



## DESIGN OF AN INTERSTITIAL STRUCTURE FOR A GRAPE SEED OIL EMULSION BY DESIGN OF EXPERIMENTS AND SURFACE RESPONSE

## DISEÑO DE UNA ESTRUCTURA INTERSTITIAL PARA UNA EMULSIÓN DE ACEITE DE SEMILLA DE UVA POR DISEÑO DE EXPERIMENTOS Y SUPERFICIE DE RESPUESTA

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### Abstract

An interstitial structure was designed to prepare a mini (nano) - emulsion of grape seed oil; the interface was composed of surface active molecules (biopolymers and surfactants) to produce the mini (nano) - emulsion by interfacial deposition of oil by displacement of acetone from the dispersed phase. Design of Experiments of mixes and Respond Surface was used to determine the best formulations for the system: F1, Surfactants: Tween 20, T, (0.68%), Dimodan, D, (0.317%) and Panodan, P, (0.0013%) and F2, Polymers system: Gum Arabic, GA, (0.029%), Maltodextrin, MD, (0.115%) and whey protein concentrate, WPC, (0.187%). The proposed formulation was prepared considering the lowest value of surface tension for surfactants and the highest value for the polymers system. A leptokurtic size distribution was obtained for the interstitial structure, prepared with a stirring rate of 10000 rpm and resulting in an average diameter of 0.185  $\mu\text{m}$  and a Z -potential of -18.23 mV. The emulsion was prepared using this structure and resulting average size and Z-potential values were 0.188  $\mu\text{m}$  and -18.55 mV respectively. These results were not significantly different from those of the interstitial structure and therefore, it was concluded that the final composition of the emulsion and preparation procedure were adequate.

**Keywords:** design of experiments, response surface, interstitial structure, grape seed oil, mini (nano)-emulsion.

### Resumen

Se diseñó una estructura intersticial para preparar una emulsión mini (nano) de aceite de semilla de uva por deposición interfacial de aceite por desplazamiento de disolvente de la fase dispersa. Las composición más adecuada de los surfactantes e hidrocoloides se determinó utilizando diseño de experimentos y superficie de respuesta: F1, surfactantes: Tween 20 (0.68%), Dimodan (0.317%) y Panodan (0.0013%) y F2, Polímeros: Goma Arábica, GA, (0.029%), Maltodextrina, MD, (0.115%) y concentrado de proteína de suero, WPC, (0.187%). La formulación propuesta se preparó considerando el menor valor de tensión superficial para los surfactantes y el mayor para la mezcla de polímeros. Se obtuvo una distribución leptocúrtica para la estructura intersticial preparada a 10000 rpm y su tamaño medio fue de 0.185  $\mu\text{m}$  y el potencial Z de -18.23 mV. La emulsión así preparada tuvo valores de tamaño medio y potencial Z de 0.188  $\mu\text{m}$  y -18.55 mV respectivamente, los que no resultaron significativamente diferentes de aquellos para la estructura intersticial confirmando que el procedimiento seguido para la formulación de la emulsión fue adecuado.

**Palabras clave:** diseño de experimentos, superficie de respuesta, estructura intersticial, aceite de semilla de uva, mini(nano)-emulsión.

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## 1 Introduction

The field of food nanotechnology has experienced significant growth over the last years and numerous food-related products have been developed taking advantage of the novel properties of the nano-sized ingredients and composites (Acosta, 2009). Emulsification is one of the fields that have been greatly influenced by nano-food related developments and modern emulsification systems and their potential application in encapsulation of food ingredients and the behavior of emulsion components in different media along with the knowledge of factors affecting the emulsions properties during emulsification is essential for the preparation of stable, high-quality emulsions. Emulsification of bioactive materials such as grape seed oil into a suitable matrix helps to protect antioxidants and other biologically active compounds contained in the oil from environmental conditions (Joshi *et al.*, 2001, Yilmaz and Toledo, 2006, Quintanilla-Carvajal *et al.*, 2011).

The understanding of the mechanisms of emulsification and the behavior of components of the emulsion as well as the knowledge of the factors affecting its properties during processing is essential for optimizing processes aiming to obtaining a stable product (Jafari *et al.*, 2007; Quintanilla-Carvajal *et al.*, 2011).

Recent advances on the knowledge of the role of interfaces on the formation and stability of micro and nano emulsions have been based on droplet size of resulting emulsions and their stability (McClements, 2005; Quintanilla-Carvajal *et al.*, 2011). The time of stability strongly depends on the characteristics of the interface separating the dispersed and continuous media and of the presence in this interface of surface-active molecules, which can be attached to the dispersed or continuous phases (McClements, 2005).

There are two classes of surface-active molecules:

1. Surfactants - detergents, emulsifiers and lipids. They may be water or oil soluble, and usually form a compact adsorbed layer with a low interfacial tension thus allowing them to migrate to regions with a reduced surfactant concentration due to perturbation during preparation. This phenomenon is known as the Marangoni mechanism (Wilde, 2000).
2. Polymers - amphiphilic macromolecules, e.g. proteins. The emulsifier stabilizes the interfacial layer between the dispersed and continuous phase which has been created through the addition of energy to the system (Flanagan and Singh, 2006) in such a way so as inducing polymers to form a visco-elastic, adsorbed

layer. This is most commonly observed in proteins, which adsorb, partially unfold and establish strong interactions which strength has been correlated with foam and emulsion stability (Wilde, 2000; McNamee *et al.*, 2001). Another factor to consider in the preparation of emulsions is the ability of a surfactant or a polymer to promptly adsorb due to competences between polymers and surfactants for attaching to the interface. The surfactants weaken the visco-elasticity of the adsorbed protein layer and the polymers retard the fluidity of the surfactants. This is known as the Orogenic mechanism (Wilde, 2000; McNamee *et al.*, 2001) which should be avoided within the context of preparation of a stable interface structure.

The arrangement of the emulsifier molecules occurs spontaneously although, often, micro and mini-(nano) emulsions might also require the presence of a co-surfactant which will attach onto the interface and maintain the low interfacial tension (Flanagan and Singh, 2006). The co-surfactant has the effect of further reducing the interfacial tension, whilst increasing the fluidity of the interface and increasing the entropy of the system (Gaonkar and Bagwe, 2003). It has been reported that the sequence of addition of ingredients played a key role in the formation of mini (nano) - emulsions or micro emulsions using ethoxylated mono- and di-glycerides as surfactants and co-surfactants (Flanagan and Singh, 2006; Flanagan *et al.*, 2006) so that the preparation of the final emulsion can be carried out by the amphiphilic solvent method (solvent displacement) in which, the elimination of the organic solvent (contained in the oil phase causes the separation of the polymers from the oil phase and a reduction of the particle size of the newly formed complex (Moinard-Chécot *et al.*, 2007). Elimination of the organic solvent is promoted by the diffusion of water into the interstitial phase (Acosta, 2009).

Some difficulties in the use of mini (nano)-emulsions and micro-emulsions as food delivery systems arise from the type of surfactant and poor solubilization of high molecular weight compounds such as triglycerides. Type of surfactant and co-surfactant (if required) and their concentration for use in food applications restrict the potential development of food-grade mini (nano) - emulsions (Flanagan *et al.*, 2006). A limitation in the production of sub-micron emulsions or mini emulsions (100 - 500 nm) in food applications is limited by the availability of surfactants that may be used for preparing these emulsions (Windharb, 2005). It is then important to evaluate how create interstitial structures and mini (nano)

emulsions using mixes of surface-active molecules through design of experiments (DoE) and surface response (RS). The application of these methodologies has many advantages over traditional trial and error protocols to produce an adequate formulation and it is then desirable, to determine an optimal combination of materials that will provide adequate emulsifying capacity and act as effective and protective barriers of a number of active compounds. In this respect, Matsuno and Adachi (1993) proposed a method for selecting the most suitable wall materials for lipid encapsulation that considers the emulsifications of lipids in a solution of hydrocolloids and further assessment of the emulsifying activity by particle size distribution. A correct construction of an emulsion may be evidenced when values of Z- potential and size distribution do not change significantly between those of the interstitial structure and the final emulsion (Jafari *et al.*, 2008).

Polymers are effective in creating interstitial structures when forming a solid visco-elastic adsorbed layer. This is most commonly observed in proteins which adsorb, partially unfold and form strong interactions with active components of a given formulation (Petkov *et al.*, 2000) which implies chemical and structural similarity of globular protein composites such as whey protein concentrate with other hydrocolloids (WPC) (Tolstoguzov, 2003). Interfacial tension ( $\gamma$ ), may be considered the main dependant variable in the preparation of surfactants and biopolymers in emulsification processes since the surfactants lower the visco-elasticity of the adsorbed protein layer and the polymers retard the fluidity of the surfactants (Wilde, 2000) thus inducing low values of  $\gamma$  for surfactant-surfactant systems (Prins, 1999) and the high values of it for polymer-polymer solutions (Mackie *et al.*, 2000). Also the interfacial tension has been reported as one of the key parameters that influence the rate of phase separation (de Hoog and Lekkerkerker, 2001).

Producing emulsions in sub-micron area with a narrow distribution (Leptokurtic) of particle sizes continues being an important technological issue (Jafari *et al.*, 2008) and involves the creation of interstitial structures which may be carried out by means of DoE and RS.

The objective of this work was to design by means of DoE and RS methodologies a mini- (nano) emulsion of grape seed oil through the solvent displacement method and by creating an adequate interstitial structure with approximately the same size distribution and Z-potential of the emulsion using food

grade surfactants (emulsifiers) and biopolymers.

## 2 Materials and methods

### 2.1 Materials

#### 2.1.1 Emulsifying agents

Tween® 20 (T), from Sigma-Aldrich Co. Saint Louis, Missouri 63103, United States, was used as surfactant agent; Dimodan® PH 300-A (D), from Danisco Emulsifiers. Vallejo, Mexico, was used as stabilizing agent; Panodan® 205 K DATEM (P), from Danisco Emulsifiers. Vallejo, Mexico was used as co-surfactant agent.

#### 2.1.2 Hydrocolloids

Arabic gum (GA), from Alfred L. Wolff GmbH Mexico, DF; Maltodextrin DE 10 (MD) from Complementos Alimenticios, State of Mexico, Mexico; Whey Protein Concentrate (WPC), from HILMAR Ingredients, CA, United States.

#### 2.1.3 Core Material and organic solvent

Grape seed oil from Olivi Hermanos, S.A. de C.V. Mexico, DF; Acetone from J.T. Baker, State of Mexico, Mexico.

### 2.2 Methodology

The experimental sequence for preparing emulsions and evaluating their quality is presented in Fig. 1. Attention was focused on the creation of an interface (interstitial structure) composed of active surface molecules (biopolymers and surfactants) which was used as stabilizing system in the continuous phase to produce the emulsions by interfacial deposition of grape seed oil due to the displacement of acetone from the dispersed phase (amphiphilic solvent method) as reported by Ribeiro *et al.*, (2008).

The interstitial structure was created by applying two different Design of Experiments (DoE) of mixes using ternary phase diagrams (Mackie *et al.*, 2000; Wilde, 2000). DoE 1, Surfactant System (F1): a mixture of T (surfactant), D (stabilizing agent) and P (co-surfactant) (Flanagan and Singh, 2006). DoE 2, Hydrocolloids (F2): a mixture of GA, MD and WPC.

Response Surface (RS) was used to define in DoE 1 and 2, the values of each one of the dependant variables (density ( $\rho$ ); pH, refractive index (IR), and

surface tension ( $\gamma$ ) to finally determine the regression models for this last parameter which was used as the key variable in the design of the emulsion as discussed earlier (Grima *et al.*, 2004; Álvarez del Castillo *et al.*, 2010; Ríos-Morales *et al.*, 2011). These variables were determined in triplicate as follows: density ( $\rho$ ) was evaluated with a Paar digital densimeter model DMA 35 N (Anton Paar, Ashland, VA, United States) at 25°C; pH with a Conductronic pH120 (LABEQUIM, S.A, Puebla, Mexico) at 25°C; refractive index with a Thermo Spectronic, ABBE model 3L 334610 at 25°C (Wilde, 2000); surface tension with a Fisher model 21 surface tensiometer Du Noüy Tensiometer fitted with a Platinum-Iridium ring (Fisher Scientific Co., United States) at 25°C (Brückner *et al.*, 2007). The interfacial tension as mentioned above was selected as the key parameter

that influences the phase separation rate (aiming for the lowest value for surfactants, F1, and for the highest value for the polymers system, F2) (Prins, 1999; Mackie *et al.*, 2000). The final formulations for F1 and F2 were calculated by means of scanning RS (MINITAB 16.0) and finding the co-ordinates in the 3D diagrams corresponding to the lowest and the highest values of  $\gamma$ .

Interstitial structure (F1 + F2) was prepared at three different stirring rates (4000, 7600 and 10000 rpm); emulsification was performed by the solvent displacement method using equal amounts (2.041% of the total formulation of the emulsion) of grape seed oil and acetone (Ribeiro *et al.*, 2008) and stirring performed with an Ultra-Turrax T-50, (Kika-Werke, IKA Works, Inc., North Carolina, United States).

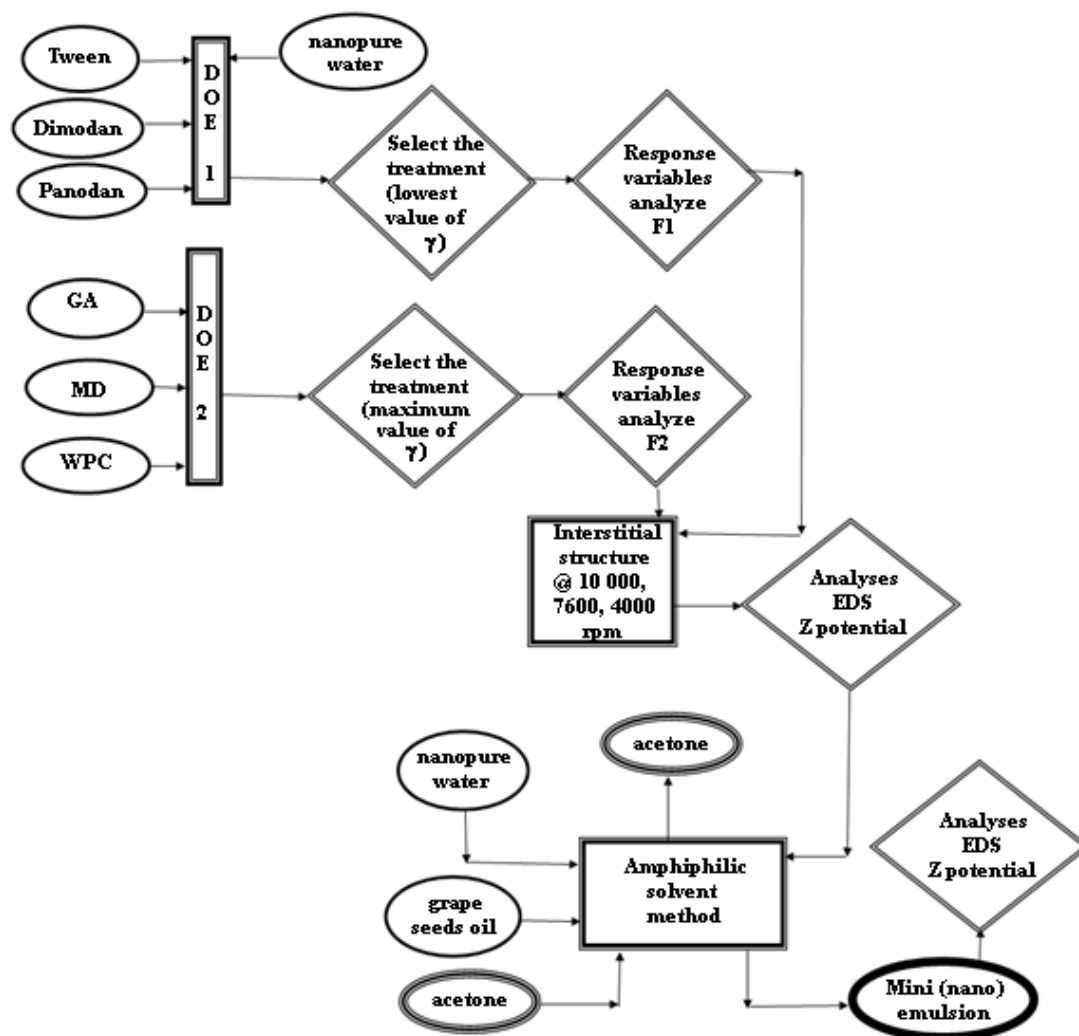


Fig. 1. Flow diagram of experimental sequence

Nanopure water (Diamond<sup>TM</sup>, Iowa, United States), was used to prepare all aqueous dispersions for F1, and F2.

### 2.2.1 Size distribution of interstitial structure and emulsion

The size distribution of the new interface and of the emulsion was determined by laser light scattering, using a Mastersizer Hydro 2000S laser diffractometer (Malvern Instruments, Worcestershire, United Kingdom) at  $\lambda = 633$  nm. All measurements were done on three freshly prepared samples and results reported as the mean value  $\pm$  standard deviation. Also, minimum and maximum diameters of resulting distributions were reported (Jafari *et al.*, 2008). Samples of the interstitial structure and resulting emulsion were stored at 4 °C and every day analyzed for determining their visual stability (Quintanilla-Carvajal *et al.*, 2011).

### 2.2.2 Z- potential of interstitial structure and emulsion

Z- potential of the interstitial structure and of the emulsion was measured by means of directly injecting diluted samples (1:2) to the measurement chamber of a ZetaPlus PW32 unit, Zeta Potential Analyzer (Zeta meter, Inc., New York, United States), according to Acedo-Carrillo *et al.*, (2006). The Z- potential values were reported as the average of 10 readings  $\pm$  standard deviation.

Size distribution and Z- potential for the interface at the three different stirring rates (10000, 7600 and

4000 rpm) were compared by means of a one way ANOVA (MINITAB V.16) to find out the stirring rate at which a monomodal size distribution with a low dispersion was obtained as well as the condition at which the value of Z- potential indicated the maximum possible stability of the interstitial structure.

After finding the most adequate stirring rate, a one way ANOVA (MINITAB V.16) was applied ( $\alpha = 0.05$ ) to compare the values of size distribution and Z- potential for both structures (interface and emulsion) and to determine if the addition of grape seed oil to the interstitial phase changed them considering that a correct construction of an emulsion is evidenced when values of Z- potential and size distribution do not change significantly between those of the interstitial structure and the final emulsion (Jafari *et al.*, 2008).

## 3 Results and discussion

### 3.1 DoE for F1: surfactants and F2: Hydrocolloids

Results of the dependant variables of DoE 1 are presented in Table 1. Values of density, pH, refractive index and surface tension are within those obtained for similar systems (Gunning *et al.*, 2004). As mentioned in Material and Methods Section, surface tension was chosen as the key parameter for obtaining the interstitial structure and for applying the RS to find, as described earlier, co-ordinates for minimum and maximum values of  $\gamma$ : lowest value for surfactants, F1, and the highest value for the polymers system, F2.

Table 1. Average values of dependant variables for F1 (surfactants)

Values of coordinates in ternary phase diagram (DoE 1)*	Average values of dependant variables			
	Density (g/cm <sup>3</sup> )	pH	Refractive index IR	Surface tension $\gamma$ (10 <sup>3</sup> N/m)
T <sub>1.00</sub>	0.9995 $\pm$ 0.003	4.79 $\pm$ 0.03	1.3341 $\pm$ 0.003	31.3 $\pm$ 0.1
P <sub>1.00</sub>	0.9993 $\pm$ 0.005	3.03 $\pm$ 0.02	1.335 $\pm$ 0.002	39.6 $\pm$ 0.2
D <sub>1.00</sub>	0.9984 $\pm$ 0.006	3.87 $\pm$ 0.00	1.333 $\pm$ 0.003	29.7 $\pm$ 0.1
T <sub>0.50</sub> P <sub>0.50</sub>	0.9993 $\pm$ 0.004	3.29 $\pm$ 0.03	1.3343 $\pm$ 0.003	28.6 $\pm$ 0.1
T <sub>0.50</sub> D <sub>0.50</sub>	0.9987 $\pm$ 0.006	5.25 $\pm$ 0.02	1.334 $\pm$ 0.001	27.8 $\pm$ 0.2
P <sub>0.50</sub> D <sub>0.50</sub>	0.9987 $\pm$ 0.006	3.19 $\pm$ 0.03	1.334 $\pm$ 0.003	33.9 $\pm$ 0.1
T <sub>0.33</sub> P <sub>0.33</sub> D <sub>0.33</sub>	0.9990 $\pm$ 0.005	3.32 $\pm$ 0.04	1.3345 $\pm$ 0.003	28.7 $\pm$ 0.2
T <sub>0.66</sub> P <sub>0.17</sub> D <sub>0.17</sub>	0.9991 $\pm$ 0.004	3.55 $\pm$ 0.02	1.335 $\pm$ 0.002	28.1 $\pm$ 0.2
T <sub>0.17</sub> P <sub>0.66</sub> D <sub>0.17</sub>	0.9990 $\pm$ 0.003	3.23 $\pm$ 0.02	1.334 $\pm$ 0.003	36.5 $\pm$ 0.1
T <sub>0.66</sub> P <sub>0.17</sub> D <sub>0.66</sub>	0.9986 $\pm$ 0.006	3.56 $\pm$ 0.03	1.3345 $\pm$ 0.002	33.5 $\pm$ 0.1

\*numeral subscripts are the mass fractions of each ingredient.



There are reports (de Hoog and Lekkerkerker, 2001; Schneider and Wolf, 2000) reporting that surfactants reduce the surface visco-elasticity of this layer. The competitive adsorption mechanism allows visualizing the structure of mixed protein-surfactant interfaces (Mackie *et al.*, 2000).

In Fig. 2, the contour 3D-plot (response surface) used to find out the lowest value of  $\gamma$  from the experimental design is shown. The co-ordinates corresponding to the lowest value of this variable on the response surface are:  $T_{0.68}P_{0.317}D_{0.0013}$ . It has been reported that low values of  $\gamma$  correspond to the formation of a compact adsorbed layer which would interact with the hydrocolloids to form a stable interstitial structure that would adequately support the core material (McNamee *et al.*, 2001).

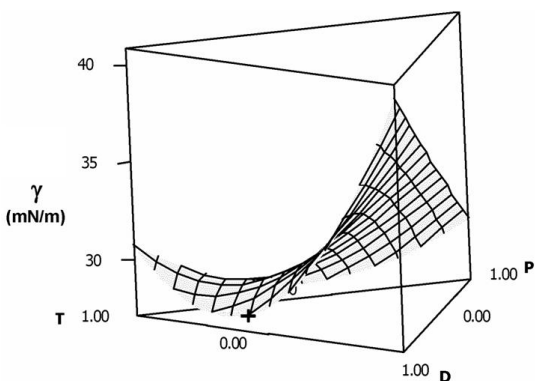


Fig. 2. Response Surface (Contour 3D-plot) for F1: Surfactants, showing the influence of ingredients on the dependant variable (Surface tension). The model for this RS is:  $\gamma = 30.83 * T + 40.12 * D + 30.37 * P$   $R^2 = 0.860$  Symbol (+) points out the lowest value of surface tension on RS corresponding to the following fractions of surfactants:  $T = 0.68, P = 0.317, D = 0.0013$ .

Results of the dependant variables of DoE 2 are presented in Table 2. As in DoE 1, values of density, pH, refractive index and surface tension fall within those obtained for similar systems (Gunning *et al.*, 2004). Also, as mentioned in Material and Methods Section, surface tension was chosen as the key parameter for obtaining the interstitial structure

and for applying the RS.

In Fig. 3, the contour 3D-plot (response surface) used to find out the highest value of  $\gamma$  from the experimental design is shown. The co-ordinates corresponding to the highest value of this variable on the response surface are:  $GA_{0.029}MD_{0.115}WPC_{0.187}$ . These conditions (high values of  $\gamma$ ) are related to the formation a visco-elastic adsorbed layer which has often been correlated with the stabilization of the interstitial structure (Wilde, 2000).

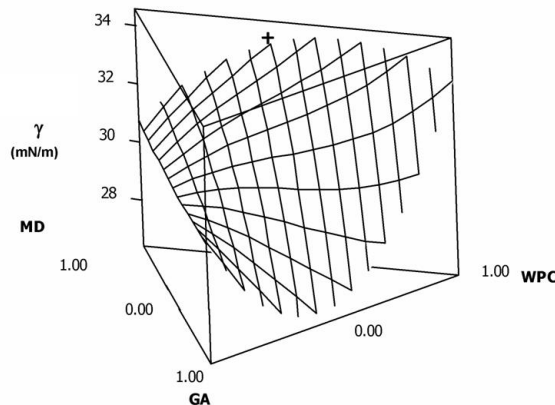


Fig. 3. Response Surface (Contour 3D-plot) for F2: Hydrocolloids, showing the influence of ingredients on the dependant variable (Surface tension). The model for this RS is:  $\gamma = 32.41 * A + 31.14 * B + 30.65 * C$ , Where: A = Mass fraction of WPC, B = Mass fraction of MD and C = Mass fraction of GA;  $R^2 = 0.818$ . Symbol (+) points out the highest value of surface tension on RS corresponding to the following fractions of hydrocolloids:  $GA = 0.029, MD = 0.115, WPC = 0.187$ .

Wilde, (2000), reported that it is possible to conceptualize interfaces formed by proteins and surfactants. The proteins formed a disordered assembly at the interface and small irregularities in the surface were thought to be responsible for surfactants to interact with the protein layer (Mackie *et al.*, 2000) which was confirmed in this work, trough results depicted in figs. 3 and 4. These results, along with those shown in Fig. 3, were the basis for applying RS for obtaining the most adequate formulation of the interstitial network.

Table 2. Average values of dependant variables for F2 (Hydrocolloids)

Values of coordinates in ternary phase diagram (DoE 2)	Average values of dependant variables			
	Density (g/cm <sup>3</sup> )	pH	Refractive index IR	Surface tension $\gamma$ (10 <sup>3</sup> N/m)
WPC <sub>100</sub>	1.0008 ± 0.005	6.52 ± 0.03	1.3350 ± 0.002	31.9 ± 0.1
MD <sub>100</sub>	1.0009 ± 0.003	5.39 ± 0.02	1.3340 ± 0.002	31.7 ± 0.3
GA <sub>100</sub>	1.0006 ± 0.005	3.77 ± 0.02	1.3345 ± 0.003	30.4 ± 0.1
WPC <sub>50</sub> MD <sub>50</sub>	1.0009 ± 0.006	5.30 ± 0.03	1.3345 ± 0.002	33.8 ± 0.1
WPC <sub>50</sub> GA <sub>50</sub>	1.0008 ± 0.006	3.61 ± 0.01	1.3350 ± 0.003	25.6 ± 0.2
MD <sub>50</sub> GA <sub>50</sub>	1.0007 ± 0.005	5.52 ± 0.02	1.3300 ± 0.002	31.3 ± 0.1
WPC <sub>33.33</sub> MD <sub>33.33</sub> GA <sub>33.33</sub>	1.0008 ± 0.005	4.95 ± 0.02	1.3345 ± 0.001	29.0 ± 0.1
WPC <sub>66.67</sub> MD <sub>16.67</sub> GA <sub>16.67</sub>	1.0008 ± 0.005	6.73 ± 0.01	1.3348 ± 0.003	34.5 ± 0.1
WPC <sub>16.67</sub> MD <sub>66.67</sub> GA <sub>16.67</sub>	1.0009 ± 0.005	6.18 ± 0.01	1.3347 ± 0.002	30.7 ± 0.2
WPC <sub>16.67</sub> MD <sub>16.67</sub> GA <sub>66.67</sub>	1.0009 ± 0.003	6.07 ± 0.03	1.3342 ± 0.003	30.9 ± 0.1

Table 3. Interstitial structure (F1 + F2) final formulation

Final Formula		
	Components	Components Total %
	Water	98.67
	<b>Surfactats</b>	
DoE 1	Tween 20	0.68
$\gamma = 27.88$ mN/m	Dimodan PH300A	0.317
(Minimum value)	Panodan 205K DATEM	0.0013
	<b>Hydrocolloids</b>	
DoE 2	GA	0.029
$\gamma = 32.93$ mN/m	MD	0.115
(Maximum value)	WPC	0.187
	<b>TOTAL</b>	<b>100</b>

### 3.2 Response Surface (RS)

In Table 3, the proportions of surfactants and hydrocolloids in the final formulation corresponding to the interstitial network are presented. Shown values are those obtained by means of RS methodology and considering the initial proposed proportions presented in DoE's 1 and 2. Stability of this interstitial formulation is also based on the compensation of chemical charges between the WPC/GA which formed complex structures whose stability may vary according to the existing proportion of protein to polysaccharide (Pr:Ps) and considering that MD helps to form robust network structures according to Klaypradit and Huang, (2008) who performed fish oil encapsulation with a structure formed using chitosan and MD with low DE. Also, GA and MD induce the formation of physical networks that are disrupted by

the presence of surfactants. This may be the case for WPC/GA/MD crosslinkage (Wilde, 2000).

The resulting overall amount of surfactants (1%) in the final formulation (DoE1) is in agreement with the proportion recommended by Ribeiro *et al.*, (2008) for the preparation of nanodispersions by the solvent displacement method.

The visco-elastic properties of the surface-active molecules have often been correlated with the functionality of the overall system; therefore, the creation of the interstitial structure and addition of the oil-acetone mixture should render a stable emulsion. Forgiarini *et al.*, (2001) found that the proper elaboration of the structure played a significant role in the formation of the interface using Tween 20 as the surfactant added in the highest amount as in the case of the present work (Table 3). According to Wang

*et al.*, (2009), the molecular structure of the emulsifier has a significant effect on the final droplet size of the emulsion; authors concluded that emulsification is also influenced by the structure of the surfactant and chain length and reported that, in the case of Tween 20 (with a saturated C12 chain), the droplet size decreased. The effect of concentration of the emulsifier has often been explained as the result of increased emulsifier adsorption around the oil-water interface of a droplet and decreased interfacial tension in the system giving place to smaller particles (McClements, 2005; Lamaallam *et al.*, 2005; Ambrosone *et al.*, 2007; Wang *et al.*, 2009).

### 3.3 Size distribution and Z- potential of the interstitial structure and emulsion

Instead of using another ternary phase diagram for evaluating conditions for including grape seed oil in the interstitial structure, it was decided to work with the conjunction of both systems (F1 and F2) because the surface properties of adsorbed protein layers is known to be important for the stabilization of foams and emulsions (Wilde, 2000).

Once the interstitial structure was prepared, the grape seed oil-acetone mixture was added and when water was incorporated to the mixture of hydrocolloids and surfactants, the displacement of the solvent was achieved (Ribeiro *et al.*, 2008). The one way ANOVA applied for evaluating uniformity of sizes and Z- potential of the interstitial structure showed that there was significant difference ( $p < 0.05$ ) between the obtained values of Z- potentials and size distributions of materials obtained by applying a stirring rate of 10000 rpm as compared with those produced using 7600 and 4000 rpm.

Emulsion distribution size (EDS) and Z- potential of the newly formed emulsion and those of the interstitial structure are presented in Fig. 4 and Fig. 5. The four curves obtained were unimodal and leptokurtic for interfaces prepared at 10000 rpm while for those prepared at 7600 and 4000 rpm distributions were bimodal and multimodal respectively (data not shown). Wang *et al.*, (2009), in spite of having used pseudo-ternary phase diagrams for designing water/emulsifier/oil systems and having proposed the inclusion of surfactants and co-surfactants, did not considered the influence of variables such as stirring rate in EDS and Z- potential distribution, which in emulsion preparing processes, strongly depend on the extent of shear (Jafari *et al.*, 2008 ) at which emulsion has been prepared thus inducing

monomodal, leptokurtic distributions and favoring a proper interaction between interstitial structure, surfactants, core material and continuous phase (Windharb *et al.*, 2005). This may also be due to the fact that emulsion prepared is a diluted one thus favoring proper water-solids emulsification interactions.

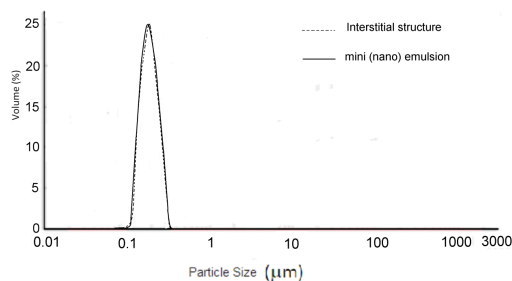


Fig. 4. Size distributions of interstitial structure and mini (nano) emulsion obtained at 10000 rpm stirring rate.

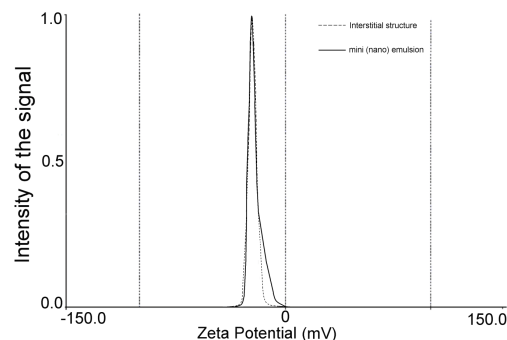


Fig. 5. Z- potentials of interstitial structure and mini (nano) emulsion obtained at 10000 rpm stirring rate.

The above results showed that 10000 rpm was a suitable stirring rate for preparing the interstitial structure and the emulsion. Values of Z- potential and EDS for these two materials were compared by means of an ANOVA which indicated that sizes of interface and emulsion were not significantly different ( $p > 0.05$ ). Sizes found for interstitial structure were in the range of 0.105 to 0.363  $\mu\text{m}$ , with a mean value of  $0.185 \pm 0.047 \mu\text{m}$  (185 nm). These sizes are classified as sub-micro or nano according to Windharb *et al.*, (2005). For the resulting emulsion, mean value of particle size was  $0.188 \pm 0.043 \mu\text{m}$  (188 nm) which was very close to that of the interface and had, approximately, in the same range (0.100 to 0.364  $\mu\text{m}$ ) than those of the interstitial structure.

Also, average values of Z- potential for the



interstitial structure and the emulsion were very close to each other:  $-18.23 \pm 0.72$  mV, and  $-18.55 \pm 0.97$  mV respectively and no significant difference between these values was observed ( $p > 0.05$ ). Similarities between the EDS and Z- potential distributions for interface and emulsion suggested that the oil was supported into the interface in such a way so as to protect it properly thus allowing limited interaction of core material with the continuous media and indicated that a correct construction of the interstitial interface and of the emulsion was achieved (Jafari *et al.*, 2008). Due to the resulting particle size distribution, it was expected that the mini (nano) emulsion created presented a high stability against creaming; visual observations indicated that interstitial structure and emulsions were stable for 48 days. Stabilizing synergic effects between surfactants and hydrocolloids such as those reported by Ribeiro *et al.*, (2008), must have had an important effect in producing a stable mini (nano) emulsion.

## Conclusions

The proportion of components of the dispersion was successfully determined by applying DoE, which allowed to prepare a suitable interface by the addition of surfactants to hydrocolloids in the proportions determined which enabled obtaining a stable mini (nano) interstitial structure and by considering surface tension as the variable to be minimized for the dispersion of surfactants and maximized for the hydrocolloids by RS methodology. The proposed process allowed the fabrication of a stable grape seed oil-water emulsion having a mean size of 188 nm typical of a mini-(nano) emulsion and Z-potential of -18.55 mV, values that were very close to those of the interstitial structure (-18.23 mV and 185 nm for Z-potential and size respectively), which allowed to confirm the creation of an adequate interface for emulsifying grape seed oil at the concentrations and preparation conditions used.

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