Research Report # 06 July 2021

Introduction

Effects on the emulsion stability can be obtained by selected protein-lecithin combinations. The influence of the components of the emulsion and the preparation technique in the homogenization process can be measured by the particle size distribution technique (PSD) andemulsifying tests, which assess the emulsion stability.

In order to evaluate the quality and stability of emulsions prepared with the Surenano emulsifier blend, emulsions were prepared and stored for a relatively short period of time. Thestability of emulsions are evaluated at two storage temperatures, 5C and 25C during 45 day ofstorage time.

The Z potential, mean particle diameter, particle distribution and the conductivity of the emulsion are evaluated to establish the stability of the emulsions. If there is no significant change inthe Z potential, no increase of the droplet size, and no loss of translucency the emulsion is considered stable.

Methods

The stability of emulsions in an experiment of storage for short term length are tested at two 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 5ºC. Particle size and Z potential are measured after several short term storage times, 0 (initial), 15days, 30 days, and 45 days.

Measurement of emulsion stability

Size determination of droplets is very useful in the evaluations of stability of oil in water emulsions. The particle size distribution and mean particle radius of diluted emulsions are measuredby 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 theinstrument attenuation factor. The samples are usually prepared by diluting

SureNano Science Ltd.
Suite 350 – 1650 West 2nd Avenue, Vancouver, BC, V6J 1H4
TEL: (604) 428-5171

the nano-emulsion, followed by filtration through 0.22 µm filters prior to analysis.

Z potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. The measurement of the Zeta potential has been introduced for the characterisation of the nanoemulsions. The Zeta potential is currently determinedby 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.

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 15mL centrifuge tubes and centrifuged at 4500rpm for 10minutes. If prepared nanoemulsions survive /are stable overthis stress condition, they are considered as thermodynamically stable.

Conductivity. Conductivity measurements are currently carried out to determine the makeup ofthe continuous phase, provided O/W emulsions are conductive, whereas W/O emulsions are nonconductive.

Results

The study of the correlation coefficients of the independent and dependent variables showedthat the storage time is negatively poorly correlated with the particle size and the particle distribution (Table 1). However, there was no change in the particle size with the storage time, andthe emulsion kept stable during the whole storage period (Table 2).

The temperature was positively correlated with the conductivity of the emulsion. The translucency and the Tyndall effect were positively highly correlated. These two variables aresubjectively evaluated.

Table 1. Correlation Coefficients of Variables in the Short Term Trial

	Temper	*Storage Time*	*Particle Size*	*Z Potential*	*PDI*	*Conduct*	*Transluc*	*Tyndall*
Temperature	1							
Storage Time	0	1						
Particle Size	0.03441	-0.5269	1					
Z Potential	-0.06507	0.15443	-0.28944	1				
PDI	0.09242	-0.5995	0.85388	-0.32070	1			
Conductivity	0.67804	0.16677	-0.22258	-0.11700	-0.0714	1		
Translucency	-0.46384	-0.2667	0.36809	-0.20048	0.41812	-0.59943	1	
Tyndall	-0.53861	-0.2148	0.29751	-0.14709	0.29242	-0.58284	0.89563	1

Neither the temperature of storage nor the storage time affected the particle size of the emulsion. The emulsion prepared with the Surenano emulsifier and formulation was stable for 45days maintaining the particle size approximately constant at 44 nm, which is smaller than the size range 50–200 nm of translucent nano-emulsions. The facts of no increase of the droplet size and no loss oftranslucency with time indicate the emulsion was adequately prepared with Surenano emulsifiers blend and stabilised against Ostwald ripening. In a similar way, the Z potential remained unaffected during most of the storage period.

Table 2. Effects of Storage Time and Temperature on the Particle Size (nm) of O/W Emulsions

Size (nm)	**Storage Time (days)**				
Temperature (C)	**0**	**15**	**30**	**45**	**average**
5	53.49	44.07	44.67	44.38	46.65 a
20	53.49	45.53	44.87	44.32	47.05 a
average	53.48 a	44.80 a	44.770 a	44.35 a	

means with the same letter do not differ significantly at $p < 0.05$

The Z potential is influenced by many factors such as surfactants, electrolyte concentration (ionic strength), particle morphology, size, pH of the solution, and state of hydration. The Z potential measurements are useful in evaluating surface charge and stability of nanoemulsions. Z potential of emulsions at the initial time, 15 days and 45days were similar and around 32mV. The stability of theemulsions did not vary along the storage of emulsion at both temperatures for 45days. The information given by the Z potential measurements states that nanoemulsions prepared with the Surenano emulsifiers blend and formulation have highly charged surface droplets that are stable andwill resist droplet aggregation.

Table 3. Effects of Storage Time and Temperature on the Z Potential of O/W Emulsions

Z potential (mV)	**Storage Time (days)**				
Temperature (C)	**0**	**15**	**30**	**45**	**average**
5	-32.77	-29.53	-24.07	-32.27	-29.66 a
20	-32.77	-31.93	-22.27	-34.07	-30.26 a
average	-32.77 a	-30.73 a	-23.17 b	-33.17 a	

means with the same letter do not differ significantly at p <0.05

Table 4. Effects of Storage Time and Temperature on the Conductivity of O/W Emulsions

Conductivity	**Storage Time (days)**				
Temperature (C)	**0**	**15**	**30**	**45**	**average**
5	0.1201	0.1203	0.1177	0.1177	0.1189 a
20	0.1201	0.1287	0.1237	0.1300	0.1256 b
average	0.1201 a	0.1245 a	0.1207 a	0.1238 a	

means with the same letter do not differ significantly at p <0.05

Table 5. Effects of Storage Time and Temperature on the Translucency of O/W Emulsions

Conductivity	Storage Time (days)			
Temperature (C)	0	15	30	45
5	4.5 a x	4.00 b y	4.5 a x	4.5 a x
20	4.50 a x	4.13 b x	3.93 c y	4.00 b c y

a,b,c compare means within the same temperature; x,y,z compare means within the same storage time at p<0.05

In order to test the thermodynamic stability of the emulsions when they were exposed to stress,the emulsions were centrifuged at 4000rpm for 10 min and the particle size, conductivity and Z potential were measured after centrifugation. There was no effect of the temperature of storage in the three variables measured before and after centrifugation (Table6). Furthermore, the size and conductivity of the emulsions did not change by effect of the centrifugation. The particle sizes of theemulsions were around 45-47nm. The only variable that was significantly different before and after centrifugation was the Z potential. However, the variable was increased by the centrifugation insteadof being reduced, so the centrifugation made the emulsions to be more stable. The Z potentials of emulsions ranged from 30 to 35mV, which are relatively high values of the Z potential indicating a good protection to the stability of the emulsions.

Table 6. Effects of Centrifugation on the Stability of O/W Emulsions

Centrifuge	Particle Size (nm)			Z Potential (mV)			Conductivity		
	5	20	avg	5	20	avg	5	20	avg
no	46.65	47.05	46.85 a	-29.66	-30.26	29.96 a	0.119	0.126	0.122 a
yes	44.71	44.89	44.8 a	-33.1	-34.975	34.04 b	0.120	0.124	0.122 a
average	45.68 a	45.97 a		31.38 a	32.62 a		0.120 a	0.125 b	

means with the same letter do not differ significantly at p <0.05

In summary, the use of the Surenano emulsifier blend allowed the preparation of nanoemulsion with a particle size in the lowest end of the nanoemulsions range. The ingredients of the emulsion and the preparation protocol resulted in emulsions with high Z potential values (-32mV) to indicate a good protection of the emulsions. The quality and stability of the emulsions did not vary along the storage of emulsion at both temperatures for 45days. The nanoemulsions prepared with the Surenano emulsifiers blend have highly charged surface droplets that make them thermodynamically stable.

SureNano Science Ltd.
Suite 350 – 1650 West 2nd Avenue, Vancouver, BC, V6J 1H4
TEL: (604) 428-5171