Anion Exchange Equilibria of Penicillin G, Phenylacetic Acid, and 6-Aminopenicillanic Acid versus Cl^- on IRA400 Ion Exchange Resin

Luuk A. M. van der Wielen,* Mark J. A. Lankveld, and Karel Ch. A. M. Luyben

Department of Biochemical Engineering, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

Binary and multicomponent exchange equilibria of the anions of Penicillin G (Pen G), 6-aminopenicillanic acid (6-APA), and phenylacetic acid (PhAc) versus Cl^- on Amberlite IRA400 resins have been measured, using batch experiments in an aqueous system. When transformed into equivalent ionic fractions, the binary exchange data for the anions of 6-APA and PhAc versus Cl^- could be correlated with a constant selectivity model. The binary exchange data of the anion of Pen G versus Cl^- showed a decreasing selectivity with increasing equivalent ionic fraction of the anion of Pen G in the liquid phase, which is common for bulky ions. The measured data are correlated satisfactorily with the Myers and Byington model. Using parameters from correlating these binary data, the Myers and Byington model predicts the multicomponent anion equilibria with an average deviation of 32%.

Introduction

Ion exchange is a working horse in the recovery and purification of biomolecules. However, reliable data for ion exchange equilibria of biomolecules as well as comprehensive thermodynamic frameworks for their interpretation are still relatively scarce. Some progress has been made in the field of carboxylic acids (Jansen *et al.*, 1995), amino acids (Saunders *et al.*, 1989; Dye *et al.*, 1990; Helfferich, 1990), and small peptides (Jones and Carta, 1993). The known approaches are based on the "classical" ion exchange reaction model, which relates the (assumed) difference in Gibbs free energy between two charged solutes in a resin and the solution phase. The difference in Gibbs energy results from differences in ionic charge, molecular interaction, and from geometric effects. The weak electrolyte nature of most biomolecules necessitates incorporation of acid−base dissociation reactions into the formulation of explicit equilibrium relations for each of the ionic forms of a specific solute (Jansen *et al.*, 1995).

Design of conventional and innovative unit operations for bioprocessing is hampered by the limited availability of reliable data (van der Wielen *et al.*, 1995). Therefore, we have focused in this work on binary and multicomponent ion exchange equilibria of relatively bulky biomolecules on strong basic anion exchange resins of the gel type. The prediction of multicomponent equilibria from binary ion exchange isotherms was our goal, and the study is limited to the pH intervals where the various species were predominantly present as monovalent ions.

Ion Exchange Equilibria

The conventional approach to ion exchange involves the identification of a constant change in the Gibbs energy ΔG_{AB} for the reversible ion exchange "reaction" between the liquid and resin (in parentheses) phases

$$A + (B) \overset{\Delta G_{AB}}{\rightleftharpoons} (A) + B \qquad (1)$$

resulting in a constant selectivity S_{AB} of the resin

$$S_{AB} = q_A c_B / q_B c_A; \quad \Delta G_{AB} = RT \ln S_{AB} \qquad (2)$$

where c_i and q_i are measures for the concentrations of species i in the liquid and resin phases, respectively. For the exchange of monovalent ions, the absolute concentrations do not matter but the ionic fractions on the resin and in the bulk of the solution do. The concentrations in eq 2 are replaced by ionic fractions for the liquid (x) and resin (y) phases

$$S_{AB} = \frac{y_A x_B}{y_B x_A} \text{ with } y_i = \frac{q_i}{Q_{max}} \text{ and } x_i = \frac{c_i}{\sum_k z_k c_k} \qquad (3)$$

with Q_{max} being the resin equivalent capacity and z_k the charge of species k. For a constant selectivity, corresponding to a uniform Gibbs energy change for all sites (Myers and Byington, 1986), the following relation is derived for the ion exchange isotherm:

$$y_A = \frac{S_{AB} x_A}{1 + x_A (S_{AB} - 1)} \qquad (4)$$

However, experimental deviations from this constant selectivity relation are observed as a rule, not as an exception (Jones and Carta, 1993). In general, for ion exchange of metal ions (Myers and Byington, 1986), amino acids (Saunders *et al.*, 1989; Dye *et al*, 1990), and peptides (Jones and Carta, 1993) on gel-type cation exchange resins, a selectivity decline or even a selectivity reversal is observed. This indicates that the functional sites throughout the resin are not uniform. Accepted reasons are the polymerization conditions during the manufacture of the resin or a variation in the acidic strength of the functional groups during the sulfonation or amidation process of the resin. It lasted until the recent works by Novosad and Myers (1982) and Myers and Byington (1986) in which a more rigorous and general thermodynamic framework for ion exchange equilibria was developed on the basis of the identification of the surface excess properties of the resin and an assumed continuous or discrete distribution of functional groups. One of the most attractive features of the Myers and Byington model is the prediction of selectivity in multicomponent mixtures using binary data only. By keeping one of the components of the binary system constant (the reference component), the required number

* To whom correspondence should be addressed. Phone: 31-15-2782361. Fax: 31-15-2782355. E-mail: Vanderwielen@stm.tudelft.nl.

0021-9568/96/1741-0239$12.00/0

of experiments can be reduced drastically when compared to evaluating all possible (binary) combinations.

It is well known that the degree of swelling and other related effects (osmotic pressure, Donnan effect) are influenced substantially by the type of counterion. For monocomponent loading, the extent of swelling is proportional to the partial molar volume of the counterions. For multicomponent equilibria, however, the situation is more complex. These effects have been neglected in this work.

In this work, the Myers and Byington model has been employed to describe binary ion exchange in batch experiments. Chloride is used as the reference component. The data from the binary exchange experiments are used for the prediction of ion exchange equilibria in a multicomponent reaction system for a broad range of experimental data.

Multicomponent Equilibrium Model for Heterogeneous Resins

The model developed by Myers and Byington (1986) assumes a binomial distribution of $n + 1$ site types, each having a characteristic energy level $E_{j,l}$ for the adsorption of ion j on site l. The site fraction of l (p_l probability) is defined as

$$p_l = \binom{n}{l} p^l (1-p)^{n-l}, \quad o < p < 1 \tag{5}$$

with the following energy level

$$E_{l,j} = \bar{E}_{l,j} + \frac{1-np}{(np(1-p))^{1/2}}\sigma_j \tag{6}$$

where $\bar{E}_{l,j}$ and σ_j are the average value and the standard deviation of the distribution of the adsorption energies and p is the skewness of the site distribution function. Myers and Byington (1986) have developed a closed-form expression for binary systems, which was extended by Carta and co-workers (Dye *et al.*, 1990; Saunders *et al.*, 1989) to a multicomponent system of N species. Taking the counterion j as the reference ion, the selectivity coefficient for ion i relative to ion j is given by

$$S_{i,j} = \bar{S}_{i,j} \frac{\sum_{k=1}^{N} (\bar{S}_{kj} x_k W_{kj}^{U+V} [(1-p) W_{i,k}^{U} + p W_{i,k}^{V}])}{\sum_{k=1}^{N} (\bar{S}_{k,j} x_k W_{k,j}^{U+V} [(1-p) W_{j,k}^{U} + p W_{j,k}^{V}])} \tag{7}$$

with

$$\bar{S}_{i,j} = \exp\left(\frac{\bar{E}_i - \bar{E}_j}{RT}\right); \quad W_{i,j} = \exp\left(\frac{\sigma_i - \sigma_j}{RT}\right) \tag{8}$$

and

$$U = \frac{-p}{(p(1-p))^{1/2}}; \quad V = \frac{1-p}{(p(1-p))^{1/2}} \tag{9}$$

The ionic fraction of species i in the resin phase can be calculated from the following equation:

$$y_i = S_{ir} x_i / \sum_k S_{kr} x_k \tag{10}$$

with index r designating the reference component. For the binary case, eq 7 simplifies to (Myers and Byington, 1986)

$$S_{12} = \bar{S} \frac{\bar{S} W^{U+V} x_1 + (W^U(1-p) + W^V p) x_2}{\bar{S}(W^V(1-p) + W^U p) x_1 + x_2} \tag{11}$$

and eq 10 into eq 4. In general, the standard deviation and the skewness are determined by geometrical constraints whereas the average selectivity is determined by the molecular properties of the solute and resin matrix. Therefore, it is expected that the extent of interaction between the solute and matrix, when expressed in terms of the Gibbs energy difference for the aqueous solution−matrix transfer, can also be related to the "hydrophobicity" scale. The selectivity extrapolated to infinite dilution S_0 which is given by

$$S_0 = \bar{S}(W^U(1-p) + W^V p) \tag{12}$$

The infinite dilution selectivity is representative for the true molecular interaction of the solutes for the best accessible and most selective functional groups in the resin, free of effects of steric hindrances on exchanged solute molecules. It has been demonstrated that the infinite dilution selectivity is directly related to similar scales for molecular interaction such as hydrophobicity scales based on partitioning (Dye *et al.*, 1990; Jones and Carta, 1992) or relative solubility (Nozaki and Tanford, 1971; Gude *et al.*, 1995a,b).

Experimental Section

Materials. Amberlite IRA 400 anion exchange resin is a commercial strong-base, gel-type resin purchased from the Rohm and Haas Co. The resin's matrix was composed of polystyrene cross-linked with 8% divinylbenzene and had quarternary ammonium functional groups. The resin capacity Q_{max} and density for the water-swollen Cl^- form have been measured using standard methods (Helfferich, 1962; Lopez *et al.*, 1992). Although the resin was supplied in its Cl^- form, the following procedure was followed to ensure complete conversion in the Cl^- form. The resin was washed three times with an equivolumetric mixture of ethanol and water to ensure complete removal of possible organic material, followed by repetitive washing with deionized water. The resin was contacted three times with a 2 N HCl solution and washed with deionized water until the effluent pH reached 6. The Potassium Penicillin G was donated by Gist-brocades and was at least 99.2% pure. The 6-aminopenicillanic acid (6-APA) and the phenylacetic acid (PhAc) have been purchased from Merck and used without further purification.

Methods. Solutions with varying concentrations of the required solute X (X = Pen G^-, 6-APA^-, $PhAc^-$) (Pen G = Penicillin G) were prepared by dissolving known masses in deionized water. The pH was adjusted with NaOH to pH 8. To study the binary and multicomponent equilibria, known masses of IRA400 (0.05−1.0 g) were prepared as described in the previous section. The resin was equilibrated with 7.5 mL of X-containing solution at 310 K for at least 2 h with continuous shaking. The initial pH was approximately 7.5 (±0.1) and decreased usually to around 7 (±0.4), which still ensures that the weak electrolyte solutes were completely in the anionic form. Then, the resin was separated from the solution. The composition of the binary systems (solute versus Cl^-) was determined with a Perkin-Elmer 3UV/VIS spectrophotometer at a wavelength of 257 nm in a 0.5 mL quartz cuvette with a path length of 1 cm. The sample could be analyzed without dilution for solute concentrations below 5 mM (10 mM for 6-aminopenicillanic acid). The experimental error of the

Table 1. Ion Exchange Isotherm of Pen G/Cl on IRA 400

x_{exp}	y_{exp}	y_{calc}	$S_{PenG,Cl}$	x_{exp}	y_{exp}	y_{calc}	$S_{PenG,Cl}$
0.076	0.147	0.155	2.23	0.449	0.525	0.515	1.30
0.080	0.144	0.161	2.21	0.456	0.535	0.520	1.29
0.097	0.212	0.188	2.15	0.484	0.541	0.539	1.25
0.137	0.284	0.242	2.02	0.508	0.581	0.556	1.21
0.140	0.277	0.246	2.01	0.509	0.551	0.556	1.21
0.212	0.310	0.326	1.80	0.514	0.590	0.560	1.20
0.235	0.372	0.348	1.74	0.564	0.555	0.594	1.13
0.299	0.396	0.404	1.59	0.578	0.654	0.604	1.11
0.332	0.311	0.430	1.52	0.582	0.636	0.607	1.11
0.336	0.429	0.433	1.51	0.609	0.649	0.626	1.08
0.347	0.410	0.441	1.49	0.672	0.725	0.672	1.00
0.386	0.436	0.470	1.41	0..683	0.720	0.680	0.99
0.400	0.478	0.480	1.39	0.685	0.696	0.682	0.99
0.417	0.464	0.492	1.36	0.826	0.727	0.800	0.84
0.419	0.513	0.494	1.35	0.844	0.745	0.818	0.83

Table 2. Ion Exchange Isotherm of 6-APA/Cl on IRA 400

x_{exp}	y_{exp}	y_{calc}	x_{exp}	y_{exp}	y_{calc}
0.321	0.104	0.127	0.631	0.284	0.344
0.340	0.104	0.136	0.634	0.370	0.347
0.370	0.148	0.152	0.647	0.342	0.359
0.427	0.179	0.186	0.648	0.398	0.361
0.437	0.175	0.192	0.666	0.420	0.380
0.462	0.215	0.208	0.675	0.377	0.389
0.484	0.244	0.223	0.716	0.423	0.435
0.496	0.238	0.232	0.717	0.417	0.438
0.538	0.261	0.263	0.749	0.483	0.478
0.567	0.302	0.286	0.753	0.488	0.483
0.587	0.260	0.304	0.787	0.547	0.531
0.595	0.312	0.310	0.790	0.559	0.536
0.602	0.250	0.317	0.860	0.684	0.653
0.608	0.332	0.322	0.872	0.715	0.675

Table 3. Ion Exchange Isotherm of PhAc/Cl on IRA 400

x_{exp}	y_{exp}	y_{calc}	x_{exp}	y_{exp}	y_{calc}
0.001	0.000	0.001	0.406	0.567	0.574
0.059	0.147	0.110	0.420	0.600	0.588
0.074	0.148	0.136	0.467	0.633	0.633
0.149	0.262	0.257	0.471	0.637	0.637
0.154	0.270	0.264	0.503	0.674	0.666
0.243	0.360	0.388	0.547	0.741	0.705
0.255	0.354	0.403	0.579	0.767	0.730
0.290	0.452	0.446	0.597	0.770	0.745
0.318	0.480	0.479	0.600	0.715	0.747
0.321	0.432	0.482	0.617	0.826	0.761
0.346	0.305	0.510	0.622	0.794	0.765
0.354	0.506	0.519	0.651	0.807	0.786
0.356	0.512	0.521	0.738	0.965	0.848
0.363	0.530	0.529	0.749	0.961	0.855
0.404	0.565	0.571	0.780	1.010	0.875

procedure was smaller than 3%. The resin phase composition is calculated from the mass balance.

Multicomponent equilibria have been measured using the same procedure, taking varying amounts of Pen G^- with stoichiometric amounts of 6-APA^- and $PhAc^-$. The sample (quaternary) solutions were analyzed by HPLC or capillary zone electrophoresis (CZE), which had a comparable accuracy (error <3%). The HPLC system was equipped with a Si-Merckosorp Si-60 precolumn, a Chrompack Chromospher C18 reversed phase column, a Waters 510 pump, a Waters WIPP 712 auto injector, and a Waters 484 UV detector, using water at pH 2 as the eluent. The CZE equipment was a Waters Quanta 4000 capillary electrophoresis system with a noncoated silica capillary of 50 cm, using UV detection at 214 nm. The eluent was a 10 mM phosphate buffer at pH 8.

Results

Physicochemical Data of the Resin. The density of the Cl^- form of the swollen resin was measured in duplicate, resulting in an average density of 1070 (±2.4) kg/m^3. This was slightly less than the value measured by Lopez *et al.* (1992; 1090 kg/m^3) and specified by the manufacturer (1110 kg/m^3). The maximum capacity of the resin Q_{max} was determined as 1.26 mequiv/g of swollen resin. This value (1.4 mequiv/mL) was substantially smaller than the value reported by Lopez *et al.* for the Cl^- form (2.0 mequiv/mL), but in better agreement with his values for the F^- (1.42 mequiv/g) and OH^- form (1.46 mequiv/g) and with the specifications of the manufacturer (1.4 mequiv/mL). The value of Q_{max} of 1.26 mequiv/g is used for further data analysis.

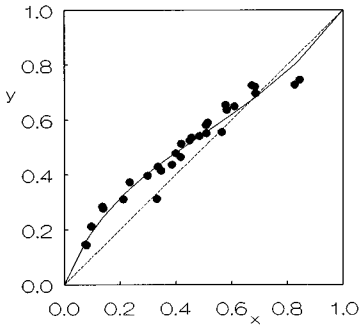

Figure 1. Ion exchange isotherm of Pen G^-/Cl^- on IRA 400 at 310 K.

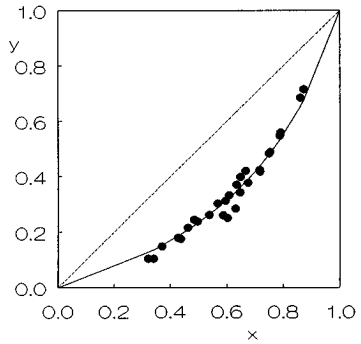

Figure 2. Ion exchange isotherm of 6-APA^-/Cl^- on IRA 400 at 310 K.

Binary Ion Exchange Equilibria. The equilibrium uptake data of Pen G, 6-APA, and PhAc by Amberlite IRA400 in its Cl^- form are given in Tables 1−3 in the form of equivalent ionic fractions. The uptake of PhAc has been measured in three series of a constant initial concentration (67, 133, and 200 mM, respectively) for varying amounts of resin. The uptake curves of Pen G and 6-APA were obtained for varying amounts of resin, using a constant initial concentration. In some cases, phenylacetate sorption was substantially larger than the resin capacity. This could probably be attributed to nonionic sorption mechanisms or precipitation in the matrix. These data have been omitted from the analysis. This phenomenon has not been observed for Pen G and 6-APA uptake.

The ion exchange isotherms are shown in Figures 1−3 for Pen G, 6-APA, and PhAc, respectively. The ion exchange isotherms of PhAc and 6-APA can be described with the constant selectivity model throughout the complete range of data. Note that the constant selectivity model and the Myers and Byington model coincide for $W = 1$ and $p = 0.5$. The curves in Figures 2 and 3 are calculated using the constant selectivity model. The average deviation of experimental and calculated resin phase composition was 7% for both cases, which was quite similar to the experimental error (5%). Correlation of the data with the more flexible, three-parameter Myers and Byington model does not further improve the correlation of the data. In contrast,

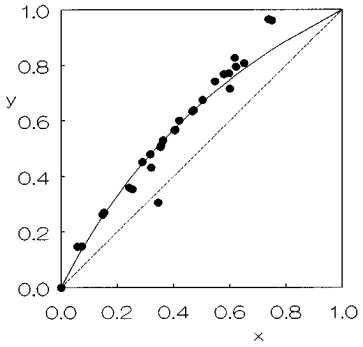

Figure 3. Ion exchange isotherm of $PhAc^-/Cl^-$ on IRA 400 at 310 K.

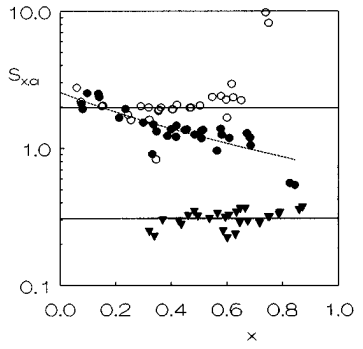

Figure 4. Experimental binary selectivities of Pen G (●), 6-APA (▼), and PhAc (○) versus Cl. Curves are calculated from eqs 9 and 10 using data from Table 4.

Table 4. Equilibrium Parameters for the Exchange of X^-/Cl^- on IRA400 at 310 K

X^-	$\bar{S}$	W	p^a	S_0
Pen G	1.338	3.519	0.5	2.544
6-APA	0.307	1[1]	0.5	0.307
PhAc	1.971	1[1]	0.5	1.971

[a] Not fitted, value taken from Dye *et al.* (1990).

the ion exchange isotherm of Penicillin G shows a decrease of the selectivity for Pen G relative to Cl^- with increasing ionic fraction of Pen G with a selectivity reversal at x_{PenG} of approximately 0.7. Of course, this behavior cannot be described adequately with a constant selectivity model, but requires an additional parameter (W) of the Myers and Byington model for improved correlation of the data. Following Dye *et al.* (1990), we have set the skewness factor p at 0.5 which corresponds to a symmetrical distribution, fitting $\bar{S}$ and W only. The curve in Figure 4 is calculated with the Myers and Byington model using the (two) optimized parameters from Table 4.

Multicomponent Ion Exchange Equilibria. Multicomponent ion exchange equilibria of the anions of Pen G, 6-APA, and PhAc have been measured for initial, cumulative concentrations ranging from 50 to 150 mol/m^3 for varying amounts of IRA 400 resin. An overview of the experimental uptake data is provided in Table 5. These data have been transformed into equivalent ionic fractions, which are given in Table 5 as well.

To demonstrate the predictive capabilities of the Myers and Byington model for multicomponent ion exchange data, the equivalent ionic fractions in the resin phase have been calculated from the solution composition using the binary parameters of Table 4. The result is given in Figure 5 in the form of a parity plot. The agreement between experimental data and predictions is reasonably good. The average deviation of calculated and experimental values was 31.6%. The ionic fraction of PhAc in the resin phase

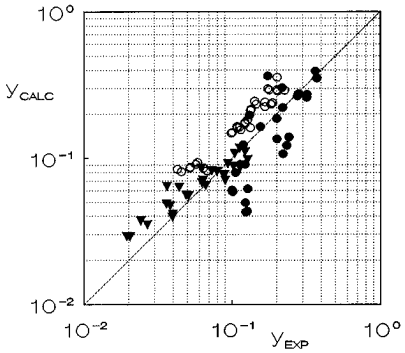

Figure 5. Parity plot of the experimental and predicted resin phase composition for the quaternary system Pen G (●), 6-APA (▼), and PhAc (○) versus Cl.

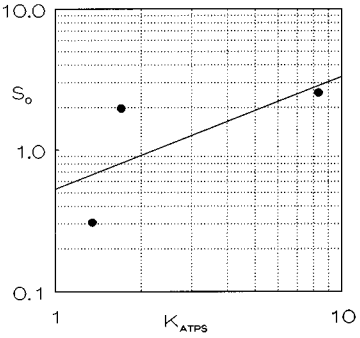

Figure 6. Correlation of partitioning data as reported by Anderson *et al.* (1984) and infinite dilution selectivities from this work (Table 4).

is generally slightly overestimated, whereas that of Pen G is usually slightly underestimated. Similar results have been calculated using parameters which were optimized for the quaternary ion exchange system. The comparable results and the relatively smaller experimental effort by using binary data did not validate a further use of parameters optimized for the quaternary system. The average deviation may be attributed to experimental errors in this complex, multicomponent electrolyte system. Saunders *et al.* (1989) observed an average deviation on the same order of magnitude for a ternary system with amino acids, supporting this statement.

Infinite Dilution Selectivities and Hydrophobicity Scales. Various researchers have demonstrated the high degree of correlation between suitable measures of the polarity of amino acids, and the infinite dilution selectivity for ion exchange (Dye *et al.*, 1990; Jones and Carta, 1993). These researchers use well-established hydrophobicity scales such as a hydrophobicity scale developed on the basis of the relative solubility in ethanol−water mixtures (Nozaki and Tanford, 1971), or on the basis of octanol−water partitioning (log P) and the distribution behavior in reversed micellar systems (Thien *et al.*, 1988). Following this approach, we have used liquid−liquid partitioning data of Pen G, 6-APA, and PhAc in the aqueous two-phase system of PEG 20000 (8.9 mass %) and potassium phosphate (7.6 mass %) at pH 7.8 and 310 K. The degree of correlation is not very impressive at present, as is shown in Figure 6, but the overall tendency encourages further research.

Conclusions

The model provided by Myers and Byington (1986) is a valuable tool for correlating ion exchange equilibria with nonconstant selectivities. We have demonstrated that multicomponent equilibria can be predicted straightfor-

Table 5. Liquid Concentrations, Experimental and Calculated Equivalent Ionic Fractions for the Quarternary Ion Exchange System PenG/6-APA/PhAc/Cl at 310 K

c_{penG}/ mM	$c_{6\text{-}APA}$/ mM	c_{PhAc}/ mM				experimental			calculated		
			x_{PenG}	x_{6APA}	xPhAc	y_{PenG}	y_{6APA}	y_{PhAc}	y_{PenG}	y_{6APA}	y_{PhAc}
6.49	6.32	1.94	0.130	0.126	0.039	0.119	0.040	0.068	0.124	0.041	0.082
5.04	6.06	2.06	0.101	0.121	0.041	0.123	0.040	0.064	0.091	0.039	0.086
10.93	8.19	3.33	0.146	0.109	0.044	0.242	0.027	0.057	0.139	0.035	0.091
15.91	8.76	3.44	0.212	0.117	0.046	0.220	0.024	0.058	0.222	0.037	0.094
24.44	9.43	4.29	0.244	0.094	0.043	0.319	0.019	0.052	0.260	0.029	0.086
25.36	9.35	4.42	0.254	0.094	0.044	0.321	0.020	0.052	0.273	0.029	0.087
39.07	11.41	5.55	0.313	0.091	0.044	0.375	0.007	0.043	0.353	0.027	0.084
42.65	11.9	5.36	0.341	0.095	0.043	0.365	0.004	0.045	0.394	0.028	0.081
0	10.64	3.78	0	0.213	0.076	0	0.090	0.133	0	0.071	0.162
0	12.1	4.24	0	0.242	0.085	0	0.079	0.127	0	0.081	0.183
5.29	14.75	6.11	0.071	0.197	0.081	0.128	0.066	0.122	0.062	0.065	0.172
7.46	15.35	6.13	0.099	0.205	0.082	0.114	0.063	0.122	0.091	0.068	0.175
12.99	16.96	7.92	0.130	0.170	0.079	0.234	0.051	0.108	0.122	0.055	0.165
18.32	17.26	7.78	0.183	0.173	0.078	0.200	0.049	0.109	0.187	0.056	0.162
31.54	19.43	9.4	0.252	0.155	0.075	0.281	0.036	0.101	0.275	0.048	0.150
30.75	18.9	9.23	0.246	0.151	0.074	0.277	0.038	0.099	0.266	0.047	0.149
0	19.43	8.22	0	0.259	0.110	0	0.113	0.183	0	0.086	0.234
0	20.06	8.4	0	0.267	0.112	0	0.112	0.188	0	0.089	0.239
5.24	23.61	11.48	0.052	0.236	0.115	0.127	0.089	0.167	0.043	0.077	0.242
5.05	23.44	10.58	0.051	0.234	0.106	0.124	0.087	0.167	0.043	0.077	0.225
14.58	27.32	14.69	0.117	0.219	0.118	0.221	0.063	0.142	0.107	0.071	0.245
17.64	27.28	14.05	0.141	0.218	0.112	0.201	0.064	0.146	0.135	0.071	0.234
40.85	30.62	16.36	0.272	0.204	0.109	0.218	0.044	0.135	0.303	0.063	0.217
47.3	31.74	16.31	0.315	0.212	0.109	0.174	0.036	0.133	0.365	0.064	0.212
0	29.59	13.72	0	0.296	0.137	0	0.127	0.227	0	0.098	0.291
0	32.92	16.95	0	0.329	0.170	0	0.104	0.202	0	0.108	0.358
8.81	33.43	17.05	0.070	0.267	0.136	0.100	0.102	0.204	0.060	0.088	0.288
8.54	34.72	17.28	0.068	0.278	0.138	0.101	0.094	0.201	0.059	0.092	0.293
24.84	38.21	21.15	0.166	0.255	0.141	0.156	0.073	0.178	0.164	0.082	0.292
28.63	39.93	21.6	0.191	0.266	0.144	0.131	0.062	0.174	0.197	0.086	0.298
5.32	30.4	6.67	0.053	0.304	0.067	0.123	0.122	0.115	0.050	0.111	0.157
7.98	31.87	6.88	0.0080	0.319	0.069	0.106	0.113	0.113	0.080	0.118	0.163

wardly and reliably from binary data for the IRA 400 system with relatively bulky β-lactam antibiotics. Further evidence in the literature for amino acids at cation exchangers (Saunders *et al*., 1989; Dye *et al*., 1990; Jones and Carta, 1993) supports the general applicability of this method. The average deviation of the model predictions is approximately 7% for binary exchange. The average deviation of predicted and experimental data for the complex quaternary PenG/6-APA/PhAC/Cl, using binary data only was smaller than 32%, which is adequate for most applications.

Infinite dilution selectivities can be correlated to some extent with hydrophobicity scales such as can be obtained from partitioning in aqueous two-phase systems, although the extent of correlation is not very impressive. However, the procedure allows the prediction of binary parameters and hence of ion exchange equilibria, based on a well-established hydrophobicity scale from the relative positions of the unknown species and a limited number of known species.

Acknowledgment

Gist-brocades N.V. is acknowledged for the supply of Penicillin G. Analytical support by C. Ras (HPLC) and H. Corstjens (CZE) is greatfully acknowledged.

Literature Cited

Anderson, E.; Mattiasson, B.; Hahn-Hägerdahl, B. Enzymatic conversion in aqueous two-phase systems: deacylation of benzylpenicillin to 6-aminopenicillanic acid with penicillin acylase. *Enzyme Microb. Technol*. **1984**, *6*, 301−306.

Dye, S. R.; DeCarli, J. P, II; Carta, G. Equilibrium sorption of amino acids by a cation exchange resin. *Ind. Eng. Chem. Res.* **1990**, *29*, 849−857.

Gude, M. T.; Meuwissen, H; van der Wielen, L. A. M.; Luyben, K. Ch. A. M. Solubilities and partition coefficients of α-Amino acids in aqueous 1-butanol. Submitted for publication, **1995a**.

Gude, M. T.; van der Wielen, L. A. M.; Luyben, K. Ch.A. M. Phase behavior of α-amino acids in multicomponent aqueous alkanol solutions. *Fluid Phase Equilib.* **1995b**, in press.

Helfferich, F. *Ion exchange* McGraw-Hill: New York, 1962.

Helfferich, F. Ion exchange equilibria of amino acids on strong-acid resins. *React. Polym.* **1990**, *12*, 95−100.

Jansen, M. L.; Straathof, A. J. J.; van der Wielen, L. A. M.; Luyben, K. Ch.A. M.; van den Tweel, W. J. J. A rigorous model for ion exchange equilibria of strong and weak electrolytes. *AIChE J*. **1995**, submitted for publication.

Jones, I. L; Carta, G. Ion exchange of amino acids and dipeptides on cation resins with varying degree of cross-linking. 1. Equilibrium. *Ind. Eng. Chem. Res.* **1993**, *32*, 107−117.

Lopez, M.; Coca, J.; Sastre, H. Anion exchange in Amberlite IRA-400 and Amberlite IRA-410. *J. Chem. Eng. Data* **1992**, *37*, 274−277.

Myers, A. L.; Byington, S. Thermodynamics of ion exchange: prediction of multicomponent equilibria from binary data. in Rodrigues, A. E. Ion exchange: science and technology. *NATO ASI Ser., Ser. E* **1986**, *107*, 119−145.

Novosad, J.; Myers, A. L. Thermodynamics of ion exchange as an adsorption process. *Can. J. Chem. Eng.* **1982**, *60*, 500−503.

Nozaki, Y.; Tanford, C. The solubility of amino acids and two glycine peptides in aqueous ethanol and dioxane solutions. Development of a hydrophobicity scale. *J. Biol. Chem*. **1971**, *246* (10), 2211−2217.

Saunders, M. S.; Vierow, J. B.; Carta, G. Uptake of phenylalanine and tyrosine by a strong acid cation exchanger. *AIChE J*. **1989**, *35* (1), 53−68.

Streat, M.; Brignal, W. Representation of ternary ion exchange equilibria. *Trans. Inst. Chem. Eng.* **1970**, *48*, T151.

Wielen, L. A. M. van der; Potters, J. J. M.; Straathof, A. J. J.; Luyben, K. Ch. A. M. Integration of bioconversion and continuous product separation by means of countercurrent adsorption. *Chem. Eng. Sci*. **1990**, *45* (8), 2397−2404.

Wielen, L. A. M. van der; Diepen, P. J.; Straathof, A. J. J.; Luyben, K. Ch. A. M. Two new countercurrent adsorptive enzyme reactors. Operational conditions for deacylation of penicillin G. *Ann. N.Y. Acad. Sci.* **1995**, *750*, 482−490.

Received for review September 8, 1995. Accepted November 11, 1995.[⊗] The Netherlands Organization for Scientific Research (NWO) is gratefully acknowledged for partial financial support.

JE950223I

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1996.