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Effect of various parameters on encapsulation efficiency of mPEG-PLGA nanoparticles: artificial neural network

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ABSTRACT

In this work we prepared curcumin loaded mPEG-PLGA nanoparticles using precipitation technique and investigated the effect of various parameters such as polyvinyl alcohol (PVA), curcumin concentrations and stirrer time on encapsulation efficiency (EE) of curcumin into mPEG-PLGA nanoparticles. Artificial neural networks (ANN) were used to model the data in order to find an ideal model which can fit the data and predict the EE with the lowest error and highest linear regression. The different samples of nanoparticles were prepared as training and testing datasets using the k-fold cross validation procedure. The best ANN design comprised 2 hidden layers with 8 and 1 nodes in each layer, respectively. Levenberg-Marquardt back propagation with log-sigmoid transfer function was the best model for our datasets. The mean square error and correlation coefficient between the observed and the predicted EE of curcumin into mPEG-PLGA nanoparticles were 0.1609 and 0.9209, respectively. In addition, three-dimensional correlation graphs showed that the most important pairs of variables which had a greater impact on EE were mPEG-PLGA/curcumin concentration and mPEG-PLGA/PVA concentration.

Keywords: *encapsulation efficiency, mPEG-PLGA, curcumin, polyvinyl alcohol, ANN.*

1. INTRODUCTION

Drug delivery vehicles offer a medium for the protection and transportation of drugs without altering the drugs' physiochemical properties. Micron and sub-micron drug carriers have been developed for the transportation of various therapeutic agents[1]. Lipid based structures such as solid lipid nanoparticles and emulsions are ideal vehicles for lipophilic drugs such as temozolomide[2]. Protein based delivery systems such as albumin and gelatin are a preferable choice for drugs with high protein binding affinity such as imatinib[3,4]. On the other hand, careful control of synthesis environment can be adapted for loading of both hydrophobic [5] and hydrophilic[6] drugs in a plethora of synthetic polymer based drug carriers. One important characteristic which is useful for the estimation of the delivery vehicles' utility is its drug EE. The EE of a drug carrier system can be defined as the percentage of the initial amount of drug present in the formulation (equation 1). For any carrier based drug delivery system, it is desirable to have a high EE in order to deliver higher amounts of drug payload. Moreover, high EE implies economical usage of drugs without a decrease in their therapeutic index[7].

$$EE\ \% = \frac{initial\ amount\ of\ drugs - amount\ of\ drugs\ in\ the\ supernatant}{initial\ amount\ of\ drugs} x100 \quad (1)$$

For most carriers, the drug loading step is achieved during the preparation. Therefore, it is difficult to control the EE as a result of many intertwining factors. Thus, in order to maximize the amount drugs entrapped by the carrier system, it is paramount to optimize all parameters which can affect the EE. The challenge is then to deduce the parameters which significantly affect the EE and the various correlations among them. This complex and tedious process will require an enormous amount of experiments to meet the minimum required for traditional statistical analysis. Alternatively, the same can be achieved with computer models which can predict the behavior of the system with a reasonable degree of accuracy using minimum experiments. The different tools that have been used to analyze patterns, correlations and forecasting include statistical methods like response surface methodology (RSM)[8], design of experiment (DOE)[9] and central composite designs (CCD)[10] among others. However, the accuracy of the model predictions are based on in built algorithms thus some data may not conform to these requirements. Biologically inspired artificial neural networks (ANN) provide an experience based (as opposed to algorithm based) forecasting model. Functionally, networks learn from experience through training and use this information to detect patterns and relationships within the data. This extracted information can then be further processed to provide more accurate forecasts[11-14]. Therefore, based on these merits, ANN has been used to predict many models. For example, Dixit et al used ANN and k-fold cross-validation method to predict transferrin receptor-targeted theranostic gold nanoparticles for drug delivery to tumor [15]. In another study, ANN was used to predict the effects of magnesium oxide nanoparticles and temperature on the thermal conductivity of water[16]. The output of the neural network shows that ANN was more accurate than correlation curve-fitting method from the experimental data. The ANN model was also used to predict the

size chitosan nanoparticles with R^2 values greater than 0.8 for various independent variables[17]. Therefore, in this work we use ANN to predict EE of curcumin into mPEG-PLGA. We further investigated the correlations among various parameters and their effect on the EE of curcumin. Curcumin is a multi-purpose therapeutic agent which is used as antioxidant[18], anticancer[19] and anti-inflammatory[20] agent among others. The mPEG-PLGA nanoparticles were prepared by the simple and reproducible single step nanoprecipitation technique.

2. EXPERIMENTAL SECTION

2.1. Materials. MPEG-PLGA with the molecular weight of 10000 g mol^{-1} was purchased from Shenzhen Esun Industrial Co. (China). PVA with the molecular weight of 60000 g mol^{-1} and ethanol were bought from Merck (Germany). Curcumin was from Alfa-Aesar (Germany). All solutions were prepared using double deionized water (DDW).

2.2. Preparation of curcumine loaded MPEG-PLGA nanoparticles by precipitation. Various PVA concentrations (0.25 – 1 %) were prepared by dissolving a specific amount of PVA in DDW. The organic solution was prepared by dissolving different amounts of mPEG-PLGA and curcumine in 5 ml of acetone. Afterwards, the organic solution was added to the aqueous PVA (20 ml) solution under magnetic stirring. Complete removal of the organic phase the solution was achieved by overnight stirring under chemical fume hood. Curcumin loaded mPEG-PLGA nanoparticles were then collected by centrifugation at 15000 rpm for 20 min. The obtained nanoparticle pellet was washed three times with DDW. The amount of drugs encapsulated within the nanoparticles was determined via UV-VIS (Cecil CE 7250, England). In addition, the mean size of nanoparticles were obtained using dynamic light scattering (Scatter Scope).

2.3. Using Artificial Neural Network. To design neural networks with different functions such as input units, output unit and various hidden layers with different nodes, the network was trained accomplishing training information and was tested using testing information. The inputs were various independent parameters including stirrer time, polymer (mPEG-PLGA), stabilizer (PVA) and drug (curcumin) concentrations (Table 1). The output unit of the ANN measured the impact of the different input units on EE. In this work, neural network toolbox of MATLAB® software was used to validate the curcumin EE of mPEG-PLGA nanoparticles.

Table1. List of the input variable of ANN modeling.

Independent input variable	Description
X_1	**MPEG-PLGA Concentration (%)**
X_2	**PVA Concentration (%)**
X_3	**Curcumin Concentration (%)**
X_4	**Stirrer time (min)**

According to the database, *k*-fold cross-validation method was used to partition our test and training data sets for obtaining better results.

2.4. Data Normalization and Network Training Using *k*-Fold Cross-Validation Procedure. Before using ANN, data normalization was performed based on equation 2.

$$y_{norm} = (y_{max} - y_{min})(x - x_{min})/(x_{max} - x_{min}) + y_{min} \quad (2)$$

where y_{min} and y_{max} are equal to −1 and 1, respectively, x is the information that should be standardized, $x_{max}$and x_{min} are the maximum and minimum values of x.

28 samples of curcumin loaded nanoparticles were prepared by the precipitation method (Table 2). The EE of the nanoparticles was analyzed using ANN models training- testing information. In this procedure, the information was divided into k (six training and testing) stages of equal subsets for testing and training data sets (Table 3). Training data was used to control the network weights and the testing dataset assessed the network function. In every stage, *k*-1 subsets were put together to create a training set and the remaining *k* subset was used as the test. The mean squared error (MSE) across all k trials was calculated (using equation 3) thereafter and it was used to assess the network soundness. In addition to the MSE, the correlation co-efficient (R) was also determined using equation 4. Therefore, the best model for predicting EE of nanoparticles which was chosen had the lowest MSE and highest R.

Table 2. List of observed and predicted EE of mPEG-PLGA nanoparticle loaded with curcuminin various sets of control parameters.

Sample	mPEG-PLGA concentration (%)	PVA concentration (%)	Curcumin concentration (%)	Stirrer time (min)	Observed EE (%)	Predicted EE (%)
1	8.00	1.00	2.00	8	98.46	98.46
2	8.00	1.00	2.00	2	98.24	96.43
3	8.00	1.00	0.50	6	96.00	98.00
4	8.00	1.00	0.50	4	92.20	95.95
5	8.00	0.25	1.50	8	93.01	91.56
6	8.00	0.25	1.00	6	93.08	92.48
7	8.00	0.25	1.00	4	91.40	91.40
8	10.00	0.75	2.00	2	92.40	95.05
9	10.00	0.75	0.50	6	92.00	74.77
10	10.00	0.75	0.50	4	85.80	85.80
11	10.00	0.50	1.50	8	98.82	98.82
12	10.00	0.50	1.00	6	97.94	95.00
13	10.00	0.50	1.00	4	97.99	75.98
14	15.00	1.00	2.00	8	98.00	96.97
15	15.00	1.00	2.00	2	98.98	96.72
16	15.00	1.00	0.50	6	93.52	97.67
17	15.00	1.00	0.50	4	94.32	97.65
18	15.00	0.25	1.50	8	98.90	98.98
19	15.00	0.25	1.50	2	98.64	97.59
20	15.00	0.25	1.00	4	99.83	99.83
21	20.00	0.75	2.00	8	98.90	98.98
22	20.00	0.75	2.00	2	97.90	98.35
23	20.00	0.75	0.50	6	90.80	64.03
24	20.00	0.75	0.50	4	89.12	92.23
25	20.00	0.50	1.50	8	97.65	60.50
26	20.00	0.50	1.50	2	84.00	84.00
27	20.00	0.50	1.00	6	93.25	91.01
28	20.00	0.50	1.00	4	92.70	92.70

Table 3. Training-testing partition pairs using 6-fold cross-validation method

Partitions	Training set	Testing set
1	Partition {1,2,3,4,5}	**Partition {6}**
2	Partition {1,2,3,4,6}	**Partition {5}**
3	Partition {1,2,3,5,6}	**Partition {4}**
4	Partition {1,2,4,5,6}	**Partition {3}**
5	Partition {1,3,4,5,6}	**Partition {2}**
6	Partition {2,3,4,5,6}	**Partition {1}**

$$MSE = \frac{100}{Nte\sigma_{d_n}{}^2}\sum_{i=1}^{i=Nte}\left(d_n(i) - d_{pn}(i)^2\right) \quad (3)$$

Where d_n and d_{pn} are observed and predicted EE of curcumin in the network, $\sigma_{d_n}{}^2$ is the variance in d_n and Nte is the number of samples.

3. RESULTS SECTION

The mean square error (MSE) and correlation coefficient (R) of test information gained from the ANN model is shown in Table 4. From this test data set, the 6-fold ANN comprised two hidden layers and 8, 1 nodes, respectively. After training the network using the 6-fold partition, the best ANN model with MSE and maximum correlation R was selected via Levenberg-Marquardt back propagation, training logarithm, and log-sigmoid transfer function (Table 5). To deduce encapsulation and standard deviation of the monitored and predicted median nanoparticles, SPSS 17 software was used. At 0.01 % (the statistically significant level), the Pearson correlation coefficient between the predicted and observed EE was 0.809 (Table 6). In addition, the mean difference between the observed and the predicted EE of nanoparticles was about 3 %, indicating the reliability of the ANN modeling (Table 7).

Table 4. The Mean square error (MSE) and Regression (R) of test data in the selected ANN network.

NETWORK	MSE	R
1	0.0495	0.964
2	0.1073	0.918
3	0.3246	0.866
4	0.1361	0.9345
5	0.1931	0.922
6	0.1549	0.921
Mean	0.1609±0.0936	0.9209±0.0318

Table 5. ANN training parameters.

Algorithm	Trainlm (Levenberg-Marquardt back propagation)
Transfer function in hidden layers	log-sigmoid and purelin
Number of epochs between showing the progress	5
Learning rate	0.01
Momentum constant	0.9
Maximum number of epochs to train	1000
Performance goal	1e-5

Table 6. The Pearson correlation between simulated predicted and observed EE of nanoparticles.

		Predicted EE	Observed EE
Predicted EE	Pearson Correlation	1	.809**
	Sig. (2-tailed)		.000
	N	30	30
Observed EE	Pearson Correlation	.809**	1
	Sig. (2-tailed)	.000	
	N	30	30

** Correlation is significant at the 0.01 level (2-tailed).

Table 7. Descriptive statistics of observed and predicted mean EE.

		Predicted EE	Observed EE
N	Valid	30	30
	Missing	0	0
Mean		89.2475	92.4483
Std. Deviation		16.26155	13.91652
Minimum		22.00	22.00
Maximum		99.83	99.83

The plot of the correlation between the observed and predicted EE of the nanoparticles is shown in Fig. 1. Considering very high degrees of complexity between the procedures and conditions of the EE of the nanoparticles, these tests show a sufficient trained model.

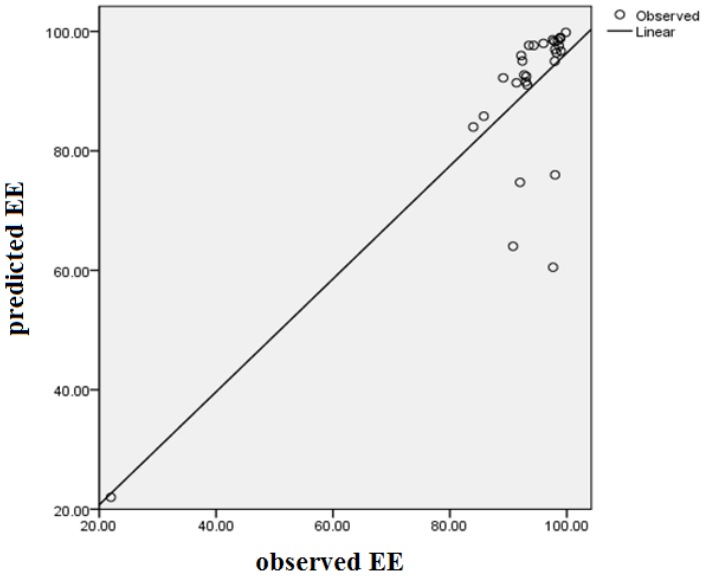


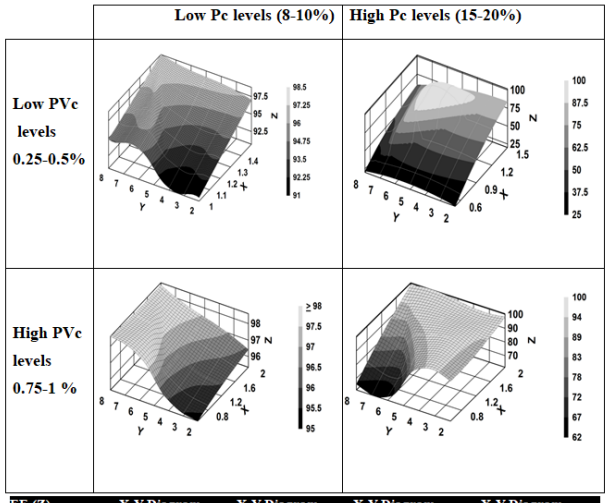
Figure 1. Regression plot between the observed and the predicted EE.

The Pearson correlation coefficients (r) between the monitored (d_n) and predicted (d_{pn}) nanoparticles EE is given by equation (4):

$$r = \frac{n(\sum d_n d_{pn}) - (\sum d_n)(\sum d_{pn})}{\sqrt{[n(\sum {d_n}^2) - (\sum d_n)^2][n(\sum {d_{pn}}^2) - (\sum d_{pn})^2]}} \quad (4)$$

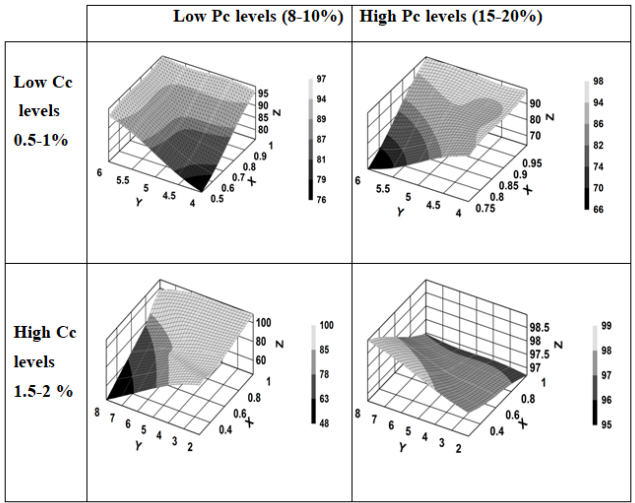
3.1. Three-dimensional plots of predicted patterns of EE. In order to investigate the effects of different chosen independent parameters (Table 1) on EE of mPEG-PLGA nanoparticle, 3D diagrams were plotted. The results show that the maximum EE of the nanoparticles is about 97% in low mPEG-PLGA concentration and high PVA concentration (Low Pc- High PVc) area, as shown in Fig. 2. On the other hand, the minimum EE of the nanoparticles is approximately 61% when mPEG-PLGA concentration is high and PVA concentration is low (High Pc-Low PVc). Generally, it has been observed that the EE increases with high polymer concentration [21-23] and low PVA concentration. However, Sharma et al showed that PVA concentration has an optimum value and hence tend to converge from either low or high values [22]. Therefore, this may partly explain the observed inverse relationship between mPEG-PLGA concentration and PVA concentration with regards to the EE of the nanoparticles. That is, decreasing PVA concentration with a corresponding increase in mPEG-PLGA concentration leads to increase in the EE of nanoparticle. For lower concentrations of PVA and mPEG-PLGA, the EE obeyed a direct relationship law. That is, increasing both PVA and mPEG-PLGA concentrations resulted in an increase in EE and the converse was also true. For both high PVA and mPEG-PLGA concentrations, an inverse relationship was observed with

regards to the EE. Increasing high PVA concentration (High PVc) and decreasing High Pc (high mPEG-PLGA) concentration resulted in higher EE, likewise, decreasing High PVc concentration (high PVA) and increasing High Pc (high mPEG-PLGA concentration) resulted in a similar outcome.



EE (Z)	X-Y Diagram Low Pc - Low PVc	X-Y Diagram High Pc - Low PVc	X-Y Diagram Low Pc- High PVc	X-Y Diagram High Pc - High PVc
Minimum Z	90.68 at X = 1, Y = 3.5	20.09 at X = 0.5, Y = 2	95.34 at X = 0.5, Y = 2	61.36 at X=0.5, Y = 6.87
Maximum Z	98.82 at X= 1.5, Y = 8	101.74 at X = 1.22, Y = 5.56	98.61 at X = 0.5, Y = 8	100.61 at X = 2, Y = 6.68
Mean	95.23%	61.52%	97.13%	89.99117
STD	1.8715	21.789	0.9057	9.9245

Figure 2. The data and 3D plots of predicted EE by ANN fixed in the mentioned levels (Pc-PVc diagram).
X= Curcumin concentration (%), Y= Stirrer time (min), Z= EE (%),
PVc= Polyvinyl alcohol concentration (%),
Pc= mPEG-PLGA concentration (%)

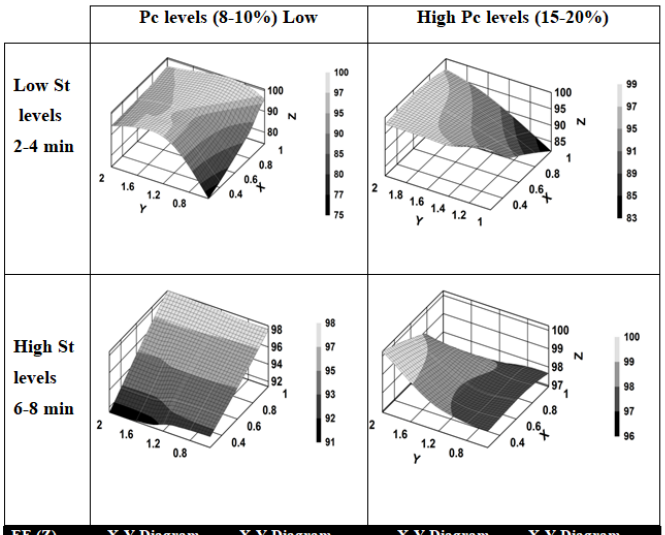


EE (Z)	X-Y Diagram Low Pc-Low Cc	X-Y Diagram High Pc-Low Cc	X-Y Diagram Low Pc- High Cc	X-Y Diagram High Pc- High Cc
Minimum Z	75.98 at X = 0.5, Y = 4	64.03 at X = 0.75, Y = 6	44.99 at X = 0.25, Y = 8	96.72 at X = 1, Y = 2
Maximum Z	98 at X = 1, Y = 6	99.04 at X = 1, Y = 5.5	99.11 at X = 1, Y = 2	98.98 at X = 0.25, Y = 8
Mean	90.60	88.95	72.56	97.52
STD	5.1898	8.1458	14.35	0.5175

Figure 3. The data and 3D plots of predicted EE by ANN fixed in the mentioned levels (Pc-Cc diagram).
X= Polyvinyl alcohol concentration (%), Y= Stirrer time (min),
Z= EE (%), Cc= Curcumin concentration (%),
Pc= mPEG-PLGA concentration (%)

The effect of Cc (curcumin concentration) and Pc (mPEG-PLGA concentration) on the EE is presented in Fig 3. In the Low Cc (low curcumin concentration) region, the mean maximum and minimum EE were about 97% and 72% respectively. For High Cc (high curcumin concentration), the mean values of EE were higher than those for Low Cc (low curcumin concentration) suggesting that higher curcumin concentrations increase EE. Further detail regarding the association of Cc (curcumin concentration) and Pc (mPEG-PLGA concentration) in all regions can be discerned from the 3D graphs of Fig 3.

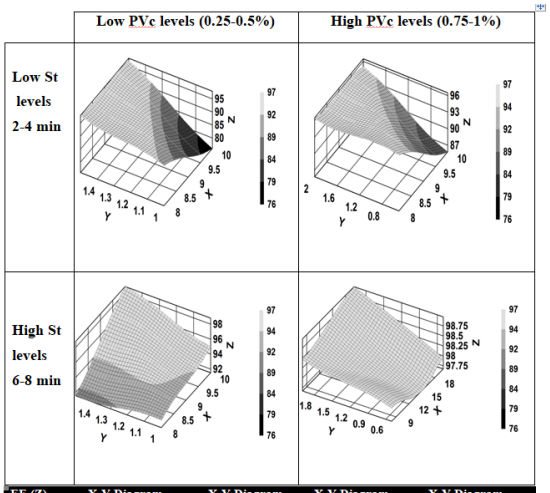
It was further observed that there is a direct relationship between mPEG-PLGA concentration and stirrer time with regards to the eventual EE (Fig 4). The highest EE of nanoparticles was observed to be about 98 % in the High St (high stirrer time) and High Pc (high mPEG-PLGA concentration). In other combinations, the EE was fairly constant at around 94%. This result may imply that stirrer time and mPEG-PLGA concentration are fairly independent with regards to EE. When mPEG-PLGA concentration was low, the EE varied following an inverse relationship between St (stirrer time) and Pc (mPEG-PLGA). Conversely, in the High Pc (high mPEG-PLGA) level, the EE followed was dependent on the direct relationship between St (stirrer time) and Pc (mPEG-PLGA).



EE (Z)	X-Y Diagram Low Pc-Low St	X-Y Diagram High Pc-Low St	X-Y Diagram Low Pc- High St	X-Y Diagram High Pc- High St
Minimum Z	74.46 at X = 0.25, Y = 0.5	82.11 at X = 1, Y = 1	91.39 at X = 0.25, Y = 2	96.934 at X = 1, Y = 1.8125
Maximum Z	99.999 at X = 1, Y = 1.0156	99.993 at X = 0.25, Y = 1.0625	98.46 at X = 1, Y = 2	99.996 at X = 0.25, Y = 2
Mean	94.41	94.65	95.17	98.20
STD	5.0782	3.6015	1.8689	0.6230

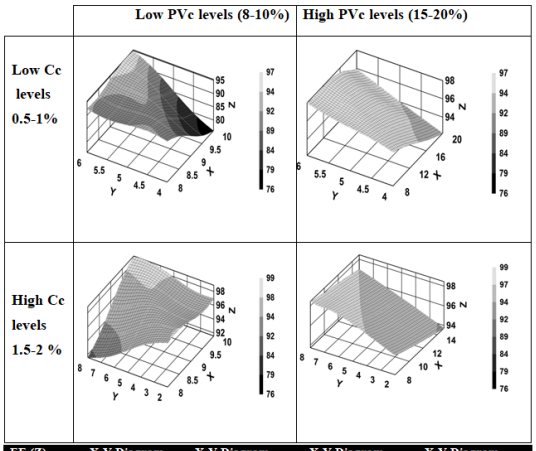
Figure 4. The data and 3D plots of predicted EE by ANN fixed in the mentioned levels (Pc-St).
X=Polyvinyl alcohol concentration (%), Y= Curcumin concentration (%),
Z= EE (%), St= Stirrer time (min),
Pc= mPEG-PLGA concentration (%)

The relationship between stirrer time and PVA concentration was also investigated and the results are shown in Fig 5. From the 3D plot, it can be deduced that the EE of nanoparticles was highest (98 %) when both St (stirrer time) was high and PVc (PVA concentration) was high. Otherwise, a variation of St (stirrer time) and PVc (PVA concentration) was fairly independent with regards to EE. The EE of nanoparticles was dependent on a direct relationship between St (stirrer time) and PVc (PVA concentration) in the Low St (low stirrer time) level. Conversely, in the High St (high stirrer time) level, EE varied according to the inverse relationship between St (stirrer time) and PVc (PVA concentration).



EE (Z)	X-Y Diagram Low PVc-Low St	X-Y Diagram High PVc-Low St	X-Y Diagram Low PVc- High St	X-Y Diagram High PVc- High St
Minimum Z	75.98 at X = 10, Y = 1	85.8 at X = 10, Y = 0.5	91.50 at X = 8, Y = 1.44	97.67 at X = 15.5, Y = 0.5
Maximum Z	97.92 at X = 9.43, Y = 1.5	96.50 at X = 8, Y = 0.922	98.82 at X = 10, Y = 1.5	98.98 at X = 20, Y = 2
Mean	91.10	93.635	94.642	98.256
STD	5.1594	2.547	1.6515	0.2925

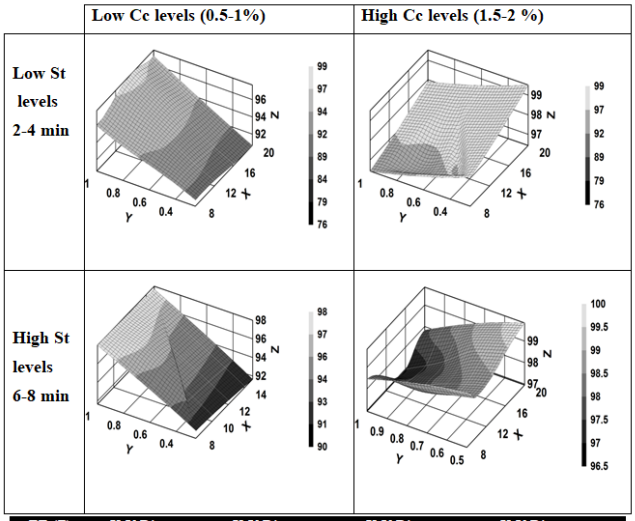
Figure 5. The data and 3D plots of predicted EE by ANN fixed in the mentioned levels (PVc-St).
X= mPEG-PLGA concentration (%), Y= Curcumin concentration (%),
Z= EE (%), St= stirrer time (min),
PVc=Polyvinyl alcohol concentration (%)



EE (Z)	X-Y Diagram Low PVc-Low Cc	X-Y Diagram High PVc-Low Cc	X-Y Diagram Low PVc- High Cc	X-Y Diagram High PVc- High Cc
Minimum Z	75.98 at X = 10, Y = 4	92.23 at X = 20, Y = 4	91.56 at X = 8, Y = 8	93.73093 at X = 15, Y = 2
Maximum Z	95.36 at X = 9.7, Y = 6	98 at X= 8,Y= 6	98.89 at X =10, Y = 7.25	98.46 at X = 8, Y = 8
Mean	89.384	95.7968	96.6202	96.30
STD	4.3224	1.344281	1.611198	1.105833

Figure 6. The data and 3D plots of predicted EE by ANN fixed in the mentioned levels (PVc-Cc).
X= mPEG-PLGA concentration (%), Y= stirrer time (min), Z= EE (%),
Cc= Curcumin concentration (%),
PVc= Polyvinyl alcohol concentration (%)

Lastly, high stirrer time is associated with higher EE (about 98 %) whilst low St has a marginally lower value of 95 % for all curcumin concentration levels. The trend for Low Cc (low curcumin concentration) is independent of stirrer time with respect to EE of nanoparticles (Fig 7). On the other hand, there is an inverse association between Cc (curcumin concentration) and St (stirrer time) in the High Cc (high curcumin concentration) level with regards to the EE.



EE (Z)	X-Y Diagram Low Cc –Low St	X-Y Diagram High Cc-Low St	X-Y Diagram Low Cc- High St	X-Y Diagram High Cc- High St
Minimum Z	90.67 at X = 20, Y = 0.25	96.43 at X = 8, Y = 1	91.66 at X = 15, Y = 0.25	96.947 at X = 15.5, Y = 1
Maximum Z	97.7 at X =15.87,Y = 1	99.52 at X = 20, Y = 0.25	98 at X = 8.656, Y = 1	99.841 at X = 20, Y = 0.5
Mean	94.05	97.68	95.232	98.449
STD	1.8128	0.7572	1.786	0.64246

Figure 7. The data and 3D plots of predicted EE by ANN fixed in the mentioned levels (Cc-St).
X=mPEG-PLGA concentration (%),
Y=Polyvinyl alcohol concentration (%), Z= EE (%),
Cc= Curcumin concentration (%), St=stirrer time (min)

Analysis of 3D graphs between curcumin concentration and PVA concentration and their impact on EE is shown in Fig 6. Apart from the Low Cc (low curcumin concentration) and Low PVc (low PVA concentration) combination, there is virtually no association between curcumin and PVA concentrations with regards to EE. The Low Cc (low curcumin concentration)/Low PVc (low PVA concentration) combination had the lowest EE (about 89 %) whilst the other three combinations averaged about 96 %. From the plots, it can be concluded that EE of nanoparticles largely varies with an inverse relationship between Cc (curcumin concentration) and PVc (PVA concentration).

4. CONCLUSIONS

In this work, 28 experiments were carried out to prepare mPEG-PLGA nanoparticles by precipitation method. The various independent parameters such as mPEG-PLGA and curcumine concentrations, stirrer time and PVA concentration were investigated with respect to their impact on the EE of the nanoparticle. Artificial neural networks were applied to the data and predicted the EE of nanoparticles with acceptable values of MSE= 0.16 and R= 0.92. This trial proves that ANN model can be used to predict the EE of mPEG-PLGA nanoparticles under well-defined conditions of curcumin, PVA, and mPEG-PLGA concentrations. In addition, 3D diagrams showed that the most significant correlationswere seen between EE and Pc/PVc (mPEG-PLGA/PVA concentrations) and Pc/Cc (mPEG-PLGA/curcumin concentrations), respectively. On the other hand, the results indicated that the stirrer time had very little impact on the EE of nanoparticles.

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6. ACKNOWLEDGEMENTS

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