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Vitamin E encapsulated nano-emulsions formulation, rheological and antimicrobial analysis

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ABSTRACT

Vitamin E is an important food ingredient that individuals ingest to help prevent numerous diseases. Nano-emulsions are frequently employed in pharmaceutical, food, and personal care applications as means of delivering a variety of lipophilic active substances, namely vitamins that are oil-soluble. Both high-energy and low-energy methods are used to create nano-emulsions. The latter, however, offers advantages including less cost, convenience of use, and increased energy efficiency. In this work, we used the emulsion phase inversion technique to create nano-emulsions containing vitamin E. We investigated the rheological and physical characteristics of nano-emulsions created at different stirring rates ranging from 30 to 110 minutes. The emulsion phase inversion approach mixes an organic phase made up of oil, vitamin E, and a surfactant with an aqueous phase. The droplet size, zeta potential, and rheology of all the nano-emulsions were measured. The size distribution of nano-emulsions was measured in the particle size examination method utilizing dynamic light scattering and average droplet diameter was observed to be within a range of 141 nm to 177 nm and to follow a sequence: 110 < 70 < 30 min. The lowest droplet size, 141 nm, with a polydispersity index of 0.234, was obtained at 110 minutes. The zeta potential of formulated nano-emulsions ranged from – 7.1 m to – 14.3 mV. The rheological properties of nano-emulsions revealed non-Newtonian flow behavior. The antimicrobial test of nano-emulsions was examined with Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus), and the emulsions were resistant to S. aureus.

Keywords: Vitamin E, Nanoemulsion, Stirring time, Mean droplet size, Viscosity.

INTRODUCTION

Aqueous colloidal dispersions, such as nano-emulsions, are made up of tiny particles with an average diameter of 20 nm to 200 nm. Because of the enhancements in physicochemical characteristics and biological performances linked to variations in particle size, nano-emulsions are being used more frequently in the pharmaceutical and food industries. Smaller droplet size in emulsion-based administration techniques has several benefits, including improved oral bioavailability, improved optical clarity, and higher resistance to aggregation of droplets and separation due to gravity [1]. In addition, nano-emulsions substantially facilitate the inclusion of lipophilic bioactive molecules, including vitamins soluble in oil or nutritional supplements, into aqueous-based goods that need to be optically transparent.

Fat-soluble vitamins collectively referred to as ”Vitamin E,” are well-known for their health advantages, including lowering the risk of diabetes, cancer, and cardiovascular disease. This has led to their application as functional additives in culinary, cosmetic, and medicinal formulations. Vitamin E acetate is popular in the food industries because of its exceptional resistance to oxidation. However, because of vitamin E’s lipophilic properties, it is not

dissolvable in an aqueous solution. Instead, it must be added to a colloidal drug carrier before dispersion. Colloidal vitamin E is now more commonly available than bulk vitamin E [2]. Adding this vitamin E to foods and beverages, pharmaceuticals, and cosmetics have received much attention. Among the problems concerning vitamin E enrichment include its poor solubility in water, chemical deterioration when subjected to the air, light, or extremely high temperature and poor oral bioavailability.

Nano-emulsions are formulated using either low-energy or high-energy techniques. Emulsion phase inversion (EPI) is one of the energy-efficient techniques for developing nanoemulsion based vitamin E delivery solutions. During this process, an organic phase composed of hydrophilic surfactant and oil is titrated with a water phase while being continuously stirred [3, 4]. The abrupt phase inversion that happens when an organic phase is exposed to a certain volume of water phase. It is the fundamental principle underpinning the EPI method [5]. With the addition of water phase, oil-in-water (O/W) emulsion develops in the system, changing it from a water-in-oil (W/O) emulsion. Researchers have proposed hypotheses that suggest that the development of tiny oil droplets within O/W emulsions depends on the oil-in water-in oil emulsion created at moderate water concentrations [6, 7]. The oil-in-water-in-oil (O/W/O) emulsions trap tiny droplets of oil by dispersing bigger water droplets across an oily continuous phase [8, 9]. The properties of nanoemulsion are regulated by factors such as polydispersity index (PDI), mean droplet size, zeta potential, oil-to-surfactant ratio, preparation methods, etc. Nano-emulsions with the lowest droplet size are more stable against aggregation, flocculation, creaming, and sedimentation [10].

It is noted that an emulsion's rheology is essential for using it in a range of industrial applications, such as the production of pharmaceuticals and food [11]. The rheological properties of emulsions are also significant since they affect their stability. The rheology of the boundary between the droplets and the continuous phase, as well as its viscosity, therefore strongly influences how the emulsions system behaves [12]. The antibacterial properties of the nanoemulsion are another critical aspect to explore since they help preserve and store the nanoemulsion delivery product. However, there is not much research that covers the rheological and bactericidal properties of vitamin E nanoemulsion in the literature. Therefore, we looked into encapsulating vitamin E utilizing O/W nano-emulsions in this work. This study aimed to investigate the properties of Vitamin E-enriched nano-emulsions generated at various stirring times using the EPI technique. The effect of different stirring periods on the viscosity, average droplet size, zeta potential, and antibacterial characteristics was studied.

MATERIALS AND METHODS

Materials

Tween 80 and Vitamin E consisting of 96% DL-alpha-Tocopherol acetate, was bought from Loba Chemie Pvt. Ltd., Maharashtra, India. Medium-chain triglyceride coconut oil was obtained from a local market. We bought sodium benzoate 99 % ultra-pure and 1 M citric acid solutions from Loba Chemie Pvt. Ltd., Maharashtra, India. In the formulations, regular distilled water was used.

Preparation of Nano-emulsions

The formulations were prepared using an EPI method of low energy approach. The oil phase (10 wt %) was made in a hot plate magnetic stirrer at room temperature (30 °C) for 30 minutes while spinning at 500 rpm with Tween 80 (5 wt %), coconut oil (2 wt %), and vitamin E (3 wt %) added. The citric acid (0.9 wt %), sodium benzoate (0.1 wt %), and distilled water (89 wt %) were used to create the aqueous phase. Emulsions were prepared by introducing the water phase into the oil phase with a flow rate of 2 drops per 1 second at 30 °C along with a constant stirring of 500 rpm. The emulsions were formulated by stirring at 30 min, 70 min, and 110 min [13].

Measurements of the droplet size and zeta potential

A dynamic light scattering device (Zetasizer Ver. 7.12, Malvern Instruments, Malvern, UK) was utilized to assess the nanoemulsion formulation's droplet diameter and polydispersity index. The average number of runs for each measurement in the instrument was 16. Before each experiment, all the formulations were diluted with distilled water to eliminate various scattering effects brought on by the viscosity of the samples. From the distribution of droplet diameter, the PDI and mean droplet diameter were determined. In addition, zeta potential measurements were also performed and reported. All measurements were made in ambient conditions.

pH Measurement

Using a calibrated pH meter, the pH of the nano-emulsions was recorded by immediately dipping the electrode into the mixture at room temperature.

Rheology Test

Information regarding the fluid's behavior, specifically its shear thinning or shear thickening nature, is provided via rheological characteristic studies. Anton Paar Rheometer was used to assess the materials' rheological characteristics at 25°C. The nano-emulsions' viscosity and shear stress were determined in relation to shear rates between 0 and 1000 s^{-1}. The rheometer was used to test the emulsion in a volume of 12.5 mL.

Antimicrobial Activity Test

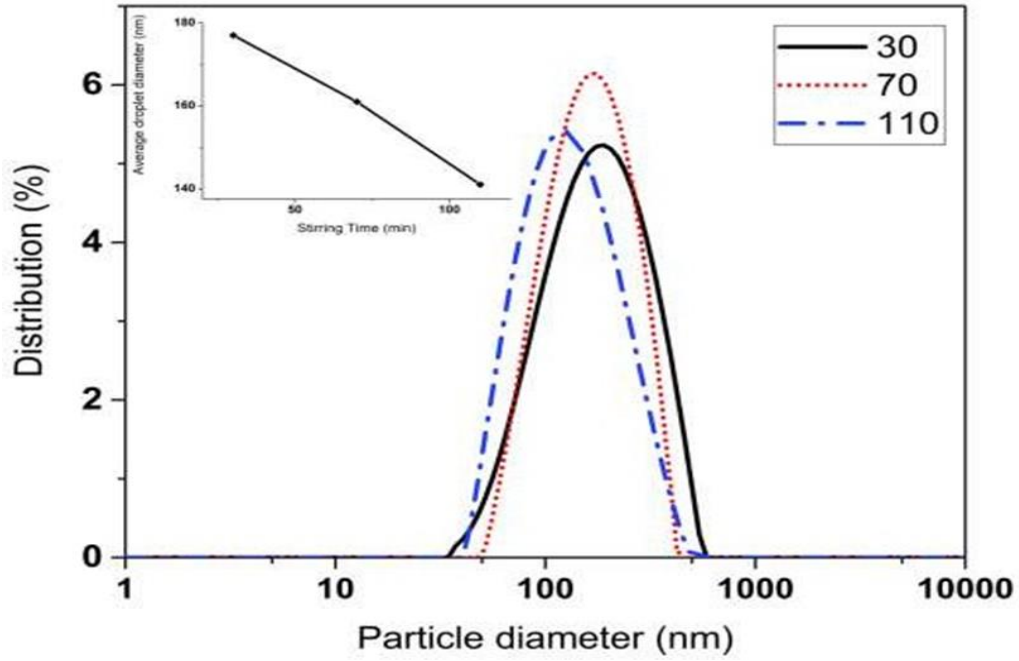
Escherichia coli (E. coli) (0335P) and *Staphylococcus aureus* (*S. aureus)* (0360P) were the bacterial strains used in the

antimicrobial testing. All strains were acquired from KwikStik. The typical disc diffusion method was used in the antimicrobial investigation, in which agar plates were seeded with a test bacterium. The nanoemulsion-containing filter paper discs were then put on the agar surface. Furthermore, Petri dishes were incubated under appropriate conditions. Following incubation, the test microorganism's growth and development were inhibited by the nanoemulsion, and the widths of the inhibitory growth zones were measured. The experiment was carried out three times [14].

RESULTS AND DISCUSSION

At room temperature, vitamin E-enhanced nano-emulsions (90 wt% aqueous phase and 10 wt% oil phase) were generated in a magnetic plate at various stirring times (30, 70, and 110 min). We determined the droplet size distributions, PDI and the mean droplet size of the nano-emulsions fabricated by utilizing EPI technique. Figure 1 shows the particle diameter distributions and average droplet meter (inset) of all the nano-emulsions prepared at various stirring times. Figure 1 depicts that the mean droplet size of all the nano-emulsions created at different stirring times ranged from 141 nm to 177 nm, and they all displayed mono modal size distributions.

Figure 1: Particle size distribution of vitamin E nano-emulsions prepared at different stirring times. Inset: The relationship between average droplet size (diameter) of nano-emulsions and stirring time.



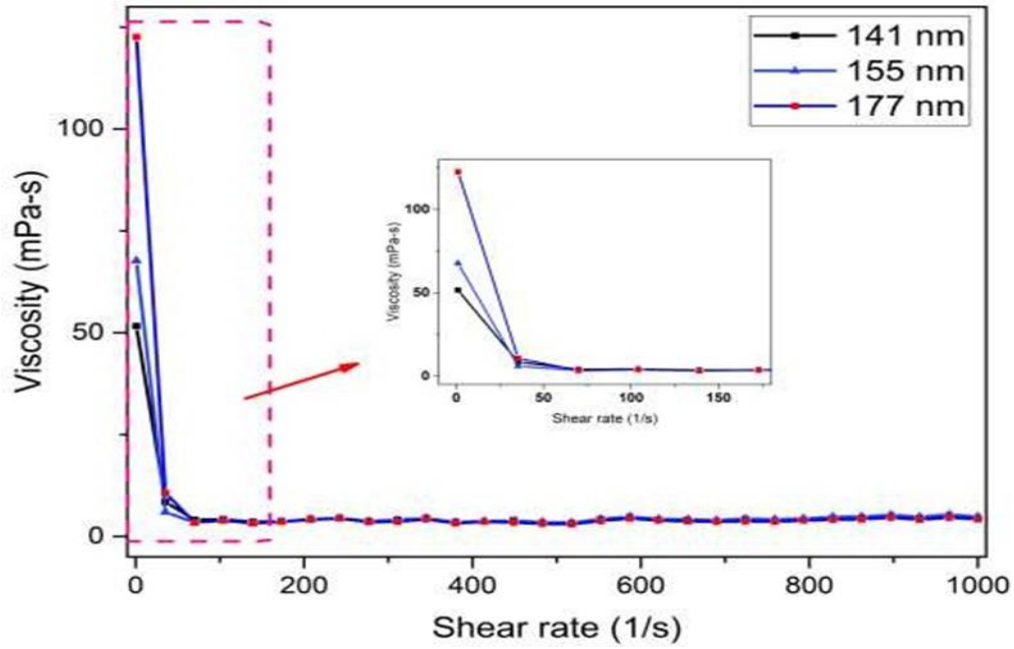
In the prepared nano-emulsions, the order of mean droplet diameter depending on stirring time (min) was 30 > 70 > 110, with mean droplet sizes of about 177, 155, and 141 nm respectively. The possible reason for the decrease in the size of nano-emulsions with the increasing stirring time could be the higher energy inputs added during emulsion preparation with stirring time, which might disrupt the particles into fine emulsions [15]. A crucial metric for determining how particles is distributed in a colloidal system is PDI, and a small range of PDI encourages system stability. Mostly, PDI values below 0.3 are considered good, which leads to ensuring system stability. Surprisingly, all prepared nano-emulsions have PDI values less than 0.3, indicating that they are of uniform size. Results of the zeta potential of fabricated emulsions ranged from – 14.3 mV to – 7.1 mV. Stirring time was found to affect the zeta potential of emulsions, and with the increasing stirring time, a decline in Zeta potential values was observed, which was significantly lower at 110 min. Negative zeta potential values were due to the presence of a negative charge on the oil droplets. The alteration to charge concentration of the electrical double layer caused by the long stirring time may have decreased the zeta potential's magnitude. Table 1 reports measurements of the nanoemulsion sizes, PDI values, and zeta potentials of the prepared nano-emulsions.

Table 1: Formulation of vitamin E nanoemulsion: mean droplet diameter, PDI value and zeta potential.

Stirring Time (min)	Droplet diameter (nm)	PDI	Zeta potential (mV)
30	177	0.255	- 14.3
70	155	0.221	- 9.3
110	141	0.234	- 7.1

The rheological properties of nano-emulsions are significant as these link to the stability and final product application. Rheological characteristics like emulsion viscosity and shear stress were evaluated under the influence of shear rate. Figure 2 depicts the flow curves for shear dependent viscosity (from shear rate 1 to 1000 s^{-1}) of nano-emulsions containing vitamin E at various stirring times at 25°C.

Figure 2: the relationship between shear rate and the viscosity of vitamin e encapsulated nano-emulsions.



It was observed that the nano-emulsions prepared at different stirring times showed the same behavior. Furthermore, the emulsions show non-Newtonian or shear thinning characteristics up to low shear rates of about 100 s^{-1}. The nanoemulsion viscosity almost remains unchanged at high shear rates, showing Newtonian behavior. The larger droplets are distorted due to shear, which accounts for the non-Newtonian features of nano-emulsions. As shown in Figure 2, the non- Newtonian aspect of dispersions is more prominent for bigger droplet nano-emulsions.

Figure 3 shows the shear stress for produced nano-emulsions of various particle sizes as a result of shear rate. All of the samples, which were produced at various stirring rates, exhibit the same tendency. These curves show linear relationships. Therefore, the collected data were subjected to Newton's law of viscosity equation given below, and viscosities were evaluated (described in Table 2). R^2 is the correlation coefficient in Table 2.

$$\tau = \mu\dot{\gamma} \qquad (1).$$

Where, τ denotes shear stress, μ stands for viscosity, and $\dot{\gamma}$ for shear rate. The table shows a linear correlation between droplet size and nanoemulsion viscosity.

Figure 3: Relationship between shear rate and shear stress for vitamin E enhanced nanoemulsion prepared at various stirring time.

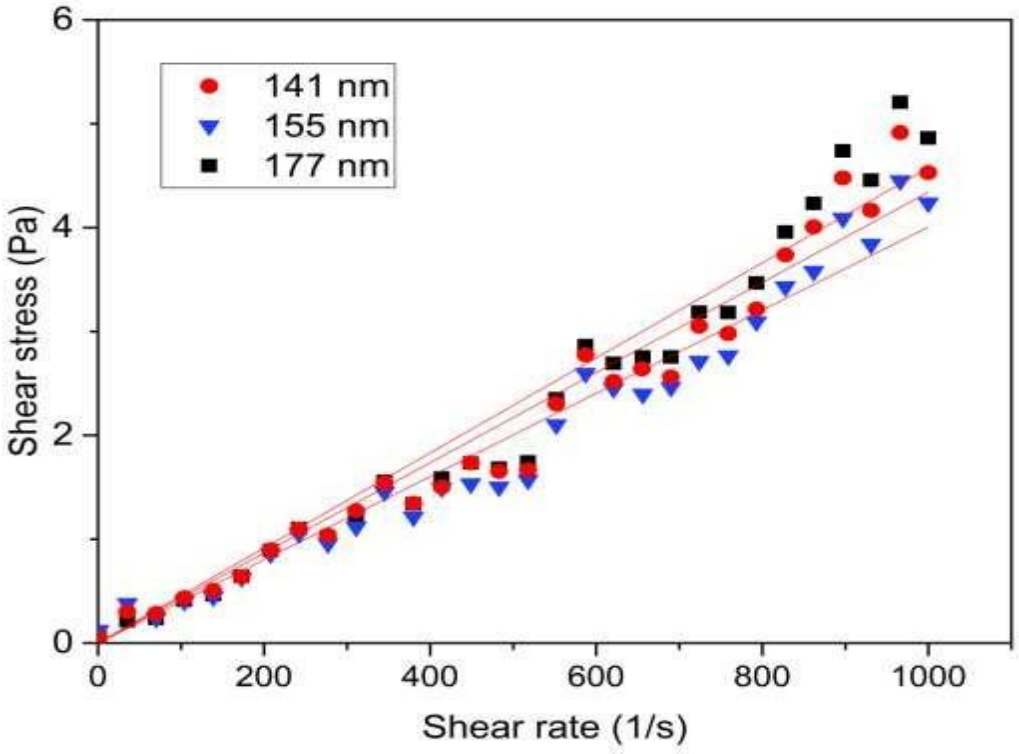
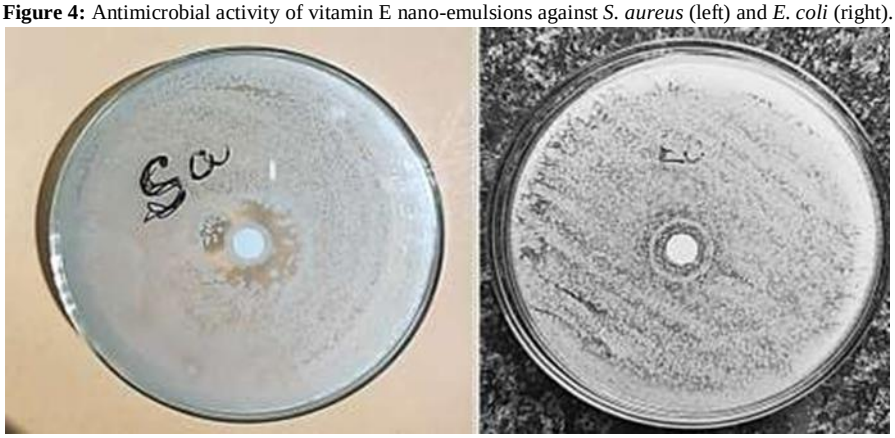


Table 2: Viscosity of vitamin E nanoemulsion

Time (min)	Droplet size (nm)	Viscosity (cP)	R2
30	177	4.58±0.098	0.986
70	155	4.34±0.088	0.987
110	141	4.01±0.077	0.988

Vitamin E has significant potential to improve targeted medication delivery due to its direct antibacterial activities and the improved antibiotic effects caused by its molecular structure. In this work, we tested the vitamin E nano emulsion's potential antibacterial properties against two commonly grown laboratory bacteria *E. coli* and *S. aureus*. The measure of antibacterial activity contained in the sample or product is often connected with the extent of the zone of inhibition. Therefore, an effectiveness of antibiotics is often shown by a greater zone of inhibition. The antibacterial activity of the vitamin E nano-emulsions against *E. coli* and *S. aureus* were noticeably different according to Figure 4. The zone inhibition diameter of all the nano-emulsions was 14±2 mm for the bacteria *S. aureus*. The nano-emulsions displayed no resistance to *E. coli*.

Figure 4: Antimicrobial activity of vitamin E nano-emulsions against *S. aureus* (left) and *E. coli* (right).



CONCLUSION

In the present study, we prepared nano-emulsions containing vitamin E at various stirring times. Size distribution results showed that all the formulations were within the nanometric size (< 200 nm). As the stirring time increases, the diameter of the nanoemulsion droplets decreases. This is due to larger energy inputs supplied during emulsion creation with stirring time, which could disrupt the particles into fine emulsions. All nano-emulsions showed negative zeta potential and PDI below 0.3. The nano-emulsions exhibit two unique flow regimes: shear thinning characteristics are seen at low shear rates, where the viscosity decreases with rising shear rates, and Newtonian behavior is seen at high shear rates, where viscosity remains independent of shear rate. According to the antimicrobial test, the nanoemulsion is effective against bacteria S. aureus.

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Conflict of interest

The authors declare no conflict of interest.

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