Formation and stability of Vitamin E nano-emulsion based delivery systems by spontaneous emulsification method: Glycerol as a co-solvent

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ABSTRACT: There is considerable interest in using nano-emulsions (NEs) as delivery systems for lipophilic bioactive ingredients. In this study, the influence of G on the properties of vitamin E (VE) enriched NEs prepared using spontaneous emulsification (SE) method, were investigated. The particle size and optical clarity of the NEs depended strongly on the co-solvent presence, absence, and concentration. The smallest droplets (mean diameter less than 23.5 and 236.71 nm) and highest transparency (lowest turbidity, 0.0069 and 0.0161 cm⁻¹) were observed in the absence of G (A formulation) and the presence of 30 wt.% G (D formulation), respectively. However, these NEs were highly unstable for droplet growth during storage, especially at high temperatures, which was attributed to coalescence and Ostwald ripening (OR). Dilution of the NEs (100× with water) before of storage considerably improved their storage stability especially at higher storage temperatures. Undiluted NEs exhibited a sharp and irreversible increase in turbidity upon heating: ≈75 °C and 70 °C for A formulation and D formulation, respectively. Diluted NEs had much better thermal stability, with a sharp increase in turbidity occurring at ≈75 °C for these systems. This study provides useful information for use in pharmaceutical, personal care, and food products.

Keywords: Co-solvent; Dynamic light scattering; Glycerol; Nano-emulsion; Spontaneous emulsification; Vitamin E.

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INTRODUCTION

The food and beverage industries are interested in the development of colloidal delivery systems to encapsulate lipophilic functional ingredients, such as bioactive lipids, flavour oils or oil-soluble-Vs (e.g. VE, VD) (Mayer, et al., 2013, Guttoff, et al., 2015). VE is a fat-soluble compound that naturally exists in eight different isomers. Among these isomers, α-tocopherol is the most naturally abundant and it has been shown to have antioxidant activity (Saberi, et al., 2013). The recommended daily intake (RDI) of VE is 15 mg/day (Yang and McClements, 2013). The utilization of α-tocopherol as a functional ingredient is currently limited owing to its heat and oxygen sensitivity, poor water-solubility,
and low and variable bioavailability. NEs are used as delivery systems (Komaiko and McClements, 2015). Reducing particle size in emulsion-based delivery systems has a number of consequences that may be beneficial for certain food applications: (i) greater stability for droplet aggregation and gravitational separation; (ii) higher optical clarity; and (iii) increased oral bioavailability of bioactive compounds (Guttoff, et al., 2015, Walkera, et al., 2015, Perrier-Cornet, et al., 2005). Two approaches can be used to fabricate NEs: high-energy and low-energy approaches (Davidov-Pardo and McClements, 2015). NEs are commonly fabricated by high-energy methods (Saberi, et al., 2015) which utilize specialized equipment capable of generating intense mechanical forces (Komaiko and McClements, 2014) such as high-pressure homogenization (Donsia, et al., 2011, Donsi, et al., 2011, Relkin, et al., 2008, Relkin, et al., 2009), micro-fluidization (Rao and McClements, 2012, Yang, et al., 2013) and ultrasonic (Kentish, et al., 2008, Silva, et al., 2012). The main variables that impact NE characteristics are the energy intensity and duration, the surfactant type and concentration, and the physicochemical properties of the oil and water phases (Komaiko and McClements, 2014). Low-energy methods are based on the spontaneous formation of nano-sized oil droplets in surfactant–oil–water mixtures when the composition or temperature of the system is changed in a particular manner (Davidov-Pardo and McClements, 2015, Saberi, et al., 2015), such a SE (Dai, et al., 1997), membrane emulsification (Sanguansri, Augustin, 2006, Nikiforidis, et al., 2011), phase inversion temperature, emulsion inversion point (Silva, et al., 2012, Anton & Vandamme, 2009) and solvent displacement (Silva, et al., 2011). The main advantage of using low-energy methods such as SE is that NEs can be produced without the need for any sophisticated or expensive equipment (Saberi, et al., 2015). Since it simply involves titration of an oil surfactants mixture into an aqueous solution with constant agitation at a fixed temperature (Saberi, et al., 2014), which is produced by different mechanisms such as budding off which it is likely that water will penetrate into the S/O phase and that surfactant will move into the water phase (Yang, et al., 2012). The major components that can be used to formulate food-grade NEs are oil phase, aqueous phase and stabilizers (McClements andRao, 2011). There are several factors, involved in emulsion stabilization mechanisms; interfacial tension, electrical charge, liquid crystal stabilization and stabilization by macromolecules and finely divided solids (Mahungu and Artz, 2009). Also, emulsion destabilization can be due to one or all of five possible mechanisms; flocculation, coalescence, sedimentation or creaming, OR, and phase inversion (Mahungu and Artz, 2009). In this study, we investigated the influence of G on the formation, properties, and stability of VE-enriched NEs. G is miscible with water co-solvent (Takamura, et al., 2012, Chanasattru, et al., 2009) that plays its role by reducing the polarity of the aqueous phase and therefore, facilitating the micro-emulsion construction...
(Amiri-Rigi and Abbasi, 2016). G has three hydroxyl groups. The hydroxyl groups of the G can robustly attach to the water molecule using hydrogen bonding (Amiri-Rigi and Abbasi, 2016). Using the G as a co-solvent in aqueous solutions changes the physical properties of aqueous solutions such as viscosity, density, interfacial tension (Takamura, et al., 2012) and the refractive index (Lide, 2004) and it showed that could alter the solubility, interfacial tension (Anton & Vandamme, 2009, Chanasattru, et al., 2009, Zeng, et al., 2014, Yaghmur, et al., 2002, Zhang, et al., 2008) and optimum curvature of some of the surfactants (Saberi, et al., 2013, Garti, et al., 2001).

The purpose of this study was to investigate the effect of the presence, absence and concentration of G, as a co-solvent on the formation, properties, and stability of VE enriched NEs prepared by SE as an isothermal low energy method.

MATERIALS AND METHODS

Materials
Vitamin E-acetate 99%, was purchased from Zhravi, Tehran, Iran, Tween 80 were obtained from Sigma-Aldrich, Tehran, Iran; Citric acid was obtained from Merck, Tehran, Iran; and Sodium benzoate was purchased from Kian Kaveh, Tehran, Iran, and they used without any purification. Deionized water was purchased from Zolal Teb Shimi, Tehran, Iran, and glycerol was purchased from Mojallali, Tehran, Iran, and they applied as solvent and co-solvent, respectively.

NE preparation
NEs were prepared by using the SE based on the method described by (Anton& Vandamme, 2009, Saberi, et al., 2013) with some minor modifications. SE was performed by the addition of an organic phase to an aqueous phase while the system was being stirred at a fixed speed using a magnetic stirrer at ambient temperature (22 °C) which it was carried out in 3 steps.

Preparation of liquid phase
An aqueous phase was prepared, containing buffer solution (80-30 wt.%)-consisting of 0.8 wt.% citric acid and 0.08 wt.% sodium benzoate, in double distilled water, pH 3, in order to simulate the aqueous phase of a beverage system- and different concentration of G (0-50 wt.%).

Preparation of organic phase
The organic phase consisted of a mixture of oil (VE) (10 wt.%) and surfactant (tween 80) (10 wt.%). At first two of the VE and tween 80 were mixed together over a 45 minute period under constant magnetic stirring (750 rpm) at ambient temperature.

Spontaneous emulsification method
The organic phase was then slowly poured into the aqueous phase over a 10 minute period with continuous stirring under constant magnetic stirring (600 rpm) at ambient temperature. After adding the organic phase, the resulting emulsion to reach equilibrium was left for another 5 min of stirring. Diluted emulsions were prepared by dilution of the VE
enriched NEs by dilution factor: 100 and using the acidic buffer solution (pH 3).

**Particle size measurements**
Particle size distributions were determined using dynamic light scattering technique. Dynamic light scattering was used to determine the particle diameters of dispersions in the submicron range (Scatteroscope1, Qudix). This instrument determines the particle size from intensity-time fluctuations of a laser beam (532 nm) scattered from a sample at a measurement angle of 90. All of these samples were diluted with the acidic buffer solution (pH 3) prior to measurement to prevent the multiple scattering effects. The foundation of this technique is based on the scattering of light by moving particles due to Brownian motion in a liquid. The movement of the particles is then related to the size of the particles (Saberi, *et al.*, 2015, Saberi, *et al.*, 2013). The instrument reports the particle size distribution. The mean particle diameter and the polydispersity index (PDI) were calculated from the particle size distribution.

**Turbidity measurements**
The turbidity of samples was determined using an Ultra Violet-Visible Spectrophotometry (RIGOL, Ultra-3660). The absorbance at 600 nm was measured as the temperature was increased from 20 °C to 80 °C, and then decreased back to 20 °C by changing in every 5 °C. We used the following equation to obtain the turbidity (eq. 1).

\[ T = \frac{(2.3 \times A)}{L} \]  

Where \( T \) is the turbidity, \( A \) is the absorbance at a wavelength of 600 nm and \( L \) is the passing path length of the beam in the cuvette (Melik and Fogler, 1982).

**Statistical analyses**
We investigated all fresh samples at least duplicate time. The results are reported as the mean and standard deviation of these measurements.

**RESULTS AND DISCUSSION**

**Effect of G concentration on the nano-emulsification and particle size of VE enriched NEs**
At first, we investigated the influence of G concentrations on the nano-emulsification and particle size distributions of VE enriched NEs. Emulsions prepared using the SE method by using 20 wt.% total organic phase (10 wt.% oil (VE) and 10 wt.% surfactant (Tween 80)) and 80 wt.% total aqueous phase (0-50 wt.% co-solvent (G) and 80-30 wt.% buffer solution (pH 3). In Table 1, had been shown different formulations of the VE enriched NEs at different concentrations of G. The effect of different concentrations of G in droplet size and size distribution had been shown in Figs. 1 and 2 NEs prepared. The droplet size distribution of NEs prepared in different concentrations of G was mono-modal (Data not have been shown). The mean diameter of the NEs particles prepared, increased significantly from 23.5 nm to 276.81 nm when the G concentration was increased from 0 wt.% to 10 wt.%. Then it increased from
276.81 nm to 308.98 nm by increasing the G concentration from 10 wt.% to 20 wt.%. Then it decreased from 308.98 nm to 236.771 nm by increasing the G concentration from 20 wt.% to 30 wt.%. Then it increased from 236.771 nm to 276.81 nm by increasing the G concentration from 30 wt.% to 40 wt.%. Then it decreased from 276.81 nm to 263.771 nm by increasing the G concentration from 40 wt.% to 50 wt.% (Fig. 1). In other hands, in the presence of G, by increasing and decreasing the G concentration from 30 wt.%, particle size was increased. As shown in Fig. 1, in this range of concentration which we studied (0-50 wt.% G), the smallest particle size in the absence of the G, was obtained, 23.5 nm and in the presence of the G (30 wt.%), the smallest particle size, was obtained, 236.771 nm. At first, the polydispersity index of the prepared NEs, increased from 0.495 to 0.6241 when the G concentration was increased from 0 wt.% to 20 wt.%. Then it decreased from 0.6241 to 0.5625 by increasing the G concentration from 20 wt.% to 30 wt.% Then it remained constant at about 0.5625 by increasing the G concentration from 30 wt.% to 50 wt.%.

Thus, in this range of concentration which we studied (0-50 wt.% G), the lowest index in the absence of G obtained, 0.49 and the highest index in the presence of G concentration 20 wt.% obtained 0.6241 (Fig. 2). In this concentration values which we investigated, our data illustrated that (0-50 wt.% G), the smallest particles (23.5 nm) with the narrowest size distribution (PDI=0.49) were formed in the absence of G (A formulation) and in the presence of G, the smallest particles (236.771 nm) with the narrowest size distribution (PDI=0.5625) were formed at the concentration of 30 wt.% (D formulation). The mean droplet diameter obtained from this study was smaller than the results of similar work by Saberi and et al., 2013, (The smallest droplet in the absence of G around 160 nm and in the presence of 30 wt.% G was smaller than 100 nm and in the optimum values 40 wt.% and 50
wt.% were smaller than 50 nm). Since commercially it is often important to increase the loading capacity of emulsion-based delivery systems, we used an oil phase consisting of pure vitamin E (100 wt.% VE) and the results obtained from this work, in the absence (0 wt.%) and lower values (30 wt.%) of G, were better than the results obtained from Saberi, et al. work (Saberi, et al., 2013). In general, different physicochemical or molecular phenomena may account for the ability of polyols and short chain alcohols to alter the properties of surfactants in aqueous solutions and emulsions. First, co-solvents may change the solubility of surfactant monomers in aqueous solutions by altering the magnitude of the hydrophobic effect, e.g., by making the aqueous phase a less thermodynamically unfavorable environment for non-polar groups. Second, amphiphilic co-solvents may be able to penetrate into the surfactant monolayer and change its optimum curvature, interfacial tension, and flexibility. Third, co-solvents may alter the hydration of the hydrophilic head-groups of surfactants, thereby also altering the packing and optimum curvature of surfactant monolayers. The mechanism of droplet formation at the boundary between the organic phase and the aqueous phase in SE has been partly attributed to the rapid diffusion of surfactant molecules from the organic phase into the aqueous phase. The solubility and optimum curvature of surfactants in surfactant-oil–water mixtures are known to play an important role in this process (Saberi, et al., 2013).

Table 1. Different formulations of the NEs enriched with VE at different concentrations of G, prepared by the SE method.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Total Amount (wt.%)</th>
<th>Organic Phase (20 Wt.%)</th>
<th>Liquid Phase (80 Wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oil (10 Wt.%), Surfactant (10 Wt.%), Co-Solvent (0-50 Wt.%), Buffer Solution (80-30 Wt.%)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>100%</td>
<td>10% VE, 10% Tween 80, 0% G, 80% Buffer Solution</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>100%</td>
<td>10% VE, 10% Tween 80, 10% G, 70% Buffer Solution</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>100%</td>
<td>10% VE, 10% Tween 80, 20% G, 60% Buffer Solution</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>100%</td>
<td>10% VE, 10% Tween 80, 30% G, 50% Buffer Solution</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>100%</td>
<td>10% VE, 10% Tween 80, 40% G, 40% Buffer Solution</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>100%</td>
<td>10% VE, 10% Tween 80, 50% G, 30% Buffer Solution</td>
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</table>

Effect of G concentration on the transparency of VE enriched NEs

The optical properties of delivery systems are of particular importance for their application in certain types of functional food and beverage products. In some situations, the final product should be optically transparent or only slightly turbid (e.g., vitamin-fortified
waters and clear juices), so the addition of any delivery system should not make them look cloudy or opaque (Saberi, et al., 2013). To prepare optically transparent NEs it is usually necessary to carefully control the initial system composition as well as the homogenization conditions (Rao, McClements, 2013). To prepare beverage emulsions that appear visually transparent the turbidity should be less than 0.05 cm\(^{-1}\) (Yang and McClements, 2013). The influence of G concentration on the appearance of VE enriched NEs was therefore investigated. A, B, C, D, E and D formulations were used in this investigation. The optical clarity of the VE enriched NEs somewhat increased by increasing the G concentration, which it can be attributed to the ability of co-solvent to decrease the size of the droplets in VE enriched NEs, thereby decreasing their scattering efficiency. It can be attributed to the ability of co-solvent to increase the refractive index of the aqueous phase, which also reduces the droplet scattering efficiency. It can be attributed to the fact that smaller particles scattering light with less intensity than the larger particles (Wooster, et al., 2008).

**Effect of G concentration on the storage stability of VE enriched NEs**

For commercial applications, it is often important that emulsion-based delivery systems remain physically stable during storage, transport, and utilization. NEs may become unstable due to a number of different physicochemical processes, including flocculation, coalescence, OR, and gravitational separation. The rate at which an NE breaks down, and the mechanism by which this process occurs, depends on its composition and microstructure, as well as on the environmental conditions it experiences during its lifetime. We, therefore, examined the influence of storage time and temperature on the stability of VE enriched NEs (Saberi, et al., 2013). Thus, in the presence of VE, the refractive index increased when the G concentration was increasing. Consequently, an increase in the aqueous phase refractive index will decrease the scattering efficiency of the oil droplets. Therefore, light scattering from an NE containing 30 wt.% G (D formulation) should be greater than other concentrations of G (Saberi, et al., 2013). The results of the turbidity measurement confirmed the results of the visual assessment. NE with high optical transparency (Data not have been shown), were corresponded to the smallest particle diameter (Fig. 3). This effect is due to the fact that smaller particles scattering light with less intensity than the larger particles (Wooster, et al., 2008).

![Fig.3. The turbidity obtained from the A, B, C, D, E and F formulations.](image)

The refractive index of aqueous G solutions has been reported to increase as the solute concentration increases. The refractive index of VE acetate has been reported around 1.50 (Saberi, et al., 2013). Therefore, light scattering from an NE containing 30 wt.% G (D formulation) should be greater than other concentrations of G (Saberi, et al., 2013). The results of the turbidity measurement confirmed the results of the visual assessment. NE with high optical transparency (Data not have been shown), were corresponded to the smallest particle diameter (Fig. 3). This effect is due to the fact that smaller particles scattering light with less intensity than the larger particles (Wooster, et al., 2008).
We investigated the storage stability of VE NEs based on the method described by Saberi and et al. in 2013 with slight changes. NEs were prepared in A formulation and D formulation in the aqueous phase. Then we stored them at 5 °C, 22 °C and 37 °C for 30 days, which were selected to represent refrigeration conditions, ambient storage in mild climates, and ambient storage in hot climates, respectively (Saberi, et al., 2013). A formulation, an increase in the mean diameter of particles in NEs prepared in all of the storage temperatures was observed (about 3 fold increases). The mean diameter of particles, increased from 23.5 nm initially to 37.571 nm at 5 °C (about 34.345% increases), 41.114 nm at 22 °C (about 74.95% increases), and 72.312 nm at 37 °C (about 207.7% increases) after 30 days storage (Fig. 4). The particle size distribution of NEs changed from mono-modal to bimodal (Data not have been shown) and it (PDI= 0.49) significantly expanded by increasing storage temperature -5 °C (PDI= 0.5476), 22 °C (PDI= 0.8464) and 37 °C (PDI= 0.9409) (Fig. 5). Since the emulsion delivery systems are often diluted in commercial applications (such as functional foods and beverages), so the effect of dilution on storage stability of A formulation was evaluated. NEs without co-solvent, diluted with water before storing (dilution factor= 100), the slight increase in mean particle diameter was observed in diluted NEs (d /d₀ increased less than about 1.5 fold). The mean diameter of particles, increased from 23.5 nm initially to 28 nm at 5 °C (about 19.15% increases), 31.571 nm at 22 °C (about 34.345% increases), and 33.86 nm at 37 °C (about 44.085% increases) after 30 days storage (Fig. 4). The particle size distribution of NEs remained mono-modal in low temperatures and then in high temperature slightly changed from mono-modal to bimodal (Data not have been shown) and it (PDI= 0.49) significantly expanded with increasing storage temperature -5 °C (PDI= 0.49), 22 °C (PDI= 0.5476) and 37 °C (PDI= 0.5041) (Fig. 5). Diluted NEs were more stable for droplet growth than undiluted NEs (Fig. 4).

![Fig. 4. The mean particle diameter obtained from changes in storage time and temperature of the undiluted (UD) and diluted (D) A formulation.](image)

![Fig. 5. The polydispersity index obtained from changes in storage time and temperature of the undiluted (UD) and diluted (D) A formulation.](image)
A formulation, an increase in the turbidity of particles in NEs prepared in all of the storage temperatures was observed (about 26 fold) (Fig. 6). The turbidity of NEs, increased from 0.0069 cm\(^{-1}\) initially to 0.023 cm\(^{-1}\) at 5 °C (about 233.34% increases), 0.0437 cm\(^{-1}\) at 22 °C (about 494.2% increases), and 0.1794 cm\(^{-1}\) at 37 °C (about 2500% increases) after 30 days storage. The turbidity of nano-emulsions significantly increased after 30 days of storage at high temperatures (Fig. 6). The turbidity of diluted NEs (dilution factor = 100) in A formulation, increased after 30 days storage and they visually had a clear appearance (Data not have been shown). The turbidity of NEs, increased from 0.0069 cm\(^{-1}\) initially to 0.023 cm\(^{-1}\) at 5 °C (about 233.34% increases), 0.023 cm\(^{-1}\) at 22 °C (about 233.34% increases), and 0.0253 cm\(^{-1}\) at 37 °C (about 266.67% increases) after 30 days storage (Fig. 6). The results of the turbidity measurement by visual observations of apparent turbidity NEs enriched with VE were similar (Data not have been shown). The system’s tendency went to transparency by reducing the turbidity (Fig. 6). The results show that a formulation NEs were sensitive to particle growth over time, in which the particle growth rate increased following increasing temperature storage and the dilution with water before storing improved storage stability. Coalescence is due to Brownian motion (Saberi, et al., 2014). Dilution with water before storing causes that reduced the particles collision to each other under Brownian motion and consequently reducing the rate of the coalescence. As a result, it is possible that coalescence is the main destabilizing mechanism in NE systems in A formulation (Saberi, et al., 2014, 2013).

In D formulation, the mean diameter of the particles in the NEs prepared in all of the storage temperatures increased (about 1.37 fold increases). The mean diameter of particles, increased from 236.771 nm initially to 295.43 nm at 5 °C (about 24.77% increases), 305.02 nm at 22 °C (about 28.82% increases), and 323.624 nm at 37 °C (about 36.68% increases) after 30 days storage (Fig. 7). The particle size distribution of NEs was monomodal (Data not have been shown) and it (PDI = 0.5625) changed by increasing storage.
The particle size distribution of NEs was mono-modal (Data not have been shown) and it (PDI= 0.5625) changed by increasing storage temperature -5 °C (PDI= 0.33), 22 °C (PDI= 0.4489) and 37 °C (PDI= 0.6724) (Fig. 8). Diluted NEs were more stable for droplet growth than undiluted NEs (Fig.7). Also, we have investigated the effect of storage time and temperature on the optical properties of undiluted (UD) and diluted (D) NEs. In D formulation, an increase in the turbidity of NEs prepared in all of the storage temperatures after 30 days were observed (about 2.86 fold).

The turbidity of NEs, increased from 0.0161 cm$^{-1}$ initially to 0.0437 cm$^{-1}$ at 5 °C (about 171.43% increases), 0.046 cm$^{-1}$ at 22 °C (about 185.7% increases), and 0.046 cm$^{-1}$ at 37 °C (about 185.7% increases) after 30 days storage. The turbidity of diluted NEs (dilution factor=100) in D formulation, slightly increased after 30 days storage (Fig. 9) and they visually had a clear appearance (Data not have been shown). The turbidity of NEs, increased from 0.0161 cm$^{-1}$ initially to 0.023 cm$^{-1}$ at 5 °C (about 42.86% increases), 0.023 cm$^{-1}$ at 22 °C (about 42.86% increases), and 0.0299 cm$^{-1}$ at 37 °C (about 85.71% increases) after 30 days storage (Fig. 9). The results of the turbidity measurement (Fig. 9) were similar by visual observations of apparent turbidity VE enriched NEs (Data not have been shown). The system’s tendency went to transparency by reducing the turbidity (Fig. 9). Also, by reducing the particle size, turbidity decreased because smaller droplets scattering light with lower intensity (Saberi, et al., 2013).

Since the emulsion delivery systems are often diluted in commercial applications (such as functional foods and beverages), so the effect of dilution on storage stability of D formulation in the aqueous phase was evaluated. NEs D formulation, diluted with water before storing (dilution factor= 100), a slight increase in mean particle diameter, was observed in diluted NEs ($d/d_0$ increased less than about 1.25 fold). The mean diameter of particles, increased from 236.771 nm initially to 260.91 nm at 5 °C (about 10.20% increases), 260.91 nm at 22 °C (about 10.20% increases), and 295.43 nm at 37 °C (about 24.77% increases) after 30 days storage (Fig. 7).
The results show that NEs D formulation were sensitive to particle growth over time, in which the particle growth rate increased following increasing temperature storage and the dilution with water before storing improved their storage stability. There are a number of possible mechanisms that might account for the observed increase in mean particle size in the NEs during storage, including flocculation, coalescence and OR (Saberi, et al., 2013, Melik and Fogler, 1982). We do not believe that flocculation was important in the NEs used in this study since; very fine droplets stabilized by non-ionic surfactants tend to be stable to flocculation due to the strong steric repulsion between the hydrophilic surfactant head groups (McClements and Rao, 2011, Saberi, et al., 2013). We, therefore, postulate that either coalescence or Oswald ripening was responsible for the droplet growth observed in this study (Saberi, et al., 2013). The rate of droplet coalescence in oil-in-water emulsions stabilized by non-ionic surfactants usually increases as the temperature is raised towards the phase inversion temperature (PIT) of the system (Anton & Vandamme, 2009, Saberi, et al., 2013). This effect has been attributed to dehydration of the surfactant head-groups leading to changes in the optimum curvature and interfacial tension at elevated temperatures (Saberi, et al., 2014). The addition of co-solvent is known to decrease the PIT of emulsions stabilized by non-ionic surfactant molecules, which is also attributed to its ability to dehydrate the surfactant head groups (Saberi, et al., 2013). The rapid droplet growth and steep turbidity increase observed in the NEs during storage at 37 °C. In the presence of high G levels may, therefore, have been due to the ability of this co-solvent to reduce the PIT of the system thereby accelerating droplet coalescence (Saberi, et al., 2013, Rao, McClements, 2013). The rate of droplet growth due to OR may also increase by increasing the temperature. The change in mean droplet diameter (d) with time (t) due to OR of emulsified oil in the steady-state regime is given by (Eq. 2) (Saberi, et al., 2013).

\[
\left(\frac{d}{d_0}\right)^2 = 1 + \frac{64\gamma V_m c D}{9RTd_0^3} t
\]  

(Eq. 2)

Where \(d_0\) is the initial diameter, \(\gamma\) is the interfacial tension, \(V_m\) is the molar volume of the oil, \(R\) is the gas constant, and \(T\) is the absolute temperature, \(c\) is the solubility of the oil in the aqueous phase, and \(D\) is the translational diffusion coefficient of the oil molecules through the aqueous phase (Anton & Vandamme, 2009, Saberi, et al., 2013). As we see in (Eq. 1) when each one of \(\gamma\), \(V_m\), \(c\), \(D\) and \(t\), increases, it caused to increase the rate of droplet growth due to OR (Ee, et al., 2008). Both water and co-solvent molecules (G) can form hydrogen bonds with polar groups and they are water soluble but the polarity of G is less than deionized water. Therefore oil droplets have more solubility in G than deionized water. Therefore \(c\), (the solubility of the oil in the aqueous phase) increases and it followed by increase the droplet growth rate due to OR (Chanasattru, et al., 2007). Also, the surfactant molecules at the oil-water interface may have inhibited OR
due to their impact on the interfacial tension or rheology. There may have been some highly hydrophobic impurities in the VE that acted as ripening inhibitors (Saberi, et al., 2013). In conclusion, the fact that we observed little droplet growth in the diluted NEs suggests that OR was not a major instability mechanism in these systems. However, OR may be contributed to instability at higher co-solvent concentrations and elevated temperatures due to an increase in the water-solubility of VE. It is also possible that droplet coalescence contributed (Saberi, et al., 2013). The rate of droplet coalescence tends to increase as the temperature approaches the PIT of the surfactant–oil–water system. The fact that droplet growth occurred more rapidly in the presence of high levels of co-solvents (undiluted NEs) than in the presence of low levels (diluted NEs), suggests that the co-solvents were able to change the properties of the surfactant monolayers (such as optimum curvature, interfacial tension and flexibility), thereby reducing their PIT. In addition, the frequency of droplet collisions decreases after dilution, which would also be expected to decrease the coalescence rate. Based on these results, we postulate that coalescence is the most likely mechanism for droplet growth at elevated temperatures (Saberi, et al., 2013).

**Effect of the absence and presence of G on the thermal stability of VE enriched NEs**

Knowledge of the thermal stability of NE-based delivery systems is important for many commercial applications since they are often exposed to elevated temperatures during their manufacture, storage, or utilization, e.g., hot filling, pasteurization, or cooking (Saberi, et al., 2013). We therefore studied the influence of the absence (A formulation) and presence of G (D formulation) on the thermal stability of the VE enriched NEs and Changes in the turbidity of selected VE enriched NEs were recorded when they were subjected to a heating and cooling cycle, as the temperature was increased from 20 °C to 80 °C, and then decreased back to 20 °C by changing in every 5 °C. To evaluate and compare the effect of absence and presence of G on the thermal stability of VE enriched NEs, at first after selecting the best examples of optimal production conditions, the effect of temperature changes by measuring the turbidity of the VE enriched NEs by measuring of absorption intensity NEs prepared by the method of SE. A formulation, in the evaluation of the thermal stability, turbidity of the NEs enriched with VE, initially remained relatively low and during the heating from 20 °C to 60 °C remained stable (T= 0.0207 cm\(^{-1}\)). Then it increased by increasing temperature from 60 °C to 65 °C (from T= 0.0207 cm\(^{-1}\) to T= 0.2208 cm\(^{-1}\)) (about 10.6 fold increases). Then it decreased by increasing the temperature from 65 °C to 70 °C (from T= 0.2208 cm\(^{-1}\) to T= 0.0391 cm\(^{-1}\)) (with ratio about 0.18 decreases). Then it increased by increasing the temperature from 70 °C to 75 °C (from T= 0.0391 cm\(^{-1}\) to T= 0.7843 cm\(^{-1}\)) (about 20 fold increases). Then it slightly decreased by increasing the temperature from 75 °C to 80 °C (from T= 0.7843 cm\(^{-1}\) to T= 0.7636 cm\(^{-1}\)) (with ratio
about 0.97 decreases) (Fig. 10). It remained relatively high during the cooling from 80 °C to 20 °C which indicates that an increase in turbidity that occurs during heating is irreversible. The increase in turbidity can be attributed to an increase in droplet size during heating. The physicochemical origin of this effect can be related to progressive dehydration of the polar head group of the non-ionic surfactant molecules (Tween 80) as the temperature is raised. This process reduces the hydration repulsion between the droplets thereby allowing them to get closer together. In addition, head group dehydration means that the optimum curvature of the surfactant monolayer moves closer to unity, which favours an ultralow interfacial tension and promotes droplet coalescence (Saberi, et al., 2013). The fact that the turbidity remained high during cooling (and the samples appeared visibly cloudy (Data not have been shown)) suggests that the oil droplets remained large after coalescence had occurred (Saberi, et al., 2013). We also studied the turbidity of diluted NEs (dilution factor= 100) in the absence of co-solvent in the thermal stability measurement. The turbidity of the NEs enriched with VE remained relatively low (T= 0.0069 cm\(^{-1}\)) and it remained stable during heating from 20 °C to 60 °C (T= 0.0069 cm\(^{-1}\)). Then it increased by increasing the temperature from 60 °C to 65 °C(from T= 0.0069 cm\(^{-1}\) to T= 0.0795 cm\(^{-1}\)) (about 11 fold increases). Then it slightly decreased by increasing the temperature from 65 °C to 70 °C (from T= 0.0759 cm\(^{-1}\) to T= 0.0207 cm\(^{-1}\)) (with ratio about 0.27 decreases). Then it significantly increased by increasing the temperature from 70 °C to 75 °C(from T= 0.0207 cm\(^{-1}\) to T= 0.3243 cm\(^{-1}\)) (about 15.6 fold increases). Then it slightly decreased by increasing the temperature from 75 °C to 80 °C (from T= 0.3243 cm\(^{-1}\) to T= 0.2990 cm\(^{-1}\)) (with ratio about 0.71 decreases) (Fig. 11). Then it remained high during cooling from 80 °C to 20 °C. The turbidity-temperature profiles of diluted NEs were, similar to the turbidity-temperature profiles of undiluted NEs in the absence of the co-solvent (Figs. 10 and 11). In D formulation, in the evaluation of the thermal stability, the turbidity-temperature profile of the VE enriched NEs was more complex than in the absence of G. During heating, turbidity initially remained relatively low and it remained stable during the heating from 20 °C to 55 °C (T= 0.0437 cm\(^{-1}\)) which it indicates that NEs are relatively stable for droplet growth in this range of temperature. Then it slightly increased by increasing temperature from 55 °C to 60 °C (from T= 0.0437 cm\(^{-1}\) to T= 0.046 cm\(^{-1}\)) (about 1.05 fold increases). Then it slightly increased by increasing the temperature from 60 °C to 65 °C (from T= 0.046 cm\(^{-1}\) to T= 0.0506 cm\(^{-1}\)) (about 1.1 fold increases). Then it significantly increased by increasing the temperature from 65 °C to 70 °C (from T= 0.0506 cm\(^{-1}\) to T= 0.5635 cm\(^{-1}\)) (about 11.14 fold increases). Then it decreased by increasing the temperature from 70 °C to 75 °C (from T= 0.5635 cm\(^{-1}\) to T= 0.0575 cm\(^{-1}\)) (with ratio about 0.1 decreases). Then it significantly increased by increasing the temperature from 75 °C to 80 °C (from T= 0.0575 cm\(^{-1}\) to T= 0.3657 cm\(^{-1}\)) (about 6.36
fold increases). Then it decreased during the cooling by decreasing the temperature from 80 °C to 75 °C (from $T = 0.3657 \text{ cm}^{-1}$ to $T = 0.0644 \text{ cm}^{-1}$) (with ratio about 0.18 decreases). Then it decreased by decreasing temperature from 75 °C to 70 °C (from $T = 0.0644 \text{ cm}^{-1}$ to $T = 0.0529 \text{ cm}^{-1}$) (with ratio about 0.82 decreases). Then it significantly increased by decreasing temperature from 70 °C to 65 °C (from $T = 0.0529 \text{ cm}^{-1}$ to $T = 0.5543 \text{ cm}^{-1}$) (about 10.48 fold increases). Then it remained high during cooling from 65 °C to 20 °C (Fig. 12). A low turbidity in concentrated oil-in-water emulsions is evidence of the presence of oil droplets that are so small that they do not scatter light strongly.

At relatively low temperatures, the non-ionic surfactant used in this study (Tween 80) would be expected to be relatively hydrophilic and to have a packing parameter ($p$) less than unity. The packing parameter is the cross-sectional area of the lipophilic tail-group divided by the cross-sectional area of the hydrophilic head-group, and determines the nature of the colloidal structures that are stable in a particular surfactant–oil–water mixture at different temperatures. For $p < 1$, the surfactant has an optimum curvature that favours the formation of O/W emulsions. As the temperature is raised, the polar head groups of the non-ionic surfactants are progressively dehydrated which reduces the water solubility of the surfactant and changes its optimum curvature toward unity. In addition, the strength of the steric repulsion between the surfactant head-groups is reduced. Overall, this leads to an increase in the susceptibility of the emulsions for droplet coalescence, which leads to an increase in turbidity. Upon further heating, even more dehydration of the surfactant head-group occurs, which changes the optimum curvature toward unity ($p = 1$) and leads to an ultralow interfacial tension and a highly flexible surfactant monolayer. These changes favor the formation of a bi-continuous micro-emulsion containing very small domains that do not scatter light efficiently, thereby leading to a reduction in turbidity. When the temperature is raised above the PIT the hydrophilic head groups are further dehydrated, which leads to an increase in surfactant hydrophobicity and to a packing parameter ($p > 1$) that favours the
formation of W/O emulsions. Since the W/O emulsions produced above the PIT contain relatively large water droplets, they can scatter light efficiently and thereby increase turbidity (Saberi, et al., 2015). After rapid cooling, NEs are created. This last step simply recreates the emulsification conditions of the SE procedure. The system is very rapidly set up at a temperature that dramatically changes the surfactant partitioning coefficients, making them fully hydrophilic. As a result, amphiphiles spontaneously move from the oil to the aqueous phase, just as occurs in SE. Finally, NEs are generated, due solely to this displacement of hydrophilic materials (Anton, Vandamme, 2009). Final system had droplets that were only slightly larger than the original emulsions, if heating the emulsions to temperatures around or above the PIT followed by rapid cooling. Similar trends were also observed for the turbidity measurements, which is to be expected because the turbidity is directly related to the droplet diameter in this particle size range. As discussed earlier, these results can be attributed to rapid droplet coalescence around the PIT due to changes in surfactant properties caused by head-group dehydration. If the PIT is not reached, any increase in droplet size due to coalescence will be preserved when the emulsions are cooled back to ambient temperature. From a practical point of view, this may be a useful means of tuning the size of the particles in an emulsion stabilized by a non-ionic surfactant with a relatively low PIT. Conversely, it may be important commercially to ensure that the emulsions are not heated to a temperature where they spend too long in the droplet coalescence zone, otherwise they will become unstable for droplet growth and phase separation (Anton & Vandamme, 2009). In this study, the cooling rate was slow, at first the turbidity of emulsion system was relatively high and it related to relatively large droplets then after cooling the turbidity of emulsion decreased and system was transparent and it was related to relatively small droplets and after keeping the cooling process the turbidity of emulsion increased and system was highly turbid and the system was containing relatively large droplets emulsion. Because of the relatively slow cooling rate, droplet coalescence probably occurred below the PIT and it caused for droplets growth. Similar results have been reported by Anton and Vandamme, in 2009, for low-energy emulsions formed using other surfactant–oil–water combinations when they were held at temperatures below their PIT. In commercial applications, the range of the droplet coalescence zone will depend on the precise nature of the surfactant–oil–water system utilized, and would have to be established for each system (Anton & Vandamme, 2009). Probably, the PIT of the VE enriched NEs free co-solvent was above 80 °C temperatures, used during the thermal stability measurement and therefore we observed the initial droplet coalescence phenomenon (Fig. 10). The presence of co-solvent in the aqueous phase of the NEs reduced the PIT of the system (Fig. 12) (Saberi, et al., 2013). This effect can be attributed to the ability of G to dehydrate the polar head-groups of the non-ionic surfactant.
molecules, thereby altering their optimum curvature and decreasing their cloud point and PIT (Saberi, et al., 2013, Anton & Vandamme, 2009, Saberi, et al., 2014). We also studied the turbidity of diluted NEs (dilution factor of 100) in the presence of 30 wt.% G in the thermal stability measurement. Dilution reduces the NE concentrations, also changes the composition of the aqueous phase and much of the water compound (0.3 wt.% G and 99.7 wt.% buffer solution). The turbidity-temperature profiles of diluted NEs were similar to the turbidity-temperature profiles of undiluted NEs in the absence of the co-solvent (Fig. 10). The turbidity of the NEs enriched with VE containing 0.3 wt.% G remained stable during heating from 20 °C to 60 °C (T= 0.0161 cm$^{-1}$). Then it increased by increasing the temperature from 60 °C to 65 °C(from T= 0.0161 cm$^{-1}$ to T= 0.0851 cm$^{-1}$) (about 5.3 fold increases). Then it decreased by increasing temperature from 65 °C to 70 °C (from T= 0.0851 cm$^{-1}$ to T= 0.0299 cm$^{-1}$) (with ratio about 0.35 decreases). Then it significantly increased by increasing the temperature from 70 °C to 75 °C(from T= 0.0299 cm$^{-1}$ to T= 0.3312 cm$^{-1}$) (about 11.08 fold increases). Then it slightly decreased by increasing temperature from 75 °C to 80 °C (from T= 0.3312 cm$^{-1}$ to T= 0.2806 cm$^{-1}$) (with ratio about 0.85 decreases). Then it remained high during cooling from 80 °C to 20 °C (Fig. 13).

We postulate that the concentration of co-solvent was so low in the diluted NEs that it had little influence on the properties of the surfactant monolayer, such as optimum curvature, interfacial tension, and flexibility (Saberi, et al., 2013). Dilution decreases the initial droplet concentration in the NEs, but it also changes the composition of the aqueous phase so that it is mainly comprised of pure water. Qualitatively, the diluted NEs behave similarly to the undiluted NEs, they initially had a low turbidity, but became cloudy when they were heated above a particular temperature, and remained cloudy upon cooling (Saberi, et al., 2013). The presence of the G decreases the PIT and so it was increased, in the absence of G. Therefore, dilution of the NEs before heating may improve their thermal stability (Saberi, et al., 2013).

**CONCLUSIONS**
Previous studies have shown that G as a polyol can greatly influence the formation and properties of emulsion-based systems (Yaghmur, et al., 2002.). Therefore, in the current study, we decided to create NE-based delivery systems for VE using a simple and inexpensive low-energy method: SE (Guttoff, et al., 2015). We investigated the effect of G on the formation, stability and properties of VE enriched NEs prepared using the SE method. Our results showed that probably the ability of G in the adjustment characteristics of the surfactant has a considerable effect on the formation, physical properties and stability of VE enriched NEs. The particle size and optical clarity of the NEs depended strongly on the G presence, absence and concentration. The smallest droplets (mean diameter less than 23.5 and 236.771) and the highest transparency (the lowest turbidity, 0.0069 cm^-1 and 0.0161 cm^-1) were observed in the absence of the co-solvent (A formulation) and the presence of 30 wt.% G (D formulation), respectively. NE with high optical transparency (in the absence of G in the presence of 30 wt.% G), were corresponded to the smallest particle diameter. This effect is due to the fact that smaller particles scattering light with less intensity than the larger particles (Saberi, et al., 2013, Ec, et al., 2008). The size of the primary particles and turbidity of the NEs formed using the SE method, decreased, in the presence of 30 wt.% G that it is probably attributed to changes in the curvature and solubility of surfactants (Saberi, et al., 2013, Anton & Vandamme, 2009, Garti, et al., 2001). The results show that NEs free of co-solvent and containing 30 wt.% G were sensitive to particle growth over time, which the particle growth rate increased following increasing temperature storage and the dilution with water before storing improved storage stability which this in accordance with the results of previous research with Saberi and et al. in 2014 (Saberi, et al., 2014). Also, the storage stability of NEs attributed to G concentration, the rapid growth of particles at high temperatures, in systems containing high concentrations of G was observed. This effect has been attributed to increasing the coalescence and OR at high temperatures due to the changes in the optimum curvature and the solubility of the surfactant in the presence of G (Saberi, et al., 2014). Thereby, dilution of NEs with water (dilution factor= 100) before storage, improved storage stability, especially at high temperatures that it could due to decreasing the frequent particles collision with each other after dilution which it causes to decreasing the rate of coalescence (Anton & Vandamme, 2009, Saberi, et al., 2014). Dilution of NEs with water (dilution factor =100) before storage, improved NEs thermal stability, especially at high temperatures. Undiluted NEs showed a significant irreversible increase in turbidity upon heating: ≈75 °C and 70 °C for the systems in the absence of the co-solvents and the presence of 30 wt.% G, respectively, which it has been attributed to this fact that G concentration significantly decreased, therefore, co-solvent molecules had slightly effect on properties of surfactant monolayer such as optimum curvature, interfacial tension and flexibility.
(Saberi, et al., 2013). This study provides important information about the effect of the presence and absence of G, as a co-solvent on the formation, stability, and physical properties of VE enriched NEs suitable for use in pharmaceutical and food products. Also, this study has important implications for optimization require conditions for the SE method as one of the best methods for NEs production and also developing stable VE enriched NEs as delivery systems suitable for use in foods, gels, fruit juices, enriched waters, soft drinks (transparency appearance) and the oil industry (increasing the shelf life of frying oils in standby phases). Therefore, we can use the information obtained from this study for the design of effective delivery systems for encapsulation and stabilization of functional compounds for their use in food, beverage and pharmaceutical and other industries (Saberi, et al., 2014).

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