Research Report # 02

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Introduction

Emulsifiers have the property to concentrate in the interface between oil and water. They have hydrophilic (water soluble) and lipophilic (oil soluble) parts, which determine their surface active properties. Within the range of food grade emulsifiers, three main groups can be distinguished on lipid basis:

- lecithins or phospholipids,
- mono- and diglycerides of edible oils and fatty acids and their diacetyl, lactic acid, citric acid, and ethoxylated esters,
- sucroglycerides and their esters and polysorbates.

The interactions at the interface of dispersions and emulsions are influenced by surface-active emulsifiers such as lecithins. Synergistic effects on the emulsion stability can be obtained by selected protein-lecithin combinations. Lecithins are modified physically and enzymatically, giving a range of food grade emulsifiers with different hydrophilic-lipophilic-balance (HLB) values. The influence of phospholipid fractions in the homogenization process can be measured by the particle size distribution technique (PSD) and emulsifying tests, which assess the emulsion stability.

Stabilization of the droplets can be achieved in three ways:

- Electrostatic stabilization: Emulsifiers, with a hydrophilic and a lipophilic part in each molecule, concentrate at the interface and reduce the interfacial tension. The surface charge on food emulsion droplets is due to adsorption of ionic surfactants, proteins, and other polyelectrolytes. Aqueous emulsions made with triglycerides and phosphatidylcholine give Zeta potentials from about 10 to 60mV, demonstrating the electrostatic repulsion by phospholipids.
- Stabilization by solid particles in the form of fat crystals is applied in foods such as butter, margarine, and ice cream. The crystals form a network, the hard stock in which liquid oil is embedded. High-melting monodiglycerides will act also as crystals in the interface.
- Steric stabilization: Nonionic emulsifiers (e.g. mono and diglycerides) and soluble polymers such as proteins cover parts of the interface and also reduce the interfacial tension. Proteins can be depleted from the interface by emulsifiers and vice-versa. In addition to steric stabilization, polymers, such as starches, hydrocolloids, and proteins with gelling activity, increase the viscosity of the emulsion. The coalescence of aggregates and creaming of the oil drops during storage of the product are retarded.

Methods

Measurement of emulsion stability

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Particle size. Size determination of droplets is very useful in evaluations of stability. The particle size distribution and mean particle radius (Z-average) of diluted emulsions are measured by a commercial dynamic light-scattering device (Nano-ZS, Malvern Instruments). Samples are diluted with external phase solution prior to analysis to avoid multiple scattering effects to reach the instrument attenuation factor. The solution/ buffers used for dilution should have the same pH and ionic composition as the samples being analysed. The samples are usually prepared by diluting the nano-emulsion with the external aqueous phase, followed by filtration through 0.45 µm filters prior to analysis. All measurements are carried out at a scattering angle of 90° and at 25 °C.

Z Potential measurement. The measurement of the Zeta potential has been introduced for the characterisation of the nano-emulsion. The Zeta potential is currently determined by the measurement of electrophoretic mobility in Malvern's Zetasizer Nano instrument (Malvern Instruments).

Influence of environmental stresses on emulsion stability
The physical and chemical stability of substrate enriched nanoemulsions to environmental stresses can be tested by a variety of methods.

Temperature: 10-15mL emulsion samples (pH 7.0) are transferred into glass tubes and stored in the dark at 5, and 20, ºC for 15days and 30days. Also, a long term stability test of storage at 5C with evaluations at 1, 2, 4, 8, and 12 months is carried on.

The influence of thermal processing on the stability of emulsions can also be studied. Emulsions prepared at the same pH are held isothermally at temperatures ranging from 30 to 90 ºC, cooled to room temperature and then stored for 24hours. The Z potential, mean particle diameter and creaming stability of emulsion are measured. If there is no evidence of creaming, no significant change in the Z potential or mean particle diameter of the emulsion it means the emulsion is stable to thermal processing.

pH: Emulsion samples are prepared in aqueous buffer solutions, and then the pH is adjusted to the desired final value (pH 3–8) using either NaOH and/or HCl solution. Emulsion samples (20 ml) are then transferred into glass tubes and stored in a dark place at ambient temperature (25º C) for 5 days.

Salt: Emulsions (pH 7.0) are diluted with different amounts of NaCl and buffer solution to form a series of samples with the same droplet concentration, but different salt concentrations (0–500 mM NaCl). The emulsions are stirred for 30 min and then transferred into glass tubes and stored in a dark place at ambient temperature for 5 days.

Transparency. A microemulsion is transparent, but this term needs to be quantified if perfect transparency is not required. A Tyndall effect can be observed and suggests that the particle diameters are on the order of 1/4 the wavelength of the incident light. Microemulsions can be translucent solutions with a slight sky-blue opalescence.

An assessment of transparency is commonly used to define the microemulsion zone in pseudo-ternary diagrams. Fixed quantity of the nanoemulsion is added to fixed quantity of suitable medium under continuous stirring (50 rpm) on magnetic plate at ambient temperature. In turbidimetry, the intensity of light transmitted through the medium, the unscattered light, is measured. In nephelometry, the intensity of the scattered light is measured, usually, but not necessarily, at right angles to the incident light beam.The formation of monophasic/biphasic system can be confirmed by visual method wherein a case where turbidity appears the emulsion is considered as biphasic but in a case where clear and transparent mixture are visualized after stirring the emulsion is considered as monophasic system.

Centrifugation. Prepared nanoemulsions are subjected to stress conditions such as centrifugation. Set a fixed volume (8-10mL) of the prepared nanoemulsion into 15mL centrifuge tubes and centrifuge for 6min at 5000rpm. If prepared nanoemulsions survive /are stable over this stress condition, they are considered as thermodynamically stable.

Conductivity. Conductivity measurements are currently carried out to determine the makeup of the continuous phase, provided O/W emulsions are conductive, whereas W/O emulsions are nonconductive. Measure of variations of conductivity during titration can be used to screen formulations.

Viscosity. The structure and type of microemulsion system can be characterised by rheological measurements as a function of the aqueous phase. If a system has low viscosity then it is O/W type. If a system has high viscosity, then it is W/O type.

Results

The use of lecithins in the preparation of O/W nanoemulsions with nonionic surfactants have been tested. Several kinds of lecithins have been employed in nanoemulsion formulations and the quality of the system was evaluated by measuring the droplet particle size, and the Z potential. One solid powder lecithin (Leci SF Supreme), and three liquid lecithins (Leci Sunflower, Leci Soy and Rapeseed) are tested alone or in combinations as co-surfactants to improve efficacy and increase the emulsion stability.

In the search for a formulation with low content of emulsifiers for different applications, but mainly to be employed in applications where low or no dilution is intended, formulations with 1.0, 1.2, and 1.4 g of a nonionic surfactant (SureNano_High_HLB 1 L) were prepared. Formulations prepared with 1.2 g of surfactant resulted in emulsions with intermediate quality with medium to low translucency and Tyndall effect. Increasing the content of surfactant in the formulations to a minimum of 1.4g resulted in very good quality emulsions with good translucency and Tyndall effect. Furthermore the particle size of these formulations were smaller than 100nm with a high Z potential (Table 1).

Table 1. Formulations of Low Content of Emulsifier with Several Combinations of Lecithins

ID	LecithinSF	LecithinSY	LecithinRS	Oil	SureNano_ Low_HLB	Emulsifier SureNano_High _HLB L	Size (nm)	Z Potential (mV)
Blend #1	0.2		0.6	0.85	0.15	1.80		-33.3
June 3 #2	0.6	0.5		0.86	0.40	1.40	48.81	-33.0
June 5 #1	0.55	0.42		0.79	0.30	1.40	49.61	-38.3
June 15 #1	0.6		0.40	0.85	0.40	1.40	47.18	-35.0
June 15 #2	0.65		0.40	0.85	0.30	1.40	44.55	-32.2
June 17 #1	0.65		0.31	0.88	0.20	1.40	43.48	-36.2

The blend #1 formulation is a selected mix of ingredients that is being manufactured by Caldic for Surenano. The blend #1 has a larger weight of the surfactant and it has been included in the Table 1 as a reference. All the other formulations have 1.4g of surfactant and about 1g of one or two lecithins. The weights of oil in these formulations have been reduced in an amount equal to the oil included in the liquid lecithin. There is not much difference in the values of both particle size and Z potential of the formulations. The smaller particle sizes are in the last two formulations (June 15 #2 and June 17 #1) which have been prepared with powder lecithinSF and liquid lecithinRS. The highest Z potential is in the formulation prepared with powder lecithinSF and liquid lecithinSY.

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In the search for a formulation with optimum particle size and stability, the best formulation from the Response Surface (RS) optimization test (formula #4) was modified by changing the amount and type of lecithin used in the formulation of the RS test. All the formulations of the RS test had the same amount and type of lecithin, 0.7 grams of Leci SF Supreme. The amount of lecithin was not an independent variable in the test so the content or type of lecithin was not optimized in the test.

In order to compare the effect of different types of lecithin in the formulation, the LecithinSF powder lecithin was replaced or combined in several formulations with liquid lecithins. The amount of LecithinSF in the formulation was replaced by an equivalent amount of liquid lecithin. The weight of carrier oil in the formulations was reduced in a weight identical to the oil contribution of each liquid lecithin. The formulations were prepared with 1.95 g of nonionic surfactant (SureNano_High_HLB 1 L).

Replacement of the Lecithin SF powder lecithin with both the sunflower and liquid lecithinRS was detrimental for the quality of the emulsion, decreasing the Z potential and increasing the particles size (Table 2). The particle size is increased from 32 to around 40 nm and the Z potential is decreased from 42mV. If rapeseed lecithin partially replaced the powder lecithin the quality of the emulsion did not change and remained similar to the formula 4 characteristics. When the Leci Supreme was replaced with LecithinSY liquid lecithin, the particles size of the formulation decreased slightly, but the Z potential increased markedly. The liquid LecithinSF lecithin is the most beneficial liquid lecithin. The Z potential increased to 49mV in the formulation with liquid LecithinSY lecithin. When the replacement of the powder lecithin with the lecithinSY was only partial, the benefits of the liquid lecithin are less evident. In fact the replacement of only 50% of the powder lecithin maintained the same particle size and Z potential of the emulsion with only LecithinSF. When the percentage of lecithinSY in the emulsion was reduced to 40% and 23% the particle size remained similar to that of the Powder lecithin but the Z potential was continuously decreased. In summary, the lecithins that best contribute to improving the quality of the emulsions are either the powder lecithin lecithinSF or the liquid lecithinSY. The three best formulations tested with lecithins are those using the lecithinSF and the lecithinSY alone or in a combination of 1 to 1 ratio.

Two formulations with very high content of surfactant were selected from the RS optimization test, the formulations #47 and #13. Emulsions prepared with these formulations resulted in very small particle size and high Z potential (Table 3). High amounts of emulsifier of about 2.4g resulted in particle size of 28nm and Z potential of 45mV. One formulation was also prepared with low content of the surfactant to have an alternative emulsion of slightly larger particle size. The formulation with 1.2g of surfactant resulted in an emulsion with a particle size of 79nm (Table4). Even though the formulation has low surfactant content, the Z potential of the emulsion was high

Table 2. Formulations of High Content of Emulsifier with Several Combinations of Lecithins

ID	LecithinSF	Liquid Lecithin SF	LecithinSY	LecithinRS	Oil	SureNano_Low_HLB	Emulsifier SureNano_High_HLB L	Size (nm)	Z Potential (mV)
Blend # 1	0.2			0.6	0.85	0.15	1.80		-33.3
formula #4	0.7	--	--	--	1.08	0.15	1.95	32.12 ± 0.39	41.9 ± 2.48
April 23 #1	--	1.0	--	--	0.68	0.15	1.95	41.42	-39.6
May 11 #1			0.92		0.72	0.15	1.95	35.61	-49.2
July 6 #1				0.90	0.74	0.15	1.95	40.20	-32.5
May 27 #1	0.35			0.36	1.04	0.15	1.95	32.9	-38.2
May 11 #2	0.35		0.38		0.94	0.15	1.95	33.51	-42.0
May 28 #1	0.40		0.28		1.05	0.15	1.95	33.13	-38.1
Jun 1st #1	0.50		0.15		1.08	0.12	1.95	33.05	-36.3

May 11 #1 is the formula #4 from optimization RS experiment, with liquid lecithin and balancing the oil weight.(lecithin contribution)
May 11 #2, May 28 #1, and June 1st #1 are similar to formula #4 but 50%, 40%, and 23% of the Leci SF Supreme weights are replaced by Leci Soy liquid lecithin, respectively.

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Table 3. Formulations with Very High Content of Emulsifier

ID	LecithinSF	LecithinSY	LecithinRS	Oil	SureNano_Low_HLB	Emulsifier SureNano_High_HLB L	Size (nm)	Z Potential (mV)
Apr 20 #1 (formula47)	0.6	--	--	1.06	0.09	2.36	28.65	-47.6
Apr 20 #2 (formula13)	0.6	--	--	1.08	0.03	2.44	27.77	-43.4

Table 4. Formulation with Low Content of Emulsifier for Larger Particle Size

ID	LecithinSF	LecithinSY	LecithinRS	Oil	SureNano_Low_HLB	Emulsifier SureNano_High_HLB L	Size (nm)	Z Potential (mV)
Apr 13 #1	0.7	--	--	1.63	0.47	1.18	79.12 ± 0.148	37.9 ± 1.94

possibly due to the contribution of the lecithin. It should be also pointed that this emulsion had a larger amount of oil which could have increased the particle size. A reduction of the amount of oil would benefit the quality of the emulsion, and reduce the particle size.

A larger amount of lecithins are tested with 1.4 and 1.8grams of surfactant (Table 5). The combination of LecithinSF with lecithinRS did not significantly improve the quality of the emulsion in the proportion of 0.65 to 0.31, respectively. However, the emulsion with 1.8g of surfactant seems to be better than the blend #1. The use of these two lecithins did not increase the Z potential and thus the two formulations confirmed the lecithinSF and lecithinRS would not improve the stability of the emulsions.

In summary, the use of lecithins as cosurfactants affected both the particle size and the Z potential of the emulsions. However, the most influencing factor on the particle size is the amount of surfactant. The amount of surfactant directly affects the particle size which can be decreased or increased by modification of the surfactant incorporated in the emulsion. The LecithinSF powder lecithin was the superior in reducing the particle size, followed by the LecithinSY liquid lecithin. These two lecithins are also the most effectives in increasing the Z potential of the emulsions. They are the best lecithins to improve the quality of the emulsions. The most significant improvement of the quality of the emulsions is achieved using the LecithinSF or the LecithinSY alone and their combination in a one to one proportion.

Table 5. Formulation with Higher Powder Leci SF Supreme Lecithin Content

ID	LecithinSF	LecithinSY	LecithinRS	Oil	SureNano _Low_HLB	Emulsifier SureNano_High_HLB L	Size (nm)	Z Potential (mV)
Blend # 1	0.2		0.6	0.85	0.15	1.80		-33.3
Mar 27 # 1	0.65		0.31	0.80	0.20	1.40	50.7	-34
Mar 27 # 2	0.65		0.35	0.80	0.22	1.80	37.0	-30