



Research Report # 07

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Introduction

The polar structure of the lecithin molecules renders them useful emulsifying agents.

Lipophilic side chains of esterified fatty acids are bound to the polar head group. The phosphates and nitrogen-containing moieties can be ionised, thereby representing negatively and positively charged residues, respectively. This allows for aqueous interactions, which form a mechanical barrier. Improved stabilization of nanodroplets is in most cases achieved by means of electrochemical or steric stabilization. Electrostatic repulsion can be achieved by introducing a high surface charge on droplets, which will consequently repel each other according to the DLVO-theory (DLVO stands for Derjaguin, Landau, Verwey and Overbeek). DLVO theory is a theory of colloidal dispersion stability in which zeta potential is used to explain that as two particles approach one another their ionic atmospheres begin to overlap and a repulsion force is developed. In this theory, two forces are considered to impact on colloidal stability: Van der Waals forces and electrical double layer forces. Lecithin moieties can be ionised improving stabilization of nanoparticles.

Since the lecithin molecule possesses a phosphate and a trimethylammonium group separated by two methylene groups, its structure allows two ionic forms. This high surface charge on droplets can result in electrostatic repulsion and improved stabilization of nanoemulsions. To prepare nanoemulsions, lecithin mixtures can be employed, which contain other phospholipids able to acquire an electrical charge. These include phosphatidic acid, phosphatidylserine, phosphatidyl-inositol and small amounts of free fatty acids. Emulsions stabilized with mixtures of phospholipids exhibit a significant increase in resistance against flocculation since an electrostatic repulsion between the droplets favors long-term stability. The droplet surface charge of such nanoemulsions is usually in the negative range due to the presence of negatively charged phospholipids, especially the free fatty acids and phosphatidic acid. The goal of this test is to evaluate the effect of the pH of the emulsion media on the preparation, quality and stability of nanoemulsions.

Methods

Three emulsions are prepared with the Surenano emulsifier in a ratio of emulsifier blend to active ingredient of 5.56:1. The emulsion formulations are 1.68g of the active ingredient, 9.34g of the Surenano emulsifier mix, 50 mL of antimicrobial solution, and 79.0g of pH 7 phosphate buffer solution (0.01M). The antimicrobial solution is prepared with sodium benzoate, ascorbic acid, and potassium sorbate in water. The final concentrations of the antimicrobials in the emulsion are 0.1% sodium benzoate, 0.125% ascorbic acid, and 0.15% potassium sorbate. Phosphate buffer 0.01M solution is prepared by mixing 291 milligrams of sodium phosphate



monobasic monohydrate and 774 milligrams of sodium hydrogen phosphate heptahydrate, dissolving in 400mL of water, adjusting the pH to 7, and making to 500mL volume with water.

Each emulsion (140mL) was divided in 7 equal volumes of approximately 20mL, which were transferred to glass test tubes for storage and further evaluations. The pH of the emulsions are adjusted to pHs 2, 4, 6, 8, and 10 with 1M sodium hydroxide solution or 1M hydrochloric acid solution. Emulsions were prepared in triplicates and three test tubes of each pH emulsion are stored at room temperature. Particle size, Z potential, particle size distribution, conductivity and pH of emulsions are measured after 5 days of storage. Samples of the initial emulsions at pH 5 are also evaluated.

Measurement of emulsion stability

Particle size determination of droplets is very useful in the evaluations of stability of oil in water emulsions. The particle size distribution, mean particle radius and the Z potential of diluted emulsions are measured by a commercial dynamic light-scattering device (Nano-ZS, Malvern Instruments). Samples are diluted (1:20) with distilled water prior to analysis to avoid multiple scattering effects to reach the instrument attenuation factor. The measurement of the Zeta potential has been introduced for the characterisation of the nanoemulsions. The Zeta potential is currently determined by the measurement of electrophoretic mobility in Malvern's Zetasizer Nano instrument (Malvern Instruments). A Z potential value in the magnitude of ± 30 mV can be taken as the arbitrary value that separates low-charged surfaces from highly charged surfaces. Hence, the Z potential values in the magnitude of ± 30 mV indicate the formation of a stable nanoparticle system.

Results

The highest correlation coefficients of the independent and dependent variables were between the particle size and the translucency, Tyndall effect, and conductivity (Table 1). Also the pH had high correlation coefficients with the particle size, conductivity, translucency and Tyndall effect.

Besides the high correlation of the conductivity with the pH and the particle size, conductivity was also correlated with the translucency and Tyndall effect.



Table 1. Correlation Coefficients of Variables in the pH Trial

	<i>pH</i>	<i>Particle Size (nm)</i>	<i>Z Potential (mV)</i>	<i>PDI</i>	<i>Conduct L</i>	<i>Translucency</i>	<i>Tyndall</i>
pH	1.00						
Particle Size (nm)	-0.82	1.00					
Z Potential (mV)	-0.56	0.0355	1.00				
PDI	-0.3960	0.6551	-0.0828	1.00			
Conduct L	-0.7259	0.9747	-0.0996	0.6123	1.00		
Translucency	0.7812	-0.9865	0.0134	-0.6481	-0.9837	1.00	
Tyndall	0.7900	-0.9879	-0.0064	-0.6644	-0.9811	0.9992	1.00

The pH was negatively correlated to the particle size, mainly because of a high increase of the particle size of about 15nm when the pH was reduced to 2 (Table 2). However, the particle size was not modified in all the range of pH from 4 to 10. Thus, the reduction of the pH to 4 or the increase of it to 10 from the initial pH 5 did not change the particle size of the emulsion. The emulsions prepared with Surenano can be used in a broad range of pH without modifying the characteristic particle size. Because of the very high correlation of the size with the translucency and Tyndall effect, the quality of the emulsion has not been modified by the pH.

The Z potential was fairly stable to the changes of pH. The potential of some lecithins in the pH range 2-8 remains unchanged, which indicates that these monolayers are effectively uncharged (nonionic) and suggests that the phosphate and trimethylammonium groups neutralize each other. Similarly other lecithins showed the properties of uncharged monolayers in a smaller pH range, 4-8, also. In this study, only emulsions at pH 4 and pH 10 had different Z potential from the initial. Most of the Z potentials were higher than 30, which indicates high stability of the emulsions. However, in the low pH end the Z potential was slightly reduced to -26.3 when the pH was reduced from 5 to 4. The increase of the pH of the emulsions to 10 increased considerably the Z potential to -45.1. It is possible the ionic solution added to increase the pH could have an effect on the stability of the emulsion by increasing the charges around the particles and the Z potential as the latter is influenced by the electrolyte concentration or ionic strength of the solution.



Table 2. Effects of pH of the O/W Emulsion on the Particle Size, Z Potential, and Conductivity

pH	Size		Z Potential		Conductivity	
	Value	Significance	Value	Significance	Value	Significance
2 (2.04)	56.86	a	-34.7	b	0.4873	a
4 (3.93)	41.61	b	-26.3	c	0.1987	b c
initial 5.06	43.16	b	-34.8	b	0.1437	d
6 (5.78)	40.33	b	-31.8	b c	0.1663	c d
8 (6.93)	40.13	b	-37.6	b	0.2073	b c
10 (7.47)	40.73	b	-45.1	a	0.2297	b

The conductivity is also affected by the electrolyte concentration, therefore the ionic solutions added to modify the pH from 5 to 2 and to 10 may have been responsible for the increase of conductivity. The lowest conductivity was recorded at the initial pH of 5, and it increased with every addition of ionic solutions while the pH was reduced to 2 and when the pH was increased to 10 (Table 2).

The particle size of nanoemulsions prepared with the Surenano emulsifier blend did not change in a wide range of pH from 4 to 10. Most of the Z potentials of emulsions were higher than 30 for several pH, which indicates high stability of the emulsions. In summary, Surenano emulsions can be used in applications with varied pH with very small particle size and high stability.