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The effect of ingredients' concentration on nanoemulsion particle size of eucalyptus essential oil

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This study aimed to determine the effect of different parameters affecting the particle size of eucalyptus-essential oil nanoemulsion using artificial neural networks. The formulation of nanomaterials used in this study included Tween 80 (as surfactant), ethanol (as co-surfactant), and eucalyptus essential oil (as internal phase), in water. To prepare the nanoemulsion, the mixtures were sonicated and the size of resulting nanoparticles were measured using dynamic light scattering (DLS). Then, the data were modelled using artificial neural networks. The results revealed that high Tween 80 concentration and low essential oil concentration had positive effects on reduction of particle size, while the change in ethanol concentration had no obvious impact on the size. The prepared nanoemulsions has the potential to be used in biomedical applications.

Keywords: Artificial neural networks, Essential oil, Eucalyptus, Nanoemulsion, Particle size.

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Introduction

Nanoemulsions are promising delivery systems which have the potential to improve stability, solubility, bioavailability, and permeability of different bioactive agents[1]. Nanoemulsions are homogeneous systems that consist of two immiscible liquids to form nanoscale droplets[2-5]. The particles can exist in oil-in-water (O/W) or water-in-oil (W/O) forms[6]. The insolubility of the oil phase in water leads to creation of interfacial tension which can destabilize the nanoemulsion. To prevent this, surfactant(s) and co-surfactant(s) are used to minimize the interfacial tension and increase nanoemulsion's stability[6,7]. The produced particles, as nanocarriers, promise bright futures in diagnosis and treatment, especially in drug delivery fields[2,8,9]. Nanoemulsions are resistant to destabilization processes such as flocculation, coalescence, and deposition[2,6]. They also have a high surface area, thereby, have a high potential for transport and delivery of drugs, especially, water-insoluble ones[5,6,10,11].

Along centuries, medicinal plants have been used for treatments of numerous diseases[1,12]. Essential oils (EOs), as compounds which are extracted from plants, are the most interesting and efficient natural products which are commonly used in pharmaceutical and biomedical applications[13]. Eucalyptus is a herb which is commonly used for treating respiratory tract diseases and common cold[14,15] and its EO is a famous EO for cold and cough treatment[16]. However, use of Eucalyptus essential oil (EcEO) in different products is limited due to its high volatility, low stability, and low solubility[12,13,17].

In this study, EcEO was used as internal phase to prepare a nanoemulsion, to be used for inhalation purposes. By formulating EOs in nanoemulsions, interesting formulations for delivery of hydrophobic drugs may be obtained. On the other hand, various studies have shown that the efficiency and uptake of nano drug depend on its size[18-20]. For instance, reduction in particle size leads to an increase in surface area, thereby increases the efficiency of drug delivery[21].

Artificial neural networks (ANNs) are computer modeling programs to examine complex and non-linear relationships between input and output parameters. They can be used for medical and pharmaceutical applications[18,22-25]. In the present study, EcEO nanoemulsion was optimized by ANNs and factors affecting the size of nanoparticles were evaluated using this modelling network.

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Materials and Methods

Materials

Eucalyptus essential oil (EcEO) was purchased from Golkaran cultivation and industry co. (Iran). Tween 80 was purchased from Sigma-Aldrich (Germany) and ethanol was purchased from Pars alcohol company (Iran).

Preparation of eucalyptus nanoemulsion and particle size measurement

The O/W nanoemulsion was formulated using EcEO, non-ionic surfactant (Tween 80), co-surfactant (ethanol 70%), and water. Various proportions of the ingredients were tested to obtain the optimal percentage to prepare the most stable product which could remain clear with no sign of phase separation (e.g. presence of particles or precipitation). Initially, the emulsion was prepared by adding ethanol to the oil and surfactant mixture using a magnetic stirrer at 3000 RPM for 10 minutes. Then, water was added and the mixture was probe-sonicated for 10 minutes at 20 W. The Sonication process was performed on the ice to prevent overheating. Afterwards, macroscopic characteristics of prepared nanoemulsions were observed for one week to monitor stability. The droplet size of nanoemulsions was analyzed using the DLS technique (Scatteroscope I, K-ONE, Korea) after 1:10 dilution[26].

ANNs modelling

Software tool

In this study, ANNs modelling was performed using INForm v4.02 (Intelligensys, UK). The INForm software is a commercial ANNs software, which uses neural communications to model the non-linear and complex relations between the inputs and the output. The results are shown as three-dimensional graphs response surfaces to illustrate the effect of two input variables on the output when the value of other inputs are fixed.

Data set, training parameters and validation

In this study, three factors including concentrations of oil, Tween 80, and ethanol were used as input variables and nanoparticles size was the single output. Twenty-one samples were experimentally prepared and their particle size was measured using DLS. Experimental data were randomly divided into three data sets: training, test and validation data set to perform the modelling procedure. The training data sets were used to train the ANNs and clarify the relationships between the input variables and the output, while test data sets were applied to prevent overtraining (Table 1). Test data were 10% of the training data sets which were randomly selected by the software. The training parameters are given in Table 2 and in more details

Table 2 — The training parameters set with INForm v4.02

Network structure	Number of inputs	3
	Number of hidden layers	1
	Number of nodes	2
Backpropagation type		Quick prop
Transfer function	Transfer type	Symmetric sigmoid
	Output transfer type	Tanh

Table 1 — The training and test data sets used in ANNs modeling

Sample no.	Eucalyptus oil (%)	Tween 80 (%)	Ethanol (%)	Observed particle size (nm) after dilution	Predicted particle size (nm) after dilution
1	1.2	7.9	0.9	10.9	12.1
2	3.1	6	3	20	17.7
3	1	9.1	6.1	13.7	12.1
4	2	8.9	7.1	13.1	12.2
5	7	10	10	62.8	61.5
6	1	5.2	7.1	14.2	13
7	5.1	8	1.1	18.9	19.8
8	2.1	5.1	4.9	14.2	16.5
9	1.1	10.1	6.1	13.1	12.2
10	1	9.9	10.1	12.2	12.3
11	6	8	7	28.9	29.7
12	2	7.1	1	13	12.6
13	5	9.1	10.4	18.7	19.6
14	4	10	4.1	12.6	13.4
15 *	4.1	5	9	38.7	32.4
16 *	2	7.8	8.3	14.1	12.3

*The last two individual data set show the “test data”.

in our previous report[23]. Moreover, 5 experiments results were excluded from training/testing processes to be used as validation or unseen data to validate the trained model (Table 3). After network training, the quality and predictability of trained models were evaluated using correlation coefficient, R-squared (R^2), for all data sets. The best model has the highest R^2 for all data sets.

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}$$

Using the equation, y is the mean of observed value and ŷ is the predicted value from the model.

Eventually, the validated model was used to assess the effect of input variables on nanoparticle size.

Results

In present study, ANNs software was used for semi-quantitative evaluation of the relationships between concentrations of Tween 80, ethanol, and EcEO as input variables on particle size (output variable). After modelling the experimental data using ANNs, the best predictive model provided R^2 values of 0.99, 0.85 and 0.81 for the training, test and validation data sets, respectively. These R^2 values revealed a satisfactory quality and predictability for the trained model. The measured particle size by DLS and predicted values by the model are shown in Tables 1 and 3. Subsequent to validation, response surfaces (3D graphs) were used to illustrate the effect of two input variables on particle size, while the third input variable was fixed at a low, mid-range or high value[27].

Initially, to evaluate the effect of Tween 80 and oil on particle size, ethanol concentration was fixed at low, mid-range, and high values (i.e. 2.5, 5.7, and 8.8 mg/mL, respectively). From Fig. 1, the rise of oil concentration leads to increased particle size, regardless of whether the ethanol value is high or low. Whereas, Tween 80 concentration has no important effect on the particle size.

Fig. 2 was used to show the effects of ethanol and Tween 80 on the particle size when oil concentration was fixed at low, mid-range and high values (i.e. 2, 4, and 6 mg/mL, respectively). As shown, the increase in Tween 80 concentration generally leads to decrease in the nanoparticle size when oil concentration was fixed. It should be noted that this size reduction when oil concentration was high, was more pronounced compared to low value of oil concentration. Also, in general, the increase in ethanol concentration did not show an important effect on the nanoparticle's size.

Table 3 — The validation data set used in ANNs modeling

Sample no.	Eucalyptus oil (%)	Tween 80 (%)	Ethanol (%)	Observed particle size (nm) after dilution	Predicted particle size (nm) after dilution
1	1.1	9.0	9.0	13.7	12.1
2	2.1	5.9	10.1	15.2	13.4
3	3.0	7.1	2.0	13.3	13.7
4	6.0	9.1	5.0	28.0	24.4
5	2.0	9.9	2.0	15.2	12.2

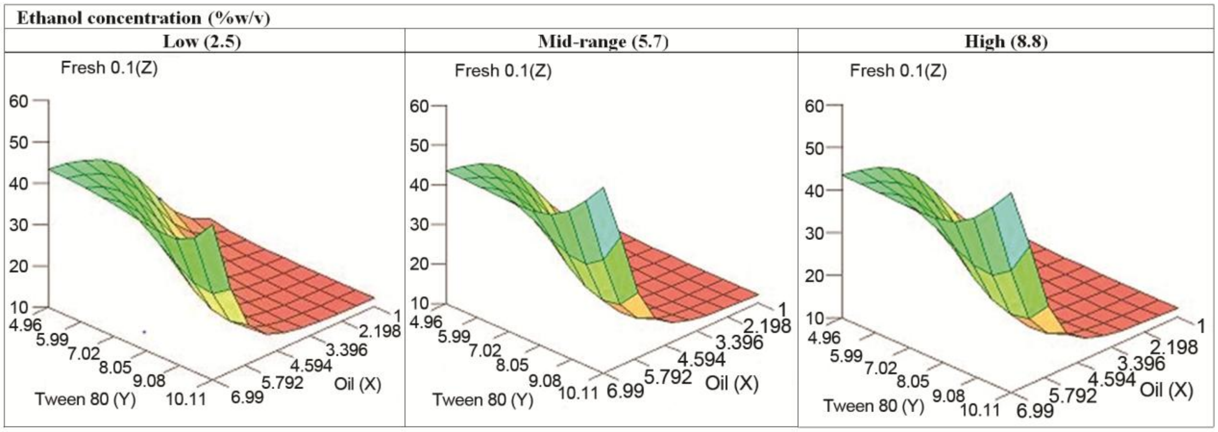

Fig. 1 — 3D plot of particle size (nm) predicted by the ANNs model against the concentration of oil and Tween 80. In each figure, the effect of two input variables on nanoparticle sizes was evaluated when ethanol was fixed at a low, mid-range, and high value.

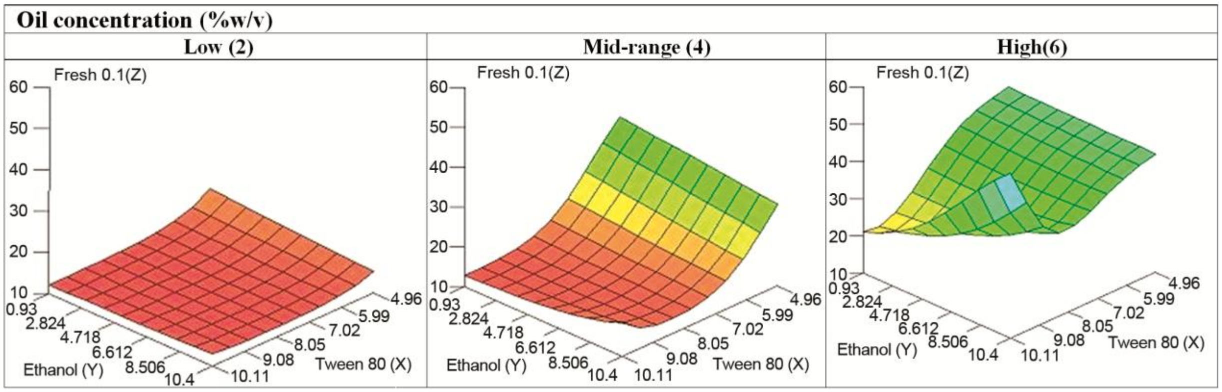

Fig. 2 — 3D plot of particle size (nm) predicted by the ANNs model against the concentration of ethanol and Tween 80. In each figure, the effect of two input variables on nanoparticle sizes was evaluated when oil was fixed at a low, mid-range, and high value.

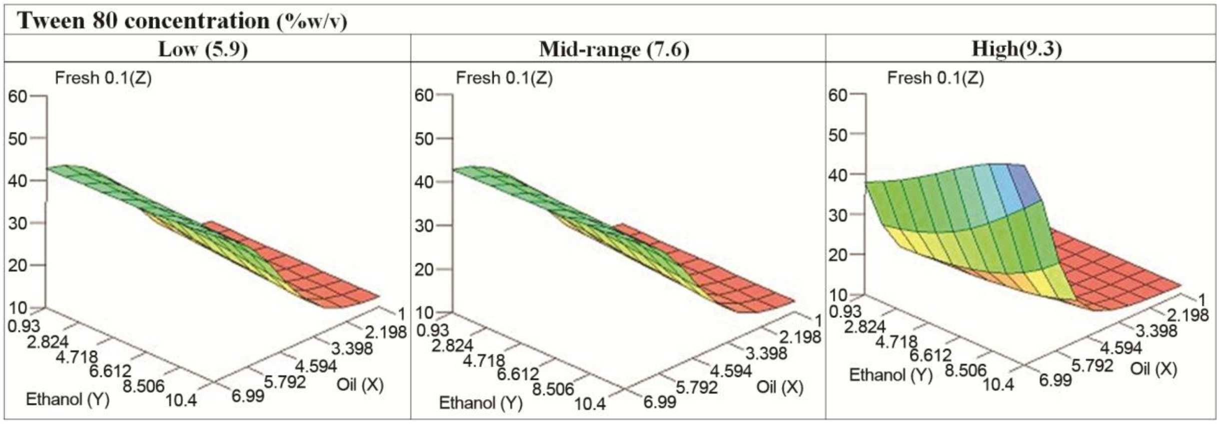

Fig. 3 — 3D plot of particle size (nm) predicted by the ANNs model against the concentration of ethanol and oil. In each figure, the effect of two input variables on nanoparticle sizes was evaluated when Tween 80 was fixed at a low, mid-range, and high value.

Moreover, the effects of ethanol and oil on particle size were evaluated when Tween 80 was fixed at low, mid-range, and high values (i.e. 5.9, 7.6, and 9.3 mg/mL, respectively). The results are shown in Fig. 3 and confirm the above findings. As mentioned above, ethanol has no considerable effect on the particle size, while high oil concentration increased the particle size.

Discussion

Nanoemulsions are being introduced as promising carriers in diagnosis, treatment, and drug delivery purposes. They have a high surface area, thereby have a high potential for transport and delivery of drugs. Moreover, it has already been reported that drug permeation enhances by decreasing particle size. Accordingly, we optimized EcEO nanoemulsion by ANNs and used this modelling network to evaluate the effects of input variables on the size of nanoparticles.

In current study, findings revealed that high concentration of Tween 80 and low concentration of oil contributes to decreased particle sizes. Tween 80 serves as a surfactant and makes the particles turn into smaller but more stable particles[28]. Oil concentration is another effective factor on particle size. It is arguable that at high oil concentration, the oil droplets agglomerate to form larger particles, leading to an increase in particle size of the nanoemulsion. Our findings are in agreement with a previous study which reported that the droplet size of nanoemulsions increased with an increase in the concentration of essential oil (i.e. from 32.45±2.84 to 142.35±10.58 nm when EO concentration increased from 12 to 28%)[29]. Another study showed that increasing the olive oil percentage led to larger droplet size of NEs[30]. In a research on palm oil esters, it was indicated that with increase in oil concentration, particle size increased whereas increase in Tween 80 concentration decreased the particle size[31]. It should be noted that results of some studies do not appear to agree with our findings. For instance, increase in the concentration of sesame oil and olive oil has been reported to decrease the particle size[32]. In summary, findings from different studies reveal that determining the size of the nanoparticles is a very complex process and depends on various factors, including the type and amount of oil[33]. Therefore, it is suggested that each nanoemulsion preparation should be investigated separately, and no comprehensive rule may be obtained for all the nanoemulsion preparations.

Conclusion

In the present study, ANNs were used to evaluate the effect of three input factors on droplet size of EcEO nanoemulsion. The model showed that oil concentration is the dominant factor in determining the particle size with a direct effect, while Tween 80 concentration showed reverse effects on particle size. Also, it was shown that ethanol had no important effect on the particles size. Considering the particle size obtained here, the preparation may be used in pharmaceutical and biomedical applications.

Conflict of interest

The authors declare that there is not any conflict of interest.

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