



# Effect of Processing/Formulation Parameters on Particle Size of Nanoemulsions Containing Ibuprofen - An Artificial Neural Networks Study

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## Abstract

**Background:** Nanoemulsions are colloidal transparent systems for the delivery of hydrophobic drugs. This study aimed to determine the effect of parameters affecting particle size of a nanoemulsion containing ibuprofen using artificial neural networks (ANNs).

**Methods:** Nanoemulsion samples with different values of independent variables, namely, concentration of ethanol, ibuprofen and Tween 80 as well as exposure (homogenization) time were prepared and their particle size was measured using dynamic light scattering (DLS). The data were then modelled by ANNs.

**Results:** From the results, increasing the exposure time had a positive effect on reducing droplet size. The effect of concentration of ethanol and Tween 80 on droplet size depended on the amount of ibuprofen. Our results demonstrate that ibuprofen concentration also had a reverse relation with the size of the nanoemulsions.

**Conclusion:** It was concluded that to obtain minimum particle size, exposure (homogenization) time should be maximized.

## Introduction

Nanoemulsion systems are promising and useful tools for delivery of hydrophobic drugs. They are colloidal transparent systems consisting of oil and aqueous phase with low viscosity in the nano-droplet size.<sup>1</sup> Nanoemulsion-based topical delivery systems are interesting formulations that employ different mechanisms to improve delivery of hydrophobic/hydrophilic drugs.<sup>2</sup> Such efficient formulations offer advantages such as enhancing drug solubilization capability, increasing shelf life, improving bioavailability of drugs and facilitating the preparation process.<sup>3</sup>

To break droplets during formation of nanoemulsions, usually, a considerable amount of energy is required. A common approach to provide such mechanical energy is use of ultrasound homogenizers.<sup>4</sup> In this process, exposure time (homogenization time) is an important factor that influences properties of the prepared nanoemulsions,<sup>5</sup> so that in most cases, droplet sizes gradually decreases when the time increases.

Nanoemulsion systems are thermodynamically/kinetically stable with a defined combination of surfactant/co-surfactant. The specifications of surfactant and co-

surfactant play substantial role in formation and properties of nanoemulsions.<sup>6</sup> The studies so far have reported that concentration of surfactant/co-surfactant must be minimum to reduce their toxic effects, while it should be high enough to provide proper surface tension<sup>7</sup> and stability<sup>8</sup> for the preparation.

Ibuprofen, a non-steroidal anti-inflammatory drug (NSAID), can be used to reduce fever and control mild/moderate pain and inflammation caused by many conditions. Topical delivery of ibuprofen can be useful for preventing its adverse effects and simultaneously avoid the first-pass metabolism. To increase permeation of ibuprofen via topical delivery, different formulations/methods have been reported. For example, use of supersaturated solutions,<sup>9</sup> and polyoxyethylene alkyl ethers (as penetration enhancer)<sup>10</sup> have been proposed while no approach has shown to be universal yet. A more recently suggested approach is application of nanoemulsions in delivering lipophilic drugs which has shown promising.<sup>11</sup>

Several factors must be considered to achieve an effective nanoemulsion-based topical delivery system, one of which is droplet size. The droplet size of nanoemulsion is an

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important parameter in determining the permeation of the cargo to the skin cells.<sup>12</sup> Smaller sizes substantially increase the interfacial area, thus, the drug can rapidly release to the target. Generally, the droplet size determines the release rate from the carrier.<sup>13</sup> Due to the role of droplet size in permeability through skin, it is necessary to find the parameters affecting the size of nanoemulsions. To the best of our knowledge, no work so far has focused on parameters changing particle size of a nanoemulsion containing ibuprofen. In present work, we aimed to study the influence of concentration of ibuprofen, Tween 80, and ethanol, as well as exposure time on droplet size of an ibuprofen-loaded nanoemulsion. Artificial neural networks (ANNs) were used to study the interactions between the four variables and their effects on the size. ANNs are computing systems that are able to map relationships between an input with the desired output.<sup>14</sup> ANNs have been shown to outperform classical statistical models in dealing with nonlinear and complex relations.<sup>15</sup>

## Materials and Methods

### Materials

Ibuprofen (pharmaceutical grade) was gifted by Sepidaj Pharmaceutical Co (Iran). Ethyl oleate, ethanol and Tween 80 were from Merck chemicals Co (Germany) and purchased locally. All the materials were used without further purification.

### Preparation of ibuprofen-loaded nanoemulsion and droplet size measurement

To prepare the nanoemulsion, a mixture of ethyl oleate, Tween 80 and ethanol (oil phase; surfactant and co-surfactant, respectively) was prepared. Ibuprofen, as a drug, was added to the mixture and homogenized for the time specified at room temperature. The droplet size of the final mixture was analyzed by Dynamic Light Scattering (DLS) technique.

### Software tool

The experimental model was modelled by INForm V4.02 (Intelligensys, UK). Then, generated 3D response surfaces

were used to show the impact of two inputs on the output when the two other inputs were fixed.

### Modelling and data set

For feeding data to our network, 32 experimental runs, having random values in the ranges determined by preliminary studies, were sorted into two sets: 24 runs as training data, 2 runs as test data and 6 runs as unseen data. The data were normalized using the in-built functions of the software where required. All runs involved four factors: concentration of ibuprofen (mg/3g), Tween 80 (mg/3g) and ethanol (mg/3g), as well as exposure time (s). Samples were prepared and followed by DLS analysis to obtain droplet size as the output. The training data were applied for training ANNs and specifying the relationships between the inputs and the output. The test data (10% of the training data) and the unseen or validation data, were used to stop overtraining and recognize the predictive capability of the trained network, respectively.<sup>16</sup> When overtraining occurs, value of coefficient of determination ( $R^2$ ) of the test data reduces and neural network stops the training process.  $R^2$  for unseen data was determined to identify the predictability and quality of the model.<sup>17</sup> The measured and predicted output data for the unseen set are shown in the Table 1. Training parameters of model, which were employed to obtain maximum values for  $R^2$  of training, test and unseen data, are summarized in Table 2. Other training parameters are reported in the previous study.<sup>18</sup>

### Results

$R^2$  values of the model were 0.94, 0.99 and 0.75 for the training, test and validation (unseen) data sets, respectively, indicator of predictability and quality of the model. Based on previous study, 3D response surfaces can be used to study the relations between the inputs and the output.<sup>19</sup> With this method, using two input factors fixed at a low, mid-range and high values, the effects of the other input factors on the output and even interactions between them may be assessed.<sup>20</sup>

**Table 1.** Measured and predicted size (using the generated ANNs model) for the prepared nanoemulsions

Ibuprofen concentration (mg/3g)	Tween 80 concentration (mg/3g)	Ethanol concentration (mg/3g)	Exposure time (s)	Measured size (nm)	Predicted size (nm)
<b>Test data</b>					
0.14	0.75	0.70	200	87	88
0.18	0.82	0.54	60	151	147
<b>Unseen data</b>					
0.09	0.66	0.50	110	28	52
0.12	0.66	0.66	200	39	69
0.11	0.63	0.52	190	36	67
0.19	0.79	0.50	150	151	163
0.11	0.54	0.63	120	38	43
0.15	0.82	0.62	130	100	79

**Table 2.** The training parameters used in training the model

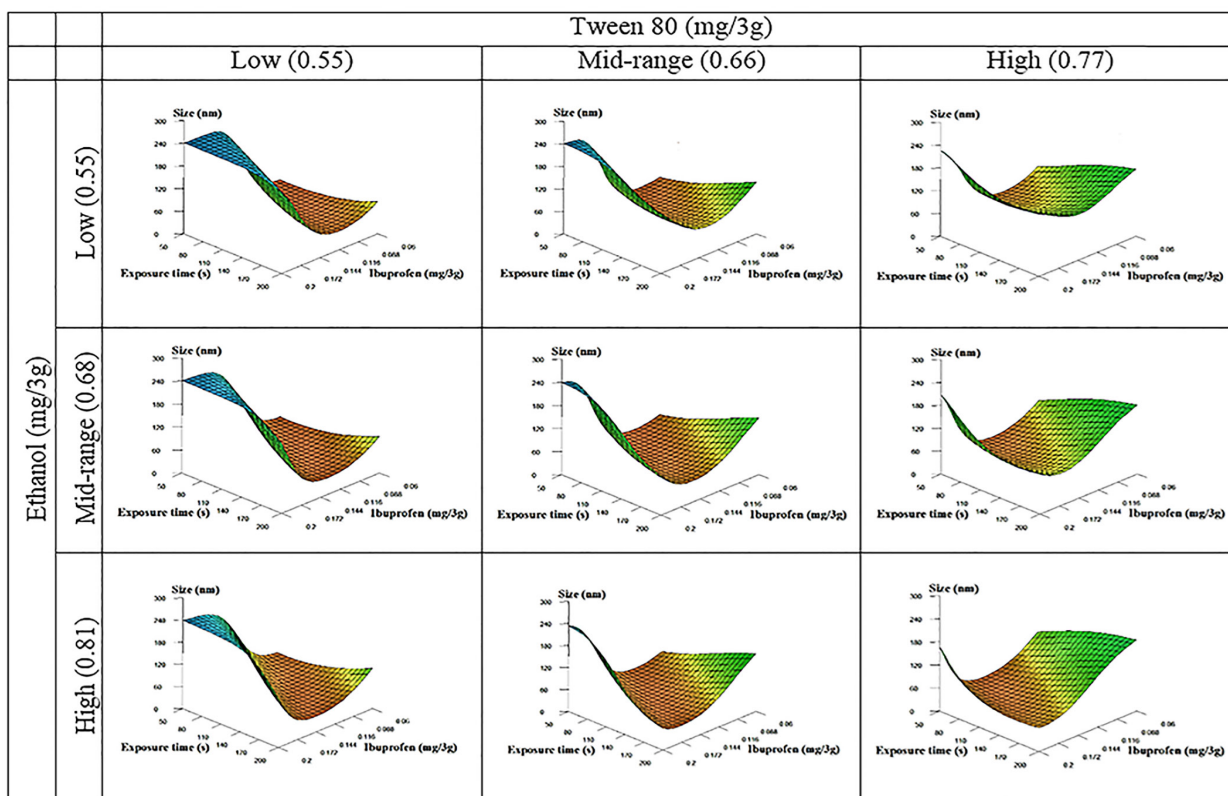
Parameters	Type/Value
Number of hidden layers	1
Number of nodes in hidden layer	3
Back propagation type	Batch
Hidden layer transfer function	Asymmetric Sigmoid
Output transfer function	Tanh
Target epochs	1000

In Figure 1, the 3D-response graphs are made to represent the effect of ibuprofen and exposure time on droplet size, when the values of ethanol and Tween 80 are fixed (i.e. 0.55, 0.68, and 0.81 mg/3g as well as 0.55, 0.66 and 0.77 mg/3g for ethanol and Tween 80 concentration, respectively). The results indicate that increasing the exposure time generally leads to decreased droplet size whereas when changing the ibuprofen concentration, different size values may be observed: first a decrease, followed by an increase in droplet size, is observed as the amount of ibuprofen increases.

Figure 2 illustrates the effects of ethanol and Tween 80 on particle size of the nanoemulsion, when the other two inputs are fixed at low, mid-range and high values (i.e. 0.08, 0.13 and 0.18 mg/3g and 75, 125 and 175 s for ibuprofen and exposure time, respectively). From the details of Fig. 2, the increase in the Tween 80 concentration leads to decrease in droplet size, when ibuprofen is high. Also, when the amount of ibuprofen is low, increasing the Tween

80 increases the size of the nanoemulsion. About the effect of ethanol, in general, when the concentration of ethanol increases, the size of the droplets decreases, except in cases where the amount of ibuprofen is low. In this case the increase in ethanol concentration does not have an important effect on the droplet size.

In Figure 3, the values of ethanol and ibuprofen concentration are fixed to show 3D graphs of droplet size against exposure time and Tween 80 concentration. The graphs related to high-value of ibuprofen (0.18 mg/3g) show that increasing the other three parameters, especially homogenization time, causes decrease in size of droplets. In medium ibuprofen level, low concentration of Tween 80 and high homogenization time are required to reduce droplet size. Also, in this range, if the exposure time is low, high concentration of Tween 80 can also result in a decrease in droplet size.



**Figure 1.** 3D plots of droplet size. The effect of exposure time and ibuprofen concentration on the output factor is shown at low, medium, and high values of concentration of ethanol and Tween 80.

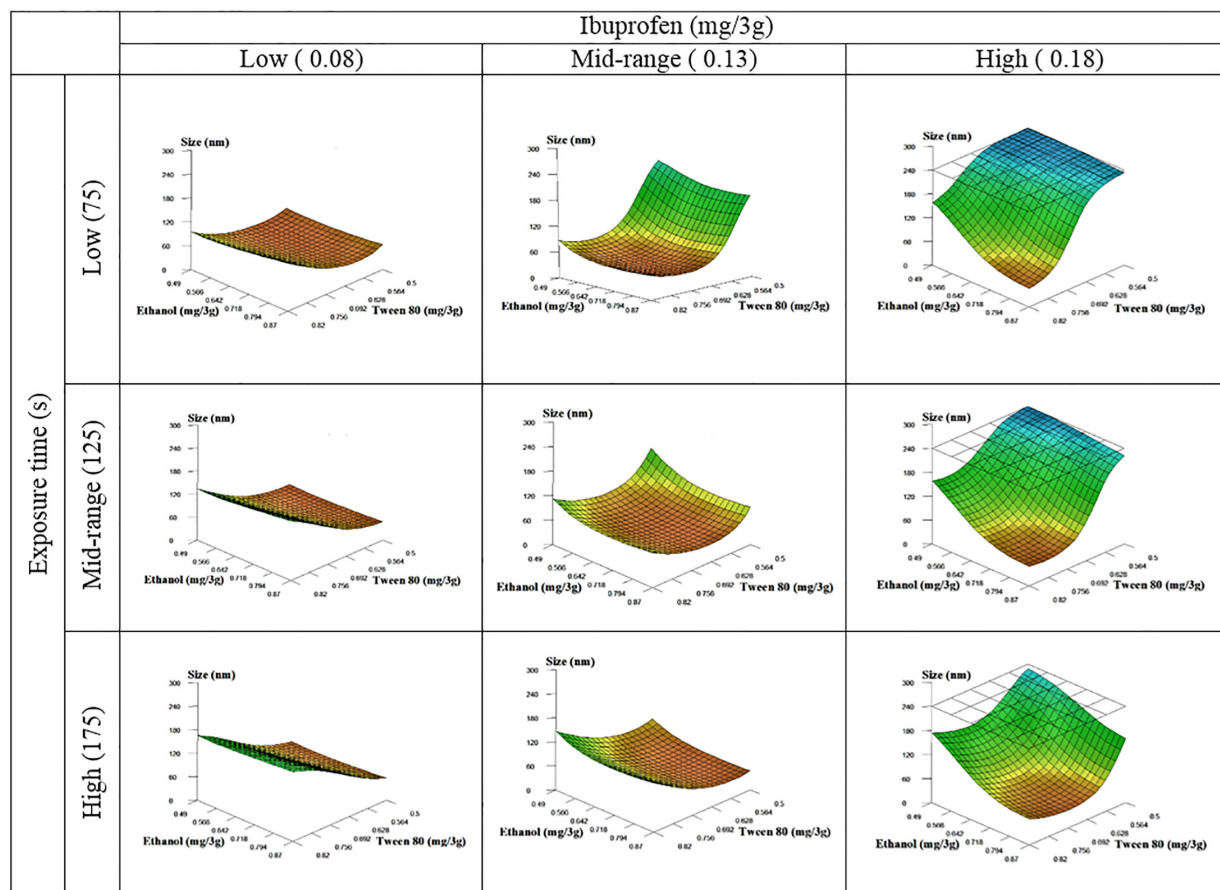


Figure 2. 3D plots of droplet size. The effect of ethanol and Tween 80 on the output factor is shown at low, medium, and high values of exposure time and ibuprofen concentration.

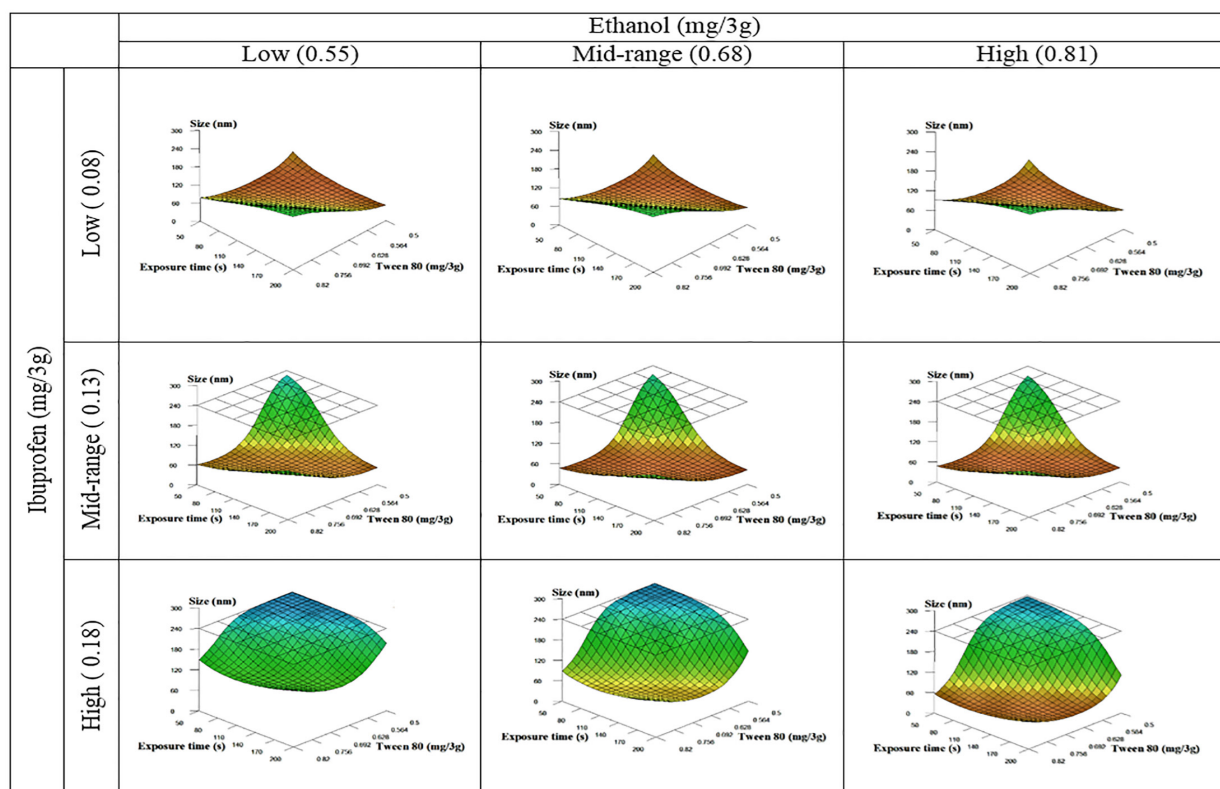


Figure 3. The effect of exposure time and Tween 80 concentration on the output factor is shown at low, medium, and high values of concentration of ethanol and ibuprofen.



Figure 4 demonstrates the effect of ibuprofen and ethanol concentration on particle size when other inputs are fixed. As has been mentioned earlier, the effect of ibuprofen is nonlinear on droplet size. Also, increasing ethanol reduces the droplet size. From the graphs, at low ibuprofen concentration, increasing the concentration of Tween 80 increases the particle size. On the contrary, at high concentrations of ibuprofen, rise of Tween 80 concentration reduces the droplet size. This effect is more pronounced when homogenization time is low. Figures 5 and 6 confirm the results obtained from Figures 1 to 4. To summarize, the results show that:

-The droplet size reduces with increasing homogenization time.

- Increasing the amount of ethanol reduces the droplet size slightly but this effect is not always important.
- Effect of ibuprofen is nonlinear. As the ibuprofen increases in the formulation, the particle size decreases slightly, then, increases.
- Effect of tween 80 concentration on the droplet size depends on the ibuprofen concentration:
  - High levels of ibuprofen: increasing the amount of Tween 80 reduces the droplet size.
  - Low levels of ibuprofen: increasing the amount of Tween 80 increases the size of the nanoemulsion.

### Discussion

In this study, droplet size of nanoemulsion was evaluated as

an important factor in drug efficacy by a simple preparation and fast analysis method. Different studies have been published to study the efficacy of topical ibuprofen.<sup>21,22</sup> It has previously been reported that interactions between drug-loaded nanoparticles and skin depend on the charge of the particles and their size which affect drug permeation.<sup>23</sup>

Here, we used an ANN model to determine effect of ingredients' concentration and exposure (homogenization) time on droplet size of nanoemulsions containing ibuprofen. We used 3D-graphs to study the interactions between the inputs and the output. The 3D response surfaces were drawn while two factors were fixed at high, mid, or low values. As stated in the results, to have smallest droplet size, the ibuprofen concentration should be medium (i.e. ~ 0.116–0.144). In a study, it has been reported that the droplet diameter of nanoemulsions increased with the increase in the drug concentration, due to higher concentration of ibuprofen present in the lipophilic core of surfactant.<sup>24</sup> However, at low concentrations of ibuprofen, inadequate reactions between the drug and the oil may be the reason for the increase in droplet size.

Considering the generated 3D-graphs, a decrease in the droplet size was obtained with increasing the exposure time. It can be deduced that exposure time (homogenization time) has an important factor on nanoemulsion size. In similar studies it has been reported that droplet/particle size mainly depends on the homogenization time so that smaller size of droplets are formed with increase in

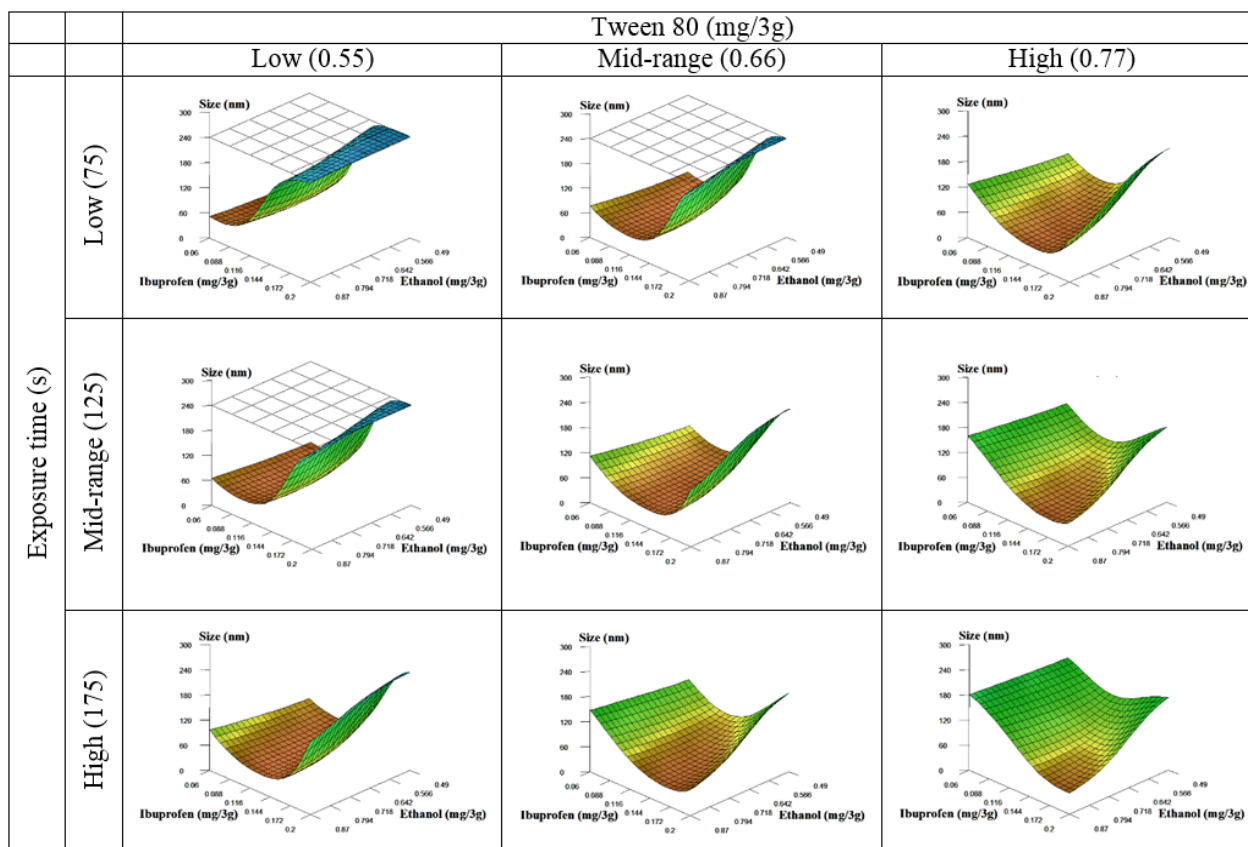


Figure 4. The effect of ethanol and ibuprofen concentration on the output factor is shown at low, medium, and high values of Tween 80 concentration and exposure time.

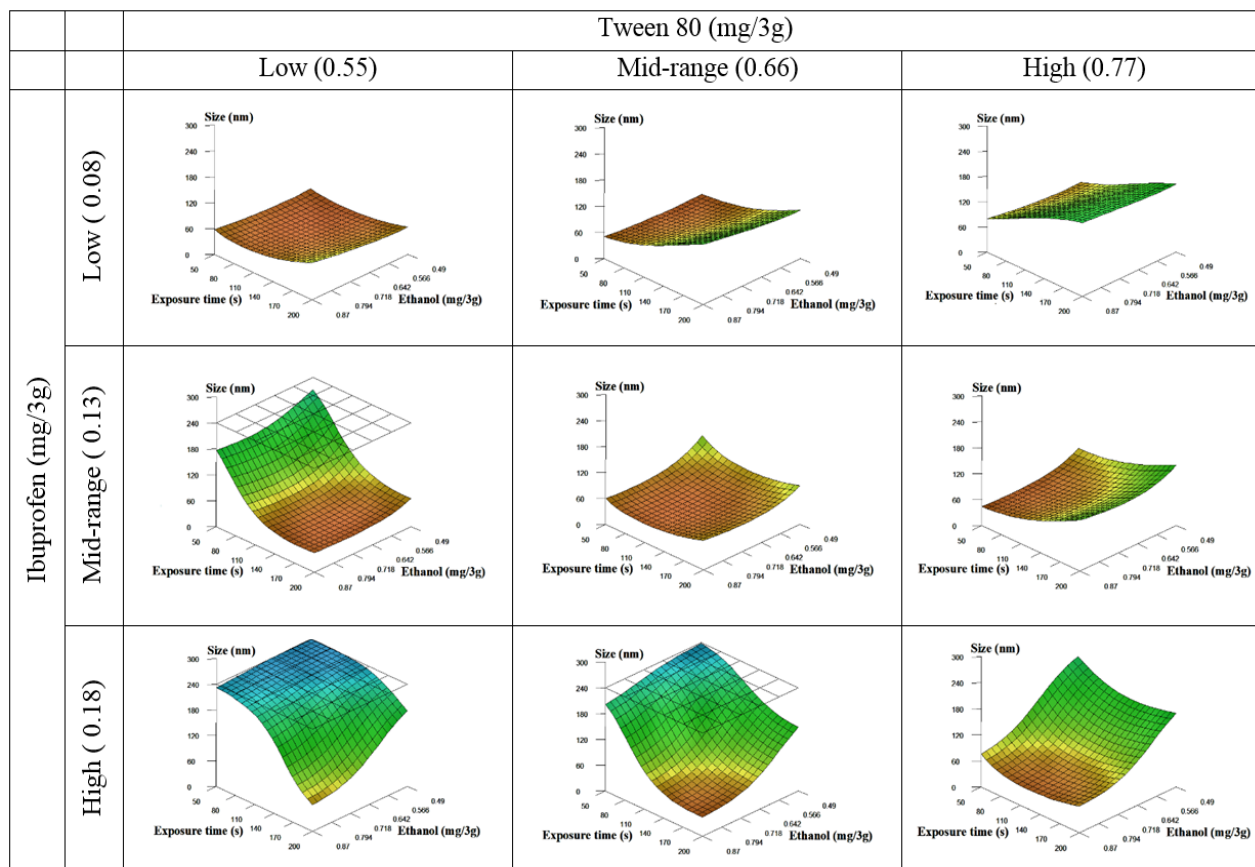


Figure 5. The effect of exposure time and ethanol concentration on the output factor is shown at low, medium, and high values of concentration of ibuprofen and Tween 80.

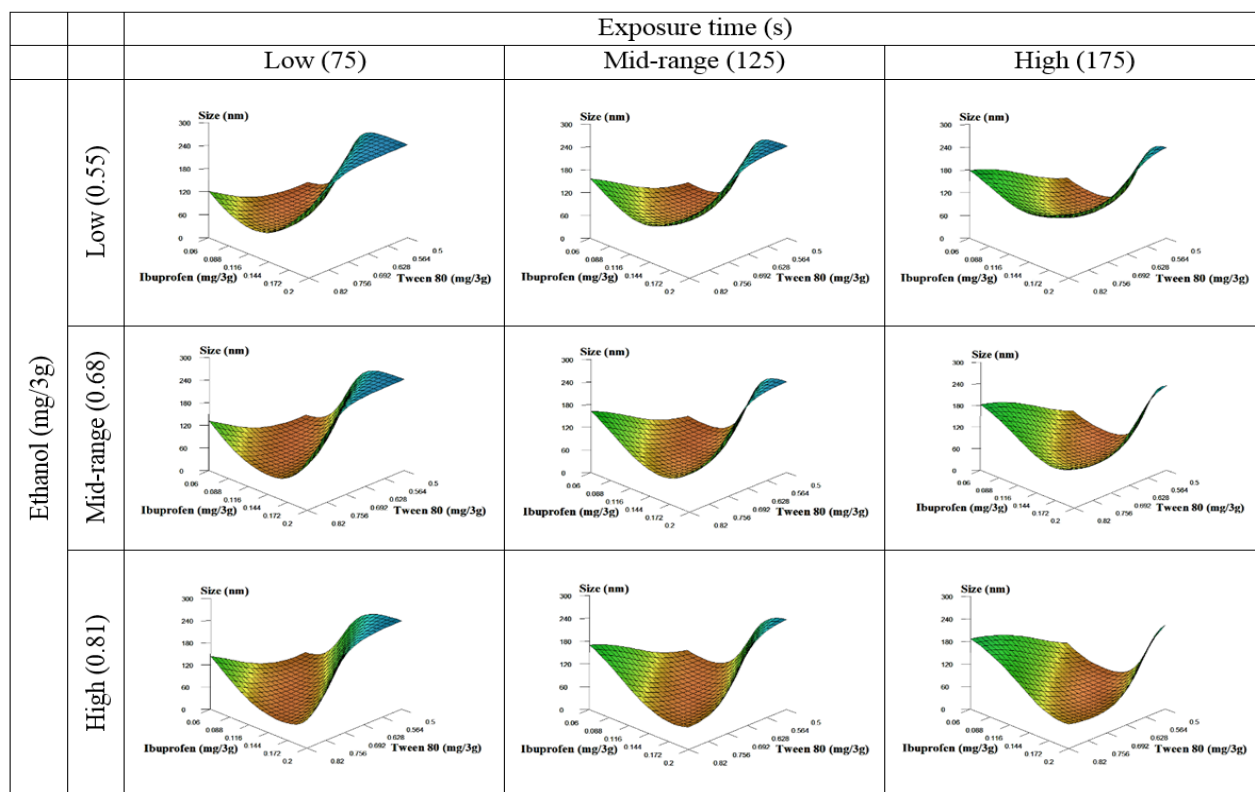


Figure 6. The effect of ibuprofen and Tween 80 concentration on the output factor is shown at low, medium, and high values of concentration of ethanol and exposure time.

time.<sup>24-26</sup> Stenger and Peukert reported that shear stress on the processed samples increases with processing time which provides size reduction.<sup>27</sup>

In this work, effects of Tween 80 and ethanol concentration on nanoemulsion's droplet diameter were investigated to achieve minimum droplet size. It is generally accepted that the increase in concentration of ethanol and Tween 80 should decrease the size of droplets. Previous studies also indicated that low values of surfactant and co-surfactant led to increase in size of nanoemulsion,<sup>28</sup> probably due to destabilizing the system. At low values of surfactant/co-surfactant, the small droplets which are produced during homogenization join together to form comparatively larger size droplets.<sup>29</sup> As an exception we observed that when concentration of ibuprofen is low, increasing concentration of Tween 80, increases the particle size. This could be due to destabilizing effect of high values of surfactants which is occasionally observed and could be a function of several phenomena such as packing the surfactant molecules around the nanoemulsion particles.<sup>30</sup>

### Conclusion

This investigation showed interactions of four input factors on droplet size of ibuprofen-loaded nanoemulsions. The present study indicated that homogenization time and ibuprofen concentration are potentially the most important parameters that influence the droplet size. In total, homogenization time can be playing a key role in nanoemulsion size, which is required to be maximum for obtaining minimum droplet size.

### Author Contributions

AH: Performing the ANN modelling, analyzing the data, drafting the manuscript, AA: Preparation of the samples, data collection, finalizing the manuscript. Authors have read and agreed to the published version of the manuscript.

### Conflicts of Interest

The authors confirm no conflicts of interest.

### References

- Jaiswal M, Dudhe R, Sharma P. Nanoemulsion: An advanced mode of drug delivery system. *3 Biotech*. 2015;5(2):123-7. doi:10.1007/s13205-014-0214-0
- Osborne D, Ward A, O'NEILL K. Microemulsions as topical drug delivery vehicles: In-vitro transdermal studies of a model hydrophilic drug. *J Pharm Pharmacol*. 1991;43(6):451-4. doi:10.1111/j.2042-7158.1991.tb03511.x
- Sharma N, Mishra S, Sharma S, Deshpande RD, Sharma RK. Preparation and optimization of nanoemulsions for targeting drug delivery. *Int J Drug Dev Res*. 2013;5(4):37-48. doi:10.1016/j.jconrel.2017.03.008
- Esmailzadeh-Gharedaghi E, Faramarzi MA, Amini MA, Rouholamini Najafabadi A, Rezayat SM, Amani A. Effects of processing parameters on particle size of ultrasound prepared chitosan nanoparticles: An artificial neural networks study. *Pharm Dev Technol*. 2012;17(5):638-47. doi:10.3109/10837450.2012.696269
- Mao L, Yang J, Xu D, Yuan F, Gao Y. Effects of homogenization models and emulsifiers on the physicochemical properties of  $\beta$ -carotene nanoemulsions. *J Dispers Sci Tech*. 2010;31(7):986-93. doi:10.1080/01932690903224482
- Aswathanarayan JB, Vittal RR. Nanoemulsions and their potential applications in food industry. *Front Sustain Food Syst*. 2019;3:95. doi:10.3389/fsufs.2019.00095
- Silva HD, Cerqueira MA, Vicente AA. Influence of surfactant and processing conditions in the stability of oil-in-water nanoemulsions. *J Food Eng*. 2015;167:89-98. doi:10.1016/j.jfoodeng.2015.07.037
- Priya S, Koland M, Kumari S. Nanoemulsion components screening of quetiapine fumarate: Effect of surfactant and co surfactant. *Asian J Pharm Clin Res*. 2015;8(6):136-40.
- Iervolino M, Cappello B, Raghavan SL, Hadgraft J. Penetration enhancement of ibuprofen from supersaturated solutions through human skin. *Int J Pharm*. 2001;212(1):131-41. doi:10.1016/s0378-5173(00)00603-7
- Park ES, Chang SY, Hahn M, Chi SC. Enhancing effect of polyoxyethylene alkyl ethers on the skin permeation of ibuprofen. *Int J Pharm*. 2000;209(1-2):109-19. doi:10.1016/s0378-5173(00)00559-7
- Ghiasi Z, Esmaeli F, Aghajani M, Ghazi-Khansari M, Faramarzi MA, Amani A. Enhancing analgesic and anti-inflammatory effects of capsaicin when loaded into olive oil nanoemulsion: An in vivo study. *Int J Pharm*. 2019;559:341-7. doi:10.1016/j.ijpharm.2019.01.043
- Haines D, Bak I, Giricz Z, Wise J, Ferdinandy P, Mahmoud F, et al. Suppression of severe airway inflammation with non-toxic dietary nutrients in histamine-challenged guinea pigs: A comparison with ibuprofen. *Iran J Pharm Res*. 2010;3(Supplement 2):24. doi:10.22037/ijpr.2010.364
- Marzuki NHC, Wahab RA, Hamid MA. An overview of nanoemulsion: Concepts of development and cosmeceutical applications. *Biotechnol Biotechnol Equip*. 2019;33(1):779-97. doi:10.1080/13102818.2019.1620124
- Hussain AS, Shivanand P, Johnson RD. Application of neural computing in pharmaceutical product development: Computer aided formulation design. *Drug Dev Ind Pharm*. 1994;20(10):1739-52. doi:10.3109/03639049409038390
- Li Y, Abbaspour MR, Grootendorst PV, Rauth AM, Wu XY. Optimization of controlled release nanoparticle formulation of verapamil hydrochloride using artificial neural networks with genetic algorithm and response surface methodology. *Eur J Pharm Biopharm*. 2015;94:170-9. doi:10.1016/j.ejpb.2015.04.028
- Shao Q, Rowe RC, York P. Comparison of neurofuzzy logic and neural networks in modelling experimental data of an immediate release tablet formulation.

- Eur J Pharm Sci. 2006;28(5):394-404. doi:10.1016/j.ejps.2006.04.007
17. Bourquin J, Schmidli H, van Hoogevest P, Leuenberger H. Application of artificial neural networks (ANN) in the development of solid dosage forms. *Pharm Dev Technol.* 1997;2(2):111-21. doi:10.3109/10837459709022616
  18. Baharifar H, Amani A. Size, loading efficiency, and cytotoxicity of albumin-loaded chitosan nanoparticles: An artificial neural networks study. *J Pharm Sci.* 2017;106(1):411-7. doi:10.1016/j.xphs.2016.10.013
  19. Aghajani M, Shahverdi AR, Rezayat SM, Amini MA, Amani A. Preparation and optimization of acetaminophen nanosuspension through nanoprecipitation using microfluidic devices: An artificial neural networks study. *Pharm Dev Technol.* 2013;18(3):609-18. doi:10.3109/10837450.2011.649854
  20. Esmailzadeh-Gharehdaghi E, Faramarzi MA, Amini MA, Moazeni E, Amani A. Processing/formulation parameters determining dispersity of chitosan particles: An anns study. *J Microencapsul.* 2014;31(1):77-85. doi:10.3109/02652048.2013.805842
  21. Jannat S, Al-Mausam M, Sharmin F, Islam S, Reza M. Formulation and evaluation of sustained release matrix type transdermal film of ibuprofen. *Bang Pharm J.* 2012;15(1):17-21.
  22. Zair MA, Prasanthi D, Chennuri A, Hanthal ZR, Lakshmi P. Effect of transcitol and stearylamine on ibuprofen hydrophilic gel for transdermal delivery. *Int J Drug Deliv Technol.* 2018;8(01):6-14. doi:10.25258/ijddt.v8i01.11901
  23. Uchechi O, Ogbonna JD, Attama AA. Nanoparticles for dermal and transdermal drug delivery. In: Sezer AD, editor. *Application of Nanotechnology in Drug Delivery.* London: InTech; 2014. p. 193-227. doi:10.5772/58672
  24. Sakeena MHF, Elrashid SM, Munavvar AS, Azmin MN. Effects of oil and drug concentrations on droplets size of palm oil esters (POEs) nanoemulsion. *J Oleo Sci.* 2011;60(4):155-8. doi:10.5650/jos.60.155
  25. Plakkot S, De Matas M, York P, Saunders M, Sulaiman B. Comminution of ibuprofen to produce nano-particles for rapid dissolution. *Int J Pharm.* 2011;415(1-2):307-14. doi:10.1016/j.ijpharm.2011.06.002
  26. Thakur G, Mitra A, Basak A, Rousseau D, Pal K. Characterization of oil-in-water gelatin emulsion gels: Effect of homogenization time. 2010 International Conference on Systems in Medicine and Biology, Kharagpur, India, 2010. doi: 10.1109/ICSMB.2010.5735392
  27. Stenger F, Peukert W. The role of particle interactions on suspension rheology—application to submicron grinding in stirred ball mills. *Chem Eng Technol.* 2003;26(2):177-83. doi:10.1002/ceat.200390026
  28. Mehmood T, Ahmad A, Ahmed A, Ahmed Z. Optimization of olive oil based o/w nanoemulsions prepared through ultrasonic homogenization: A response surface methodology approach. *Food Chem.* 2017;229:790-6. doi:10.1016/j.foodchem.2017.03.023
  29. Anarjan N, Mirhosseini H, Baharin BS, Tan CP. Effect of processing conditions on physicochemical properties of astaxanthin nanodispersions. *Food Chem.* 2010;123(2):477-83. doi:10.2147/IJN.S72835
  30. Seyedhassantehrani N, Karimi R, Tavoosidana G, Amani A. Concurrent study of stability and cytotoxicity of a novel nanoemulsion system—an artificial neural networks approach. *Pharm Dev Technol.* 2017;22(3):383-9. doi:10.1080/10837450.2016.1212878