# Thermodynamic Modeling of Multicomponent Ion-Exchange Equilibria of Amino Acids

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*A thermodynamic model was developed to describe phase equilibria for aqueous amino acid/polyelectrolyte gel systems. In the gel phase, activities of all exchangeable species (charged and neutral components) are calculated with the generalized Flory-Huggins model. In the surrounding solution, a numerical procedure is used to allow for partial dissociation phenomena combined with a modified UNIFAC model associated with the Pitzer-Debye-Hückel term. The osmotic pressure difference between the two phases is also taken into account in the equations. The model enables the prediction of resin-phase composition, gel swelling and intraparticle pH. It can be used for various biochemicals in a wide range of concentrations. Its validity was successfully tested using binary and multicomponent exchange equilibria data of several amino acids (phenylalanine, alanine, proline, and glutamate) and HCl on a strong-acid cation-exchange resin (Amberjet 1200H).*

## Introduction

Ion-exchange resins are nowadays commonly used in the recovery and purification on a large scale of carboxylic acids, amino acids, and peptides. Therefore, the optimal design of fixed-bed exchangers that may operate over wide range of concentrations is necessary and this requires, among other things, the accurate prediction of the multicomponent ion-exchange equilibria between the biochemicals and the charged resin.

The modeling of ion-exchange uptakes for weak acids has been recently revisited by Jansen et al. (1996), who proposed an interesting approach that departs significantly from the conventional models developed in the last decade. These latter still remain of widespread use (Carta and Dinerman, 1994; Melis et al., 1996a,b; van der Wielen et al., 1996), but their description of ion-exchange processes will be definitely restricted and limited by the basic hypotheses of their framework. Indeed, the main principle of the exchange in these conventional models is the displacement of an adsorbed solute followed by the stoichiometric adsorption of the displacing molecule in such a way as to maintain the electroneutrality of the resin phase (Saunders et al., 1989; Jones and Carta, 1993; Dye et al., 1990; Melis et al., 1996a,b; Myers and Byington, 1986). Therefore, except for a few investigations (Helfferich, 1990; Yu et al., 1987; Wang et al., 1989), no mechanism has been clearly proposed to account for a possible sorption of neutral or zwitterionic species. Furthermore, ions are supposed to form well-defined compounds with a polyelectrolyte, which is considered as a solid solution from which co-ions are completely excluded (Vink, 1985). As a result, key operating variables like, for instance, intraparticle pH remain difficult and even impossible to define.

To tackle such problems and to get a greater insight into the phenomena involved in the ion-exchange process, Jansen et al. (1996) proposed a rigorous model based on thermodynamics and, more precisely, Donnan equilibria. The gel is here considered as a homogeneous phase, that is, a bulk fluid phase encaged in an elastic structure that behaves like a semi-permeable membrane allowing the partitioning of only selected compounds (neutral, zwitterionic, and positively or negatively charged species) and leading to Donnan equilibria

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(Donnan, 1925; Maurer and Prausnitz, 1996). This new framework is of greater interest compared to the conventional models, and it has been proven by Jansen et al. (1996) to give a convenient description of ion exchange of both strong and weak electrolytes over a wide range of concentration and pH.

Nevertheless, this model still fails to predict the network elastic properties that play crucial roles in many processes (Maurer and Prausnitz, 1996). Furthermore, since an ideal behavior is still assumed for both coexisting phases, the gel and the surrounding aqueous phase, the modeling may lead to erroneous conclusions or results for liquid-phase equilibria calculations (Achard et al., 1994), as well as for the prediction of intraparticle properties, such as pH or other component activity (Sassi et al., 1996).

The objective of the work reported here is to extend and develop the model of Jansen et al. (1996) to actual electrolyte systems and to account for the elastic properties of the network structure. The activity of each component in the liquid phase has been evaluated for the short-range interactions by using the modified Universal Functional Activity Coefficient (UNIFAC) group-contribution model proposed by Larsen et al. (1987) and completed with the new groups introduced by Kuramochi et al. (1997), which are best suited to represent the activity coefficients of biochemicals like amino acids. In the case of electrolytes, the extended form of the Debye-Hückel equation proposed by Pitzer (1980) was added to the UNIFAC equation to take the long-range electrostatic interactions into account. A procedure developed by Achard et al. (1994) was also implemented with the above formalism to predict the real concentrations of the electrolytes involved in partial dissociation equilibria. The behavior of the polymeric phase has been described in the framework of the extended Flory-Huggins model used by Mazzoti et al. (1996, 1997) and Maurer and Prausnitz (1996). Finally, the model is tested using binary and multicomponent exchange equilibria data of several amino acids (phenylalanine, alanine, proline, and glutamate) on a strong-acid cation-exchange resin.

## Materials and Experimental Methods

### *Materials*

Amberjet 1200H is a commercial strong-acid, gel type resin purchased from Rohm and Haas. The resin matrix is composed of polystyrene cross-linked with 8% divinylbenzene and is functionalized with sulfonic groups ($SO_3^-$). It is supplied in its hydrogen form, and its average particle size is $650 \pm 50$ $\mu$m. The resin density ($\rho_r$) is equal to 1,520 kg·m$^{-3}$. Its ion-exchange capacity and water content were determined experimentally as explained below.

The amino acids studied in this work were L-phenylalanine (L-Phe), L-alanine (L-Ala), L-proline (L-Pro), and L-glutamic acid (L-Glu). They were purchased from Sigma Chemical Co. (St. Louis, MO). They are given in Table 1 along with some of their relevant properties. Their purity was greater than 99%, and they were used without further purification. Other chemicals (NaOH Normapur and HCl Normapur) were purchased from Prolabo (Paris, France). U.H.Q. water was purified by a Milli-Q Reagent Water System unit (Millipore, Bedford, MA).

**Table 1. Amino Acid Properties in Solution at 25°C*: $pK_i = -\log_{10} K_i^{id}(c)$**

| Amino Acid | $pK_1$ | $pK_2$ | $pK_3$ | pI |
|---|---|---|---|---|
| L-Glu | 2.19 | 4.25 | 9.67 | 3.22 |
| L-Phe | 2.11 | 9.13 | | 5.62 |
| L-Ala | 2.34 | 9.69 | | 6.02 |
| L-Pro | 2.0** | 10.6** | | 6.3** |

*Values from Dye et al. (1990) unless indicated.
**CRC Handbook of Chemistry and Physics, 1978.

### *Resin characterization and uptake equilibrium measurements*

The total ion-exchange capacity of the resin ($Q_x$, mol/g of dry resin) was determined by equilibrating a sample of the resin (3–5 g) in the hydrogen form with an excess volume of a 0.1 mol·l$^{-1}$ solution of NaOH. When equilibrium is reached, the excess sodium hydroxide left in the solution is measured by titration with a 0.1 mol·l$^{-1}$ HCl solution, and the total capacity is calculated from a material balance (Dye et al., 1990). The resin capacity was found to be 0.00486 mol/g of dry resin.

The dry weight fraction ($\omega$) of the hydrated resin in the hydrogen form was obtained from the weight loss of a hydrated sample upon drying it in an oven at 110°C. Prior to drying, the resin sample was vacuum filtered in a Büchner funnel to remove interstitial water (Dye et al., 1990). The value of 0.529 g of dry resin/g of hydrated resin was obtained, which gives a water content of 0.0493 mol/g of dry resin.

The equilibrium uptake of amino acids was determined as follows. Varying amounts of known weights (1–2 g) of the hydrated resin in the hydrogen form were placed in sealed Erlenmeyer flasks in contact with solutions (100 mL) containing known initial concentrations of amino acids and chloride ions, these latter being introduced in the form of HCl solutions. The flasks were placed in a temperature-controlled shaker (25°C) for 30 h, which conditions were proved experimentally (see Dye et al., 1990) to be sufficient to reach equilibrium. After this period, the solutions in the flasks were sampled to determine the equilibrium amino acid concentrations and pH. The equilibrium amino acid concentration in the resin phase $Q_A$ (mmol/g of dry resin) was finally obtained from the following mass balance equation (Melis et al., 1996a; Dye et al., 1990)

$$Q_A = \frac{V \cdot (C_A^o - C_A)}{M \cdot \omega} \tag{1}$$

where $V$ (mL) stands for the volume of the liquid phase, $M$ (grams of hydrated resin) the mass of the hydrated resin sample initially introduced in the flasks, $C_A$ (mol·l$^{-1}$) the equilibrium total concentration of the particular amino acid, and $C_A^o$ (mol·l$^{-1}$) the initial total concentration of the amino acid.

Total concentrations of amino acids in the aqueous phase (including all ionic forms) were determined with an Amino-Quant 1090 high-pressure liquid chromatograph (Hewlett-Packard) after derivation by *ortho*-phthalaldehyde in the presence of 3-mercaptopropionic acid. Separation is performed with a $C_{18}$ column, and the spectrophotometric de-

tection is at 338 nm. Finally, the solution pH was determined with a Tacussel apparatus (Model PHN81) and a Tacussel electrode (TC200).

### *Numerical tools*

Aqueous phase equilibria were calculated with a simulation program written in FORTRAN (WATCOM FORTRAN 77 by WATCOM International Corporation). All computations were made using the PROPHY software from PROSIM S.A. (Toulouse, France). PROPHY software is dedicated to the computation of physical properties and thermodynamic equilibria. It includes a data bank of components, but provides no value for interaction parameters. PROPHY software is associated with the process simulator PROSIM software.

The multicomponent phase equilibria calculations were performed with Mathcad PLUS 6.0 Professional version (MathSoft), combined with a Numerical Recipes Function Pack. This routine uses the Newton-Raphson method to find the zero of a set of nonlinear equations.

## Model Development

The objective of the model is to describe the thermodynamic equilibrium distribution of amino acids and salts between a liquid phase and an ion-exchange resin, considered here as an homogeneous phase. As we will see in a next section, most of the complex problems relative to the behavior of a polyelectrolyte were simplified to avoid an increased description of experiments (Sassi et al., 1996). Finally, as will be discussed later, all the components under study (solvent, amphoteric species, and salts), if not size-excluded, may freely partition between the surrounding bulk phase and the gel phase.

When equilibrium is obtained between the two phases, we measure the liquid-phase pH and $C_A$ ($\text{mol}\cdot\text{l}^{-1}$), the total equilibrium concentration in the liquid phase of the amino acid under study. With these two experimental values, we can calculate the concentrations and activities of all ionic and uncharged species in the bulk. These latter values are then used in conjunction with the conditions of multicomponent phase equilibria to finally obtain the resin-phase concentrations of all exchangeable species.

### *Description of the liquid phase*

*Activity Coefficients Model.* The investigation of electrolyte solutions has been a growing area of study in the last two decades, and this interest has led to the development of reliable methods for phase equilibrium calculations (Renon, 1986, 1996; Maurer, 1983). However, these models were essentially restricted to salts/water systems, and, since the pioneering work of Kirkwood (1934, 1939), attention has only recently been given to the modeling of phase behavior of actual electrolyte systems containing biochemicals like amino acids. Nass (1988) first proposed an activity coefficient model based on the Wilson equation and applied it to the description of the aqueous solubility of three amino acids. Gupta and Heidemann (1990) correlated UNIFAC group interaction contributions from experimental activity data of amino acids in the aqueous solution and presented solubility models which have the advantage of being predictive. However, as for the work published by Nass (1988), the calculations were performed at the isoelectric point of the amino acids, so that no long-range contributions to the activity coefficient model were considered. To complete these approaches, Perez and Macedo (1994) and Pinho et al. (1994) developed models (UNIFAC or UNIQUAC) combined with a Debye-Hückel term to account for the electrostatic interactions. However, when used as predictive tools, all these models suffer from a lack of precision and may give incorrect estimations of activity coefficients. Recently, Kuramochi et al. (1996, 1997) presented a more accurate model, based on Larsen's UNIFAC model with new assignments for the UNIFAC groups and combined with the Pitzer-Debye-Hückel term to take the long-range electrostatic interactions into account. As this work resulted in good representations of the behaviors of various amino acids/salts/water systems, we decided to use their approach to describe the aqueous-phase systems for our study.

A brief presentation of the activity coefficient model is now proposed and, for a complete overview of its different parts, see Kuramochi et al. (1997) and Achard et al. (1994). To estimate the activity coefficients, one uses the excess Gibbs energy $G^E$ defined by

$$G^E = G - G^{id} \tag{2}$$

where $G$ is the Gibbs energy of the real solution at $T$ and $P$, containing all the components under study, and $G^{id}$ is the Gibbs energy of the ideal mixture, at the same $T$ and $P$ and with the same composition as in the real mixture. Then, from Eq. 2 it can easily be shown that (see, for instance, Zerres and Prausnitz, 1994)

$$RT \ln \gamma_i = \left( \frac{\partial G^E}{\partial n_i} \right)_{T, P, n_{j \neq i}} \tag{3}$$

where $\gamma_i$ is the activity coefficient of component $i$ and $n_i$, its mole number.

The excess Gibbs energy is assumed to result from two terms, one resulting from short-range ($S$-$R$) interactions and the second from long-range ($L$-$R$) electrostatic ion-ion interactions

$$G^E = G^E_{S\text{-}R} + G^E_{L\text{-}R} \tag{4}$$

and similarly for activity coefficients

$$\ln \gamma_i^* = \ln \gamma_i^{*,\,S\text{-}R} + \ln \gamma_i^{*,\,L\text{-}R} \tag{5}$$

where the asterisk ($*$) refers to the unsymmetric convention (infinite dilution basis). For neutral species (the zwitterion for instance), this equation reduces to

$$\ln \gamma_i^* = \ln \gamma_i^{*,\,S\text{-}R} \tag{6}$$

The selected model to account for the short-range contribution in Eq. 5 is the modified form of the UNIFAC equation proposed by Larsen et al. (1987). For a detailed definition of its structure, see the Appendix. This model gives symmetric activity coefficients in the mole fraction scale that have to be

normalized to the standard state for the ions. This normalization is achieved by

$$\ln \gamma_i^{*,S\text{-}R} = \ln \gamma_i^{S\text{-}R} - \ln \gamma_i^{\infty,S\text{-}R} \tag{7}$$

where $\gamma_i^{S\text{-}R}$ is the activity coefficient according to the UNIFAC model (mole fraction scale and symmetric convention), and $\gamma_i^{\infty,S\text{-}R}$ is the infinite dilution UNIFAC activity coefficient of $i$, based on the symmetric convention. We used this model with the new group assignments proposed by Kuramochi et al. (1996, 1997), and which allow a correct extension of Larsen's UNIFAC model to biomolecules such as amino acids, peptides, or sucrose. Amino acids can be here divided into several main groups (see Table 2): $\alpha$-CH, $NH_2$, COOH, $CH_2NH$, $\Phi$ (the aromatic residue) and sc-$CH_2$ (side chain-$CH_2$). Furthermore, it is assumed that all UNIFAC groups which constitute the anionic and cationic species are the same as those of the zwitterion (Pinho et al., 1994; Kuramochi et al., 1997). The inorganic ion ($Cl^-$) was also used as another UNIFAC group taken from the work of Kuramochi et al. (1997). See the Appendix for a listing of the main numerical parameters used for the calculations.

Finally, the hydrogen and hydroxide ions $H^+$ and $OH^-$ were also introduced as UNIFAC groups with their structural parameter and their group interaction energies (with water or other cations or anions) as they were defined and calculated by Achard et al. (1994).

For a quantitative description of the long-range electrostatic contribution in the activity model, the extended form of the Debye-Hückel equation proposed by Pitzer (1973, 1980) was used. This formulation is generally presented as the Pitzer-Debye-Hückel (PDH) equation

$$\ln \gamma_i^{*,L\text{-}R} = -\sqrt{\frac{1{,}000.0}{M_s}} \cdot A_\Phi \cdot \left[ \left( \frac{2 \cdot z_i^2}{\rho} \right) \cdot \ln\left(1 + \rho \cdot \sqrt{I_x}\right) + \frac{z_i^2 \cdot \sqrt{I_x} - 2 \cdot I_x^{3/2}}{1 + \rho \cdot \sqrt{I_x}} \right] \tag{8}$$

where $M_s$ ($g \cdot mol^{-1}$) stands for the molecular mass of the solvent which is water in our case. $\rho$ is the closest approach parameter, which has, for the sake of simplicity, a fixed value for all ionic species. Pitzer (1980) and Chen et al. (1982) reported a value of 14.9 to be satisfactory. $I_x$ ($= 0.5 \sum_i z_i^2 \cdot x_i$) is the ionic strength on a mole fraction basis, and $z_i$ represents the charge of ion $i$. $A_\phi$ is the usual Debye-Hückel parameter and can be calculated by the expression used by Achard et al. (1994), or the correlation proposed by Chen et al. (1982), which gives its temperature dependence. Note that Eq. 8 refers to a specific standard state for ion $i$, which is a hypothetical ideal dilute solution in water when its mole fraction equals one, that is, $x_{ion} = 1$.

**Table 2. Relevant Structure and Group Decomposition of the Amino Acids Considered in this Study**

| Amino Acid | Structure | Groups |
|---|---|---|
| L-Ala | $CH_2(NH_2)COOH$ | $\alpha$-$CH_2$*, $NH_2$, COOH |
| L-Phe | $CH(CH_2)\Phi(NH_2)COOH$ | $\alpha$-CH*, sc-$CH_2$*, $NH_2$, $\Phi$**, COOH |
| L-Glu | $CH(CH_2)_2(NH_2)(COOH)_2$ | $\alpha$-CH*, 2×sc-$CH_2$*, $NH_2$, 2×COOH |
| L-Pro | $CH(CH_2)_3(NH)COOH$ | $\alpha$-CH*, 2×sc-$CH_2$*, $CH_2NH$*, COOH |

*New groups introduced by Kuramochi et al. (1996, 1997).
**Aromatic residue.

*Dissociation Equilibria of Amino Acids.* Amino acids, generally represented as $NH_2CH\,RCOOH$, are amphoteric molecules which, therefore, take several ionic forms when dissolved in water. They may be classified into three main groups according to the chemical nature of their side chain group, designated $R$ in the above formula. Neutral amino acids are those with an uncharged $R$ group, acidic are those with a negatively charged $R$ group at physiological pH, and basic are those with a positively charged $R$ group at physiological pH.

In the following, we will develop the case of a single amino acid with no ionizable $R$ group. This will simplify the reasoning even if the extension to more complex systems does not raise a specific problem.

In aqueous solution, the following two acid-base dissociation equilibria take place simultaneously for an amino acid [for a complete description of the reaction scheme for acidic and basic amino acids, see Melis et al. (1996b)]

$$NH_3^+ RCOOH \Leftrightarrow NH_3^+ RCOO^- + H^+ \tag{9}$$

$$NH_3^+ RCOO^- \Leftrightarrow NH_2 RCOO^- + H^+ \tag{10}$$

All the species involved in these reactions appear to be electrically charged, except for the neutral zwitterion ($NH_3^+ RCOO^-$). Each of the above equilibria may be characterized by a thermodynamic equilibrium constant ($K_a$) defined as the ratio of activities and independent of the composition of the mixture, when $T$ and $P$ are fixed. However, as quoted by Achard et al. (1994), no such constants are available in the literature. Indeed, the conventional constants one can find in every handbook refer to concentration ratios determined experimentally near *infinite dilution* for a solution assumed to be *ideal* (Achard et al., 1994). In our case, these concentration based equilibrium constants, $K_{a1}^{id}(c)$ and $K_{a2}^{id}(c)$, respectively, for reactions 9 and 10, are written as follows, to conform to Achard's notation

$$K_{a1}^{id}(c) = \frac{C_{H^+} \cdot C_{A^\pm}}{C_{A^+}} \tag{11}$$

$$K_{a2}^{id}(c) = \frac{C_{H^+} \cdot C_{A^-}}{C_{A^\pm}} \tag{12}$$

where $C_{H^+}$ stands for the concentration of $H^+$, and $C_{A^+}$, $C_{A^-}$ and $C_{A^\pm}$ represent the concentrations of the different ionic forms of the concerned amino acid.

To estimate now the concentration of the different ionic forms present in solution, the classical procedure is to as-

**Table 3. Expression of Ionization Constants $K_i(c)$ as a Function of $K_i^{id}(c)$**

$$K_{a1}(c) = \frac{\nu_w^o}{\nu_m^L} \cdot \frac{\gamma_{A^+}^*}{\gamma_{H^+}^* \cdot \gamma_{A^\pm}^*} \cdot K_{a1}^{id}(c)$$

$$K_{a2}(c) = \frac{\nu_w^o}{\nu_m^L} \cdot \frac{\gamma_{A^\pm}^*}{\gamma_{H^+}^* \cdot \gamma_{A^-}^*} \cdot K_{a2}^{id}(c)$$

$$K_w(c) = \left(\frac{\nu_w^o}{\nu_m^L}\right)^2 \cdot \frac{a_w}{\gamma_{H^+}^* \cdot \gamma_{OH^-}^*} \cdot K_w^{id}(c)$$

sume an *ideal behavior for the liquid phase* (Saunders et al., 1989; Dye et al., 1990; Melis et al., 1996b; Jansen et al., 1996). The problem is that neglecting the effects of solution nonidealities may lead to an estimation of ionic concentrations that differs widely from the actual situation, and, finally, large discrepancies may be observed between the ideal concentration ratios $K_i^{id}(c)$ and the actual or *true* ones $K_i(c)$, which should not be mistaken with the thermodynamic constant $K_{\text{the}}$ of the equilibrium, which is only dependent on temperature (Achard et al., 1994). To allow for such nonidealities and to perform accurate calculations, one has to consider and use the framework originally developed by Achard et al. (1994), which defines the actual concentration ratios $K_i(c)$ that have first to be calculated to give the liquid-phase composition. In Table 3 are listed the expressions from Achard et al. (1994) for $K_i(c)$ corresponding to the acid-base dissociation equilibria (Eqs. 9–10), and the *true* product dissociation of water $K_w(c)$. The expressions given in Table 3 enable one to recalculate $K_i(c)$ as a function of $K_i^{id}(c)$, the activity coefficients in the unsymmetric convention $\gamma_i^*$, and the molar volumes of either the solution ($\nu_m^L$) or the pure liquid water ($\nu_w^o$). Finally, water activity, $a_w$ refers to a pure liquid standard state at system temperature and pressure.

With these new definitions, the calculation of the actual concentrations in the mixture is now possible. This approach corresponds to an iterative process where the initialization has been done by using the ideal solution hypothesis and *two* experimental data, that is, the liquid-phase pH and the total concentration of the amino acid ($C_A$) in the aqueous phase (the iterative scheme is slightly different from the one proposed by Achard et al., 1994).

Six concentrations in the liquid phase are therefore determined (three ionic forms for the amino acid, the hydrogen and hydroxide ions, and the chloride anions), the water concentration being assumed to be constant, 55.56 $\text{mol}\cdot\text{l}^{-1}$.

### *Activity model in the polymer phase*

In this study, the resin is considered as a fluid phase enclosed in an elastic structure, that is, the polyelectrolyte fully dissociated (Maurer and Prausnitz, 1996). This gel phase allows all species (neutral and charged molecules) to penetrate inside its structure, for we have assumed that steric hindrance was not expected for the small-sized solutes that we have used. The gel is expected to swell or to shrink until mechanical equilibrium is achieved and, in the case of a multicomponent mixture, the components are sorbed, however, each are sorbed to a different extent (Mazzoti et al., 1997). All these complex behaviors deserve particulate attention if one wants to describe accurately the partitioning of the liquid components between the polymeric and the liquid phases.

In the literature, it is common practice to evaluate the activity of sorbed species in a polymer phase by using the Flory-Huggins model (Maurer and Prausnitz, 1996; Bisschops et al., 1998), which describes simply and with sufficient accuracy the change in Gibbs energy of the resin upon mixing. Within this framework, the activity, $a_i^P$ of a single component inside the gel phase is given by

$$\ln a_i^P = \ln(1-\upsilon_P) + \upsilon_P + \chi \upsilon_P^2 \tag{13}$$

where $\upsilon_P$ (which equation is given later for the general case of $N$ constituents) is the volume fraction of the polymer ($\upsilon_P = 1-\upsilon_i$) and $\chi$ is a binary interaction parameter between the component $i$ and the gel matrix. The first two terms in the righthand side of Eq. 13 refer to an entropic contribution, and the last term ($\chi \upsilon_P^2$) is predominantly determined by enthalpic contributions, but contains also entropic contributions (Prausnitz, 1994). The generalized version of Eq. 13 for $N$ components is given as follows (Mazzoti et al., 1996, 1997)

$$\ln a_i^P = 1 + \ln \upsilon_i - \sum_{j=1}^{N+1} m_{ij} \cdot \upsilon_j + \sum_{j=1}^{N+1} \chi_{ij} \cdot \upsilon_j - \sum_{j=1}^{N+1} \sum_{k=1}^{j-1} m_{ik} \cdot \upsilon_j \cdot \upsilon_k \cdot \chi_{kj} \tag{14}$$

where $\upsilon_i$ is the volume fraction of the $i$th component, and can be expressed through mole numbers

$$\upsilon_i = \frac{n_i^P \cdot \upsilon_i^o}{\sum_{j=1}^{N} n_j^P \cdot \upsilon_j^o + V_p} \tag{15}$$

where $n_i^P$ is the mole number of the $i$th molecule in the gel phase, $V_p$ is the volume of the dry resin, and $\upsilon_i^o$ is the molar volume of component $i$. $N$ is the number of the species allowed to partition between the gel and the liquid phase, the $(N+1)$th component referring to the polymer, quoted as indice $P$ in the following. $m_{ik}$ is the ratio of the molar volume of component $i$ to that of component $k$ ($m_{ik} = \upsilon_i^o/\upsilon_k^o$), with $m_{iP} = 0$ (Mazzoti et al., 1997). As specified by Mazzoti et al. (1996), the denominator of Eq. 15 represents the total volume of the swollen polymer particle.

Finally, $\chi_{ij}$ is the Flory interaction parameter. This parameter may incorporate short-range interactions, such as hydrogen bonds, between the components, especially the solvent, and the charged matrix (Bisschops et al., 1998). Furthermore, since the matrix we have used is a strong-acid gel type resin, its network will present the same charge density whatever the operating conditions. As a result, electrostatic interactions between components $i$ and the network will be assumed to be lumped into these Flory parameters to give an approximate and, with regard to the scope of this study, a sufficiently correct description of the energetic behavior of the gel phase. Finally, we will consider these parameters as adjustable vari-

ables that will be estimated through a direct fitting procedure of the equilibrium data. Therefore, because of all these simplifying assumptions, one has to keep in mind that Eq. 14 finally represents a vast simplification of the original Flory-Huggins model. Nevertheless, more complex approaches are still possible which detail all the contributions to the activity coefficient of sorbed species (Sassi et al., 1996).

When considering these Flory parameters, two additional relations have to be specified (Mazzoti et al., 1997)

$$\chi_{ij} = \chi_{ji} \cdot m_{ij} \tag{16a}$$

$$\chi_{ii} = 0 \tag{16b}$$

### *Equilibrium relationships between the two phases*

We are now at the core of our model development. In the preceding sections we have presented the way to estimate the relationships between concentrations and activities of all species in the liquid phase, and their corresponding activities in the resin. The final step is to use these models in the expression of the chemical potential $\mu_i$ of the component under consideration in order to obtain the resin-phase composition. For this, the key relationship is given by the condition for phase equilibrium with the equality of chemical potentials

$$\mu_i^P = \mu_i^L \tag{17}$$

where superscript $P$ stands for the gel (polymer) phase and $L$ stands for the liquid phase. As detailed by Maurer and Prausnitz (1996), Eq. 17 is applied only for uncharged components $i$, water, zwitterion ($A^{\pm}$), and neutral salts of type $MX$, that is, HCl and ACl, the positive amino acid combined with the chloride anion (Maurer and Prausnitz, 1996).

In a very general approach, we have specified that all the molecules encountered in the liquid phase are allowed to partition between the two phases. Nevertheless, as a first and reasonable approximation when considering the low pH values measured in our experiments, it was assumed that the hydroxide ions and the negatively charged amino acid will be present to a very small extent in the resin so that they will not be considered in the equilibrium relationships. Therefore, in the following, we will restrict the calculations to the uptake of *five* species, that is, the hydrogen ion, the positive and neutral amino acid, the chloride anion, and water. This hypothesis simplifies, of course, the description of the system, but it should not undermine our scientific approach in so far as it is always possible to reconsider this assumption for systems with higher pH values.

*Chemical Potentials in the Liquid Phase.* For neutral species, the zwitterion and water, the chemical potential at liquid-phase temperature ($T$) and pressure ($P$) will be written as follows

$$\mu_i^L(T,P) = \mu_i^o(T,P) + RT \ln\left(\gamma_i^{S\text{-}R} \cdot x_i\right) \tag{18}$$

where $\mu_i^o(T,P)$ is the standard chemical potential of component $i$. The standard state is that of pure liquid water or pure supercooled fused zwitterion, and the mole fraction-based activity coefficients ($\gamma_i^{S\text{-}R}$) are normalized by $\gamma_i^{S\text{-}R} \to 1$ as $x_i \to 1$.

We consider now the electrically neutral salt $CA$, which dissociates *completely* into *one* cation $C$ and *one* anion $A$. Its chemical potential is related to those of the ions as defined by Zerres and Prausnitz (1994), and can be expressed by

$$\mu_{CA}(T,P) = \mu_{CA}^{o*}(T,P) + RT \ln\left(\gamma_A^* \cdot x_A\right) + RT \ln\left(\gamma_C^* \cdot x_C\right) \tag{19}$$

where $\mu_{CA}^{o*}(T,P)$ $[= \mu_A^{o*}(T,P) + \mu_C^{o*}(T,P)]$ is the standard chemical potential of the salt. The standard state for the anion $\mu_A^{o*}(T,P)$ and the cation $\mu_C^{o*}(T,P)$ is a hypothetical ideal dilute solution in water when $x_A = 1$ or $x_C = 1$, respectively. Activity coefficients $\gamma_i^*$ are unsymmetric activity coefficients in the mole fraction scale as they were defined by Eq. 5. They are normalized by $\gamma_i^* \to 1$ as $x_i \to 0$ and $x_w \to 1$.

*Chemical Potentials in the Gel Phase.* As pointed out by Maurer and Prausnitz (1996), the pressure ($P'$) in the gel phase is different from the one encountered in the surrounding bulk phase ($P$), and because this external pressure ($P$) is held constant, it is preferable to express the chemical potential in the resin at the pressure of the liquid phase. This is done with the following expression [for details concerning on how to obtain this equation, see Maurer and Prausnitz (1996) and Gusler and Cohen (1994)]

$$\mu_i^P(P,T) = \mu_i^o(P,T) + RT \ln a_i^P + RT\eta \cdot v_i^o \cdot \left(\frac{5}{3} \cdot v_P^{1/3} - \frac{7}{6} \cdot v_P\right) \tag{20}$$

where $\mu_i^P(P,T)$ is the chemical potential in the polymer at system temperature ($T$), and external pressure ($P$), and $\mu_i^o(P,T)$ is the standard chemical potential referring to the pure component: pure solvent and uncharged species and pure completely dissociated liquid electrolyte. The electrolyte reference state may be hypothetical, or it may actually exist as a pure fused salt (Chen et al., 1982). $v_i^o$ is the molar volume of component $i$, and $v_P$ is the volume fraction of the polymer. $\eta$ is called the elasticity parameter, and represents the number of moles of active elastic chains of the network per unit volume (Mazzoti et al., 1997).

In Eq. 20 we have to quote the separability of two phenomena in two different terms (Gusler and Cohen, 1994). First, we note the term related to the activity of the neutral component ($RT \ln a_i^P$), which accounts for *entropic* and also *enthalpic* contributions (see Eq. 13) to the chemical potential (Prausnitz, 1994). Secondly, the last term on the righthand side of Eq. 20 accounts for the network *elastic deformation* contribution. Among the several models proposed in the literature to describe these elastic-retractive properties of the resin, we have chosen the expression developed by Gusler and Cohen (1994) and used by Mazzoti et al. (1996, 1997) for resins similar to the one we used in our study.

Equation 20 is valid for neutral molecules such as the zwitterion and water. When considering neutral salts, it is rearranged to give

$$\mu_{CA}^P(P,T) = \mu_{CA}^o(P,T) + RT \ln\left(a_C^P\right) + RT \ln\left(a_A^P\right) + RT\eta \cdot v_{CA}^o \cdot \left(\frac{5}{3} \cdot v_P^{1/3} - \frac{7}{6} \cdot v_P\right) \tag{21}$$

*Phase Equilibria Relationships.* We conclude now this theoretical section. To obtain the five unknown mole numbers in the gel phase ($n^P_{A^\pm}$, $n^P_{A^+}$, $n^P_{Cl^-}$, $n^P_{H^+}$ and $n^P_w$), the system description requires five equations. The first one is given by the electroneutrality condition in the resin, and is expressed as follows

$$n^P_{Cl^-} + Q_x = n^P_{H^+} + n^P_{A^+} \tag{22}$$

where $Q_x$ is the total ion-exchange capacity of the resin, determined in the experimental section ($Q_x$, mol/g of dry resin), and where all mole numbers are expressed per gram of dry resin. The remaining four equations are given by the phase equilibrium conditions for the zwitterion, water, HCl, and ACl.

For water, Eq. 17, combined with the definition of its chemical potential in the liquid and resin phases (Eqs. 18 and 20) gives

$$\ln\left(\frac{a^P_w}{a^L_w}\right) + \eta \cdot v^o_w \cdot \left(\frac{5}{3}\cdot v_P^{1/3} - \frac{7}{6}\cdot v_P\right) = 0 \tag{23}$$

where $a^L_w$ ($= \gamma^{S\text{-}R}_w \cdot x_w$) is the water activity in the liquid phase. Since standard chemical potentials are defined in the same way in both phases, they have cancelled each other out. For the zwitterion we have

$$\ln\left(\frac{a^P_{A^\pm}}{a^L_{A^\pm}}\right) + \eta \cdot v^o_{A^\pm} \cdot \left(\frac{5}{3}\cdot v_P^{1/3} - \frac{7}{6}\cdot v_P\right) = 0 \tag{24}$$

with $a^L_{A^\pm}$ ($= \gamma^{S\text{-}R}_{A^\pm} \cdot x_{A^\pm}$). Phase equilibria conditions for neutral salts are obtained by combining Eqs. 17, 19, and 22.

$$\ln\left(\frac{a^P_{H^+}\cdot a^P_{Cl^-}}{a^{L,*}_{H^\pm}\cdot a^{L,*}_{Cl^-}}\right) + \eta \cdot v^o_{HCl} \cdot \left(\frac{5}{3}\cdot v_P^{1/3} - \frac{7}{6}\cdot v_P\right) + A_{HCl} = 0 \tag{25}$$

$$\ln\left(\frac{a^P_{A^+}\cdot a^P_{Cl^-}}{a^{L,*}_{A^\pm}\cdot a^{L,*}_{Cl^-}}\right) + \eta \cdot v^o_{ACl} \cdot \left(\frac{5}{3}\cdot v_P^{1/3} - \frac{7}{6}\cdot v_P\right) + A_{ACl} = 0 \tag{26}$$

where all $a^{L,*}_i = \gamma^*_i \cdot x_i$ are activities calculated in the unsymmetric convention. The constants $A_{HCl}$ and $A_{ACl}$ are defined as follows

$$A_{HCl} = \frac{\mu^o_{HCl}(T,P) - \mu^{o^*}_{HCl}(T,P)}{RT} \tag{27}$$

$$A_{ACl} = \frac{\mu^o_{ACl}(T,P) - \mu^{o^*}_{ACl}(T,P)}{RT} \tag{28}$$

These two constants $A_{HCl}$ and $A_{ACl}$ are not known, and will have to be determined by data regression.

Equations 23–28, which constitute the fundamental relations of our model, should now describe with sufficient precision the multicomponent equilibria between the two phases, and should enable one to determine the gel-phase composition.

## Results and Discussion

### *Preliminary remarks*

The model we have developed in the above sections presents a new approach to multicomponent ion-exchange equilibria, but requires an excessive number of model variables (the two constants $A_{HCl}$ and $A_{ACl}$, $\eta$, and above all, the binary interaction parameters $\chi_{ij}$, of Eq. 14), which may impede its general use. In order to obtain an easy to handle model, we have first to make several assumptions and to clarify the physical meaning of most of these parameters.

We first consider the solvent-polymer interaction parameter $\chi_{w,P}$. The conventional procedure is to obtain its numerical value by independent measurements for a solution of the *linear* polymer in solution (Kim and Prausnitz, 1994); it can also be estimated from a relation used by Gusler and Cohen (1994). We lack physical data to use this latter method, and in so far as the gel matrix used was a commercial one (purchased from Rohm and Haas), we were unable to experimentally perform the necessary measurements required for the first method. Finally, as a last resort, instead of handling $\chi_{w,P}$ as an adjustable parameter (which raises numerous problems since its influence is not distinguishable from the effects of other variables), we decided to choose for this parameter the reasonable value of 0.2 (Maurer and Prausnitz, 1996). For typical polymer solutions, $\chi_{w,P}$ is in the region 0.2–1.2 (Prausnitz, 1994).

Our approach, although open to criticism, should not jeopardize the general validity of our model. Nevertheless, it has to be said that the choice of this parameter value of 0.2 will mean that the other model variables will be relative to this numerical variable, and, accordingly, will give estimations of species activities in the resin phase closely linked to our choice. With respect to our objectives, this drawback is not important since *we are presenting a general framework that we have tested specifically as applied to the prediction of gel compositions*. However, we must notice that for other applications of the model, where water activity or $H^+$ activity in the resin phase has to be known in a precise way (heterogeneous bioconversions, for example), further experiments should be necessary to obtain $\chi_{w,P}$ [see Gusler and Cohen (1994), who analyzed the swelling equilibrium of a cross-linked polymer network by a pure solvent].

The parameter $\eta$ can be estimated on physical grounds (Mazzoti et al., 1997; Gusler and Cohen, 1994; Kim and Prausnitz, 1994). It is defined for a perfect network as $\eta = \rho_r/M_c$, where $\rho_r$ is the resin density and $M_c$ is the average molecular weight between junctions. Following the same reasoning as that given by Mazzoti et al. (1997), when considering the degree of cross-linking and the concentration of sulfonic acid sites in our resin, our estimation yields $\eta = 600 \cdot 10^{-6}$ mol·cm$^{-3}$. This is an approximate value, and if one wants to obtain a more precise estimation, Kim and Prausnitz (1994) indicate how to calculate $\eta$ for networks prepared by bulk or solution polymerization, but the basic equations they presented require the knowledge of specific parameters that we could not obtain since our resin was a commercial one.

Nevertheless, as for $\chi_{w,P}$, $\eta$ should be evaluated precisely in order to calculate the activity of the components inside the resin. In our case, the approximate value we obtained will be sufficient to test the potential of our model.

Finally, for the sake of simplicity and since chloride ions are supposed to penetrate slightly into the resin, we neglected the $Cl^-$-*polymer* and $Cl^-$-*ion* interactions. We also neglected all *ion-water* interactions and all interactions involving the zwitterion. Thus, there remains only three binary interaction parameters to be determined, $\chi_{H^+,P}$, $\chi_{A^+,P}$, $\chi_{A^+,H^+}$. Because of their relative importance in the gel, we considered that the hydrogen ion and the positive amino acid may interact with the polymer to influence significantly the amino acid uptake. This argument seems reasonable since the same amino acid is known to behave differently with respect to ion-exchange resins with varying degrees of cross-linking (see Figure 11 in Jones and Carta, 1993), and only two adjustable values $\chi_{w,P}$ and $\eta$ cannot explain by themselves these various observed behaviors.

Finally, it must be said that, since the Flory-Huggins model is chosen for the gel phase, it is not common practice, to our knowledge, to consider other binary interaction parameters apart from the one involving the solvent and the polymer (Gusler and Cohen, 1994; Maurer and Prausnitz, 1996; Brannon-Peppas and Peppas, 1991). Nevertheless, our even more complex approach may lead to a more detailed and precise understanding of amino acid equilibrium uptakes (see below).

All these assumptions gave us finally five variables to be determined, that is, three binary interaction parameters and the two constants $A_{HCl}$ and $A_{ACl}$. However, once $\chi_{A^+,H^+}$ and $A_{HCl}$ are obtained, they will be common to all the other experiments that we have done, reducing in this way the number of adjustable variables to three ($\chi_{A^+,P}$, $\chi_{A^+,H^+}$ and $A_{ACl}$), which is acceptable. Note that the most commonly used model for ion-exchange equilibria developed by Myers and Byington (1986) requires also three parameters (Saunders et al., 1989; Dye et al., 1990; Jones and Carta, 1993; van der Wielen et al., 1996; Melis et al., 1996b).

## Results

*Alanine Uptake Equilibrium.* The equilibrium uptake of alanine by the hydrogen form of the resin is shown in Figure 1a. The data were obtained for various concentrations of $Cl^-$. $Q_{Ala}$ is the total uptake of amino acid (including all ionic forms, except the negative one which was assumed to be negligible in the gel), and $C_{Ala}$ is the total equilibrium concentration of alanine in solution. Figure 1a shows a typical behavior for alanine whose uptake approaches, in the high $C_{Ala}$, the total exchange capacity of the resin $Q_x = 0.00486$ mol/g of dry resin. As already explained in the literature, it is worth noting that the uptake of alanine is strongly dependent on the chloride concentration, and, therefore, on the pH (Saunders et al., 1989; Dye et al., 1990; Jones and Carta, 1993; van der Wielen et al., 1996; Melis et al., 1996b). For these experiments, the water content of the resin was measured as explained in the experimental section for the resin in the hydrogen form. These water content values could not be obtained from repeated measurements and may suffer from a lack of accuracy. Nevertheless, since we have numerous values, they provide valuable information and give an average

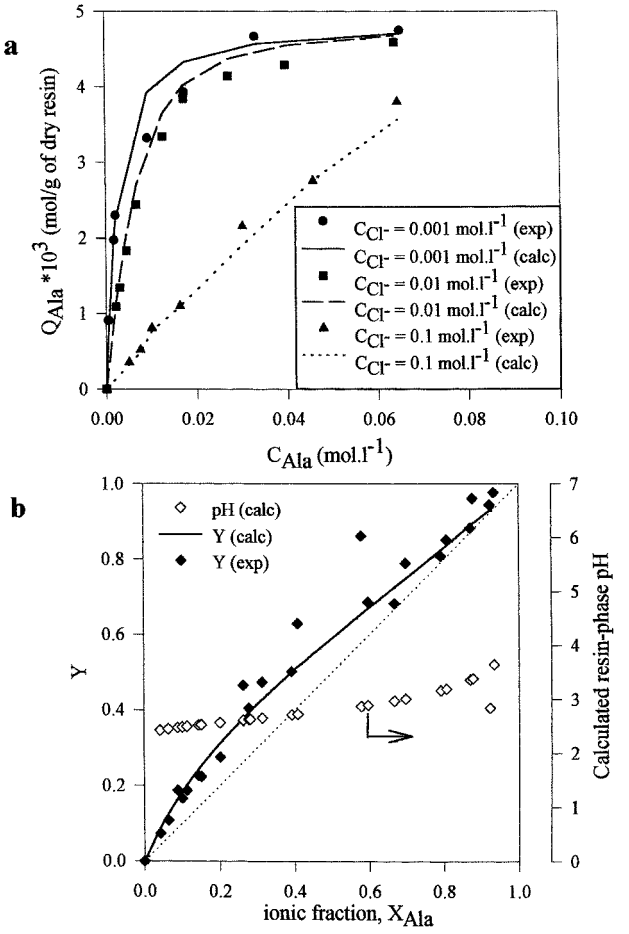


**Figure 1. (a) Equilibrium uptake of alanine; (b) reduced equilibrium uptake of alanine and calculated intraparticle pH.**

value of 0.045 mol/g of dry resin (no increase or decrease in this water content was observed).

The objective function used in the fitting procedure is given by the squared relative deviations of the experimental and calculated values of the resin-phase concentrations $Q_{Ala}$. As shown in Figure 1a for all the chloride concentrations used, our model gives good agreement between experimental and calculated uptakes indicating that it does have some physical significance. Tables 4 and 5 give the parameters of the model obtained by regression.

The uptake data for alanine shown in Figure 1a are replotted in Figure 1b in the form of a X-Y diagram, normally used

**Table 4. Values of the Adjustable Variables for the Activity Model in the Polymer Phase**

| $\chi_{i,j}$ | $j$ | |
|---|---|---|
| $i$ | Polymer | $H^+$ |
| Water | 0.2 | 0 |
| L-Ala | −11.1 | −3.02 |
| L-Glu | −10.5 | −4.2 |
| L-Phe | −9.9 | −15.1 |
| L-Pro | −11 | −16.5 |
| $H^+$ | −7.5 | 0 |

**Table 5. Values of Adjustable Constants $A_{MCl}$**

| $M$ | $A_{M\,Cl}$ |
|---|---|
| L-Ala | 4.15 |
| L-Phe | −0.35 |
| L-Glu | 4.5 |
| L-Pro | 4.05 |
| $H^+$ | 3.7 |

for *conventional* ion-exchange models where Y, the ionic fraction of alanine in the resin ($Y = Q_{Ala}/Q_x$), can be directly related to the *ionic fraction of positively charged* alanine $X_{Ala} = [C_{Ala^+}/(C_{Ala^+} + C_{H^+})]$ in the liquid phase (Myers and Byington, 1986; Saunders et al., 1989). In our calculations, we have shown that the amino acid resin-phase concentration was essentially the positively charged alanine, and even if the zwitterion was allowed to partition, the amount of the latter in the gel was calculated to be very small. The alanine uptake is, therefore, directly related to the amount of positively charged alanine in the liquid phase, and this is the reason why, as can be observed, our model also gives a good agreement for this type of X-Y plot. As can be observed on this X-Y plot, alanine (and especially the other amino acids studied below) does not display a symmetric behavior. To explain such data, conventional models for ion exchange refer to variable selectivity coefficients that can be attributed to inhomogeneities in the structure of the polymer and in the strength of the functional groups (Myers and Byington, 1986; Saunders et al., 1989; Dye et al., 1990; van der Wielen et al., 1996). Part of the originality of our approach is that it proposes another explanation based essentially on the nonidealities in the liquid and in the gel phases, these latter resulting from interactions between different components (water, positively charged amino acids, and hydrogen ions) and the polymer. This is a new way to account for amino acid behavior and, of course, at first sight, it is not possible to decide whether one explanation is better than the other. Nevertheless, our approach offers more fundamental physical information about the system than the conventional one (gel phase activities, swelling ratio, and so on).

Finally, our model enables one to calculate the intraparticle hydrogen ion activity with the Flory-Huggins expression (Eq. 14). This value, just like the other component activities calculated with Eq. 14, may be important, for instance, to model heterogeneous bioconversions. Knowledge of internal pH is also of value when dealing with immobilized enzyme kinetics and stability. Unfortunately, the proton activity obtained cannot be used as such to estimate the internal $pH^{int}$ $[-\log_{10}(a_{H^+})]$ since the conventional definition of pH in electrolyte solutions refers to concentration-based activities and to a reference standard state which is a hypothetical ideal dilute solution in water when $C_{H^+} = 1$ M. As far as we know, conversion from one convention (the symmetric convention of the Flory-Huggins framework) to the other is not straightforward for the hydrogen ion, and may lead to erroneous results. We propose here an approximate solution by considering the following phase equilibrium conditions for HCl, where the term correcting for the osmotic-pressure difference has been omitted (see also Kim and Prausnitz, 1994)

$$\mu^P_{HCl}(P,T) = \mu^L_{HCl}(P,T) \tag{29}$$

Gel-phase and aqueous-phase activities may be expressed in the same reference state

$$\mu^{o,*}_{HCl}(P,T) + RT\ln\left(a^{P,*}_{H}\cdot a^{P,*}_{Cl}\right) = \mu^{o,*}_{HCl}(P,T) + RT\ln\left(a^{L,*}_{H^+}\cdot a^{L,*}_{Cl^-}\right) \tag{30}$$

Since the standard state is the same in both phases, Eq. 30 is identical to

$$a^{P,*}_{H^+}\cdot a^{P,*}_{Cl^-} = a^{L,*}_{H^+}\cdot a^{L,*}_{Cl^-} \tag{31}$$

This last relation can be expressed in term of concentrations

$$C^P_{H^+}\cdot C^P_{Cl^-}\cdot\gamma^{*,P}_{H^+}\cdot\gamma^{*,P}_{Cl^-} = C^L_{H^+}\cdot C^L_{Cl^-}\cdot\gamma^{*,L}_{H^+}\cdot\gamma^{*,L}_{Cl^-} \tag{32}$$

If we assume that the activity coefficients in the gel phase of the chloride and the hydrogen ion $\gamma^{*,P}_{Cl}$ and $\gamma^{*,P}_{H}$, respectively, are equal, we obtain

$$C^P_{H^+}\cdot C^P_{Cl^-}\cdot\left(\gamma^{*,P}_{H^+}\right)^2 = C^L_{H^+}\cdot C^L_{Cl^-}\cdot\gamma^{*,L}_{H^+}\cdot\gamma^{*,L}_{Cl^-} \tag{33}$$

Since we know the gel-phase composition, the total volume of the swollen polymer particle, and the salt activity in the surrounding aqueous solution, Eq. 33 enables one to estimate $\gamma^{*,P}_{H^+}$ and, in the process, to calculate the intraparticle $pH^{int}$ $[-\log_{10}(\gamma^{*,P}_{H^+}\cdot C^P_{H^+})]$. Figure 1b shows the variation of the calculated $pH^{int}$ vs. the alanine ionic fraction in the aqueous phase, and Figure 2 provides for each of the alanine experiments of Figure 1a the predicted pH difference between resin and liquid phases. As can be observed in Figure 1b, $pH^{int}$ is described by a single line and increases with increasing values of $X_{Ala}$, thus denoting the decreasing uptake of hydrogen ions, repelled from the resin by the positively charged alanine. As expected, the intraparticle $pH^{int}$ plotted in Figure 2 is estimated to be different from its respective value in the aqueous phase (Maurer and Prausnitz, 1996; Jansen et al.,

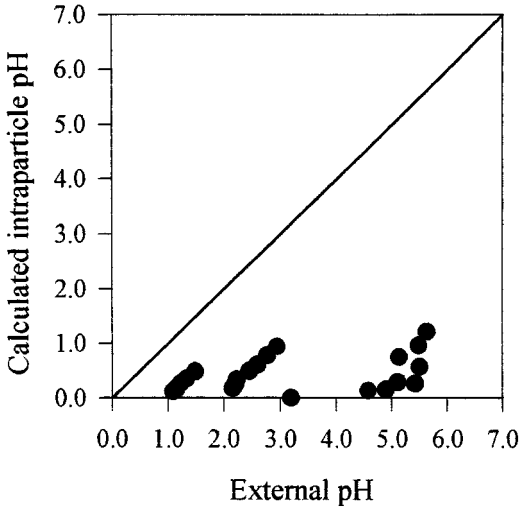


**Figure 2. Calculated intraparticle pH vs. liquid-phase pH for alanine experiments.**

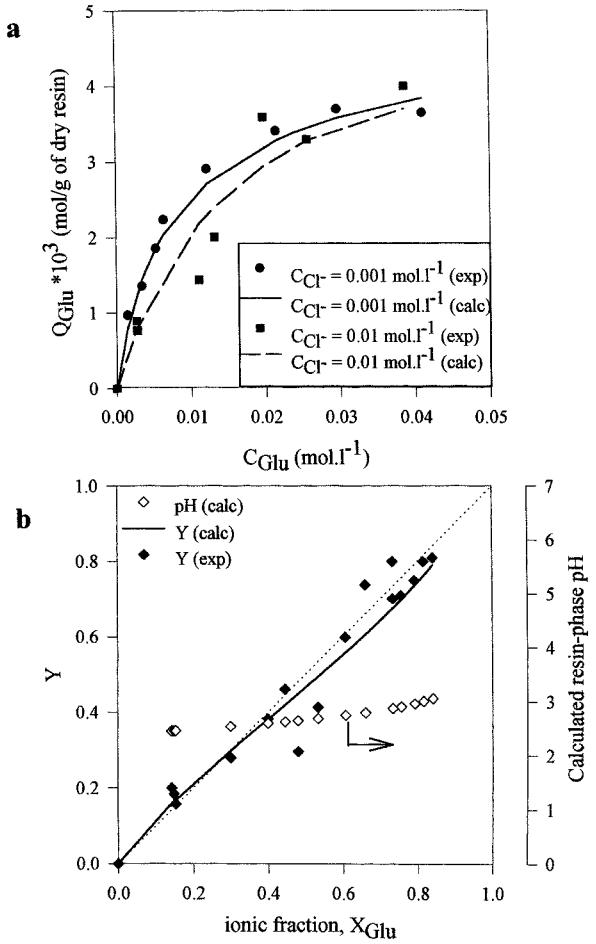


**Figure 3. (a) Equilibrium uptake of glutamate; (b) reduced equilibrium uptake of glutamate and calculated intraparticle pH.**

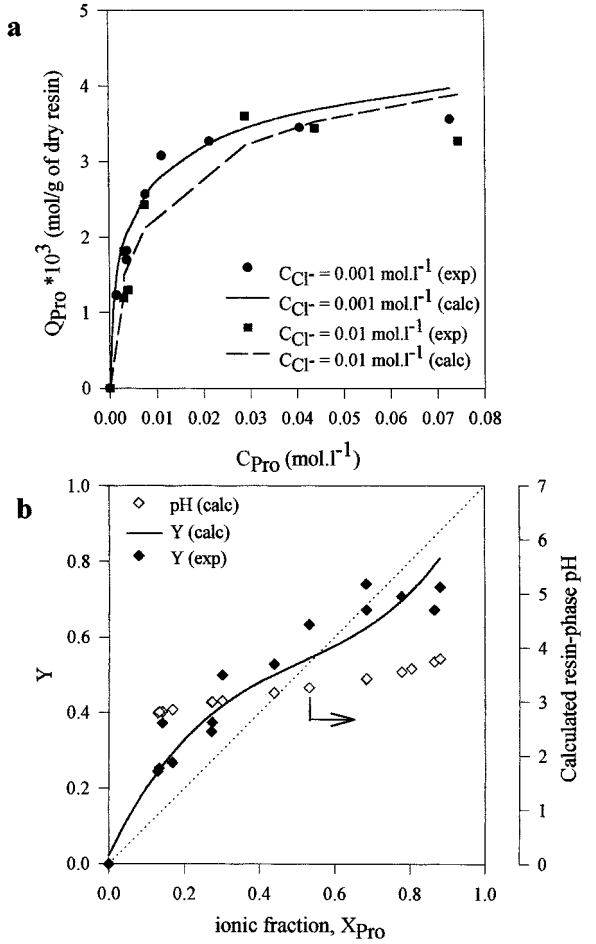


**Figure 4. (a) Equilibrium uptake of proline; (b) reduced equilibrium uptake of proline and calculated intraparticle pH.**

1996). Since the resin is highly functionalized with negative groups ($SO_3^-$), an excess of hydrogen ions in the gel phase is expected, leading in every situation to a higher activity compared to the one calculated outside the resin. Indeed, $pH^{int}$ remains very low, and the pH difference between the two phases is even estimated to be as large as four pH units. This illustrates once more the importance of a correct description of phase equilibria to avoid misleading confusion between calculated activities, that is, pH, in the aqueous and gel phases. Finally, further experimental work will be necessary to validate this theoretical study on intraparticle pH, but, at this time, we think that our approach offers a better estimation than the one proposed by Jansen et al. (1996) who limited their study to ideal systems.

### *Glutamate, proline and phenylalanine uptake equilibria*

Figure 3 shows the results of the experimental and calculated uptakes for glutamate. Since two variables have been determined previously from alanine experiments $\chi_{H^+,P}$ and $A_{HCl}$, the model requires now only three adjustable parameters, that is, $\chi_{A^+,P}$, $\chi_{A^+,H^+}$ and $A_{GluCl}$ (see Table 4). As can be observed in Figures 3a and 3b, our model yields a good fit to these data also. Resin-phase pH was also calculated (see Figure 3b). The measured water content was approximately equal to that observed with alanine experiments (the same is true for proline and phenylalanine experiments).

Results concerning proline are shown in Figure 4. As can be observed (see Figure 4b), this amino acid exhibits a complex behavior that widely differs from the previous ones, especially with an inversion of selectivity at high ionic fractions. Despite this complexity, our model still gives a good agreement between experimental and simulated uptakes, which proves its great flexibility.

Uptake experiments with phenylalanine are shown on Figure 5, and, once again, good agreement is observed between all experiments and calculations.

### *Multicomponent ion-exchange equilibria*

Multicomponent uptake measurements were also carried out for the system Ala/Glu/$H^+$. To demonstrate the predictive capabilities of our model for multicomponent ion-exchange data, we calculated resin-phase compositions using the

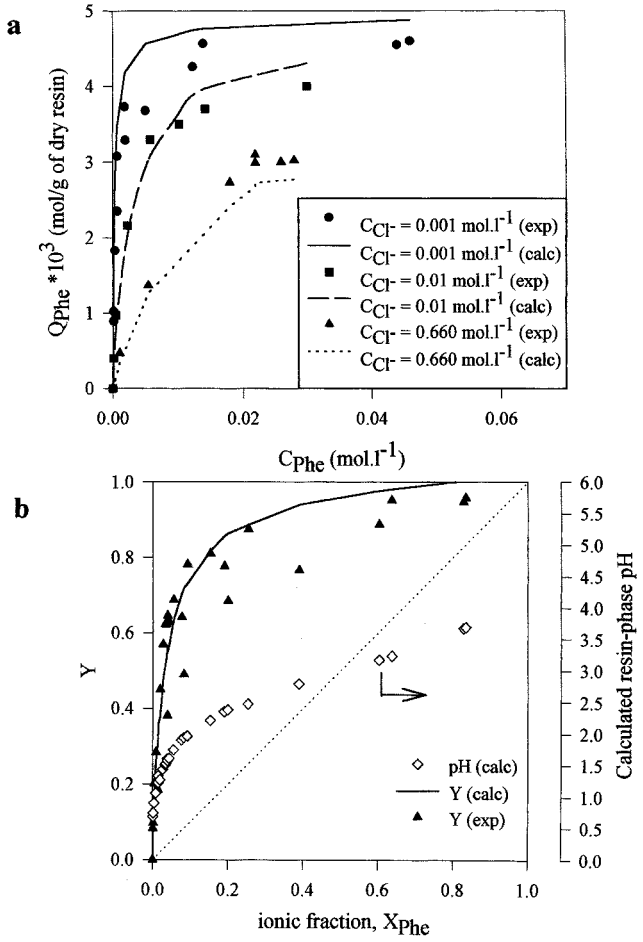


**Figure 5. (a) Equilibrium uptake of phenylalanine; (b) reduced equilibrium uptake of phenylalanine and calculated intraparticle pH.**

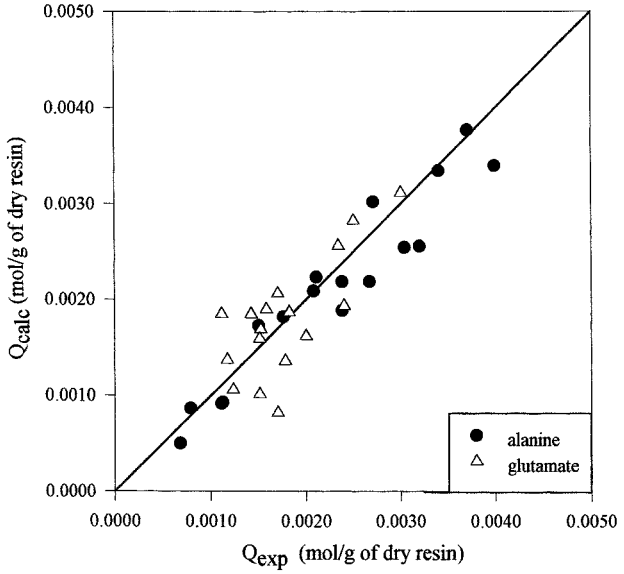


**Figure 6. Comparison between experimental and calculated resin-phase composition for the ternary system Glu/Ala/$H^+$.**

single-solute parameters of Tables 4 and 5. It means that no model variable was adjusted to these ternary data and, as can be observed in Figure 6, the agreement between experimental data and prediction is reasonably good. The average deviation between calculated and experimental values is 15.2%. Therefore, when single-solute data are available, they can be used to predict multicomponent behavior with sufficient accuracy. Similar results can be obtained with conventional ion-exchange models (van der Wielen et al., 1996; Dye et al., 1990), but, as we said previously, our approach proposes a new understanding of the phenomena and enables one to get information about resin-phase activities and, therefore, internal pH.

## Conclusion

The framework of this study provides a thermodynamic procedure for calculating multicomponent ion-exchange equilibria of strong and weak electrolytes on water-swollen polyelectrolyte hydrogels. The approach that we have developed here proposes a new way to understand the complex uptake behaviors of amino acids, and is essentially based on the description of the strong nonidealities encountered both in the liquid and resin phases. In the liquid phase, we have used the UNIFAC model of Kuramochi et al. (1996, 1997) combined with the approach developed by Achard et al. (1994). In the gel phase, we have used the generalized Flory-Huggins expression in which we have included interactions between the polymer and the other components of interest (positively charged amino acid, hydrogen ion, and the solvent). Binary interaction parameters between the positive ionic form of the amino acid and the proton were also included to ensure a precise description of the system.

By introducing some reasonable assumptions, the number of parameters has been reduced to five, that is, three binary interaction parameters in the resin and two other constants. Most of these adjustable parameters can be easily obtained from uptake measurements and, once two of them are determined, which are common to all the other experiments, only three variables remain necessary to the calculation of the multicomponent phase equilibria. Nevertheless, if one wants to go further in the use of the model, and wants to predict, with sufficient accuracy, resin-phase activities of some exchangeable species, one has to obtain accurately from independent measurements the binary interaction parameter between water and the polymer $\chi_{w,P}$.

Our model has been proven to be very flexible and to give very good agreement with experimental amino acid uptakes. It can be used to predict various multicomponent equilibria, and may be easily extended to account for the uptake of other numerous biochemicals. It enables one to estimate intraparticle pH and the swelling of the resin. This latter characteristic was only slightly influenced by the nature of the amino acid in solution and, therefore, further experiments with other different salts will be necessary to define more exactly the capabilities of our model to estimate correct swelling ratio.

The UNIFAC model that we have used for the prediction of liquid-phase activities should enable one to consider very

highly concentrated solutions for it that have been proved to give good estimates of activity coefficients up to 6 $mol \cdot kg^{-1}$ (Kuramochi et al., 1996, 1997). Further works will be performed in our laboratory to ensure the ability of the model to predict correct equilibria for these types of operating conditions.

Finally, we believe that our thermodynamics procedure can be especially designed to simulate numerous biochemical operations using charged resins. Indeed, it may be included in a more general model to simulate ion-exchange preparative chromatographic separations, where it is often observed that resin shrinks or swells to a large extent. It can be also used to simulate heterogeneous bioconversions with immobilized enzymes, since such operations require the knowledge of internal pH and water activity, and must account for the selective partitioning of the involved components.

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**Table A1. UNIFAC Groups and Size Parameters Studied by Kuramochi et al. (1997)**

| Group Number | | | | |
|---|---|---|---|---|
| Main | Subgroup | Name | $R$ | $Q$ |
| 1 | 1 | $H_2O$ | 0.9200 | 1.400 |
| 2 | 2 | $\alpha$-$CH_2$ | 0.6744 | 0.540 |
| 2 | 3 | $\alpha$-CH | 0.4469 | 0.228 |
| 3 | 4 | sc-$CH_2$ | 0.6744 | 0.540 |
| 4 | 5 | $NH_2$ | 0.6948 | 1.150 |
| 5 | 6 | COOH | 1.3013 | 1.224 |
| 6 | 7 | $CH_2NH$ | 1.2070 | 0.936 |
| 7 | 8 | $Cl^-$ | 0.9860 | 0.992 |

**Table A2. Group Interaction Parameters ($a_{nm}$) of the UNIFAC Model (Kuramochi et al., 1997)**

| | $m$ | | | | | | |
|---|---|---|---|---|---|---|---|
| $n$ | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | 0.0 | −1385 | 85.7 | −66.39 | 8.62 | −1572 | −1431 |
| 2 | −401.4 | 0.0 | −167.3 | −960.5 | −573.2 | 2070 | 0.0 |
| 3 | 49.97 | −896.5 | 0.0 | 1554 | 218.6 | −200.6 | 0.0 |
| 4 | −244.5 | −603.4 | 3085 | 0.0 | −489.0 | 2908 | −2393 |
| 5 | 86.44 | 921.8 | 1360 | 867.7 | 0.0 | 1085 | 0.0 |
| 6 | −286.9 | −1923 | 1455 | −867.6 | −648.7 | 0.0 | n.a. |
| 7 | −378.8 | 0.0 | 0.0 | −759.2 | 0.0 | n.a. | 0.0 |

n.a. = not available

## Appendix

*Group-contribution expression for short-range interactions, Larsen et al. (1987)*

For a mixture of $N$ components, the combinatorial contribution to the activity coefficient is given by

$$\ln \gamma_i^C = \ln\left(\frac{\Phi_i}{x_i}\right) + 1 - \frac{\Phi_i}{x_i} \tag{A1}$$

with $\Phi_i$ the molecular volume fraction of component $i$ defined as follows

$$\Phi_i = \frac{x_i r_i^{2/3}}{\sum_j x_i r_j^{2/3}} \tag{A2}$$

$$r_i = \sum_k \upsilon_k^{(i)} R_k \tag{A3}$$

where $\upsilon_k^{(i)}$ is the number of groups $k$ present in molecule $i$, and $R_k$ is the relative van der Waals volume of subgroup $k$

$$R_k = \frac{V_{wk}}{5.17} \tag{A4}$$

with $V_{wk}$ the van der Waals volume ($cm^3 \cdot mol^{-1}$). (See Table A1 for the numerical values used in this work.)

The contact number parameter of subgroup $k$ is defined as

$$Q_k = \frac{A_{wk}}{2.510^9} \tag{A5}$$

with $A_{wk}$ the van der Waals surface area ($cm^2 \cdot mol^{-1}$). (See Table A1 for the numerical values used in this work.)

The expression for the residual activity coefficient is

$$\ln \gamma_i^R = \sum_k \upsilon_k^{(i)} \left(\ln \Gamma_k - \ln \Gamma_k^{(i)}\right) \tag{A6}$$

with

$$\ln \Gamma_k = Q_k \cdot \left[1 - \ln\left(\sum_m \theta_m \psi_{mk}\right) - \sum_m \left(\frac{\phi_m \psi_{km}}{\sum_p \theta_p \psi_{pm}}\right)\right] \tag{A7}$$

$$\theta_m = \frac{Q_m X_m}{\sum_p Q_p X_p} \tag{A8}$$

$$X_m = \frac{\sum_j \upsilon_m^{(j)} x_j}{\sum_j \sum_p \upsilon_p^{(j)} x_j} \tag{A9}$$

$$\psi_{mn} = \exp\left[\frac{-(u_{mn} - u_{nn})}{RT}\right] = \exp\left[\frac{-a_{mn}}{RT}\right] \tag{A10}$$

where $u_{ij}$ is a UNIFAC group contribution energy (K), and $a_{ij}$ a UNIFAC binary group interaction parameter (K). (See Table A2 for the numerical values used in this work.)

$\Gamma_k^{(i)}$ is also calculated with relations A7 to A10.

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