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Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: Factors affecting particle size and stability

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ABSTRACT

Oil-in-water nanoemulsions are particularly suitable for encapsulation of lipophilic nutraceuticals because of their ability to form stable and transparent delivery systems with high oral bioavailability. In this study, the influence of system composition and preparation conditions on the particle size and stability of vitamin D nanoemulsions prepared by spontaneous emulsification (SE) was investigated. SE relies on the formation of small oil droplets when an oil/surfactant mixture is titrated into an aqueous solution. The influence of oil phase composition (vitamin D and MCT), surfactant-to-oil ratio (SOR), surfactant type (Tween 20, 40, 60, 80 and 85), and stirring conditions on the initial particle size of vitamin D nanoemulsions was studied. Nanoemulsions with small droplet diameters ($d < 200$ nm) could be formed using Tween 80 at SOR $\geqslant 1$ at high stirring speeds (800 rpm). These systems were relatively stable to droplet growth at ambient temperatures (<10% in diameter after 1 month storage), but unstable to heating ($T > 80$ °C). The thermal stability of the nanoemulsions could be improved by adding a cosurfactant (sodium dodecyl sulphate (SDS)). The spontaneous emulsification method is simple and inexpensive to carry out and therefore has great potential for forming nanoemulsion-based delivery systems for food, personal care, and pharmaceutical applications.

1. Introduction

Nanoemulsions are colloidal dispersions that contain small particles (typically around 20–200 nm in diameter) dispersed within an aqueous medium (McClements, 2012; McClements & Rao, 2011; Silva, Cerqueira, & Vicente, 2012). There is increasing interest in the utilization of nanoemulsions in the food industry because of the changes in physicochemical properties and biological performance associated with alterations in particle size. Reducing particle size in emulsion-based delivery systems has a number of 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 (McClements, 2011, 2013). In particular, nanoemulsions may be particularly useful for encapsulating lipophilic bioactive components, such as oil-soluble vitamins or nutraceuticals, into aqueous-based food products that should be optically clear, such as fortified waters, soft drinks, or juices.

In this study, we examined the potential of using oil-in-water nanoemulsions to encapsulate an oil-soluble vitamin, *i.e.* vitamin D. This substance is essential for the proper functioning of the human body, but cannot be synthesized endogenously. Vitamin D has been reported to play a critical role in bone, teeth, and cartilage development (Cranney, Weiler, O'Donnell, & Puil, 2008; Hark & Deen, 2005), as well as in preventing cancer, heart disease, and immune diseases (Haham et al., 2012; Holick, 2004). This oil-soluble vitamin typically comes in two different molecular forms: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_2 is naturally present in low amounts in certain foods, whereas vitamin D_3 is typically synthesized in the human skin after exposure to sunlight (Holick, 2007; Lee, O'Keefe, Bell, Hensrud, & Holick, 2008). Individuals in many countries are deficient in vitamin D due to lack of exposure to the sun, extensive use of sun cream, or poor dietary intake (Haham et al., 2012; Holick, 2004; Tsiaras & Weinstock, 2011). Dietary restrictions such as veganism or lactose intolerance could significantly impact an individual's vitamin D status, as well as a mother's lack of supplementation if exclusively breastfeeding their child. A vitamin D deficiency typically manifests itself as the development of a bone disease, such as rickets or osteomalacia.

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Table 1
Comparison of molecular and physicochemical properties of vitamin D_3 and E (data taken from ChemSpider.com).

Property	Vitamin D	Vitamin E
Name	Cholecalciferol	α-tocopherol
Formula	$C_{27}H_{44}O$	$C_{29}H_{50}O_2$
Appearance	White crystals	Viscous liquid
Molar mass	384.64 g/mol	430.7 g/mol
Melting point	83–86 °C	4 °C
log *P*	9.1	11.9
Surface tension	37.1 dyne/cm	34.7 dyne/cm
Molar volume	396.9 cm^3	462.8 cm^3
Density	0.969 g/cm^3	0.900 g/cm^3

Those at risk of a poor vitamin D status are infants breastfed exclusively, older adults, those with limited sun exposure, those with dark skin, and individuals who are obese (or who have undergone gastric bypass surgery, as the portion of the intestine that absorbs this vitamin may be impacted), and those who have a fat malabsorption condition (Medicine, 2010). Consequently, there is considerable interest in fortifying food and beverage products with this vitamin (Yang, Laillou, Smith, Schofield, & Moench-Pfanner, 2013). Some of the challenges associated with vitamin D fortification are: poor water solubility; chemical degradation when exposed to light, oxygen, or elevated temperatures; and variable oral bioavailability (Haham et al., 2012; Tsiaras & Weinstock, 2011).

In the current study, we aimed to create nanoemulsion-based delivery systems for vitamin D using a simple and inexpensive low-energy method: spontaneous emulsion (McClements, 2011; Solans & Solé, 2012). This method simply involves titrating an organic phase (surfactant, carrier oil, and bioactive) into an aqueous phase (Anton, Benoit, & Saulnier, 2008; Vandamme & Anton, 2010). The surfactant used should be primarily hydrophilic, *i.e.* it should have a high hydrophilic-to-lipophilic balance (HLB) number. The hydrophilic surfactant rapidly moves into the aqueous phase after the organic phase is added to it, which leads to spontaneous formation of very fine oil droplets at the phase boundary.

A similar spontaneous emulsification method has previously been used in our laboratory to form nanoemulsions enriched with other oil-soluble vitamins, *i.e.* vitamin E (Saberi, Fang, & McClements, 2013a, 2013b, 2013c). In general, delivery systems must be specifically designed for each application depending on the characteristics of the active agent to be encapsulated, *e.g.*, polarity, solubility, physical state, and chemical stability. Vitamin D and E are both highly non-polar bioactive molecules that are essential for maintaining human health and wellness, however, they do have different physicochemical characteristics (Table 1). In particular, vitamin D is crystalline at ambient temperature, whereas vitamin E is liquid. Consequently, vitamin D must be dissolved in a suitable carrier oil prior to incorporation into nanoemulsion-based delivery systems. A major objective of the current research was therefore to determine whether the spontaneous emulsification that was successfully used for encapsulating vitamin E in previous studies, could be used to encapsulate vitamin D dissolved in a carrier oil (medium chain triglycerides).

2. Materials and methods

2.1. Materials

Vitamin D (VD) (cholecalciferol) was kindly donated by BASF (Ludwigshafen, Germany). This source of the bioactive component consisted of vitamin D (2.5 wt%) dissolved within medium chain triglycerides (MCT). Pure MCT oil (*MIGLYOL*® 812) was also purchased from Warner Graham Company (Sasol Germany GmbH). Non-ionic surfactants (TWEEN® 20, 40, 60, 80, and 85) were purchased from Sigma–Aldrich Co. (St. Louis, MO). Citric acid and sodium benzoate were provided by PepsiCo (Valhalla, NY). Double distilled water was used in the preparation of all solutions and emulsions.

2.2. Emulsion preparation

2.2.1. General

A protocol previously used to fabricate vitamin E nanoemulsions by spontaneous emulsification was used to create vitamin D nanoemulsions in this study (Saberi et al., 2013c). Initially, an organic phase was prepared consisting of vitamin D, MCT and/or surfactant, and an aqueous phase was prepared containing buffer solution (0.8% citric acid, 0.08% sodium benzoate, in double distilled water, pH 3). The organic phase was then slowly titrated (15×500 μl injections made one per minute) into an aqueous phase that was being stirred at a fixed speed using a magnetic stir bar. Unless otherwise stated, a set of standard conditions were used to prepare the nanoemulsions: composition = 10 wt% surfactant, 10 wt% oil, and 80 wt% aqueous buffer; stirring speed = 500 rpm; and temperature = 25 °C. Nevertheless, a number of parameters were systematically varied to determine their influence on nanoemulsion formation.

2.2.2. Surfactant type

A series of non-ionic surfactants (TWEEN 20, 40, 60, 80, and 85) was tested to establish the influence of surfactant type on nanoemulsion formation. The standard conditions mentioned above were utilized to prepare the nanoemulsions in these experiments using both vitamin D preparation (vitamin D in MCT) and pure MCT as the oil.

2.2.3. Surfactant concentration

The surfactant-to-emulsion ratio was varied to determine the most suitable surfactant concentration to prepare the nanoemulsions. The total oil content (vitamin D in MCT) was fixed at 10 wt%, while the surfactant-to-emulsion ratio was varied according to the following equation:

$$\mathrm{SER} = 100 \times m_S/(m_O + m_W + m_S) \quad (1)$$

where m_S, m_O and m_W are the masses of the surfactant, oil, and water, respectively. This was achieved by varying the relative amounts of surfactant and water present in the system.

2.2.4. Stirring speed

The influence of agitating the aqueous solution during titration was determined by varying the stirring speeds while maintaining a constant SER and temperature (25 °C).

2.3. Particle size measurements

Dynamic light scattering was used to analyse the particle sizes of the nanoemulsions (Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at 25 °C. This equipment reports the data as an average of 13 runs for the intensity-time relationship. The mean particle diameter (Z-average) and polydispersity index (PDI) were calculated from the particle size distribution. All of these samples were diluted with buffer solution prior to measurement, and were measured both one hour and 24 h after the nanoemulsion were fabricated.

3. Results and discussion

3.1. Influence of surfactant type

Initially, we examined the influence of surfactant type on the formation of vitamin D nanoemulsions containing small droplets. The spontaneous emulsification method was used to form a series of systems with the same overall composition (10% surfactant, 10% oil, and 80% aqueous phase), but using different kinds of non-ionic surfactant (Tween 20, 40, 60, 80 and 85) and different kinds of oil (vitamin D in MCT or just MCT). The size of the droplets produced and the width of the particle size distribution (polydispersity index) depended strongly on surfactant type, with the smallest droplets and polydispersity indices being produced using Tween 80 (Fig. 1). Similar results have previously been reported for vitamin E nanoemulsions produced by both spontaneous emulsification (Saberi et al., 2013c) and emulsion phase inversion methods (Mayer, Weiss, & McClements, 2013).

One of the most important factors influencing the spontaneous formation of nanoemulsions is believed to be the packing of the surfactant molecules at the oil–water boundary, which can be characterized by their molecular geometry (Israelachvili, 2011). A surfactant's molecular geometry is classified by a packing parameter (p), which is the cross-sectional area of the tail group relative to that of the head group: $p = a_T/a_H$. Differences in surfactant packing at oil–water boundaries influence interfacial characteristics such as surface energy and dynamics, which are likely to play an important role in the spontaneous formation of ultrafine oil droplets using spontaneous emulsification. Tween 20 (C12:0), 40 (C16:0) and 60 (C18:0) all have saturated linear chains, whereas Tween 80 (C18:1) has an unsaturated kinked one. Thus, Tween 80 would be expected to have a higher packing parameter (due to a larger a_T) than Tween 20, 40 or 60. Tween 80 has a single unsaturated tail (monooelate – C18:1) whereas Tween 85 has three unsaturated tails (triooleate – 3 × C18:1) and so one would expect Tween 80 to have a smaller packing parameter (due to a lower a_T) than Tween 85. Presumably, there is an optimum surfactant geometry that is required to promote the spontaneous formation of very small droplets at the oil–water boundary using low energy methods.

Oil type had little influence on the size of the droplets produced, which can be attributed to the fact that the bioactive oil consisted of about 2.5 wt% vitamin D dissolved in MCT, and therefore behaved fairly similarly to just MCT. For this reason, we only report the results for the vitamin D enriched oil phase in the remainder of the experiments.

3.2. Influence of surfactant concentration

In this series of experiments we examined the influence of surfactant concentration on the formation of vitamin D nanoemulsions. Ideally, one would like to be able to form small droplets using the lowest amount of surfactant possible for economic, taste, and safety reasons. In these experiments we used Tween 80 as the surfactant since this gave the smallest droplets using the spontaneous emulsification method. The total amount of oil in the final systems was kept constant (10 wt%), while the amount of surfactant was varied (5–17.5%) with the remainder being buffer solution (85–72.5%).

The mean droplet diameter of the systems, as measured by dynamic light scattering, decreased as the surfactant concentration increased (Fig. 2). The polydispersity index was lowest at intermediate surfactant concentrations (SER = 10%). These results indicate that nanoemulsions containing relatively small oil droplets (d < 200 nm) can be formed at a surfactant-to-oil ratio of 1:1. This value is much higher than the value typically needed to fabricate vitamin-enriched nanoemulsions using high energy methods such as high pressure homogenization (Yang & McClements, 2013), but is similar to that reported for the formation of nanoemulsions by low energy methods such as spontaneous emulsification (Saberi et al., 2013c) and emulsion phase inversion (Mayer et al., 2013). Even smaller droplets could be formed at a surfactant concentration of 17.5% (SOR = 1.75), but when the surfactant level was further increased small droplets could not be formed. The need for such high surfactant concentrations may be related to the phase behaviour of the surfactant-oil–water (SOW) mixtures used to form nanoemulsions.

It has been proposed that the formation of ultrafine droplets using low energy methods requires the SOW system to rapidly pass through a state that consists of an oil-in-water microemulsion (Gutierrez et al., 2008; Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005; Solans & Solé, 2012; Sole et al., 2010). If the surfactant concentration is too high then a liquid crystalline SOW state is formed, which is difficult to disperse thereby leading to large droplets. On the other hand, if the surfactant concentration is too low then a microemulsion SOW state is not formed, which

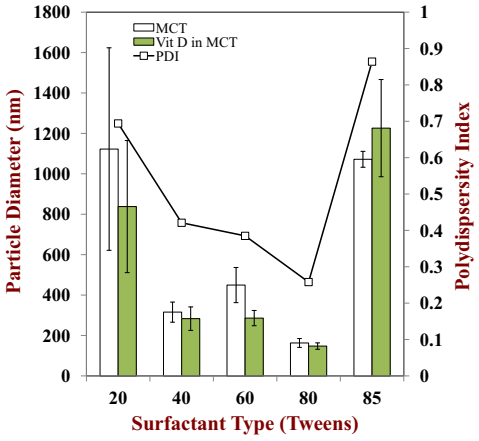

Fig. 1. Effect of surfactant type on mean particle diameter and polydispersity index of emulsions produced by spontaneous emulsification. Emulsions were prepared using 10 wt% oil phase (vitamin D in MCT or only MCT), 10% surfactant phase, and 80 wt% water (pH 3 buffer solution) at a stirring speed of 500 rpm at ambient temperature. The PDI is only shown for the sample containing vitamin D – the other sample gave similar results.

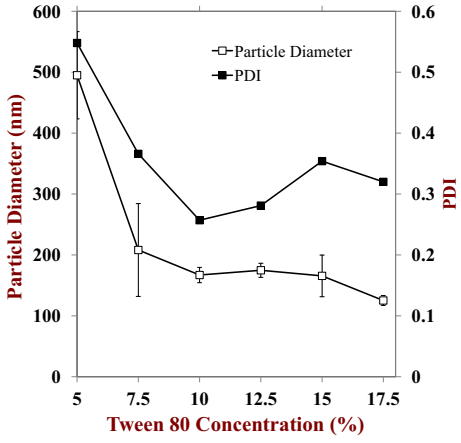

Fig. 2. Effect of tween 80 concentration on mean particle diameter and polydispersity index of emulsions produced by spontaneous emulsification. Emulsions were prepared using 10 wt% oil phase (vitamin D), surfactant phase (TWEEN 80), and water (pH 3 buffer solution) at a stirring speed of 500 rpm at ambient temperature.

also leads to the formation of large droplets. Consequently, there is an optimum surfactant concentration required to form very small droplets, which must be determined for each surfactant, oil, water combination.

3.3. Influence of stirring speed

The spontaneous emulsification method does not require any specialized homogenization equipment, such as a high pressure valve homogenizer, microfluidizer, or sonicator. However, it does require some stirring during the titration of the organic phase into the aqueous phase, presumably to ensure even mixing conditions. In this section, therefore, the influence of stirring speed on the size of the droplets produced by spontaneous emulsification was examined. The effect of stirring speed was investigated for systems produced using either 10 or 17.5 wt% surfactant at a fixed oil level (10 wt% oil phase).

At both surfactant levels, the droplet size produced by spontaneous emulsification clearly decreased with increasing stirring speed (Fig. 3). Presumably stirring ensures an even distribution of the surfactant/oil phase in the aqueous phase, which facilitates the spontaneous formation of small droplets. Previous studies have also reported that the mean droplet size of nanoemulsions produced by a low energy method decreases with increasing stirring speed (Mayer et al., 2013; Saberi et al., 2013b), which highlights the importance of controlling this parameter.

3.4. Isothermal and thermal stability

For practical applications it is important than emulsion-based delivery systems remain stable during storage and utilization. We therefore examined the influence of storage at ambient temperature and of exposure to thermal treatments on the stability of vitamin D enriched nanoemulsions. A nanoemulsion was prepared that contained 10% vitamin D in MCT oil, 10% surfactant (Tween 80) and 80% buffer solution.

The increase in droplet size of the nanoemulsions was measured after storage at 25 °C for one month: *Particle growth* = $100 \times d(t)/d(0)$, where $d(0)$ and $d(t)$ are the mean droplet diameters before and after storage. Previous studies have shown that the storage stability of low-energy nanoemulsions can be improved by diluting them with water prior to storage (Saberi, Fang, & McClements, 2013a, 2013b, 2014a), and therefore the influence of dilution with water (2- or 6-fold dilution) or with cosurfactant solution (2-fold dilution) was examined. The cosurfactant solution consisted of 0.5% anionic surfactant (SDS) dissolved in buffer solution since this surfactant has previously been shown to improve the stability of nanoemulsions to droplet growth (Rao & McClements, 2010). In all samples, only a slight (<10%) increase in mean droplet diameter was observed after storage for 1 month (Fig. 4a) alongside little change in the particle size distribution (Fig. 4b), which suggests that they were relatively stable to droplet growth under these conditions. Dilution of the nanoemulsions with buffer or cosurfactant prior to storage did not lead to any major improvement in their isothermal storage stability.

The stability of the nanoemulsions to thermal treatment was also examined, since oil droplets coated by non-ionic surfactants are known to be unstable to heating due to dehydration of their head groups leading to changes in their solubility and optimum curvature. In this case, measurements were carried out in diluted samples in the absence and presence of cosurfactant, since cosurfactant has previously been shown to appreciably improve the thermal stability of nanoemulsions (Saberi, Fang, & McClements, 2014b). In the absence of cosurfactant (0% SDS), there was an appreciable increase in the turbidity when the samples were heated above about 80 °C, which suggests that some droplet

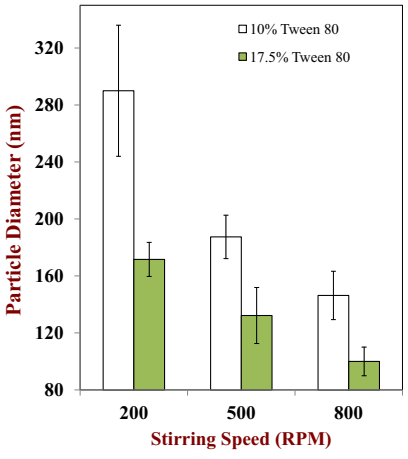

Fig. 3. Effect of stirring speed on mean particle diameter of emulsions prepared by spontaneous emulsification. 10 wt% oil-in-water emulsions (pH 3) were prepared at room temperature using 10% TWEEN 80, 10% oil phase (vitamin D in MCT), and 80% buffer solution.

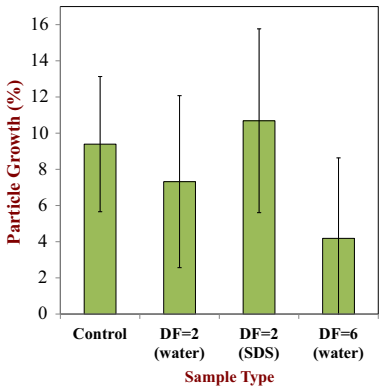

Fig. 4a. Effect of dilution in buffer solution or cosurfactant solution (0.5% SDS) on increase in mean particle diameter of nanoemulsion containing vitamin D, Tween 80, and buffer solution (pH 3) after storage at 25 °C for one month. DF = dilution factor.

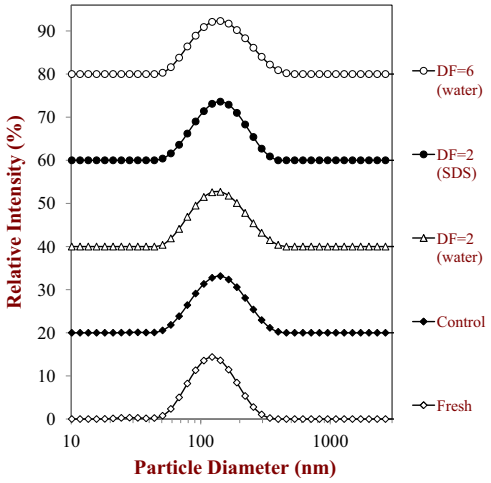

Fig. 4b. Effect of dilution and cosurfactant (SDS = 0.5%) on mean particle diameter of nanoemulsions containing vitamin D, Tween 80 and buffer solution (pH 3) after storage at 25 °C for one month. For more information see Fig. 4a.

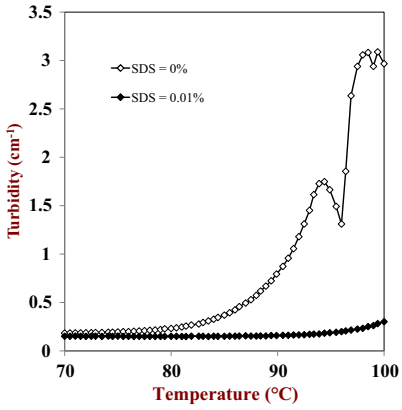

Fig. 5. Effect of temperature on the turbidity of diluted VD-emulsion in the presence and absence of SDS. SDS = 0%: diluted emulsion containing 0.1% oil phase and 0.1% surfactant (Tween 80); SDS = 0.01%: diluted emulsion containing 0.1% oil phase and 0.1% surfactant (Tween 80), and 0.01% SDS. Initial emulsion was prepared using spontaneous emulsification approach and stirring speed of 800 rpm.

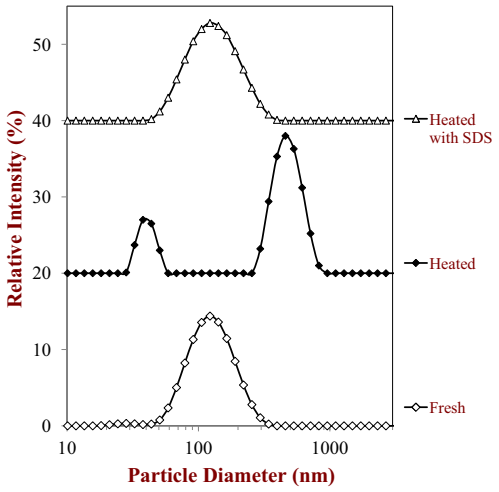

Fig. 6. Effect of thermal processing and cosurfactant (SDS) addition on the particle size distributions of diluted Vitamin D-emulsions. The fresh emulsion contained 10% oil phase (vitamin D), 10% surfactant (Tween 80); The heated emulsion diluted with water contained 0.1% oil phase and 0.1% surfactant (Tween 80); The heated emulsion diluted with SDS solution contained 0.1% oil phase and 0.1% surfactant (Tween 80), and 0.01% co-surfactant (SDS).

growth occurred leading to greater light scattering. As mentioned earlier, this effect can be attributed to progressive dehydration of the non-ionic surfactant head groups causing the packing parameter of the surfactant to tend towards unity ($p \approx 1$). As a result there was a decrease in interfacial tension and an increase in interfacial flexibility, which will promote droplet coalescence (Israelachvili, 2011; Shinoda & Friberg, 1986). On the other hand, in the presence of cosurfactant this effect was largely suppressed, with the turbidity remaining relatively low across the entire temperature range (Fig. 5). Presumably, incorporation of cosurfactant into the oil–water interface altered the optimum curvature of the surfactant and increased the repulsive interactions between droplets, thereby reducing the tendency for coalescence to occur (Fig. 6).

4. Conclusions

This study showed that nanoemulsions formed by spontaneous emulsification offer a simple and inexpensive means of encapsulating vitamin D. The initial size of the droplets depended on surfactant type, surfactant level, and stirring conditions. Nanoemulsions containing small droplets ($d < 200$ nm) could be formed using a non-ionic surfactant (Tween 80) at a surfactant-to-oil ratio of $\geqslant$1:1 with constant stirring during the titration process. These nanoemulsions remained physically stable during storage for one month at ambient temperature, but were susceptible to droplet growth when exposed to elevated temperatures (>80 °C). The thermal stability of the nanoemulsions could be improved by adding an anionic cosurfactant (SDS) prior to heating. To our knowledge, this is the first published work that utilizes low-energy homogenization to fabricate vitamin D-rich nanoemulsions. Nevertheless, the same method has previously been used to encapsulate another oil-soluble vitamin (vitamin E), which suggests that it may have more general application for this purpose.

The nanoemulsions developed in this study may be useful as vitamin D delivery systems for utilization in functional food and beverage products. These products will vary in their pH, ionic composition, ingredient interactions, storage conditions, and preparation procedures. Consequently, it will be important to test the stability of the vitamin D and the delivery systems under the precise conditions under which they will be utilized in commercial products.

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References

Anton, N., Benoit, J. P., & Saulnier, P. (2008). Design and production of nanoparticles formulated from nano-emulsion templates – a review. *Journal of Controlled Release, 128*(3), 185–199.

Cranney, A., Weiler, H. A., O'Donnell, S., & Puil, L. (2008). Summary of evidence-based review on vitamin D efficacy and safety in relation to bone health. *American Journal of Clinical Nutrition, 88*(2), 513S–519S.

Gutierrez, J. M., Gonzalez, C., Maestro, A., Sole, I., Pey, C. M., & Nolla, J. (2008). Nano-emulsions: New applications and optimization of their preparation. *Current Opinion in Colloid & Interface Science, 13*(4), 245–251.

Haham, M., Ish-Shalom, S., Nodelman, M., Duek, I., Segal, E., Kustanovich, M., et al. (2012). Stability and bioavailability of vitamin D nanoencapsulated in casein micelles. *Food & Function, 3*(7), 737–744.

Hark, L., & Deen, D. (2005). *Nutrition for life*. London, U.K.: Dorling Kindersley.

Holick, M. F. (2004). Vitamin D: Importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *American Journal of Clinical Nutrition, 79*(3), 362–371.

Holick, M. F. (2007). Vitamin D deficiency. *New England Journal of Medicine, 357*(3), 266–281.

Israelachvili, J. (2011). *Intermolecular and surface forces* (3rd ed.). London, UK: Academic Press.

Lee, J. H., O'Keefe, J. H., Bell, D., Hensrud, D. D., & Holick, M. F. (2008). Vitamin D deficiency: An important, common, and easily treatable cardiovascular risk factor? *Journal of the American College of Cardiology, 52*(24), 1949–1956.

Mayer, S., Weiss, J., & McClements, D. J. (2013). Vitamin E-enriched nanoemulsions formed by emulsion phase inversion: Factors influencing droplet size and stability. *Journal of Colloid and Interface Science, 402*, 122–130.

McClements, D. J. (2011). Edible nanoemulsions: Fabrication, properties, and functional performance. *Soft Matter, 7*(6), 2297–2316.

McClements, D. J. (2012). Advances in fabrication of emulsions with enhanced functionality using structural design principles. *Current Opinion in Colloid & Interface Science, 17*(5), 235–245.

McClements, D. J. (2013). Edible lipid nanoparticles: Digestion, absorption, and potential toxicity. *Progress in Lipid Research, 52*(4), 409–423.

McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: Formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical Reviews in Food Science and Nutrition, 51*(4), 285–330.

Medicine, I. o. (2010). Dietary reference intakes for calcium and vitamin D. In F. a. N. B. National Institute of Health (Ed.). Washington, DC: National Academy Press.

Rao, J. J., & McClements, D. J. (2010). Stabilization of phase inversion temperature nanoemulsions by surfactant displacement. *Journal of Agricultural and Food Chemistry, 58*(11), 7059–7066.

Saberi, A. H., Fang, Y., & McClements, D. J. (2013a). Effect of glycerol on formation, stability, and properties of Vitamin-E enriched nanoemulsions produced using spontaneous emulsification. *Journal of Colloid and Interface Science, 411*, 105–113.

Saberi, A. H., Fang, Y., & McClements, D. J. (2013b). Fabrication of vitamin E-enriched nanoemulsions by spontaneous emulsification: Effect of propylene glycol and ethanol on formation, stability, and properties. *Food Research International, 54*(1), 812–820.

Saberi, A. H., Fang, Y., & McClements, D. J. (2013c). Fabrication of vitamin E-enriched nanoemulsions: factors affecting particle size using spontaneous emulsification. *Journal of Colloid and Interface Science, 391*, 95–102.

Saberi, A. H., Fang, Y., & McClements, D. J. (2014a). Stabilization of vitamin E-enriched mini-emulsions: Influence of organic and aqueous phase composition. *Journal of Agricultural and Food Chemistry* (in press).

Saberi, A. H., Fang, Y., & McClements, D. J. (2014b). Stabilization of vitamin E-enriched nanoemulsions: Influence of post-homogenization cosurfactant addition. *Journal of Agricultural and Food Chemistry, 62*(7), 1625–1633.

Shinoda, K., & Friberg, S. (1986). *Emulsions and solubilization*. New York: Wiley Interscience.

Silva, H. D., Cerqueira, M. A., & Vicente, A. A. (2012). Nanoemulsions for food applications: Development and characterization. *Food and Bioprocess Technology, 5*(3), 854–867.

Solans, C., Izquierdo, P., Nolla, J., Azemar, N., & Garcia-Celma, M. J. (2005). Nano-emulsions. *Current Opinion in Colloid & Interface Science, 10*(3–4), 102–110.

Solans, C., & Solé, I. (2012). Nano-emulsions: Formation by low-energy methods. *Current Opinion in Colloid & Interface Science, 17*(5), 246–254.

Sole, I., Pey, C. M., Maestro, A., Gonzalez, C., Porras, M., Solans, C., et al. (2010). Nano-emulsions prepared by the phase inversion composition method: Preparation variables and scale up. *Journal of Colloid and Interface Science, 344*(2), 417–423.

Tsiaras, W. G., & Weinstock, M. A. (2011). Factors influencing vitamin D status. *Acta Dermato-Venereologica, 91*(2), 115–124.

Vandamme, T. F., & Anton, N. (2010). Low-energy nanoemulsification to design veterinary controlled drug delivery devices. *International Journal of Nanomedicine, 5*, 867–873.

Yang, Y., & McClements, D. J. (2013). Encapsulation of vitamin E in edible emulsions fabricated using a natural surfactant. *Food Hydrocolloids, s30*(2), 712–720.

Yang, Z., Laillou, A., Smith, G., Schofield, D., & Moench-Pfanner, R. (2013). A review of vitamin D fortification: Implications for nutrition programming in Southeast Asia. *Food and Nutrition Bulletin, 34*(2), S81–S89.