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Factors Affecting the Production and Stability of Optimal Formulation of Nanoemulsion Containing Vitamin A and D by Spontaneous Production

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Abstract
Due to their unique functional properties, using nanoemulsions for the enrichment of many hydrophobic compounds in beverages is beneficial. In this study, vitamins A and D nano emulsions produced by spontaneous formation using various surfactants (tween 60, 80 and 85) and corn oil and miglyol 812 as carrier oils; various types of vitamins A and D (2:1, 1:1 and 1:2) and various surfactant emulsion ratios (SER). Measurement of the average diameter of the droplets, zeta potential, rheology, turbidity and stability of system during storage time were done. Analysis of the results by laser light dispersion in completely randomized design showed the particle size in formulation with SER=17.5%, using tween 80 and miglyol 812 including 5% vitamin A and D, was equal to 81 nm with -27.9 mv zeta potential was gained. The optimum sample was stable at a temperature of 23 °C during storage time (3 months). The images of the electron microscopy also confirmed the results of measuring the size of droplets by particle size analyzer. The study of the rheological behavior of optimum nano emulsion also indicated the Newtonian behavior and the non-dependence viscosity by shear-shear rates.

Keywords: Miglyol 812, Nanoemulsion, Stability, Tween, Vitamin A & D

Introductıon
Nanoemulsions are among the most important nano carrier systems, which are clear systems with droplet size in the range of 20-200 nm. Extensive researches have been done to usenanoemulsions for food enrichment and development of the functional foods containing bioactive components (Pezeshki *et al*., 2016; Saberi, *et al*., 2013; Ozturk, *et al*., 2015; Komaiko & McClements, 2014). The aim of this study was to investigate the factors influencing the production and stability the droplets and also survey on the physicochemical properties of

vitamin A-D nanoemulsion (with optimal size and stability) using spontaneous method for use in food and beverages fortification.

Material and methods

Preparation of Vitamin A-D nanoemulsion

We produced nanoemulsion of vitamin A-D using low enegy spantaneus method (Anton & Vandamme, 2009). During the formation of the emulsion the mixture is continuously stirred by the magnetic stirrer (500 rpm at 25 °C).

Particle size and size distribution

The average diameter and span value of the particles were determined using particle size analyzer (Wing SALD 2101, Shimadzo, Japan), at 25 °C (Pezeshki *et al*., 2016).

Morphology characterization

Morphology of the nanoemulsions was observed using transmission electron microscopy (TEM) (Klang *et al*., 2012).

Surfactant concentration

The influence of surfactant concentration on the droplet size was studied by variation the surfactant-to-emulsion ratio (SER), while keeping the total oil content (MCT+Vitamin A-D) constant (10 wt.%). In generated nanoemulsions based on tween 80 and Mygliol 812 as a carrier oil, containing vitamin A–D 5 different percentages of SER were examined.

Influence of oil type

A series of emulsions were prepared with similar overall compositions (%SER=17.5%), but using different oils including medium chain triglycerides, (Mygliol 812; corn oil and different ratios of vitamin A and D.

Effect of surfactant type

We restricted ourselves to a range of food-grade non-ionic surfactants (Tween 60, 80 and 85) since this type of surfactant is generally considered as the most suitable surfactants for the formation of emulsions by low energy methods.

Rheological analysis

Measurement of rheological characteristics of the formulation of sample one day after preparation with three replication was done at 25 °C using Physical Anton Paar rheometer model MCR 301, made in Austria. To determine the flow behavior of the samples, the linear viscoelastic region the shear rate increased from 2^{S-1} to 100^{S-1} within the interval of 10 min.

Statistical analysis

Statistical analysis was designed based on a complete randomized optimization after 3 repetitions. One-way ANOVA and Duncken’s mean comparison tests were used at 5% with SPSS version 16.0.

Results and discussion

Effect of surfactant concentration on the droplet size

At a concentration of SER=10%, the size distribution of droplets was broad (Figure 1). By increasing the concentration of surfactant by SER=15%, % 17.5 the size of obtained droplets decreased to less than 100 nm and these droplets almost had the same size. In SER=15% the size distribution of droplets was bimodal, while the nanoemulsions with SER=17.5% had monomodular size distribution. The reason of decreasing the droplet size by increasing the surfactant concentration may be related to the absorption of surfactant molecules on the oil/water surface and further reduction of interfacial tension. Also increasing the surfactant concentration would result in a greater number of surfactant molecules emigrating from the oil phase to the aqueous phase of the emulsion and nano droplets would be produced (Saberi *et al*., 2013).

Unexpectedly by increasing surfactant concentration up to SER=20% droplet size increased and particles had broad, non-uniform size distribution (Figure 1). By increasing the concentration of surfactants, they can form multiple structures such as double layer structure which can cover the droplets. This causes droplets to get away from each other and reduces the vanderwals attractive force between them. For approaching the droplets to each other, these surfactant structures which are liquid crystal structures, must be driven along and with this mechanism, the emulsion would get consistent (Komaiko & McClements, 2014).

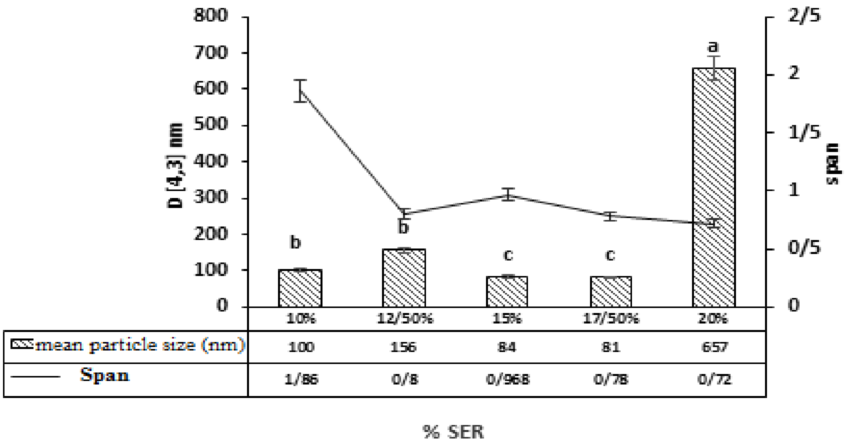

Figure 1. Effect of surfactant emulsion ratio (SER) on the particle size and particle size distribution of vitamin A-D nanoemulsion.

Droplet morphology using transmission electron microscopy (TEM)

Images obtained by TEM, showed spherical droplets in nanoemulsions system, without the occurrence of phase separation, aggregation and also, the results of the size measurement of nano particles obtained from measurement devices, were confirmed (Figure 2).

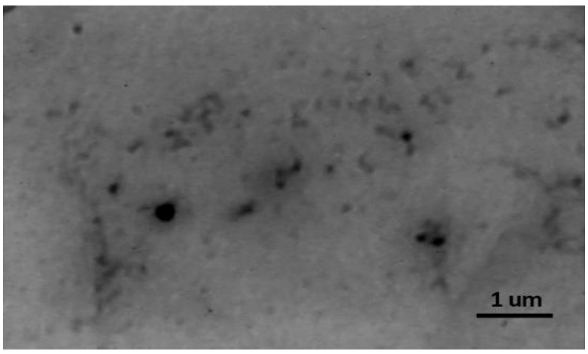

Figure 2. TEM morphology of vitamin A -D nanoemulsion

The effect of surfactant type on size and distribution of droplets

The smallest droplet size produced by tween 80 (HLB=15), while the tween 60 with HLB equal to 16.7 (the higher rank shows it is more hydrophilic), produced larger droplets. In tween 80, presence of double bond of the oleate ester in its hydrophobic chain, induced more mobility for it rather than other tweens with saturated chains. In addition, high molecular weight of some tweens (tween 85) reduces mobility of the surfactant from organic phase to aqueous so they show a poor emulsifier property (Ostertag *et al*., 2012).

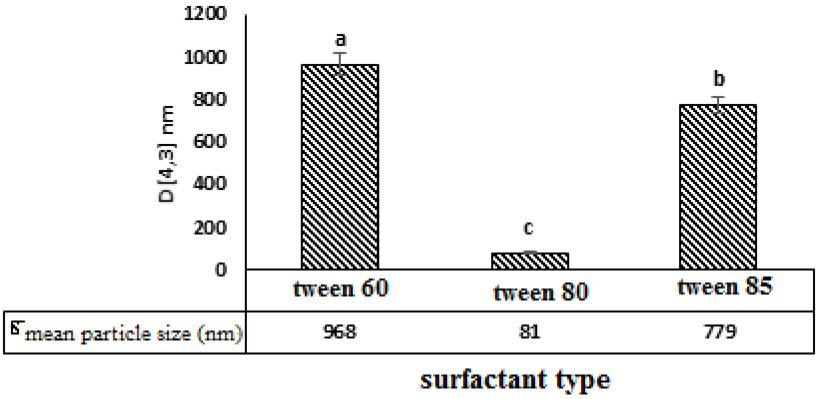

Figure 3. Effect of surfactant type on the particle size and particles size distribution of vitamin A-D nanoemulsion.

Stability of optimized nanoemulsion

Long term stability of optimal nanoemulsion was checked out by measurement of changes in the size and also appearance of samples (see phase separation) during storage at room temperature Over a period significant changes were not observed between samples (P>0.05) and the size distribution for all samples were uniform and monomodular (Figure 4). Reduction in the size of the droplets overcome the brawian motion to the gravitational force and also increases the viscosity, then two important instability mechanisms, gravitational separation and aggregation (flocculation) are prevented.

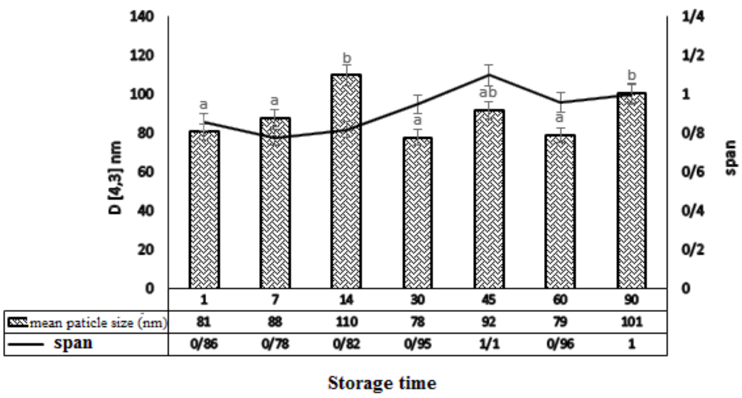

Figure 4. particle size and particles size distribution of vitamin A -D nanoemulsion during storage time.

Influence of the oil carrier phase

There is significant difference between size of droplets when different oil carriers are used, and using Mygliol 812 as carrier oil compared to other oil phases, had significant reduction in the size of the nanoemulsion droplets (Figure 5). Migliol 812 belongs to MCT (Median Chain Triglyceride) groups (Rowe *et al*., 2009). MCTs are food grade oils and MCTs are known to have high stability against oxidation (Hippalgaonkar *et al*., 2010).

Viscosity of the oil is effective on the move of the organic phase to the aqueous phase, and with reducing the viscosity of oil, surfactant molecules move faster and therefore produce smaller droplets (Israelachivilbi, 2011).

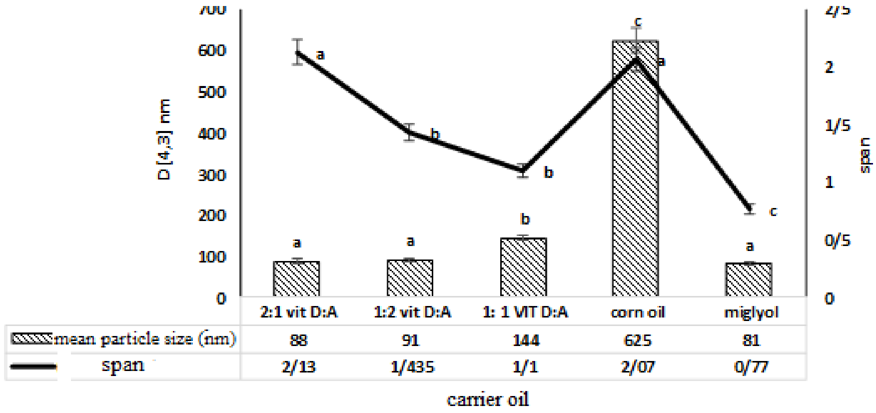

Figure 5. Effect of carrier oil type on the particle size of vitamin A-D nanoemulsion.

Rheological properties of the nanoemulsion

As can be seen in Figure 6, there was a relatively linear relationship between shear stress and shear rate. This behavior shows the simplest flow behavior of solutions, the Newton behavior. The relationship between shear stress and viscosity as a function of shear rate was measured at 10 min shear rate 2-100^{S-1}, to determine the flow behavior of samples. Here the viscosity of the fluid is measured from the slope of shear stress- shear rate curve. So that by increasing the viscosity of the solution, the slope of the curve increases.

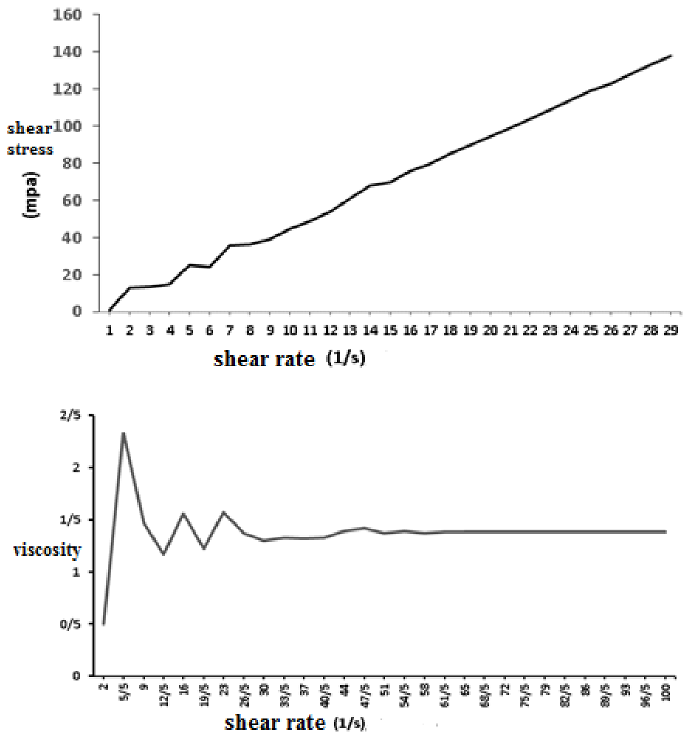

Figure 6. Rheological Properties of the vitamin A-D nanoemulsion

Conclusion

Deiffernt physicochemical factors have important influence on the production, rheological properties and stability of A-D nanoemulsion. The using of tween 80 and mygliol 812, produced the smallest droplet sizes, also there was an optimum surfactant concentration. Initially by increment of the concentration up to SER=15 and 17.5%, the droplet size was decreased but after passing a spicial value, Optimal nanoemulsion had a high stability during 90 days of storage. The factors affecting stable nanoemulsion production include the type and concentration of surfactant and type of carrier oil.

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