, *alpha*-tocopherol loaded NLC dispersion and *alpha*-tocopherol loaded NLC-gel at 37°C were investigated by using Automated Franz Diffusion Cell System (Microette Plus Autosampling System, Hanson Research Co., USA) with diffusate chamber volume of 4 mL and effective diffusion area of 0.636 cm^2. The Franz diffusion cells were fitted with pre-hydrated regenerated cellulose membranes with 10 kDa molecular weight cut-off. Ethanol: Phosphate Buffer Solution (PBS) (76:24 v/v) was filled into the receptor chambers to ensure pseudo-sink conditions by increasing the solubility of the active compound in the receiving phase. Similar receptor medium had been used for release studies of vitamin E in previous studies (Sharipova *et al.,* 2016; Shylaja *et al.,* 2016; Yenilmez *et al.,* 2011). The receiving phase was stirred continuously at 400 rpm and thermostated at 37±1°C. Approximately 1 mL of sample was loaded into each donor chamber and the experiments were run for 24 hours. Samples were withdrawn and replaced with fresh medium from the reservoir at each predetermined time interval. The amount of *alpha*-tocopherol can be determined by employing the spectrophotometric method (Cary 50 UV-Vis Spectrometer, Agilent Technologies, USA). The experiment was then repeated with *alpha*-tocopherol gel and *alpha*-tocopherol loaded NLC-gel at 30°C.

Rheological characterization of gel

A strain rate/stress controlled Rheometer MCR 301 (Anton-Paar, Austria) was employed to carry out the rheological measurements of blank gel, NLC-gel and *alpha*-tocopherol loaded NLC-gel. All the tests were done at 25±1°C (except temperature sweep) with measuring geometry cone and plate 1°/ 50 mm and a gap of 0.100 mm. Viscometry test was first performed at controlled shear rate ranged from 0.01 - 100 s^{-1}. After that, oscillatory tests were started with amplitude sweep at controlled strain mode within strain range 1 - 1000% at constant frequency 1 Hz to obtain the linear viscoelastic region (LVR) for the following frequency and temperature sweep tests. Frequency sweep was then performed at constant strain 5% at frequency range 10 - 0.1 Hz. Temperature sweep was carried out by heating the sample from 20°C to 80°C at a rate of 10°Cmin⁻¹, at single frequency 1 Hz and constant strain 5%. Solvent traps were used to minimize the evaporation of sample.

RESULTS AND DISCUSSION

Mean particle size and zeta potential of NLC

Amount of *alpha*-tocopherol added was found to affect the mean particle size of NLC and the stability of the formulation. The mean particle size and polydispersity index (PDI) were rising with the increasing amount of *alpha*-tocopherol (Fig. 1a). Sedimentation was observed in NLCs with 0.4% and 0.6% of *alpha*-tocopherol after 3 weeks, plausibly due to the denser particles formed during lipid solidification. The high amount of *alpha*-tocopherol, which possesses high viscosity, produced a highly viscous nanoemulsion hence forming dense nanoparticles upon solidification. Even though NLCs with 0.1 % and 0.2% of *alpha*-tocopherol were having similar mean particle size after 1 month, 0.1% was considered more stable for its lower and steadier increment in mean particle size and PDI. The fluctuating mean particle size and PDI of NLCs with loaded *alpha*-tocopherol more than 0.1% was probably caused by the expulsion of *alpha*-tocopherol from the lipid matrix where the free *alpha*-tocopherol might form nanoemulsion with excess surfactant and consequently contributing to the higher PDI (Woo *et al.*, 2014).

On the other hand, the *alpha*-tocopherol loaded NLCs were having an insignificant difference of average zeta potential with the unloaded NLC and there are no much changes across the analysis time. This showed consistent condition on the surface structure of the particles. In addition, the zeta potential for all formulations was found lower than -30 mV whereby it was generally accepted as a stable colloidal dispersion (Müller *et al.,* 2001). Nevertheless, steric stabilizer present in the system was not taken into account in this prediction. For instance, Tween 80 as a nonionic surfactant could not ionize in aqueous system but it was able to adsorb onto the surface of particles, forming steric hindrance between the particles (Han *et al.,* 2008).
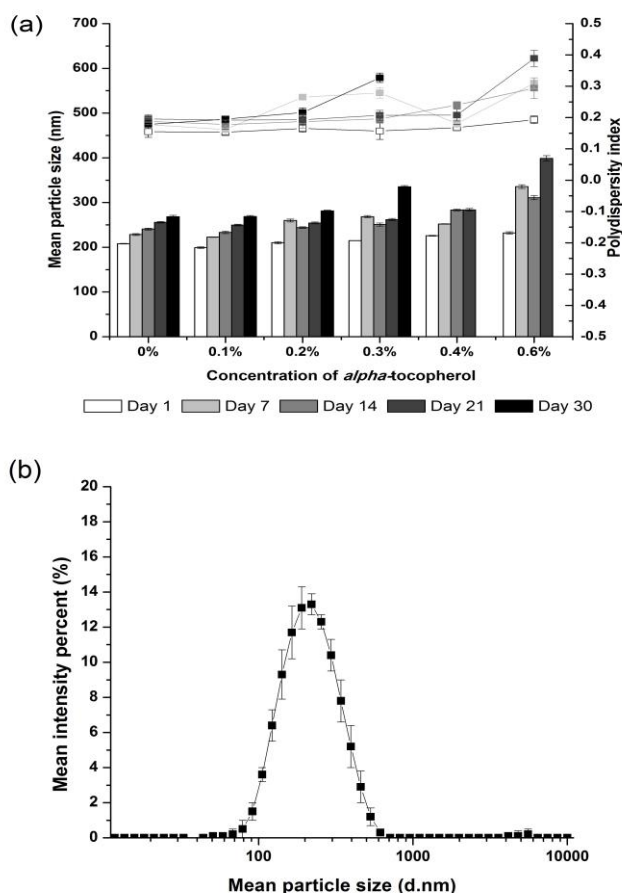

Fig. 1 (a) Mean particle size and PDI of NLCs as a function of concentration of *alpha*-tocopherol; (b) Mean intensity percent of 0.1% *alpha*-tocopherol loaded NLC as a function of mean particle size.

Morphology of NLC

TEM analyses of the *alpha*-tocopherol loaded NLCs revealed spherical shape which is in accordance with the results of particle size measurement. As shown in Fig. 2(a), NLC with 0.1% *alpha*-tocopherol had a monodisperse particle size and no evident aggregation. Nevertheless, NLC with a high amount of *alpha*-tocopherol showed potent aggregation that explains the sedimentation occurred after storage for 3 weeks (Fig. 2(b)).
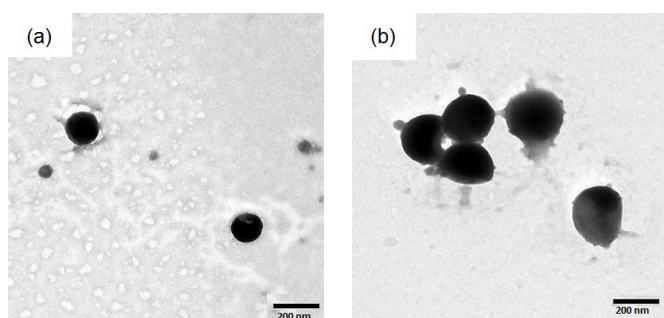

Fig. 2 TEM micrographs of NLCs loaded with (a) 0.1% and (b) 0.6% *alpha*-tocopherol.

Encapsulation efficiency of *alpha*-tocopherol

Only stable formulations with no sedimentation and acceptable increment in particle size were evaluated the encapsulation efficiency of *alpha*-tocopherol. Encapsulation efficiency of *alpha*-tocopherol was high, more than 90% for all formulations under investigation. This observation might be attributed to its hydrophobicity to stay in the lipid matrix rather than the aqueous dispersing medium. In addition, incorporation of oleic acid into the solid lipid perturbed the crystalline matrix of the solid lipid matrix and hence increasing the loading space for active compound (Woo *et al.,* 20.14). Therefore, the main criteria for selecting the optimum formulation were not based on the encapsulation efficiency but the stability of the formulation during storage.

In vitro release

NLC with 0.1% *alpha*-tocopherol was chosen as the optimized formulation to undergo the release studies at 37˚C. The release of *alpha*-tocopherol solution, *alpha*-tocopherol gel and *alpha*-tocopherol loaded NLC-gel were investigated as comparisons. With respect to the thermoresponsive behavior of the gel system, release profiles of the gel samples were also studied at 30˚C as well. The release of *alpha*-tocopherol was supposed only from the lipid phase of NLC due to its poor solubility in the aqueous phase (Jenning *et al.,* 2000). The cumulative *alpha*-tocopherol released from the samples at 37˚C (Fig. 3(a)) and 30˚C (Fig. 3(b)) were plotted against time, respectively.
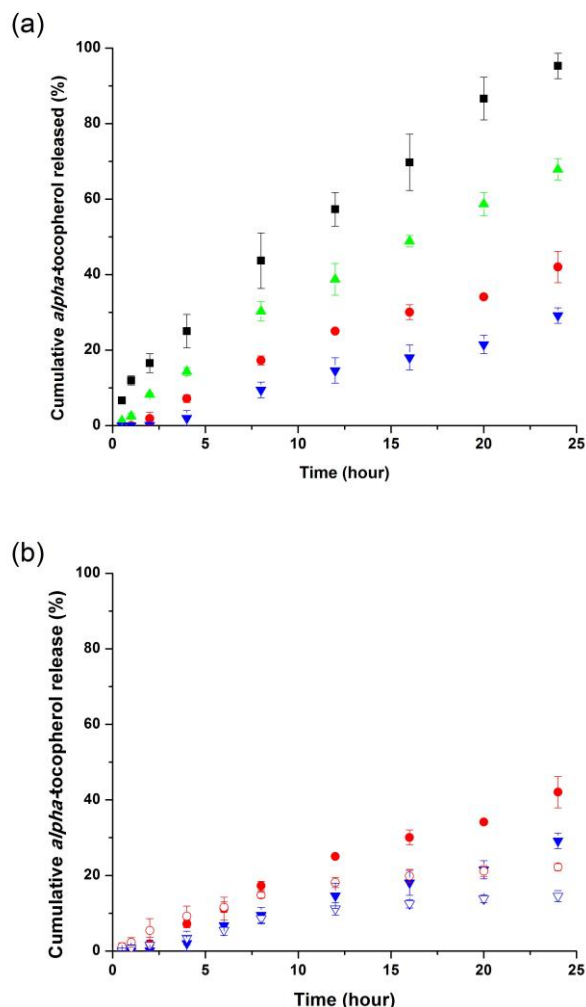

Fig. 3 *In vitro* release of (a) *alpha*-tocopherol solution (■), *alpha*-tocopherol gel (●), *alpha*-tocopherol loaded NLC dispersion (▲) and *alpha*-tocopherol loaded NLC-gel (▼) over a period of 24 hours at 37˚C; (b) *alpha*-tocopherol gel (●) and *alpha*-tocopherol loaded NLC-gel (▼) at 37˚C (solid symbol ●) and 30˚C (open symbol ○).

As shown in Fig. 3(a), the release pattern of *alpha*-tocopherol from solution and NLC dispersion were similar but NLC dispersion was

proven its slow release property. The mobility of the active ingredient was limited by solid lipid matrix of NLC, compared with free motion in solution. Surprisingly, the *alpha*-tocopherol gel was released even slower than the *alpha*-tocopherol loaded NLC dispersion, indicating that the *alpha*-tocopherol was easier to diffuse through the lipid matrix compared with the aqueous gel matrix. The fast diffusion in NLC might be attributed to the fact that *alpha*-tocopherol enriched the outer shell of the particles instead of the lipid core (Fig. 4). This is in agreement with the previous study where the shell enrichment model was proposed for hot homogenization production method (Üner *et al.*, 2007). In addition, the gel samples had higher viscosity, making the active compound more difficult to diffuse. As expected, the *alpha*-tocopherol loaded NLC-gel gave the lowest cumulative release after 24 hours since the active compound had to diffuse through both the lipid matrix and gel matrix before entering the receiving phase.

On the other side, cumulative release of *alpha*-tocopherol from the gel samples at 30˚C was significantly lower than that at 37˚C (Fig. 3(b)). At 30˚C, the gel samples in the donor chamber behave like solid with rigid gel network. The high viscosity increased the difficulty for the active ingredients to diffuse through the gel matrix. In contrast, the liquid-like property became dominant in the gel samples at 37˚C, leading to faster diffusion of *alpha*-tocopherol. This observation

corresponds with the hypothesis made earlier that the optimized thermoresponsive gel system posesses a better flowing profile and hence better release of the active ingredients at body temperature.
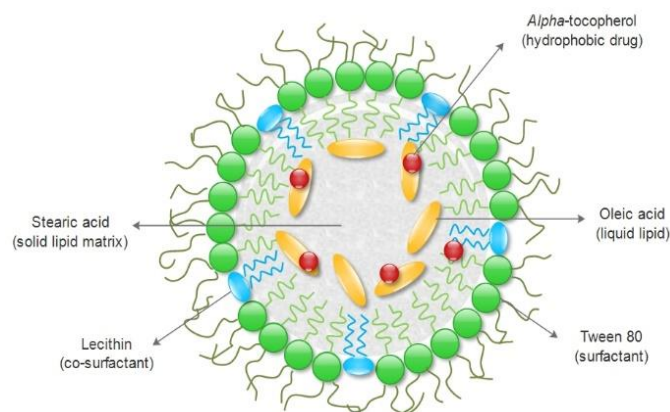

Fig. 4 Schematic illustration of *alpha*-tocopherol NLC.
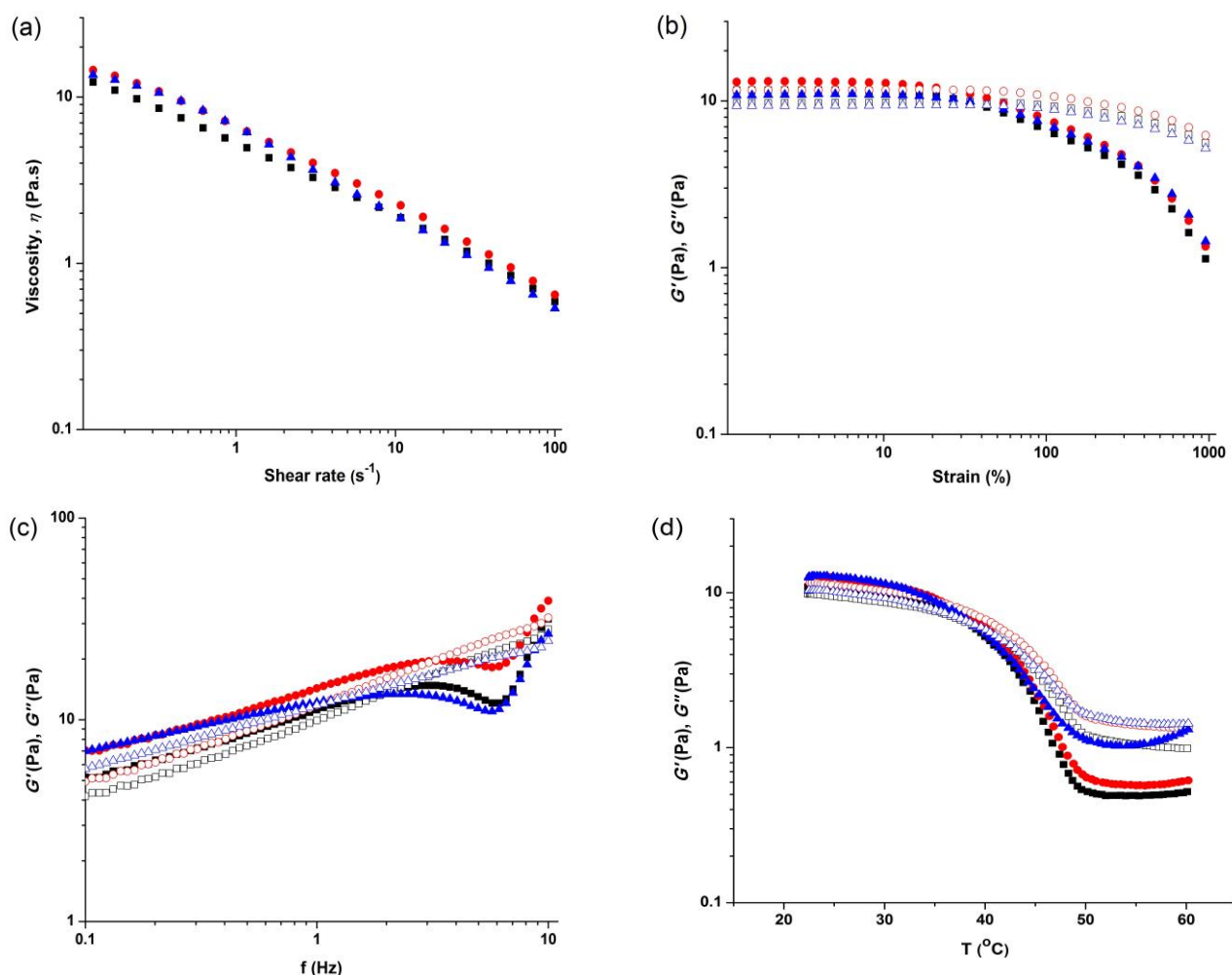

Fig. 5 Rheological measurement of sample blank gel (■), NLC-gel (●) and *alpha*-tocopherol loaded NLC-gel (▲): (a) *η* as a function of shear rate in viscometry; *G'* (solid symbol ■) and *G''* (open symbol □) (a) as a function of strain in amplitude sweep; (b) as a function of frequency in frequency sweep and (d) as a function of temperature in temperature sweep.

Rheological characterization of gel

Understanding the rheological properties is vital to reveal the microstructural environment and mobility, hence deduce the drug diffusion and compatibility. From Fig. 5(a), the flowing curves of the gel samples displayed shear thinning behavior where the viscosity, *η* decreased with increasing shear rate. This non-Newtonian behavior is desired to provide slow flow from the bottle and easy distribution on the skin (Islam *et al.*, 2004). At higher shear rates, the disentanglement

of polymer occurred due to the deformation applied, consequently the active compound entrapped was able to diffuse following the direction of flow.

The oscillation tests were initiated with amplitude sweep to obtain the linear viscoelastic region (LVR) of the samples which indicates the maximum strain capable by the structure before it collapsed. All the samples exhibited solid-like properties at lower strain but viscous components dominate when strain applied reached approximately 30%

(Fig. 5(b)). Therefore, a strain value of 5 % was chosen for the subsequent frequency and temperature sweep tests to ensure the gel structure was not destroyed by the strain applied.

In frequency sweep (Fig. 5(c)), storage modulus, *G'* were found predominant at the lower frequency, showing the good stability of the gel at long time scale, i.e. during storage. However, a weak structure was characterized by high loss modulus, *G''* over *G'* at the higher frequency. This represents its good spreadability at short time scale, i.e. application on the skin. Furthermore, the rheological gelling point determined by the crossover point of *G'* and *G''* happened at a temperature between 35°C – 40°C for all the samples (Fig. 5(d)). This phase transition from solid-like to liquid-like at a temperature in the vicinity of body temperature 37°C again contributes to better spreadability of the gel upon application on the skin.

Basically, the rheological measurements indicated that all the gel samples behave similarly. Nevertheless, the NLC-gel had slightly higher *η* and *G'* compared with the blank gel, owing to the presence of lipid nanoparticles. This suggested that NLC was able to store the applied energy in their elastic components under shear stress applied. The interactions between the nanoparticles and the polymer chains of the gel might also lead to the increased elastic behavior. It was proposed that hydrogen bonds were formed between the oxygen atoms in the fatty acid and the hydroxyl groups (O-H) at the backbone of the polymer chains in the gel matrix (Chieng *et al.*, 2010; Tan *et al.*, 2014). Despite that, the difference between *alpha*-tocopherol loaded NLC-gel and non-loaded NLC-gel was negligible. This revealed that the *alpha*-tocopherol was encapsulated within the lipid matrix, without disturbing the gel network.

CONCLUSION

In the present study, NLC made of stearic acid as solid lipid, oleic acid as liquid lipid, Tween 80 and lecithin as surfactants, was prepared by employing hot homogenization method. Since the encapsulation efficiency of *alpha*-tocopherol in the NLC was high for all formulations, the optimized formulation was determined from the stability profile according to the mean particle size and PDI measurements. 0.1% *alpha*-tocopherol loaded NLC was then incorporated into a thermoresponsive gel consists of *ι*-C and CMC. As expected, NLC released the active compound at a slower rate compared with the free *alpha*-tocopherol solution. Incorporation of *alpha*-tocopherol loaded NLC into the gel matrix even delayed the release rate. Rheological measurements indicated that the gel was beneficial in topical use for its shear thinning behavior under shear stress applied. The thermally activated phase transition from gel-like to liquid-like at a temperature close to the body temperature could improve spreadability of the gel and hence provide better delivery of the active compound, in line with the release profile of the gel samples at 30°C and 37°C. At the meanwhile, the rigidity of the gel was more pronounced with the incorporation of NLC, suggesting enhanced stability of NLC in the gel system. Considering the results obtained in the study, the thermoresponsive gel mixture of *ι*-C and CMC is likely to be developed as a topical formulation while lipid nanoparticles is a promising carrier system for *alpha*-tocopherol.

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