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Stability of the Oil-in-water Type Triacylglycerol Emulsions

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Abstract Lipid emulsions with saturated triacylglycerols (TAGs) with 4 to 10 carbons in each acyl chain were prepared to study how the oil component alters the stability of the lipid emulsions when phosphatidylcholines were used as emulsifiers. The average droplet size of the emulsions became smaller as the chain length of the TAG increased. For a given oil, emulsion with smaller droplets was formed with an emulsifier having higher HLB value. The influence of HLB values on the droplet size was biggest for the tributyrin (C4) emulsions. For the tricaprylin (C8) emulsions, droplet size was identical at given emulsifier concentrations regardless of HLB values. The HLB value and the concentration of the emulsifiers also affect the droplet size of the emulsions. The emulsions with smaller average droplet size were more stable than with bigger size for 20 days. The oil and water (o/w) interfacial tension is inversely proportional to the initial droplet size of the emulsion.

Keywords: egg PC, emulsifier, HLB, hydrophobicity, phosphatidylcholine, tributyrin, tricaproin, tricaprylin, tricaprin

INTRODUCTION

Lipid emulsion is a thermodynamically unstable dispersion where immiscible oil and water are dispersed by the use of an emulsifier. In oil-in-water (o/w) type lipid emulsions, oil forms spherical droplets surrounded by one or more layers of emulsifiers. Water forms the continuous phase. Since the oil constitutes the core of the droplets, it is often called the core-oil. Oil is an essential part in constituting the lipid emulsions. In this paper, we have investigated the correlation between the average droplet size of the emulsion and the physical properties of the core oils. [1] A homologous series of saturated triacylglycerols (TAGs) were used as the oil phase of the emulsions. Longer chain TAGs are more hydrophobic and, therefore, have higher oil/water (o/w) interfacial tension than shorter chain ones [1]. A notation C*n* represents a triacylglycerol that has three identical acyl chains with *n* carbons per chain in this paper.

Since the reduction of the o/w interfacial tension by the emulsifier is considered the main driving force to form the emulsion [2], it is expected that oils that have different hydrophobicities will change the emulsion stability. There are indications in the literature that the stability of the emulsion changes greatly by changing the oil [3,4]. The o/w interfacial tension [3,4] or the intrinsic viscosity [4] of the oils has a correlation with the droplet size of the emulsions. In these studies, mixtures of oils that could partition into the surface of the emulsion were used as the oil phase. For instance, in the ternary mixtures of castor oil/medium chain glyceride (MCT)/oleic acid, oleic acid could partition preferentially into the surface [5]. To avoid the complications in interpreting the results by using oil mixtures, we have chosen single-component oil systems to elucidate how the physical properties correlate with the emulsion stability. The stability of the emulsion was quantified in terms of the emulsion droplet size and/or its change with time in this paper.

We also investigated the stability relationship between the hydrophobicity of the oils and the choice of the emulsifiers having different hydrophile-lipophile balance (HLB) values at different concentrations. The emulsifying capacity is quantified by the HLB values [2] There are many studies to investigate the correlation between the physicochemical properties, such as the droplet size and the stability, of the emulsion and the emulsifying capacity and the concentration of the emulsifier [6,7]. We have investigated how the emulsifiers with varying HLB values affects the emulsion stability when different TAGs are used as the oils.

MATERIALS AND METHODS

Materials

Tributyrin (C4), tricaproin (C6), tricaprylin (C8) and tricaprin (C10) were purchased from Sigma Chemical Company (St. Louis, MO, USA). L-α-Phosphatidylcholine from dried egg-york (egg PC), 1,2-dinonanoyl-*sn*-glycero-3-phosphocholine (DNPC) and 1,2-dilauryl-*sn*-glycero-3-phosphocholine (DLPC) were also from Sigma Water was purified by using a water purification system (Milli-Q Plus; Millipore Corp., Bedford, MA, USA).

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Preparation of Emulsions

The o/w emulsions contained 100 μL/mL of oil and various concentrations (1-24 mg/mL) of emulsifier. Emulsifier (egg PC, DNPC or DLPC) was weighed and mixed with deionized distilled water. The lecithin/water mixture was sonicated until clear in an ice/water bath by using a probe type sonicator (High intensity ultrasonic processor, 600 W model, Sigma). The aqueous phase was added to the oil phase and sonicated in an ice/water bath for *ca.* 4 min to form emulsions.

Determination of the Emulsion Size and Droplet Number

The size and droplet number measurements were performed by using photon correlation spectroscopy (Malvern Instruments Ltd., UK). Emulsion was diluted by 300 times in water for these measurements. The measurements were repeated 3 times at least for a single emulsion system. Relative number of droplet particles was obtained in the unit of kilo counts per seconds (kCps). Absolute number of particles was not calculated.

In this paper, the average particle size and standard deviation will represent the size distribution function of an emulsion system as described previously [8]. Briefly, the size distribution of the droplets follows a log-normal distribution. Log-normal distribution function shows a Gaussian distribution in the logarithmic axis mode. The variance of the function is called polydispersity. Therefore, the standard deviation corresponds to (polydispersity)$^{1/2}$ and is positively-skewed in the normal axis mode.

Interfacial Tension

Interfacial tension between oil and aqueous subphase was measured by using du Nouy type surface tensiometer (Fisher Surface Tensiomat, Model 21, Fisher Scientific Company, Pittsburgh, PA, USA). The interfacial tension was measured from more dense liquid, aqueous solution to less dense liquid, oil by slowly lifting the platinum-iridium ring while increasing the scale to maintain the zero reading. The scale reading at the breaking point of the interfacial film was the apparent interfacial tension. In this paper, apparent values are reported. The measurement was performed at 22 ± 2°C. The lower and upper phases were deionized distilled water and pure oil, respectively, without emulsifiers.

RESULTS AND DISCUSSION

Triacylglycerol Emulsions Emulsified by Egg PC

The emulsions were prepared with a homologous series of saturated triacylglycerols (TAG) as the core using egg PC (12 mg/mL) as an emulsifier. The size of the emulsions in 1 and 4 days after the preparation is shown in Fig. 1. Unstable emulsion, which phase-separated in a few days, was formed by using tributyrin (C4) as the core oil. Tricaproin (C6) also produced very unstable emulsions with the average droplet size of *ca.* 900 nm. Big aggregates were observed immediately after the preparation. The size of these unstable emulsions grew further upon storage at room temperature. The smallest emulsions (*ca.* 200 nm) were made with tricaprylin (C8) and tricaprin (C10). When stored at room temperature, the sizes and their distributions did not change beyond the error range in these systems for the first 4 days.

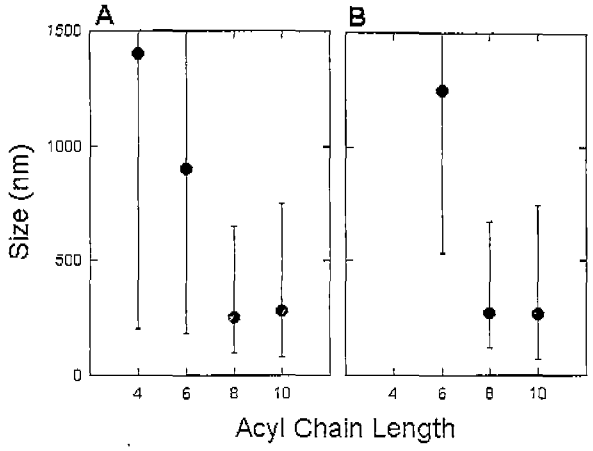

Fig. 1. Average droplet size of the emulsions in A) 1 day and B) 4 days after preparation. Acyl chain length in the X-axis indicates the number of carbon atoms in each acyl chain of the TAGs. The concentration of TAG and emulsifier (egg PC) were 100 μL/mL and 12 mg/mL, respectively, in the emulsions. The error bar is the standard deviation of the log-normal distribution function (See Ref 1 for detail).

Triacylglycerol Emulsions Emulsified by DNPC or by DLPC

Saturated triacylglycerols, tributyrin (C4), tricaproin (C6) and tricaprylin (C8) were selected as the core oils of the emulsions. As emulsifiers, DNPC and DLPC were selected to study the effect of acyl chain length of the lecithins on emulsion stability. DNPC and DLPC have 9 and 12 carbons, respectively, in each acyl chain.

As the DLPC concentration increased, the size of the emulsions decreased (Fig. 2). Tributyrin (C4) did not form stable emulsions in the emulsifier concentration range studied. Size of the emulsion was bigger than 1 μm even at the DLPC concentration of 24 mg/mL. Tricaproin (C6) formed emulsions with bigger droplet size than tricaprylin (C8) in the emulsifier concentration range studied. The droplet size increased greatly for the tributyrin emulsions in 20 days. For all the tributyrin emulsions, phase separation was observed in a few days. More interesting result was obtained in case of the tricaproin emulsions. After 20 days from the emulsion formation, the size of the emulsion increased to *ca.* 600 -800 nm, regardless of the emulsifier concentration ex-

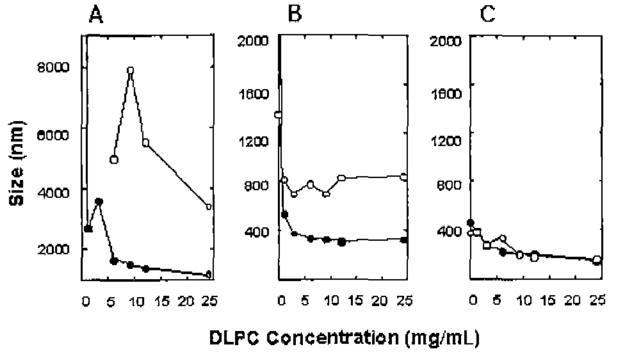

Fig. 2. Average droplet size of the A) tributyrin (C4), B) tricaproin (C6) and C) tricaprylin (C8) emulsions at different DLPC concentrations. Filled and open circles indicate the average droplet size immediately and in 20 days, respectively, after preparation.

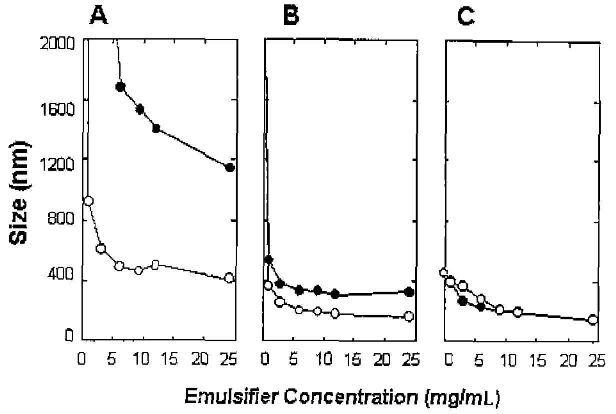

Fig. 3. Average droplet size of the A) tributyrin (C4), B) tricaproin (C6) and C) tricaprylin (C8) emulsions at different emulsifier concentrations immediately after preparation. Filled and open circles indicate the average droplet size of the emulsions when DLPC and DNPC, respectively, were used as emulsifiers. The droplet size of the tricaprylin (C8) emulsions was virtually identical for the DNPC and DLPC at the same emulsifier concentrations.

cept the sample without an emulsifier. For the tricaprylin emulsions, the emulsion size did not change beyond the statistical error range from the original value for 20 days.

When DNPC was used as an emulsifier instead of DLPC, the emulsions having smaller average droplet size were obtained (Fig. 3). When emulsifying tributyrin, both of the emulsifiers did not function well (Fig. 3(A)). However, DNPC at 24 mg/mL could form moderately stable emulsions with the average droplet size of *ca.* 400 nm. Similar behavior was also observed with the tricaproin emulsions, although the particle size was much smaller than the tributyrin emulsion (Fig. 3(B)). For the emulsion with more hydrophobic tricaprylin core, there was no statistical difference in the emulsion sizes when emulsified by either DNPC or DLPC (Fig. 3(C)). Another notable point is that the sizes of the tricaproin and tricaprylin emulsions were similar at same emulsifier concentrations when DNPC was used as an emulsifier. For instance, the average droplet size of both emulsions at 24 mg/mL DNPC was *ca.* 150 nm for both systems.

Interfacial Tension between Oil and Water

Interfacial tension between TAG and water was measured at *ca.* 22°C. The interfacial tension between tributyrin, tricaproin, tricaprylin and tricaprin and water were 3.6, 7.1, 13.3 and 16.6 dyne/cm, respectively. The o/w interfacial tension was higher for TAGs with longer chains indicating that the increasing hydrophobicity.

Droplet Number and Size Changes in Tributyrin/DNPC Emulsion

Tributyrin (100 mg/mL) was emulsified by using DNPC (12 mg/mL) as an emulsifier. The number and the size of the droplets in the emulsion were measured. The number of droplets in the emulsion was normalized by dividing the number at time *t* with the initial droplet number. The number of droplets in the emulsion decreased rapidly for the first few hours after the preparation and became steady after this time (Fig. 4(A)). The size of the emulsion grew from 540 nm (t = 5 min) to 1500 nm (t = 4 h) (Fig. 4(B)). The droplet size grew further to *ca.* 2200 nm after 28 h.

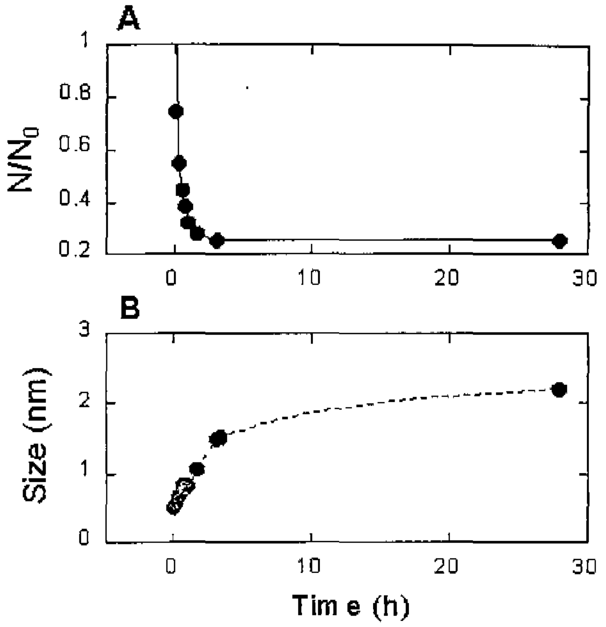

Fig. 4. The changes in (A) the number and (B) the size of the droplets in tributyrin emulsions. DNPC at 12 mg/mL was used as the emulsifier. Solid line in (A) was drawn to guide eyes only.

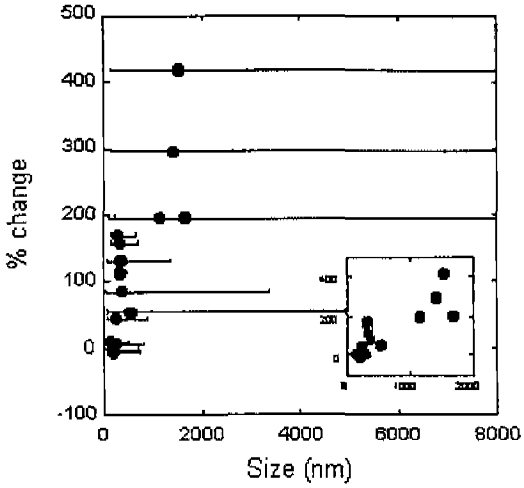

Fig. 5. The droplet size change in 20 days after the emulsification. To make this plot, data were gathered from all the different TAG emulsions that contained three different emulsifiers. The emulsifier was selected from egg PC, DLPC or DNPC.

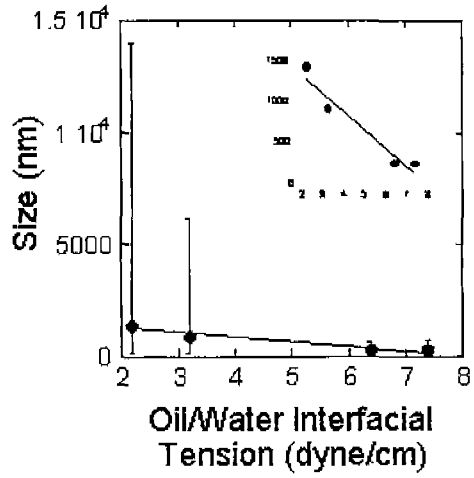

Fig. 6. Correlation between the droplet size of the TAG emulsions in 1 day after the preparation and the o/w interfacial tension. Egg PC (12 mg/mL) was used as an emulsifier. The insert shows the same plot without the error bars. A linear fit (R = 0.97) was drawn to guide eyes only.

Emulsions with Smaller Initial Droplet Size are More Stable

It has been stated that the emulsions with smaller average droplet size are more stable than with bigger size[2]. To evaluate the direct relationship between the stability and the size of the emulsion droplets, the droplet size of different TAG emulsions one day after the preparation was plotted against the percent change in the droplet size 20 days after the preparation (Fig 5). Emulsions had different emulsifiers (egg PC, DLPC and DNPC) at various concentrations (3-24 mg/mL). The % change was calculated by the following formula,

$$\%\,\text{Sixe change} = \frac{\text{Size at day 20} - \text{Size at day 1}}{\text{Size at day 1}} \times 100 \qquad [1]$$

There is a strong correlation between the initial droplet size and the % size changes. Correlation coefficient, R was 0.81 by a linear fitting. We can conclude that the emulsions with smaller droplets are more stable than with bigger droplets.

Relationship between Emulsion Droplet Size and o/w Interfacial Tension

TAGs (C4:0-C10:0) that have longer acyl chains form emulsions with smaller droplets (Fig. 1). To evaluate the correlation between the hydrophobicity of the oil and the droplet size of the emulsion, the droplet size was plotted against the o/w interfacial tension when egg PC at 12 mg/mL was used as an emulsifier (Fig. 6). The droplet size and the interfacial tension have a strong inversely-proportional relationship (R=0.98). We have reported a similar observation in the natural oil/egg PC emulsions [8]. Such a behavior has also been observed when castor oil and medium chain triacylglycerol (MCT) mixtures were used as emulsion core oils [3]. When mixtures of castor oil and MCT were used as core-oils, the droplet size of the emulsion was inversely-proportional to the o/w interfacial tension. Similar relationship was obtained by using a ternary oil mixture system, oleic acid oleylester/castor oil/MCT [4]. There was little correlation, however, between the o/w interfacial tension and the droplet size of the emulsion when oleic acid/castor oil/MCT or oleic alcohol/castor oil/MCT mixtures were used as the ternary oil phases [4]. Instead, there was a strong relationship between the viscosity of the oil and the droplet size of the emulsions. When the ternary system is used as the oil phase, however, data interpretation could be complicated. One or more components in the oil phase could partition preferentially into the emulsifier layers. Oleic acid or alcohol is often used as a co-surfactant [10]. For instance, in phosphatidylcholine-triolein emulsions, oleic acid acts more like an emulsifier than a component constituting the core [5]. Therefore, most of the oleic acid could partition into the emulsifier layers in the above ternary oil systems. Oleic alcohol, which is more hydrophilic than oleic acid [4], probably partitions more in the surface of the emulsion. By adding oleic acid or alcohol as a third component of the oil phase, therefore, probably they have changed inadvertently the nature of the emulsifiers as well as of the oil phase. When more hydrophobic oleic acid oleylester was used as a third component, interfacial tension was inversely proportional to the droplet size in the ternary system. Therefore, to correlate the physical properties of oils with the emulsion stability, it is much simpler to use a single component oil system.

Emulsion Stability: Oil and Emulsifier Effect

The emulsifying capacity of these emulsifiers could be quantified by using the concept of HLB values [9]. When formulating an emulsion, it is well-known to choose an emulsifier with a proper HLB value. HLB value is expressed as follows,

$$\begin{aligned} \text{HLB} = 7 + \Sigma\ &(\text{hydrophilic group numbers}) \\ -\ \Sigma\ &(\text{lipophilic group numbers}). \end{aligned} \quad [2]$$

The HLB value of egg PC is *ca*. 7 [7]. The components and composition of the hydrocarbon chains of egg PC is are listed in the Catalog of the Avanti Polar Lipids, Inc. The contribution of the lipophilic groups to the HLB value, therefore, could be calculated for egg PC and was 16.0. The contribution of the phosphatidylcholine headgroup (hydrophilic moiety) was calculated as 16.0 using Eq. [2]. Therefore, for lecithins the equation reduces to

$$\text{HLB} = 23.0 - \Sigma\ (\text{lipophilic group numbers}). \quad [3]$$

The lipophilic group number for each carbon unit (CH, CH_2 or CH_3) is 0.475 (2). Since the numbers of carbon units for DNPC and DLPC are 18 and 24, respectively, the corresponding HLB values are 14.5 and 11.6, respectively according to Eq. [3]. From above, DNPC is the best emulsifier in this group followed by DLPC and egg PC.

The difference in the emulsifying capacity is illustrated well in Fig. 3. DNPC (HLB =14.5) has a bigger emulsifying capacity than DLPC (HLB = 11.6) since the former has shorter acyl chains than the latter. More hydrophilic oils require a better emulsifier (higher HLB value) to form stable emulsions. Good emulsifiers are known to decrease the interfacial tension effectively, and therefore could generate a big interfacial tension gradient when two emulsion droplets approach.

In this paper, we have investigated the relationship between the hydrophobicity of the core oil and the stability of the corresponding emulsion. Many studies have been performed on the effect of the emulsifier in emulsion stability. However, it seems that there are few systematic studies in the field to elucidate the correlation between the choice of the core oils and the emulsion stability. In this paper, we show that the more hydrophobic saturated TAGs (such as tricaprin, C10) form more stable emulsion with small droplets than less hydrophobic ones (such as tributyrin, C4), which is similar to our previous findings [8].

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