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Prediction of permeability of FD-4 through porous poly (2-hydroxyethyl methacrylate) membrane by multiple linear regression and artificial neural network

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Received October 20, 2008, accepted November 6, 2008

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Pharmazie 64: 311–315 (2009) *doi: 10.1691/ph.2009.8305*

The aim of this study was to predict the permeability through porous poly (2-hydroxyethyl methacrylate) (pHEMA) membranes of fluorescein isothiocyanate-labeled dextran molecular weight 4400 (FD-4) as a model of peptide and protein drug movement. Homogeneous standard membranes were prepared by redox polymerization. Permeability data were predicted by an artificial neural network (ANN) as a function of polymerization factors, and the accuracy was compared with that of conventional multiple linear regression (MLR). Good linearity was observed with each model, with the correlation coefficient of a leave-one-out cross-validation (R_{cross}) being 0.857 for the MLR model and 0.876 for the ANN model. The mean bias and mean accuracy for the ANN were somewhat smaller than those of the MLR. The ANN method provides an accurate quantitative approximation of the permeability coefficient of FD-4, as judged by conventional MLR, and could be applied to prediction of the non-linear relation between polymerization factors and the permeability of FD-4.

1. Introduction

Poly(2-hydroxyethyl methacrylate) (pHEMA) has been studied extensively in the biomedical and pharmaceutical fields for a variety of applications, including soft contact lenses (Tighe 1987) and drug delivery (Mack and Kim 1987). Significant attention has also been paid to porous pHEMA membranes because of their potential use as biofunctional materials for cellular and tissue engineering (Oxley et al. 1993; Skelly and Tighe 1979; Haldon and Lee 1972). The mechanism of drug permeation through a porous polymer such as pHEMA membrane is not clear in detail, although it can be expressed generally by Fick's diffusion theorem (Higuchi 1963). The combined effects of various parameters such as porosity, tortuosity, and microscopic viscosity of water in the pore structure on drug permeation (Yamane et al. 1998) through a porous pHEMA membrane (Yanagawa et al. 2006) are too complicated to be recognized in detail. Nevertheless, prediction of drug permeation (Lipinski et al. 1997; Simon and Fernandes 2004) through the membrane (Hosoya et al. 2004) is important in the formulation of pharmaceuticals.

One of the difficulties in the quantitative approach to the prediction of drug permeation through a porous polymer is approximating the actual relation between causal factors and pharmaceutical responses (Takahara et al. 1997; Takayama et al. 1998). In this regard, several experiments were carried out to determine the relation between factors acting on the system and the responses or properties of the system, and to represent the relation by using software techniques (Lewis et al. 1999; Hussain et al. 1991). One of the most popular techniques for the prediction of pharmaceutical responses, the artificial neural network (ANN), has been used to solve various problems ranging from interpretation of analytical data, drug and dosage form design, and biopharmacy to clinical pharmacy (Surini et al. 2003; Takayama et al. 1999). Artificial neural networks can be applied to quantify a non-linear relation between causal factors and pharmaceutical responses by means of iterative training with experimental data.

The aim of this study was to predict the permeation through a porous pHEMA membrane using fluorescein isothiocyanate-labeled dextran molecular weight 4400 (FD-4) as a model of peptide or protein drugs. Homogeneous standard pHEMA membranes were prepared by redox polymerization. Permeability data were predicted by the ANN as a function of polymerization factors. The homogeneity and accuracy of these standard samples has been verified in the literature. The accuracy of the ANN method was compared with that of conventional multiple linear regression (MLR).

2. Investigations, results and discussion

2.1. Prediction of the permeability coefficient of FD-4 by MLR and ANN

Figure 1 shows typical surface morphology of porous pHEMA membranes observed by scanning electron microscope (SEM). Numerous pores with irregular shapes were visible. Fig. 2 shows the permeation profiles of FD-4 through the porous pHEMA membranes and the perme-

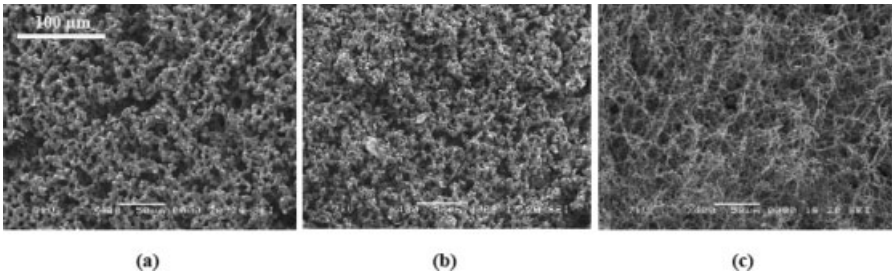

Fig. 1:
Typical SEM micrographs of porous pHEMA membranes. (a: No. 3, b: No. 6, c: No. 7)

ability coefficient of FD-4 determined from the slope of the permeation profiles (Table 1). The permeation profiles and permeability coefficient were significantly different for the membranes prepared with various amounts of the initiator and the crosslinker. The larger the amount of initiator, the greater the permeability coefficient. On the other hand, a larger amount of crosslinker led to a smaller permeability coefficient.

To understand and estimate the actual relation between polymerization factors such as the amounts of crosslinker and initiator and the permeability coefficient of FD-4, a representation of the linear and non-linear relation was needed. First, we applied MLR to represent the linear relation between two causal polymerization factors and the permeability coefficient as the response variable. From the MLR analysis relevant to the overall combination of causal factors, the optimal regression equation was obtained:

$$Y = 0.221\ (\pm\ 0.048)\ X_2 - 0.130\ (\pm\ 0.037)\ X_1X_2 + 0.191\ (\pm\ 0.108)$$

$$n = 27,\quad s = 0.129,\quad F\ (2, 24) = 44.1,\quad R = 0.887 \quad (1)$$

where Y is the permeability coefficient of FD-4, X_1 is the amount of crosslinker, X_2 is the amount of initiator, n is the number of data pairs, s is the standard deviation of the estimates, F (ϕ1, ϕ2) is the observed F value, ϕ1 is the first degree of freedom, ϕ2 is the second degree of freedom, and R is the multiple correlation coefficient. Although the independent term of X_1 was not included in the optimal equation, the Y value was given as a linear combination of X_2 and X_1X_2, and the multiple correlation coefficient (R) was good enough. Thus, the permeability coefficient of FD-4 (Y) correlated negatively with the amount of crosslinker (X_1) and positively with the amount of initiator (X_2).

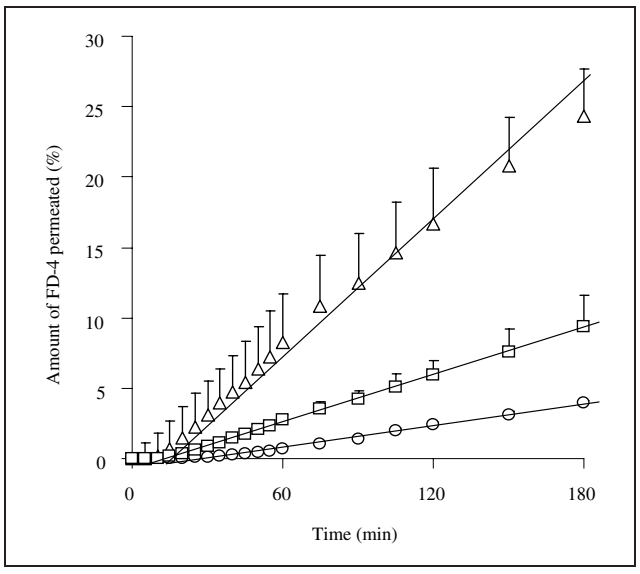

Fig. 2: Typical permeation profiles of FD-4 through porous pHEMA membranes (△: No. 3, □: No. 6, ○: No. 7)

Second, we applied the ANN to represent the non-linear relation between two causal polymerization factors and the permeability coefficient of FD-4 as the response variable. As in the MLR approach, when using X_1 and X_2 in the input layer and Y as a node in the output layer, the number of the nodes in the hidden layer was optimized with Akaike's information criterion (AIC) as a judging index together with the "leave-one-out (LOO)" technique (Takayama et al. 2000). The AIC values of ANN that were trained for the prediction of the permeability coefficient of FD-4 as a function of the number of nodes in the hidden layer are shown in Fig. 3. The ANN learns an approximate non-linear relation by a procedure called "training," which involves varying weight values. Training is a search process for the optimized set of weight values, which can minimize the squared error between the estimation and experimental data of units in the output layer (Takayama et al. 2003). As a result, two units in the hidden layer were selected as an optimal structure for the ANN.

Table 1: LOO-predicted permeability coefficient of FD-4 by MLR and ANN

Formulation No.	Permeability coefficient $\times 10^3$ cm^2/min[a]	MLR		ANN	
		Mean ± SD[a]	Range[a]	Mean ± SD[a]	Range[a]
1	3.54 ± 0.31	3.45 ± 0.03	3.42–3.49	4.31 ± 0.09	4.23–4.40
2	7.91 ± 2.41	6.46 ± 0.21	6.23–6.65	7.88 ± 0.82	7.02–8.65
3	9.86 ± 0.92	9.64 ± 0.25	9.37–9.86	9.51 ± 0.29	9.19–9.76
4	2.91 ± 0.66	2.80 ± 0.07	2.72–2.84	2.24 ± 0.05	2.18–2.28
5	3.65 ± 0.23	4.66 ± 0.01	4.65–4.67	4.10 ± 0.05	4.06–4.16
6	4.99 ± 0.93	6.58 ± 0.10	6.51–6.69	5.47 ± 0.40	5.17–5.91
7	1.72 ± 0.13	2.21 ± 0.02	2.19–2.22	2.16 ± 0.03	2.13–2.20
8	2.79 ± 1.40	2.66 ± 0.12	2.55–2.79	2.79 ± 0.10	2.68–2.89
9	4.18 ± 0.91	2.90 ± 0.24	2.63–3.10	3.13 ± 0.14	2.98–3.24

[*] Mean ± SD and range of three determinations

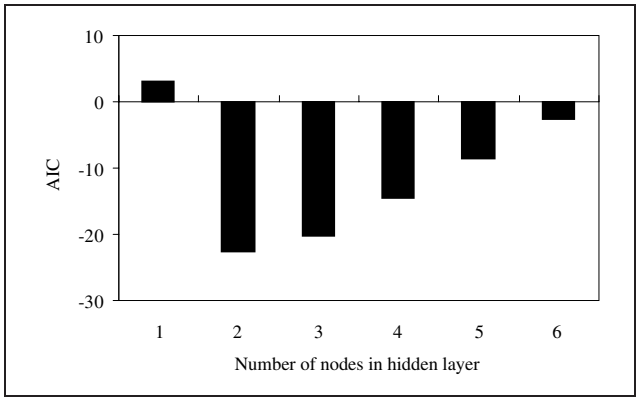

Fig. 3: Akaike's information criterion (AIC) values of ANN trained for prediction of permeability of FD-4 through porous pHEMA membranes based on number of nodes in hidden layers

Figure 4 shows the three-dimensional diagram of the permeability coefficient of FD-4 as a function of the amounts of crosslinker and initiator. The linear or non-linear relation between the causal polymerization factors and the permeability coefficient of FD-4 was represented with the response surfaces predicted by the MLR and ANN (Fig. 4). It can be seen that the response surface generated by ANN was smoother and more concave than produced by the MLR. Although differences in the shapes of the response surfaces were apparent, the two surfaces were equivalent in that the larger the amount of crosslinker, the smaller the permeability coefficient. On the other hand, the larger the amount of initiator, the higher the permeability coefficient. A larger amount of crosslinker and a smaller amount of initiator may cause a higher dense three-dimensional internal structure of a pHEMA membrane.

2.2. Predictive ability of ANN method compared with MLR

The LOO method of cross-validation (R_{cross}) was used to assess the predictive ability of the MLR and ANN methods. The results for the MLR and ANN models are summarized in Table 1 and the results of the correlation coefficient (R) and correlation coefficient of the R_{cross} in Table 2. The approximation of ANN was significantly greater, with $R = 0.930$, than that of MLR, with $R = 0.887$ (Fig. 5). Additionally, a good linearity for each model was observed, with the correlation coefficient of an R_{cross} of 0.857 for the MLR model and 0.876 for the ANN model (Fig. 6).

Table 2: Statistic indices for evaluating reliability estimated by MLR and ANN

Statistic index	MLR	ANN
R	0.887	0.930
R_{cross}	0.857	0.876
B_m (%)	7.570	3.709
A_m (%)	23.126	19.361

Table 3: Amounts of crosslinker and initiator used to prepare porous pHEMA membranes

Formulation No.	Crosslinker mol/L	Initiator mg/mL
1	0.5	1.0
2	0.5	3.0
3	0.5	5.0
4	1.0	1.0
5	1.0	3.0
6	1.0	5.0
7	1.5	1.0
8	1.5	3.0
9	1.5	5.0

The accuracy of the ANN method was compared with that of the MLR using the mean bias and the mean accuracy, as determined by Eqs. (2) and (3), respectively (Okumura et al. 2006):

$$B_m = \frac{\sum (X_p - X_t)/X_t}{n} \times 100 \qquad (2)$$

$$A_m = \frac{\sum |X_p - X_t|/X_t}{n} \times 100 \qquad (3)$$

where B_m is the percentage mean bias, A_m is the percentage mean accuracy, X_p is the permeability coefficient predicted by the LOO method, X_t is the actual permeability coefficient, and n is the number of experiments. The results for the MLR and ANN models are summarized in Table 3. Both B_m and A_m were somewhat smaller for the ANN model owing to the ANN's superior sensitivity to the fluctuations of variables where greater non-linearity exists. Because porous pHEMA membranes are prepared by two causal polymerization factors, most of the relation between the response variables and the causal factors are not linear. These results indicate that the ANN method provides an accurate quantitative approximation of the permeability coefficient of FD-4 through porous pHEMA membranes compared with the conventional MLR method.

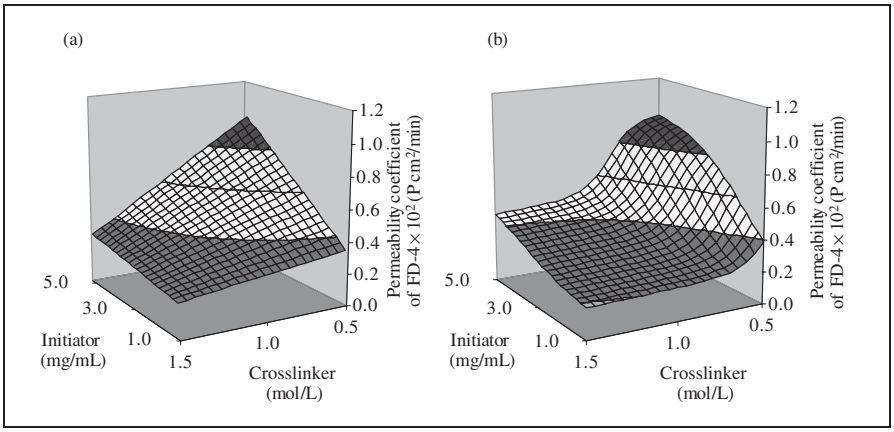

Fig. 4:
Response surfaces of permeability of FD-4 through porous pHEMA membrane (Y) as function of amount of TEGDMA as crosslinker (X_1) and APS or SMBS as initiator (X_2). Response surface predicted by (a) MLR and (b) ANN methods

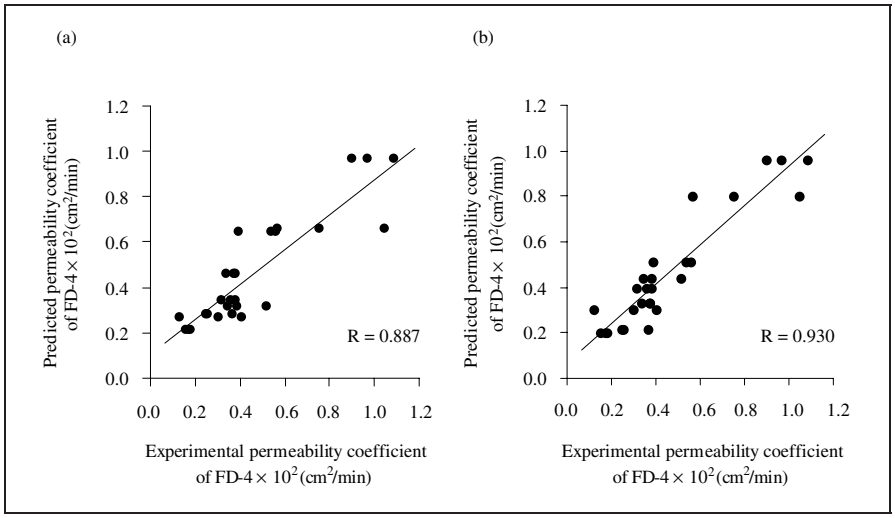

Fig. 5:
Correlation between experimental and predicted permeability coefficient of FD-4 through porous pHEMA membranes obtained by (a) MLR and (b) ANN

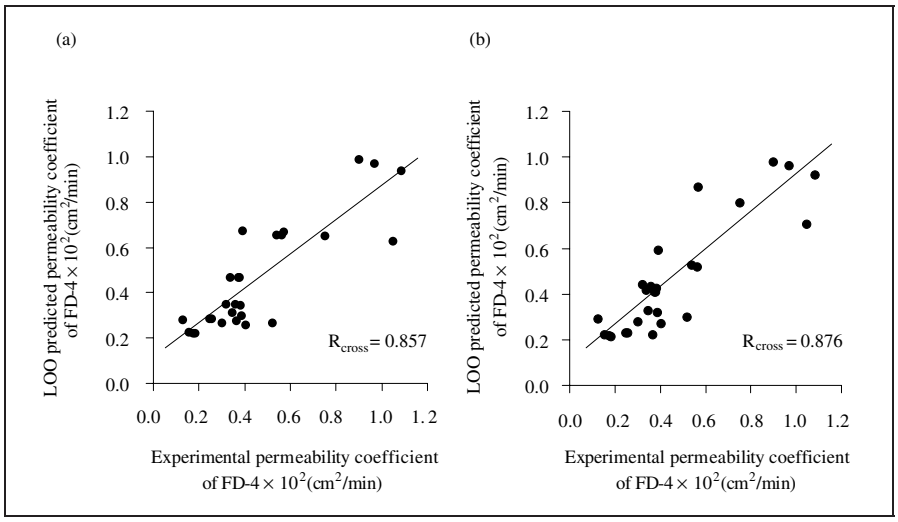

Fig. 6:
Correlation between experimental and LOO-predicted permeability coefficient of FD-4 through porous pHEMA membranes obtained by (a) MLR and (b) ANN

Although MLR is an effective approach for predicting the permeability of FD-4 through porous pHEMA membranes, somewhat better results were observed with a non-linear approximation using the ANN method. It was concluded that the ANN method can be a powerful means for predicting the permeability of drug through porous pHEMA membranes.

3. Experimental

3.1. Materials

2-Hydroxyethyl methacrylate (HEMA) and FD-4 were purchased from Sigma Chemical Co., St. Louis, MO, USA. Tetraethylene glycol dimethacrylate (TEGDMA) and quat amine divinylbenzene/styrene copolymer (De-Hibit 200) were purchased from Polysciences Inc., Warrington, PA, USA. Ammonium persulfate (APS) and sodium metabisulfite (SMBS) were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Other chemicals used were of reagent grade.

3.2. Preparation of porous pHEMA membrane for quantitative analysis

In order to remove the inhibitor 4-methoxyphenol (MEHQ), the HEMA was passed through an exchange column packed with De-Hibit 200 before pHEMA synthesis. Crosslinked porous pHEMA membranes were synthesized by redox polymerization. The amounts of crosslinker and initiator used for various preparations are listed in Table 3. A monomer of HEMA (1.0 mL) was mixed with TEGDMA as a crosslinker and dissolved in a mixture with APS and SMBS as an initiator, each in phosphate buffered saline (PBS; 4.0 mL). Then the mixture was kept for 24 h at 25 °C.

3.3. Permeation studies

The permeability of the model penetrant, FD-4, through porous pHEMA membranes was evaluated using a water-jacket-type two-chamber diffusion cell with an available diffusion area of 0.7928 cm^2, with each half-cell volume being 3.0 mL at 37 °C. The cells were clamped to prevent leakage. A volume of PBS and the FD-4 solution (0.1 mg/mL as the concentration of FD-4 and 3.0 mL of the total amount) were applied to the receiver and donor compartment, respectively. A 200 μL sample from the receiver solution was withdrawn at each interval and replaced by an aliquot volume of fresh PBS. The concentration of FD-4 in the sample was analyzed using a microplate luminometer (Mithras LB840, Beltold Japan Co., Ltd., Osaka, Japan) at an excitation wavelength of 485 nm and emission wavelength of 535 nm. Permeability coefficients were calculated from the slope of the plot of cumulative amounts of harvested FD-4 as a function of time.

3.4. Data analysis and computer programs

3.4.1. Multiple linear regression

The best combination of causal factors was selected from among $2^q - 1$ kinds of regression equations (where q is the number of causal factors); i.e., the overall combination of factors was evaluated. The coefficient of determination, which had been doubly adjusted by degrees of freedom (R^{**2}), was used as a judging standard for selection of the optimal combination of factors (Okumura et al. 2006; Wu et al. 2001):

$$R^{**2} = 1 - \frac{(p-1)(p+q+1)}{(p+1)(p-q-1)}(1-R^2) \quad (4)$$

where p is the number of data pairs, q is the number of causal factors, and R is the multiple correlation coefficient. Computer program ALCORA (Takayama et al. 2003), written by us to be executable in Windows XP, was used for the MLR.

3.4.2. Artificial neural network

A general ANN structure has one input layer, one or more hidden layers, and one output layer. Each layer has some units corresponding to neurons. The units in neighboring cells between two units are called "weights". In each hidden and output layer, the processing unit sums its input from the previous layer and then applies the sigmoidal function to compute its output to the following layer according to the following equations (Takayama et al. 2003):

$$y_p = \sum w_{pq} x_p \tag{5}$$

$$f(y_q) = \frac{1}{1 + \exp(-\alpha y_q)} \tag{6}$$

where w_{pq} is the weight of the connection between unit q in the current layer and unit p in the previous layer; x_p is the output value from the previous layer, $f(y_p)$, that is conducted to the following layer as an output value; and α is a parameter relating to the shape of the sigmoidal function. Nonlinearity of the sigmoidal function is strengthened with an increase in α. In this study, a hierarchical ANN with one input, one hidden, and one output layer was employed with the amount of TEGDMA or APS and SMBS as a node in the input layer and the permeability coefficient of FD-4 as a node in the output layer.

To enable reasonable prediction of each response variable by an ANN, AIC was applied to evaluate in the optimality of ANN (Takayama et al. 2000):

$$AIC = n_s \times \ln(SS) + 2 \times n_w \tag{7}$$

where n_s is the number of data pairs, n_w is the number of weights in the ANN, and SS is the residual sum of squares between the actual and predicted response variables. An extended Kalman filter was employed as a training algorithm (Okumura et al. 2006; Murase et al. 1991). This filter enables the number of training runs to be greatly reduced, and thus the iteration of training was set at the most to 1000 to avoid over-training problems. Computer program ANNA (Takayama et al. 2003), written by us to be executable in Windows XP, was used for the ANN.

3.4.3. Leave-one-out method

The predictabilities of the MLR and ANN models were evaluated by an R_{cross} procedure (Aoyama et al. 1990; Lim et al. 2002). As in the MLR model, this method systematically removes one data pair from the calibration data set. A MLR model was then constructed on the basis of the reduced data set and subsequently used to predict the removed data. This process was repeated for all data so that a complete set of predicted values was obtained. The ANN model was treated likewise: one data pair was removed from the training data set, and the ANN was then trained using the reduced data set. The trained ANN was adopted to predict the removed data. This process repeated for all data, as with the MLR.

References

Aoyama T, Suzuki Y, Ichikawa H (1990) Neural networks applied to quantitative structure-activity relationship analysis. J Med Chem 33: 2583–2590.

Haldon HR, Lee BE (1972) Structure and permeability of porous films of poly(hydroxyethyl metharylate). Br Polym J 4: 491–501.

Higuchi T (1963) Mechanism of sustained action medication, Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 52: 1145–1149.

Hosoya O, Chono S, Saso Y, Juni K, Morimoto K, Seki T (2004) Determination of diffusion coefficients of peptides and prediction of permeability through a porous membrane. J Pharm Pharmacol 56: 1501–1507.

Hussain AS, Yu X, Johnson RD (1991) Application of neural computing in pharmaceutical product development. Pharm Res 8: 1248–1252.

Lewis GA, Mathieu D, Phan-Tan-Lu R (1999) Pharmaceutical experimental design. In: Swarbrick J, (ed.) Drug and The Pharmaceutical Sciences. Vol. 92., Marcel Dekker, New York, p. 1–22.

Lim CW, Fujiwara S, Yamashita F, Hashida M (2002) Prediction of human skin permeability using a combination of molecular orbital calculations and artificial neural network. Biol Pharm Bull 25: 361–366.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Del Rev 23: 3–25.

Mack EJ, Kim SW (1987) Biomedical applications of poly(2-hydroxyethyl methacrylate) and its copolymers. In: Peppas NA (ed.) Polymers, Vol. II., CRC Press, p. 65–93.

Murase H, Koyama S, Honami N, Kuwabara T (1991) Kalman filter neuron training. Bull Univ Osaka Pref Ser B 43: 91–101.

Okumura T, Nakazono M, Otsuka M, Takayama K (2006) An accurate quantitative analysis of polymorphs based on artificial neural networks. Colloids Surf B Biointerfaces 49: 153–157.

Oxley H, Corkhill PH, Fittonm JH, Tighe BJ (1993) Macroporous hydrogels for biomedical applications: Methodology and morphology. Biomaterials 14: 1064–1072.

Simon L, Fernandes M (2004) Neural network-based prediction and optimization of estradiol release from ethylene-vinyl acetate membranes. Comput Chem Eng 28: 2407–2419.

Skelly P, Tighe B (1979) Novel macroporous hydrogel adsorbents for artificial liver support perfusion systems. Polymer 20 : 1051–1052.

Surini S, Akiyama H, Morishita M, Nagai T, Takayama K (2003) Release phenomena of insulin from an implantable device composed of a polyion complex of chitosan and sodium hyaluronate. J Control Release 90: 291–301.

Takahara J, Takayama K, Nagai T (1997) Multi-objective simultaneous optimization technique based on an artificial neural network in sustained release formulations. J Control Release 49: 11–20.

Takayama K, Fujikawa M, Nagai T (1998) Artificial neural networks as a novel method to optimize pharmaceutical formulations. Pharm Res 16: 1–6.

Takayama K, Fujikawa M, Obata Y, Morishita M (2003) Neural network based optimization of drug formulations. Adv Drug Del Rev 55: 1217–1231.

Takayama K, Morva A, Fujikawa M, Hattori Y, Obata Y, Nagai T (2000) Formula optimization of theophylline controlled-release tablet based on artificial neural networks. J Control Release 68: 175–186.

Takayama K, Takahara J, Fujikawa M, Ichikawa H, Nagai T (1999) Formula optimization based on artificial neural networks in transdermal drug delivery. J Control Release 62: 161–170.

Tighe BJ (1987) Hydrogels as contact lens materials. In: Peppas NA (eds.) Properties and Applications, Vol. III., CRC Press, p. 65–93.

Wu PC, Obata Y, Fujikawa M, Li CJ, Higashiyama K, Takayama K (2001) Simultaneous optimization based on artificial neural networks in ketoprofen hydrogel formula containing O-ethyl-3-butylcyclohexanol as percutaneous absorption enhancer. J Pharm Sci 90: 1004–1014.

Yamane A, Takayama K, Nagai T (1998) Effect of fractal dimension on drug permeation through porous ethylcellulose films. J Control Release 50 : 103–109.

Yanagawa F, Onuki Y, Morishita M, Takayama K (2006) Involvement of fractal geometry on solute permeation through porous poly(2-hydroxyethyl methacrylate) membranes. J Control Release 110: 395–399.