Int. J. Bio-Inorg. Hybr. Nanomater., 9(3): 179-193, Autumn 2020

Investigation the effect of the Propylene Glycol as a Co-solvent on VE-enriched nano-emulsion properties: Spontaneous Nano-emulsification

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Received: 22 June 2020; Accepted: 25 August 2020

ABSTRACT: There is considerable interest in using nano-emulsions (NEs) as delivery systems for lipophilic bioactive ingredients. In this study, we investigated the influence of propylene glycol (PG) on the properties of VE enriched NEs prepared using the spontaneous emulsification (SE) method. The particle size and optical clarity of the NEs depended strongly on the co-solvent concentration. The smallest droplets (mean diameter less than 23.5, and 262.96) and highest transparency (lowest turbidity, 0.0069 cm^{-1}, and 0.023 cm^{-1}) were observed in the absence of the PG (A formulation) and the presence of 10 wt.% PG (B formulation), respectively. However, these NEs were highly unstable to droplet growth during storage, especially at high temperatures, which was attributed to coalescence and Ostwald ripening. Dilution of the NEs (100× with water) before 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 50 °C for the systems in A formulation and B 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 the pharmaceutical, foods, and beverages industries

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

INTRODUCTION

The food and beverage industries are interested in the development of colloidal delivery systems to encapsulate lipophilic functional ingredients, such as bioactive lipids, flavor oils, or oil-soluble Vs (e.g. VE, VD) [1,2]. 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 [3]. The recommended daily intake (RDI) of VE is 15 mg/day [4]. 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 [5]. Reducing particle size in emulsion-based delivery systems has some consequences that may be beneficial for certain food applications: (i) greater stability to droplet aggregation and gravitational separation; (ii) higher optical clarity; and, (iii) increased oral bioavailability

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(*) Corresponding Author - e-mail: alaleh.sadr64@yahoo.com of bioactive compounds [2,6,7]. Two approaches can be used to fabricate NEs: high-energy and low-energy approaches [8]. NEs are commonly fabricated by high-energy methods [9] which utilize specialized equipment capable of generating intense mechanical forces [10] such as high-pressure [11,12,13,14], micro-fluidization [15,16], and ultrasonic [17,18]. 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 [10]. 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 [8,9], such a SE [19], membrane emulsification [20,21], phase inversion temperature, emulsion inversion point [18,22] and solvent displacement [23]. 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 [9]. On the other hand, the low-energy method takes an advantage of the chemical energy of emulsifier to change the spontaneous curvature of droplets, resulting in formation of nanoemulsion with a small amount of external mechanical energy input. The most widely used low-energy method is spontaneous emulsification method, where components are mixed in a specific order and proportion. This approach has a low requirement on external temperature and external energy input, satisfying the formation condition of in-situ nanoemulsion [24]. Since it simply involves titration of an oil surfactants mixture into an aqueous solution with constant agitation at a fixed temperature [25], which is produced by different mechanisms such as budding off which water will likely that penetrate the S/O phase and that surfactant will move into the water phase [26]. The major components that can be used to formulate food-grade NEs are the oil phase, aqueous phase, and stabilizers [27]. There are several factors, involved in emulsion stabilization mechanisms; interfacial tension, electrical charge, liquid crystal stabilization, and stabilization by macromolecules and finely divided solids [28]. Also, emulsion destabilization can be due to one or all of five possible mechanisms; flocculation, coalescence, sedimentation or creaming, OR, and phase inversion [28]. In this study, we investigated the influence of PG on the properties of VE-enriched NEs. PG has two hydroxyl groups [29], as a hydrogen-donor and hydrogen-acceptor compound is miscible with water in all proportions and has been studied in particular as a possible co-solvent in liquid pharmaceutical dosage forms design for several drugs [30]. Using the less polar co-solvents in aqueous solutions changes the physical properties of aqueous solutions such as viscosity, density, interfacial tension [31], and the refractive index [32] and it showed that could alter the solubility, interfacial tension [22,29,33,34] and optimum curvature of some of the surfactants [3,35]. The purpose of this study was to investigate the effect of the presence, absence, and concentration of PG, 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 propylene glycol 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 *et al.* in 2009 and Saberi and *et al.* in 2013 [22,36] 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 ºC) which it was carried out in 3 steps.

Preparation of liquid phase

An aqueous phase was prepared to contain a buffer solution (80-30 wt.%) -consisting of 0.8 wt.% citric acid and 0.08 wt.% sodium benzoate, in double distilled water, pH 3, to simulate the aqueous phase of a beverage system- and different concentration of PG (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 over 45 minutes under constant magnetic stirring (750 rpm) at ambient temperature.

Spontaneous emulsification method

The organic phase was then slowly poured into the aqueous phase over 10 minutes 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 the 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) before 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 [9,36]. The instrument reports the particle size distribution. The mean particle diameter and polydispersity index (PDI) were calculated from the particle size distribution.

Turbidity measurements

The turbidity of samples was determined using 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).

$$T = \frac{(2.3 \times A)}{L} \qquad \text{(Eq.1)}$$

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 [37].

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 PG concentration on nano-emulsification and particle size of VE enriched NEs

At first, we investigated the influence of PG 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 (PG) and 80-30 wt.% buffer solution (pH=3). In Table 1, had been shown the different formulations of the VE enriched NEs at different con-

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

Formulation	Total Amount (Wt.%)	Organic Phase (20 Wt.%)		Liquid Phase (80 Wt.%)	
		Oil (10 Wt.%)	Surfactant (10 Wt.%)	Co-Solvent (0-50 Wt.%)	Buffer Solution (80-30 Wt.%)
		VE	Tween 80	PG	
A	100	10	10	0%	80%
B	100	10	10	10%	70%
C	100	10	10	20%	60%
D	100	10	10	30%	50%
E	100	10	10	40%	40%
F	100	10	10	50%	30%

centrations of PG, prepared by the SE method. The effect of different concentrations of PG in droplet size and size distribution had been shown in Figs 1 and 2 NEs prepared. The droplet size distribution of NEs prepared in 0-40 wt.% concentrations of PG was mono-modal and the droplet size distribution of NEs prepared in 50 wt.% the concentration of PG was bi-modal (Data not have shown).

The mean diameter of the NEs particles prepared, increased significantly from 23.5 nm to 262.96 nm when the PG concentration was increased from 0 wt.% to 10 wt.%. Then it increased from 262.96 nm to 307.44 nm by increasing the PG concentration from 10 wt.% to 20 wt.%. Then it slightly decreased from 307.44 nm to 292.65 nm with increasing the PG concentration from 20 wt.% to 30 wt.%. Then it increased from 292.65 nm to 342.2 nm by increasing the PG concentration from 30 wt.% to 50 wt.%. (Fig. 1). On the other hand, in the presence of PG, with increasing the PG concentration from 10 wt.%, particle size was increased. As shown in Fig. 1, in this range of concentration which we studied (0-50 wt.% PG), the smallest particle size in the absence of the PG, was obtained, 23.5 nm and, in the presence of the PG (10 wt.%), the smallest particle size, was obtained, 262.96 nm.

At first, the polydispersity index of the prepared NEs, slightly decreased from 0.49.5 to 0.4489 when the PG concentration was increased from 0 wt.% to 20 wt.%. Then it increased from 0.4489 to 0.5041 by increasing the PG concentration from 20 wt.% to 30 wt.%. Then it increased from 0.5041 to 0.5625 with increasing the PG concentration from 30 wt.% to 40 wt.%. Then it decreased from 0.5625 to 0.5041 with increasing the PG concentration from 40 wt.% to 50 wt.%. Thus, in this range of the concentrations which we studied (0-50 wt.% PG), the lowest PDI obtained, 0.4489 in the lowest PG concentrations (10, 20 wt.%) (Fig. 2).

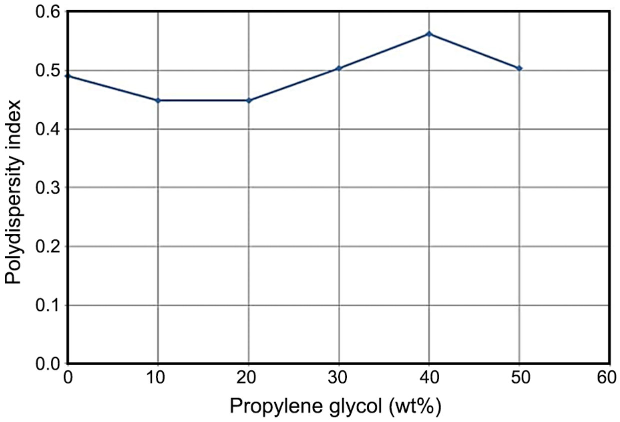

Fig. 2. The polydispersity index obtained from the A, B, C, D, E and F formulation.

In these concentration values which we investigated (0-50 wt.% PG), our data illustrated that smaller particles (23.5 nm) with the narrowest size distribution (PDI= 0.49) were formed in A formulation and in the presence of PG, the smallest particles (262.96 nm) with the narrowest size distribution (PDI= 0.4489) at the concentration of 10 wt.% (B formulation) was formed. The mean droplet diameter obtained from this study was smaller than the results of similar work by Saberi and *et al.* in 2013, (The smallest droplet in the absence of PG around 160 nm and the presence of 10 wt.% PG was smaller than 160 nm and in the optimum

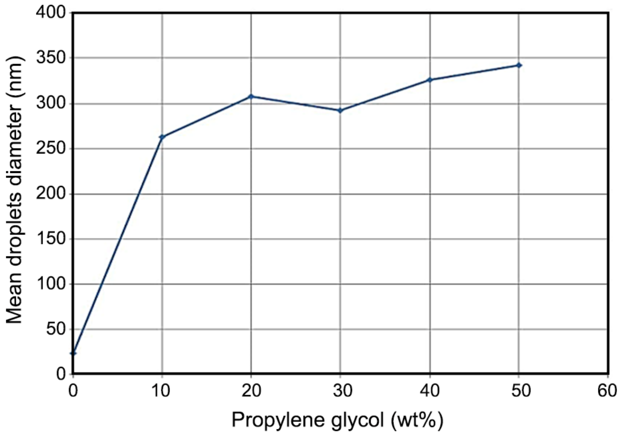

Fig. 1. The mean droplet size was obtained from the A, B, C, D, E, and F formulation.

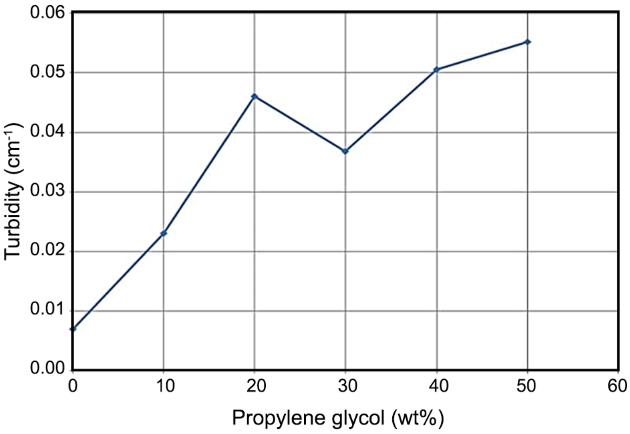

Fig. 3. The turbidity obtained from the A, B, C, D, E and F formulation.

values 30 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, A.H; *et al.* work [36].

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 less thermodynamically unfavourable environment for non-polar groups. Second, amphiphilic co-solvents may be able to penetrate 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 [36].

Effect of PG 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 [36]. To prepare optically transparent NEs it is usually necessary to carefully control the initial system composition as well as the homogenization conditions [38]. To prepare beverage emulsions that appear visually transparent the turbidity should be less than 0.05 cm^{-1} [4]. The influence of PG concentration on the appearance of VE enriched NEs was investigated. A, B, C, D, E and D formulations were used in this investigation. The optical clarity of the VE enriched NEs, at first, somewhat increased with increasing the PG concentration and then significantly decreased and then it increased.

On the other hand, in these concentrations which we studied, when PG concentration was increased from 0 wt.% to 10 wt.%, they were transparent. Then, when PG concentration was increased from 10 wt.% to 20 wt.%, they were opaque. Then, when PG concentration was increased from 20 wt.% to 30 wt.%, they were slightly transparent. Then, when PG concentration was increased from 30 wt.% to 50 wt.%, they were quite opaque. Which their transparency can be attributed to the ability of the co-solvent to decrease the size of the droplets in VE enriched NEs, thereby decreasing their scattering efficiency. Therefore, light scattering from the NE containing 10 wt.% PG (B formulation) should be greater than other concentrations of PG [36]. The results of the turbidity measurement confirmed the results of the visual assessment (Data not have shown). NE with high optical transparency (in A formulation, in B formulation), were corresponded to the smallest particle diameter (Fig. 3). This effect is since smaller particles scattering light with less intensity than larger particles [39].

Effect of PG 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 some different physicochemical processes, including flocculation, coalescence, Ostwald ripening, and gravitational separation. The rate at which a 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. We investigated the storage stability of VE NEs based on the method described by Saberi and *et al.* in 2013 with slightly changing [36]. NEs were prepared in A formulation and B formulation at the aqueous phase. Then we stored them at 5 ºC , 22 ºC and 37 ºC for 30 days, which were selected to represent refrigeration conditions, ambient storage in mild climates, and ambient storage in hot climates, respectively [36]. In 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 shown) and it (PDI= 0.49) significantly expanded with increasing the storage temperature 5 °C (PDI= 0.5476), 22 °C (PDI= 0.8464) and 37 °C (PDI= 0.9409) (Fig. 5). Since emulsion delivery systems are often diluted in commercial applications (such as functional foods and beverages), so the effect of dilution on the storage stability of NEs containing 0 wt.% co-solvent (A formulation) in the aqueous phase was evaluated. NEs free of the co-solvent, diluted with water before storing (dilution factor= 100), a slight increase on the 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 at the lower temperatures and then at a high temperature slightly changed from mono-modal to bimodal (Data not have shown) and it (PDI= 0.49) significantly expanded with increasing the storage temperatures 5 °C (PDI= 0.49), 22 °C (PDI= 0.5476) and 37 °C (PDI= 0.5041) (Fig. 5). Diluted NEs were more stable to the droplet growth than undiluted NEs (Fig. 4).

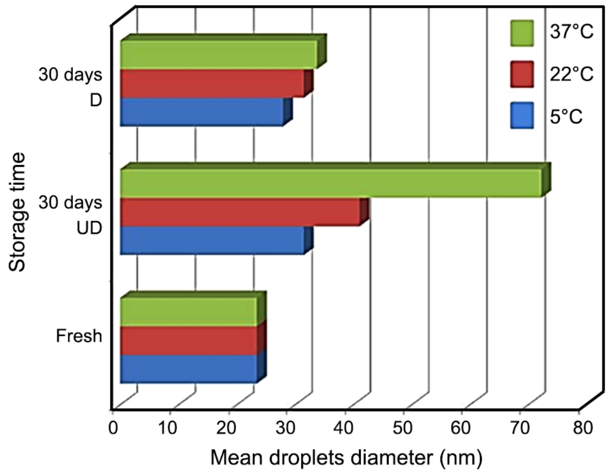

Fig. 4. The mean particle diameter obtained from changes in the storage time and temperature of the undiluted (UD) and diluted (D) A formulation.

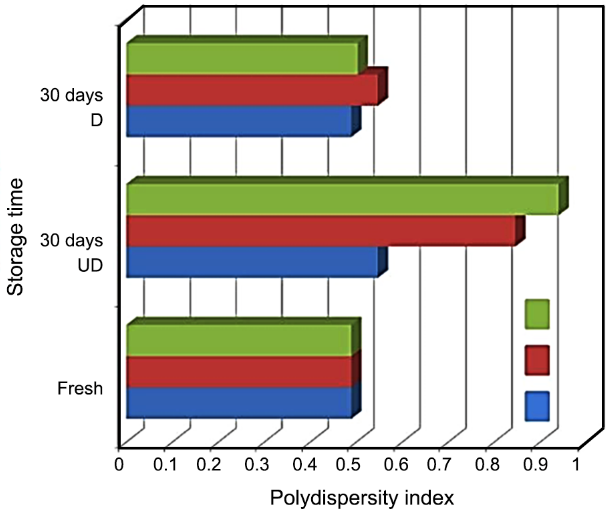

Fig. 5. The polydispersity index obtained from changes in the storage time and temperature of the undiluted (UD) and diluted (D) B formulation.

In A formulation, the turbidity of the NEs prepared in all of the storage temperatures were increased (about 26 fold) (Fig. 6). The turbidity of NEs, increased from 0.0069 cm^{-1} initially to 0.023 cm^{-1} at 5

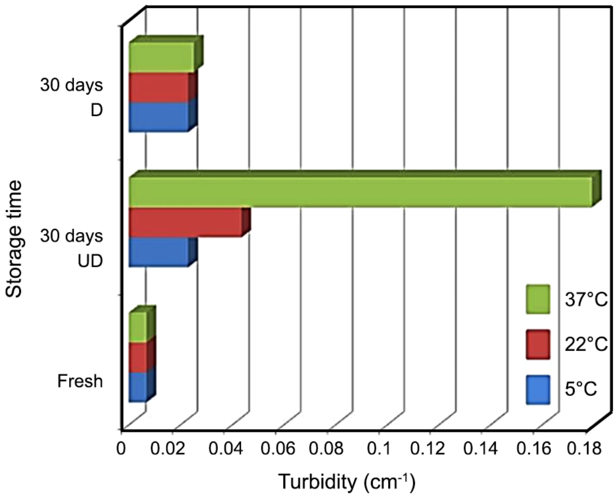

Fig. 6. The turbidity obtained from changes in the storage time and temperature of the undiluted (UD) and diluted (D) A formulation.

°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 of 100) in the absence of co-solvents, increased after 30 days of storage and they visually had a clear appearance (Fig. 7). 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] 37 °C (about 266.67% increases) after 30 days storage (Fig. 7).

The results of the turbidity measurement by visual observations of the apparent turbidity NEs enriched with VE were similar (Data not have shown). The system's tendency went to transparency by reducing the turbidity (Data not have shown). The results show that A formulation NEs 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. Coalescence is due to Brownian motion [25]. Dilution with water before storing causes reduced the collision of the particles to each other under Brownian motion and consequently reducing the rate of the coalescence.

As a result, coalescence may be the main destabilizing mechanism in NE systems in A formulation [25,36]. In B 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 262.96 nm initially to 326.2 nm at 5 °C (about 24.05% increases), 326.2 nm at 22 °C (about 24.05% increases) and 359.45 nm at 37 °C (about 36.69% increases) after 30 days storage (Fig. 8) [37]. The particle size distribution of the NEs was bi-modal (Data not have shown) and it (PDI= 0.4489) changed with increasing the storage temperature 5 °C (PDI= 0.5625), 22 °C (PDI= 0.5625) and [37] 37 °C (PDI= 0.4489) particle size distribution got wider (Fig. 9). Since emulsion delivery systems are often diluted in commercial applications (such as functional foods and beverages), so the effect of dilution on the storage stability of NEs containing 10 wt.% PG (B formulation) in the aqueous phase was evaluated. NEs containing 10 wt.% PG (B formulation), diluted with water before storing (dilution factor of 100), a slight increase in the mean particle diameter, was observed in diluted NEs (d /d0 increased less than about 1.13 fold). The mean diameter of particles increased from 262.96 nm initially to 265.12 nm at 5 °C (about 0.82% increases), 292.65 nm at 22 °C (about 11.29% increases), and 297.78 nm at 37 °C (about 13.24% increases) after 30 days storage (Fig. 8) [37]. The particle size distribution of the NEs was monomodal (Data not have shown) and it (PDI= 0.4489) changed with increasing the storage temperature 5 °C (PDI= 0.6241), 22 °C (PDI= 0.5041) and 37 °C (PDI= 0.6724) (Fig. 9) [37]. Diluted NEs were more stable to droplet growth than undiluted NEs (Fig. 8). Also, we have investigated the effect of storage time and temperature on the optical properties of undiluted (UD) and diluted (D) NEs. In B formulation, the turbidity of the NEs prepared in all of the storage temperatures after 30 days increased (about 6.4 fold). The turbidity of NEs, increased from 0.023 cm^{-1} initially to 0.0713 cm^{-1} at 5 °C (about 210% increases), 0.0897 cm^{-1} at 22 °C (about 290% increases), and 0.1472 cm^{-1} at 37 °C (about 540% increases) after 30 days storage [37]. The turbidity of diluted NEs (dilution factor of 100) in A formulation, slightly increased after 30 days of storage (Fig. 10) and they visually had a somewhat clear appearance (Data not have shown). The turbidity of NEs, increased from 0.023 cm^{-1} initially to 0.0299 cm^{-1} at 5 °C (about 30% increases), 0.046 cm^{-1} at 22 °C (about 100% increases), and 0.0621 cm^{-1} at 37 °C (about 170% increases) after 30 days storage (Fig. 10) [37]. The results of the turbidity measurement were similar (Fig. 10) by visual observations of the apparent turbidity VE enriched NEs (Data not have shown). The system's tendency went to transparency by reducing the turbidity (Data not have shown).

Also, by reducing the particle size, turbidity decreased because smaller droplets scattering light with lower intensity [36]. The results show that NEs B formulation was sensitive to particle growth over time, which the particle growth rate increased following increasing temperature storage and the dilution with water before storing improved their storage stability. Some possible mechanisms that might account for the observed increase in mean particle size in the NEs

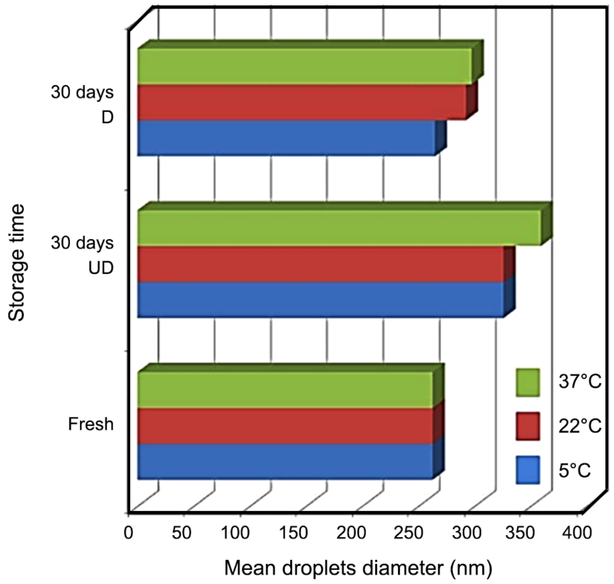

Fig. 8. The mean particle diameter obtained from the changes in the storage time and temperature of the undiluted (UD) and diluted (D) B formulation.

during storage, including flocculation, coalescence and OR [3,37]. We do not believe that flocculation was important in the NEs used in this study since; very fine droplets stabilized by nonionic surfactants tend to be stable to flocculation due to the strong steric repulsion between the hydrophilic surfactant head groups [27,36].

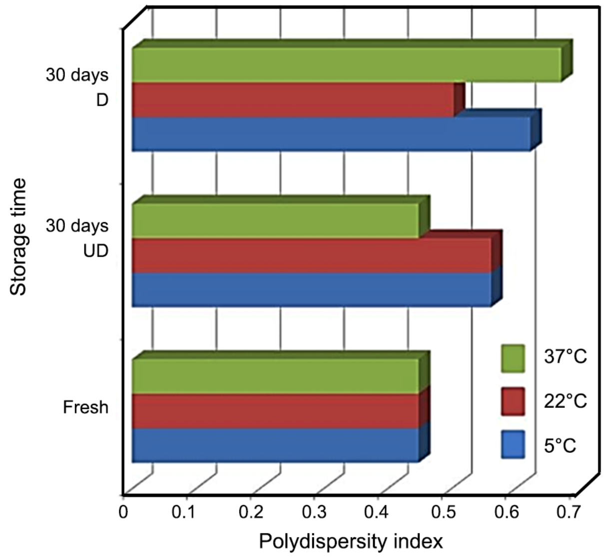

Fig. 9. The polydispersity index obtained from the changes in the storage time and temperature of the undiluted (UD) and diluted (D) B formulation.

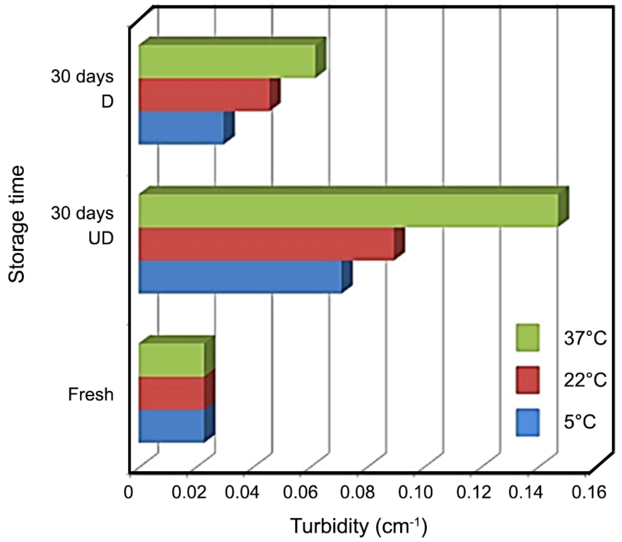

Fig. 10. The turbidity obtained from the changes in the storage time and temperature of the undiluted (UD) and diluted (D) B formulation.

We, therefore, postulate that either coalescence or Oswald ripening was responsible for the droplet growth observed in this study [36]. The rate of droplet growth due to OR may also increase with increasing the temperature. The change in the mean droplet diameter (d) with time (t) due to Ostwald ripening of emulsified oil in the steady-state regime is given by (Eq. 2) [36]:

$$(d^3 - d_0^3) = 1 + \frac{64V_m cD}{9} t \qquad \text{(Eq.2)}$$

Where d_0 is the initial diameter, γ 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 [22,36]. As we see in (Eq. 2) when each one of γ, Vm, c, D and t, increases, it caused increases in the rate of droplet growth due to OR [40]. PG is miscible with water [30]. Both water and co-solvent molecules (PG) can form hydrogen bonds with polar groups and they are water-soluble but the polarity of PG is less than deionized water. Therefore oil droplets have more solubility in PG than deionized water. Therefore c, (the solubility of the oil in the aqueous phase) increases and it followed by an increase in the droplet growth rate due to OR [41]. 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 [36]. 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 maybe 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 [36]. 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 [36].

Effect of the absence and presence of PG 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 [36]. We, therefore studied the influence of the absence (A formulation) and presence of PG (B formulation) on the thermal stability of the VE enriched NEs and the changes in the turbidity of the 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 the absence and presence of PG 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 with measuring of absorption intensity NEs prepared by the method of SE. In A formulation, the evaluation of the thermal stability, the turbidity of the NEs enriched with VE, initially remained relatively low and then during heating from 20 °C to 60 °C remained stable (T= 0.0207 cm^{-1}). Then it increased with increasing the 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 with 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 with 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 with 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. 11). It remained relatively high during cooling from 80 °C to 20 °C which indicates that an increase in the turbidity that occurs during heating is irreversible. The increase in turbidity can be attributed to an increase in the 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 [36]. The fact that the turbidity remained high during cooling (and the samples appeared visibly cloudy (Data not have shown)) suggests that the oil droplets remained large after coalescence had occurred [36]. We also studied the turbidity of diluted NEs (dilution factor= 100) in the absence of co-solvents on 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 with 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 with 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 with increasing the temperature from 70 °C to 75 °C (from T= 0.0207

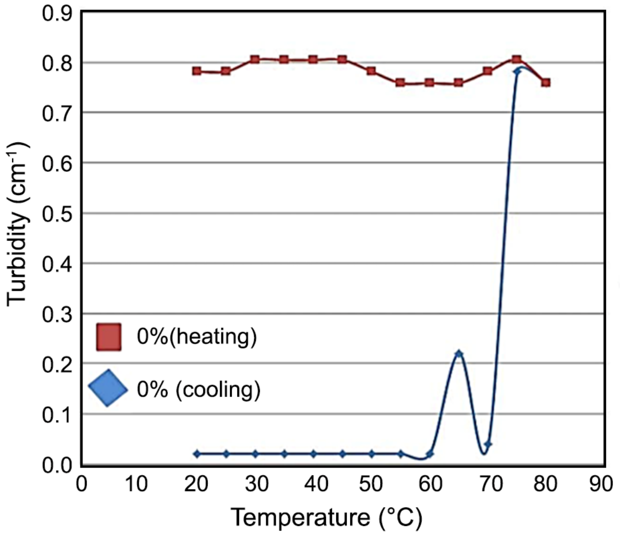

Fig. 11. The turbidity obtained from the changes in the temperature of the undiluted (UD) A formulation.

cm^{-1} to T= 0.3243 cm^{-1}) (about 15.6 fold increases). Then it slightly decreased with 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. 12). Then it remained high during cooling from 80 °C to 20 °C. The turbidity-temperature profiles of the diluted NEs were, similar to the turbidity-temperature profiles of the undiluted NEs in the absence of co-solvents (Figs 11, 12). In B formulation, the evaluation of the thermal stability, the turbidity-temperature profile of the VE enriched NEs was the same as the absence of PG. During heating, the turbidity initially remained relatively low and it remained stable during heating from 20 °C to 25 °C (T= 0.0644 cm^{-1}) which indicates that NEs are relatively stable to the droplet growth in this range of the temperature. Then it slightly increased with increasing the temperature from 25 °C to 45 °C (from T= 0.0644 cm^{-1} to T= 0.1725 cm^{-1}) (about 2.68 fold increases). Then it increased with increasing the temperature from 45 °C to 50 °C (from T= 0.1725 cm^{-1} to T= 0.4416 cm^{-1}) (about 2.56 fold increases). Then it remained high with increasing the temperature from 50 °C to 80 °C. Then it remained high by decreasing the temperature from 80 °C to 20 °C (Fig.13).

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 solu-

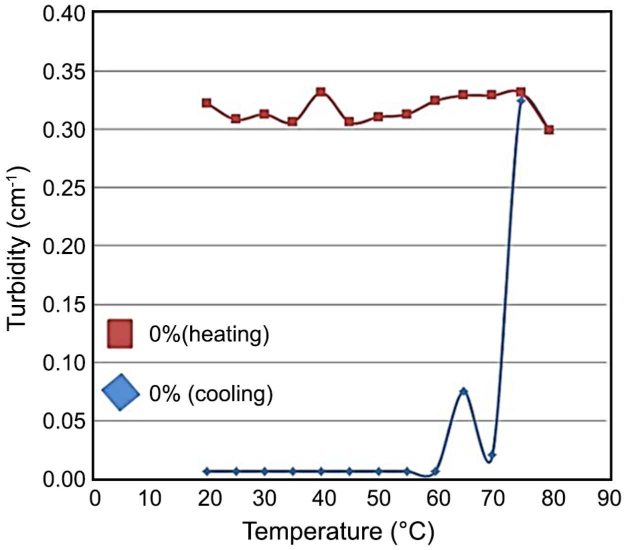

Fig. 12. The turbidity obtained from the changes in the temperature of the diluted (D) A formulation.

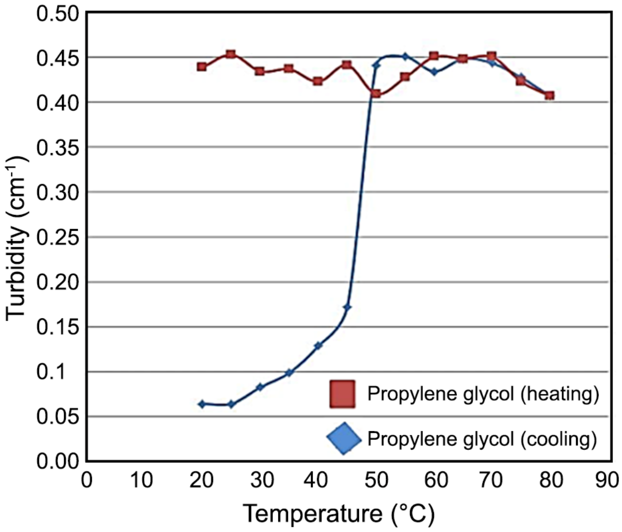

Fig. 13. The turbidity obtained from the changes in the temperature of the undiluted (UD) B formulation.

bility 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 to 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 favour 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 [42]. 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 [22]. The 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 to droplet growth and phase separation [22]. In this study, the cooling rate was slowly, the turbidity of the emulsion system remained relatively high during cooling. Probably, the PIT of the VE enriched NEs free of co-solvents was above 80 °C temperature, used during thermal stability measurement and therefore we observed the initial droplet coalescence phenomenon (Fig. 11). We expected that the presence of co-solvents at the aqueous phase of the NEs reduced the PIT of the system (Fig. 13) [36] because this effect could be attributed to the ability of PG to dehydrate the polar head-groups of the non-ionic surfactant molecules, thereby altering their optimum curvature and decreasing their cloud point and PIT [43]. However, we only observed the initial droplet coalescence stage during heating for both diluted and undiluted NEs in the presence of PG but did not reach temperatures high enough to observe the clearing stage [36] and they have similar profiles of turbidity-temperature that it probably was because of the low concentration of PG (10 wt.%) in undiluted NEs which it makes that relatively the same as diluted NEs. We also studied the turbidity of the diluted NEs (dilution factor of 100) in the presence of 10 wt.% PG on the thermal stability measurement. Dilution reduces the NE concentrations, also changes the composition of the aqueous phase and much of the water compound (0.1 wt.% PG and 99.9 wt.% buffer solution). The turbidity-temperature profiles of the diluted NEs are similar to the turbidity-temperature profiles of the undiluted NEs in the absence of co-solvents (Fig. 11).

The turbidity of the NEs enriched with VE is containing 0.1 wt.% PG remained stable during heating from 20 °C to 60 °C ($T = 0.023\ cm^{-1}$) which indicates that NEs are relatively stable to the droplet growth in this range of temperature. Then it increased with increasing the temperature from 60 °C to 70 °C (from $T = 0.023\ cm^{-1}$ to $T = 0.0529\ cm^{-1}$) (about 2.3 fold increases). Then it significantly increased with increasing the temperature from 70 °C to 75 °C (from $T = 0.0529\ cm^{-1}$ to $T = 0.299\ cm^{-1}$) (about 5.65 fold increases). Then it increased with increasing the tem-

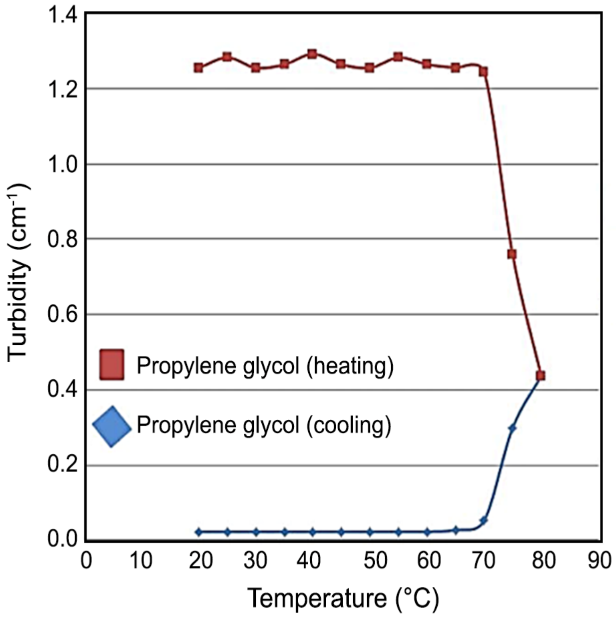

Fig. 14. The turbidity obtained from the changes in temperature of the diluted (D) B formulation.

perature from 75 °C to 80 °C (from T= 0.299 cm^{-1} to T= 0.4 cm^{-1}) [37] (about 1.46 fold increases). Then it remained high during cooling from 80 °C to 20 °C (Fig.14).

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 [36]. 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 low turbidity, but became cloudy when they were heated above a particular temperature, and remained cloudy upon cooling [36]. However, there were important qualitative differences between their thermal behaviour (diluted and undiluted NEs). The temperature in which turbidity significantly increased was different. It was Obtained 50 °C (T= 0.4416 cm^{-1}) for undiluted NE and 70 °C (T= 0.299 cm^{-1}) for diluted NE. The presence of the PG decreases the PIT and so it was increased, in the absence of PG. Therefore, dilution of the NEs before heating may improve their thermal stability [36].

CONCLUSIONS

Previous studies have shown that G as a polyol can greatly influence the formation and properties of emulsion-based systems [44]. Therefore, in the current study, we decided to create NE-based delivery systems for VE using a simple and inexpensive low-energy method: SE [2]. We investigated the effect of PG on the formation, stability and properties of V E enriched NEs prepared using the SE method. Our results showed that possibility the ability of PG 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 PG presence, absence and concentration. The smallest droplets (mean diameter less than 23.5 and 262.96) and the highest transparency (the lowest turbidity, 0.0069 cm^{-1} and 0.023 cm^{-1}) were observed at the absence of co-solvents (A formulation) and the presence of 10 wt.% PG (B formulation), respectively. NE with high optical transparency (in the absence of PG in the presence of 10 wt.% PG), were corresponded to the smallest particle diameter. This effect is since smaller particles scattering light with less intensity than the larger particles [36,40]. The size of the primary particles and the turbidity of the NEs formed using the SE method, decreased, in the presence of 10 wt.% PG that which is possibly attributed to changes in the curvature and solubility of surfactants [3,22,35]. The results show that NEs free of co-solvents and containing 10 wt.% PG 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 following results of previous research with Saberi and *et al.* in 2014 [25]. Also, the storage stability of NEs attributed to PG concentration, the rapid growth of particles at high temperatures, in the systems containing 10 wt.% PG was observed. NEs were unstable to the droplet growth especially at elevated temperatures due to unstable mechanisms such as the coalescence and Ostwald ripening, thereby, dilution of NEs with water (dilution factor= 100) before storage, improved storage stability especially at high temperatures that it could due to decreasing to the collision of the frequent particles each other after dilution which it causes to decreasing the rate of coalescence [22,25]. Dilution of NEs with water (dilution factor of 100) before storage, improved NEs thermal stability, especially at high temperatures. Undiluted NEs showed a significantly irreversible increase in the turbidity upon heating: ≈75 °C and 50 °C for the systems in the absence of co-solvents and the presence of 10 wt.% PG, respectively, which it has been attributed to this fact that PG concentration significantly decreased, therefore, the co-solvent molecules had slightly effect on the properties of surfactant monolayer such as optimum curvature, interfacial tension and flexibility [36]. 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 industry.

ACKNOWLEDGEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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