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Introduction

Effects on the emulsion stability can be obtained by selected protein-lecithin combinations. Lecithins give a range of food grade emulsifiers with different hydrophilic-lipophilic-balance (HLB). 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. It has been demonstrated in previous trials that the lecithins that best contribute to improving the quality of the emulsions are either the powder lecithin Leci Supreme or the liquid lecithin Leci soy. These two lecithins alone or combined contributed to more stable nanoemulsions which hold larger Z potentials. A test of two new formulations with the mentioned lecithins is carried to confirm the concept.

Two experiments are set to evaluate the quality and stability of emulsions prepared with the emulsifier blend prepared by Caldic. Stability of emulsions prepared with the Surenano blend are evaluated at two storage temperatures, $5C$ and 25C during a short term storage time. Besides, the stabilities of Surenano emulsions are tested in a long term storage time of one year.

Experiments are set to evaluate the quality and stability of the emulsions prepared with the Surenano emulsifier. Emulsions prepared at the same pH are held isothermally at ambient and refrigeration temperatures. The Z potential, mean particle diameter and 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 for the storage time at the tested temperatures.

Methods

In the short term trial of physical and chemical stability of nanoemulsions, the stability of emulsions are tested at two storage temperatures. 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 0.84g of the active ingredient, 4.67g of the Surenano emulsifier mix, 25 mL of antimicrobial solution, and 39.49g of water. 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.

Each emulsion (70mL) was divided in 7 equal volumes of approximately 10mL which were transferred to glass test tubes for storage and further evaluations. Three test tubes of each emulsionare stored either at room temperature or in a refrigerator at approximately 5°C. Particle size and ZPotential are measured after several short term storage times, 0 (initial), 15days, 30 days, and 45 days.

In a second experiment of stability of nano emulsions, the stability of emulsions during long periods of storage time are tested at one refrigeration temperature. Three emulsions are prepared with the Surenano emulsifier in the same ratio of emulsifier blend to active ingredient as the ratio ofthe first trial. The formulations are identical to the formulations of the first test, but the quantities ofthe ingredients were increased to produce a total of 105g of each emulsion.The three emulsions werefractionated in six equal amounts which were transferred into glass test tubes. Tubes were stored in the dark in a refrigerator at 5 °C. The quality and stability of nano emulsions during long term storage, were evaluated after 1, 2, 4, 8, and 12 months.

Measurement of emulsion stability

Size determination of droplets is very useful in evaluations of stability. The particle size distribution and mean particle radius 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 samples are usually prepared by diluting the nano-emulsion, followed by filtration through 0.22μm filters prior to analysis. 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 Malvem's Zetasizer Nano instrument (Malvern Instruments).

Influence of environmental stresses on emulsion stability

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.

Centrifugation. Prepared nanoemulsions are subjected to stress conditions such as centrifugation. Set a fixed volume (8-10mL) of the prepared nanoemulsion into l 5mL centrifugetubes and centrifuge for 6 min at 5000rpm. If prepared nanoemulsions survive /are stable over thisstress 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.

Results

Lecithins with nonionic surfactants have been tested in the preparation of O/W nanoemulsions.

The use of lecithins as cosurfactants either alone or in combinations has been tested to prepare nanoemulsions with the Surenano emulsifier. The most significant improvement of the quality of the nanoemulsions with Surenano blend is achieved using the Leci Supreme or the Leci soy alone and their combination in a one to one proportion. Two new formulations with these lecithins and the same amount of the Surenano emulsifier are prepared (Table 1).

Table 1. Formulations of Emulsifier with Combinations of Lecithins

Ingredient	Formulation May8 #1	Formulation May8 #2
Active Ingredient	0.84	0.84
SureNano_Low_HLB	0.15	0.15
Oil	0.94	0.94
LecithinSF	0.65	0.35
LecithinSY	0.36	0.36
SureNano_High_HLB L	1.80	1.80
Preservative	25.0	25.0
Water	40.2	40.5

Both formulations yield very clear and translucent emulsions with laser light going strong through the emulsions in the beakers. The formulation May 8 #1 which contains more content of lecithins had a slightly darker color, but it was still clear. Both emulsions were filtered through PSE 0.22 μm syringe filters very easily, being only one filter required to finish the filtration. The quality of the emulsions was very good with particle size of 37-38nm and Z potential of -30 and 34mV.

Table 2. Particle size and Z potential of Formulations with Combinations of Lecithins

Ingredient	Size (nm)	Z Potential (mV)
Formulation May 8 #1	37.38	-34.0
Formulation May 8 #2	38.76	-29.6

In summary, the use of lecithins as cosurfactants affected both the particle size and the Z potential of the emulsions. The combination of Leci Supreme powder lecithin with Leci Soy liquid lecithin resulted in emulsions with very small particle size in the range of 30 run and good stability and Z potential around -30mV. There was not a difference of quality between the two formulations,so it may be possible to reduce the amount of lecithin as in the formulation #2 without increasing the particle size or decreasing the stability of the emulsion.