

Effect of pH, ionic strength, temperature and sugar concentration on orange peel essential oil/ T60: propanol and water microemulsion zone using Response Surface Methodology

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Abstract

In this study, microemulsification of orange peel oil (OPO) using Tween 60:propanol with the ratio of 1:1 was studied under different conditions of pH, ionic strength, and sugar concentration. Results showed that critical temperature (the temperature in which one-phase microemulsion system was still stable) for the microemulsions with higher sucrose concentrations (in the range between 0 to 30%) was lower while by decreasing in sugar concentration, critical temperature shifted to higher temperatures, as it reached to 90°C for the samples without sugar. The prepared microemulsions were stable at 5 and 25°C for seven days, but samples with higher concentrations of sugar (25 and 30%) became turbid at 45°C, whereas all other samples exhibited a one-phase microemulsion system at this temperature. Microemulsions were not stable at -3°C (freezing temperatures). In sensory evaluation, it was observed that the microemulsified OPO was dissolved in water as soon as it was added into the medium, in contrast to free essential oil as it was spreading on the surface of the solution. Encapsulation of OPO caused lower release of aroma, resulting a milder odor and taste (lower intensity) in samples which were preferred by the panelists. The overall acceptability of all samples containing OPO microemulsion was significantly higher than samples with free essential oil.

Keywords: Microemulsion, Orange peel oil, Response surface methodology, Modeling

Introduction

Orange peel essential oil is a by-product of sweet orange that is extracted mostly by cold press from the peel of the fruit (Ashurst, 1999). As the most compounds of orange peel oil are more lipophilic, therefore they have normally lower solubility in aqueous and sugar-containing aqueous beverages due to lower hydrogen bonding. Orange peel oil is a popular flavoring agent in food, pharmaceutical or cosmetic formulations due to its specific aroma and low cost. In addition, it has other beneficial effects such as anti-inflammatory, anti-depressant, anti-spasmodic, anti-septic, aphrodisiac, carminative, diuretic, tonic, sedative and cholagogue effects (Duke *et al.*, 2002). Apart from these advantages, its low solubility in water

and aqueous media, high volatility and degradation during processing and storage, which leads to changes in the sensory properties of the product, are some of the major concerns which has limited its applications. In order to overcome the abovementioned limitations, over the past few decades many efforts have been done (Ponce Cevallos *et al.*, 2010; Haroldo *et al.*, 2010), to entrap the essential oil within a protective layer of coating material known as “encapsulation” by different techniques. Emulsification is a process which can be considered as a technique of encapsulation; because, an emulsion normally consists of two immiscible liquids (usually oil and water), and that one liquid is dispersed as small spherical droplets in the other one (McClements, 1999). In this regard, microemulsions have several advantages over macroemulsions, such as enhanced solubility, better thermodynamic stability, ease of manufacturing, stability against oxidation and controlled release (Zhong *et al.*, 2009). In the last years microemulsions has attracted interests for being used in cosmetic, pharmaceutical and food industries to encapsulate and deliver compounds or allow solubility of high polar molecules (Polizelli *et al.*, 2006). Where stability and homogeneity of the finished product is desired, the stability and small droplet size of

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microemulsions can be helpful (Bidyut and Satya, 2001). Microemulsions are homogeneous, clear, and thermodynamically stable solutions, which consist of different ratios of oil, surfactant, co-surfactant and water (McClements, 1999; Radomska *et al.*, 2000; Feng *et al.*, 2009). They usually have spherical droplets with a radius less than 50 nm (Feng *et al.*, 2009). Microemulsions can be used for coating of volatile compounds such as essential oils to increase their stability during storage or processing (Zhong *et al.*, 2009). In terms of food systems, their applications are restricted mostly due to several factors such as the surfactant types which are allowed as additive in foods (Flanagan, 2006) as well as the high amount of surfactant needed for formation of microemulsions (Zhong *et al.*, 2009). An important factor which limits the application of microemulsions in food industry is its sensitivity to formulation. In other words, after formulation of a stable microemulsion and its addition into a food medium, the microemulsion might be broken due to the high or low pH, sugar or salt concentration and temperature. Few studies have been performed concerning the stability of microemulsions in media with different concentrations of electrolyte, sugar as well as pH. In this regard, Abbasi & Radi (2016) showed that the presence of salts (NaCl and CaCl₂) slightly increased the W/O areas of canola oil/ lecithin:*n*-propanol/ water microemulsions, whereas pH variation was not effective on the microemulsion formation. Ahmad *et al.* (2008) studied the effect of electrolytes on the ζ potential of vegetable oil microemulsions stabilized by a non-ionic surfactant where its value was negative for some tested electrolytes (Na⁺, Ca²⁺ and Mg²⁺). Hsu and Nacu (2003) also showed that ζ potential was dependent on pH and the higher concentrations of Na⁺ and K⁺ was able to separate the oil phase from soybean oil-in-water emulsion. Furthermore, Rajib and Bidyut (2005) showed that addition of NaCl (up to 0.2 mol dm⁻³) increased the microemulsion area while its further addition (0.5 mol dm⁻³) decreased the microemulsion formation area in Brij-35 and AOT [Sodium bis-2 (ethylhexyl) sulfosuccinate]/ eucalyptus oil/ butanol microemulsion. Similar observations were also recorded for glucose addition in the same system. Radi and Abbasi (2018) showed that the single phase microemulsion of canola oil/ lecithin:propanol/ water areas were increased by increasing the temperature. A good freeze-thaw, pasteurization and short UV exposure stability was

recognized for lycopene microemulsion (Amiri-Rigi & Abbasi, 2017).

As it was mentioned, to the best of our knowledge, the effects of ionic strength, pH, temperature and sucrose concentration on the microemulsion area of edible flavors had not been studied yet. Therefore, the main objectives of this study were to evaluate the influence of these variables on the formation of orange peel oil microemulsion stabilized with Tween 60/propanol using response surface methodology (RSM).

Materials and methods

Orange peel oil (OPO) (100%, w/w) was obtained from Giah Esanse Company (Gorgan, Iran). Propanol, CaCl₂, sucrose, NaOH, HCl, and Tween 60 (T60) [polyoxyethylene (20) sorbitan monostearate] purchased from Merck Chemical Co. (Darmstadt, Germany). All other chemicals were analytical grade and commercially available. Distilled water was used for microemulsion formation.

Preparation of microemulsion using RSM

The phase diagrams were constructed to evaluate the effect of pH, ionic strength and sugar concentration on the microemulsion zones of T60: Propanol (1:1). Response surface methodology (RSM) was used to design experiments as well as to model the above mentioned variables. Each independent variable was coded at three levels between -1 and +1. Therefore, the pH over the range 3 (-1) to 7 (+1), ionic strength 0 (-1) to 70 (+1), and sugar concentration 0 (-1) to 60 (+1), were given to the Design Expert 7.0 software (Stat-Ease Inc., Minneapolis, USA) to model different combinations of three variables (Table 1). Twenty experiments were planned and their responses [microemulsion area (%)] were measured. The statistical software package (Design-Expert, Stat-Ease Inc., Minneapolis, USA) was also used to plot response surface. Therefore, pH, ionic strength and sugar concentration of water phase was adjusted according to Table 1. The pH of formulating water was adjusted using HCl and NaOH 0.5 M. Sucrose, as the most applicable sugar in food systems, and calcium chloride were used for tuning the sugar concentration and ionic strength, respectively. It is noteworthy that the ionic strength, *I*, of a solution is a function of the concentration of all ions present in that solution (Skoog *et al.*, 2004).

$$I = \frac{1}{2} \sum_{i=1}^n c_i z_i^2 \quad (1)$$

Where c_i is the molar concentration of ion (mol dm^{-3}), z_i is the valence of that ion, and the sum is taken over all ions in the solution.

Afterwards, the microemulsions were prepared using formulated water, T60: propanol (1:1) and OPO. For OPO/ formulated water/ T60:propanol (1:1) microemulsion preparation, mixtures of formulated water (or OPO) with T60:propanol were made with the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1, respectively. The prepared mixtures were diluted by adding aliquots ($10 \mu\text{l}$) of OPO (or formulated water) stepwise, until the transparency disappeared. Then, the microemulsions were kept for 24 h at room temperature to ensure equilibrium (Flanagan *et al.*, 2006; Zhong *et al.*, 2009). Therefore, the microemulsion regions were determined based on the visual transparency of the mixtures. In the next step, the phase diagram was constructed to find the points that represent transparent, one-phase systems (microemulsions).

The particle size distribution of a microemulsion (containing T60: propanol (1:1) 28, water 71 and OPO 1% wt.) was also measured using dynamic light scattering technique (Zetasizer, Nano ZS, 4mW He-Ne laser, Malvern Instrument Ltd., UK) at ambient temperature (wavelength of 633 nm, detection angles 70 and 90°, dynamic viscosity of sample 8.76 mPa.s).

Validation experiment

The mathematical model created by RSM implementation was validated by conducting some experiments on some selected conditions.

The effect of temperature and sugar concentration on orange peel oil/ T60: propanol and water microemulsion zone

For evaluating the combined effect of sugar concentration and temperature, different levels of sugar concentration (0, 5, 10, 15, 20, 25 and 30% w/w) were prepared and their microemulsions were constructed with OPO/T60: propanol under 5, 25 and 45 °C. For this purpose, microemulsions were prepared using water formulated with different concentrations of sugar, OPO and T60: propanol (1:1), then their phase diagrams were constructed. Microemulsifications were operated at constant temperatures of 5, 25 and 45°C.

Heat stability measurement

For evaluating the heat stability of microemulsions prepared at different sugar concentration, a point from the stable systems

[containing T60: propanol (1:1) 34, sugar containing water 65 and OPO 1% w/w] was selected using their phase diagrams and the heat stability of the prepared microemulsions were evaluated. The temperature, in which the transparency of emulsions changed to turbid, was considered as the point of instability.

In another experiment, the same microemulsion systems were kept at 5, 25 and 45°C for two months and their stability (visual appearance, phase separation) was assessed.

Sensory analysis

A non- flavored carbonated water was purchased from a local supermarket, in which OPO (free and in the microemulsion form) was added to give a final concentration of 10 ppm and the sensory properties of samples were evaluated. Samples were evaluated for color, viscosity, taste and odor by 12 experienced panelists selected from members and students of Azad University of Yasooj. Assessors evaluated the intensity of odor and taste in each sample and rated their liking score (0 to 10) for odor intensity, taste intensity (0 to 10) and overall acceptability of each sample.

Results and Discussion

The influence of different parameters on the microemulsion area

The phase diagrams of OPO microemulsions are shown in Fig.1a. The transparent, one phase regions were considered as the microemulsion regions (38.66%) whereas the OPO, T60, propanol and water were soluble (Liu *et al.*, 1998; Zhong *et al.*, 2009). The boundaries between three regions of O/W, bicontinuous and W/O for OPO, water and T60 with propanol microemulsion area were determined (Fig. 2), measuring the electrical conductivity and the viscosity of the system along with three dilution lines of 10:90, 20:80 and 30:70 OPO/ surfactant: cosurfactant (data not shown) (Kim *et al.*, 2009). As it can be seen, the W/ O region of T60 in combination with propanol was significantly wider than those of others. Moreover, the O/ W region in T60 was almost evident. Therefore, it can be concluded that T60 was likely efficient in terms of OPO entrapment.

According to the Dynamic light scattering (DLS) results (Fig. 3), the mean particle size of over 90% of particles was 2.495 nm and only about 10% had greater size (18.44 nm) for a sample from the O/W region of T60:propanol microemulsion. The polydispersity index was 0.394, indicating the homogeneity of the microemulsion. The

polydispersity index is the indicator of particle homogeneity and varies from 0 to 1. When the value is closer to zero, the system is more homogenous (Amiri *et al.*, 2013). The small sizes

of the micelles obtained in this study can be related to high ratio of surfactant to OPO, as demonstrated by Polizelli *et al.* (2009).

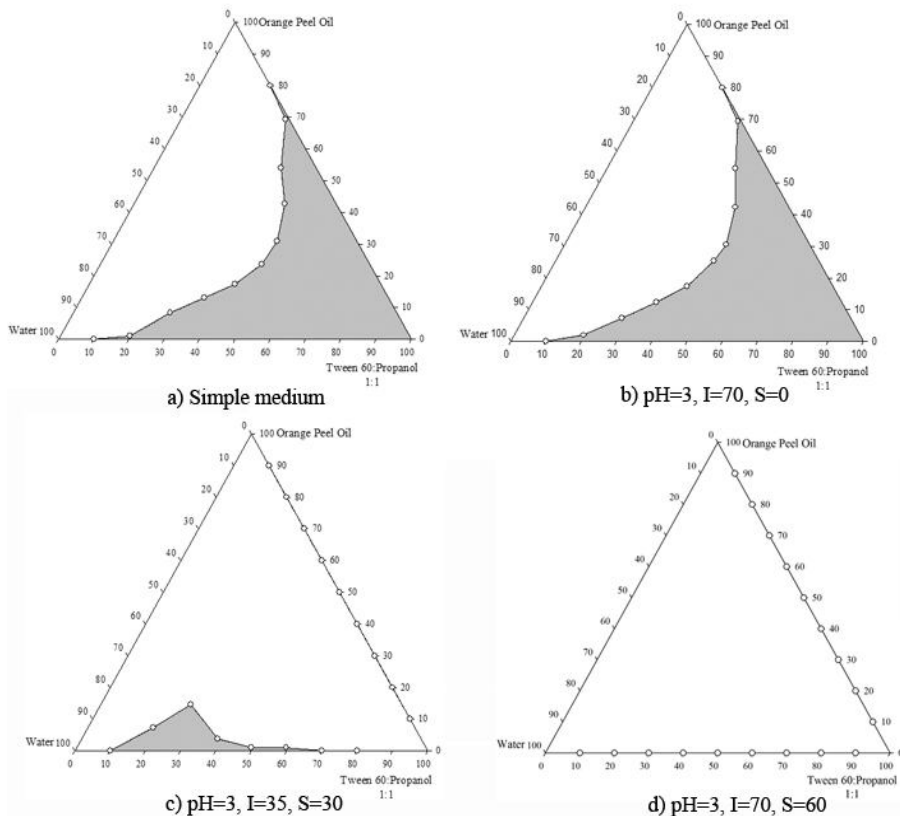


Fig. 1. Phase diagrams of OPO microemulsions with the surfactant:cosurfactant ratio of 1:1 at ambient temperature under different conditions of ionic strength (mM) (I) and sugar concentration (%) (S). The grey zones show the microemulsion areas. All ratios were by weight.

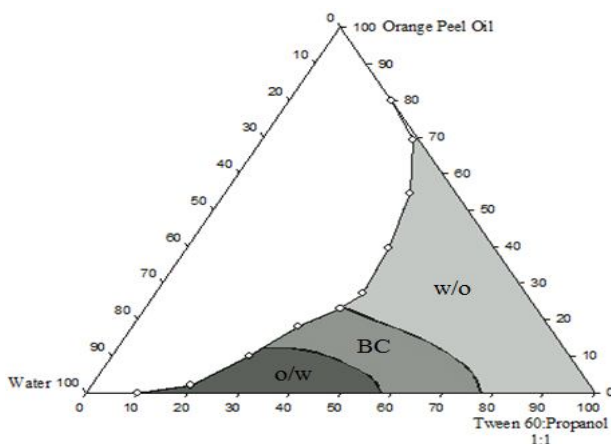


Fig. 2. Phase diagram of OPO microemulsions with the surfactant:cosurfactant ratio of 1:1 at ambient temperature. The grey, dark and light colors show the W/O, bicontinuous and O/W microemulsion areas. All ratios were by weight

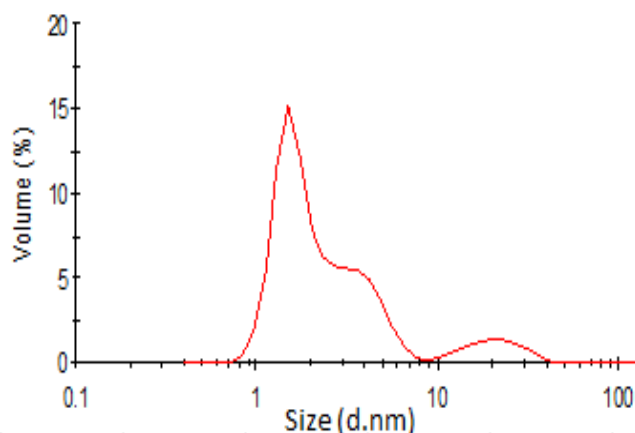


Fig. 3. Size distribution of OPO microemulsion (71 % water/1% OPO/28% surfactan:cosurfactant).

Apart from the above mentioned debate, the response surface methodology was used to evaluate the influence of pH, ionic strength, sugar concentration as well as their interactions on the microemulsion area of OPO/ T60:propanol. Central composite design (CCD) was used to design the experiment. The phase diagrams of

OPO microemulsion under different conditions of pH, ionic strength, sugar concentration and their interactions are depicted in Figs.1b, and c (all data not shown) and their microemulsion areas (%) in CCD experimental design were calculated (Table 1). The software predicted a Quadratic Model for three examined factors.

Table 1. Effect of pH, ionic strength and sugar concentration on mono-phase microemulsion area using central composite design.

| Runs | Variables | | | Response |
|------|-----------|---------------------|-------------------------|------------------------|
| | pH | Ionic Strength (mM) | Sugar concentration (%) | Microemulsion area (%) |
| 1 | 5 | 35 | 0 | 37.89 |
| 2 | 3 | 70 | 0 | 39.26 |
| 3 | 3 | 0 | 0 | 38.66 |
| 4 | 5 | 35 | 30 | 5.35 |
| 5 | 5 | 35 | 30 | 4.89 |
| 6 | 5 | 35 | 30 | 5.23 |
| 7 | 5 | 35 | 30 | 5.18 |
| 8 | 5 | 35 | 30 | 5.07 |
| 9 | 5 | 35 | 30 | 5.75 |
| 10 | 5 | 70 | 30 | 5.30 |
| 11 | 5 | 0 | 30 | 5.66 |
| 12 | 7 | 35 | 30 | 5.66 |
| 13 | 7 | 70 | 0 | 38.13 |
| 14 | 7 | 0 | 0 | 38.56 |
| 15 | 3 | 35 | 30 | 5.40 |
| 16 | 3 | 70 | 60 | 0.00 |
| 17 | 3 | 0 | 60 | 0.00 |
| 18 | 5 | 35 | 60 | 0.00 |
| 19 | 7 | 70 | 60 | 0.00 |
| 20 | 7 | 0 | 60 | 0.00 |

The equation given by the software was:

$$\begin{aligned} \text{Microemulsion Area (\%)} = & +40.12 - 1.28\text{E} - \\ & 003 \text{ Ionic Strength} - 0.647 \text{ pH} - 1.56 \text{ Sugar Conc.} - \\ & 1.84\text{E} - 003 \text{ Ionic Strength} \times \text{pH} - 2.02\text{E} - 005 \text{ Ionic} \\ & \text{Strength} \times \text{Sugar Conc.} + 2.56\text{E} - 003 \text{ pH} \times \text{Sugar} \\ & \text{Conc.} + 1.51\text{E} - 004 \text{ Ionic Strength}^2 + 0.06 \text{ pH}^2 + \\ & 0.02 \text{ Sugar Conc.}^2 \end{aligned}$$

The statistical significance of the model equation was evaluated by the F-test for analysis of variance (ANOVA). The F-value (5221.30) of obtained model shows the significance of the quadratic model. In this model, sugar concentration and sugar concentration² were found as the most significant parameters. In addition, the obtained Lack of Fit F-value of 1.33 implies the Lack of Fit was not significant relative to pure error. Furthermore, Fig. 4 depicts combined effect of pH and ionic strength on microemulsion area (%) at a constant sugar concentration as a response surface plot. The microemulsion area of T60:propanol system was not affected by pH, ionic strength or their interactions. Since these parameters and their interactions were detected to be not significant in the model, therefore their results appeared in a flat form (Fig. 4). Consequently, the three-dimensional

response surface curves for microemulsion area (%) had two stationary parts along with two dimensions of pH and ionic strength, which means that microemulsion area was not altered when pH or ionic strength were changed. The Brij-35 stabilized eucalyptus oil/butanol system was also indifferent toward salinity of the system with NaCl, but inorganic salts had strong effects on the behavior of microemulsions which stabilized by ionic surfactants as it was observed for NaCl in Rajib and Bidyut studies (Rajib & Bidyut, 2005). It is reported that the shielding effect of NaCl on polar head group of an O/W microemulsion droplet increases the cohesive interaction among the droplets and subsequently could result in the coagulation of droplets and decreasing the water solubility of AOT and cause the phase separation of the microemulsion (Rajib & Bidyut, 2005). T60, as a powerful steric stabilizer with no effect on electrostatic stabilization due to its non-ionic nature, is a valuable polysorbate emulsifier for preparing emulsions or concentrates that must be diluted by water with unknown hardness (Whitehurst, 2004; Polizelli *et al.*, 2006).

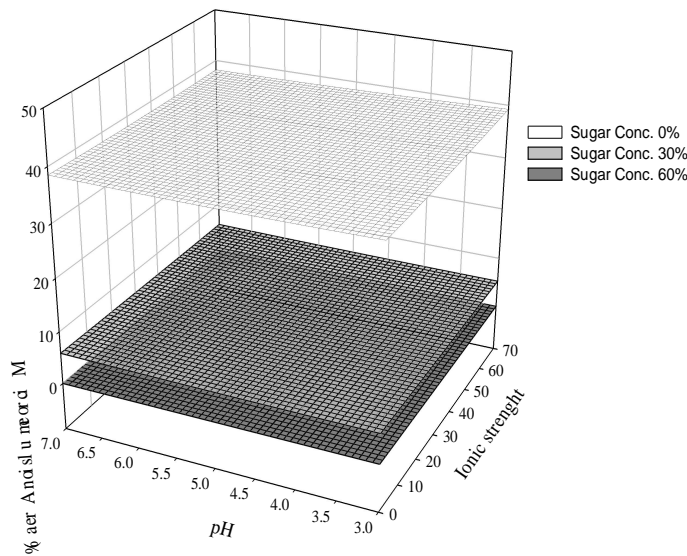


Fig. 4. Response surface for microemulsion area (%) as a function of ionic strength (mM) and pH at constant sugar concentration.

The three-dimensional response surface curve for microemulsion area (%) had a decreasing part in one dimension with a stationary part in another dimension (Fig. 5). The stationary point is the

point at which the slope of the response surface is zero when taken in all directions. The response surface decreased as sugar concentration was increased as at sugar concentration of 60%, no

microemulsion zone was observed. Therefore, sugar concentration played an important role in the microemulsion formation and area. The response surface had a stationary region in different pH

(Fig. 5) or ionic strength (not shown) variations, indicating that pH or ionic strength had no effect on microemulsion area.

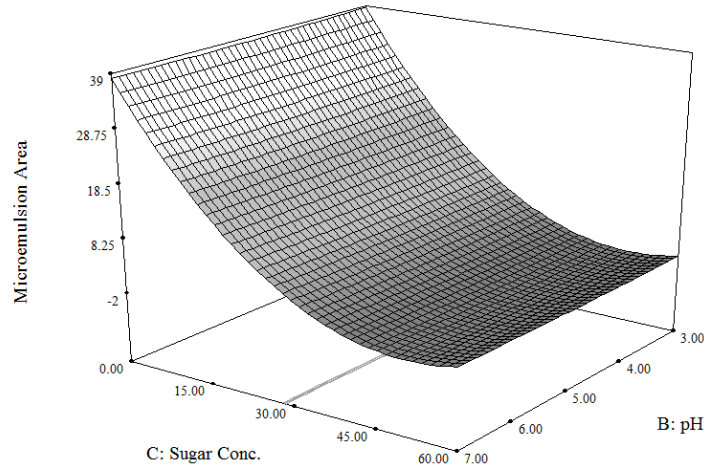


Fig. 5. Response surface for microemulsion area (%) as a function of sugar concentration (%) and pH at constant ionic strength of 35%.

Validation of optimized results

In order to verify the model, several experiments (5%, 10% and 20% sugar concentration) were performed under the predicted conditions. The experimental values did not agree with the results obtained from the model, showing the unsuitability of the model for prediction of microemulsion area (%) due to weak prediction on sugar concentration. As a result, in the next step for strict modeling of sugar concentration effect,

the pH and ionic strength parameters were omitted and the sugar concentration was given to Design expert software between two levels of -1 (0) and +1 (40%). Therefore, one factor design was applied by the software. The variety of values chosen by the software for sugar concentration was much more than the previous one (Table 2). Ten experiments were planned and the response, microemulsion area (%), was measured.

Table 2- Effect of sugar concentration on microemulsion area.

| Runs | Sugar concentration (%) | Microemulsion area (%) |
|------|-------------------------|------------------------|
| 1 | 0.00 | 38.66 |
| 2 | 25.00 | 8.15 |
| 3 | 30.00 | 4.38 |
| 4 | 5.00 | 38.99 |
| 5 | 40.00 | 0.27 |
| 6 | 0.00 | 38.13 |
| 7 | 15.00 | 32.19 |
| 8 | 40.00 | 0.26 |
| 9 | 20.00 | 23.53 |
| 10 | 20.00 | 23.04 |

Software suggested a cubic model as follows:

$$\text{Microemulsion Area (\%)} = +37.83 + 1.47 \text{ Sugar Conc.} - 0.17 \text{ Sugar Conc.}^2 + 2.64\text{E-}003 \text{ Sugar Conc.}^3$$

The ANOVA results of cubic model for the one dependent variable (sugar concentration) are presented in Table 3. The F-value (152.62) of model for sugar concentration implied that the cubic model was significant. There is only a 0.01% chance that a "Model F-Value" could occur due to noise. Values of "Prob>F" less than 0.05 indicate that the model terms are significant and hence the cubic model can be used for prediction of microemulsion area when sugar concentration is changing from 0 to 40%. In this case, A and A^3 (Table 3) were significant model terms and values greater

than 0.1000 indicate that the model terms were not significant. The "Lack of Fit F-value" of 116.33 also implies the significance for sugar concentration. Moreover, there was only a tiny chance (0.13%) that a "Lack of Fit F-value" could occur due to noise. The normal % probability versus studentized residuals graphs for responses of microemulsion area yielded fairly straight line (Fig. 6), showing normal distribution of the data. Hence, the residual plots indicated a normal distribution and there was no apparent problem with normality in all cases.

Table 3- ANOVA analysis for response "mono-phasic microemulsion area" with one factor at a time (sugar concentration).

| Source | Sum of squares | DF | Mean square | F value | Prob > F | |
|--------------------|----------------|----|-------------|---------|----------|-------------|
| Model | 2321.09 | 3 | 773.70 | 152.62 | < 0.0001 | significant |
| A^a | 544.81 | 1 | 544.81 | 107.47 | < 0.0001 | |
| A^2 | 17.48 | 1 | 17.48 | 3.45 | 0.1127 | |
| A^3 | 129.67 | 1 | 129.67 | 25.58 | 0.0023 | |
| Residual | 30.42 | 6 | 5.07 | | | |
| Lack of Fit | 30.16 | 3 | 10.05 | 116.33 | 0.0013 | significant |
| Pure Error | 0.26 | 3 | 0.086 | | | |
| Cor Total | 2351.51 | 9 | | | | |

^aA=Sugar concentration

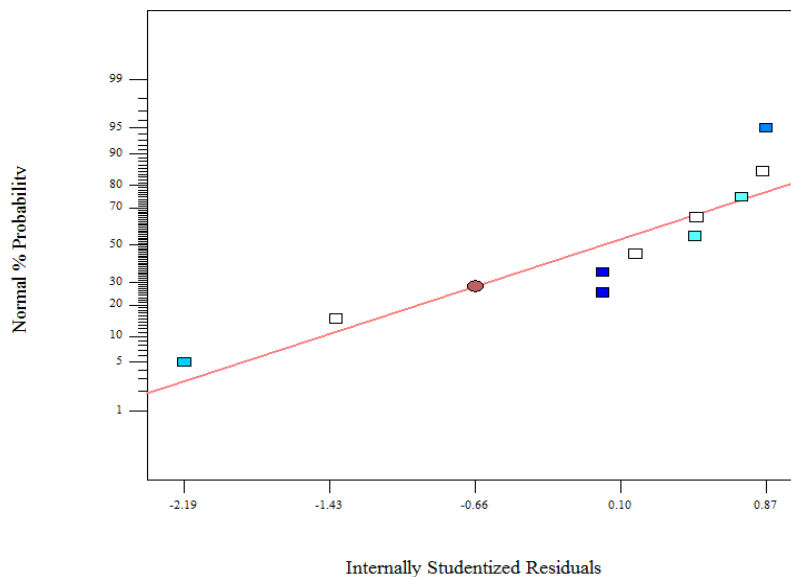


Fig. 6. Normalized % probability versus studentized residuals for microemulsion area (%) with one factor.

Fig. 7 depicts actual microemulsion areas, the measured response data obtained by experiments and the predicted values

obtained by cubic models. High correlation coefficient of 0.9865 was indicating good

microemulsion area prediction of model with variation in sugar concentration.

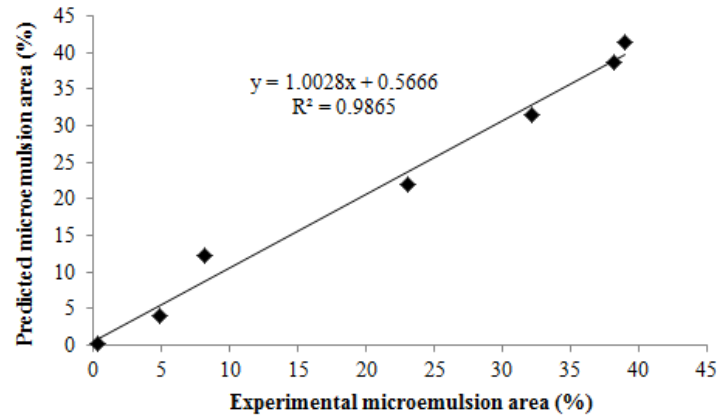


Fig. 7. Correlation between experimental and predicted microemulsion areas in the presence of different sugar concentration.

The model given by RSM for predicting the combined effect of sugar concentration and temperature on the microemulsion area (%) (the following model), was recognized unsuitable, as the experimental data did not confirm the predicted model (data not shown). It was concluded that RSM is not suitable for predicting the effect of factors on the response, where response (Table 4)

doesn't have a constant trend (a decreasing or increasing trend). Consequently, the combined effect of sugar concentrations (0, 5, 10, 15, 20, 25 and 30%) and temperature (5, 25 and 45°C) on the microemulsion area were evaluated in which its 3D surface plot is shown in Fig. 8.

$$\text{Microemulsion Area (\%)} = +44.56599 - 1.16820 \times \text{Sugar Conc.} - 4.65090 \times 10^{-3} \times \text{Tem}$$

Table 4- Microemulsion areas (%) of OPO microemulsions with the surfactant:cosurfactant ratio of 1:1 under different sugar concentrations (%) (S) and temperatures (°C) (T).

| Microemulsion (Sugar conc.) | Microemulsion area (%) | | |
|-----------------------------|------------------------|-------|-------|
| | 5°C | 25°C | 45°C |
| 0% | 35.76 | 38.66 | 39.98 |
| 5% | 38.05 | 38.99 | 44.64 |
| 10% | 36.18 | 38.15 | 38.82 |
| 15% | 33.87 | 32.19 | 23.40 |
| 20% | 27.71 | 23.04 | 11.52 |
| 25% | 12.18 | 8.15 | 1.23 |
| 30% | 5.11 | 4.89 | 0.53 |

Based on our findings, low concentration of sugar (<5%) had no effect on the microemulsion area, whereas at higher concentrations (>5%) microemulsion area decreased. However, this decrease was very low at 10% sucrose. Regarding the justification of these behaviors it can be said that sucrose most likely affects the hydration capacity of the surfactant head group as high sugar concentrations attract the water and takes water away from the access of surfactant.

That is why with increasing sucrose concentration, the W/O type microemulsion was impeded till completely disappeared (at 25% sucrose). Indeed, the water of high sugar aqueous solutions is highly bonded with sucrose molecules. Such phenomenon is true for both O/W and W/O microemulsion particularly for W/O microemulsion due to its lower amount of water which results in sugar precipitation. Similar results were obtained in Brij-35 stabilized eucalyptus oil/butanol

system in the presence of glucose (Rajib & Bidyut, 2005).

Evaluating the combined effect of temperature and sugar concentration showed that both parameters are effective on the microemulsion area (Fig. 9). With no sugar addition or at low concentrations of sugar (up

to 5%), the microemulsion area was increased slightly with increasing temperature from 5 to 45°C. This was due to decrease in the surface tension of water with temperature (Shahin & Servet, 2006), which results in wider microemulsion zones.

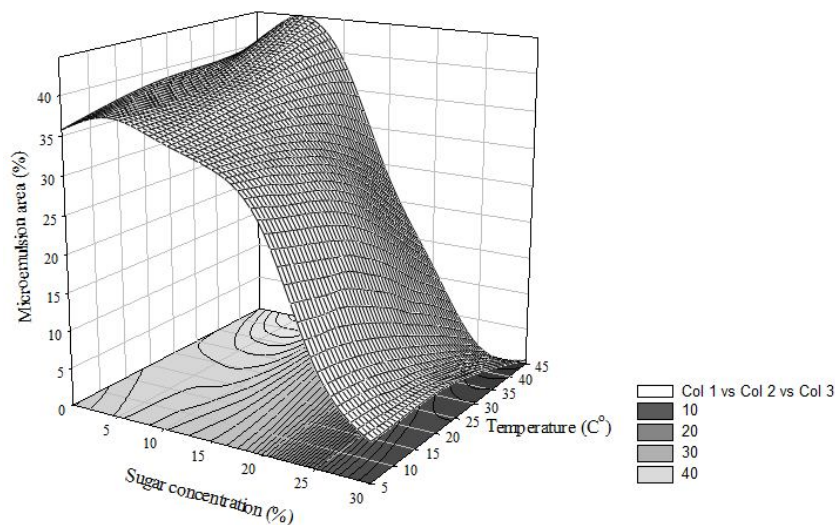


Fig. 8. Response surface for microemulsion area (%) as a function of temperature (C°) and sugar concentration (%).

At 10% sugar concentration, less microemulsion zone was constructed at 5°C due to higher surface tension of sucrose solutions (Shahin & Servet, 2006), but a slight increase was observed in the microemulsion area when temperature was increased from 25 to 45°C (Fig. 8). It seems that in 10% sugar concentration, the concentration is not such high for immobilizing the water, but can form hydrogen bonds with polyoxyethylene chains of T60, and hence increased the hydrophilicity of the surfactant (Rajib & Bidyut, 2005). Increasing in the hydrophilicity of the surfactant results in W/O microemulsion region reduction, as simultaneously increases the O/W microemulsion zone. Therefore, increasing the extent of a region in the microemulsion area and decreasing another part of the microemulsion area, causes no significant increase in the microemulsion area from 25 to 45°C.

At higher sugar concentrations (>10%), a significant decrease in the microemulsion area was observed with increasing temperature. As it was described before, at higher sugar concentrations, the amount of water in which forms hydrogen bonds with polyoxyethylene chain of T60, decreases as it was bonded with sugar molecules. The amount of free water which is available for microemulsion formation diminished more with increasing temperature due to breakage of hydrogen bonds between the water molecules and the hydrophilic head of the surfactant molecules. This results in decreasing the microemulsion area. Such reduction is more pronounced in W/O microemulsion regions as the low amount of their water becomes inaccessible. Consequently, the O/W region is less affected than W/O or bicontinuous zones. In such systems (with high sugar concentrations), precipitation of sugar was observed when breakage of microemulsion occurred.

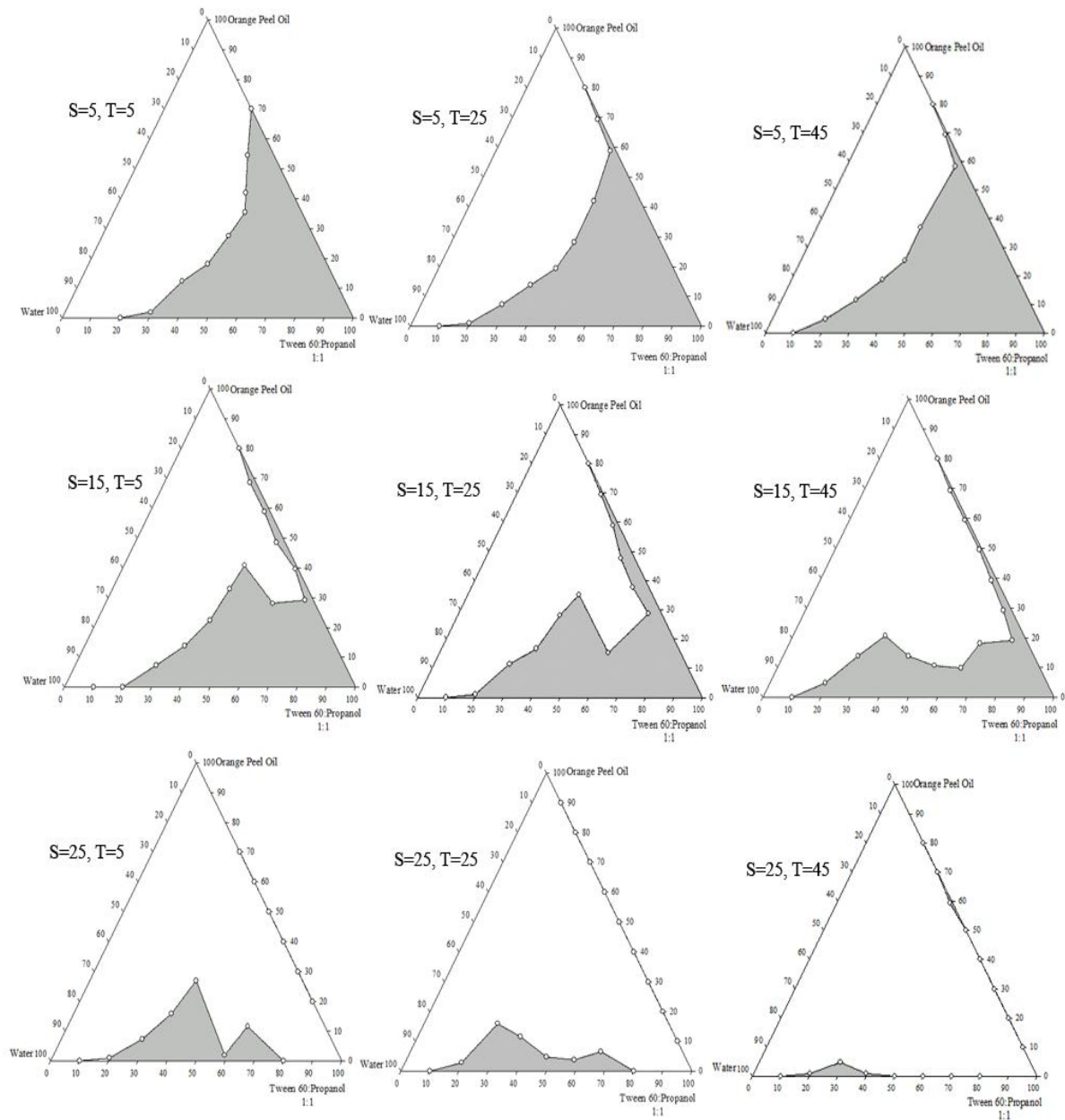


Fig. 9. Phase diagrams of OPO microemulsions with the surfactant:cosurfactant ratio of 1:1 under different sugar concentrations (%) (S) and temperatures (C°) (T). All ratios were by weight.

Heat stability

The effect of sucrose concentration on the critical temperature, above which microemulsion becomes turbid, was examined (Fig. 10). This critical temperature is defined as the cloud point of the surfactant at a particular concentration. In the formulated

microemulsion systems, the concentration of sugar was changed from 0 to 30% while the other formulation components such as OPO (1%), T60: propanol (34%) and water (65% wt.) were fixed. Systems with higher sucrose concentrations became turbid at lower temperatures while by decreasing in sugar

concentration, the maximum temperature in which one-phase microemulsion system was still stable, shifted to higher temperatures as reached to 90°C for the samples without sugar. By further heating the solutions above the critical temperature, the emulsions became turbid. Cloudiness is associated with a rapid increase in the micelle aggregation, with the formation of long cylinders (Tadros, 2005). It is also worthy to note that the temperature ranges in which microemulsion formation occurred on heating overlap well with the temperature ranges in which microemulsion formation occurred on cooling. Temperature

increase, significantly changes the hydrophile-lipophile balance (HLB) of nonionic surfactant, due to the dehydration of the oxyethylene group which results in more lipophilicity of the surfactant and phase separation of the microemulsion. On the other hand, the effect of temperature is less evident for ionic surfactants (Rajib & Bidyut, 2005). Rajib and Bidyut (2005) found the Brij-35 stabilized eucalyptus oil/butanol system highly dependent on temperature, whereas the AOT and mixed (AOT+ Brij-35) stabilized systems were insensitive to temperature.

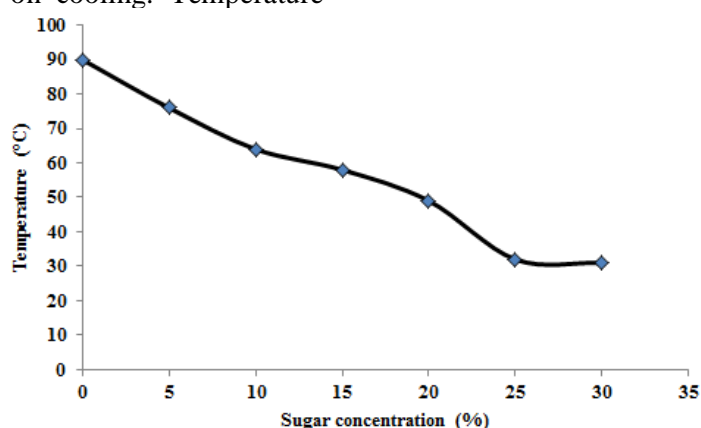


Fig. 10. Effect of sugar concentration on the critical temperature of OPO microemulsion formation.

The stability of OPO microemulsions at 5, 25 and 45°C

The stability of the prepared microemulsions was also examined at three

temperatures of 5, 25 and 45°C for seven days (Table 5). All samples were stable at 5 and 25°C.

Table 5- Influence of storage temperature and sugar concentration on the stability of OPO (1%)/ T60: propanol (1:1) (34%) and water (65% wt.) microemulsion for seven days.

| Sugar conc. (%) | Storage temperature (°C) | | | |
|-----------------|--------------------------|--------|--------|----------|
| | -3 | 5 | 25 | 45 |
| 0 | unstable | stable | stable | stable |
| 5 | unstable | stable | stable | stable |
| 10 | unstable | stable | stable | stable |
| 15 | unstable | stable | stable | stable |
| 20 | unstable | stable | stable | stable |
| 25 | unstable | stable | stable | unstable |
| 30 | unstable | stable | stable | unstable |

The samples which contained high concentrations of sugar (25 and 30%) were turbid at 45°C, whereas all other samples exhibited a one-phase microemulsion system at this temperature. On the basis of our

findings, microemulsions were not stable at freezing temperatures (-3°C). At subzero temperatures, the water is frozen and the essential oil is solidified. These factors result in separation of the aqueous and oily phases

from the microemulsion system and therefore the breakage of microemulsion.

Sensory profile

The microemulsified OPO were dissolved in the solutions as fast as adding to the medium. It was completely in contrast with what happened for the free essential oil as it was spreading on the surface of the solutions. Presence of CO₂ did not influence the solubility of the microemulsion. Statistical analysis revealed significant differences between samples containing free and encapsulated OPO. No significant differences were recognized in color and viscosity of all samples for the free and encapsulated essential oil in comparison with control (without OPO).

Although the odor and taste intensity of free oil in all samples were higher than the capsulated ones, samples containing OPO microemulsions were recognized as preferred samples in comparison with samples containing free OPO ($p < 0.05$). Encapsulation of OPO caused lower release of aroma, resulting a milder odor and taste (lower intensity) in samples which were preferred by

the panelists. The lower intensity of odor and taste in samples containing microemulsified OPO were verified for all samples by the assessors. The overall acceptability of all samples containing OPO microemulsion was significantly higher than the samples with free oil ($p < 0.05$).

Conclusions

Results obtained from this study showed that sugar concentration and temperature affect the microemulsion area of OPO/ T60: propanol system, significantly. Moreover, microemulsion area and the heat stability of the OPO microemulsions were also affected by sucrose addition as the heat stability was decreased with increasing sucrose concentration. The effectiveness of microemulsions in encapsulating of OPO was proved by panelists who confirmed the lower odor and taste intensity of carbonated water containing microemulsified OPO in comparison with sample containing free essential oil.

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تأثیر pH، قدرت یونی، دما و غلظت شکر بر شکل‌گیری میکروامولسیون اسانس پوست پرتقال / توئین 60: پروپانل و آب با استفاده از روش سطح پاسخ

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چکیده

میکروامولسیون‌ها سامانه‌هایی هموزن هستند که قطر ذرات فاز پراکنده در آن‌ها کمتر از 100 نانومتر می‌باشد. از آن‌جا که شکل‌گیری این سامانه‌ها، تحت تأثیر پارامترهای مختلفی قرار می‌گیرد، در این تحقیق تشکیل میکروامولسیون اسانس پوست پرتقال با استفاده از توئین 60 و پروپانل (با نسبت 1:1) تحت شرایط مختلف pH، قدرت یونی، غلظت شکر و دما مورد مطالعه قرار گرفت. برای این منظور، از روش سطح پاسخ برای تعریف تعداد آزمون‌ها و ترکیب فاکتورهای مورد بررسی استفاده گردید. نتایج نشان داد که pH و قدرت یونی و تعامل آن‌ها در شکل‌گیری میکروامولسیون موثر نیستند و سطح میکروامولسیون تحت تأثیر پارامترهای مذکور قرار نمی‌گیرد. غلظت شکر به‌طور معنی‌داری بر شکل‌گیری میکروامولسیون موثر بود و با افزایش غلظت شکر از صفر به 30 درصد، منطقه میکروامولسیون از 38% به حدود 4/5% کاهش یافت. مدل کیوبیک تأثیر غلظت شکر بر سطح میکروامولسیون را به خوبی پیش‌بینی کرد. علاوه بر این، منطقه میکروامولسیون با افزایش دما در غلظت‌های پایین شکر (تا 10%) افزایش یافت اما در غلظت‌های بالاتر شکر کاهش معنی‌داری را نشان داد. همچنین پایداری حرارتی میکروامولسیون اسانس پوست پرتقال / توئین 60: پروپانل / آب با افزایش غلظت شکر به شدت کاهش یافت. نتایج آنالیز حسی نشان داد که استفاده از میکروامولسیون اسانس پوست پرتقال در آب گازدار در مقایسه با اسانس آزاد، انحلال و پذیرش محصول را خصوصاً از نظر فاکتورهای طعم و آروما به‌طور معنی‌داری بهبود بخشید.

واژه‌های کلیدی: میکروامولسیون، اسانس پوست پرتقال، روش سطح پاسخ، مدل کردن

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