Ferulic Acid Lecithin-Based Nano-Emulsions Prepared by Using Spontaneous Emulsification Process

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ABSTRACT: *Ferulic acid (4-hydroxy-3-methoxycinnamic acid), an effective component of medical plant, is a phenolic acid with low toxicity that can be absorbed and easily metabolized in the human body. The solubility of Ferulic Acid (FA) is very low in aqueous solutions which can cause problem in preparation of pharmaceutical products, but it can easily be dissolved in oil/water interface of nano-emulsions. In the present work, a kind of ferulic acid nano-emulsion was prepared using the spontaneous emulsification method which occurs when an organic phase and an aqueous phase are mixed. A chemometrics approach has been used to optimize the size of FA nanoemulsions. The experiments were performed according to a Box-behenken experimental design, one of the most suitable experimental design for modeling studies. The effect of three experimental parameters on droplet size was studied using multivariate analysis. The factors studied and the related levels were the concentration of lecithin (0.7-2 % w/w, in aqueous phase), the concentration of tween-80 (2-8 % w/w, in aqueous phase) and sounication time (10-45 minutes). In all the experiments, the water phase was added to the organic phase including lecithin, tween-80 and FA in ethanol solvent. Then experimental droplet sizes were fitted to the polynomial model. Good descriptive and predictive ability of the model was verified as high values of the statistics R^2 (0.996) and F (112.5) were obtained for the linear relationship between predicted and experimental values of the dependent variable.*

KEY WORDS: *Multiple Linear Regression (MLR), Ferulic Acid (FA), Lecithin-based nanoemulsion, Spontaneous emulsification, Box-behenken experimental design.*

INTRODUCTION

Flavonoids are a widely distributed group of polyphenolic compounds with health-related properties [1,2]. These properties include anticancer, antiviral, anti-inflammatory activities, effects on capillary fragility and

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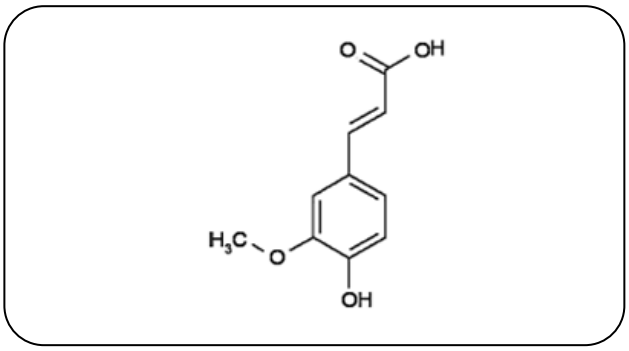


Fig. 1: The chemical structure of FA.

ability to inhibit human platelet aggregation [3, 4]. Ferulic Acid [(E)-3-(4-hydroxy-3-methoxy-phenyl) prop-2-enoic acid)] (Fig. 1) is a common and the most abundant polyphenolic compound with significant pharmacological action especially immunomodulatory activity and antioxidant effect which occur in vegetables, especially artichokes, eggplants (~90% of total polyphenols) and in maize bran (~3.1% of total polyphenol content) [5-6]. For hydrophobic bioactive compounds such as Ferulic Acid (FA), poor water solubility is a major limiting factor for their use in different applications in the field of food industry or pharmacy. For this reason, they are administrated as emulsions, in which the substance is dissolved in an organic compound, which is dispersed in an aqueous phase as droplets stabilized by a surfactant. Emulsion formulations offer an appealing alternative for the administration of poorly water soluble drugs due to their effectiveness for drug solubilization, potential for improved efficacy and anticipated patient acceptance and compliance due to the reduced side effects [7]. Nevertheless the use of safe and skin friendly ingredients is desirable for such colloidal formulations. As already reported, lecithin, a natural mixture can fulfill these requirements, generally it is non-irritating and non-sensitizing for animal and human skin [8-9]. Moreover, lecithin compounds present an affinity to cellular membranes thus leading to an increased absorption of several drugs [9]. The incorporation of poorly soluble drugs in nanoemulsions has received increasing attention as colloidal carriers for topical delivery [10-11] that could increase the skin permeation rate and enhance the topical effects due to prolonged residence time in the uppermost skin layers due to the large surface area and low surface tension of the oil droplets [12-14]. It also causes to administrate of less amount of drug to the patient.

Nano-emulsions are emulsions in which the droplet sizes range typically from 20 to 500 nm, which are intermediate between normal emulsions and microemulsions [15]. Nano-emulsions are also referred to as mini-emulsions, fine-dispersed emulsions, submicron emulsions and so forth, but are all characterized by a great stability in suspension due to their very small size, essentially the consequence of significant steric stabilization between droplets [16-17]. However, the long- term physical stability of nano-emulsions (with no apparent flocculation or coalescence) makes them unique and they are sometimes referred to as "approaching thermodynamic stability" [18-19]. Nano-emulsions possess numerous advantages including the possibilities of controlled drug release and drug targeting and the incorporation of a great variety of therapeutic actives [20]. Unlike micro-emulsions, they are stabilized by non-ionic surfactants and/or polymers exhibiting a steric stabilization effect. The droplets size and size distribution are depending on the spontaneity of emulsification [21-23]. The spontaneity of the emulsification is poorly defined, since it should account not only for the rate of the emulsification process, but also for the volume and the particle size distribution of the produced emulsion. The spontaneity of the emulsification process depends mainly on the following variables: interfacial tension, interfacial and bulk viscosity, phase transition region and surfactant structure and concentration [24-33]. In the spontaneous emulsification method, the addition of the solvent-oil solution results in the emulsification of the oily phase into nano-droplets, due to some kind of interface instability originating from lipid diffusion of the solvent across the interface and decrease of the interfacial tension [34-35]. This method is receiving increased attention and is interesting for formulation studies as it is easy to perform in laboratory scale, does not require sophisticated equipment nor the use of high temperature and generally leads to the formation of small droplet size formulations, among other advantages.

In this study, for solving problems related to ferulic acid solubility in water, a kind of nano- emulsion was prepared through spontaneous emulsification, in order to increase of skin absorption in treatment of some skin disorders such as psoriasis and atopic dermatitis. To reach this aim, response surface methodology was applied in nanotechnology science. It develops an empirical model

which evaluates the relationship between a set of controllable experimental factors and observed results. The formation of water-in-oil nano-emulsions was studied in a system containing the mixture of nonionic and lipophilic surfactants (tween 80-lecithin). The droplet size was optimized by chemometric approaches of experimental design (Box-Behenken design), Multi Variate Analysis (MLR). Since the application of ultrasound to the creation of nano- emulsions has been considered in several works [36-40], the ability of ultrasonic homogenizer was also assessed to produce nano-emulsions. The three experimental variables considered were: concentration of tween-80 in aqueous phase (% w/w), concentration of lecithin in aqueous phase (% w/w) and sonication time. In order to select the most important effects and to calculate the coefficients relating these effects to droplet size, the method of backward Multiple Linear Regression (MLR) was used in modeling. The results obtained indicated that the droplet size and size distribution are strongly dependent on these factors.

EXPERIMENTAL SECTION

Chemical and reagent

Ethanol and trifluoracetic acid were purchased from Merck (Darmstadt, Germany). The nonionic surfactant poly (oxyethylene) (80) sorbitan monolaurate (tween-80) was provided from Sigma Chemical Company (St. Louis, MO). Egg-Lecithin and HPLC grade methanol and all other chemicals used in the study were obtained from Merck (Darmstadt,Germany). Ferulic Acid (FA) was purchased from Fluka (Germany). All reagents were analytical grade and were used without further modifications. Ultrapure water was obtained from a Millipore, Direct-Q® Water purification system (Millipore Co., Bedford, MA, France).

Preparation and characterization of nanoemulsions

Nanoemulsions were prepared by mixing the separately prepared oily and water phases through spontaneous emulsification method [41-44]. The experiments were performed according to the box-behenken experimental design that were prepared with different combinations of the values of the three factors. The three experimental variables considered were: the contents of tween-80 (TW) and lecithin (L) in aqueous phase (% w/w) and sounication time (t). The sequence of experiments was randomized. In this work, the organic phase containing egg-lecithin, tween-80 and FA was first dissolved in ethanol and then mixed with de-ionized water using a conventional homogenizer (Heidolph Diax-900, Schwabach, Germany) for 15 min to produce a coarse oil-in-water emulsion. The resulting coarse pre-emulsion was immediately homogenized using an ultrasonic bath (Bondelin Sonorex equipment) under a controlled certain time. Solvent was then removed from the fine emulsion completely by a rotary evaporator under a reduced pressure at approximately 40°C. Fifteen batches were prepared, using the mentioned steps, by varying the lecithin concentration (0.7-2% w/w, final concentration in aqueous phase), the concentration of tween-80 (2-8% w/w, final concentration in aqueous phase) and sounication time (10-45 minutes). Table 1 shows the feasible region of the mentioned variables in which the experimental optimization could be carried out. All the performed experiments, the variable names and their final values in aqueous phase have been presented in Table 2. The FA concentration was 1 mg/mL (in aqueous phase) at all experiments. Each dispersion sample was then characterized for droplet size and size distribution and for further storage evaluation (4°C with sampling 4 weeks intervals for a period of 12 weeks). Particle size analysis measurements were performed using Zetasizer Nano ZS (Malvern Instrumen Ltd, Malvern, UK) after dilution in water at 25°C. The PolyDispersity Index (PDI) is a measure for the width of the distribution. It is a measure for the width of the distribution ranging from 0 (monodispersed) to 0.5 (relatively broad distribution). The pH values of nanoemulions were directly determined in samples (CyberScan 510 Ion pH meter). The final pH value of all these emulsions was between 5.0 to 5.5.

Table 1: Experimental factors and the corresponding three level settings.

Level	L (%, w/w)[a]	TW (%, w/w)[a]	t (min.)
-1	0.7	2	10
0	1.35	5	35
+1	2	8	60

[a] *Final content of the component in aqueous phase after evaporating organic solvent*

Table 2: Box-Behenken design for three factors studied and Experimental results (size distribution and droplet size, nm) obtained by design[a].

Experiment	L[b]	TW[b]	t[b]	particle size (nm)	PDI[b]
1	-1	-1	0	76.16	0.329
2	+1	-1	0	112.70	0.265
3	-1	+1	0	12.19	0.230
4	+1	+1	0	90.89	0.455
5	-1	0	-1	15.47	0.340
6	+1	0	-1	93.09	0.252
7	-1	0	+1	14.18	0.307
8	+1	0	+1	183.30	0.288
9	0	-1	-1	99.63	0.282
10	0	+1	-1	13.88	0.288
11	0	-1	+1	122.20	0.176
12	0	+1	+1	14.81	0.318
C	0	0	0	88.76	0.399
C	0	0	0	89.54	0.300
C	0	0	0	90.82	0.408

[a] *FA is %0.1 (w/w in aqueous phase) in all experiments*

[b] *L, Lecithin concentration; TW, Tween concentration; t, sonication time, PDI, polydispersity index*

Storage stability

Prepared nanoemulsions were placed in duplicate in amber bottles and stored at 4°C for 90 days. The samples were used to determine the droplet size and PDI at interval of 4 weeks.

Statistical analysis

All experiments and measurements were duplicated. The statistical analysis of the multiple regression was also performed on range scaled factor values of [-1, +1] using backward method in SPSS software (The statistical package for IBMPC, Quiad software, Ontario). The significant difference level was set at 0.05. Each reported value was the mean of three analyses from two replications.

RESULTS AND DISCUSSION

At first, several batches were prepared, using the mentioned steps, by varying the order of adding organic and aqueous phase. The rate of addition was kept approximately constant, stirring at 700 rpm, all experiments were run at 25 °C. The aim of this part of study was to evaluate the order of adding two phases together allowing the formation of a nano-emulsion with the smaller particle size and size distribution. Each dispersion sample was then characterized for droplet size and PDI. Fig. 2 shows the intensity vs. droplet size for one of the studied solutions. The results for all the batches studied showed that droplet size and PDI increased as the organic phase added to aqueous phase. Therefore, the water phase was added to organic phase in all experiments.

Preparation applying Box-behenken design

The properties of nano-emulsions, as non-equilibrium systems, to be optimized, for example droplet size and polydispersity, will depend on composition variables and could depend on preparation variables such as emulsifying path, agitation, emulsification or sounication time. Therefore, optimization can be carried out with respect to these two types of variables. In other words, direct application of nano-emulsions requires optimization studies for achieving the best properties

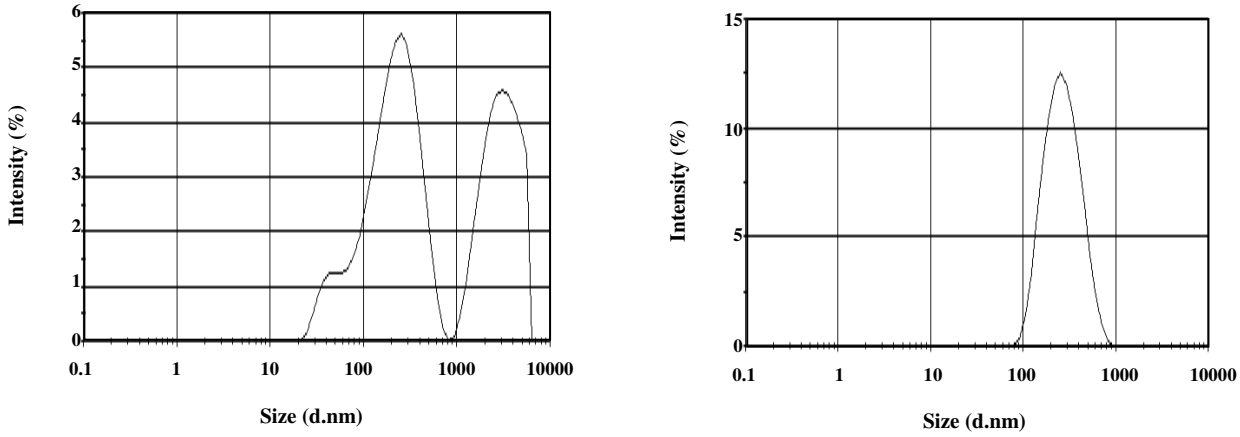


Fig. 2: Intensity vs. droplet size, in a nanoemulsion solution containing of 0.2% lecithin, 0.2% tween-80 and 35 minutes sounication time. A) After adding organic phase to aqueous phase; B) After adding aqueous phase to organic phase

for specific applications which can be carried out by experimental designs. It allows reducing the number of experiments needed. Statistical analysis of results will allow to know which variables have a significant influence, and to correlate desired response with variables by polynomial equations.

In this study, experiments used for modeling droplet size were performed on three levels of three factors, using Box-behenken experimental design. This design is one of the experimental designs suitable for modeling and optimizing. The effect of three experimental factors on the droplet size was studied using multivariate analysis. In order to define experimental domain, a preliminary study was performed on the factor levels. The experimental values of droplet size and size distribution for all the experiments were also reported in Table 2.

Modeling of droplet size

The droplet size values for the complete set of 15 experiments were fitted to the polynomial model. An ordinary least square method was used by a variable selection algorithm (backward search) in SPSS software to find a model that describes efficiently the dependence of droplet size on the experimental parameters. The obtained model and its statistics were reported in Table 3. Criteria for the evaluation of the descriptive capability of the model were Fisher-ratio value (F), squared correlation coefficient (R^2), and standard error of the estimate (SE). Different polynomials with all possible combinations of the factors were generated. It was found that the simplest polynomial that successfully described the system under study was third order. The low value of SE and the high value of R^2 and F statistics indicate that the model is quite successful in calculating droplet size. The model obtained indicated that two factors, lecithin and tween-80 concentrations have significant effect on the droplet size value. It is also noteworthy that the terms of TW^2 and t^2 showed negative contributions to the dependent variable. This means that the respective response hypersurfaces in the multidimensional factor space are curved in the sphere of the experimental design. Determination of the importance of the factors in the model by the standardized coefficient [31] demonstrated that the interaction between L and t and/or L and TW have positive contribution on the droplet size. Therefore, the existence of interaction terms between main factors in conditions of our experiments emphasizes once the necessity to carry out active multifactor experiments for determining the optimal condition of FA nanoemulsions. Figs. 3, 4 and 5 also show response surface variations for droplet size versus lecithin, tween-80 concentration and sonication time.

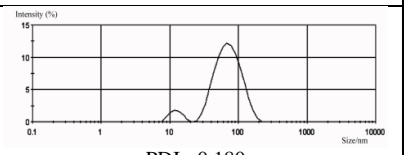
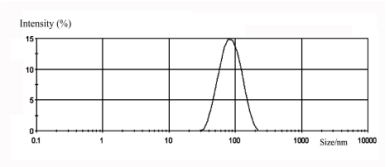
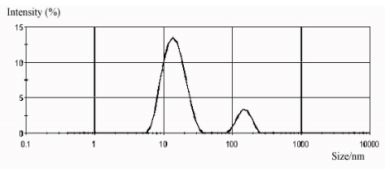
To find the optimum condition in the preparation of FA nanoemulsions, a grid search was performed in Excel software. In this software, the droplet size was predicted from the model within the feasible factor space. Prediction was performed for all conditions necessary to obtain a grid with a 0.325% (w/w), 1.5% (w/w) and 12.5 min. intervals in the lecithin, tween-80 contents and sounication time, respectively. Therefore, 125 different

Table 3: Specification of the best polynomial model for prediction of the size of prepared nanodispersions.

Varible	Coefficient	Standardized coefficient (mean effect)
Intercept	88.832	-
L	45.247 (0.003)	0.677
TW	-34.865 (0.009)	-0.521
t	14.052	0.210
L×TW	10.540	0.111
L×t	22.875	0.242
TW×t	-5.410	-0.057
TW^2	-15.192	-0.155
t^2	-11.667	-0.119
Statistics R	0.940	
F-value	5.717 (0.024[a])	
SE	26.28	

[a] *number in parenthesis is p-value of regression.*

Table 4: Predicted and experimental droplet size in some of the best conditions found for the preparation of FA nanodispersions.

L (%, w/w)	TW (%, w/w)	t (min)	Pred. size (nm)	Exp. Size (nm)	Graph
1.09	2	34.5	94.40	76.06	 PDI= 0.180
1.35	4	17.0	81.30	96.91	 PDI= 0.270
1.48	8	10.0	25.04	14.82	 PDI= 0.264

experimental conditions were predicted using the obtained model. The efficiency of prediction of the polynomial model was confirmed by performing the experiment under some of the proper conditions with the lowest droplet size (Table 4). The droplet size graphs under these optimal conditions have also been shown in Table 4. The results also show a good agreement between predicted and experimental droplet size under the selected conditions. Therefore, the developed model was suitable for interpreting the experimental space and was confirmed for indicating the optimum experimental condition.

Poly Dispersity Index (PDI) and Stability

The droplet diameter and size distribution are important parameters affecting emulsion stability. Table 4 shows PDI values in optimal conditions. PDI values

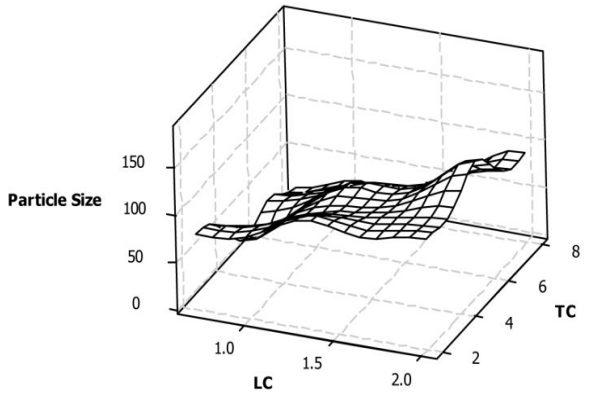


Fig. 3: 3D response surface graph for droplet size versus lecithin and tween-80 concentration.

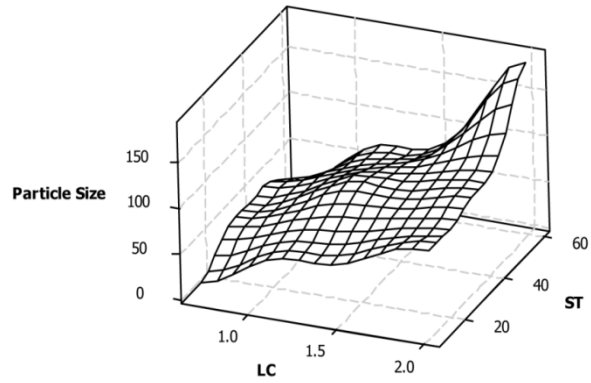


Fig. 4: 3D response surface graph for droplet size versus lecithin concentration and sonication time.

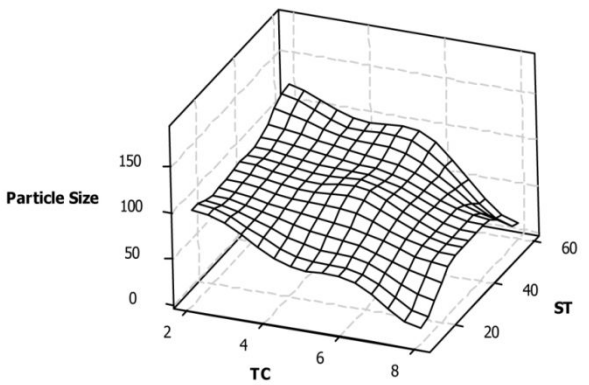


Fig. 5: 3D response surface graph for droplet size versus tween-80 concentration and sonication time.

lower than 0.25 indicate a closed size distribution providing good stability of nanoemulsions due to the reduced Ostwald ripening [32]. The stability of this nanodispersions produced under optimal condition was assessed by measuring the variation of droplet sizes and PDI at interval of 4 weeks for a period of 90 days at fixed temperature (~25°C). The prepared nanodispersions presented a good stability without phase separation during weeks, but with a slight increase of droplet size with time. The results also showed that the lower the PDI value, the more stable nanodispersions and the droplets in the emusions experiences slower growth rates.

CONCLUSIONS

A chemometric approach has allowed us to find the optimum conditions in preparation of FA nanodispersions with a limited number of experiments. The results showed that droplet size regularly changes as a function of lecithin and tween-80 concentrations and sounication time. Furthermore, the Box-behenken design permits to determine the regression model, which describes the dependence of droplet size to the experimental parameters in preparation of FA nanodispersions. Results also indicated that the emulsification-evaporation technique proved to be an efficient way to stabilize the droplet size of the prepared nanodispersions. FA nanodispersions were physically stable throughout the 12 weeks of storage period at 4°C.

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REFERENCES

[1] Salah N., Miller N.J., Paganga G., Tijburg L., Bolwell G.P., Rice-Evans C., Polyphenolic Flavanols as Scavengers of Aqueous Phase radicals and as Chain-Breaking Antioxidants, *Arch. Biochem. Biophys.* **322**(2), p. 339 (1995).

[2] Morel I., Lescoat G., Cogrel P., Sergent O., Pasdeloup M., Brissot P., Cillard P., Cillard J., Antioxidant and Iron-Chelating Activities of the Flavonoids Catechin, Quercetin and Diosmetin on Iron-Loaded Rat Hepatocyte Cultures, *Biochem. Pharmacol.* **45**(1), p. 13 (1993).

[3] Middleton E.Jr, Kandaswami C., Potential Health-Promoting Properties of Citrus Flavonoids, *Food Technol.* **48**(11), p. 115 (1994).

[4] Benavente-García O., Castillo J., Marin F.R., Ortuño A., Del Río J.A., Uses and Properties of Citrus Flavonoids, *J. Agric. Food Chem.* **45**(12), p. 4505 (1997).

[5] Rechner A.R., Pannala A.S., Rice-Evans C.A., Caffeic Acid Derivatives in Artichoke Extract are Metabolised to Phenolic Acids in Vivo, *Free Radic. Res.* **35**(2), p. 195 (2001).

[6] Saulnier L., Vigouroux J., Thibault J.F., Isolation and Partial Characterization of Feruloylated Oligosaccharides from Maize Bran, *Carbohydr. Res.* **272**(2), p. 241 (1995).

[7] Kelmann R.G., Kuminek G., Teixeira H.F., Koester, L.S., Carbamazepine Parenteral Nanoemulsions Prepared by Spontaneous Emulsification Process, *Int. J. Pharm.* **342**(1-2), p. 231 (1995).

[8] Fiume Z., Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin, *Int. J. Toxicol.* **20**(1 suppl.), p. 21 (2001).

[9] Paolino D., Ventura C.A., Nistico S., Puglisi G., Fresta M., Lecithin Microemulsions for the Topical Administration of Ketoprofen: Percutaneous Adsorption Through Human Skin and in Vivo Human Skin Tolerability, *Int. J. Pharm.* **244**(1-2), p. 21 (2002).

[10] Piemi M.P.Y., Korner D., Benita S., Marty J.P., Positively and Negatively Charged Submicron Emulsions for Enhanced Topical Delivery of Antifungal Drugs, *J. Control. Release* **58**(2), p. 177 (1999).

[11] Fernandez C., Marti-Mestres G., Ramos J., Maillols H., LC Analysis of Benzophenone-3: II Application to Determination of 'In Vitro' and 'In Vivo' Skin Penetration from Solvents, Coarse and Submicron Emulsions, *J. Pharm. Biomed. Anal.* **24**(1), p. 155 (2000).

[12] Klag S.H., Parnas M., Benita S., In "Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs", Muller R.H., Benita S., Bohm B. H.L. (Eds.), Medpharm Scientific Publishers, Stuttgast, pp.31-65 (1998).

[13] Benita S., Prevention of Topical and Ocular Oxidative Stress by Positively Charged Submicron Emulsion, *BioMed. Pharmacother.* **53**(4), p. 193 (1999).

[14] Tadros T., Esquena P., Solans C., Formation and Stability of Nano-Emulsions, *Adv. Colloid Interface Sci.* **108-109**, p. 303 (2004).

[15] Lin Ee Sh., Duan X., Liew J., Dzuy Nguyen Q., Droplet Size and Stability of Nano-Emulsions Produced by the Temperature Phase Inversion Method, *Chem. Eng. J.* **140**(1-3), p. 626 (2008).

[16] Ugelstad J., El-Aasser M.S.. Vanderhoff J.W., Emulsion Polymerization: Initiation in Monomer Droplets, *J. Polym. Sci.,Polym. Letters Ed.*, **11**, p. 5030 (1973).

[17] El-Aasser M.S., Lack C.D., Vanderhoff J.W., Fowkes F.M., The Miniemulsification Process-Different form of Spontaneous Emulsification, *Colloid Surf.*, **29**(1), p. 103 (1988).

[18] Tadros Th.F., Vandamme A., Levecke B., Booten K., Stevens C.V., Stabilization of Emulsions Using Polymeric Surfactants Based on Inulin, *Adv. Colloid. Interf. Sci.,* **108/109**, p. 207 (2004).

[19] Girard N., Tadros Th.F., Bailey A.I., Styrene and Methylmethacrylate Oil-in-Water Microemulsions, *Colloid. Polym. Sci.*, **275**(7), p. 698 (1997).

[20] Calderilla-Fajardo S.B., Cazares-Delgadillo J., Villalobos-Garcia R., Quintanar-Guerrero D., Ganem-Quintanar A., Robles R., Influence of Sucrose Esters on the In Vivo Percutaneous Penetration of Octyl Methoxycinnamate Formulated in Nanocapsules, Nanoemulsion, and Emulsion, *Drug dev. Ind. Pharm.*, **32**(1), p. 107 (2006).

[21] Gopal R.E.S., In "Principles of Emulsion Formation", Sherman, P. (Ed.), Emulsion Science, Academic Press: London, pp.1–75 (1968).

[22] Becher D.Z., In "Application in Aagriculture, Encyclopaedia of Emulsion Technology", Becher, P. (Ed.), first ed. Marcel Dekker, New York, pp. 239-320 (1983).

[23] Shahidzadeh N., Bonn D., Aguerre-Chariol O., Meunier J., Spontaneous Emulsification: Relation to Microemulsion Phase Behaviour, *Colloid Surf. A: Physicochem. Eng. Aspects,* **147**(3), p. 375 (1999).

[24] Lopes-Montilla J.C., Herrera-Morales P.E., Pandeya S., Shah D.O., Spontaneous Emulsification: Mechanisms, Physicochemical Aspects, Modeling, and Applications, *J. Dispers. Sci. Technol.,* **23** (1-3), p. 219 (2002).

[25] Davies J.T., Rideal,E.K., In "Diffusion through Interfaces", Willmer, H., Ed., Interfacial Phenomena, First ed. Academic Press, New York, pp. 343–450 (1961).

[26] Aveyard R., Binks B.P., Clark S., Mead J., Interfacial Tension Minima in Oil-Water-Surfactant Systems. Behaviour of Alkane-Aqueous NaCl Systems Containing Aerosol OT, *J. Chem. Soc. Faraday Trans.*, **82**, p. 125 (1986).

[27] Aveyard R., Binks B.P., Mead J., Interfacial Tension Minima in Oil-Water-Surfactant Systems. Effects of Alkane Chain Length and Presence of n-Alkanols in Systems Containing Aerosol OT, *J. Chem. Soc. Faraday Trans.*, **82**, p. 1755 (1986).

[28] Miller C.A., Spontaneous Emulsification Produced by Diffusion-A Review, *Colloid Surf.,* **29**(1), p. 89 (1988).

[29] Miller C.A., Raney K.H., Solubilization-Emulsification Mechanisms of Detergency, *Colloids Surf. A: Physicochem. Eng. Aspects,* **74**(2-3), p. 169 (1993).

[30] Miller C.A., Solubilization and Intermediate Phase Formation in Oil-Water-Surfactant Systems, *Tenside Surf. Det.*, **33**, p. 191 (1996).

[31] Hackett J.L., Miller C.A., Microemulsions to Liquid-Crystal Transition in Two Anionic Surfactant Systems. *SPE Res. Eng.* **3**, p. 791 (1988).

[32] Lide D.R., In "CRC Handbook of Chemistry and Physics", 74th ed., CRC Press, USA (1993).

[33] Davies J.T., Haydon D.A., Spontaneous Emulsification, *Int. Congr. Surf. Act.* **2** (1), p. 417 (1957).

[34] Vitale S.A., Katz J.L., Liquid Droplet Dispersions Formed by Homogeneous Liquid-Liquid Nucleation: The Ouzo Effect, *Langmuir*., **19**, p. 4105 (2003).

[35] Bouchemal K., Briancon S., Perrier E., Fessi H., Nano-Emulsion Formulation Using Spontaneous Emulsification: Solvent, Oil and Surfactant Optimization, *Int. J. Pharm.*, **280**(1-2), p. 241 (2004).

[36] Abismail B., Canselier J.P., Wilhelm A.M., Delmas H., Gourdon C., Emulsification by Ultrasound: Drop Size Distribution and Stability, *Ultrasonics Sonochem.,* **6**(1-2), p. 75 (1999).

[37] Freitas S., Hielscher G., Merkle H.P., Gander B., Continuous Contact- and Contamination-Free Ultrasonic Emulsification-a Useful Tool for Pharmaceutical Development and Production, *Ultrasonics Sonochem.*, **13**(1), p. 76 (2006).

[38] Jafari S.M., He Y., Bhandari B., Production of Sub-Micron Emulsions by Ultrasound and Microfluidization Techniques, *J. Food Eng.* **82**(4), p. 478 (2007).

[39] Maa Y.F., Hsu C.C., Performance of Sonication and Microfluidization for Liquid-Liquid Emulsification, *Pharm. Dev. Tech.*, **4**(2), p. 233 (1999).

[40] Gaikwad S.G., Pandit A.B., Ultrasound Emulsification: Effect of Ultrasonic and Physicochemical Properties on Dispersed Phase Volume and Droplet Size, *Ultrasonics Sonochem.* **15**(4), p. 554 (2008).

[41] Davies J.T., Haydon D.A., An Investigation of Droplet Oscillation During Mass Transfer. ii. A Dynamical Investigation of Oscillating Spherical Droplets, Proc. 2nd, Int. Congr. Surf. Act. London, Vol. 1, pp. 417 (1957).

[42] Becher D.Z., In "Encyclopedia of Emulsion Technology", Marcel Dekker, New York, Vol. 2 (1985).

[43] Pouton C.W., Formulation of Self-Emulsifying Drug Delivery Systems, *Adv. Drug Deliv. Rev.* **25**(1), p. 47 (1997).

[44] Kawashima Y., Yamamoto H., Takeuchi H., Hino T., Niwa T., Properties of a Peptide Containing Dl-Lactide/Glycolide Copolymer Nanospheres Prepared by Novel Emulsion Solvent Diffusion Methods, *Eur. J. Pharm. Biopharm.*, **45**(1), p. 41 (1998).