

ORIGINAL RESEARCH PAPER

IMPROVED SOLUBILIZATION OF CURCUMIN WITH A
MICROEMULSIFICATION FORMULATION

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Received 15 July 2011

Revised 6 October 2011

Due to the large number of bioactive substances, with low and very low solubility in water, new and improved investigation methods were developed. Researches in this area have shown that lipid systems in lipophilic substances formulation increase their bioavailability and prevent or reduce the toxicological risk because most of the components involved in the formulation are of natural origin, with a structure compatible with biological membranes components.

Among the lipid systems used in the leaching, transport and release of lipophilic substances there are: liposomes, solid lipid nanoparticles, double and single emulsions, autoemulsionante and auto-microemulsionante lipid systems. The last are the subject of the present research and meet specialists in concern for the harmonization of cost-benefit-risk in order to improve population health. Curcumin [(1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a yellow pigment derived from the rhizome of the plant *Curcuma Longa* with phenol groups and conjugated double bounds which is unstable at light and basic pH, degrading within 30 minutes.

The aim of this study is curcumin solubilization used as alimentary dye in automicroemulsionante systems. Dye/oil/surfactant/cosurfactant mixing ratio was made, based on quaternary phase diagrams. Mesofazice structures were revealed by conductivity and viscosimetric analysis. A curcumin solubilization system in aqueous medium was obtained. On the other hand, this paper studies the colour evolution of these automicroemulsionante systems comparing with hexane dye solution. The use of the chromatic attributes L^* , a^* and b^* and L^* , C^* and h_{ab} , suggested by the Commission Internationale de l'Eclairage (CIE) (i.e., the CIELAB system), obtained from direct transmittance measurements, which made it possible to follow the evolution of colour.

Keywords: self microemulsifying oil formulation, cosolvents, surfactants, quaternary phase diagrams

Introduction

Recently research on the behavior and properties of nanomaterials have found numerous applications in food industry (Moraru *et. al.*, 2003; Moraru *et. al.*, 2009). Thus, more and more published papers have shown the role of food nanotechnology in areas such as: food safety and security (Bäumner, 2004), sensory analysis and food nanotexture, fortification of some foods with bioactive components (McClements *et. al.*, 2009).

Taking into account that in the period 2007-2012 the functional foods market increased annually by 5.7% (<http://www.foodsciencecentral.com>), one of the most important application of food nanotechnology is to design new functional foods, contributing in this way to the increase of consumers' health.

A major research activity is the use of plant components (phytochemicals) as food supplements or as support for the fortified foods. Due to poor solubility in water and even oil, these phytochemicals have low bioavailability and present difficulty to be included in food. Therefore, the research in recent years was oriented towards using different methods and techniques of biocomponents encapsulation. Thus, the systems have achieved high nutritional capacity. One of the solubilization methods of the phytochemicals is their inclusion in nanoemulsions.

Nanoemulsions, known as microemulsions, are liquid systems, stable thermodynamically, transparent, with low viscosity and dispersed phase droplets between 50-100 nm.

Due to the small size of droplets, the solubilised components can be transported easily by cell membrane causing an increase of their concentration in the blood.

Studies have shown that the use of lipid systems in lipophilic substances solubilisation increases their bioavailability and prevents or reduces the toxicological risk because most of the components involved in formulation are of natural origin, with a structure compatible with the components of biological membranes. Among the lipid systems used in solubilisation, transport and release of lipophilic substances are mentioned: liposomes, solid lipid nanoparticles (SLN), single and double emulsions, autoemulsified (SEOF) and automicroemulsionante (SMEOF) lipid systems. These last ones make the subject of some recent scientific researches and subscribe to specialists concern for cost-advantage-risk equilibrium in order to increase the population health (Pegg and Shahidi, 2007).

The use of autoemulsified and automicroemulsionante lipid systems in solubilisation of lipophilic substances has several advantages, such as:

- they have a high solubilization capacity and dispersion of active principles;
- autoemulsification and automicroemulsification are spontaneous processes occurring at ambient temperature;
- it offers the possibility of system sterilization by filtration;
- it offers the possibility of active substances protection against the attack of biological liquids.

However, a recent research (Pegg and Shahidi, 2007) in the field of emulsified lipid systems emphasizes some restrictions on:

- the small field of lipophilic active substances at which is applied the emulsification method ($\log P > 4$)
- the excessive use of surfactants, which involves a toxicological problem;
- the small number of automicroemulsified lipid system toxicity studies.

Many of these functional foods are colored with various natural dyes.

Curcumin, the yellow pigment in turmeric and curry, is a dicinnamoylmethane dye authorized as a food additive in the EU and consists of three principal colouring components. It consists essentially of curcumins i.e. the colouring principle (1E, 6E)-1, 7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1, 6-diene-3, 5-dione and its desmethoxy- and bis-desmethoxy-derivatives. Curcumin is used extensively in the food and chemical industry as a colouring, flavouring and preservative agent. It has also been found to exhibit anti-oxidative (Sharma, 1976) and anti-inflammatory properties in clinical trials (Sharma *et. al.*, 1994). Curcumin with polyphenolic structure is water insoluble and scarcely dissolved in the organic phase.

The aim of this paper was to investigate the optimization of curcumin solubilization conditions in order to obtain emulsified systems used in food coloring. In this respect the procedure was the following:

- reporting on Oil / Surfactant / Cosurfactant mixing and realization of the pseudoquaternary phase diagrams;
- automicrostructure study of the prepared emulsified systems;
- colour characteristics evaluation, in model systems, of the solubilised curcumin.

Materials and methods

Reagents

Micromulsification was performed in the presence of synthetic emulsifier Tween 80 (Sigma-Aldrich Chemie GmbH, Germany). As dye curcumine (E 100, Germany) was used. Sunflower oil, linseed oil, soybean oil were obtained from the supermarket, being refined by BUNGE technology.

Deionized water (EasypureRF, compact ultrapure RF, UK) was used to prepare all solutions.

The pH has been ensured with the help of buffer solution (Sigma Chemical Co. - St. Louis, MO): the citric acid, $C_6H_8O_7 \cdot H_2O$, 0.1 M and sodium acid phosphate, $Na_2HPO_4 \cdot 2 H_2O$, 0.2 M (pH=2.2–8).

Glycerol and ethanol (purity $\geq 99\%$) were obtained from Fluka. The n-Hexane (Prolabo, Bucharest) used has the following characteristics: molecular weight: 86.18 and purity $\geq 99\%$.

Apparatus

A UV-Visible spectrophotometer (DOUBLE BEAM PC 8 SCANNING AUTO CELL UVD-3200 LABOMED, INC) was used to perform the transmittance measurements. For that purpose, quartz cells with a path length of 10 mm were used.

Dye solution and microemulsion were observed using optic microscope and microscope images were captured with a video camera (VP-L907, Samsung) and transferred for analysis to the USB port of a Pentium IV, 1200 MHz computer.

For ultrasonication (45% Amplitude, 0.05 pulse) the Bandelin, Sonopuls, Germany, equipped with a titanium horn (3 mm diameter) mounted at the top of the cylindrical glass cell was used.

Solubility of curcumin

To study the curcumin solubility, the following solvents were used: deionized water, sunflower oil, soybean oil and linseed oil. For this, in four glass tubes, 10 g solvent was taken in which 1 mg of curcumin was added.

Each sample was ultrasonicated for 3 minutes at 25°C. To determine solubility, the ultrasonication continued for another 3 minutes at the same temperature, adding curcumin until the solvent was disturbed, and at the bottom of the tube unsolubilised curcumin crystals appeared.

Phase diagrams construction

The phase pseudoternary diagrams are constructed to find the mixing ratio of the the corresponding microemulsion state components.

The oil phase formed of oil and curcumin is mixed with a surfactant / cosurfactant mixture with 3:1 mass ratio, in the mass reports: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. These mixtures are titrated with deionized water at temperature of 25°C.

Samples are shaken gently and left in stand for 10 hours. Phase diagrams, presented in Figure 1, are obtained on macroscopic and microscopic observations.

Preparation of oil-in-water microemulsions and automicromulsified systems with curcumin

Depending on the appearance of phase diagram were prepared and studied two groups of microemulsions with curcumin, one in which ethanol was used as cosurfactant, and another one which used glycerol. Because the mixtures prepared with sunflower oil and soybean oil are turbulent irrespective of surfactant and cosurfactant concentration; for study of microemulsions the linseed oil was used.

Curcumin was solubilised into oil in different concentrations. The Surfactant: Cosurfactant mass ratio for each sample was 3:1 (wt / wt).

Formed mixtures were ultrasonicated for 3 minutes at amplitude of 30% and a frequency of 20 kHz and 25°C. Prepared microemulsions were stored at room temperature for 6 hours to establish steady state before investigation. Microemulsions were stable, remaining clear and transparent for 6 months.

Viscosity measurement

An Ubbelohde type viscometer (ASTM, Capillary no. III, constant K = 1) was used to measure the viscosity of the samples to 25°C. For temperature control a thermostated water bath was used.

Electrical conductivity and pH measurements

Electrical conductivity was measured at a temperature of 25°C with Consort 868 multiparameter device, with electrode conductometric type SK10B and 0.10 cm² cell constant. Conductivity measurements were performed to the titration of surfactant-cosurfactant-oil mixture - curcumin with the aqueous phase along the dilution line AB. Error limit of conductivity measurements was $\pm 0.02 \mu\text{S}\cdot\text{cm}^{-1}$.

Digital pH Meter equipped with a combined glass-calomel electrode and 1 mm cells were also used for the experimentation (the pH values were measured at 25°C).

Determination of colour characteristics of the prepared model systems

The spectral transmittances in the visible spectra (the spectra was recorded between $\lambda = 380\text{-}780 \text{ nm}$) were measured with a Spectro UV-Vis Double Beam PC 8 Auto Scanning cell UVD-3200, Lobomed, INC). For that purpose, quartz cells with a path length of 10 mm were used. The measurements were made at 24 h after microemulsion preparation.

To obtain the tristimulus values, recommendations made by the Commission International de l'Eclairage: CIE 1964 (x, y) system (CIEXYZ), CIE 1976 (L*a*b*) space (CIELAB), CIE 1986 and CIE 1995 were applied, using as references the CIE Standard Illuminant C (2° visualizing field), CIE Standard Illuminant D65 (10° visualizing field) and deionized water as reference blank (Crețu et al., 2006; C.I.E., 1986; C.I.E., 1995).

All determinations were performed in triplicate and the results were presented as average.

Results and discussion

Curcumin solubility, at the temperature of 25°C, was determined using three types of oil. The results show that curcumin is insoluble in deionized water. In sunflower oil, soybean oil and flax oil solubilities are 0.015%, 0.02% and 0.035%.

Phase diagrams were used to determine the concentration of the components and to establish the area corresponding to each microemulsion. In microemulsions preparation, surfactants are designed to minimize, in addition to surfactants, interfacial tension.

Cosurfactants adsorbed at O / W interface, change the interfacial membrane favoring the formation of microemulsions.

In our experiment, the ethanol is more efficient in the formation of microemulsions. This is shown by phase diagrams where the area corresponding to microemulsions is higher when using ethanol than in the case of glycerol (Figure 1).

If the samples corresponding of AB line (Figure 1.a) are analyzed, it can be observed that the first 10 samples, obtained by successively adding water at concentrations of 5%, are homogeneous, transparent, slightly yellow - orange colored. These samples correspond to the state of microemulsion (Garti et al., 2001; Flanagan et al., 2005; Flanagan and Singh, 2006; Chuan-Chuan et al.,

2009). With increasing water content above 60% at lower concentrations of surfactant, the samples begin to lose their transparency becoming more and more turbulent and there is separation among the two phases.

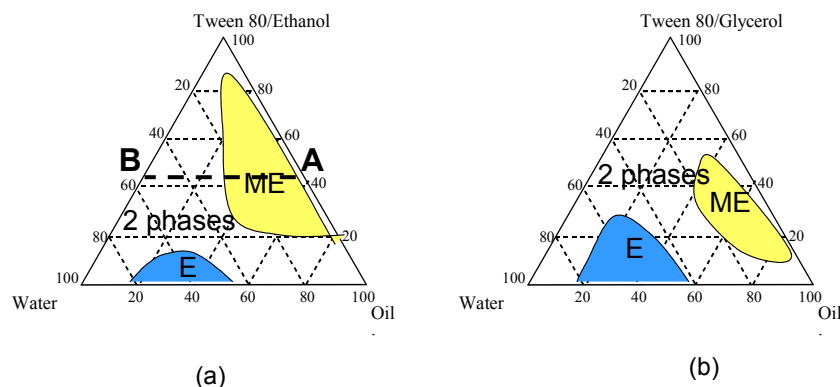


Figure 1. Pseudoternary phase diagrams: a- Tween 80/Ethanol 3:1; b- Tween 80/Glycerol 3:1

Areas with a high concentration of surfactant have a high viscosity and a homogenous translucent aspect, which corresponds to some micellar structures of amphiphilic molecules. The use of both ethanol and glycerol results in an area characteristic to conventional emulsions (E), with milky aspect, at which drops can be seen at the optical microscope.

Prepared microemulsions were mixtures formed of Linseed oil: Tween 80/alcohol: water =1:3:6. Emulsified systems contain: Linseed oil: Tween 80: Alcohol = 5:3:2. Solubility in microemulsions and automicroemulsified systems is 3.5 and 4 mg / mL which is almost ten times higher than in free oil.

Viscosity

Viscosity largely depends on the structure of microemulsions, type and form of micellar aggregates, concentration and interactions of the dispersed particles. Therefore, the viscosity measurements offer useful information about phase alternation and structural transformations into a microemulsion (Garti, 2001). All analyzed samples showed a Newtonian flow.

Analyzing the AB line from the point of view of viscosity corresponding samples reveal that in 0-30% (wt/wt) water viscosity values are relatively small and slightly increasing from 8.77 to 10.12 mPa·s, while in 30% - 50% (wt/wt) water, viscosity increases from 9.37 to 13.65 mPa·s, then fall back to 11.21 mPa·s. These variations are due to structural changes in the microemulsions.

When the mass fraction of aqueous phase is small, microemulsions consist in water dispersed globules and isolated in continuous oil phase and interactions between water-in-oil microemulsions globules are minimal. Viscosity increase is due to continuous phase structuring of the microemulsions which corresponds to some higher interactions between component molecules.

The transition from bicontinuous phase to oil-in-water (O/W) microemulsion is accompanied by a decrease in viscosity, but at higher values than corresponding water-in-oil (W/O) microemulsions (Figure 2).

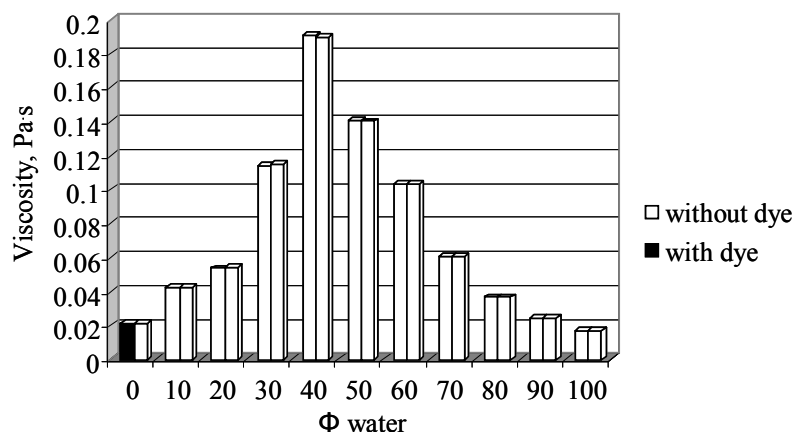


Figure 2. Variation of viscosity with water content of microemulsions with and without curcumin

Electrical conductivity

The absence of ions in the samples analyzed has led to very low values of electrical conductivity. Nevertheless there have been highlighted some variations in electrical conductivity depending on the water content. At low water content 0-10% wt / wt low conductivity is about 1.15 $\mu\text{S}/\text{cm}$, following a slight increase to 30% wt / wt water and a more pronounced increase of the conductivity up to 6.18 $\mu\text{S}/\text{cm}$ above 35% wt / wt water (Figure 3).

These variations, which correspond approximately to the variations registered for viscosity, are the expression of transition from microemulsions W/O, with low conductivity, to O/W microemulsions where aqueous continuous phase increases the conductivity.

Colour evaluation

These aspects regarding the solubilization of curcumin by microemulsification formulation are sustained by the chromatic evaluation. In Table 1 are shown the chromatic parameter values for curcumin solution and microemulsion with curcumin in the CIEXZY system. Colour coordinate L represents the lightness, while a^* and b^* indicate the change in hue from red to green and from yellow to blue, respectively. Also, C^* represent chroma, a correlate for saturation and h_{ab} is the hue angle, a useful quantity in specifying hue numerically.

Table 2 presents the values of chromatic parameters for curcumin solution and microemulsion with curcumin in CIELAB system. Also, yellow index (YI) values are presented.

As shown in Table 1 dominant wavelength varies from 574.8 to 574.5 nm when curcumin is solubilized by emulsification. These values correspond to the region yellow-orange in the chromatic diagram. Very small variation presents the excitation purity (1.63%). Also, analyzing the trichromatic parameters L^* , a^* and b^* (Table 2) it is noted that they do not present significant variations.

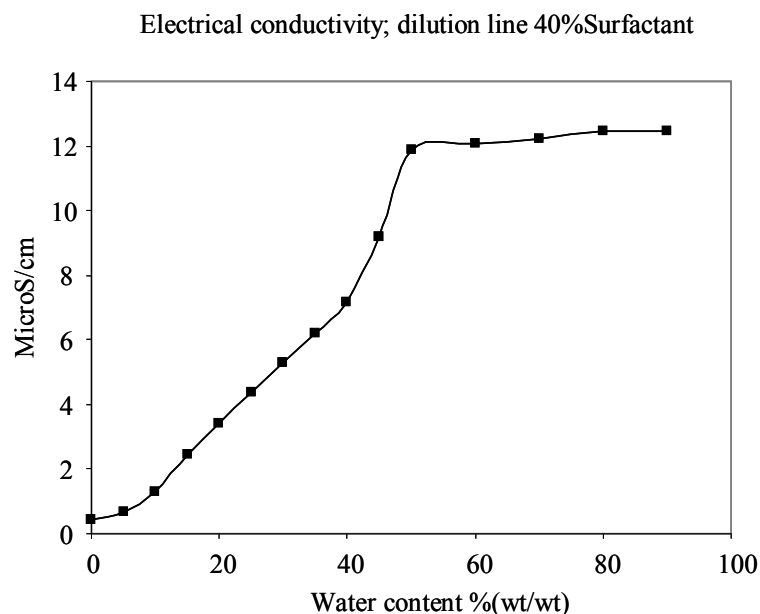


Figure 3. Variation of electrical conductivity with water content of microemulsions with curcumin

Table 1. Experimental results of 0.01 wt% hexane curcumin solution and microemulsion with dye obtained according to CIE colour system (CIEXYZ) / illuminant C/2°

System	Tristimulus values			Chromaticity coordinates			λ_d	p_e
	X	Y	Z	x	y	z		
Dye solution	75.502	81.973	1.386	0.4753	0.516	0.009	574.8	97.9
Dye microemulsion	71.68	77.475	1.53	0.4757	0.514	0.010	574.5	96.3

λ_d -dominant wavelength; p_e -excitation purity (%)

However, there is a slight decrease of the yellow index with curcumin solubilisation.

The same variation can be seen in time for chrome, which is correlated very intensively with the hue angle ($C^* ab = 0.62537 + 0.9646 \cdot hab$, correlation: $r^2 = 0.997$ for dye solution and $C^* ab = 0.605 + 0.6807 \cdot hab$, correlation: $r^2 = 0.972$ for dye microemulsion).

While hue angle variation is insignificant, in the case of chrome this decreases by 4% approximately from dye solution to dye microemulsion.

Table 2. CIELAB coordinates of 0.01 wt% hexane curcumin solution and microemulsion with dye obtained according to CIELAB and CIEL*C*h_{ab}/illuminant D65/10°

System	CIEL* a* b*			YI	L* C* h _{ab}		
	L*	a*	b*		L*	C* _{ab}	h _{ab}
Dye solution	92.563	-4.493	140.252	216.462	92.563	140.324	91.835
Dye microemulsion	90.540	-3.729	135.192	213.314	90.540	135.243	91.580

Conclusions

Pseudoternary phase diagrams were realized and have been emphasized reports of mixed O / W / S / CoS corresponding states of microemulsion. The domain corresponding to microemulsions is higher for ethanol than for glycerol, used as cosurfactants.

Through viscosity and conductimetry analysis, it has been emphasized the transition state of microemulsions oil-in-water, water-in-oil and bicontinuous structures.

A coloring stable premix for soft drinks, based on curcumin automicroemulsification was obtained.

The CIEXYZ system and CIELAB colour diagram in the cases of the microemulsion with curcumine show that trichromatic parameter values do not change significantly from those in the case of dye solution, which shows a very good stability of their colour.

Acknowledgements

This work has benefited from financial support through the 2010 POSDRU/89/1.5/S/52432 project, „Organizing the National Interest Postdoctoral School of „Applied Biotechnologies” with Impact on Romanian Bioeconomy”, project co-financed by the European Social Fund through the Sectoral Operational Programme Human Resources Development 2007-2013.

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