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Kinetic studies for sorption of amino acids using a strong anion-exchange resin
Effect of ionic strength

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Abstract

This work deals with the influence of the ionic strength on the sorption of L-phenylalanine and L-tyrosine by a strong basic anion-exchange resin, converted to the hydroxide form with sodium hydroxide. Equilibrium uptake isotherms were obtained for phenylalanine and tyrosine by carrying out batch experiments at different ionic strength values of the solution. The model used to correlate these results is the modified Langmuir equation which has been applied with success to biological systems. Batch kinetic experiments were performed using a packed bed of differential length inserted in a liquid circulation loop and in which the ionic strength of the solution was varied. Moreover, an experiment at variable pH for tyrosine was also performed. Experimental transient concentration profiles were compared to those predicted by the pore diffusion model and enabled the estimation of the intraparticle diffusivities for phenylalanine and tyrosine.

Keywords: Ionic strength; Ion exchange; Amino acid; Kinetics

1. Introduction

Sorption processes are receiving increased attention as efficient tools in modern industrial-scale biotechnology for the separation, purification and recovery of amino acids. One of these processes that have deserved particular interest is the ion exchange [1–3]. Since the net charge of the amino acid molecules may vary in magnitude and sign with the pH of the solution in which they are present, this behaviour can be exploited to separate and concentrate mixtures of these compounds on ion exchange resins by means of the parametric pumping technique. For example, an anion-exchange resin that has been loaded with an amino acid at a pH at which the amino acid is predominantly in the negatively charged form, can easily be regenerated by changing the solution pH to a new value at which the predominant form of the amino acid is positively charged. Thus the change of pH displaces the partition equilibrium of the amino acid between the two phases. This effect, in combination with periodic changes in the flow direction of the mixture to be separated through a fixed bed, is the basic principle of parametric pumping and results in the separation of the desired components. However, if the mixture is formed by amino acids with very close isoelectric points (pI), as the case of the phenylalanine (pI = 5.48) and tyrosine (pI = 5.64), it is difficult to achieve an efficient separation by a single pH parametric pump. Therefore, an additional variable other than pH, such as ionic strength, is needed for improving the separation. The technique pH-ionic strength parametric pumping for the separation of enzyme mixtures was experimentally investigated in some works [4,5].

Several studies have addressed the description of the effect of the pH on the equilibrium and rate of uptake of amino acids by ion exchange resins [6–12]. However, few researches have been carried out on the effect of variations of solution ionic strength on the sorption of these molecules by ion-exchange resins. In the present work, the effect of this variable on the equilibrium and kinetics of the sorption of phenylalanine and tyrosine by a strong anion-exchange resin, PA 316, is investigated. Experimental results were analysed and correlated by means of appropriate models.

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Table 1
Physical properties of the resin

Ionogenic group	$-N^+(CH_3)_3$
Degree of crosslinking (%)	8
Ion exchange capacity (mmol/g_{wr})	1.15
Intraparticle porosity	0.54
Water content (%)	49
Density (g_{wr}/cm^3_{wr})	1.05

2. Material and methods

2.1. Resin and chemicals

The resin used in this study, Diaion PA316 from Mitsubishi Chemical Corporation (Milan, Italy) is a strong anionic exchange resin, based on sulfonated crosslinked polystyrene and with trimethylammonium exchange group ($R–N^+(CH_3)_3$). The physical properties of this sorbent are summarized in Table 1.

The resin was pre-treated by repeated washes with 2 M HCl and 2 M NaOH solutions, and then converted to the hydroxide form by elution with 2 M NaOH and rinsed at neutral pH with deionised water (Table 2).

The ion-exchange capacity was determined by equilibrating a resin sample in the hydroxide form with an excess of 0.1 mol/l HCl containing 50 g/l of NaCl. After equilibrium was reached, the excess HCl left in solution was titrated with 0.1 M NaOH and the capacity of the resin determined from a material balance.

Pure crystalline forms of L-phenylalanine (Phe) and L-tyrosine(Tyr) from Riedel-deHaen (Seelze, Germany) were used as solutes in all the experiments.

The concentrations of Phe and Tyr were determined by an UV spectrophotometer, model DU 650 from Beckman instruments, Inc. (California, USA) at 257 and 273 nm, respectively. The pH in the liquid phase was determined by a pH meter (WTW 540 GLP) using a glass combined electrode (Mettler Toledo).

2.2. Equilibrium experiments

Ion exchange isotherms were determined from batch experiments at constant pH and ionic strength. These experiments were carried out by equilibrating over 2 days a given mass of dry resin (0.05–5 g) in the hydroxide form with 75 cm^3 of amino acid solution (≈2 mM) in sealed Erlenmeyer flasks. The flasks were placed in a constant-temperature shaker bath kept at 25 ± 1 °C. Achievement of equilibrium in these experiments was determined by monitoring the amino acid concentration from a flask used as reference.

Table 2
Equilibrium parameter values for the modified Langmuir equation

Amino acid	a_0	b_0	z_a	z_b
Phe	6.15×10^1	2.15×10^2	1.76	2.23
Tyr	8.33×10^2	5.61×10^3	2.62	3.65

The pH and ionic strength of the solutions were controlled by a mixture of boric acid and borax. In all the experiments the pH was kept approximately constant and equal to 8.

2.3. Batch kinetic experiments

Batch kinetic measurements were carried out using a packed bed of differential length inserted in a liquid circulation loop in an apparatus similar to that described by Saunders et al. [6]. A shallow layer of resin, approximately 0.6 cm thick (corresponding to about 0.45 g of dry resin) was packed in a glass column (2.0 cm internal diameter, 26 cm length) between two layers of inert glass beads (0.46 mm diameter). At sufficiently high circulate rates, i.e., pumping the solution at 180 cm^3/min, the experimental arrangement used simulates the behaviour of a stirred batch system. Before starting one sorption experiment at constant pH, the resin in OH^- form was equilibrated with an acid boric/borax solution to yield the desired pH and ionic strength. Then the reservoir was charged with a known volume of solution containing an initial concentration of amino acid and the same pH and ionic strength as the previous solution. After this, the amino acid solution was pumped through the column and the bed effluent was recirculated to the mixing reservoir. In the case of the experiment at variable pH, the column was initially equilibrated with NaOH solution of desired concentration for the run. During the recirculating period, the amino acid concentration and the pH in liquid phase were monitored with the UV spectrophotometer and a pH meter using a glass combined electrode, respectively.

3. Modelling

3.1. Equilibrium model

To describe quantitatively the experimental data obtained by the batch technique, we used the modified Langmuir equation that has been successfully applied in ion-exchange chromatography under salt gradients [13,14],

$$q_i = \frac{a_o I^{-z_a} C_i^-}{1 + \sum_{j=1}^{n} b_o I^{-z_b} C_j^-} \; i = 1, 2, \ldots n \text{ (species)} \qquad (1)$$

where C_i^- is the concentration of the amino acid, in negatively charged form, I (mmol/l) is the ionic strength; a_o, b_o, z_a and z_b are the parameters of the equation.

The value of C_i^- was calculated by simultaneously solving Eqs. (2)–(4). Eqs. (2) and (3) are derived taking into account the two equilibrium dissociation reactions of the amino acid in solution ($NH_3^+CHRCOOH \rightleftarrows^{k_1} NH_3^+CHRCOO^- + H^+$, $NH_3^+CHRCOO^- \rightleftarrows^{k_2} NH_2CHRCOO^- + H^+$) and Eq. (4) represents the electroneutrality condition. So we

have,

$$C_i^+ = \frac{C_i}{1 + k_1/C_H^+ + k_1k_2/C_H^{+2}} \quad (2)$$

$$C_i^- = \frac{C_i}{1 + C_H^+/k_2 + C_H^{+2}/k_1k_2} \quad (3)$$

where C_i is the total concentration of amino acid, C_i^+ is the concentration of positively charged amino acid and C_H^+ is the hydrogen ion concentration; k_1 and k_2 are dissociation equilibrium constants.

The electroneutrality condition can be expressed as follows,

$$\sum_i C_i^+ + C_H^+ + C_{sal}^+ = \sum_i C_i^- + C_{OH}^- + C_{sal}^- \quad (4)$$

where C_{OH}^- is the concentration of hydroxyl ion and C_{sal}^+ and C_{sal}^- are the total concentration of cations and anions, respectively, caused by the acid boric/borax dissociation in aqueous solution.

Note that for tyrosine, one more ionic species with concentration C_i^{2-} is also present in solution resulting from a third dissociation reaction ($pK_a = 10.1$). This concentration was neglected because its value is almost zero under pH 8 of the experiments performed at constant ionic strength.

3.2. "Pore-diffusion" model

The "pore-diffusion" model, Eq. (5), together with the mass balance in the bulk liquid phase, Eq. (10), were used to simulate the transient uptake of phenylalanine and tyrosine by the resin PA316 during the batch kinetic experiments. The model is based on the following assumptions: diffusion through the macropores of the particle and instantaneous equilibrium for the solute distribution between pores and gel phase. Moreover, on the basis of experimental evidence it was assumed that the species resulting from the boric acid/borax were not adsorbed by the resin. With these assumptions the mass transport of amino acid into the particle may be written as follows,

$$\varepsilon_p C_0 \frac{\partial x_{A,P}(u,\theta)}{\partial \theta} + q_0 \rho_p \frac{\partial y_A(u,\theta)}{\partial \theta} = \varepsilon_P C_0 \left(\frac{2}{u} \frac{\partial x_{A,P}(u,\theta)}{\partial u} + \frac{\partial x_{A,P}^2(u,\theta)}{\partial u^2} \right) \quad (5)$$

in which $y_A = q_A/q_0$ is the normalised concentration of total amino acid in the gel phase, $x_{A,P} = C_{A,P}/C_0$ is the normalised macropore solute concentration, $u = r/R_P$ is the normalised radial coordinate and $\theta = t/\iota_d$ ($\iota_d = R_p{}^2/D_p$) is the normalised diffusion time constant; C_0 is the initial solute concentration, q_0 is the resin capacity, ε_P is the particle porosity and ρ_p is the density of the wet resin. y_A is locally related to $x_{A,P}$ by the equilibrium isotherm corresponding to Eq. (1) that is only valid for the runs at constant ionic strength. For the run performed at variable pH, the Myers and Byngton model tested elsewhere by Moreira and Ferreira [15] was applied.

Initial and boundary conditions are given by,

$$\theta = 0 \quad x_{A,P}(u \neq 1, \theta = 0) = 0 \quad (6)$$

$$x_{A,P}(u = 1, \theta = 0) = 1 \quad (7)$$

$$u = 0 \quad \left.\frac{\partial x_{A,P}(u,\theta)}{\partial u}\right|_{u=0} = 0 \quad (8)$$

$$u = 1 \quad \left.\frac{\partial x_{A,P}(u,\theta)}{\partial u}\right|_{u=1} = B_{A,m} \frac{V_{sol}}{3V_{res}\varepsilon_P} (x_A(\theta) - x_{A,P}(u = 1, \theta)) \quad (9)$$

In the bulk liquid phase, the amino acid concentration was obtained by using the material balance,

$$\frac{d\, x_A(\theta)}{d\theta} = -B_{A,m}(x_A(\theta) - x_{A,P}(u = 1, \theta)) \quad (10)$$

where $B_{A,m} = \iota_d/\iota_f$ is the mass Biot number and in which ι_d and $\iota_f = V_P/(V_S k_f\, ap)$ are the diffusion and the film time constants, respectively. The film mass transfer coefficient k_f was estimated from the Wakao and Funazkri correlation [16]:

$$Sh = 2.0 + 1.1 Sc^{1/3} \times R_{e0}^{0.6} \quad (11)$$

where R_{e0} and Sc are the Reynolds and Schmidt numbers, u is the fluid superficial velocity, and ε is the void fraction of the resin bed. The values of k_f calculated for Phe and Tyr are 7.258×10^{-3} and 6.980×10^{-3} cm/s, respectively.

Numerical solution of Eqs. (5)–(10) was performed by discretizing Eq. (5) in the radial direction using orthogonal collocation on finite elements with cubic Hermite polynomials [17]. This led to an implicit system of NE ODE's that was integrated using the computer package-DDASSL [18].

4. Results and discussion

4.1. Analysis of equilibrium studies

The equilibrium isotherms for sorption of phenylalanine and tyrosine on PA316 are shown in Figs. 1 and 2. The amount of amino acid taken up by the resin (q_i, i = Phe, Tyr) was determined from a material balance: $q_i = (C_{o,i} - C_i)V/W$, where $C_{o,i}$ and C_i are the initial and equilibrium concentrations in the liquid phase, respectively. V and W are the volume of the solution and the weight of the wet resin, respectively. The equilibrium data obtained at three different ionic strengths (I) and at same pH value show that a much larger amount of the amino acid is up taken by the resin for lower values of I.

The experimental data were well correlated by the Eq. (1). The fitting of the experimental equilibrium data was carried out by using a nonlinear optimization routine GREG [19]. The parameter values obtained with this procedure are given

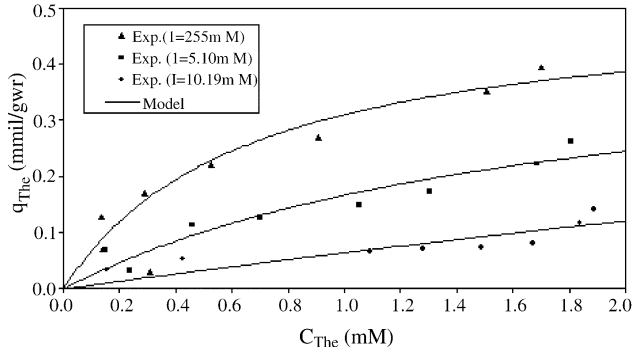

Fig. 1. Uptake equilibrium of phenylalanine as a function of total concentration of amino acid in solution at various ionic strengths.

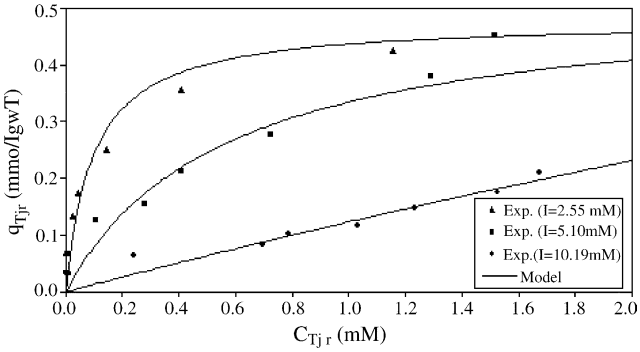

Fig. 2. Uptake equilibrium of tyrosine as a function of total concentration of amino acid in solution at various ionic strengths.

in Table 2. The equilibrium parameters involved in the Myers and Byington model are: average binary separation factor for ion i (Tyr^{2-}) relative to ion j (OH^-), $S_{ij} = 113.89$, heterogeneity parameter, $W = 6.05$, and skewness parameter, $p = 0.60$.

4.2. Analysis of batch kinetic studies

Experimental and simulated results on the kinetics of ion exchange of the amino acids under study are plotted in Figs. 3–5. It can be seen that the model predicts the transient adsorption profiles well. The best fitting values of the intra-

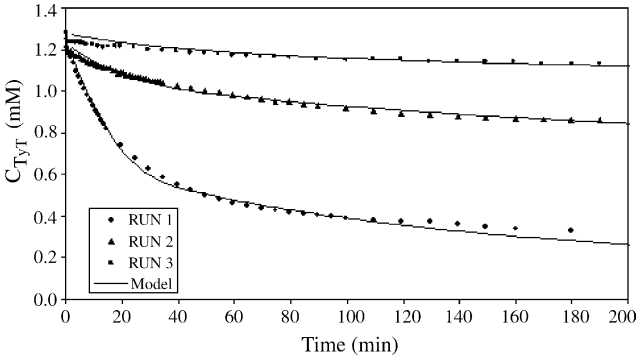

Fig. 3. Experimental results and model simulation for transient uptake of tyrosine.

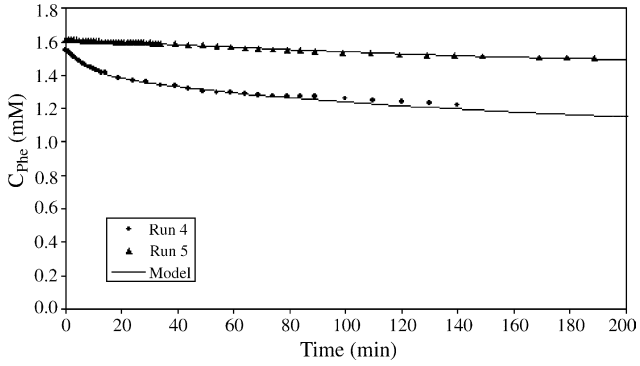

Fig. 4. Experimental results and model simulation for transient uptake of phenylalanine.

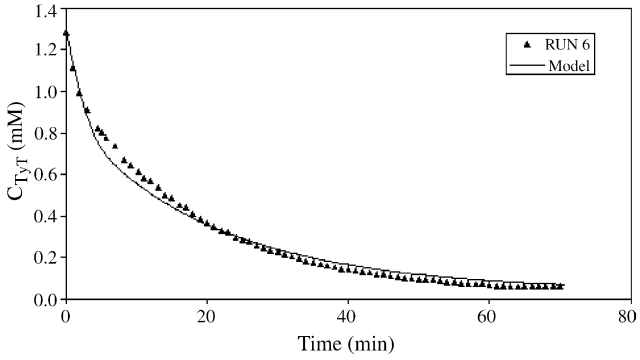

Fig. 5. Experimental results and model simulation for transient uptake of tyrosine at variable pH.

particle diffusivity (D_P) for Phe and Tyr were determined by comparing the numerical solution with the experimental data. The experimental conditions and the D_P values obtained are shown in Tables 3 and 4, respectively. The results listed in Table 4 suggest strong dependence of adsorption kinetics on the solution ionic strength. This dependence

Table 3
Experimental conditions for batch kinetic studies

Run	Amino acid	C_0 (mM)	I^a/C_{Nao} (mM)
1	Tyr	1.25	2.55
2	Tyr	1.23	5.10
3	Tyr	1.28	10.20
4	Phe	1.55	2.55
5	Phe	1.62	5.10
6	Tyr	1.29	10.00

[a] Runs 1–5. Resin mass (g dry resin) = 0.6651; flow rate (cm^3/min) = 180; V_{Sol} (cm^3) = 345.

Table 4
Parameter values used in the simulations

Run	D_P (cm^2/s) × 10^6
1	2.43 ± 0.11
2	0.49 ± 0.04
3	0.17 ± 0.02
4	0.62 ± 0.04
5	0.04 ± 0.002
6	2.57 ± 0.07

for phenylalanine and tyrosine are distinctly different. For example, increasing I from 2.55 to 5.10 mM results in a significant reduction in the diffusivity of Phe from 0.62×10^{-6} to 0.04×10^{-6} cm^2/s, i.e., D_p is reduced by ≈94 percentage points. For tyrosine, the same increase in the ionic strength causes a reduction in the diffusivity of about 80 percentage points. The relationship between D_P for Tyr and ionic strength can be correlated by the following equation with a correlation factor of 0.99: $D_p = 13.38 \times 10^{-6}/I^{1.92}$ ($I \geq 2.55$ mM). In the case of the experiment carried out at variable pH (run 6), without the presence of buffer solution, we found a diffusivity similar in magnitude to the value determined for the run with lower ionic strength (run 1). However, it should be mentioned that the ultimate solution concentration of tyrosine in run 6, which is obtained for $t \to \infty$, is lower than those in run 1. This is so because the equilibrium conditions concerning the run 6 enable higher adsorption uptake of the amino acid.

5. Conclusions

This work was mainly devoted to the study of the sorption of phenylalanine and tyrosine by a strong anion-exchange resin converted to the hydroxide form and in the presence of a buffer whose concentration determines the ionic strength of the solution.

A modified Langmuir equation that considers the effect of the ionic strength is able to predict well the equilibrium uptake of amino acids by the anion-exchange resin. The results show that a change in that variable has a significant effect on the amount of amino acid taken up by the resin. For example, at high buffer concentrations, the ionic strength is high, and the resin takes up a much lower amount of the amino acid present in solution. This probably happens due to the binding of buffer ions to the amino acid resulting in lesser solute ions that effectively participate in the exchange reaction with the resin.

Regarding the kinetics, it was shown that this process is affected by the solution ionic strength changes. Pore-diffusion model was useful for describing the intraparticle mass transfer of amino acid anions through the resin and allowed a good representation of the experimental results. Intraparticle diffusivities were obtained for each run from the fitting of the model solution to the experimental data. It was found that at high ionic strength the diffusivity value is low, thus suggesting a decrease in the mobility of the solute in the resin.

The information obtained is useful for the design of cyclic fixed bed operations, such as the parametric pumping for amino acid purification/recovery.

6. Nomenclature

a_0	isotherm parameter
a_P	$(3/R_P)$interfacial area per unit volume
b_0	isotherm parameter
B_{im}	(ι_d/ι_f) mass Biot number
C	concentration (mmol/dm^3)
D_P	intraparticle diffusion coefficient (cm^2/s)
I	ionic strength (mmol/dm^3)
k_f	film mass transfer coefficient (cm/s)
k_1, k_2	dissociation equilibrium constants (mol/dm^3)
Phe	phenylalanine
q	resin equilibrium uptake (mmol/g wet resin)
q_0	resin ion-exchange capacity (mmol/g wet resin)
R_P	particle radius (cm)
t	time (s)
Tyr	tyrosine
u	normalised radial coordinate
V	volume (dm^3)
x	normalised solute concentration
y	normalised gel phase solute concentration
z_a	isotherm parameter
z_b	isotherm parameter

Greek symbols

ε	bed void fraction
ε_p	particle macroporosity
θ	normalized time coordinate
ρ_p	wet resin density (g wet resin/cm^3 of particle)
ι_d	$(R_P{}^2/D_{P)}$ diffusion time constant
ι_f	$(V_P/(V_S k_f$ ap)) film time constant

Subscripts

0	initial conditions
P	particle
A	amino acid
sol	solution
res	resin

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References

[1] Y. Xie, C.A. Farrenburg, C.Y. Chin, S. Mun, N.-H.L. Wang, AIChE J. 49 (2003) 2850.
[2] S.M. Cramer, V. Natarajan, in: M.C. Flickinger, S.W. Drew (Eds.), Encyclopedia of Bioprocess Technology, John Wiley & Sons, Inc., 1999, p. 612.
[3] D.-J. Wu, Y.Z. Ma, N.-H.L. Wang, Ind. Eng. Chem. Res. 37 (1998) 4023.
[4] Z.M. Ahmed, Ph.D. Thesis, New Jersey Institute of Technology, 1981.
[5] H.T. Chen, Y.W. Wong, S. Wu, AIChE J. 26 (1980) 839.
[6] M.S. Saunders, J.B. Vierow, G. Carta, AIChE J. 35 (1989) 53.
[7] I.L. Jones, G. Carta, Ind. Eng. Chem. Res. 32 (1993) 107.
[8] I.L. Jones, G. Carta, Ind. Eng. Chem. Res. 32 (1993) 117.
[9] M. Agosto, L. Wang, P. Wankat, Ind. Eng. Chem. Res. 32 (1993) 2058.

[10] S. Melis, J. Markos, G. Cao, M. Morbidelli, Ind. Eng. Chem. Res. 35 (1996) 3629.
[11] G. Simon, G. Grevillot, L. Hanák, T. Szánya, G. Marton, Chem. Eng. Sci. 52 (1997) 467.
[12] A. Zammouri, S. Chanel, L. Muhr, G. Greviollot, Ind. Eng. Chem. Res. 39 (2000) 1397.
[13] F.D. Antia, C. Horváth, J. Chromatogr. 484 (1989) 1.
[14] R.D. Whitley, J.A. Berninger, N. Rouhana, L. Wang, Biotechnol. Prog. 7 (1991) 544.
[15] M.J. Moreira, L.M.G.A. Ferreira, Chem. Eng. Sci. (2004) submitted for publication.
[16] S. Yamamoto, K. Nakanishi, R. Matsuno, Ion Exchange Chromatography of Proteins, Marcel Dekker, Inc, New York, 1988.
[17] B.A. Finlayson, Non-Linear Analysis in Chemical Engineering, McGraw-Hill, New York, 1980.
[18] L.R. Petzold, A Description of DASSL: a Differential/Algebraic System Solver, Sandia Tech. Re 82-8637. 1982.
[19] W.E. Stewart, M. Caracotsios, J.P. Sørensen, Software Documentation of GREG-General Regression Software Package for NonLinear Parameter Estimation, University of Wisconsin-Madison, Madison.WI, 1993.