AAPS PharmSciTech, Vol. 13, No. 4, December 2012 (© 2012)
DOI: 10.1208/s12249-012-9859-3

Research Article

The Use of Artificial Neural Networks for Optimizing Polydispersity Index (PDI) in Nanoprecipitation Process of Acetaminophen in Microfluidic Devices

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Received 7 April 2012; accepted 12 September 2012; published online 21 September 2012

Abstract. Artificial neural networks (ANNs) were used in this study to determine factors that control the polydispersity index (PDI) in an acetaminophen nanosuspension which was prepared using nanoprecipitation in microfluidic devices. The PDI of prepared formulations was measured by dynamic light scattering. Afterwards, the ANNs were applied to model the data. Four independent variables, namely, surfactant concentration, solvent temperature, and flow rate of solvent and antisolvent were considered as input variables, and the PDI of acetaminophen nanosuspension was taken as the output variable. The response surfaces, generated as 3D graphs after modeling, were used to survey the interactions happening between the input variables and the output variable. Comparison of the response surfaces indicated that the antisolvent flow rate and the solvent temperature have reverse effect on the PDI, whereas solvent flow rate has direct relation with PDI. Also, the effect of the concentration of the surfactant on the PDI was found to be indirect and less influential. Overall, it was found that minimum PDI may be obtained at high values of antisolvent flow rate and solvent temperature, while the solvent flow rate should be kept to a minimum.

KEY WORDS: acetaminophen; artificial neural networks; microfluidic devices; nanoprecipitation; nanosuspension; polydispersity index.

INTRODUCTION

Nanosuspensions, compared with conventional drug delivery systems, have shown a variety of advantages, including increased dissolution velocity and saturation solubility, reduced administered dose (1), improved biological performance, capability of being scaled up, and possible enhancements in stability and versatility (2). They have also shown a great ability to work with poorly water-soluble drugs (1). Such preparations are composed of drug particles with colloidal dispersions below 1 μm in size (3,4) which are usually prepared by two common approaches: precipitation of drug molecules in a solution using an antisolvent and creation of smaller particles from larger ones by high shear forces (*i.e.*, milling) (5).

Microfluidic instruments are commonly known as miniaturized versions of macroscale devices showing two attractive properties—increasing the ratio of surface area to volume and presence of laminar flow (6). In these instruments, liquids flow in channels with internal diameters typically <1 mm. Liquids flowing in microfluidic channels are linear, forming a diffusion interface in the central part of the channel. When a solution of drug in a solvent gets in touch with an antisolvent, the drug molecules in the solvent diffuse across the interface (*i.e.*, diffusion layer) and start to nucleate and grow in size (7).

The increased ratio of surface area to volume could be beneficial for processes such as enzymatic reactions or extraction of active components. Additionally, such systems are useful in dealing with micro- and nanoliter volumes of solutions with their interesting performance. Moreover, microfluidic reactors compared with other methods for preparation of nanosuspensions offer a low-cost technique without producing considerable residues (8), while a monodispersed product is commonly obtained (9–11). In such instruments, to prevent uncontrolled growth and precipitation of created drug nuclei in the solution, the growth/precipitation rate can be controlled using surfactants or polymers (12).

Artificial neural networks (ANNs) are nowadays being introduced as methodologies that are able to deal with nonlinear and complex relations, particularly when the nature of relations between the experimental data is unknown (13). ANNs can learn and recognize relations between independent variables (*i.e.*, input data set) and corresponding dependent variable(s) (*i.e.*, output parameter(s)) (14). In recent years, ANNs have been successfully used in various areas of applications such as image processing, medicine, pharmaceutics (14,15), and nanotechnology (16), where statistical methods may not be efficient due to complex relations commonly observed between the data.

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Considering the literature, a few works so far have reported the factors affecting the particle size of nanosuspensions prepared by microfluidic reactors (17–19). However, no comprehensive work so far has reported the possible factors influencing the polydispersity of the nanoparticles prepared through this approach. Polydispersity having been mentioned as an important factor which can substantially affect the quality of such preparations (20). Additionally, the existence of particles with low size distribution in nanosuspensions slows the effect of Ostwald ripening, thus making the preparation more stable (21). Our previous work detailed the physical stability of a nanosuspension of acetaminophen prepared by microfluidic reactors. In brief, results of this experiment showed that increasing the ratio of antisolvent flow rate to solvent flow rate results in higher physical stability of acetaminophen nanosuspension. Also, increasing the temperature of the solvent and the surfactant content of the preparation causes an increase in the stability of the nanosuspension. To recognize the possible mechanisms, a preliminary study with limited number of experiments was performed. The effects of solvent/antisolvent flow rate as well as temperature were found to influence the PDI of the nanosuspension which could be an explanation for changes in physical stability. Nevertheless, a more detailed assessment appeared to be necessary to confirm the effect of the input parameters on the PDI (22). In the current work, we applied ANNs to study relations between parameters affecting the PDI on the nanoprecipitation process of the acetaminophen nanosuspension prepared with microfluidic devices.

MATERIALS AND METHODS

Materials

Acetaminophen powder (pharmaceutical grade) was a gift from Mehrdarou Pharmaceutical, Iran. Ethanol 96% and Tween 80 (polysorbate 80) were of analytical grade and purchased from Sigma-Aldrich, Germany.

Nanoprecipitation Process in the Microfluidic Instrument

The nanoprecipitation process was attained by streams of supersaturated ethanolic solutions of acetaminophen as solvent and distilled water as antisolvent, pumped through the microfluidic instrument. The details of the instrument have been given previously (22). Stream rates of the solvent (0.5–1 ml/min, 30–80°C) and antisolvent (0.5–2.5 ml/min) were controlled by hydrodynamic micropumps. Solvent and antisolvent solutions were injected in the microfluidic instrument at controlled temperatures. The antisolvent system was maintained at a controlled lab temperature (*i.e.*, 24±2°C) and contained different concentrations of Tween 80 (10–270 mg/ml). The obtained samples were maintained at the controlled lab temperature, and their PDI measured freshly using DLS. Subsequently, the obtained PDI data were used to study the effect of four input parameters on the PDI of the nanosuspension according to the model obtained from ANNs modeling.

ANNs Studies

In the present study, a commercially available ANNs software (INForm v4.02, Intelligensys, UK) was employed to model relationships between input and output parameters. The response surfaces from the model were illustrated as 3D graphs. In fact, as described previously (23,24), response surfaces were produced to show the changes in the output as a function of variations of two variables while the other two variables are fixed at predetermined values.

Experimentally, 38 samples were prepared under different conditions in order to train, test, and validate the ANNs software. As explained above, the process of making nanoparticles involved four input variables which were randomly designed and prepared for each test: solvent flow rate (in milliliters per minute), antisolvent flow rate (in milliliters per minute), solvent temperature (in degrees Celsius), and Tween 80 concentration (in milligrams per milliliter). Furthermore, the obtained PDI was set as the output variable. From the samples prepared

Table I. The Training Parameters Used with INForm v4.02

Network structure	No. of hidden layers	1
	No. of nodes in hidden layer	4
Backpropagation type		Incremental
Backpropagation parameters	Momentum factor	0.8
	Learning rate	0.7
Targets	Maximum iterations	1,000
	MS error	0.0001
	Random seed	10,000
Smart stop	Minimum iterations	20
	Test error weighting	0.1
	Iteration overshoot	200
	Auto weight	On
	Smart stop enabled	On
Transfer function	Output	Symmetric sigmoid
	Hidden layer	Asymmetric sigmoid

MS mean squared

Table II. The Unseen Data Sets Utilized in ANNs Modeling

Tween 80 concentration (mg/ml)	Solvent flow rate (ml/min)	Antisolvent flow rate (ml/min)	Solvent temperature (°C)	Observed PDI	Predicted PDI
43.2	0.6	1.1	60	0.37	0.38
194.4	0.5	1.0	60	0.30	0.37
54.0	0.6	1.0	30	0.42	0.44
86.4	1.0	1.3	50	0.41	0.43
86.4	0.8	1.0	50	0.40	0.44
270.0	0.5	1.0	70	0.29	0.31
259.2	0.5	2.5	60	0.16	0.16

PDI polydispersity index

experimentally, 27 were utilized as "training data" to train the network in order to establish the input–output relationships. In addition, three data sets were utilized as "test data" (*i.e.*, 10% of the training data as offered by the software) to stop overtraining during the learning process. The remaining sets were used as "unseen data" or "validation data" to evaluate the model predicted by the software. Table I includes the training parameters utilized during ANN modeling.

The developed model was then qualified using the determination coefficient (R^2) for unseen data (see Table II).

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

where $\hat{y}$ and $\overline{y}$ represent the value predicted and the mean of the variable. A value closer to 1 shows a better predictability for the model (23,24).

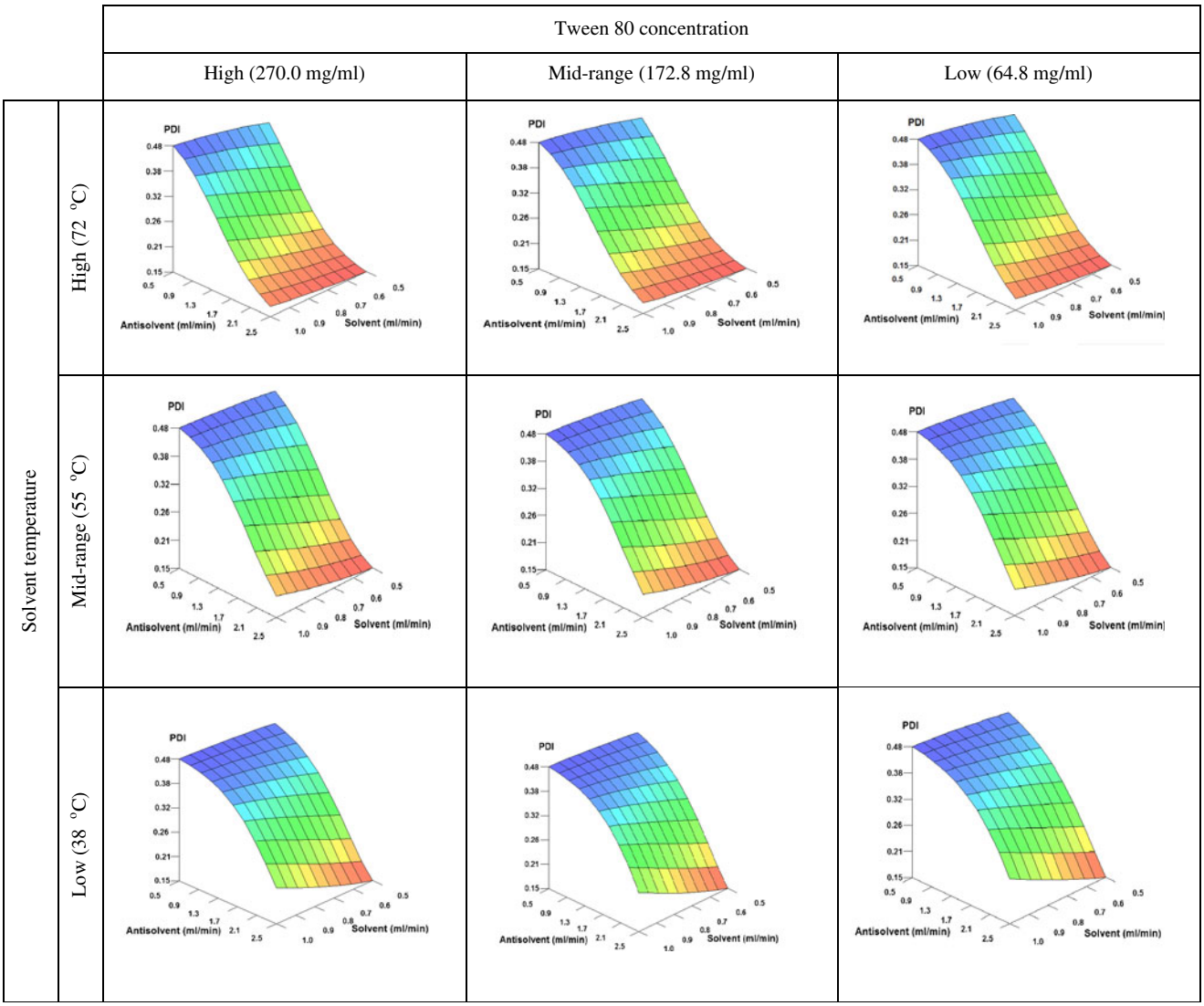

Fig. 1. 3D plots of the nanosuspensions' PDI predicted by the ANNs model fixed at low, mid-range, and high values of Tween 80 and solvent temperature

Measurement of the Nanosuspensions' PDI

PDI of the obtained formulations was measured using a Zetasizer (Malvern, UK), equipped with the Malvern PCS software (version 1.27). The temperature of the samples was set to 24°C, and measurement was done for all samples after preparation without any dilution.

RESULTS

After modeling using the ANNs software, the obtained model represented an R^2 value of 0.81 for validation data indicating an acceptable prediction ability of the model. Then, this model was employed to study the effect of input variables on the PDI in the nanoprecipitation process. The first option to study the obtained ANNs model can be the sensitivity analysis approach. Here, we used a systematic method for a "semiquantitative" investigation of relationships between the input–output variables (23,24). Briefly, in this approach, response surfaces are used to study the effect of two input variables on the output variable (*i.e.*, PDI), while the other input variables are fixed at values of low/mid-range/high (17,25).

Reviewing the graphs obtained from the software (*i.e.*, Figs. 1, 2, 3,4, 5, and 6) determines that the values obtained for the PDI are in the range of 0.15–0.48. This shows the potential of microfluidic reactors in preparing monodispersed dispersions, as has been reported previously (9,11), having mentioned that the maximum obtained value for the PDI (*i.e.*, 0.48) is still not very heterodispersed.

Following the approach expressed above, at first, the effects of solvent and antisolvent flow rate on the PDI of the acetaminophen nanosuspension were evaluated, while the two other input variables, namely, Tween 80 concentration and temperature of solvent, were fixed at 64.8, 172.8, and 270.0 mg/ml and 38°C, 55°C, 72°C, respectively (*i.e.*, low, mid-range, and high values). The obtained 3D graphs are illustrated in Fig. 1. The details in Fig. 1 show that increasing

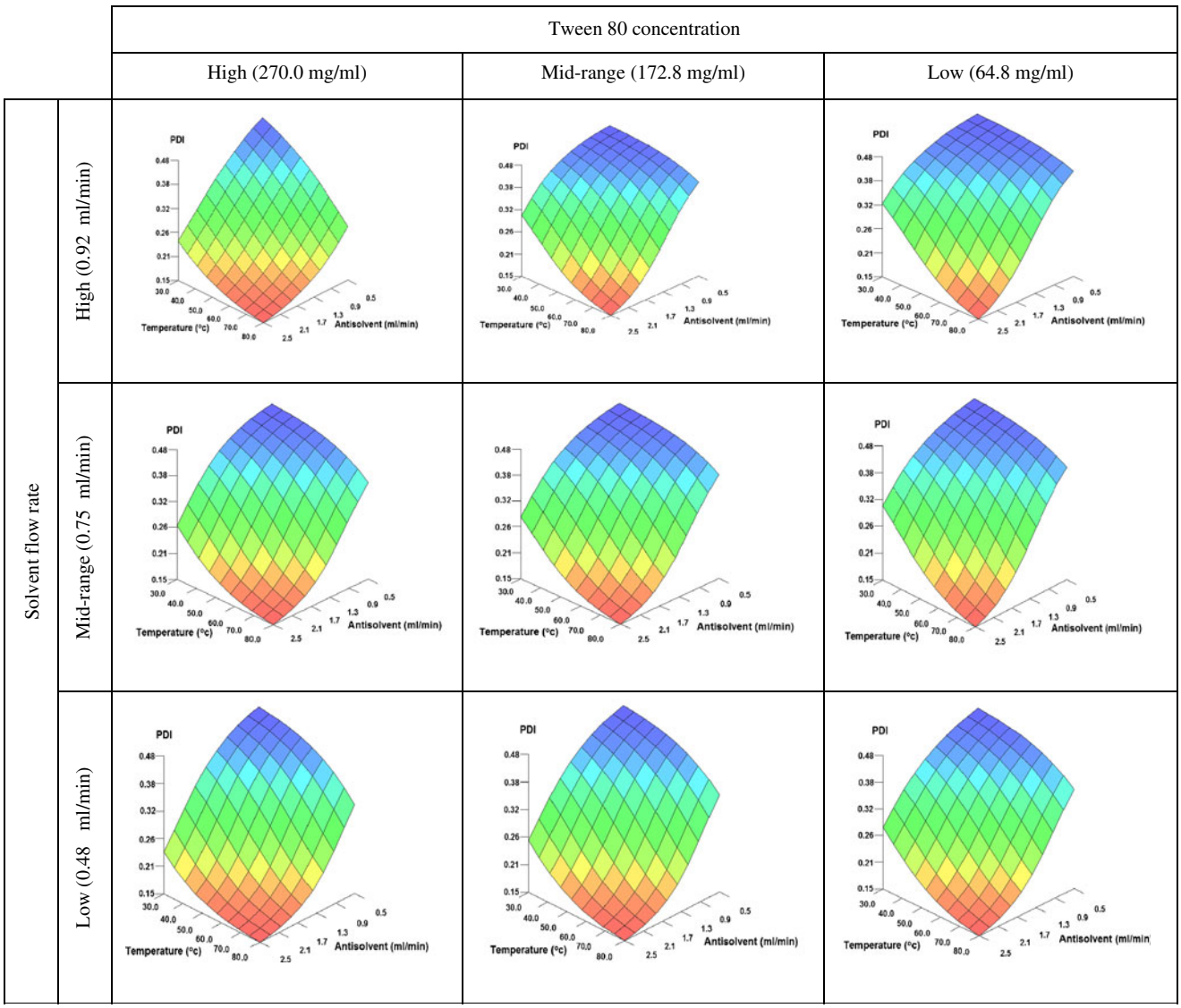

Fig. 2. 3D plots of the nanosuspensions' PDI predicted by the ANNs model fixed at low, mid-range, and high values of Tween 80 and solvent flow rate

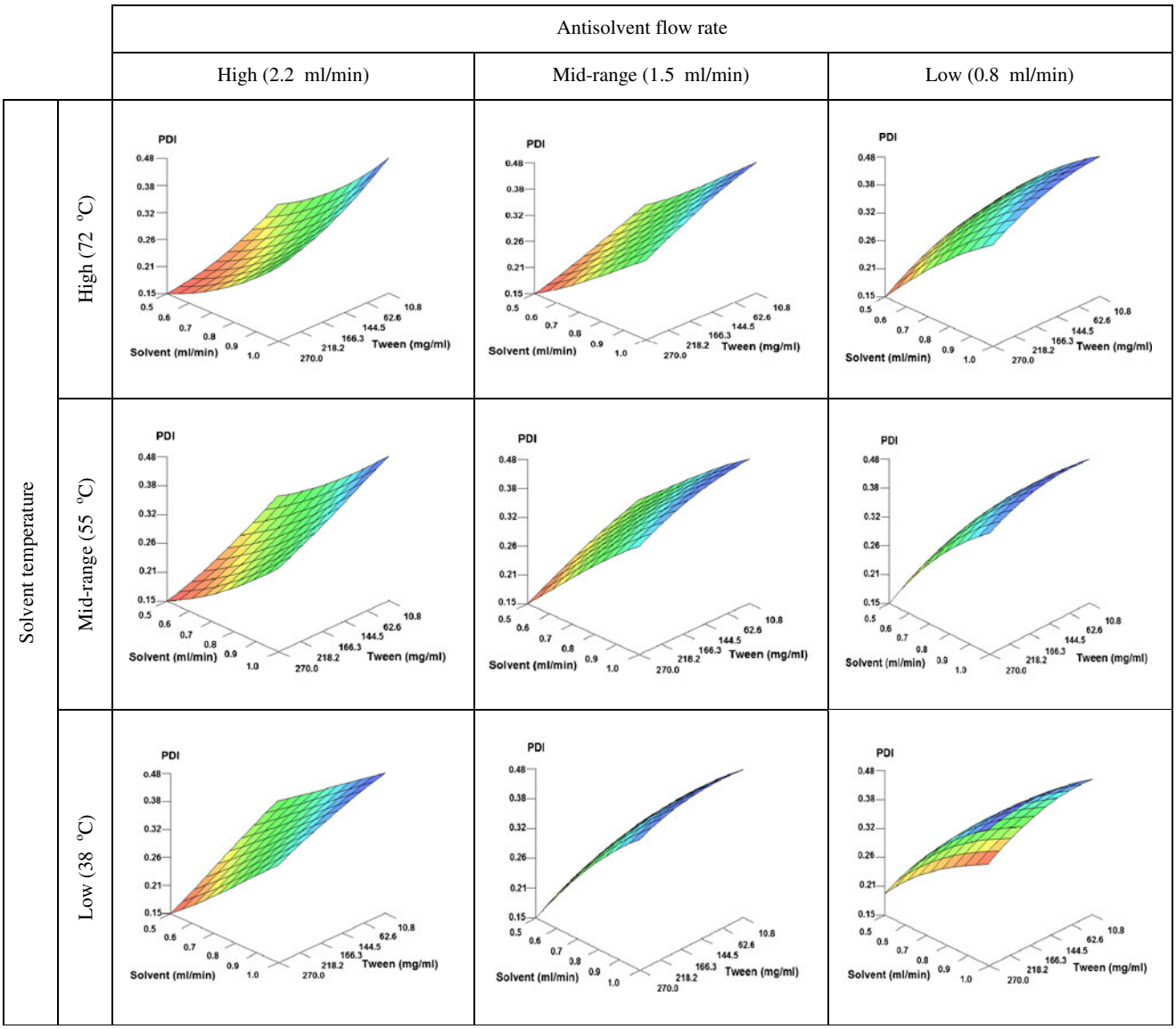

Fig. 3. 3D plots of the nanosuspensions' PDI predicted by the ANNs model fixed at low, mid-range, and high values of the antisolvent flow rate and solvent temperature

the antisolvent flow rate results in a substantial reduction in the PDI of the acetaminophen nanosuspension. It also indicates that by increasing the solvent flow rate, the PDI increases slowly.

In Fig. 2, to study the effect of solvent temperature and antisolvent flow rate on the PDI, we fixed Tween 80 concentration and solvent flow rate at 64.8, 172.8, and 270.0 mg/ml, and 0.48, 0.75, and 0.92 ml/min for low, mid-range, and high values, respectively. Here, the effect of solvent temperature on PDI for each set of fixed data clearly shows that increasing the solvent temperature causes a considerable decrease in PDI of nanosuspensions. Also, increasing the antisolvent flow rate causes a decrease in PDI, as expressed above.

In Fig. 3, the effect of Tween 80 concentration and solvent flow rate on the PDI of nanosuspensions is shown, in which the temperature of the solvent and antisolvent flow rate at low, mid-range, and high values have been fixed. As the details in this figure show, solvent flow rate has a direct and nearly linear relation with PDI, as expressed above. Furthermore, higher values of the antisolvent flow rate and solvent temperature cause smaller PDI. From Fig. 3, it is also clear that increase in the Tween 80 concentration makes PDI slightly smaller with more pronounced effect at high antisolvent flow rates.

With regard to the details in Figs. 4, 5, and 6 that show the effects of antisolvent flow rate and Tween 80 concentration, temperature of solvent and Tween 80 concentration, and solvent flow rate and temperature of solvent on the PDI, respectively, and using the aforementioned results, the following rules can be concluded:

1. In general, increase in antisolvent flow rate or decrease in solvent flow rate causes a reduction of the PDI in nanosuspension.
2. By increasing the temperature of the solvent, the PDI decreases considerably.
3. The concentration of the surfactant does not seem to be considerably effective on the PDI except when the antisolvent flow rate is high.

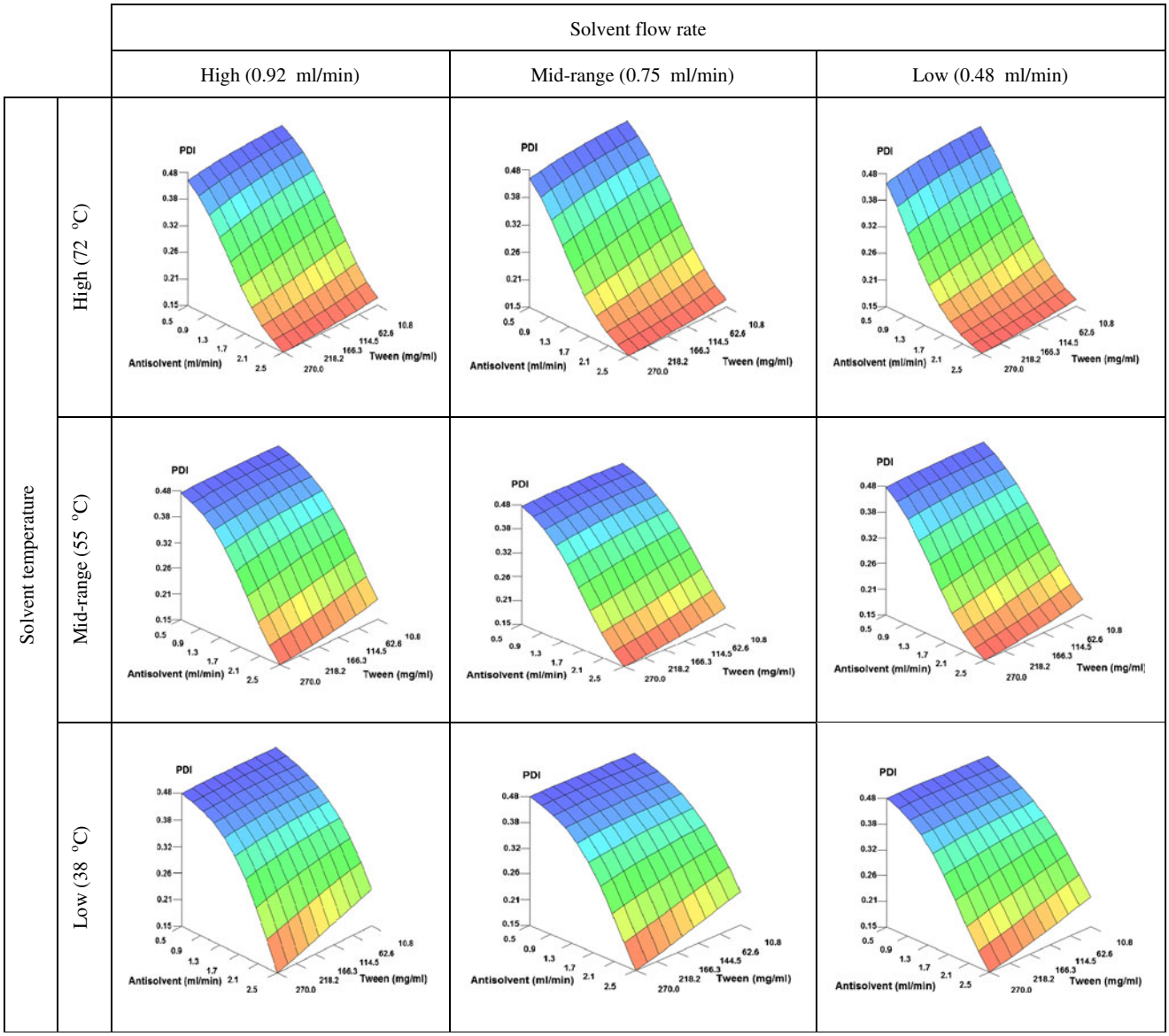

Fig. 4. 3D plots of the nanosuspensions' PDI predicted by the ANNs model fixed at low, mid-range, and high values of the solvent flow rate and solvent temperature

DISCUSSION

The linear patterns, which are commonly observed in microfluidic channels, direct a controlled diffusion of the drug molecules across the interface. This usually ends up in limited variations in the dispersity of samples, as noted above. The formed drug nuclei in the interface, which are a result of mixing solvent and antisolvent (*i.e.*, supersaturation), serve as nucleation sites and start to grow in size. Whereas, controlling the growth of the particles and preventing the drug sedimentation is usually performed by addition of surfactants and/or polymers (6) (see Fig. 7).

Our previous work on the factors affecting the physical stability of a nanosuspension of acetaminophen, prepared in microfluidic instrument, showed that antisolvent flow rate and temperature of solvent had a direct relation with physical stability, while the solvent flow rate showed reverse effect on the stability (22). In that study, it was suggested that the changes in the stability may be related to changes in polydispersity of the preparation, as examined in a preliminary study. Findings in the present work are in correlation with our previous hypothesis.

From the results expressed above, it can be concluded that the increasing temperature of the solvent results in decreasing particle size dispersity in the final product (*i.e.*, more uniform nanosuspension). It could be due to the fact that an increase in temperature of the solvent leads to more rapid diffusion of the solute from the solvent stream into the antisolvent stream. As a result, before leaving the instrument, more drug molecules get in touch with the antisolvent stream and form more drug nuclei in the diffusion layer (*i.e.*, formation of more nucleation sites). This phenomenon ends up in the formation of particles with smaller sizes and less polydispersity.

With regard to the effect of solvent/antisolvent flow rates, it has been determined that increasing the antisolvent flow

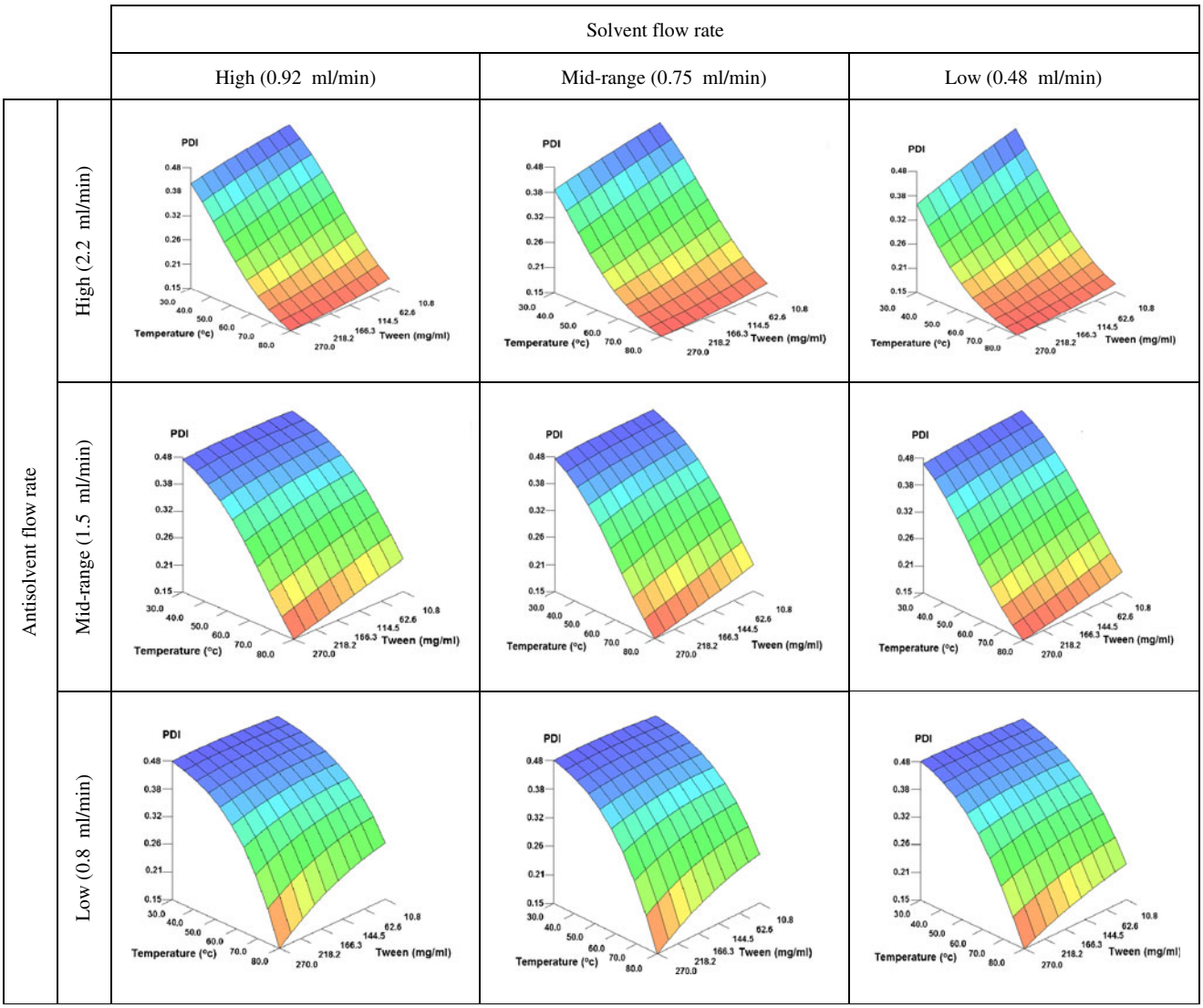

Fig. 5. 3D plots of the nanosuspensions' PDI predicted by the ANNs model fixed at low, mid-range, and high values of the solvent and antisolvent flow rate

rate makes the PDI smaller which is in good agreement with the former studies by Chen *et al.* and Wang *et al.* (26,27). In fact, with increasing antisolvent flow rate, fewer drug molecules diffuse to the flow of antisolvent per unit volume of antisolvent. Consequently, presence of the solute decreases around growing drug particles (17,19). Therefore, a nanosuspension with less PDI value will be formed. A second reason for this phenomenon could be the formation of more nucleation sites per unit volume of antisolvent. This causes the precipitation of less drug molecules per nucleation site, thus causing a more uniform distribution for the particle size (*i.e.*, less PDI). Similarly, current findings show that a higher solvent flow rate causes an increase in PDI. It is arguable that with increasing solvent flow, diffusion of drug molecules per unit volume of antisolvent to antisolvent flow will increase. This results in a product with more polydispersity, as explained above.

The details also show that the Tween 80 concentration has a small impact on particle size distribution which is in agreement with a previous report (28). Furthermore, the model showed that increasing the antisolvent flow rate promotes the effect of surfactant concentration. This is most probably due to the fact that in this experiment, the surfactant was dissolved in water (*i.e.*, antisolvent). Upon increasing the antisolvent flow rate, more surfactant molecules enter the microfluidic device. This causes a further increase in the content of Tween 80 in the final preparation and accordingly makes the preparation more monodispersed.

Overall, this study suggests that antisolvent flow rate and temperature of the solvent are possibly the dominant factors influencing polydispersity of the nanosuspension of acetaminophen. To obtain the minimum PDI—as the optimized formulation—the flow rate of the antisolvent as well as temperature of the solvent need to be kept high while the solvent flow rate should be minimum.

CONCLUSION

In this research, a quality ANN model was obtained that showed the impact of solvent and antisolvent flow rate, solvent

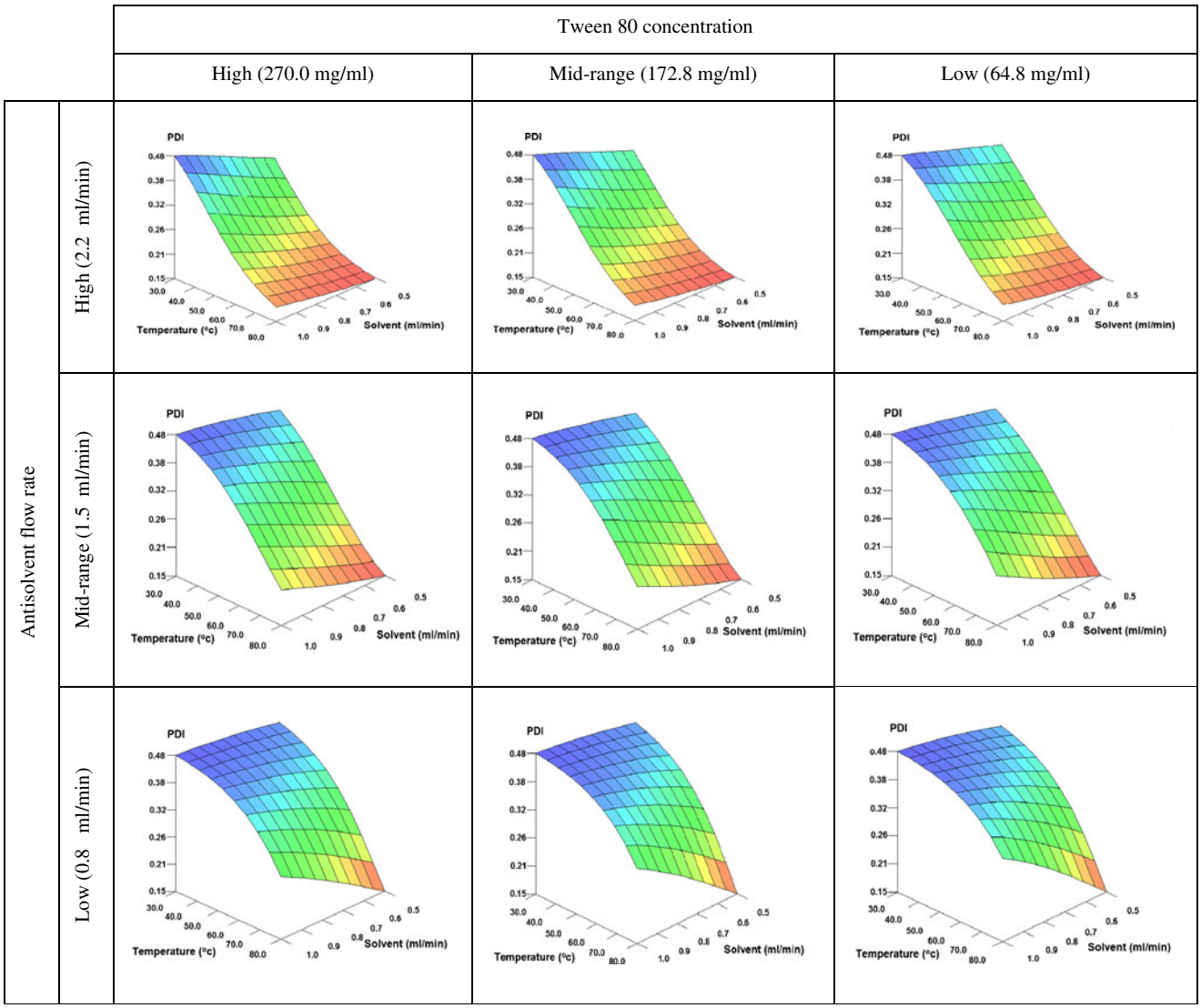

Fig. 6. 3D plots of the nanosuspensions' PDI predicted by the ANNs model fixed at low, mid-range, and high values of Tween 80 and antisolvent flow rate

temperature, and concentration of Tween 80 on the polydispersity in a nanosuspension of acetaminophen prepared in a microfluidic instrument. The response surfaces obtained from the model illustrated that all the four variables have some effects on the PDI. In general, increasing the antisolvent flow rate and solvent temperature resulted in decreasing PDI, while the solvent flow rate had a direct relation with polydispersity. It was also found that normally, the concentration of the surfactant had a reverse but less important effect on the PDI.

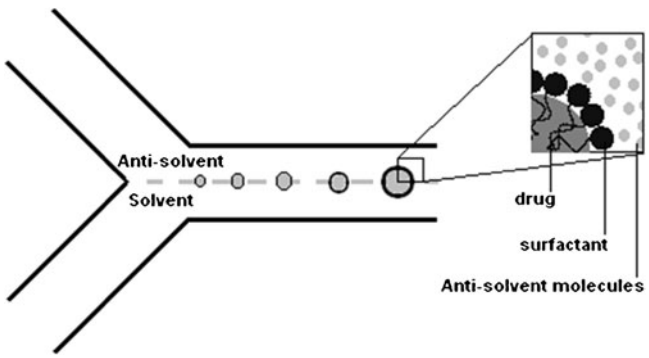

Fig. 7. A schematic of the precipitation process in microfluidic reactors

ACKNOWLEDGMENTS

This research has been supported by Tehran University of Medical Sciences and Health Services grant no. 88-04-87-9701. The authors would like to thank Dr. Reza Fardi-Majidi for his support during the study.

Conflict of Interest The authors declare that they have no conflicts of interest to disclose.

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