

Conditional equilibrium constants in multicomponent heterogeneous adsorption: The conditional affinity spectrum

Josep L. Garcés, Francesc Mas, and Jaume Puy

Citation: *J. Chem. Phys.* **124**, 044710 (2006); doi: 10.1063/1.2162876

View online: <http://dx.doi.org/10.1063/1.2162876>

View Table of Contents: <http://jcp.aip.org/resource/1/JCPSA6/v124/i4>

Published by the [American Institute of Physics](#).

Additional information on J. Chem. Phys.

Journal Homepage: <http://jcp.aip.org/>

Journal Information: http://jcp.aip.org/about/about_the_journal

Top downloads: http://jcp.aip.org/features/most_downloaded

Information for Authors: <http://jcp.aip.org/authors>

ADVERTISEMENT

Instruments for advanced science

Gas Analysis



- dynamic measurement of reaction gas streams
- catalysis and thermal analysis
- molecular beam studies
- dissolved species probes
- fermentation, environmental and ecological studies

Surface Science



- UHV TPD
- SIMS
- end point detection in ion beam etch
- elemental imaging - surface mapping

Plasma Diagnostics



- plasma source characterization
- etch and deposition process
- reaction kinetic studies
- analysis of neutral and radical species

Vacuum Analysis



- partial pressure measurement and control of process gases
- reactive sputter process control
- vacuum diagnostics
- vacuum coating process monitoring

contact Hiden Analytical for further details

HIDEN
ANALYTICAL

info@hideninc.com
www.HidenAnalytical.com

CLICK to view our product catalogue



Conditional equilibrium constants in multicomponent heterogeneous adsorption: The conditional affinity spectrum

Josep L. Garcés and Francesc Mas

Research Center of Theoretical Chemistry and Physical-Chemistry Department, Universitat de Barcelona, Martí i Franqués 1, 08028 Barcelona, Spain

Jaume Puy^{a)}

Departament de Química, Universitat de Lleida, Avenida Rovira Roure 191, 25198 Lleida, Spain

(Received 6 October 2005; accepted 5 December 2005; published online 30 January 2006)

The concept of conditional stability constant is extended to the competitive binding of small molecules to heterogeneous surfaces or macromolecules *via* the introduction of the conditional affinity spectrum (CAS). The CAS describes the distribution of effective binding energies experienced by one complexing agent at a fixed concentration of the rest. We show that, when the multicomponent system can be described in terms of an underlying affinity spectrum [integral equation (IE) approach], the system can always be characterized by means of a CAS. The thermodynamic properties of the CAS and its dependence on the concentration of the rest of components are discussed. In the context of metal/proton competition, analytical expressions for the mean (conditional average affinity) and the variance (conditional heterogeneity) of the CAS as functions of *pH* are reported and their physical interpretation discussed. Furthermore, we show that the dependence of the CAS variance on *pH* allows for the analytical determination of the correlation coefficient between the binding energies of the metal and the proton. Nonideal competitive adsorption isotherm and Frumkin isotherms are used to illustrate the results of this work. Finally, the possibility of using CAS when the IE approach does not apply (for instance, when multidentate binding is present) is explored. © 2006 American Institute of Physics. [DOI: 10.1063/1.2162876]

I. INTRODUCTION

Binding of small molecules to heterogeneous surfaces and macromolecules is a matter of fundamental interest in several fields of theoretical and applied chemistry. For instance, in the field of environmental chemistry, the bioavailability of nutrients and contaminants is determined by the complexation/adsorption properties of such substances to different particles, colloids, organic matter, and small ligands.^{1–7} Adsorption phenomena in industrial processes of gas separation have led to an increasing research activity on the role of heterogeneity in the adsorption properties of the surfaces of interest.^{8–10} In several other disciplines, such as biochemical substrate-ligand interactions,¹¹ chromatography,¹² or polymer science,^{13,14} the heterogeneous binding also plays a relevant role.

Although several theoretical approaches to describe the heterogeneous adsorption have been proposed,¹⁵ we focus here in the so-called affinity spectrum or integral equation (IE) approach. The IE approach is based on characterizing the ligand (a surface or a macromolecule) by a distribution of binding constants called affinity spectrum, which provides the fraction of sites with binding equilibrium constant lying in between the values *k* and *k*+*dk*. Hence, the coverage, i.e., the total fraction of occupied sites, is given by a weighted superposition of Langmuirian isotherms,^{8,9,16}

$$\theta(c) = \int_{-\infty}^{\infty} p(\log k) \frac{kc}{1+kc} d \log k. \quad (1)$$

Usually, as indicated in Eq. (1), the affinity spectrum is given in terms of $\log(k)$, which is proportional to the binding free energy.

Although such an approach has been widely used, in many systems of interest two or more components compete for the same macromolecular/surface sites. For instance, in natural waters the competition between the metal ion and the proton is in most cases unavoidable. A straightforward generalization of the affinity spectrum methodology to multicomponent systems relies on the definition of a multidimensional affinity spectrum.^{9,17,18} The binding properties of the system are then described by a superposition of multicomponent Langmuirian isotherms,

$$\begin{aligned} \theta_i(c_1, c_2, \dots, c_N) = & \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \cdots \int_{-\infty}^{\infty} p(x_1, x_2, \dots, x_N) \\ & \times \frac{k_i c_i}{N} dx_1 dx_2 \cdots dx_n, \\ & 1 + \sum_{j=1}^N k_j c_j \end{aligned} \quad (2)$$

where *k_i* and *c_i* represent, respectively, the concentration and the binding stability constant for component *i*, and *x_i* = $\log(k_i)$. Once a particular functional form for the affinity spectrum is suggested, the integrals appearing in (1) and (2)

^{a)}Author to whom correspondence should be addressed. Electronic mail: jpuy@quimica.udl.es

can, in principle, be performed and a binding isotherm can be obtained.^{9,15,17}

On the other hand, sometimes one could be interested in the inverse problem: the finding of the affinity spectrum embedded in a suitable binding isotherm. For monocomponent systems, the inverse problem was analytically solved by Sips in his classical analysis of binding to heterogeneous surfaces.¹⁶ Numerical regularization techniques have also been successfully applied to solve the inverse problem from experimental binding data.^{19,20} For multicomponent systems, the n -dimensional nature of the integrals appearing in (2) complicates the interpretation of the binding isotherms in terms of an underlying affinity spectrum. However, the use of a multidimensional affinity spectrum is especially interesting, since it allows characterizing the binding in terms of the moments of this distribution. In addition to the mean values, which characterize the mean binding affinity, the variance provides information on the heterogeneity of the binding, i.e., on the dispersion of the binding affinities. Finally, the covariance measures the correlation between the affinity of the different components for a given site. For instance, in the context of metal-macromolecule complexation, when large values of the affinity of the proton occur together with large values for the affinity of the metal, the correlation is positive. When a large affinity value for the proton does not imply a large or low affinity value for the metal there is no correlation and we say that the two binding energies are independent.^{17,21}

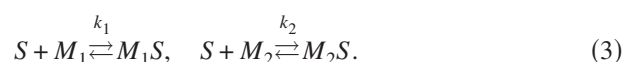
An analytical solution of the integral equation (2) has been reported in a recent paper.²² Thus, an analysis of some multicomponent isotherms, such as nonideal consistent competitive adsorption^{17,23} (NICA) and Frumkin isotherms,²⁴ in terms of an underlying affinity spectrum has been reported. In the same paper, a way to obtain the main statistics (mean, variances, and covariances) of the multidimensional affinity spectra avoiding the detailed knowledge of the multidimensional spectrum was described.

Here we present an alternative (and complementary) approach to the study of multicomponent adsorption. A classical methodology in analytical chemistry to deal with multicomponent binding lies in the definition of the so-called conditional equilibrium constants.²⁵ Conditional equilibrium constants allow considering a multicomponent system as a monocomponent one whenever the concentrations of the rest of components are kept constant. This is particularly useful for those systems in which only the concentrations of one (or more) of the species is, in equilibrium, present in a large range of values. For instance, in some natural waters, calcium and proton concentrations can approximately be taken as constants, while the concentrations of contaminants and heavy metal ions vary strongly depending on the presence or not of a pollution source.^{1,2} Conditional binding properties have indeed been used in a number of works. For instance, stability constants restricted to some fixed pH at a given ionic strength value have been reported in the literature for the binding of metal ions to synthetic or natural macromolecules.^{7,26} However, the theoretical extension of this procedure to deal with heterogeneous ligands is still lacking.

The aim of the present work is to explore the possibility of generalizing the concept of conditional equilibrium constant to heterogeneous systems. This generalization leads to the definition of the conditional affinity spectrum (CAS). Although the ideas here presented can be easily extended to other experimental situations, we will use the environmental terminology and restrict ourselves to metal/proton competition. In Sec. II, the CAS is defined as a natural generalization of a conditional equilibrium constant and the physical interpretation of such distribution is provided. The results are further applied to obtain the CAS associated to the NICA isotherm. In Sec. III, we analyze the main statistical properties of the CAS, i.e., its mean and variance, and their dependence on the pH value. In Sec. IV, we present a new methodology to obtain the main statistics of the multidimensional affinity spectra from extrapolation of the statistics of the CAS. In this way, the computing of multiple integrals can be avoided, and we are able to analytically obtain the statistics corresponding to NICA and Frumkin competitive isotherms. Finally, Sec. V shows that, although the existence of the multidimensional affinity spectrum guarantees the existence of the CAS, the CAS can also be used in cases where there is no multidimensional affinity spectrum, as defined in Eq. (2). Two of such situations are analyzed: (i) some sites bind selectively to only one component, and (ii) mono- and multidentate metal bindings coexist.

II. THE CONDITIONAL AFFINITY SPECTRUM

Let us first consider the simplest situation in which an arbitrary site, S , of the surface or macromolecule can be occupied by either the proton, M_1 , or a metal ion, M_2 present in the solution. When the sites are identical and independent, two equilibrium constants, k_1 and k_2 , can be associated with these binding processes,



The metal coverage, i.e., the fraction of sites occupied by the metal, is given by the well-known competitive Langmuirian isotherm,

$$\theta_2 = \frac{k_2 c_2}{1 + k_1 c_1 + k_2 c_2}, \quad (4)$$

where c_1 and c_2 are the free proton and free metal concentrations, respectively. Dividing by $1 + k_1 c_1$ both the numerator and the denominator, Eq. (4) can be rewritten as

$$\theta_2 = \frac{[k_2/(1 + k_1 c_1)]c_2}{1 + [k_2/(1 + k_1 c_1)]c_2} = \frac{k'(c_1)c_2}{1 + k'(c_1)c_2}, \quad (5)$$

where $k'(c_1) = k_2/(1 + k_1 c_1)$ is the well-known conditional stability constant commonly used in analytical chemistry. Notice that $k'(c_1)$ evidences that the “effective” affinity of a given site for a metal ion at some fixed pH depends on the pH value. Equation (5) indicates that, by using k' , the multicomponent system has been transformed, from a practical point of view, into an equivalent effective monocomponent one.

As usual in the literature, it is convenient to rewrite the expressions in terms of logarithms of the stability constants, since the resulting terms can be easily related to free energy by simply multiplying it by a factor $-RT \ln 10$. Accordingly, $\log k'(c_1)$ can be rewritten as

$$x' = \log k'(c_1) = x_2 - \log(1 + k_1 c_1). \quad (6)$$

Two terms can be clearly identified in expression (6). The first one, x_2 , is associated to the “intrinsic binding free energy,” that is, the free energy of the binding of a metal ion in absence of protons. (Notice that the second term vanishes when $c_1=0$, i.e., at high enough pH values.) On the other hand, the term $-\log(1+k_1 c_1)$ can be related to the average work needed to perform the necessary exchange of the proton by the metal in order to complex the macromolecular site. This exchange work [given by $RT(\ln 10)\log(1+k_1 c_1)$] increases as the proton concentration increases, and, as a consequence, the conditional equilibrium constant is a decreasing function of c_1 .

Let us obtain the distribution $p'(x'; c_1)$ of the effective stability constants, seen by a metal ion in the presence of a heterogeneous ligand, i.e., the density of sites with effective affinity $x' = \log k'$ at a given pH. From now onwards, we will refer to this distribution as the CAS.

The probability of finding a macromolecular site having affinity for the proton lying in between x_1 and $x_1 + dx_1$ and affinity for the metal ion in between x_2 and $x_2 + dx_2$ is, according to (2), given by

$$p(x_1, x_2) dx_1 dx_2. \quad (7)$$

Writing x_2 in terms of the conditional affinity x' , as given by Eq. (6),

$$p(x_1, x_2) dx_1 dx_2 = p(x_1, x' + \log(1 + k_1 c_1)) dx_1 dx'. \quad (8)$$

Since we are interested in the metal affinity, regardless of the proton affinity, we integrate (8) for all the x_1 values. This operation produces the conditional affinity spectrum which can then be written as

$$p'(x'; c_1) = \int_{-\infty}^{\infty} p(x_1, x' + \log(1 + k_1 c_1)) dx_1. \quad (9)$$

Equation (9) relates the CAS to the multidimensional affinity spectrum underlying multicomponent complexation. Notice that, according to (9), the existence of multidimensional affinity spectrum ensures the existence of the CAS. Therefore, the existence of the latter is a necessary condition for the existence of the former. However, the converse assumption might not be true: conditional affinity spectra could

be realistic for conditions under which a multidimensional affinity spectrum, as defined in Eq. (2), cannot be assumed. This possibility will be investigated in Sec. V.

In terms of $p'(x'; c_1)$, the metal coverage, using Eqs. (2) and (9), can be rewritten as

$$\theta_2(c_1, c_2) = \int_{-\infty}^{\infty} p'(x'; c_1) \frac{k' c_2}{1 + k' c_2} dx', \quad (10)$$

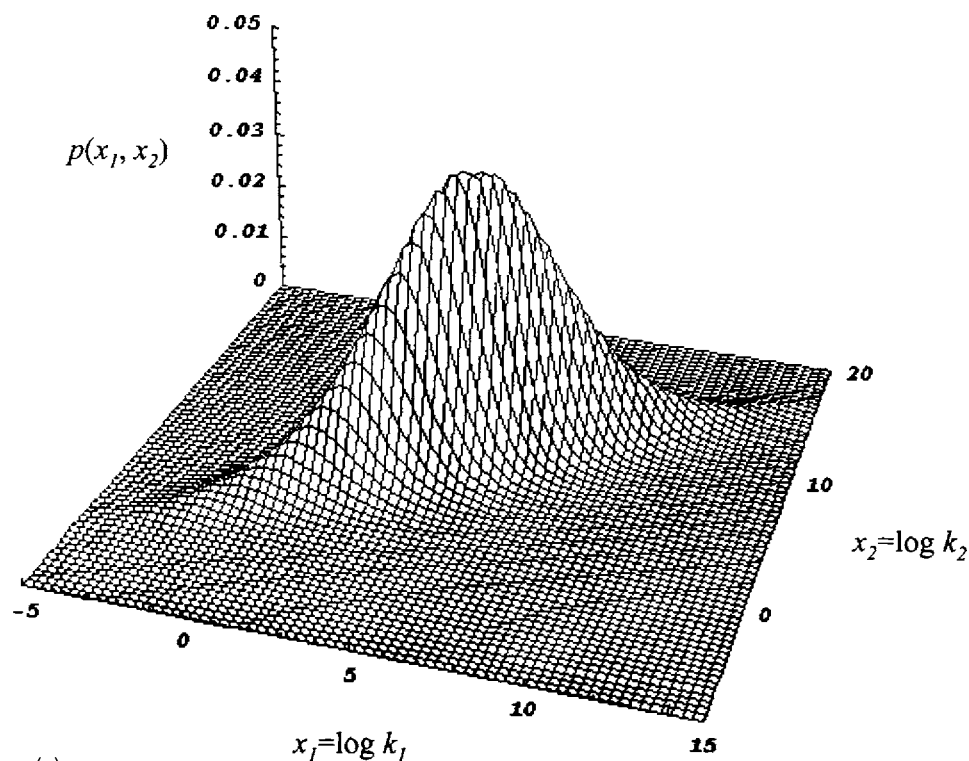
i.e., the metal coverage of the multicomponent system has been expressed by an integral equation corresponding to a monocomponent one. As a consequence, all the methods, both numerical and analytical, suggested in the literature to solve the integral equation (1) can then be applied to find the conditional affinity spectrum, bearing in mind that the resulting spectrum depends on the proton concentration, c_1 . This indicates, for instance, that the CAS can be obtained directly from the experimental binding data without the knowledge of the underlying multidimensional affinity spectrum.

Figure 1 illustrates the computation of the conditional affinity spectrum from the multidimensional one. The starting point is the multidimensional affinity spectrum depicted as a three-dimensional (3D) plot in Fig. 1(a) and as a contour plot in Fig. 1(b). As $p'(x'; c_1)$ is the density of sites of a given fixed effective affinity x' , we have to integrate the multidimensional spectrum over the curve $x_2 - \log(1 + e^{x_1 + \mu_1}) = x'$, where x' is kept constant, to obtain $p'(x'; c_1)$, as Eq. (9) indicates. For a fixed c_1 , lines for different constant x' values are plotted in Fig. 1(b). Notice that for low enough x_1 values, all the lines are parallel with ordinate at $x_1 \rightarrow -\infty$ given by the constant x' . Increasing x_1 , the curves move towards higher x_2 , this indicating that stronger complexing sites are required in order to compensate for the increased work needed to extract the proton.

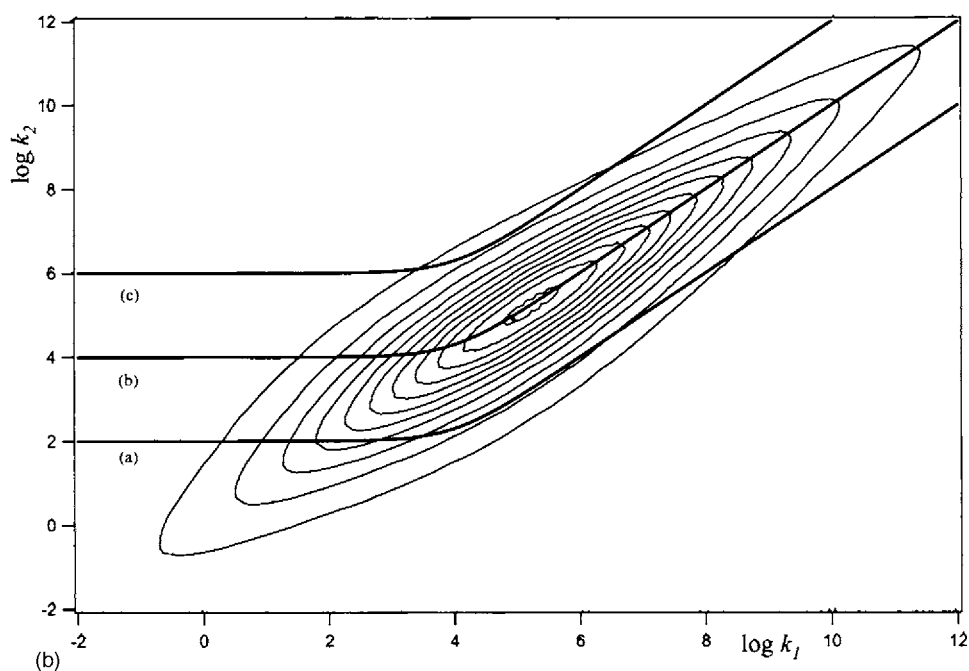
Let us apply the procedure above developed to find the CAS associated to the NICA. This isotherm is widely used to describe the competitive binding of metal ions to natural organic matter in environmental chemistry. The metal coverage is then expressed as^{23,27,28}

$$\begin{aligned} \theta_2(c_1, c_2) &= \frac{n_2}{n_1} \frac{(\bar{k}_2 c_2)^{n_2}}{(\bar{k}_1 c_1)^{n_1} + (\bar{k}_2 c_2)^{n_2}} \frac{((\bar{k}_1 c_1)^{n_1} + (\bar{k}_2 c_2)^{n_2})^p}{1 + ((\bar{k}_1 c_1)^{n_1} + (\bar{k}_2 c_2)^{n_2})^p}. \end{aligned} \quad (11)$$

We will restrict ourselves to the case $n_1 = n_2 = n$, a condition required for the existence of a multidimensional affinity spectrum underlying NICA isotherm (see, for instance, Refs. 17 and 20). So proceeding, we attach to the interpretation of the NICA isotherm where both n_i and p are heterogeneity parameters related to the particular ion and to the ligand, respectively.^{21,28} In order to obtain the CAS corresponding to the metal we have to consider the proton concentration as a fixed parameter of the system, and further solve the integral



(a)



(b)

FIG. 1. (a) Three-dimensional plot of the multidimensional affinity spectrum underlying NICA isotherm. Parameters: $\bar{k}_1=10^5$, $\bar{k}_2=10^7$, $n_1=n_2=0.5$, $p=0.5$. (b) Affinity spectrum of (a) represented as a contour plot. Iso- x' curves for $\log k'=2$ (a), $\log k'=4$ (b), $\log k'=6$ (c), and $pH=4$ are also depicted in the figure.

equation (1) for $p(x)$ with $\theta(c)$ given by (11). By using complex variable analysis, the inversion of a monocomponent isotherm is straightforward. Using the inversion formula proposed in Refs. 16 and 29, bearing in mind that proton concentration must be taken as a constant, the underlying affinity spectrum is given by¹⁶

$$p'(x'; c_1) = (\ln 10)k' \left| \operatorname{Im} \frac{\theta_2(c_1, z)}{z} \right|_{z=-1/k'}, \quad (12)$$

where $x'=\log k'$, z is a variable in the complex plane and “Im” represents the operation “take the imaginary part.” Af-

ter some algebra, Eq. (12) applied to the NICA isotherm leads to

$$p'(x'; c_1) = \frac{\ln 10}{\pi} \frac{(\bar{k}_2/k')^n M^{p-1}}{1 + M^{2p} + 2M \cos(p\phi)} \times [\sin(\pi n - (1-p)\phi) + M^p \sin(\pi n - \phi)], \quad (13)$$

where

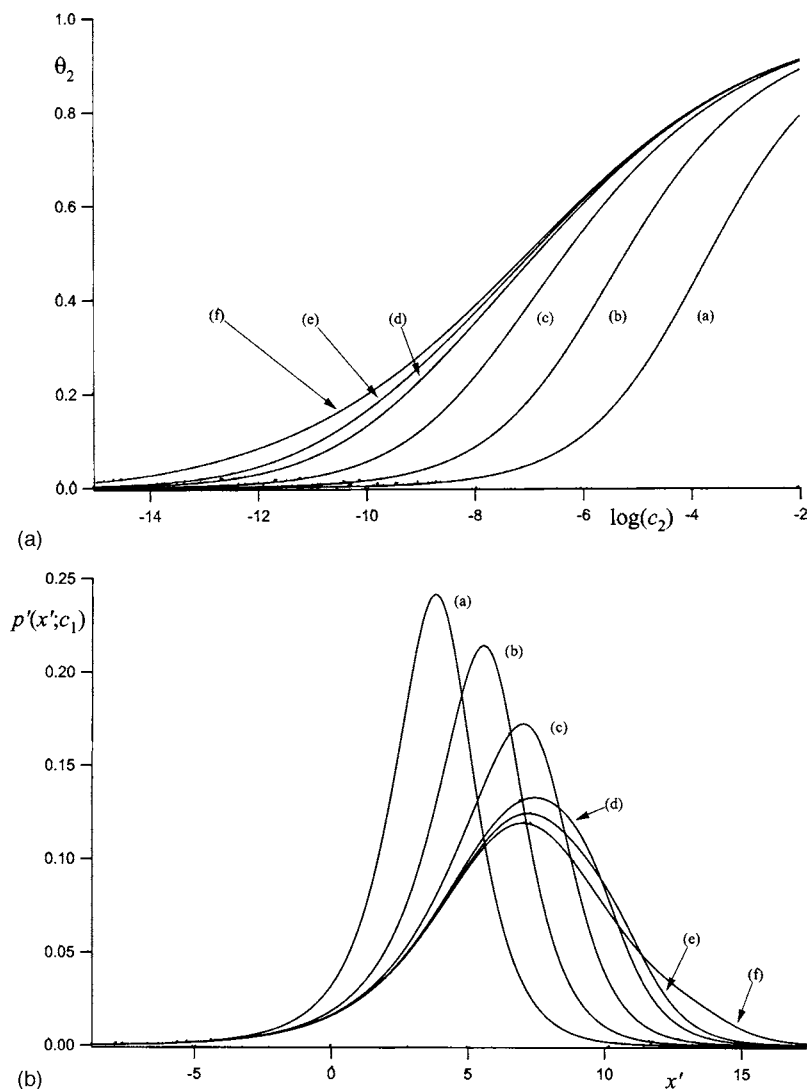


FIG. 2. (a) Binding curve θ_2 vs $\log(c_2)$ in a system where two cations (proton denoted as 1 and a metal ion denoted as 2) compete for the binding sites at different pH values: pH=2 (a), pH=4 (b), pH=6 (c), pH=8 (d), pH=9 (e), and pH=12 (f). The binding curves correspond to a NICA isotherm with $\bar{k}_1=10^5$, $\bar{k}_2=10^7$, $n_1=n_2=0.4$, and $p=0.5$. (b) Conditional affinity spectra underlying the binding curves depicted in (a) indicating the effective distribution of affinities seen by the metal ion at the corresponding proton concentration.

$$M(c_1, k') = [(\bar{k}_1 c_1)^{2n} + (\bar{k}_2/k')^{2n} + 2(\bar{k}_1 c_1)^n (\bar{k}_2/k')^n \cos(\pi n)]^{1/2} \quad (14)$$

and

$$\cos(\phi) = \frac{(\bar{k}_1 c_1)^n + (\bar{k}_2/k')^n \cos(\pi n)}{M}. \quad (15)$$

A pictorial representation of the procedure is given in Fig. 2: the initial information consist in a set of metal binding curves for different pH values [see Fig. 2(a)]. In the illustrated case we have used the NICA isotherm with parameters $n_1=n_2=n=0.4$. Each of such binding curves can now be regarded as a monocomponent binding isotherm for the metal, to which the usual inversion procedures can be applied. Using Eq. (13), a set of affinity spectra, one for each pH value [see Fig. 2(b)], have been computed. It can be observed that, in lowering the pH value, the CAS is shifted towards lower affinities indicating that the work required to exchange protons is increasing. Finally, for high enough pH values, the resulting spectra tend to collapse converging to a Sips distribution, since only one component (the metal ion) is being complexed. Actually, taking $c_1=0$ in expression (13), with

$n_1=n_2$, a Sips distribution function with average affinity \bar{k}_2 and heterogeneity parameter $m=n_2 p$ is recovered,

$$p'(x'; c_1=0) = \frac{\ln 10}{\pi} \frac{\sin(\pi m)}{(\bar{k}_2/k')^{-m} + (\bar{k}_2/k')^m + 2 \cos(\pi m)} \quad (16)$$

in agreement with the fact that NICA reduces to a Langmuir-Freundlich isotherm when only one component is present.¹⁷

III. MEAN AND VARIANCE OF THE CAS: CONDITIONAL AFFINITY AND CONDITIONAL HETEROGENEITY

In the previous section we have seen that a multicomponent system can be treated as an equivalent monocomponent one by keeping constants the concentrations of the rest of components. This has led to the definition of the conditional affinity spectrum of this ion. On the other hand, as it has been pointed out previously,³⁰ the mean and the variance of a one-dimensional affinity spectrum are the parameters that almost determine the coverage at a wide range of concentrations of the complexing ion, i.e., very different affinity spectra sharing a common mean and variance lead to the same

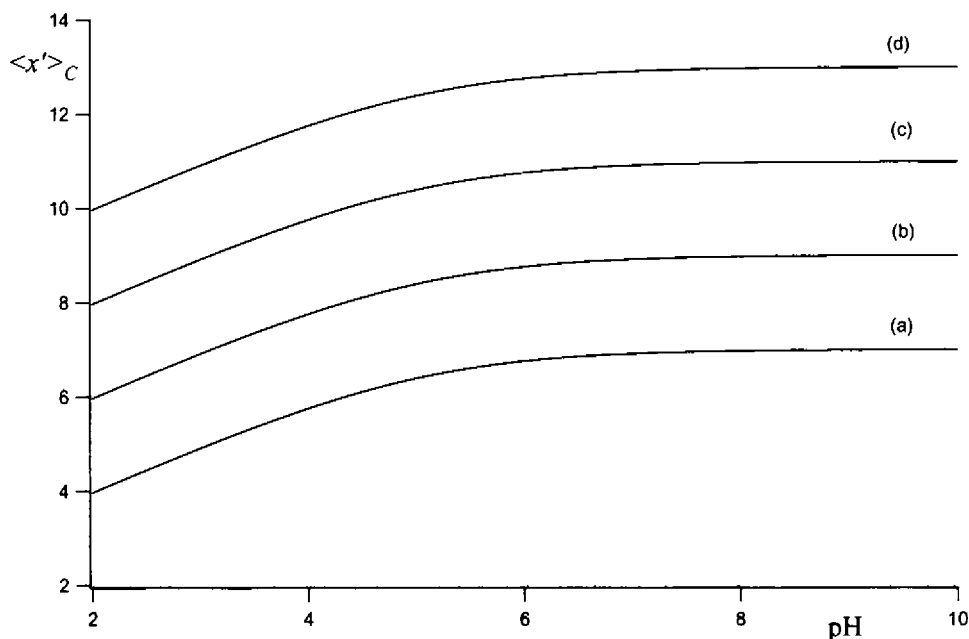


FIG. 3. Mean of the CAS corresponding to a NICA isotherm as a function of pH . Parameters: $n=0.5$, $p=1$, $\bar{k}_1/M^{-1}=10^5$ and $\bar{k}_2=10^7$ (a), $\bar{k}_2=10^9$ (b), $\bar{k}_2=10^{11}$ (c), $\bar{k}_2=10^{13}$ (d).

coverage at a very wide range of concentrations. In the same way, for a fixed pH and concentration of the rest of competing binding ions, the CAS distribution exhibits some mean affinity and a certain heterogeneity. The variance of the CAS distribution is a measure of this heterogeneity. Let us analyze the dependence of the mean and the variance of the CAS on the pH (the same procedure can be used to obtain the dependence of these statistics on the concentration of the other competing ions) and how this dependence can be interpreted in terms of the underlying binding events.

A. Mean of the CAS

The mean of the CAS can be calculated as

$$\langle x' \rangle_C = \int_{-\infty}^{\infty} x' p'(x'; c_1) dx', \quad (17)$$

where here and in the rest of this work, the symbol $\langle \rangle_C$ is used to denote the average over the conditional affinity spectrum while $\langle \rangle$ will indicate the average over the two-dimensional affinity spectrum.

Replacing the expression for the CAS, (9), in (17),

$$\langle x' \rangle_C = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} x' p(x_1, x' + \log(1 + k_1 c_1)) dx' dx_1. \quad (18)$$

By using the change of variables $x_2 = x' + \log(1 + k_1 c_1)$, and further integrating over x_2 , Eq. (18) becomes

$$\langle x' \rangle_C = \langle x_2 \rangle - \int_{-\infty}^{\infty} \log(1 + k_1 c_1) p_1(x_1) dx_1, \quad (19)$$

where $\langle x_2 \rangle$ is the mean affinity of the metal, $\langle x_2 \rangle = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} x_2 p(x_1, x_2) dx_1 dx_2$, and $p_1(x_1) = \int_{-\infty}^{\infty} p(x_1, x_2) dx_2$ is the affinity spectrum of the proton in absence of metal ions. On the other hand, Eq. (19) can be rewritten as

$$\langle x' \rangle_C = \langle x_2 \rangle - \int_{-\infty}^{\log c_1} \left[\int_{-\infty}^{\infty} \frac{k_1 c_1}{1 + k_1 c_1} p_1(x_1) dx_1 \right] d \log c_1. \quad (20)$$

Finally, noting that the term between brackets in Eq. (20) is the proton coverage in absence of metal ions, $\theta_1(c_1, 0)$, we obtain the sought expression for the conditional average affinity,

$$\langle x' \rangle_C = \langle x_2 \rangle - \int_{-\infty}^{\log c_1} \theta_1(c_1, 0) d \log c_1. \quad (21)$$

The resulting expression has a general validity, restricted only to the suitability of the IE approach to describe the thermodynamic properties of the system. A clear physical interpretation of the conditional average affinity emerges from Eq. (21). It indicates that the mean affinity seen by the metal ion at some pH is lower than the mean affinity that would experience in absence of protons. The subtracting term, the second term of the right-hand side (rhs) of (21), increases as the proton concentration increases and just reflects the increasing work that the metal has to do to be bound, i.e., the interchanging energy.

Furthermore, expression (21) has a remarkable property. Notice that in order to know the average affinity for the metal at a given pH , we only need a constant, $\langle x_2 \rangle$, and the proton titration curve of the ligand in absence of metal. As a consequence, the mean conditional affinity of any metal is, up to an additive constant, independent of the nature of the metal, provided that Eq. (2) is accepted to describe the thermodynamic properties of the system. This fact is illustrated in Fig. 3, where we have plotted the conditional average affinity as a function of the pH , for systems in which proton binding is described by a Langmuir-Freundlich isotherm. The different curves correspond to different ions, whose binding properties can be, in principle, completely different, and thus be characterized by very different multicomponent isotherms. However, in agreement with the result (21), the

curves only differ in a constant value, characteristic of the metal ion under study. Finally, it is worth noting that, since $\langle x' \rangle_C$ and the $\int_{-\infty}^{\log c_1} \theta_1(c_1, 0) d \log c_1$ can be experimentally determined in an independent way, Eq. (21) can be regarded as a test of the validity of the integral representation (2), by simply noting that the addition $\langle x' \rangle_C + \int_{-\infty}^{\log c_1} \theta_1(c_1, 0) d \log c_1$ should be independent of the pH and equal to $\langle x_2 \rangle$.

B. Variance of the CAS

A similar approach can be applied to obtain the variance of the conditional affinity spectrum, which is defined as

$$\sigma'^2(c_1) = \langle x'^2 \rangle_C - \langle x' \rangle_C^2 \quad (22)$$

and measures the spread of the conditional affinity values. As it happened with the conditional average affinity, $\langle x' \rangle_C$, the quantity $\sigma'^2(c_1)$ can be obtained directly from experimental data or from the multicomponent isotherm under study. In a previous paper, we showed that the variance of any affinity spectrum can be directly obtained from the isotherm, without the detailed knowledge of the underlying affinity spectrum.²²

$$\sigma^2 = \int_{-\infty}^{\infty} (\log c)^2 \frac{\partial \theta(c)}{\partial \log c} d \log c - \frac{(\pi/\ln 10)^2}{3}. \quad (23)$$

By trivially adapting this result to the present situation, σ'^2 can be expressed as

$$\sigma'^2(c_1) = \int_{-\infty}^{\infty} (\log c_2)^2 \frac{\partial \theta_2(c_1, c_2)}{\partial \log c_2} d \log c_2 - \frac{(\pi/\ln 10)^2}{3}. \quad (24)$$

Although useful from a practical point of view, expression (24) does not provide a physical interpretation of the apparent heterogeneity reflected by $\sigma'^2(c_1)$. With this aim, let us recall the definition of $p'(x', c_1)$ to calculate $\sigma'^2(c_1)$. By using Eq. (9), the computation of the first term in (22) leads to

$$\begin{aligned} \langle x'^2 \rangle_C &= \int_{-\infty}^{\infty} x'^2 p'(x'; c_1) dx' \\ &= \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} (x_2 - \log(1 + k_1 c_1))^2 p(x_1, x_2) dx_1 dx_2 \\ &= \langle x_2^2 \rangle - 2 \langle x_2 \log(1 + k_1 c_1) \rangle + \langle \log^2(1 + k_1 c_1) \rangle. \end{aligned} \quad (25)$$

Inserting Eqs. (19) and (25) in (22), the CAS variance can be expressed as

$$\sigma'^2(c_1) = \sigma_2^2 - 2\sigma_{\text{corr}}^2(c_1) + \sigma_{\text{first component}}^2(c_1), \quad (26)$$

with

$$\sigma_2^2 = \langle x_2^2 \rangle - \langle x_2 \rangle^2, \quad (27)$$

$$\sigma_{\text{corr}}^2(c_1) = \langle x_2 \log(1 + k_1 c_1) \rangle - \langle x_2 \rangle \langle \log(1 + k_1 c_1) \rangle, \quad (28)$$

and

$$\sigma_{\text{first component}}^2(c_1) = \langle \log^2(1 + k_1 c_1) \rangle - \langle \log(1 + k_1 c_1) \rangle^2. \quad (29)$$

Each one of the three terms appearing in (26) can be regarded as coming from a different source of apparent or conditional heterogeneity.

- (i) σ_2^2 is the variance of the affinity spectrum for the metal in absence of protons, i.e., it is a measure of the intrinsic heterogeneity of the ligand for the metal ion and hence it is independent from pH.
- (ii) $\sigma_{\text{first component}}^2(c_1)$ concerns the variance of the exchange work needed to bind metals at a given pH. As the exchange work requires the release of protons from the complexing sites, the variance of the exchange work will depend on the heterogeneity of the proton binding energy. As a result, $\sigma_{\text{first component}}^2(c_1)$ increases the variance of the conditional affinity spectrum and it can be obtained from data of the system containing only protons.
- (iii) Finally, $\sigma_{\text{corr}}^2(c_1)$ is a very interesting term which depends on the correlation between the binding energy of proton and metal and which tends to decrease the variance of the conditional affinity spectrum. Correlation is a measure of the linear association between the affinity of the metal and that of the proton for the sites. When a large affinity value for the proton does not imply a large or low affinity value for the metal there is no correlation, and we say that the two binding energies are independent, i.e., $p(x_1, x_2) = p_1(x_1)p_2(x_2)$. In this case, σ_{corr}^2 is zero, and $\sigma'^2(c_1)$ is the result of adding two independent contributions coming from the two components, i.e.,

$$\sigma'^2(c_1) = \sigma_2^2 + \sigma_{\text{first component}}^2(c_1). \quad (30)$$

On the other hand, when the metal and proton binding energies are fully correlated, i.e., $p(x_1, x_2) = p_1(x_1)\delta(x_2 - x_1 - \alpha)$, it is easy to show that σ_{corr}^2 becomes

$$\sigma_{\text{corr}}^2 = \langle x_1 \log(1 + k_1 c_1) \rangle - \langle x_1 \rangle \langle \log(1 + k_1 c_1) \rangle, \quad (31)$$

which only depends on information concerning the proton binding. So we conclude that, when the binding is fully correlated or fully uncorrelated, the apparent heterogeneity only depends, up to an additive constant, on information that can be obtained using only the proton titration curve. Actually, this means that Eqs. (26), (30), and (31) can be used to experimentally assess whether the competitive binding is correlated and to quantify the correlation of the binding energies of metal and proton as we will see in more detail in the next section.

In Fig. 4 we have plotted $\sigma'^2(c_1)$ in terms of the pH for bicomponent systems whose binding properties are described by means of a NICA isotherm with $n_1 = n_2 = n$. The product np has been kept constant while different values of the parameter p have been used in the different curves. Besides, we have plotted two limiting cases: the fully correlated and the

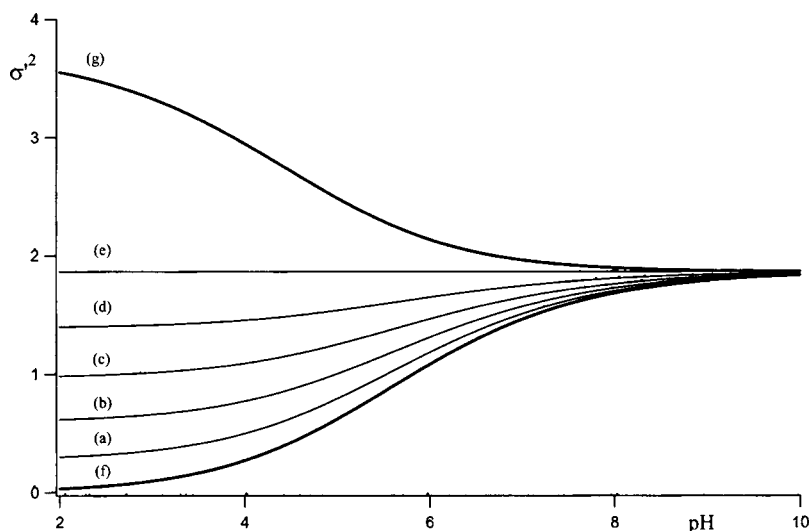


FIG. 4. Variance of the CAS corresponding to a NICA isotherm as a function of pH. Parameters: $\bar{k}_1=10^5$, $\bar{k}_2=10^7$, $np=0.5$ and $p=0.6$ (a), $p=0.7$ (b), $p=0.8$ (c), $p=0.9$ (d), $p=1$ (e). Curves (f) and (g) correspond to the fully correlated and fully uncorrelated cases, respectively.

fully uncorrelated systems. We can see that for noncorrelated systems, $\sigma'^2(c_1)$ is an increasing function of the proton concentration. In effect, according to (30), there are only two independent, and thus additive, sources of heterogeneity, σ_2^2 and $\sigma_{\text{first component}}^2(c_1)$, the latter being related to the fluctuations of the exchange work. Conversely, in presence of some correlation, the variance of the conditional spectrum decreases. We could say that correlation introduces some additional ordering in the metal binding, resulting in a lower heterogeneity, which increases with c_1 . As a consequence, increasing the n value, while the product np is kept constant, $\sigma'^2(c_1)$ progressively tends to the conditional variance corresponding to the fully correlated system, in agreement with the role of parameter p as a common source of heterogeneity for all the ions.^{17,22}

IV. QUANTIFYING THE BINDING CORRELATION BY USING THE CONDITIONAL AFFINITY SPECTRUM

A. The covariance matrix corresponding to an arbitrary multicomponent isotherm

We have shown that the analysis presented for the variance of the CAS leads to the conclusion that apparent heterogeneity and binding correlation are closely related. Let us provide some insight in this general assertion in the framework of the IE approach. The quantity of interest is the covariance of the affinity spectrum, C_{12} , which is defined as the multiple integral,

$$C_{12} = \langle x_1 x_2 \rangle - \langle x_1 \rangle \langle x_2 \rangle, \quad (32)$$

where

$$\langle x_1 x_2 \rangle = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} x_1 x_2 p(x_1, x_2) dx_1 dx_2. \quad (33)$$

It is useful to express the binding correlation in terms of the correlation coefficient, a dimensionless quantity defined as $R = C_{12}/(\sigma_1 \sigma_2)$, $-1 < R < 1$.

According to (32), the computation of R implies the computation of the multidimensional affinity spectrum, by inverting Eq. (2) and further performance of the multiple integrals appearing in (32). The main difficulty of this pro-

cedure is that the affinity spectrum for most of isotherms is not known. An alternative strategy was provided in Ref. 22. There, we showed that R can be directly computed from the expression of the multicomponent isotherm without the knowledge of the underlying affinity spectrum. The following expression for C_{12} was reported:

$$C_{12} = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \log c_1 \log c_2 \frac{\partial \theta(c_1, c_2)}{\partial \log c_1 \partial \log c_2} \times d \log c_1 d \log c_2 - \langle x_1 \rangle \langle x_2 \rangle - \frac{(\pi/\ln 10)^2}{6}, \quad (34)$$

with $\theta = \theta_1 + \theta_2$. Following this way, an analytical expression for the correlation of the NICA isotherm with $n_1 = n_2 = n$ has been obtained²²

$$R = \frac{2 - p^2 - n^2 p^2}{2(1 - n^2 p^2)}, \quad (35)$$

which indicates that NICA isotherm always considers some degree of positive correlation between proton and metal binding. Notice that for $n=1$, we have $R=1$, which implies full correlation, in agreement with the analysis performed in Sec. III.

B. Finding the covariance matrix from the CAS

Nevertheless, the use of Eqs. (32)–(34) to obtain analytical expressions for the binding correlation is limited by the fact that, for most multicomponent isotherms, the double integrals appearing in (34) cannot be performed analytically. This problem is especially relevant in the cases where the coverages cannot be explicitly expressed in terms of the concentrations, as it happens in most multicomponent isotherms (Frumkin, Temkin, etc.). Here we propose a new method which allows the computing of the binding correlation by profiting from the properties of the CAS variance.

Taking the limit $c_1 \rightarrow \infty$ in expressions (28) and (29) and noting that, in this limit, $k_1 c_1 \gg 1$, we have

$$\lim_{c_1 \rightarrow \infty} \sigma_{\text{first component}}^2 = \langle (x_1 + \log c_1)^2 \rangle - \langle x_1 + \log c_1 \rangle^2$$

$$= \langle x_1^2 \rangle - \langle x_1 \rangle^2 = C_{11} = \sigma_1^2, \quad (36)$$

and

$$\lim_{c_1 \rightarrow \infty} \sigma_{\text{corr}}^2 = \langle x_2(x_1 + \log c_1) \rangle - \langle x_2 \rangle \langle x_1 + \log c_1 \rangle$$

$$= \langle x_1 x_2 \rangle - \langle x_1 \rangle \langle x_2 \rangle = C_{12}, \quad (37)$$

where σ_1^2 represents the intrinsic heterogeneity for the proton and C_{12} is the covariance between the binding energies of both components.

Thus, in the limit $c_1 \rightarrow \infty$, the variance of the conditional affinity spectrum becomes

$$\lim_{c_1 \rightarrow \infty} \sigma'^2 = \sigma_1^2 + \sigma_2^2 - 2C_{12}. \quad (38)$$

The covariance C_{12} can hence be expressed as

$$C_{12} = \frac{1}{2} \left(\sigma_1^2 + \sigma_2^2 - \lim_{c_1 \rightarrow \infty} \sigma'^2 \right). \quad (39)$$

Equation (39) is the sought expression for the binding correlation. All the terms in the right-hand side of (39) can be directly computed by using just the properties of monocomponent systems: the calculation of the variance of the affinity spectrum for monocomponent systems implies the calculation of single integrals, as indicated by Eq. (23), and σ_1^2 (and σ_2^2) can be directly obtained by taking $c_2 \rightarrow 0$ (and $c_1 \rightarrow 0$) in the multicomponent isotherm,

$$\lim_{c_1 \rightarrow 0} \sigma'^2 = \sigma_2^2, \quad \lim_{c_2 \rightarrow 0} \sigma'^2 = \sigma_1^2. \quad (40)$$

Moreover, expressions (38) and (40) indicate that the difference between the limiting σ'^2 values at low and high enough pH values gives $\lim_{c_1 \rightarrow \infty} \sigma'^2 - \lim_{c_1 \rightarrow 0} \sigma'^2 = \sigma_1^2 - 2C_{12}$. Thus, the computation of σ' from experimental metal binding data in the limit of very low and very high pH range together with the proton titration curve can provide a way of determining the correlation.

Let us apply the result (39) to provide analytical expressions for the binding correlation in the case of the multicomponent Frumkin isotherm.

C. Binding correlation in Frumkin isotherm

The Frumkin isotherm was originally derived to take into account the interaction between the binding agents by means of the introduction of some interaction parameters ρ_{ij} . For a bicomponent system, it reads

$$b_1 c_1 = \frac{\theta_1}{1 - \theta_1 - \theta_2} \exp(\rho_{11} \theta_1 + \rho_{12} \theta_2), \quad (41)$$

$$b_2 c_2 = \frac{\theta_2}{1 - \theta_1 - \theta_2} \exp(\rho_{12} \theta_1 + \rho_{22} \theta_2). \quad (42)$$

The calculation of the correlation coefficient in a bicomponent Frumkin isotherm will clarify the advantages of the new approach based on the properties of the CAS and outlined in Sec. IV A. First, it is convenient to rewrite (41) as

$$1 - \theta_1 - \theta_2 = \frac{\theta_1}{b_1 c_1} \exp(\rho_{11} \theta_1 + \rho_{12} \theta_2). \quad (43)$$

Clearly, in the limit $c_1 \rightarrow \infty$ the right-hand side of (43) tends to zero. Hence,

$$1 - \theta_1 - \theta_2 \approx 0 \Rightarrow \theta_1 \approx 1 - \theta_2. \quad (44)$$

On the other hand, dividing Eq. (41) by (42) and replacing (43) in the resulting equation we have

$$\frac{b_1 c_1}{b_2 c_2} = \frac{1 - \theta_2}{\theta_2} \exp((\rho_{11} - \rho_{12}) \theta_1 + (\rho_{12} - \rho_{22}) \theta_2). \quad (45)$$

Rearranging terms in (45), the macromolecular coverage for the metal becomes

$$\theta_2 = \left(\frac{b_2}{b_1 c_1} e^{(\rho_{11} - \rho_{12})} \right) c_2 (1 - \theta_2)$$

$$\times \exp(-(\rho_{11} + \rho_{22} - 2\rho_{12}) \theta_2), \quad (46)$$

which can be recognized as a monocomponent Frumkin isotherm with affinity parameter $\tilde{b} = (b_2/b_1 c_1) e^{(\rho_{11} - \rho_{12})}$ and interaction parameter $\tilde{\rho} = \rho_{11} + \rho_{22} - 2\rho_{12}$,

$$\theta_2 = \tilde{b} c_2 (1 - \theta_2) \exp(-\tilde{\rho} \theta_2). \quad (47)$$

The variance of the spectrum underlying the monocomponent Frumkin was reported in Ref. 30. Applied to (47) it yields $\sigma^2 = (\tilde{\rho}^2/12 + \tilde{\rho})/(\ln 10)^2$. Likewise, σ_1^2 and σ_2^2 are given by $\sigma_i^2 = (\rho_{ii}^2/12 + \rho_{ii}^2)/(\ln 10)^2$. With these results at hand, the limit in (38) becomes

$$\lim_{c_1 \rightarrow \infty} \sigma'^2 = \left[\frac{(\rho_{11} + \rho_{22} - 2\rho_{12})^2}{12} + (\rho_{11} + \rho_{22} - 2\rho_{12}) \right] / (\ln 10)^2 \quad (48)$$

and replacing (48) in Eq. (39), the correlation coefficient corresponding to the affinity spectrum for the multicomponent Frumkin isotherm is given by

$$R = \frac{12\rho_{12} + 2\rho_{12}(\rho_{11} + \rho_{22}) - \rho_{11}\rho_{22} - 2\rho_{12}^2}{(\rho_{11}^2 + 12\rho_{11})^{1/2}(\rho_{22}^2 + 12\rho_{22})^{1/2}}. \quad (49)$$

It is worth noticing from (48) that, since the conditional variance is a positive quantity, i.e., $\sigma'^2 > 0$, there is a restriction on the values of the Frumkin parameters for the existence of the CAS. This condition is

$$\rho_{12} < \frac{\rho_{11} + \rho_{22}}{2}, \quad (50)$$

i.e., the interaction energy between different components has to be smaller than the average of the intrinsic monocomponent interaction energies. Furthermore, since the existence of the CAS is necessary for the existence of the affinity spectrum, (50) can also be seen as a necessary condition for the existence of a multidimensional affinity spectrum underlying a Frumkin isotherm.

Figure 5 plots the variance of the CAS corresponding to a Frumkin isotherm as a function of the pH for different ρ_{12} values. Inside the figure, we have depicted the correlation

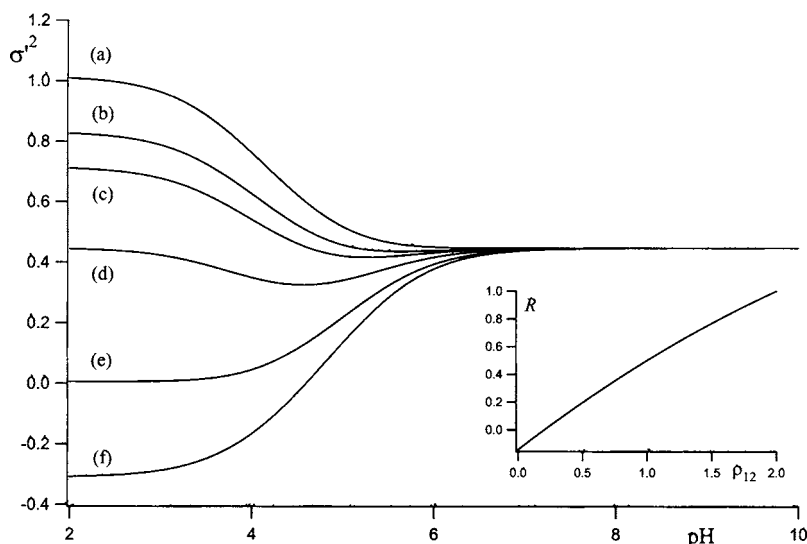


FIG. 5. Variance of the CAS underlying the Frumkin isotherm as a function of pH . Parameters: $\rho_{11}=2$, $\rho_{22}=2$ and $\rho_{12}=0$ (a), $\rho_{12}=0.3$ (b), $\rho_{12}=0.5$ (c), $\rho_{12}=1$ (d), $\rho_{12}=2$ (e), and $\rho_{12}=3$ (f). Inset: correlation coefficient, R , for the same isotherm with $\rho_{11}=2$, $\rho_{22}=2$ as a function of ρ_{12} .

coefficient as a function of ρ_{12} . Notice that the correlation coefficient increases from $R=0$ to $R=1$ as ρ_{12} increases from 0 to $\rho_{12}=\rho_{11}+\rho_{22}/2$. A further increase of ρ_{12} yields negative values of σ'^2 . These values do not have physical meaning [see curve (f) in the figure], this indicating the nonexistence of the CAS (and, thus, of affinity spectrum) for this set of parameters ($\rho_{11}=\rho_{22}=2.0$ in the figure). Notice that there are three parameters determining the Frumkin isotherm, just the number of independent elements of the covariance matrix. Thus, there is no restriction on the values of these elements and all the range of R values can be covered. Figure 5 also shows that all ρ_{12} values lead to a common σ'^2 value at high pH range. This σ'^2 value is just $\sigma_2^2=(\rho_{22}^2/12+\rho_{22})/(\ln 10)^2$, the intrinsic variance of the binding energy of the metal according to (40). The σ'^2 value at low pH range is given by (48) and decreases as the correlation increases in agreement with (38).

The procedure outlined in this section can be applied to any multicomponent isotherm and, among them, to the NICA isotherm. As expected, expression (35) for the correlation coefficient, previously obtained by performing the multidimensional integrals involved in Eq. (34), is recovered.

V. CONDITIONAL AFFINITY SPECTRA IN SOME CASES WHERE THE INTEGRAL EQUATION APPROACH IS NOT SUITABLE: SPECIFIC AND MULTIDENTATE BINDING

Up to now, we have dealt with systems which can be described by an underlying multidimensional affinity spectrum (IE approach). We have shown that, if Eq. (2) is accepted as a good thermodynamic description of the system, a CAS can always be defined. However, the fulfillment of this condition is too restrictive for the existence of the CAS, so that a conditional spectrum could in some cases be defined even when Eq. (2) does not apply.

Although a general CAS formulation is out of the scope of the present work, there are at least two kinds of systems of interest for which the IE approach does not apply. We will use these cases to generalize now the CAS. Let us first con-

sider the case of a ligand which has some selective sites for one of the competing cations, i.e., sites to which only one of the cations, for instance, the cation i , is able to be bound. Another case of special interest is the treatment of competitive systems where the competing cations bind with different stoichiometries to the functional groups of the ligand. Actually, Eq. (2) requires that both components bind to the sites in a one-to-one stoichiometry, so that Eq. (2) does not account for the possible formation of chelates. However, in metal binding to macromolecules, multidentate complexation has been widely reported^{26,31,32} and exchange ratios higher than 1 have been recorded.²³ Let us analyze these two situations in the framework of the conditional affinity spectrum.

A. A system where not all the ions compete for all the sites

When some sites are able to selectively bind a metal cation, while protons cannot be bound to these sites, the metal coverage can be written as

$$\theta_2(c_1, c_2) = q_{ns} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} p_{ns}(x_1, x_2) \frac{k_2 c_2}{1 + \sum_{j=1} k_j c_j} dx_1 dx_2 + q_s \int_{-\infty}^{\infty} p_s(x_2) \frac{k_2 c_2}{1 + k_2 c_2} dx_2, \quad (51)$$

where q_{ns} and $q_s=1-q_{ns}$ represent the fraction of sites which have nonselective and selective bindings for the metal ions, respectively. The first term of the rhs of (51) specifies the occupation of the nonselective sites. The structure of this term indicates that the nonselective sites fulfill all the properties given in Secs. II and III for the CAS underlying a multidimensional affinity spectrum. Thus the global CAS of this system consists in the CAS distribution associated to the nonselective binding sites plus the affinity distribution of the selective sites. The resulting distribution is depicted in Fig. 6 for different pH values. The parameters of the figure have been selected so that the distribution is bimodal in order to evidence the effect of pH . Notice that the distribution asso-

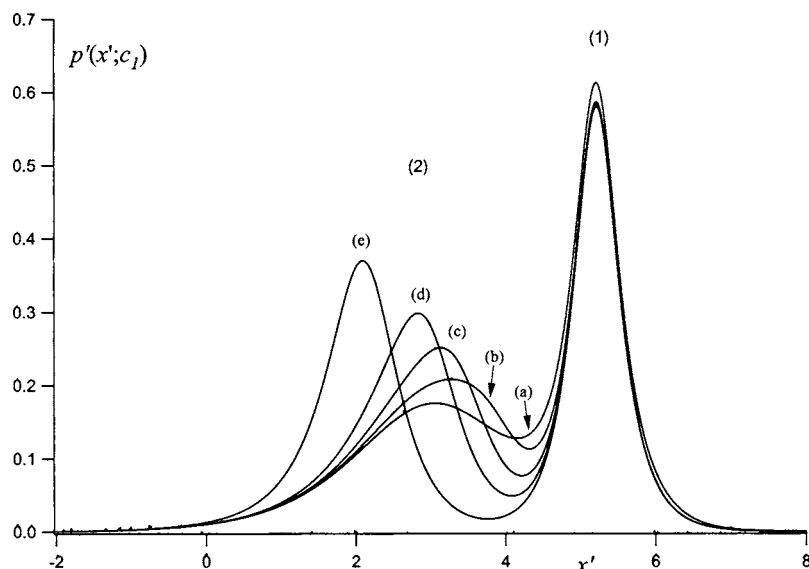


FIG. 6. CAS of a system with selective (1) and nonselective sites for the metal at different pH values: (a) pH=12, (b) pH=7, (c) pH=6, (d) pH=5, and (e) pH=3. The peak (1) corresponds to the selective sites with weight $q_s=0.5$ described by a Sips distribution and parameters $m=0.5$, $\bar{k}=10^{12}$. The peak (2) corresponds to the nonselective sites of weight $q_{ns}=0.5$ described by the CAS underlying the NICA isotherm with $k_1=10^5$, $\bar{k}_2=10^5$, and $n=p=0.5$.

ciated to the competitive sites (those corresponding to the metal binding sites able to be protonated) is shifted when changing the pH value as described in Sec. III, while the affinity of the sites able to bind only metals is not affected by a pH change. Finally, we remark that Eq. (51) assumes that there is no interaction between metals bound to selective and nonselective sites. If interactions were present, some changes in the selective binding distribution should be observed as a response to a pH change. The mean conditional affinity can then be written as

$$\langle x' \rangle_C = \langle x_2 \rangle - q_{ns} \int_{-\infty}^{\log c_1} \theta_1(\mu_1, 0) d \log c_1, \quad (52)$$

which differs from the average conditional affinity provided in Sec. III [see Eq. (21)]. This is a consequence from the fact that Eq. (21) was deduced under the hypothesis of the suitability of the IE approach which now does not apply.

B. A system with multidentate complexation

Let us address the question of multidentate complexation considering a linear chain able to bind protons and metal ions either in monodentate or bidentate bonding as depicted in Fig. 7. This system will be used to exemplify the application of the CAS in describing multidentism phenomena. Interactions between occupied sites are assumed to be negligible. In a recent paper, Koper *et al.*³³ provided a general method to deal with chelate complexation of metals to linear

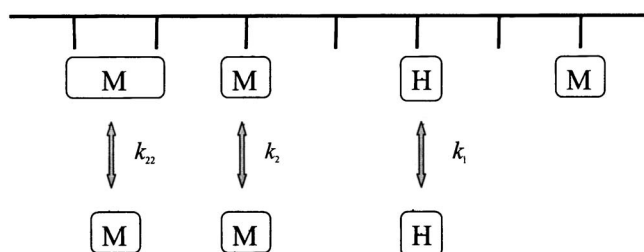


FIG. 7. Linear chain able to bind protons (with stability constant k_1) and metals. The metals can be bound in two different forms: single bond (with stability constant k_2) and bidentate bond (with stability constant k_{22}).

chains. The method is based on generalizing the classical transfer-matrix techniques used in Ising-type models. By using these techniques, the resulting isotherm for the present binding system is given by

$$\theta_2(c_1, c_2) = \frac{1}{2\lambda} \left(k_2 c_2 + \frac{k_2 c_2 (1 + k_2 c_2 + k_1 c_1) + 2k_{22} c_2}{[4k_{22} c_2 + (1 + k_2 c_2 + k_1 c_1)^2]^{1/2}} \right), \quad (53)$$

where k_1 is the equilibrium constant for the proton, k_2 and k_{22} the equilibrium constants for the metal in the monodentate and bidentate forms, respectively, and λ is the highest eigenvalue of the transference matrix,

$$\lambda = \frac{1}{2} (1 + k_1 c_1 + k_2 c_2 + [4k_{22} c_2 + (1 + k_2 c_2 + k_1 c_1)^2]^{1/2}). \quad (54)$$

Under a constant proton concentration, Eq. (53) becomes a monocomponent isotherm which is plotted in Fig. 8(a). Notice that while at low metal concentration the bidentate binding predominates, at high metal concentration the metal binding becomes monodentate independently of the values of the binding parameters k_2 and k_{22} .

Applying the inversion formula (12) to Eq. (53), the CAS corresponding to a given pH value can be obtained. However, we will proceed in a different way. Simple algebra shows that isotherm (53) can be rearranged in the alternative form,

$$\theta_2(c_1, c_2) = \frac{k^{\text{eff}} c_2}{2\lambda^{\text{eff}}} \left(u^{\text{eff}} + \frac{4 - 2u^{\text{eff}} + 2(u^{\text{eff}})^2 k^{\text{eff}} c_2}{2[4k^{\text{eff}} c_2 + (1 - u^{\text{eff}} k^{\text{eff}} c_2)^2]^{1/2}} \right), \quad (55)$$

where

$$\lambda^{\text{eff}} = \frac{1}{2} (1 + u^{\text{eff}} k^{\text{eff}} c_2 + [4k^{\text{eff}} c_2 + (1 - u^{\text{eff}} k^{\text{eff}} c_2)^2]^{1/2}). \quad (56)$$

The parameters k^{eff} and u^{eff} , which are functions of c_1 , are defined as

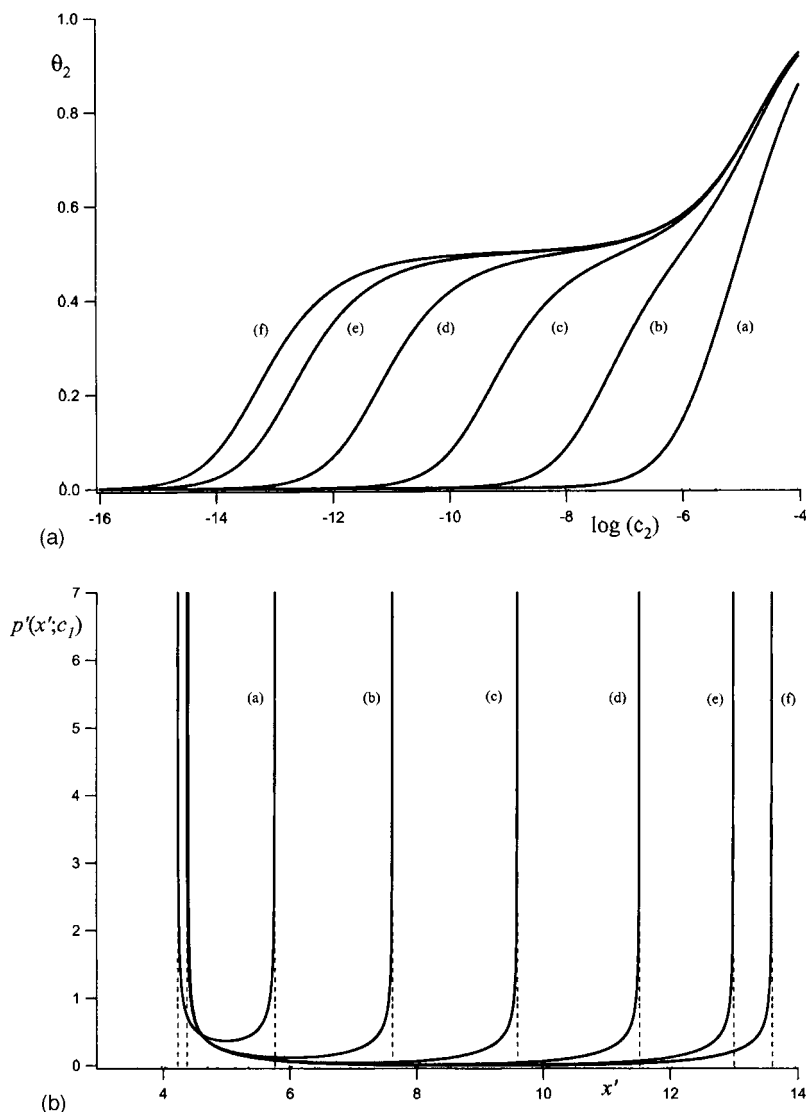


FIG. 8. (a) θ_2 vs $\log(c_2)$ curves as given in Eq. (53) and corresponding to the model depicted in Fig. 7 for different pH values: (a) pH=2, (b) pH=3, (c) pH=4, (d) pH=5, (e) pH=6, (f) pH=8, and (g) pH=12. Parameters: $k_1=10^{15}$, $k_2=10^8$, and $k_{22}=10^{12}$. CAS corresponding to the binding curves depicted in (a).

$$k^{\text{eff}} = \frac{k_2}{1 + k_1 c_1} + \frac{k_{22}}{(1 + k_1 c_1)^2}, \quad (57)$$

$$u^{\text{eff}} = \frac{1}{1 + k_{22}/[k_2(1 + k_1 c_1)]}. \quad (58)$$

For a fixed pH, the isotherm (53) so expressed, is equivalent to that of a system which merely consists in a linear chain to which a metal can be bound by only monodentate binding but showing an effective interaction energy between occupied sites given by $\varepsilon^{\text{eff}} = -kT \ln u^{\text{eff}}$. The affinity spectrum of this system, previously reported in Ref. 29 reads

$$p'(x'; c_1) = \frac{\ln 10}{2\pi} \frac{u^{\text{eff}} k^{\text{eff}}/k' + 1}{((4k^{\text{eff}}/k') - ((u^{\text{eff}} k^{\text{eff}}/k') + 1)^2)^{1/2}}. \quad (59)$$

Equation (59) indicates that this affinity spectrum is symmetric with respect to $\log k^{\text{eff}} + \log u^{\text{eff}}$, being its existence always warranted by the fact that $u^{\text{eff}} < 1$. Using Eq. (59) for the cases depicted in Fig. 8(a), we obtain the CAS spectra depicted in Fig. 8(b). One can see that two peaks appear, the distance between them being dependent on the value of u^{eff} : the smaller u^{eff} , the wider the spectrum. Since the parameters k^{eff} and u^{eff} are pH dependent, Eqs. (57) and (58) explain the

dependence of the CAS on the pH. Notice in Fig. 8(b) that the apparent heterogeneity increases as the ratio k_{22}/k_2 increases. For a better understanding of this behavior, it is useful to analyze the situation that appears in absence of protons. The interaction parameter u^{eff} reduces to

$$u^{\text{eff}} = \frac{1}{1 + k_{22}/k_2}, \quad (60)$$

indicating that even in this case ($c_1=0$), there is some heterogeneity that can be explained in terms of entropy changes in the binding process when multidentate complexation is present. It is known³⁰ that the decrease of the number of microstates (and thus the increasing of entropy) when a new metal is added to the macromolecule is lower in the case of chelate complexation than in monodentate binding, so that, when multidentism is present, the equilibrium constants decrease faster with metal loading. This is reflected in the spectrum through a wider distribution of affinities.

On the other hand, when the proton concentration increases the spectrum shifts towards smaller free energies (as expected), and the apparent heterogeneity becomes smaller. A simple explanation can again be found in Eqs. (57) and (58): the system behaves with an effective equilibrium con-

stant which is the sum of a term due to bidentate binding $[k_{22}/(1+k_1c_1)^2]$ and a term corresponding to monodentate binding $[k_2/(1+k_1c_1)]$. Clearly, the first term is much more affected by a change in the proton activity than the second, since the binding of a bidentate metal can imply, in principle, the removing of two protons. In other words, the chelate contribution, responsible for the heterogeneity of the system, decreases as the pH decreases. In the limiting case of very acidic media ($c_1 \rightarrow \infty$), the system becomes fully monodentate and homogeneous ($u^{\text{eff}} = 1$).

Finally, it is worth to explore the limiting case in which monodentate binding is absent ($k_2 \rightarrow 0$). Then, the low affinity peak simply disappears from the spectrum. Now it can be shown that the spectrum normalizes to 1/2 instead of 1, due to the fact that only half of the sites can be occupied. The resulting spectrum becomes

$$p'(x'; c_1) = \frac{\ln 10}{2\pi((4k^{\text{eff}}/k') - 1)^{1/2}}. \quad (61)$$

Furthermore, it is easy to show that the average affinity is given by

$$\langle x' \rangle_c = \langle x_2 \rangle - 2 \int_{-\infty}^{\log c_1} \theta_1(c_1, 0) d \log c_1, \quad (62)$$

which, as happened in the case discussed in Sec. V A, does not correspond to the expression reported in Sec. III [Eq. (21)] as a consequence of the unsuitability of IE approach in the case of multidentate complexation.

VI. CONCLUDING REMARKS

The concept of conditional equilibrium constant, a fundamental tool in the study of multicomponent binding, has been extended to describe the competition in systems where the complexing agents can be bound to the ligand according to a distribution of affinities. In this case, the extension of the conditional affinity constant has led to the conditional affinity spectrum (CAS). The CAS can be understood as an “effective” or “apparent” affinity spectrum experienced by a particular component when the concentrations of the rest of components are kept constant. It has been shown that the CAS always exists if the competitive binding can be described by means of a multicomponent affinity spectrum (IE approach). However, CAS can also exist even in some cases for which the IE approach does not apply.

Since, via the CAS, a multicomponent system is contracted into an equivalent monocomponent one, all the properties of the monodimensional affinity spectra can be applied. For instance, for a system with two binding cations (metal and proton), one can obtain the conditional affinity spectrum of the NICA or Frumkin isotherms for the metal ions by simply applying the Sips inversion formula to the multicomponent isotherm, keeping constant the proton concentration. The CAS obtained will now be a function of the pH of the system.

As a result, the mean and the variance of the CAS are functions of the proton concentration. When the IE applies, the mean of the CAS, which we call “mean conditional affinity,” can be expressed in terms of the mean free binding

energy characteristic of the metal ion (corresponding to the system in absence of protons), and a term only depending on the proton titration curve. This fact implies that if one plots the apparent affinity as a function of the pH , the curves obtained for different metal ions must be parallel, i.e., they differ just in an additive constant. The verification of this property could be a first test of validity of the affinity spectrum approach for multicomponent systems.

On the other hand, the CAS variance represents a measure of the “conditional heterogeneity” of the reduced system. In contrast with the apparent affinity, the apparent heterogeneity can be expressed as a sum of a constant plus a term coming from the proton titration curve only if there is no correlation between the binding of the metal and protons. If the multicomponent system presents binding correlation, an additional term appears. This dependence of the CAS variance on the binding correlation provides a procedure to compute the correlation matrices corresponding to multicomponent affinity spectra. By using this procedure, analytical expressions for the binding correlation of NICA and Frumkin isotherms were obtained. In this way, the binding parameters of such isotherms can be interpreted in terms of the heterogeneity properties of the system.

Finally, the possibility of extension of CAS to cases for which IE approach fails in describing the system is highlighted. Two cases are considered: selective binding (some sites bind only one of the components) and multidentism. In both cases the CAS are obtained. Proper dependences of the conditional average affinity on the pH are reported for these cases highlighting that the properties of these CAS spectra are different from those in which the IE approach applies.

ACKNOWLEDGMENTS

The authors gratefully acknowledge support of this research by the Spanish Ministry of Education and Science (DGICYT: Project No. BQU2003-9698), by the European Community under Contract No. EVK1-CT2001-86, and from the “Comissionat d’Universitats i Recerca de la Generalitat de Catalunya.”

¹J. Buffle, *Complexation Reactions in Aquatic Systems: An Analytical Approach* (Ellis Horwood, Chichester, 1988).

²M. M. Nederlof, W. H. van Riemsdijk, and L. K. Koopal, *Heavy Metals in the Environment, Trace Metals in the Environment* (Elsevier, Amsterdam, 1991), pp. 365–396.

³B. Prelot, R. Charmas, P. Zarzycki, F. Thomas, F. Villieras, W. Piasecki, and W. Rudzinski, *J. Phys. Chem. B* **106**, 13280 (2002).

⁴R. Charmas, W. Rudzinski, W. Piasecki, B. Prelot, F. Thomas, F. Villieras, and W. Janusz, *Appl. Surf. Sci.* **196**, 331 (2002).

⁵M. Cernik, M. Borkovec, and J. C. Westall, *Environ. Sci. Technol.* **29**, 413 (1995).

⁶J. Bouanda, L. Dupont, J. Dumonceau, and M. Aplincourt, *Anal. Bioanal. Chem.* **373**, 174 (2002).

⁷M. A. Ramos, S. Fiol, R. Lopez, J. M. Antelo, and F. Arce, *Environ. Sci. Technol.* **36**, 3109 (2002).

⁸M. Jaroniec and R. Madey, *Physical Adsorption on Heterogeneous Solids* (Elsevier, Amsterdam, 1988).

⁹K. Nieszporek and W. Rudzinski, *Colloids Surf., A* **196**, 51 (2002).

¹⁰T. Panczyk and W. Rudzinski, *Langmuir* **19**, 1173 (2003).

¹¹C. Bruni, A. Germani, G. Koch, and R. Strom, *J. Theor. Biol.* **61**, 143 (1976).

¹²P. Szabelski, K. Kaczmarek, A. Cavazzini, Y. B. Chen, B. Sellergren, and G. Guiochon, *J. Chromatogr. A* **964**, 99 (2002).

- ¹³R. J. Umpleby, S. C. Baxter, Y. Z. Chen, R. N. Shah, and K. D. Shimizu, *Anal. Chem.* **73**, 4584 (2001).
- ¹⁴T. Takeuchi, T. Mukawa, J. Matsui, M. Higashi, and K. D. Shimizu, *Anal. Chem.* **73**, 3869 (2001).
- ¹⁵W. Rudzinski, K. Nieszporek, H. Moon, and H. K. Rhee, *Chem. Eng. Sci.* **50**, 2641 (1995).
- ¹⁶R. Sips, *J. Chem. Phys.* **16**, 490 (1948).
- ¹⁷U. Rusch, M. Borkovec, J. Daicic, and W. H. van Riemsdijk, *J. Colloid Interface Sci.* **191**, 247 (1997).
- ¹⁸H. Moon and C. Tien, *Chem. Eng. Sci.* **43**, 2967 (1988).
- ¹⁹M. Cernik, M. Borkovec, and J. C. Westall, *Langmuir* **12**, 6127 (1996).
- ²⁰J. L. Garcés, F. Mas, and J. Puy, *Environ. Sci. Technol.* **32**, 539 (1998).
- ²¹L. K. Koopal, W. H. van Riemsdijk, J. C. M. Dewit, and M. F. Benedetti, *J. Colloid Interface Sci.* **166**, 51 (1994).
- ²²J. L. Garcés, F. Mas, and J. Puy, *J. Chem. Phys.* **120**, 9266 (2004).
- ²³D. G. Kinniburgh, W. H. van Riemsdijk, L. K. Koopal, M. Borkovec, M. F. Benedetti, and M. J. Avena, *Colloids Surf., A* **151**, 147 (1999).
- ²⁴R. H. Fowler and E. A. Guggenheim, *Statistical Thermodynamics* (Cambridge University Press, Cambridge, 1960).
- ²⁵K. A. Connors, *Binding Constants: The Measurement of Molecular Complex Stability* (Wiley, New York, 1987).
- ²⁶A. M. Puziy, O. I. Poddubnaya, A. Martinez-Alonso, F. Suarez-Garcia, and J. M. D. Tascon, *Carbon* **40**, 1493 (2002).
- ²⁷L. K. Koopal, W. H. van Riemsdijk, and D. G. Kinniburgh, *Pure Appl. Chem.* **73**, 2005 (2001).
- ²⁸L. K. Koopal, T. Saito, J. P. Pinheiro, and W. H. van Riemsdijk, *Colloids Surf., A* **265**, 40 (2005).
- ²⁹G. J. M. Koper and M. Borkovec, *J. Chem. Phys.* **104**, 4204 (1996).
- ³⁰J. L. Garcés, F. Mas, J. Cecflia, E. Companys, J. Galceran, J. Salvador, and J. Puy, *Phys. Chem. Chem. Phys.* **4**, 3764 (2002).
- ³¹J. Arago, A. Bencini, A. Bianchi, E. GarciaEspana, M. Micheloni, P. Paoletti, J. A. Ramirez, and P. Paoli, *Inorg. Chem.* **30**, 1843 (1991).
- ³²R. S. Bai and R. T. Yang, *J. Colloid Interface Sci.* **239**, 296 (2001).
- ³³G. Koper and M. Borkovec, *J. Phys. Chem. B* **105**, 6666 (2001).