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Research Article

OPTIMIZATION OF CHITOSAN BASED DOXORUBICIN MICRO PARTICLES BY NN (NEURAL NETWORK)

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ABSTRACT

The present work was aimed to formulate and evaluate the micro particles of doxorubicin(Dox) - chitosan using neural network.Chitosan doxorubicin micro particles prepared by emulsion crosslinking method. A 3^3 full factorial design with Neural network was employed to study the influence of three variables namely phase volume ratio , emulsification time and stirring speed on percentage practical yield, drug content, time required for $t_{50}$and t_{90} of doxorubicin(Dox) release and particle size. The microparticles were prepared by dropping the chitosan solution containing Dox into mechanically stirred mineral oil solution.Furthermore, the desirability function was employed in order to optimize the process under study. Topographical characterization was carried out by Scanning Electron Microscopy. The production yield and drug content of microparticles were in the range of 72.28 - 92.56 % and 69.96 – 92.26% respectively. Microparticles were obtained in range of 9.6-20.25 micrometer size with porous surface. The desirability function resulted to optimum values of factors at which produced microparticles could control particle size, which influence on t_{50} and t_{90}.It was found that the proper selection of variable level had significantly influenced on particle size, and drug release profile. ANOVA results and desirability function could serve better optimization and reduced trial based experiments.

Keywords: Doxorubicin-chitosan, Neural network.

INTRODUCTION

Neural network involves a network of simple processing elements (neurons) which can exhibit complex global behaviour, determined by the connections between the processing elements and element parameters. The original inspiration for the technique was from examination of the central nervous system and the neurons which constitute one of its most significant information processing elements, brain is an excellent pattern recognition tool. In a neural network model, simple nodes are connected together to form a network of nodes. Currently, the term ANN (artificial neural network) tends to refer mostly to neural network models employed in statistics and artificial intelligence. In modern software implementations of artificial neural networks inspired by biology has more or less been abandoned for a more practical approach based on statistics andsignal process.They are characterized by architecture, transfer function and learning paradigm.[1-3]The Microsoft Windows-based NN software NeuroSolutions Version 6.12 (NeuroDimension, Inc) was used. Neural Network (NN) models might generalize better than regression models, because regression analyses are dependent on predetermined statistical significance levels (i.e. less significant terms are not included in the model). [4-5] The use of at least 1 hidden layer enables the NNs to describe nonlinear systems. One layer is usually sufficient to provide an adequate prediction, even if continuous variables are adopted as the units in the output layer. Additionally, there is a little evidence to suggest that a larger number of hidden layers improve performance. [6]The MLP with single hidden-layer architecture was chosen. The experimental matrix of 32 input-to-desired output data sets was introduced in to the model, with 3 input neurons (process variables), 1 hidden layer, and 3 output neurons (response variables). Various adjustable parameters, like the number of neurons in the hidden layer, the step size, the momentum of the hidden layer and the output layer, and so forth, were optimized. At the start of the training run, weights were initialized with random values. During training, 5 additional data sets of input-to-desired output ratio were used for the cross-validation and were back-propagated through the network to evaluate the trained network. The training termination criterion was the rise in minimum standard error of the cross-calibration set compared with that of the training set for 25 continuous epochs. The network trained under optimum conditions was used to predict the responses at different factor values and response surfaces were generated for interpretation. The three most common criterions to stop training are: to cap the number of iterations, to threshold the output mean square error, or to use cross validation.The multilayer perception (MLP) with back propagation algorithm is one of the most widely implemented NN topologies and is important in the study of nonlinear dynamics.[7] Two important characteristics of the MLP are its smooth nonlinear neurons (sigmoidal function) and its massive interconnectivity(i.e. any element of a given layer feeds all the elements of the next layer).

NN has been successfully applied to many pharmaceutical areas in recent years, [8] such as quantitative structure activity relationship analysis and drug modeling,[9] pharmacokinetic-pharmacodynamic studies,[10-11]optimization and pharmaceutical formulation development, [6]powder flow,[12]compound determination using high-performance liquid chromatography,[13]analysis of nuclear magnetic resonance spectra,[14]prediction of drug release profile,[15]prediction of physicochemical properties,[16]prediction of octanol-water partition coefficient,[17]prediction of solubility[18]and so forth.

NN consists of a number of processing elements (neurons) which are interconnected forming input and output layers and one or more hidden layers (Figure 1).

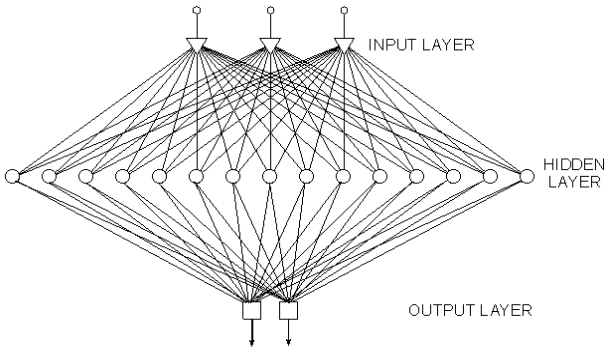

Fig. 1: Architecture of three-layer neural network

Doxorubicin (former generic name, Adriamycin), a highly effective anticancer drug, produces cardio toxicity, which limits its therapeutic potential. The mechanism of this cardio toxicity has remained elusive. Significant and persistent cardiac dysfunction develops in more than 60% of patients receiving maximal acceptable

cumulative dosages of doxorubicin (430-600 mg/m^2). According to a popular hypothesis, doxorubicin impairs cardiac function by generating oxygen free radicals that bind to and disrupt membrane proteins and phospholipids. Chitosan [poly(1,4-β-D-glycopyrano-s-amine)] is one of the polysaccharides which show antimicrobial and biocompatible behavior Toxic side effects associated with the administration of anticancer drugs makes them ideal candidates for targeted drug delivery. The present work was aimed to formulate and evaluate the microparticles of DOX-chitosan using neural network.

MATERIALS AND METHOD

Materials

Chitosan was procured from Central Institute of Fisheries Technology, Cochin. Doxorubicin Hydrochloride(DOX) was obtained as a gratis sample from Sun Pharmaceuticals Ind. Ltd. Liquid paraffin, Glacial acetic acid, n-hexane, Na_2HPO_4, Glutaraldehyde, concentrated Hydrochloric acid and all other reagents used were of AR grade and were procured from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of microparticles

Concentrated chitosan solution was prepared by dissolving in 1.5 % V/V acetic acid solution. Required quantity of drug Doxorubicin hydrochloride was dispersed in polymer solution. Final concentration of chitosan was adjusted to required concentration 1-3 % W/V and was used after being degassed under a vacuum.[19] The microparticles were prepared by dropping of the chitosan solution (10 ml) containing DOX from the dropping device such as syringe (18gauge) to mechanically stirred mineral oil. Emulsion was stirred for 20 min for uniform particle size reduction and distribution. Microparticles were hardened with hardening agent glutaraldehyde for 3 hours. The microparticles were harvested by decanting oil followed by washing with n–hexane and dried until constant weight. Products were stored in desiccators for further characterization (Table 4).[20]

Characterization of Microparticles

Percentage Yield

$$\%Yield = \frac{Weight\ of\ microparticles}{Weight\ of\ (Drug + Polymer)} \times 100$$

Particle size analysis and Scanning Electron Microscopy (SEM)

The mean particle size of the prepared microparticles was measured using optical microscope. The purpose of SEM study was to obtain a topographical characterization of microparticles. The microparticles were mounted on brass stubs using double- sided adhesive tape. Photomicrographs were taken using a Philips (FE l company) SEM – XL-30 TMP.

Analysis of Drug content

A weighed quantity of chitosan- DOX microparticles were extracted with 0.1N Hydrochloric acid in glass stoppered conical flask at 37° for 6 h. The mixture was filtered and absorbance was measured at 480 nm after appropriate dilution. The amount of DOX present was determined from Beer's plot.

In-Vitro Drug Release

The *in-vitro* DOX release from microparticles was conducted in 7.4 pH phosphate buffer saline (PBS) at 37°± 0.5°. Drug loaded microparticles were placed in the dialysis tube, which was covered with semi permeable membrane (molecular weight is 12,000-14,000). Samples were analyzed using Shimadzu 1601 UV-spectrophotometer. Each determination was carried out in triplicate and the release results were plotted as the cumulative percentage of drug content in dissolution media Vs time.

Determination of t_{50} and t_{90}

Time required for 50 (t_{50}) and 90 (t_{90}) percentage of drug release are the parameters for drug release study. For optimization purpose, dissolution study was carried out as mentioned above*in-vitro*drug release t_{50} and t_{90} were found from cumulative percentage drug release versus time plot by drawing a projection line on the time axes at 50 % and 90 % release, respectively.

Factorial design and the desirability function[21-25]

Factorial design is a useful tool in order to characterize multivariable process. It gives the possibility to separate the important factors from these which are not identifying any possible interactions between them.[23]Traditionally pharmaceutical formulations are developed by changing one variable at a time approach. The method is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to evolve an ideal formulation using this classical technique since the joint effect of independent variables is not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial designs.[24-25]

The number of experiments required for these studies is dependent on the number of independent variables selected. The response is measured for each trial and then either simple linear equation, or interactive equation orquadratic model is

$(Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3)$

$(Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3)$

$(Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_1^2X_{11} + b_2^2X_{22} + b_3^2X_{33} + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{123}X_1X_2X_3)$

To study all the possible combinations of all factors at all levels, three factors and three levels full factorial design was constructed and were conducted in a fully randomized order.

To study all the possible combinations of all factors at all levels, a three factor, three levels full factorial design was conducted in a fully randomized order. Three independent factors, phase volume ratio (X_1), emulsification time (X_2), stirring speed (X_3), were set at three different levels. High and low levels of each factor were coded as 1 and -1, respectively, and the mean value as zero(Table 1). The dependent variables were particle size, t_{50}, t_{90} as shown in Table 2.

Table 1: Factorial 3^3: factors and their levels

Independent Variables	Levels		
	Low(-1)	Medium(0)	High(1)
X_1= phase volume ratio	1 : 5	1: 10	1: 15
X_2 = emulsification time (min)	10	15	20
X_3 = stirring speed (rpm)	500	1000	1500

RESULTS AND DISCUSSION

The particle size value was minimized in the optimization procedure. The desirability functions of this response were calculated using the following equation: $Y_{max}$and Y_{target} are 20.13 µ and 7.25 µ respectively.

$$d_1 = \frac{Y_{\max} - Y_i}{Y_{\max} - Y_{target}}$$

The values t_{50} and t_{90} were maximized in the optimization procedure, as suitable microparticles should have high longer t_{50} and t_{90}.The desirability function of these responses were calculated using the following equation:d_2, d_3,$Y_{min}$and Y_{target} for Y_{min} and Y_{target} for t_{50} and $t_{90}$of are 6.38, 9.41and11.36 ,24.83 hrs respectively ($d_2$and d_3).

$$d_2 or d_3 = \frac{Y_i - Y_{\min}}{Y_{target} - Y_{\min}} \qquad \text{for } Y_i < Y_{target}$$

$$= 1 \qquad \text{for } Y_i > Y_{target}$$

The overall desirability values were calculated from the individual values by using the following equation:

$$D = (d_1 d_2 d_3)^{1/3}$$

The statistical evaluation of the results was carried out by analysis of variance (ANOVA) using Microsoft Excel Version-2000. The ANOVA results (p value) for the effect of the variables on particle size, t_{50}, t_{90} of microparticles is depicted in Table.2.

Table 2: For the factorial 3^3design and results for the measured responses and over all desirability

Batches	X_1	X_2	X_3	Particle size	t_{50}(hrs)	t_{90}(hrs)	Overall Desirability
D_1	-1	-1	-1	20.13	8.90	23.83	0.000
D_2	-1	-1	0	19.93	8.50	22.12	0.338
D_3	-1	-1	1	19.12	7.93	21.85	0.166
D_4	-1	0	-1	19.72	9.21	24.83	0.416
D_5	-1	0	0	18.84	8.45	22.13	0.368
D_6	-1	0	1	18.58	7.54	20.83	0.302
D_7	-1	1	-1	18.14	9.41	20.21	0.434
D_8	-1	1	0	18.32	8.92	21.42	0.426
D_9	-1	1	1	16.42	8.54	20.54	0.467
D_{10}	0	-1	-1	15.58	8.15	20.13	0.475
D_{11}	0	-1	0	14.64	7.68	19.79	0.447
D_{12}	0	-1	1	15.69	7.05	19.12	0.317
D_{13}	0	0	-1	16.63	9.34	21.23	0.553
D_{14}	0	0	0	10.42	8.11	20.34	0.616
D_{15}	0	0	1	9.98	6.91	18.53	0.371
D_{16}	0	1	-1	12.32	7.88	20.83	0.562
D_{17}	0	1	0	11.83	6.75	17.79	0.282
D_{18}	0	1	1	10.53	7.12	18.66	0.408
D_{19}	1	-1	-1	14.73	6.98	16.49	0.233
D_{20}	1	-1	0	16.71	6.54	16.2	0.120
D_{21}	1	-1	1	9.81	6.38	16.35	0.000
D_{22}	1	0	-1	15.23	7.79	16.88	0.279
D_{23}	1	0	0	13.38	7.10	15.39	0.174
D_{24}	1	0	1	11.11	6.82	15.12	0.113
D_{25}	1	1	-1	10.88	7.49	16.41	0.337
D_{26}	1	1	0	8.90	6.73	15.63	0.188
D_{27}	1	1	1	7.25	6.51	11.36	0.000

Table 3: Anova results (P value) effect of the variables on particle size, T_{50}, T_{90}

Factors	Particle Size		t_{50}		t_{90}	
	Coefficient	P Value	Coefficient	P Value	Coefficient	P Value
intercept	13.450759	1.781E-11	7.7725789	2.08418E-16	19.75611	7.57E-19
X1	-3.41748613	1.091E-07	-0.8311214	6.52496E-07	-2.98542	3.14E-11
X2	-1.78137502	0.000234	0.0744341	0.489420637	-0.49097	0.018686
X3	-1.36418053	0.0023489	-0.5805453	4.67538E-05	-0.85736	0.000315
X12	2.3485972	0.0024069	0.0922325	0.618657836	-0.67792	0.052728
X22	-0.3630694	0.5857098	-0.2877675	0.132819168	-0.48792	0.151615
X32	-0.2097360	0.7521017	0.1272325	0.493844047	0.257083	0.439164
X1X2	-0.6337708	0.1906826	-0.0674845	0.608382908	0.253124	0.287914
X2X3	-0.1362291	0.7727421	0.0133179	0.919131585	0.036043	0.877576
X1X3	-0.7595625	0.1209949	0.0883179	0.503766879	0.241043	0.310746
X1X2X3	0.3147503	0.6222956	0.1272325	0.575160378	-0.57251	0.084352
R^2	0.894216		0.8602921		0.949497	
F	13.52527334		9.852463		30.0813	

Regression coefficients *, statistically significant (P<0.05)

The significant factors in the equations were selected using a stepwise forward and backward elimination for the calculation of regression analysis. The terms of full model having p value non significant (p>0.05) have negligible contribution in obtaining dependent variables and thus neglected.

The equations represent the quantitative effect of the formulation variables on responses.

Particle size = 13.45 - 3.41 X1 - 1.78 X2+ 1.364 X3 + 2.34 X12

R^2 = 0.8945, D.F. = 4,22 F= 13.52 , P< 0.05

t_{50} = 7.772 – 0.83X1 -0.5806 X3

R^2 = 0.86, D.F. =3,23 F= 9.8 , P < 0.05

t_{90} = 19.756 - 2.98 X1 - 0.496 X2 - 0.86X3

R^2 = 0.935, D.F. =4, 22 F =194.85, P< 0.05

Table 4: Experiments and NN-Predicted responses

S. No.	Measured Responses			NN-Predicted Responses		
	Particle size	t_{50}(hrs)	t_{90}(hrs)	Overall Desirability	t_{50}(hrs)	t_{90}(hrs)
1	20.13	8.90	23.83	0.000	9.11	19.27
2	19.93	8.50	22.12	0.338	8.61	19.09
3	19.12	7.93	21.85	0.166	7.88	18.77
4	19.72	9.21	24.83	0.416	9.15	18.51
5	18.84	8.45	22.13	0.368	8.71	18.27
6	18.58	7.54	20.83	0.302	7.97	17.89
7	18.14	9.41	20.21	0.434	9.16	17.38
8	18.32	8.92	21.42	0.426	8.72	17.03
9	16.42	8.54	20.54	0.467	8.00	16.54
10	15.58	8.15	20.13	0.475	8.47	18.62
11	14.64	7.68	19.79	0.447	7.55	18.09
12	15.69	7.05	19.12	0.317	6.99	17.37
13	16.63	9.34	21.23	0.553	8.54	17.56
14	10.42	8.11	20.34	0.616	7.57	16.92
15	9.98	6.91	18.53	0.371	6.97	16.11
16	12.32	7.88	20.83	0.562	8.53	15.99
17	11.83	6.75	17.79	0.282	7.59	15.27
18	10.53	7.12	18.66	0.408	7.00	14.42
19	14.73	6.98	16.49	0.233	7.26	17.01
20	16.71	6.54	16.2	0.120	6.83	16.07
21	9.81	6.38	16.35	0.000	6.68	15.11
22	15.23	7.79	16.88	0.279	7.27	15.56
23	13.38	7.10	15.39	0.174	6.85	14.58
24	11.11	6.82	15.12	0.113	6.70	13.65
25	10.88	7.49	16.41	0.337	7.33	13.81
26	8.90	6.73	15.63	0.188	6.91	12.92
27	7.25	6.51	11.36	0.000	6.76	12.14

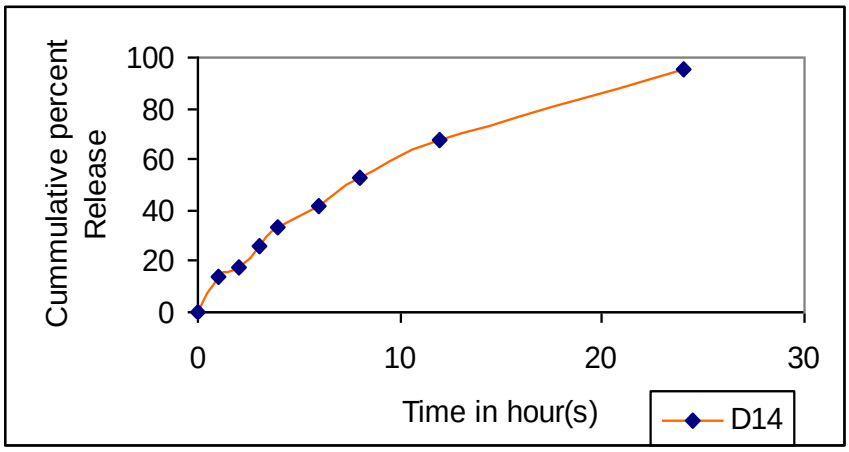

Fig. 2: In-vitro release profile of optimized batch in D_{14}7.4PH PBS

Table 5: NN-Predicted anova results (P value) effect of the variables on particle size, t_{50}, t_{90}

Factors	Particle Size		t_{50}		t_{90}	
	Coefficient	P Value	Coefficient	P Value	Coeffi-cient	P Value
Intercept	14.659	0.000	7.653	0.910	16.853	0.000
X1	-3.117	0.000	-0.818	0.000	-1.772	0.000
X2	-1.729	0.000	0.034	0.000	-1.328	0.000
X3	-1.331	0.000	-0.548	0.438	-0.651	0.000
X12	-0.265	0.138	-0.004	0.000	-0.262	0.000
X22	-0.052	0.761	0.003	0.938	-0.036	0.202
X32	-0.143	0.414	0.154	0.963	-0.293	0.000
X1X2	-0.129	0.599	0.082	0.010	-0.394	0.000
X2X3	-0.061	0.804	-0.004	0.290	-0.178	0.000
X1X3	-0.014	0.955	0.061	0.954	-0.043	0.278
X1X2X3	0.566	0.015	-0.007	0.432	0.071	0.046
R^2	0.979		0.9705		0.999	
F	76.28		52.706		1135.33	

Optimum number of neurons in hidden layer was found to be 5, while optimum step size for hidden layer and output layer was 0.1 (out of 0.1 to 1.0). Optimum momentum for hidden layer and output layer was found to be 0.7 (out of 0.1 to 1.0). For prediction purpose, the neural network was constructed using the optimum conditions and trained (n=3) along with cross validation data set. The matrix of the experiments and neural network predicted responses are given in Table 6. The predicted responses were plotted to generate the contour plots for interpreting the effect of various process factors.

The relation between process variables and response factors was derived using multi-linear regression (MLR) which can be represented as:

Bias was calculated by the following equation:

Particle size = $14.659 - 3.117X_1 - 1.729X_2 - 1.331X_3 - 0.265X_1^2 - 0.052X_2^2 - 0.143X_3^2 - 0.129X_1X_2 - 0.061X_2X_3 - 0.014X_1X_3 + 0.566X_1X_2X_3$

$T_{50} = 7.653 - 0.818X_1 + 0.034X_2 - 0.548X_3 - 0.004X_1^2 + 0.003X_2^2 + 0.154X_3^2 + 0.0.082X_1X_2 - 0.004X_2X_3 + 0.061X_1X_3 - 0.007X_1X_2X_3$

$T_{90} = 16.853 - 1.772X_1 - 1.328X_2 - 0.651X_3 - 0.262X_1^2 - 0.036X_2^2 - 0.293X_3^2 - 0.394X_1X_2 - 0.178X_2X_3 - 0.043X_1X_3 + 0.071X_1X_2X_3$

Evaluation of model

In order to assess the reliability of the model, five check point batches were conducted by varying the process variables at values other than that of the responses were estimated by using the equations and experimental procedure. In Table the comparison between the experimental and predicted values of the responses for these additional experiments is presented depicted in Table 6. Bias was calculated by following equation.

$$Bias = \frac{(\text{Predicted value} - \text{experimental value})}{\text{Predicted value}} \times 100$$

It can be seen that in all cases there was a reasonable agreement between the predicted and experimental value, since low value of the bias were found.

It can be seen that in all cases there was a reasonable agreement between the predicted and the experimental value, since low value of the bias were found. For this reason it can be concluded that the NN predicted responses describe adequately the influence of the selected process variables on the responses under study and NN can be used successfully as a predictive and optimizing tool.

Table 6: Comparison between predicted and experimental values for check point batches

Response	Check batch	Factors/Levels			Predicted values	Experimental Values	% Bias
		A	B	C			
Particle size	1	-1	+1	+1	17.16	16.85	1.8
	2	+1	-1	+1	12.88	12.66	1.7
	3	0	0	+1	11.88	11.73	1.2
	4	-0.6	+0.2	0.6	10.61	10.53	1.5
	5	-0.4	0.6	-0.2	16.77	16.58	1.1
t_{50}	1	-1	+1	+1	7.87	7.68	2.3
	2	+1	-1	+1	6.24	6.09	2.4
	3	0	0	+1	7.19	7.06	1.8
	4	-0.6	+0.2	0.6	7.86	7.69	2.1
	5	-0.4	0.6	-0.2	8.32	8.10	1.75
t_{70}	1	-1	+1	+1	20.72	20.34	1.8
	2	+1	-1	+1	16.02	15.85	1.05
	3	0	0	+1	19.15	18.75	2.1
	4	-0.6	+0.2	0.6	20.89	20.63	1.23
	5	-0.4	0.6	-0.2	19.95	19.54	2.05

Stability Studies of Doxorubicin Hydrochloride Containing Chitosan Microparticles

The stability studies of the prepared films were done at the conditions as per ICH guideline. The intermediate stability studies of the microparticles were carried out at temperature of 30°C and at humidity of RH 65% RH. The accelerated stability studies were carried out at the temperature of 40°C and at humidity of RH 75%. To maintain the RH of 60% and 75 % saturated solutions of magnesium chloride and magnesium nitrate respectively were prepared. These solutions were filled in separate glass desiccators. Ten lots with same process and product parameters of 200 mg placed in a petridish were kept in each desiccator for six months.

The microparticles were tested at 0, 1, 2, 3, 4, 5, 6 months time intervals for drug content, degree of cross linking, and *in-vitro* dissolution study in triplicate. The results of the stability study of DOX microparticles are shown in Table 7.

Table 7: Results of the stability studies of doxorubicin hcl microparticles.

Test	Time (month)												
	0	1		2		3		4		5		6	
		I	A	I	A	I	A	I	A	I	A	I	A
Drug Content %	92.23 ±2.6	91.15 ± 2.45	91.23 ±1.82	91.03 ±2.3	89.90 ±1.65	89.89 ± 2.56	88.23 ± 2.1	89.11 ± 3.01	87.40 ± 2.22	88.13 ±3.4	86.24 ±3.1	87.76 ±3.2	85.53 ±3.33
t_{50} hr	8.11 ±0.06	8.10 ±0.08	8.0 ±0.88	7.95 ±0.069	7.80 ±0.97	7.83 ±0.071	7.66 ±0.21	7.75 ±0.09	7.65 ±0.16	7.63 ±0.1	7.590 ±.11	7.5 ±0.12	7.48 ±0.14
t_{90} hr	20.34 ±0.88	20.21 ±0.89	20.06 ± 0.64	20.0 ±0.53	19.98 ± 0.49	19.94 ± 0.53	19.62 ± 0.63	19.71 ±0.59	19.41 ± 0.58	19.43 ± 0.88	18.68 ±0.78	18.88 ±0.71	18.22 ±0.69

I = Intermediate condition (30 °C/60%)

A= Accelerated condition (45°C/75 %)

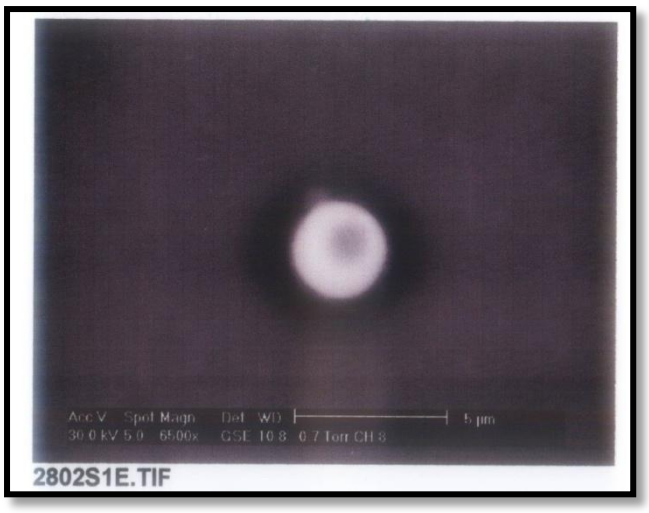

Fig. 3: Scanning electron photomicrographoptimized batch D14 (6500X)

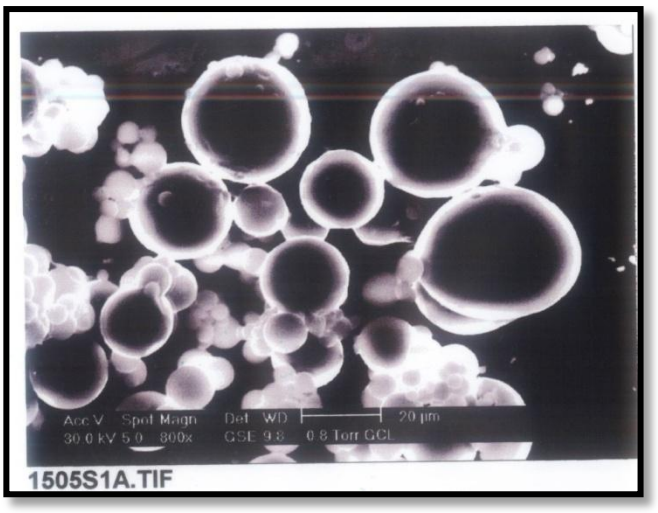

Fig. 4: Scanning electron photomicrographoptimized batch D14 (800 X)

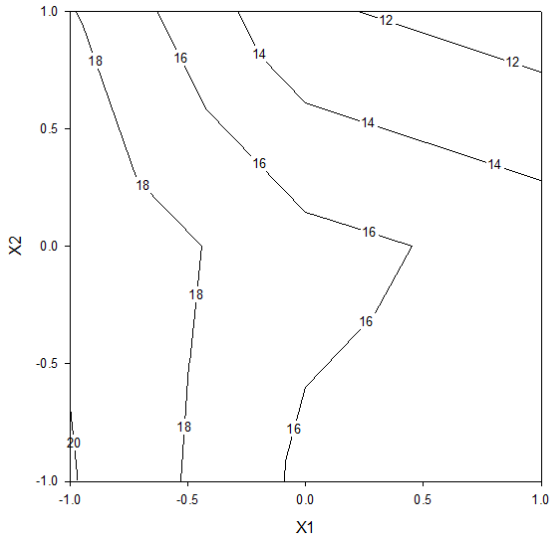

Fig. 5:Contour plot for particle size at -1 level of X_3

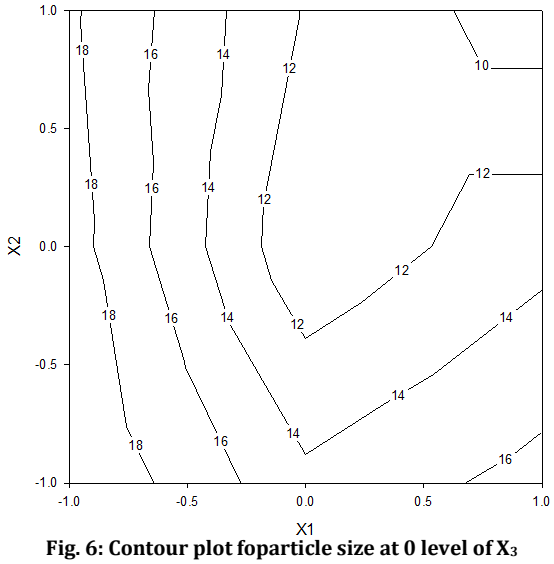

Fig. 6: Contour plot foparticle size at 0 level of X_3

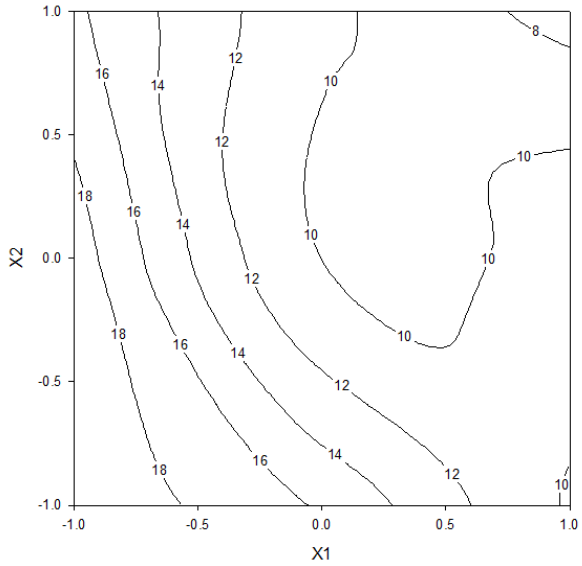

Fig. 7: Contour Plot For Particle Size AT +1 level OF X_3

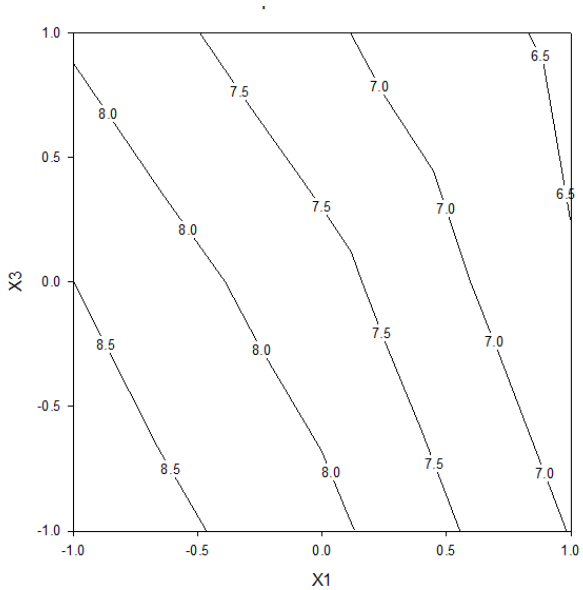

Fig. 8: Contour plot for t_{50} at -1 level of X_2

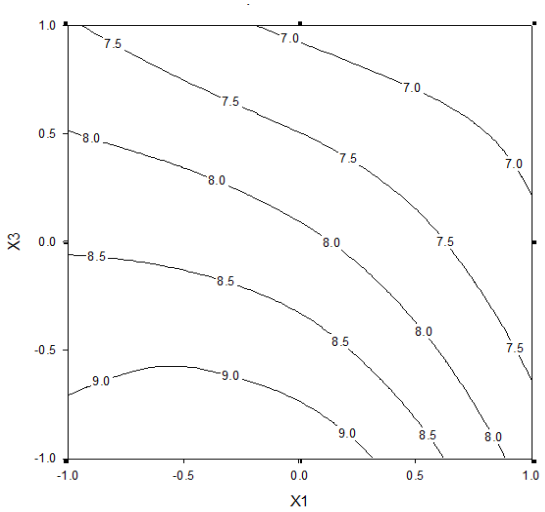

Fig. 9: Contour plot for t_{50} at 0 level of X_2

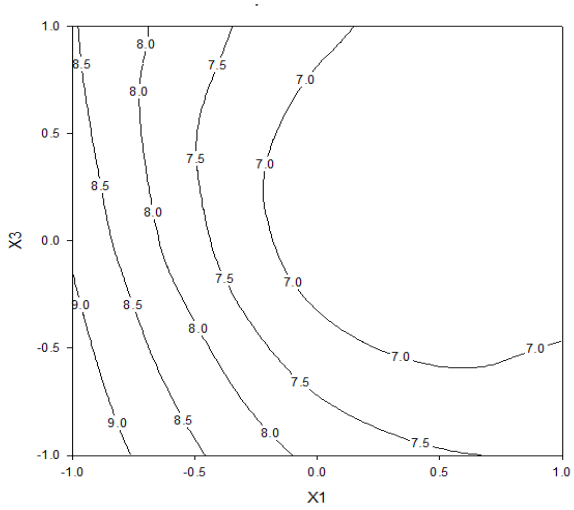

Fig. 10 : Contour plot for t_{50} at +1 level of X2

CONCLUSION

Results showed that it is possible to predict response factors more accurately using neuralnetworks than using regression models.Regression analyses are dependent on pre determined statistical significance levels and less significant terms are usually not included in the model.With ANN methods, all data are used potentially, making the models more accurate.An ANN method was successfully applied for modelling and process optimization. ANNs can be used as a powerful tool in pharmaceutical product formulation, as well as other areas in the pharmaceutical industry, so that the development tasks can be performed rapidly and efficiently with an increase of productivity, consistency and quality.Increasing attention being paid to the development of polymeric microparticles for the controlled delivery of drug. The optimization of the process using the factorial design, and NN-predicted responses resulted in formulation with the necessary characteristics with better reproducibility. This combination of statisticdesign with neural modeling act as significant tool for optimization.

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