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IDENTIFICATION AND PREDICTION OF PROTEIN ADSORPTION BREAKTHROUGH, DESORPTION, AND FRACTIONATION IN A PACKED COLUMN USING A NEURAL NETWORK

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ABSTRACT

A simple neural network with a three-node hidden layer has been used to identify and predict protein adsorption, desorption and fractionation profiles in a 25 x 1 cm ID Productiv™ CM ion-exchange column. To predict the effect of flow rate on the adsorption breakthrough curve, two sets of data obtained at the maximum and minimum of the full range of flow rates used were sufficient to train the neural network which was then able to predict the effects of flow rate changes within the training range on the adsorption breakthrough curve. This training method was also applied to explore the effects of flow rate on desorption and fractionation. It was found that the network training algorithm performed satisfactorily if the flow rate data for desorption and fractionation were scaled in the form of logarithm.

INTRODUCTION

Modeling protein chromatographic processes in a packed column is of great importance for performance optimization and scale-up. Yet the physical and thermodynamic phenomena involved in the protein adsorption or desorption process are still not fully understood (1). A mechanistic model may give good predictions for a linear adsorption or desorption process, but the use of such a model is limited for nonlinear adsorption processes. Simplifying assumptions are usually needed to develop a model which handles the problem more easily and to reduce the computation time. Prior or on-line determination of the isotherm parameters, maximum capacity, q_m, and dissociation constant, K_d, and adsorption or desorption rate constants is usually required by a mechanistic model. These parameters are often determined by separate measurements, usually taking days. If a model includes the factors of mass transfer, the values of bed voidage, particle

porosity, diffusion, and axial dispersion coefficients will be required. This consequently requires the use of correlations which need validating or separate measurements, which are time consuming. Moreover, the reliability of such parameter determination is often questionable. Tsou and Graham (2) developed a two-phase resistance model, but their model could not predict the early part of the breakthrough curve well. Skidmore and Chase (3) and Skidmore *et.al.* (4) developed a simple kinetic rate constant model and a film and pore diffusion model for lysozyme and bovine serum albumin (BSA) adsorption in a column packed with Sepharose FF ion-exchanger. In order to give a good curve fit, the values of q_m and k_1 estimated from the isotherm and a batch uptake experiments needed to be readjusted for each case. This shows that a mechanistic model may give reasonable predictions, but at a greater expense of a process mechanism investigation and parameter predetermination.

For a nonanalytic or preparative packed column, stepwise desorption is a nonlinear process. Desorption, whose mechanism is still not fully understood, usually occurs first as soon as the eluent contacts the adsorbent. This results in almost instantaneously reaching a maximum column output concentration, followed by a gradual decline in output concentration as the desorbing molecules diffuse slowly to the surface of the adsorbent particles and are diluted in the eluting stream. In the case of a Productiv CM packed column, there is a double peak in desorption rates, giving a "bobsled" desorption curve. To predict this "bobsled" desorption curve using a mechanistic model is rather difficult. Few reports on modeling a protein desorption process have been found in the literature to date.

This paper demonstrates how a simple neural network model can be used effectively for process identification and prediction of protein adsorption and desorption in a packed column. The main advantage of a neural network model is that it does not require (1) any process assumption; (2) any insight or deep understanding of the process; (3) predetermination of any model parameter. Conversely, the use of a neural network model adds no insight into the mechanism or validity of any process assumptions. A significant drawback of a neural network model, in comparison

with mechanistic methods, is that training a network usually requires a great deal of experimental data, which can be larger by orders of magnitude than required for a mechanistic model (5). Fortunately, modern computer acquisition of data readily generates a large quantity of usable information suitable for training. In this study, we trained the neural network model using a "full range" training method, which required two sets of data obtained at the maximum and minimum of the range of operating parameters used. This gave an acceptably short training time.

EXPERIMENTAL METHOD

A 250 x 10 mm-ID Productiv™ CM ion-exchange (6) column (BPS Separation Ltd, County Durham, UK) was used to adsorb 3 g lysozyme (Fluka Chemika-Biochemika, UK) dissolved in 0.02 M sodium acetate buffer, pH 5. After washing with 0.02 M sodium acetate buffer (pH 5), the column was eluted with 0.7 M NaCl. For the case of fractionation, the Productiv CM column was loaded with 60 mL of 38 mg/mL egg-white protein solution at pH 4.7 and then was fractionated using gradient elution of 250 mL sodium acetate buffer, pH 4.7, and 250 mL 0.7 M NaCl (7). An LKB Uvicord II monitor was used on-line to detect the protein concentration from the column outlet at 280 nm. Data were collected and subsequently processed by a Viglen IV/25 computer (Viglen Ltd, London) through a data logging programme.

NEURAL NETWORK MODEL AND TRAINING METHOD

The neural network model used in this study is similar to that used in previous work (8) shown in Figure 1. The weights were randomly initialized. Dynamic column elution volume (product of flow rate and processing time) was the input x_1. Dynamic loaded protein or salt in weight [product of flow rate, feedstock concentration (protein or salt) or salt concentration slope, K, and processing time] was the input x_2. Input x_3 was a bias which was a normalization of feedstock concentration. The network training corrected the weights by a standard back propagation of errors algorithm using an "inhouse" program written in Turbo Basic. The training time was 3 h for identification of the adsorption breakthrough curve, 1 h for identification of the desorption process and 10 h for identification of the

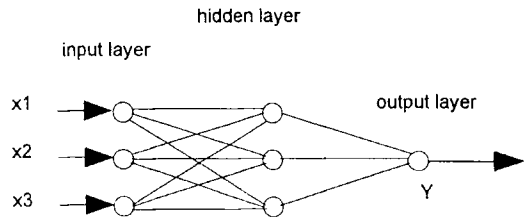

FIG. 1 Neural network structure used in this study

fractionation process by using a Viglen IV/25 486 computer. The subsequent predictions took only a few seconds.

Full-range training meant that two sets of data obtained at the maximum and minimum of flow rates and a single feedstock concentration, or otherwise, were used for the network training.

RESULTS AND DISCUSSION

Identification and Prediction of the Effect of Flow Rate on Column Adsorption Breakthrough Curve

Figure 2 shows the training fits given by a neural network model, coded as N-WF1, trained with data obtained at a maximum flow rate (17 mL/min) and a minimum flow rate (1.3 mL/min). This model was then used to predict the effects of flow rates on column breakthrough curves at other flow rates. Test results shown in Figure. 3 used flow rates of 5.8, 7, and 10 mL/min, which were randomly chosen within the training range. These results indicate that the model N-WF1 predicts most parts of the breakthrough curve well except for the tailing. This can be improved if one more set of data was introduced into the training. In practice, the tail region of a breakthrough curve is less important than the initial region or the first half of curve, as loading is normally stopped as initial breakthrough occurs. In this case, the network trained with the data obtained only at the maximum and minimum flow rates can follow the effect of flow rate well and gives a satisfactory prediction for the first half of the column breakthrough curve.

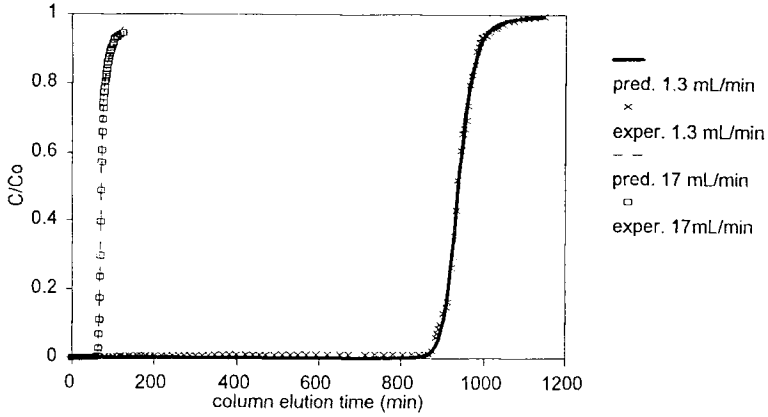

FIG. 2 Training fits given by network N-WF1

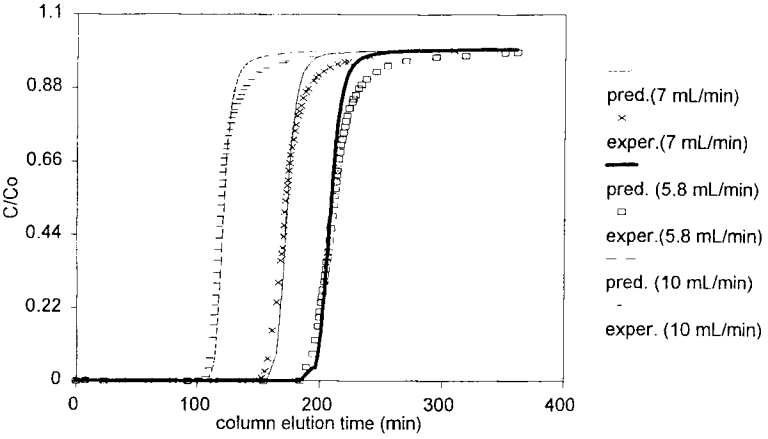

FIG. 3 Prediction of effect of interpolated flow rate on breakthough curve using network N-WF1 (feed concentration: 1.5 mg/mL)

It was also possible to predict the effect on the breakthrough curve of some flow rates beyond the training range. Figure 4 shows that a network model, coded as N-WF3, trained with data obtained at flow rates of 5.8 and 10 mL/min, was able to predict the breakthrough curves for flow rates both within (6.8 mL/min) and outside (1.3 mL/min and 17 mL/min) the training range. The lowest flow rate used (1.3 mL/min) did not result in a very good prediction of the first half of the breakthrough curve.(Figure 4). The range of flow rates used are at the high end of normal operation of preparative chromatographic columns which tend to be used up to linear flows of 1 m/h. This corresponds to the 1.3 mL/min with the flow of 17 mL/min being over 12 m/h. This is the region where flow rates are considered very high and where operators have concern that breakthrough will not be easily predicted.

Identification and Prediction of the Effect of Feed Concentration on Breakthrough Curve

For prediction of the effect of feedstock concentration, the neural network trained with only two sets of data obtained at the maximum and minimum feedstock concentrations failed to give a satisfactory prediction in spite of the good training fits. When full ranges of both flow rates (1.3 and 17 mL/min) and feedstock concentrations (0.5 and 3 mg/mL) were used to train the neural network, the neural network model, coded as N-WFC, was able to give a satisfactory prediction for the column breakthrough curve following changes in feedstock concentration in flow rate, or in both, as can be seen in Figures. 5. This suggests that to identify the effects of both flow rate and feed concentration at least four data sets are required for the network training.

Identification and Prediction of Desorption and Fractionation Processes

In this case, only the effect of flow rate was considered. Simply applying the full range of flow rate training method to the desorption, the network gave a poor prediction with considerable time shifts (Figure 6). To improve the network performance alternative scaling algorithms were attempted.

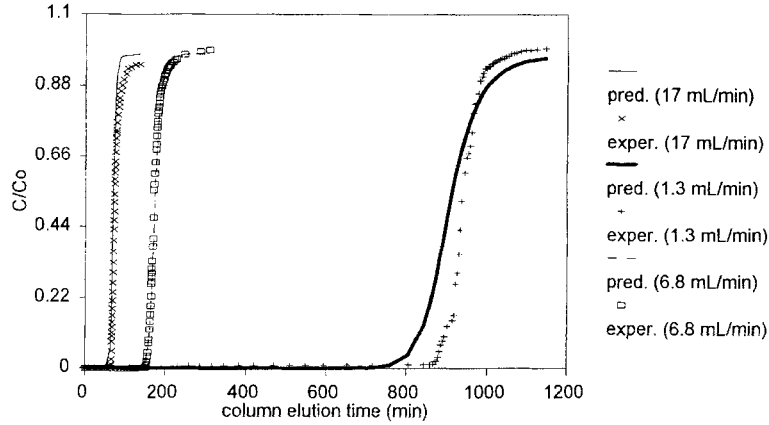

FIG. 4 Prediction of effect of interpolated and extrapolated flow rate on breakthrough curve using network N-WF3 (feed concentration: 1.5 mg/mL)

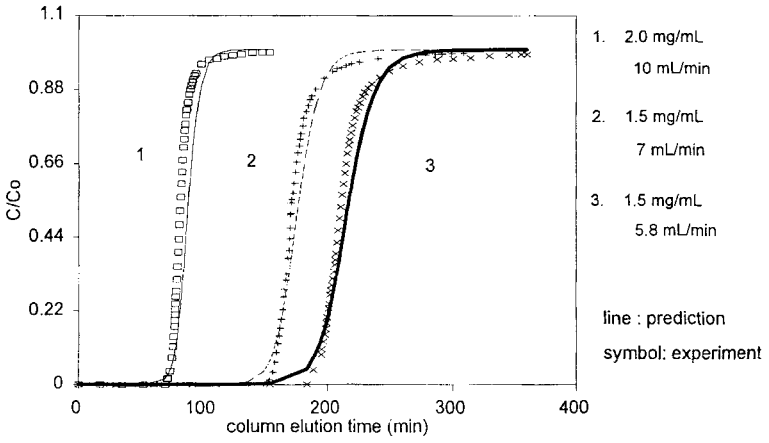

FIG. 5 Prediction of effects of both flow rate and concentration on breakthrough curve using network N-WFC

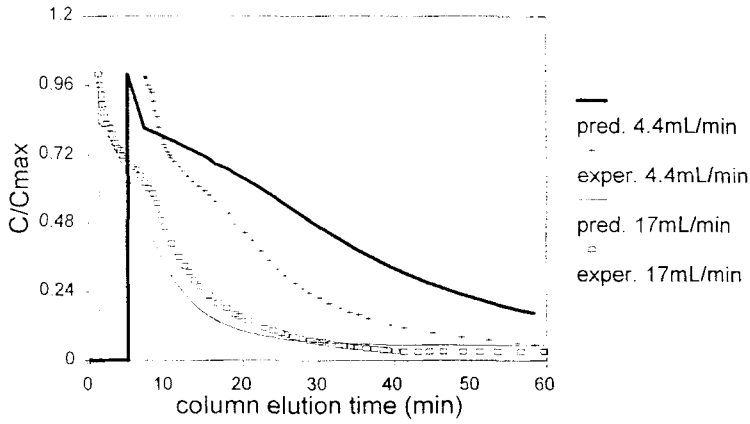

FIG. 6 Desorption identification using the flow rate data scaled linearily

Firstly the flow rate data were scaled in its nth root form. The attenuation of the flow rate range appeared to be helpful and the prediction error was thus considerably reduced. A trial and error method was carried out to find the optimum value of "n", however this was found to be time consuming. A logarithm scaling method was then suggested, as it was thought that the factor of nth root would end up as parts of the weights developed during the network training in view of the nature of the logarithm function. Figure 7 shows the significantly improved predictions given by the trained network (coded as N-Log1) using this scale method.

The same method was applied to the case of fractionation, the trained network (coded as N-log2) can give reasonable predictions (Figure 8).

The neural networks used are summarized in Table 1

CONCLUSIONS

In the case of prediction of the flow rate effect on adsorption breakthrough curve, two sets of data obtained at

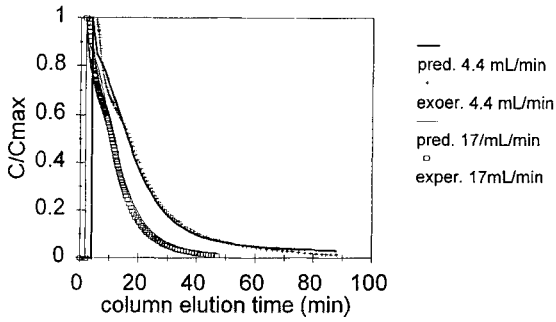

FIG. 7 Desorption identification using the flow rate data scaled in the form of logarithm

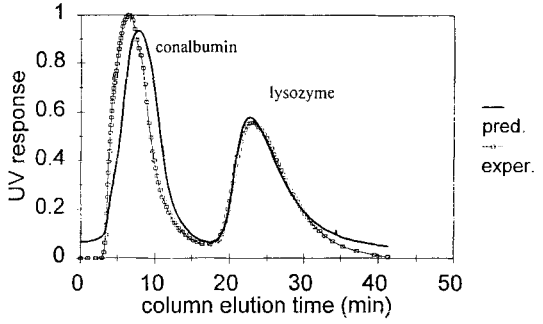

FIG. 8 Prediction of egg-white protein fractionation using network N-Log2

TABLE 1. NEURAL NETWORKS USED IN THIS PAPER

Network	Training Data Conditions	Data Scale Method	Figures
N-WF1	(1.3 mL/min, 1.5 mg/mL) (17 mL/min, 1.5 mg/mL)	linear	2 & 3
N-WF3	(5.6 mL/min, 1.5 mg/mL) (10 mL/min, 1.5 mg/mL)	linear	4
N-WFC	(1.3 mL/min, 1.5 mg/mL) (17 mL/min, 1.5 mg/mL) (10 mL/min, 0.5 mg/mL) (10 mL/min, 3 mg/mL)	linear	5
N-LOG1	(1.3 mL/min), (22 mL/min)	logarithm	7
N-LOG2	(2.4 mL/min), (18 mL/min)	logarithm	8

different flow rates were sufficient to train the neural network model. Such a trained network can be used to predict the effect of flow rate on the breakthrough curve within the flow rate range used in the network training and even beyond this range, albeit with reduced accuracy. In the case of predictions of the flow rate effect on the column desorption and fractionation, the flow rate input should be input in the form of logarithm.

For following the effects of feedstock concentration on the column breakthrough curve, two sets of data were not sufficient for the network training. It required at least four sets of training data. If these were used from a star design, prediction was successful. This gave at least three separate values of each variable within the training sets.

In comparison with mechanistic modeling, the neural network modeling applied in this study was easy to develop and robust to use. It would seem to be useful to allow optimization of the loading and elution protocols by varying flow rates and then predicting the overall productivity of the system.

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