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Prediction of the isotherms of human IgG adsorption on Ni(II)-IDA-PEVA membrane using artificial neural networks

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Abstract The use of artificial neural networks (ANNs) to predict the adsorption isotherms of human immunoglobulin G on immobilized Ni(II) affinity hollow fiber membranes was studied. Neural networks were trained using the Levenberg–Marquardt algorithm combined with Bayesian regularization technique and experimental data from different temperatures. The resulting neural network demonstrated to be able to interpolate the behavior of the maximum adsorption capacity and equilibrium concentration in the temperature range (4, 37 °C) with correlation coefficients higher than 0.96. Results demonstrated to be very similar to those achieved with traditionally Langmuir model adjustment. The advantage of interpolation ability of ANNs was also showed.

Keywords Artificial neural networks · Adsorption isotherms · Immunoglobulin G · Affinity hollow fiber membranes

1 Introduction

Affinity chromatography is a widely used method for the identification, purification and separation of macromolecules. It is based on specific and reversible binding ability of molecules in solution to complementary molecules, the ligands, usually immobilized on a solid chromatographic media, such as agarose gels, membranes, monoliths, cryogels or alternative novel support materials (Labrou and Clonis 1994; Serpa et al. 2005; Tscheliessnig and Jungbauer 2009; Prasanna and Vijayalakshmi 2010; Gondim et al. 2012; Doğan et al. 2012; Rajak et al. 2012; Borsoi-Ribeiro et al. 2013; Pavan et al. 2014).

The most used support materials, polysaccharides based gels such as agarose, have disadvantageous characteristics such as the compressibility and greater resistance to mass transfer due to the diffusion. For instance, when affinity membranes were used, the mass transfer is governed by convection, which facilitates the macromolecule transport to the adsorption site, providing higher flow rates, lower pressure drops, higher productivities and lower costs in several cases (Klein 1991; Thommes and Kula 1995; Bueno et al. 1995; Aquino et al. 2006; Varadaraju et al. 2011; Gagnon 2012).

Irrespective of the support material used, in the immobilized metal ion affinity chromatography (IMAC), the adsorption involves coordination interactions between a target macromolecule in solution and metal ions chelated via a chelating ligand immobilized on a solid phase, the adsorbent (Porath et al. 1975; Chaga 2001; Sharma and Agarwal 2002; Ueda et al. 2003; Gutiérrez et al. 2007; Bresolin et al. 2009). However, electrostatic, hydrophobic and hydrogen bonding may also contribute with the adsorption (Gaberc-Porekar and Menart 2005; Gutiérrez et al. 2007; Todorova and Vijayalakshmi 2006).

In order to optimize the adsorption process is necessary to know the behavior of the adsorbent–adsorbate pair. For IMAC, this approach involves the nature of the chelating ligand, the metal ion, the composition of the mobile phase, the support material and the temperature. For modeling purposes in IMAC protein adsorption, this interaction is commonly represented by means of isotherms models, e.g. Langmuir, Freundlich, Langmuir–Freundlich and Temkin, in which the equation parameters are adjusted in order to fit

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the experimental data (Sharma and Agarwal 2001; Bayramoglu et al. 2006; Ribeiro et al. 2008; Bresolin et al. 2010; Borsoi-Ribeiro et al. 2013; Pavan et al. 2014).

Several mathematical techniques have been used for modeling of adsorption isotherms. In the last years, artificial neural networks (ANN) proved to be an efficient alternative, since they are computational methodologies that perform multivariate analysis (Yetilmezsoy and Demirel 2008; Morse et al. 2011; Çelekli et al. 2013; Dutta and Basu 2013). ANNs have been developed to mimic, mathematically, networks of biological neurons. They operate as single layers of interconnected non-linear devices, called neurons. The interactions between neurons are quantified by means of the values of synaptic weights set during the training procedure. A successful training can result in ANNs that can perform tasks such as predicting an output value, classifying an object, a function approaching, and recognizing a pattern in multivariate data (Dayhoff and DeLeo 2001).

Morse et al. (2011) indicated the use of neural networks for modeling adsorption isotherms due to the inherent complexities of adsorption process, in which the adsorbent-adsorbate interaction may depend on several conditions like temperature, pH, compositions and/or the nature of adsorbent material. As confirmed by De Laurentiis and Ravdin (1994), ANNs are able to deal with these kinds of complex interactions, such as protein adsorption. Also, it must be noted that neural networks could deal well with imprecise data (Jagielska et al. 1999).

The purpose of this work is the application of neural networks to predict the adsorption isotherms of the human Immunoglobulin G (IgG) onto nickel affinity polyethylene vinyl alcohol (PEVA) hollow fiber membranes with iminodiacetic acid (IDA) as chelating ligand. For this, the experimental data provided by Ribeiro et al. (2008) at 4, 15, 25 and 37 °C were used. ANNs were trained using the Levenberg–Marquardt algorithm combined with Bayesian regularization technique in order to be able to predict the behavior of the adsorption in the temperature range of 4–37 °C.

2 Experimental

2.1 Experimental data

The descriptions of materials and experimental procedures were presented by Ribeiro et al. (2008). The data collection consisted of the adsorbent IgG capacity (Q*, mg g^{-1}) for different IgG liquid-phase equilibrium concentrations (C*, mg mL^{-1}) at 4 °C (22 points), 15 °C (18 points), 25 °C (19 points) and 37 °C (20 points).

2.2 Artificial neural network (ANNs)

Artificial neural networks, or simply neural networks, consist of a collection of processing nodes. These nodes, also called neurons, are interconnected and arranged in layers. So, when a stimulus is presented to the ANN, a unidirectional signal is propagated from the input layer to the output layer. The signals that reach each neuron are amplified or suppressed accordingly to a synaptic weight and a bias, processed by means of an activation function and emitted to the next layer. The propagation of the signal takes place until the output layer, where the ANN response to the stimulus is obtained. However, is necessary that the ANN learn to represent adequately the input–output behavior. This is done in the training stage. In this stage a set of input–output patterns are presented to the ANN and, based in the output error, the synaptic weights and bias are adjusted. The ANN obtained after the training must be able to represent the input–output behavior assimilated from the training data and generalize it for unseen data sets.

The effective use of ANN involves basically the determination of its structure, including the number of layers and the number of neurons on each layer, of the activation function and algorithm of training. The multilayer perceptron is the ANN structure most used for engineering purposes. They are designed with one input layer, one output layer and hidden layers. Their layers are connected in a way that the layer output signal is emitted just for the subsequent layer. The number of neurons in the input and output layers equals the number of inputs and outputs, respectively. The determination of the ideal number of neurons in the hidden layer(s) is a complicated task. Designing an ANN with few neurons in the hidden layers produces an ANN with no sufficient capacity and low precision is achieved. On the other hand, with an excessive number of neurons the capacity of generalization is affected and overfitting can occur. However, the use of generalization improvement techniques such as Bayesian regularization could overcome this problem (MacKay 1992).

The Bayesian regularization technique could be combined with the Levenberg–Marquardt algorithm, resulting in a efficient training method. The objective function used by this method is a linear combination of the sum of the squares of the synaptic weights and bias (SSW) and of the sum of the squares of the errors (SSE). Therefore, the synaptic weights and bias are enclosed to small values.

The activation functions most used in the hidden layer are nonlinear sigmoid functions (logistic and hyperbolic tangent functions). The nonlinearity imposed by these activation functions leads to local minima in the error surface. Thus, for certain initial sets of synaptic weights a

local minimum could be found and the solution attained is not optimal (Demuth et al. 2008).

2.3 Isotherm modelling by means of artificial neural networks

The Mathworks™ neural networks toolbox was used to train the neural networks. The trainings were carried out using the Levenberg–Marquardt algorithm combined with the Bayesian regularization technique. In order to find the best topology neural networks trainings were performed varying the number of neurons in the hidden layer trained (just single hidden layers were considered in this study). The data set was normalized in the range [−1, 1] and every different neural network training was repeated fifty times, therefore the chance of obtaining a model that had converged to local minima was reduced. This also reduces the importance of the choice of the activation function. Thus, only the hyperbolic tangent function was used. After the trainings the neural networks that achieved the smallest sum of squared error was chosen.

The neural network output vector, **y**, is calculated by means of Eq. (1):

$$\mathbf{y} = \mathbf{O}\mathbf{h} + \mathbf{c} \tag{1}$$

where **O** is the matrix of synaptic weights connecting the hidden layer to the output layer (number of neurons in the output layer × number of neurons in the hidden layer), **c** is the vector containing the biases values of the output layer (number of neurons in the output layer × 1) and the vector **h** is calculated using an activation function, as:

$$h_i = \frac{2}{1 + e^{-2v_i}} - 1 \tag{2}$$

where the vector **v** (number of neurons in the hidden layer × 1), Eq. (3), represents the output of the hidden layer.

$$\mathbf{v} = \mathbf{W}\mathbf{p} + \mathbf{b} \tag{3}$$

In Eq. (3), **W** is the matrix of synaptic weights connecting the input layer to the hidden layer (number of neuron in the hidden layer × number of neurons in the input layer), **p** is the vector of inputs (number of inputs × 1) and vector **b** contains the values of biases added to the hidden layer (number of neurons in the hidden layer × 1).

3 Results and discussion

3.1 Predicting an adsorption isotherm using ANN

In the first experiment a neural network was used to predict the adsorption isotherm of human IgG on a Ni(II)-PEVA-IDA membrane at 4 °C. In this case, the neural network have one input, the equilibrium concentration (C*) and one output, the adsorption capacity (Q*). The data set was divided in three subsets, 14 points were used in the training, 4 in the tests and 4 in the validation.

The training proceed until the neural network convergence, that is, until the training set error becomes constant or the error of validation set begins to increase between an epoch to the next. Also, the number of maximum epochs was set to 200. Trainings were performed for 2–15 neurons in the hidden layers and the smaller sum of squared error achieved was 2018.0 (mg g^{-1})^2 in the 11th training with 9 neurons in the hidden layer, the deviations of all 22 points were considered in this calculation. It was observed that the neural networks had always converged before reach the maximum of epochs.

The 22 points were fitted to the Langmuir isotherm model (Eq. 4) using the Mathworks™ curve fitting toolbox. Better results were achieved by means of the Levenberg–Marquardt algorithm.

$$Q^* = \frac{Q_m C^*}{K_d + C^*} \tag{4}$$

For a confidence level of 90 %, the fitted parameters Q_m (the maximum IgG binding capacity) and K_d (the apparent dissociation constant) achieved the following values:

$Q_m = 262.5$ mg g^{-1} (the confidence bounds were 246.9 and 278.0 mg g^{-1});

$K_d = 2.528$ mg mL^{-1} (the confidence bounds were 1.885 and 3.171 mg mL^{-1}).

Equation (4) leads to the coefficient of determination, $R^2 = 0.973$ and SSE = 4,585 (mg g^{-1})^2. The comparison of the results obtained by neural network and by the Langmuir isotherm model is presented in Fig. 1.

Besides, the maximum deviation obtained by ANN was 20.73 mg g^{-1} against 25.59 mg g^{-1} of the isotherm model. It must be pointed out that only 14 of the 22 points were effectively used to prepare the neural model. The R^2 value achieved by ANN was 0.988.

3.2 Including the effect of temperature

In a second experiment, the effect of the temperature was included. Experimentally, affinity increases at higher temperature while the binding capacity decreases. At lower temperature the coulombic forces are the main forces prevailing during the adsorption process (Finette et al. 1997; Ribeiro et al. 2008). Thus, there were two inputs, the IgG liquid-phase equilibrium concentration, C*, and the temperature. In this case, all experimental points obtained at 4 and 37 °C were used in the training. The achieved neural model was tested to the interior points, that is, to predict the isotherms at 15 and 25 °C. No validation set

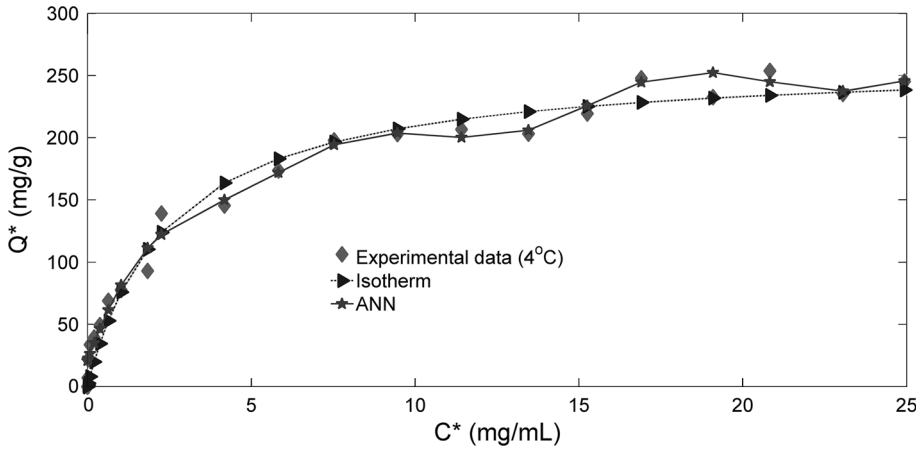

Fig. 1 Comparison of adsorption isotherm at 4 °C obtained using artificial neural network (ANN) and Langmuir isotherm model

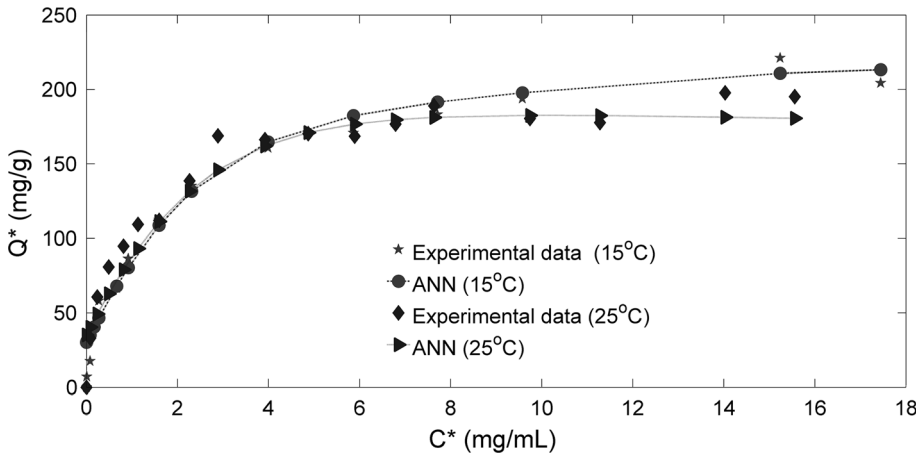

Fig. 2 Human IgG surface concentration calculated using the neural model for unseen data

was considered during the training, so the maximum of epochs has been reached several times.

The performance of the neural networks was measured using the Eq. 5:

$$P = \min\left\{\frac{\sum_{T=1}^{2}\left[\sum_{i=1}^{nd}\left(Q^{*}_{i,T} - Q^{*}_{N,i,T}\right)^{2}\right]}{2}\right\} \quad (5)$$

where P is the performance index, nd is the number of experimental points at temperature set T ($nd = 18$ for 15 °C, i.e., $T = 1$ and $nd = 19$ for 25 °C, i.e., $T = 2$) and Q^{*}_{N} is the IgG surface concentration calculated by the ANN.

The P value of 3010.0 (mg g^{-1})^2 was obtained for the 26th training with 12 neurons in the hidden layer. The adsorption isotherms at 15 and 25 °C obtained using this ANN are illustrated in the Fig. 2.

In Fig. 3 are presented the adsorption isotherms at 4 and 37 °C obtained using the neural model. These two sets of data were used in the training.

The correlation coefficients (R) achieved for Langmuir model and ANN are presented on Table 1. R values for Langmuir model demonstrated to be slightly better, however it must be pointed that the best ANN was not chosen based on R value, but on performance index, P. So, ANN's presenting larger R values could be trained, but certainly their P values will be worst.

It could be observed in Figs. 1, 2 and 3 that ANNs were able to describe the isotherms adequately including the effect of temperature. As expected, the Levenberg–Marquardt algorithm combined with Bayesian regularization technique was able to avoid overfitting problems even for an excessive number of neurons. These results are in accordance with that presented by Morse et al. (2011).

3.3 Predicting adsorption isotherms using ANN trained trained without experimental data

In the last experiment, the ANN's were trained using a dataset generated using the Langmuir isotherm models (Eq. 4), instead of using the experimental data. The values of maximum IgG binding capacities and the apparent dissociation constants, presented in Table 2, were used to calculate the IgG surface concentrations at different temperatures.

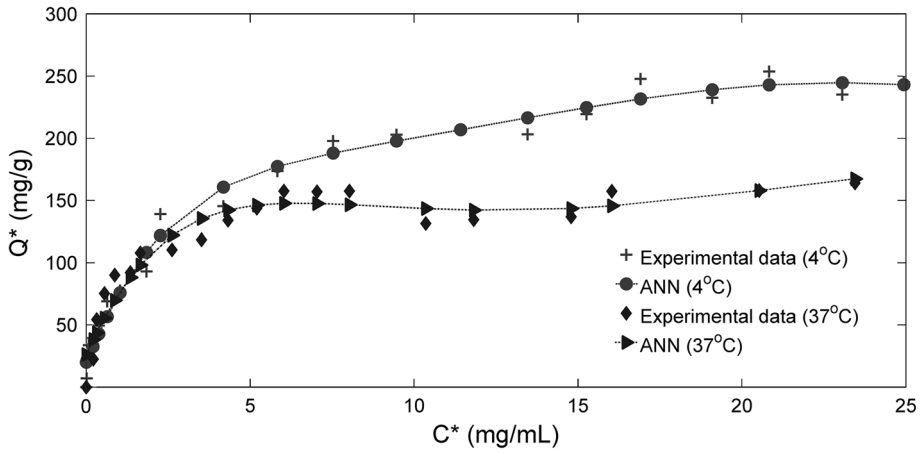

Fig. 3 Human IgG surface concentration calculated using the neural model for training data

Table 1 Correlation coefficients achieved by ANN and by Langmuir model and Langmuir model parameters calculated for a 90 % confidence level

Temperature (°C)	Langmuir			ANN
	Q_m (mg g^{-1})	K_d (mg mL^{-1})	R	R
4	2.528 ± 0.643	262.4 ± 15.5	0.986	0.992
15	1.547 ± 0.407	227.4 ± 14.8	0.988	0.988
25	0.836 ± 0.182	199.9 ± 9.0	0.986	0.973
37	0.786 ± 0.209	159.4 ± 7.9	0.974	0.961

Table 2 Maximum IgG binding capacity, Q_m, and apparent dissociation constant, K_d, values used to generate the dataset used to train the ANN

Temperature (°C)	4	15	25	37
Q_m (mg g^{-1})	2.528	1.548	0.836	0.786
K_d (mg mL^{-1})	262.5	227.4	199.9	159.4

As in the second experiment, dataset on the temperatures of 4 and 37 °C were used in the training and that on 15 and 25 °C for testing. The training set was composed by values of Q* calculated, using Eq. (4), for C* values in the range (0–17 mg mL^{-1}) with increments of 0.001 mg mL^{-1} and its corresponding temperature. Besides, in this case a Nonlinear Autoregressive network with Exogenous inputs (NARX) architecture was used. This approach is normally used for time series data, but considering that knowledge about the isotherm is used as a whole, the data could be sorted in a sequence and is possible to use it (Demuth et al. 2008; Pollastri et al. 2002; Çoruh et al. 2014). Therefore, the input vector included also the last output value, that is, $Q*(k) = f[C*(k), T(k)$ and $Q*(k-1)]$ (Fig. 4).

The training procedure is the same as the other ANN's. However, in this case the dataset was composed by thousands of input–output pairs, so the training required more

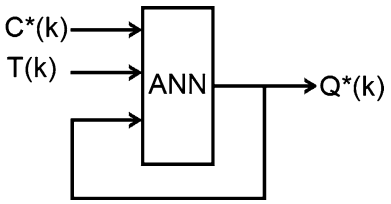

Fig. 4 Nonlinear autoregressive with exogenous inputs architecture used to describe the isotherms

computational effort. This huge quantity of data is necessary to describe adequately the fast changes of Q* in low IgG liquid-phase equilibrium concentrations. For this reason, only topologies with 2 neurons in the hidden layer were tested. In the Figs. 5 and 6 are presented the isotherms achieved for 15 and 25 °C, respectively.

Good performance of ANN's for interpolate the isotherms could be observed again, the mean absolute error (the error was calculated as Q* calculated using Langmuir model minus Q* calculated using ANN) was 4.07 mg g^{-1}. It must be pointed that no experimental data was used in this case.

This study could be expanded by including the effect of different chelating ligands, pH, or buffer systems in the analysis. Furthermore, the proposed approach could be extended to enable prediction of apparent dissociation constant, K_d, at different temperatures. Therefore, making it possible to calculate thermodynamic parameters needed for the design of industrial separation processes.

4 Conclusions

Artificial neural networks were successfully used to predict the adsorption isotherms of human IgG on Ni(II)-IDA-PEVA hollow fiber membranes. The resulting neural

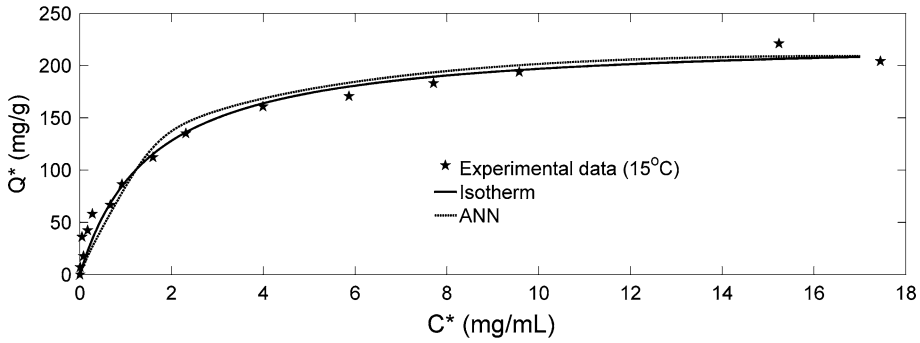

Fig. 5 Human IgG surface concentration at 15 °C calculated using the neural model trained with data from Langmuir isotherm model

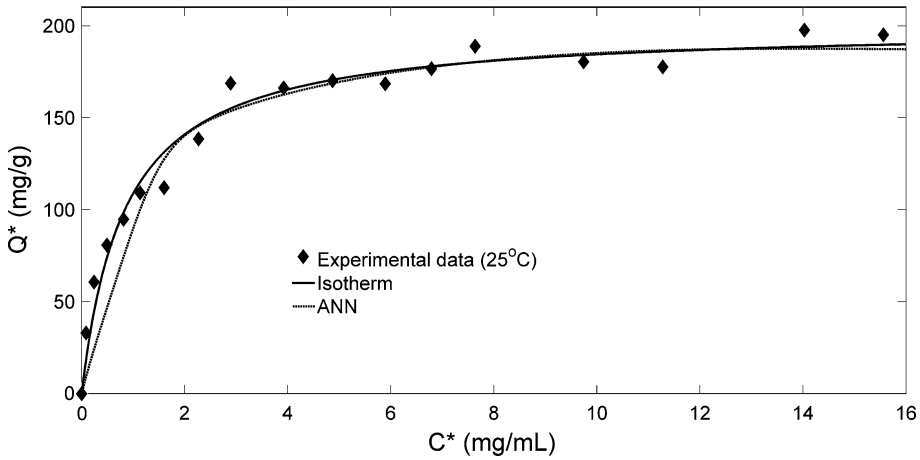

Fig. 6 Human IgG surface concentration at 25 °C calculated using the neural model trained with data from Langmuir isotherm model

network was able to interpolate the behavior of the adsorption process in the temperature range used in the training. The results were similar to that of the traditionally used Langmuir model. However, ANN possesses unquestionable advantages, such as the interpolation ability and the capability of deal well with imprecise data.

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