Equation Chapter 1 Section 1

Modelling proton and metal binding to humic substances with the NICA-EPN model

Andrea C. Montenegro, Silvia Orsetti† and Fernando V. Molina*

Instituto de Química Física de Materiales, Ambiente y Energía (INQUIMAE) and Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

†Present address: Institut für Geowissenschaften, Zentrum für angewandte Geowissenschaften, Eberhard-Karls Universität Tübingen, Germany

*CORRESPONDING AUTHOR: INQUIMAE, Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Ciudad Universitaria, pabellon II, C1428EHA Buenos Aires, Argentina. Tel.: +54-11-4576-3378/80 ext 230. FAX: +54-11-4576-3341. E-mail: fmolina@qi.fcen.uba.ar

ENVIRONMENTAL CONTEXT

Trace metals such as lead, copper or cadmium, frequently present in waters and soils, are harmful to health. Natural organic matter plays a key role due to its ability to bind these metals (forming complexes) and consequently to fix them in immobile, non bioavailable forms. Sound mathematical models are important to reliably predict the behaviour of such pollutants, an how they affect organic matter or other environmental colloids; here a new model is discussed and compared with precedent ones.

ABSTRACT

The mathematical modelling of metal cation-natural organic matter interactions is a fundamental tool in predicting the state and fate of pollutants in the environment. In this work, the binding of protons and metal cations to humic substances is modelled applying the Elastic Polyelectrolyte Network (EPN) electrostatic model with the Non Ideal Competitive Adsorption (NICA) isotherm as the intrinsic part (NICA-EPN model). Literature data of proton and metal binding to humic substances at different pH and ionic strength values are analysed, discussing in depth the model predictions. The NICA-EPN model is found to describe well these phenomena. The electrostatic contribution to the Gibbs free energy of adsorbate-humic interaction in the EPN model is lower than that predicted by the Donnan phase model; the intrinsic mean binding constants for protons and metal cations are generally higher, closer to independent estimations and to the range of acid-base and complexation equilibrium values for common carboxylic acids. The results for metal cations are consistent with recent literature findings. The model predicts shrinking of the humic particles with increased metal binding, as a consequence of net charge decrease.

1. Introduction

The interaction of natural organic matter (NOM) with ionic species, specially metal cations, has attracted the interest of researchers for several decades[1–3] due, among other aspects, to the environmental concerns involved; this ability, in particular for binding of trace metals, has an important role in the fate of pollutants in the environment. Soil organic matter (SOM) has a fundamental role in nutrient availability for plants, among other important properties,[4,5] and contribute to the fixation of cationic pollutants; on the other hand, aquatic NOM, generally referred to as dissolved organic matter (DOM) facilitates metal mobility in the environment.[6] Thus, knowledge of the interaction of cations with NOM is highly desirable, including the capability to predict the extension of cation binding to NOM as well as its effect on NOM properties. Humic substances (HS) constitute an important part of NOM, specially of SOM, and have been widely studied, particularly in their interaction with metal cations.[2,7] The binding of ions to NOM is, at first sight, akin to metal ion complexation to small organic molecules, where cations like Cu(II), Cd(II) or Pb(II) are coordinated to Lewis basic groups like $–COO^-$, $-NH_2$, etc. However, HS are relatively large molecules or, more properly, molecular associations[4,8] with colloidal properties, consequently they also retain cations electrostatically, showing cation exchange properties, as well as softness and penetrability.[9,10] Modelling of metal sorption to HS has interested researchers for many years,[11–15] because models describing adequately that sorption are required for predictive purposes. Such models, similarly to mineral colloids like oxides and clay minerals, generally comprise chemical (intrinsic) and electrostatic contributions. In the case of HS, the intrinsic interaction has been modelled as chemical equilibriums (chemical modelling)[14,16] or as adsorption competitive isotherms, as in the Non-Ideal Competitive Adsorption (NICA) isotherm equation.[17] The electrostatic contribution modelling has also followed two basic lines: one treated the humic substances as rigid particles,[13] applying standard colloid chemistry models, whereas other authors applied the concept of Donnan equilibrium.[12,18] In Tipping´s Windermere Humic Aquatic Model (WHAM)[12,16] a Donnan phase (DP) is assumed, surrounding the humic particles and containing only positively charged counterions, the electrostatic effect on binding being accounted by an empirical

Boltzmann factor; here the humic particles are assumed to be of fixed size. In the NICA-Donnan (ND) model[17,18] a Donnan phase, termed "Donnan volume", of solution around and including the humic particle is assumed to have a volume large enough so as to be electrically neutral and in Donnan equilibrium with the bulk, the volume being dependent on the ionic strength; this model has been applied to proton and metal ion binding.[19] Both approaches (WHAM and ND) lead to good fit of cation binding data;[20] however, HS have been show to be soft, permeable, with pH-dependent size,[10,21] and to undergo aggregation/disaggregation processes;[8,22] thus, considerations for swelling and shrinking in electrostatic models turns to be important. Recently, a new electrostatic model, the Elastic Polyelectrolyte Network (EPN) model was introduced and combined with the NICA equation in the NICA-EPN model for proton binding to HS;[23] a first application to metal binding was also shown recently.[24] The EPN model assumes that a fraction of the HS particles behaves as a gel in Donnan equilibrium with the bulk solution, with swelling/shrinking properties; at variance with other DP models, the humic particles themselves (more precisely, a fraction of them) are treated as a gel. Here, the NICA-EPN model for competitive proton and metal binding to HS is discussed and evaluated through application to literature data; the results are analysed and compared with other models, mainly the NICA-Donnan one, as in other cases (such as WHAM or the Stockholm humic model (SHM)) the different schemes assumed for the intrinsic contribution makes difficult the comparison of the electrostatic contribution.

2. Theory: The NICA-EPN model

The EPN model has been introduced elsewhere,[23] thus here we will present a slightly reduced account, focusing on metal cation binding. In the EPN model, the HS particles are considered as divided in two fractions: an inner fraction g_f, which behaves as a gel in Donnan equilibrium with the bulk solution, and an external fraction 1 - g_f, as shown schematically in Fig. 1. The gel is treated, in a mechano-statistical sense, as an elastic polymeric network formed by N_2 equivalent chains which are cross-linked and/or

entangled; this network is a model of the structure of humic particles, generally considered as a supramolecular arrangement of relatively small molecules bound by a variety of interactions including hydrogen bond, van der Waals and electrostatic forces and metal complexation.[4,8] Thus, HS are treated here as soft gel-like particles independently of its size, even in the case of fulvic acids (FA), which are composed of relatively small molecules (of a few kDa), because they are expected to be associated in natural environments. The gel can exchange ions and solvent with the surrounding solution, swelling and shrinking as a consequence of humic charge changes; the equilibrium volume for a given condition is found by minimization of the free energy of swelling:

where A is the Helmholtz free energy, T the temperature and V the gel volume. Three independent contributions are considered in Eqn : the free energy of mixing between N_2 chains and N_1 solvent molecules (the solution is assumed to have the bulk properties of water), ΔA_{mix}, the network deformation contribution, ΔA_{def}, and the electrostatic interaction free energy ΔA_{el}.

The electrostatic contribution to the free energy (repulsion between humic particle negative charges) can be obtained by assuming that the humic charged groups are initially in their equilibrium sate (i.e. free or bound) but uncharged, and calculating the work to reversibly charge the network:[25]

where G is the Gibbs free energy, q is the humic net charge and ψ the electrostatic potential. Eqn requires knowledge of the electric potential profile inside the gel, which in principle should be found through the Poisson-Boltzmann equation; in the EPN model it is assumed a constant potential inside the gel, equal to ψ_D, neglecting border effects (which also makes the model independent of the particle size), thus the Poisson-Boltzmann equation can be written for this case:[23]

$$\varepsilon_0 \varepsilon_r \sum_{i=1}^{N} \frac{(1-\varepsilon_r)\varepsilon_0 \varepsilon_r}{\varepsilon_0 \varepsilon_r} \frac{\varepsilon_0 \varepsilon_r}{\varepsilon_0 \varepsilon_r}$$

where F is Faraday's constant, ε_r is the relative dielectric constant of the medium (taken as water), ε_0 is the vacuum permittivity, ρ is the humic charge density (in molar units), given by

$$\rho = \frac{Z}{V} = g_f Q \delta \varphi_2$$

where Z is the net humic charge of the humic particle (mol), V the hydrated humic particle volume, Q is the net specific charge (mol kg^{-1}) and δ the density of the dry humic substance (following Dinar[26] δ = 1.5 g cm^{-3}). φ_2 is the volume fraction of the humic substance in the gel:

$$\varphi_2 = \frac{V_2}{V}$$

where V_2 is the volume of dry humic substance. The factors (1-φ_2) in Eqn and φ_2 in Eqn account for the charge density changes due to swelling (mixing of HS and electrolyte). Note that Eqn is the electroneutrality condition; it has no analytical solution for ψ_D in the general case, thus it should be solved numerically. In the case of a 1:1 electrolyte, as it is well known, the Donnan potential can be found, being:[23]

$$\psi_D = \frac{RT}{F} \operatorname{arcsinh}\left(\frac{\rho}{2(1-\varphi_2)}\right)$$

where I is the ionic strength, equal to the electrolyte concentration in the 1:1 case; from Eqns and it is found that:[23]

$$\frac{\partial \psi_D}{\partial \rho} = \frac{\partial \left[\frac{\rho}{2(1-\varphi_2)}\right]}{\partial \rho} \left[\frac{\partial \left(\frac{\rho}{2(1-\varphi_2)}\right)}{\partial \rho}\right]^{-1} = \frac{\partial \left(\frac{\rho}{2(1-\varphi_2)}\right)}{\partial \rho}$$

This equation is strictly valid for 1:1 electrolytes; for proton binding experiments, this is generally the situation found in the literature,[23,27,28] thus Eqns and ara appropriate. In the presence of di- and

trivalent metal salts, in principle this is not true. For low metal concentrations, the ionic strength can be approximated well by the supporting electrolyte concentration, but as the metal concentration increases this approximation becomes increasingly inexact. Fortunately, as the cation concentration increases so does the amount bound, decreasing the humic charge and consequently the Donnan potential, thus the error introduced by using Eqn effectively decreases. The consistency of the results was verified, after fitting of the experimental data using the EPN model results based on Eqn , by computing ψ_D through numerical solution of Eqn using the electrostatic parameters found in fitting, and comparing with the results of Eqn , resulting that the differences, for ionic strength values $I \geq 0.01$, amounted to at most 1-2 mV. Consequently, Eqn was kept, using the full ionic strength in all cases, in Eqns and below. There is no experimental data available on metal binding at lower ionic strengths; however in some natural environments ionic strengths of the order of 1 mM can be found. Estimations obtained through simulations suggest that, at low pH values (pH < 6) differences of the order of 10 mV can arise for ψ_D at $I = 1$ mM.

The mixing and deformation contributions are found quite straightforwardly following Flory and Hill,[25,29,30] resulting in:[23]

where v_1 is the water molar volume, χ is a Van Laar interaction parameter, R is the gas constant, N_0 is Avogadro's number and f_P is the Flory functionality giving the degree of cross-linking of the statistical polymer segments. Here, a value $f_P = 3$ is assumed,[23] which is expected to reflect better the structure of the humic particles. From Eqns , and , the swelling equilibrium condition is found:

The humic binding sites outside the gel are in equilibrium with the bulk solution, whereas those inside the gel are in equilibrium at the internal ionic activity of species j, a_j^{int}, which is given by

$$a_j^{\text{int}} = a_j \exp\left(-z_j F \psi_{\text{int}} / \Delta_{\text{int}}\right)$$

The amount bound, in the NICA-EPN model, of species i per unit of humic mass in the presence of species j is given by

$$\theta_{i,k} = \frac{\left(K_{i,k} a_i\right)^{n_{i,k}}}{\sum_{j=1}^{N} \left(K_{i,k} a_j\right)^{n_{i,k}}} \times \frac{\left[\sum_{j=1}^{N} \left(K_{i,k} a_j\right)^{n_{i,k}}\right]^{p_k}}{1 + \left[\sum_{j=1}^{N} \left(K_{i,k} a_j\right)^{n_{i,k}}\right]^{p_k}}$$

where $\theta_{i,k}$ is the fraction of type k sites occupied by species i, given by the NICA equation:

$$\theta_{i,k}(a_1, a_2, \ldots, a_N) = \frac{\left(K_{i,k} a_i\right)^{n_{i,k}}}{\sum_{j=1}^{N} \left(K_{i,k} a_j\right)^{n_{i,k}}} \times \frac{\left[\sum_{j=1}^{N} \left(K_{i,k} a_j\right)^{n_{i,k}}\right]^{p_k}}{1 + \left[\sum_{j=1}^{N} \left(K_{i,k} a_j\right)^{n_{i,k}}\right]^{p_k}}$$

In Eqns and , N is the number of species binding to the HS, M the number of site types, $Q_{\max}$ the total number of sites per unity of mass, $Q_{\max,k}$ the same for type k sites (assuming 1:1 binding), q_k the fraction of type k sites ($Q_{\max,k} = q_k\, Q_{\max}$), $n_{i,k}$ the nonideality parameter for species i on type k sites, ($n_{1,k}$ for H^+), p_k is the heterogeneity parameter of type k sites and $K_{i,k}$ is the mean binding constant of species i on type k sites. Two site types are often considered: low affinity sites (LAS, $k = 1$) and high affinity sites (HAS, $k = 2$)[15,27,31] so $M = 2$ (albeit some studies propose three types[32]), and for binding of a metal cation in the presence of protons $N = 2$; low and high affinity sites are often referred to as carboxylic and phenolic, respectively. $Q_{i,el}$ is the excess amount of species i electrostatically bound in the gel phase:

$$Q_{i,el} = \left(c_i^{\text{int}} - c_i\right) \frac{q_i}{\delta_{\text{gel}}}$$

where c_i^{int} is the concentration of species i in the gel.

3. Data analysed and methods

Table 1 presents the datasets analysed, mostly coming from Milne et al.;[19] some data was also obtained from recent works by Companys et al.[33] and Christl.[34] Instead of attempting to fit all data available, selected datasets were processed and the results analysed. In the selection process, it was considered that a high number of data points, including proton titration data, should be available and, where possible, that different ionic strength values, besides pHs, were measured. Relatively few datasets fulfilled those requirements; in some instances, data from different authors for the same HS and metal, covering different ionic strengths, were collated; in doing so, it was attempted to integrate all data available for a single system (e.g, Ca-PPHA, Pb-PPHA, etc.), however in some cases it was found that a particular dataset was not consistent with the rotation, as revealed by large differences in the root mean square error (RMSE) between inclusion and exclusion of the dataset, and visible discrepancies in the fitting plot; these datasets were not considered further. For several cases, the proton binding data were already analysed with the NICA-EPN model in a previous study.[23] The Davies equation[35] was employed to calculate activity coefficients.

The analysis procedure was already outlined;[24] it involved as a first step fitting the proton binding curves alone as described previously;[23] H^+ binding is described through the NICA equation for binding of a single ion which, in the NICA-EPN model, reads:

$$Q_H(a_H) = Q_{Hk}\left\{(1-q)\left[q\frac{(K_{1,H}a_H)^{n_1}}{1+(K_{1,H}a_H)^{n_1}} + (1-q)\frac{(K_{1,2,H}a_H)^{n_2}}{1+(K_{1,2,H}a_H)^{n_2}}\right] + q\left[q\frac{(K^{in}_{1,H}a_H)^{n_1}}{1+(K^{in}_{1,H}a_H)^{n_1}} + (1-q)\frac{(K^{in}_{1,2,H}a_H)^{n_2}}{1+(K^{in}_{1,2,H}a_H)^{n_2}}\right]\right\} + Q_{Hh}$$

with

$m_k = n_{\mathrm{H},k} p_k$

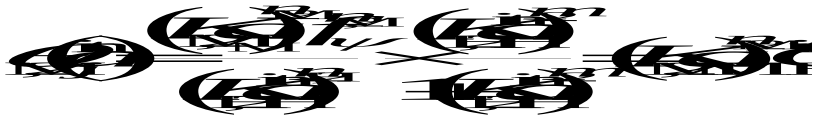
Briefly, the most general procedure to determine the H^+ binding parameters started obtaining the conditional distribution for the highest ionic strength by means of the CONTIN code;[7] this distribution was fitted to a bimodal Sips distribution, and the resulting parameters were used as initial values of $K_{\mathrm{H},k}$ and m_k to simultaneously fit the Q_A vs. pH curves at all ionic strengths to the NICA-EPN model; however, this initial step was sometimes omitted, starting the procedure with typical mean values for log $K_{\mathrm{H},k}$ and m_k. The fitting to the EPN model was done using a restricted Levenberg-Marquardt algorithm[36] with χ, v_2, g_f and the intrinsic constants $K_{\mathrm{H},k}$ and m_k as adjustable parameters. The allowable range of these parameters was restricted based on the literature observed values[19] for the NICA constants and on previous results[23] for the EPN ones. The calculations (for each iteration) involved, for a given set of parameters, solving numerically Eqn for φ_2, then Eqn to find ψ_D and Eqn to find $a_{\mathrm{H}}^{\mathrm{int}}$ so that Q_{H} can be computed through Eqn , and finally $Q_A = Q_{\max} - Q_{\mathrm{H}} + Q_0$, with Q_0 the humic charge at the initial pH of the experiment, which was adjusted for each curve individually.

Then, curves of metal binding as a function of its activity were also fitted to the NICA-EPN model, Eqn , taking the proton binding and EPN parameters from the proton experiments which were kept unmodified; the parameters adjusted here were the intrinsic parameters $K_{\mathrm{M},k}$, $n_{\mathrm{M},k}$ and p_k, keeping these last constant for a given HS for different metals; the m_k were also kept constant. In the cases where several metal cations were studied for the same HS (SRFA, PPHA and PAHA), the procedure was iterated varying the p_k parameters until all metal curves were satisfactorily fitted with the same p_k values. The procedure was coded in the FORTRAN programming language, and was checked using the Mathematica package (Wolfram Research, inc., Champaign, IL, USA) by simulating curves with assumed parameters and checking that the program output produced the same values; also curves were simulated for some fitting result parameters and compared with the program calculated curves. The code is available from the authors.

4. Results and Discussion

Table 2 collects the results of EPN parameters, proton binding constants and heterogeneity parameters for the HS considered here. The p_k values were obtained in the analysis of metal binding curves; consequently, the $n_{\mathrm{H},k}$ values were obtained form Eqn . Table 3, in turn, presents the corresponding metal binding parameters. In general, very good agreement is obtained for proton binding, and for metals good agreement is found as shown in Fig. 2 for some examples; other plots can be found in Fig. S1 (Supporting material). In just one case (Ca(II) binding to PUHA) the proton binding parameters had to be found by simultaneous fitting of the metal and proton curves. The model predicts well the behaviour either for parallel binding curves, such as Al(III) on SRFA (Fig. 2a), or those showing a tendency to converge at high metal activity, for example Cd(II) binding to PPHA (Fig. 2d). The Pb(II)-PPHA curves (Fig. 2e), instead, show deviations at low Pb(II) activities; in a previous study on Pb(II) binding to HA[24] similar deviations are observed. Furthermore, in the low activity range the experimental log Q vs. log a curves are not parallel, which is also observed in other cases (see for example Fig. 2d). The NICA-EPN model predicts parallel curves for low activities; in fact, from Eq. it can be written, for the high affinity sites (k = 2) and the non-gel fraction, when $a_{\mathrm{M}} \rightarrow 0$,

Here, C_{H} is a coefficient depending only on pH. For the gel fraction, it should be noted that at low Q_{M} values and fixed pH the net humic charge Q will be constant, and consequently ψ_D and φ_2 will also be constant, thus:



where $f_\psi = \exp(-F\psi_D/RT)$; C_H^{int} is dependent on pH and I. For the total metal binding, considering that at low a_M only the high affinity sites will contribute, it results from Eqns , and :

Thus, a plot of log Q_M vs. log a_M would have a slope equal to $n_{M,2}$. The lack of fulfilment of this behaviour could be indicative that the NICA equation, at least in its present form, does not predict well the metal binding in the very low activity range for some metal cations.

In the following sections, the intrinsic proton and metal binding parameters are discussed. In the analysis of metal binding (Section 4.2 below), the resulting values of the mean constants (as log $K_{M,1}$ and log $K_{M,2}$) from Table 3 are compared with complex formation constants for common organic acids (see Table S1, Supporting material); these are collected mostly from the MINTEQA2 database included in Gustafsson's visual minteq code[37] plus some additional data.[38,39] Also, to analyse metal binding relative to proton binding, the differences Δ_i = log $K_{M,i}$ - log $K_{H,i}$ for NICA-EPN and NICA-Donnan (generic values) are also presented in Table 3.

4.1. Proton binding analysis

In Table 4 the NICA-EPN results for proton binding are compared with values reported in the literature as well as some estimated ones. In general, fitted values are based on data corresponding to different HS, but several authors[12,23,40] have employed equal or similar datasets from the compilation of Milne.[27] The present study yields, for $K_{H,1}$, average results of 4.10 for FA and 4.31 for HA, close to the values for common carboxylic acids, specially unsaturated aliphatic and aromatic ones. It is evident that the ND model results for $K_{H,1}$, as reported by Milne et al.[27], are generally lower than these and other results: it gives for FA values of 2.3-2.8 for log $K_{H,1}$ whereas all other results fall in the range 3.2-4.0; for HA, the values are 2.9-3.7 for ND and 4.1-4.4 for the rest. Recently, Lenoir et al.[41] reported different results for the ND model based on a revised optimization procedure; these authors observed strong correlations

between ND parameters in standard optimization algorithms, leading to equally good fitting with different parameter sets. The revised procedure yielded average results of 3.54 for FA and 3.87 for HA, which are closer to other literature values. The results of Lenoir et al. and others compare reasonably well with different, model independent estimations: the empirically estimated range of 3.5-4.5 (Avena et al.[42]), the IR deduced result of 3.3-3.5 (Hay and Myneni[43]), the semiempirical calculations of Matynia et al.[28] giving 3.8 for FA and 3.7 for HA and the estimations by linear free energy combinations of Atalay et al.[44] for aquatic HS, giving 3.9 for FA and 4.0 for HA; Atalay et al. also presented a soil HA log $K_{H,1}$ of 3.0, a value lower than all other estimations, which can be due to the limited range of molecular structures considered (in fact, the distribution shown in Fig. 4 of Ref.[44] is consistent with two separate mean values for LAS and other two for HAS). For the high affinity sites, there is a wider range of reported values: for FA, the log $K_{H,2}$ found in the NICA-EPN model is 8.75 whereas for other models the range is 7.0-9.8; for HA, the present result is 9.63, the general range being 7.7-9.7; the ND results lying again in the lower part of the range. The semiempirical calculations[28] give results of about 9.8 for both types of HS, whereas Avena et al.[42] deduced a range of 7.5-8.5, and Atalay et al.[44] inform results between 8.0 and 9.5. Overall, the results of the present work lie in the higher part of the range for both site types; for the high affinity case, these results are close to the pK_a for phenol (9.8); it has been reported that in HS most phenolic OH groups are isolated[45,46] which is consistent with that value.

Considering the average fractions q_1 and q_2 of low and high affinity types, in general a higher proportion of carboxylic types is found in fitted results (except WHAM and SHM models, where they are fixed), which is in agreement with chemical analysis methods.[47]

4.2. Metal binding analysis

4.2.1. Aluminium

The fitting of the Al(III)-SRFA curves to the NICA-EPN model resulted, for the logarithm of mean binding constants, in values of 3.0 and 12.8 for the low and high affinity sites, respectively. Milne et al.[19] report -4.11 and 12.16 as recommended generic values for Al(III) to FA. The log $K_{M,2}$ value found here is close to Milne's, but log $K_{M,1}$ is quite different; it should be noted that, in the course of the computations, the fitting was found to have little sensitivity to log $K_{M,1}$; in fact, R^2 did not change appreciably for log $K_{M,1} < 2$ (down to at least -10), increased slightly from 2 to 3 and increased markedly for higher log $K_{M,1}$ values. Thus, 3.0 was adopted as a reasonable value because lower ones did not improve fitting. This behaviour would indicate that low affinity sites have little influence on Al binding to FA. Pinheiro et al.[48] recalculated Al parameters by analysis of Pb-Al competitive binding curves, obtaining log $K_{M,1} = 2.0$, which is in fair agreement with the present result. The relatively low values for $K_{M,1}$ are of the order of monodentate Al(III) binding with monocarboxylic acids (see Table S1, Supporting material); on the other hand, the high value of $K_{M,2}$ is in the range (Table S1) of tetracoordinate complexes such as those with oxalate (Ox), $Al(Ox)_2^-$ or citrate (Ci), $Al(Ci)_2^{3-}$, or chelates with salicylate or catechol type groups.

For PPHA, parameter values were deduced from the analysis of competition experiments, with data obtained by digitalization of Figs. 1 and 3 from Pinheiro et al.[48]; experimental data for Al-PPHA was not available. The fitting of Pb-Al and Cd-Al competition curves was iterated until a set of Al(III) binding parameters describing satisfactory both sets of curves was found. It should be noted that the number of pH values and ionic strengths studied is limited, so these results should be taken with caution. Here, high $K_{Al,i}$ values are found for both site types: $K_{Al,2}$ is also in the range of tetracoordinate species mentioned above, whereas $K_{Al,1}$ is similar to the values for bi-tridentate complexes with oxalate or citrate.

4.2.2. Calcium

One FA and three HA, two of these using recently reported data,[34] have been analysed for Ca(II); as it can be observed for example in Fig. 2b and Fig S1h (Supporting material), good agreement is found for

both pH and ionic strength variations. Calcium is reported to bind in a relatively weakly way with HS: this is reflected in low values for the NICA mean constants: for FA, -0.10 and 2.25 are found here (Table 3) for LAS and HAS, respectively; for HA similar values for both site types are found (between about 1.3-2.5 for LAS, 2.4 and 3.1 for HAS), which fall in the range of mono- and dicarboxylic complexes with Ca(II) (Table S1, Supporting material), as well as the HAS value for FA, suggesting that only carboxylic groups are involved in binding. These values are noticeably higher than the recommended values of Ref.[19] for FA, of -2.13 and -3.0 (this last value was assumed, not fitted).

4.2.3. Cadmium

In the case of cadmium, two HA have been analysed: PPHA (Milne datasets HCd-03 and 07) and PAHA (from a more recent study[33]). In the first case, intermediate values of the binding constants (log $K_{M,1}$ and $K_{M,2}$) are found (2.12 and 6.12), whereas in the second case lower results are found (0.87 and 2.5); it should be noted that the experimental Cd activity range is smaller in the last case. The Milne et al.[19] results are lower at -0.20 and 2.37, comparable with those of Cd-PAHA, but far from the present results for PPHA; Kinniburgh et al.[31] reported for Cd-PPHA (HCd-03), fitted to the ND model, log $K_{M,1}$ = 0 and log $K_{M,2}$ = 2.30. It is interesting to note that simple Cd(II) complexes with monocarboxylic acids have log K values of about 2 (Table S1), whereas either tetracoordinate carboxylate complexes, or phenolic chelate complexes, such as those with salicylate and catechol, have log K values in the range 5-8. Cd(II) binding to HS has been quite intensely studied by spectroscopy, mainly ^{113}Cd NMR measurements; Otto et al.[49] studied in this way Cd(II) binding to fulvic acids, concluding that there are two clearly differentiated sites: one strong binding site best represented by a polydentate carboxylate model (albeit an hydroxycarboxylate site could also be possible), and another weaker site, attributed to monocarboxylate binding. These results are consistent with the attribution of HAS of PPHA to complexes like $Cd(Ox)_2^{2-}$ or $Cd(Ci)_2^{4-}$, and of LAS to monocarboxylate complexes.

4.2.4. Zinc

For this metal, only one HS, PAHA, has been analysed. The binding constants found (0.47 and 3.2) are similar to those for Cd with the same HA, lying in the range of single and double coordination to carboxylic groups (Table S1), and comparable to the generic ND values, 0.11 and 2.39. Other studies have reported somewhat higher values for log $K_{M,2}$, using different methods.[50]

4.2.5. Copper

Two FA and two HA have been analysed in Cu(II) binding. The mean constants for low affinity sites are rather similar, ranging from 2.85 to 4.12 (Table 3); it is interesting to note that the generic ND values are lower, being 0.26 for FA and 2.23 for HA. The present values are comparable to constants for complex formation with monocarboxylic acids and some dicarboxylic ones, such as phtalic or glutaric. For the high affinity sites, however, most results are high values, except for PUFA, where log $K_{M,2}$ = 4.65 is near to the low affinity value of 3.49. For the remaining HS, log $K_{M,2}$ are in the range 10-13, which is well above the Milne generic values of 8.26 for FA and 6.85 for HA. Also, Kinniburgh et al.[31] found, fitting to ND model the dataset HCu-04 values of 0.69 for the LAS and 7.41 for HAS, which are in the same range of generic ND values. The results found here (except for PUFA) for the HAS are comparable to the constants for $Cu(Ox)_2^{2-}$, $Cu(Ci)_2^{4-}$, Cu(Sal) and Cu(Cat) (Sal standing for salicylate and Cat for catecholate). There is a large number of spectroscopic studies on Cu(II) binding to HS.[7,19] Xia et al.[51] in X-ray absorption studies of Cu(II) binding to HS in the pH range 4-6, found the Cu ions in a tetragonally distorted octahedral environment with 4 O atoms in the first coordination shell and 4 C atoms forming the second coordination shell; Karlsson et al.[52] found EXAFS evidence of chelate structures in Cu(II) complexation to soil and natural dissolved organic matter, finding 4 O/N atoms in the first shell, a second coordination shell with an average of 2-3.8 C atoms, and a third coordination shell with 2-3.8 O/C atoms, suggesting that Cu(II) is forming one or two five-membered chelate rings. These results suggest in turn that Cu(II) could be coordinated to four organic ligand sites; there is no indication as if those sites would be carboxylic of phenolic, but the magnitude of the high affinity constants is closer to carboxylate tetracoordinate structures, than phenolic ones (see Table S1,

Supporting material). It is also conceivable that tetracoordinate Cu(II) ions could be bridging together two humic molecules.

4.2.6. Lead

In the case of Pb(II), Table 3 presents parameters derived here for two FA and one HA, plus two other HA from a previous study[24]; Fig. 2d and Figs. S1d and i (Supplementary material). For PPHA, three different datasets were combined (HPb-05, 06 and 07); as mentioned above, there are some differences in the low binding range (Fig. 2d), nevertheless the agreement is good. For the low affinity sites, two of these HS gave results for log $K_{M,1}$ of 2.2-3.5 whereas the other two resulted to be about 0.5; the Milne et al. recommended values lie at -1.16 for FA and 1.25 for HA, thus poor coincidence is observed. For the high affinity sites, two groups are found in the present results (but not the same as for the low affinity case): two HS have log $K_{M,2}$ of 5.6-5.8, whereas the other two show higher values: 8.6 and 9.8. In Ref.[19] the recommended values are 6.92 for FA and 4.84 for HA, Kinniburg et al. found by fitting to the ND model log $K_{M,2}$ = 6.26 for Pb-PPHA; thus, in the case of LSFA and FHA the NICA-EPN results can be considered as roughly coincident with the ND results, but for PPHA and ESHA the differences are important; other studies claimed that Milne generic parameters did not describe adequately Pb binding by soil HS.[24,53] Comparing with simple organic ligands, values about 2.5-3.5 are typical of coordination of Pb(II) to carboxylate groups, whereas higher values usually correspond to coordination with oxalate or salicylate-type ligands (Table S1, Supporting material); however the $K_{M,k}$ values cannot be considered proof of the coordination number. Lead sorption by humic substances has been also widely studied,[7,19] however there is less spectroscopic evidence available. Xia et al.[51] studied the interaction of Pb(II) with a humic extract from a silt loam soil suggesting the presence of two C atoms in the second coordination shell, which would indicate bidentate binding. Recently, Xiong et al.[53] performed a XAFS study of Pb binding to soil HS, finding evidence, at high loading, of bidentate binding to salicylate- and catechol-like groups. On the other hand, Puy et al.[54] obtained conditional affinity spectra for Pb(II) binding to purified Aldrich HA, interpreted in terms of two contributions:

carboxylic at lower log K_M values and phenolic at higher log K_M values. This interpretation could be consistent with that given above if the phenolic types were mainly involved in bidentate binding, in salicylate-type groups. Manceau et al.[55] interpreted EXAFS measurements in lead contaminated soils suggesting also the presence of salicylate- and catecholate-Pb complexes; Arai et al.[56] in similar experiments suggested the same type of complexes. It should be noted that, due to the complex nature of the soil, with a potentially high number of different contributions to the XAS signal, these results are not conclusive at present. In a recent study,[24] analysing the fluorescent response in Pb binding to HA, the quenching of the emission upon Pb binding was found to be consistent with metal-induced aggregation of the humic molecules, that is bridging of HS molecules by the Pb(II) cations.

4.2.7. Competitive metal binding

In order to examine the ability of the NICA-EPN model to predict competitive metal binding, some literature experiments were analysed. It should be noted that experimental studies of that type are not abundant, and the range of pH and ionic strength employed is limited. Fig. 3 shows the results; in Fig. 3a the effect of Ca(II) on the binding of Cd(II) to PPHA at different pH values, studied by Kinniburgh et al.[17] is modelled with the NICA-EPN model, using the parameters found for Ca(II)-PPHA and Cd(II)-PPHA individually, without further adjustment. A very good prediction is observed at pH = 10, and a reasonable agreement is found in the other two cases. In Figs. 3b and c the effect of Al(III) on the binding of Pb(II) and Cd(II) to PPHA, respectively, measured by Pinheiro et al.[48] is presented. In these cases, the parameters for Al(III) binding to PPHA cannot be found independently, thus the two sets of curves were fitted varying the Al parameters ($K_{Al,k}$ and $n_{Al,k}$), keeping constant the Pb-PPHA and Cd-PPHA parameters found previously; this procedure resulted in the values shown in Table 3; because the limited number of pH and ionic strengths studied, these results should be taken with caution. Nevertheless, a very good prediction is observed in the case of Pb, also a fair agreement is found for Cd. Presumably, fitting more extensive experimental datasets for competitive binding along with single

metal experiments would lead to improved parameter sets; however, new experimental data is required to that end.

4.2.8. Metal binding constants relative to proton constants

In Table 3, the differences $\Delta_i = \log K_{M,i} - \log K_{H,i}$ for NICA-EPN and ND models (in the last case, using the Milne et al.[19] generic values, due to the lack of sufficient HS-specific parameters) are tabulated. In principle, as metal cation binding always competes with proton binding, it can be expected that these differences would give a better comparison of metal binding between the two models. However, no clear tendencies can be found in Table 3. Considering EPN results for different HS (fulvic or humic, separately) with the same cation, in some cases the Δ values lie within about ± 1 log unit (for example, Ca-HAs or LAS for Cu-FA, Pb-FA) whereas in other cases there are large differences (HAS for Cu-FA or Pb-HAs). Comparing EPN with Donnan results, taking into account that the last are only generic, nevertheless no clear tendency stems here. For most cations there are large differences (such as Al, or HAS for Ca) whereas in some cases (for example LAS for Cd) the values are comparable. More insight into the nature of the binding with the diverse HS appears to be necessary for a better interpretation of the binding constants results.

4.3. Electrostatic contribution

4.3.1. Model predictions and comparisons

Comparing NICA-EPN with NICA-Donnan results, it is observed that in most cases the former model yields higher values for the mean constants for both proton and metal binding, closer to values for complex formation with organic ligands. To compare the two electrostatic submodels (EPN and DP), it is worth starting to consider that, either explicitly or implicitly, modelling the interaction between ions and colloids generally involves separating the Gibbs free energy change of ion binding, ΔG_b, in two contributions, assumed independent:

$$\Delta G_{B} = \Delta G_{in} + \Delta G_{el}$$

where ΔG_{in} is the intrinsic part and ΔG_{el} is the electrostatic contribution. The last one can be evaluated by assuming that the system is "frozen" in the final humic-ion configuration but uncharged, and performing a gradual charging of both the humic particle and the electrolyte in the gel:[25]

$$\Delta G_{el} = \int_{0}^{2\pi} \psi(q)\, dQ$$

where q is the colloid charge. In the EPN model, the Donnan potential is given by Eqn where $g_f Q$ is the humic charge (in mol kg^{-1}), equal and opposite in sign to that of the net gel ionic charge, thus the interaction energy for a given charge is $-\psi_D(g_f Q) g_f Q$, consequently Eqn results in

$$\Delta G_{el}^{EPN} = \int_{0}^{2\pi} \frac{RT}{F} \operatorname{arsinh}\left[\frac{(g_f Q)(\delta_2)}{2(1+\varepsilon_2)}\right] R\, d(g_f Q) =$$

$$= RT\left\{ g_f Q \operatorname{arsinh}\left[\frac{g_f Q(\delta_2)}{2(1+\varepsilon_2)}\right] + \sqrt{(g_f Q)^2 + \left[\frac{2(1+\varepsilon_2)}{\delta_2}\right]^2} - \frac{2(1+\varepsilon_2)}{\delta_2} \right\}$$

Similarly, for the DP model the humic charge Q is neutralized by a charge $-Q$ contained in the Donnan volume V_D, which is a function of the ionic strength:[31]

$$V_D = 10^{k(\log I - 1) - 1}$$

where the Donnan potential is now given by

$$\psi_D^{DP} = \frac{RT}{F} \operatorname{arsinh}\left(\frac{Q}{2V_D}\right)$$

and consequently the electrostatic free energy change is

$$\Delta G_{el}^{DP} = \int_{0}^{2\pi} \frac{RT}{F} \operatorname{arsinh}\left(\frac{Q}{2V_D}\right) R\, d(Q) = \left[\operatorname{arsinh}\left(\frac{Q}{2V_D}\right) + \sqrt{\left(\frac{Q}{2V_D}\right)^2 + 1} \right] \frac{RT}{F} \left(\frac{Q}{2V_D}\right)$$

To evaluate the predictions of Eqns and the electrostatic parameters (b in the DP model and χ, g_f and v_2 in the EPN case) should be consistent; thus the comparison will be done for the case of PPHA, which has been quite extensively studied. The EPN parameters are presented in Table 2, whereas for the ND model fitted to PPHA proton data Kinniburgh et al.[31] found b = 0.334. Fig. 4 shows the predicted electrostatic contribution to the free energy of binding as a function of ionic strength for different values of the humic charge. It is clearly visible that the ND model gives (in absolute value) a higher electrostatic energy than the EPN model. This in turn will result in lower values for the intrinsic binding constants, because a higher value of ΔG_{el} (in absolute value) forces a lower value for ΔG_{in}. The fact that the ND model tends to produce values for the log $K_{i,k}$ lower than other models and estimations (see Table 3 and Refs.[28,41]) suggests that in the Donnan volume model the electrostatic contribution could be effectively overestimated. On the other hand, there is no enough evidence at present to ascertain the accuracy of the EPN predictions.

Regarding the other electrostatic variables, φ_2 is inversely proportional to the volume of the humic gel fraction, thus a plot of $\varphi_2^{-1} = V/V_h$ shows the changes in the gel volume resulting from the electrostatic effects. Fig. 5 shows calculated curves of V/V_h for PPHA as a function of Q for different I values. These curves go through a common minimum at Q = 0: the model predicts a minimum volume, independent of I, for the case of zero net charge in the HS, increasing as the charge increases in either direction, the effect being less marked as I increases; the gel swells due to the electrostatic repulsion of ionized groups. The DP model, on the other hand, postulates a Donnan volume dependent only on I, Eqn . It should be noted that, for multivalent cation binding, the net charge can be positive at high loadings. Fig. 6 shows the Donnan potential as given by Eqns and for EPN and DP models, respectively; again the DP model predicts a higher value for ψ_D, specially at high charges, because in the EPN model for high Q the humic particle swells, causing an effective decrease of the charge density and consequently a relative decrease in ψ_D (Fig. 6b).

4.3.2. Experimental results for metal binding

The behaviour predicted by the NICA-EPN model for several experimental systems is analysed next. Fig. 7 shows the results of ρ, ψ_D and V/V_h for Cu(II) binding to ESLHA (dataset HCu-08),[57] plotted as a function of the Cu(II) activity. The volume curves clearly merge at a minimum at $a_{Cu} \sim 10^{-3}$ M, because the humic charge vanishes at this point, where ψ_D also vanishes, as expected. In the limit of lower Cu activities the Donnan potential is constant, because the amount of metal bound has a negligible effect on the net humic charge. As Q_{Cu} increases, ρ becomes less negative, ψ_D follows the tendency and volume decreases (the gel shrinks) due to decreased electrostatic repulsion. The effect of pH and I is observed: at higher pH and lower I the volume changes are more marked due to the increased electrostatic effect, because of higher charge and lower screening, respectively. In the case of the Donnan potential, the ionic strength has a noticeably stronger effect; the higher charge at high pH is partially compensated by the gel swelling, effectively limiting the charge density; noticeably the effect of I is markedly different comparing ρ and ψ_D: at low I, ρ is lower because there is smaller screening of the electrostatic repulsion and so the gel swells significantly; at higher I there is more screening and less swelling. Conversely, the Donnan potential follows essentially the magnitude of the electrostatic repulsion, and the volume behaves similarly. An interesting result is that all curves merge at the same point in a_{Cu}, not only those at different ionic strength (as expected) but also those at different pHs; this fact would indicate that the pH has little effect on the humic charge, which should be dominated by Cu binding to the HAS due to the high value of $K_{Cu,2}$ (Table 3). In Fig. 2c it can be seen that all the Cu binding curves tend to merge at high Cu activities. In most cases analysed here, the same features are observed; Fig. 8 shows similar results for binding of Pb(II) to Fluka humic acid.[24] Additionally, in Figs. S2 to S4 (Supporting material) some other cases are presented; even when the range of metal activities are more restricted, the tendency of the curves at different pHs to merge at high metal activity is observed.

In Fig. 9 results of ρ and V/V_h for Al(III) binding to SRFA are presented (the curves for the Donnan potential are very similar in shape to ρ). Here, at variance with Fig. 8 the curves do not appear to merge

at $\rho = 0$, but instead tend to diverge as the Al activity increases; at higher pH, the curves rise more markedly. The cation Al^{3+} undergoes hydrolysis at pH ≥ 4 (Fig. S5, Supporting material), and in this process, as it is well known, the formation of polynuclear complexes takes place (polymerization);[58] the onset of that process lies where the curves in Fig. 9 start to cross over those at lower pHs. Thus, this behaviour is attributable to the formation of Al-OH^- "polymers" upon binding, increasing the positive charge bound to the HS, which shows up in the rapid rise of ρ (and also ψ_D) and decrease of volume observed. It should be noted that this behaviour is not included in the model; as remarked in the theoretical section, the model in its present form is not sensitive to the particle size; also, multiple cation binding to a single site is not considered.

4.3.3. Final remarks

The NICA-EPN model is shown to explain with good accuracy the binding of metal cations to HS. It predicts a moderate electrostatic interaction free energy (as compared with the Donnan volume model), which leads to log $K_{H,k}$ values closer to the range of common carboxylic acids. The electrostatic parameters predict volume changes in the gel fraction accompanying the binding of protons and/or metal cations; at present time there is little experimental information to compare with such predictions, only in the case or proton binding[23] electrophoretic mobility experiments are reported which are consistent with model predictions.[10] The available spectroscopic data suggest that low affinity sites should correspond to single binding to carboxylate groups, whereas high affinity sites should be related to multidentate binding, in chelate type complexes, possibly involving phenol groups in salicylate or catechol groups, including bridging together different humic molecules through ligand groups of each molecule. It should be noted, however, that the NICA isotherm does not consider multidentate binding.

It is worth to remark here that the NICA-EPN model shows good behaviour for both fulvic and humic acids. It is important to take into account that FAs are composed by relatively small molecules (a few kD), thus they are not expected in principle to behave as a gel. However, in natural environments these small molecules are normally associated in larger entities; in the laboratory experiments modelled here,

it is also possible that FA molecules were associated to some extent. On the other hand, it should be noted that the most important feature here is the increased electric potential inside the model gel; even for a not very large FA molecule, it can be expected the presence of an increased electric field in the molecular neighbourhood, which on average would be represented by the EPN Donnan potential. Also, molecular size increase upon pH increase is shown by FA similarly to HA as verified, for example, by viscosity[21] and mobility[10] measurements, thus the gel expansion models this behaviour. Clearly, direct experimental evidence of size changes associated to metal binding (other than particle agglomeration) for fully unaggregated fulvic acids is required to check the model but, unfortunately, this is not presently available. It should be also noted that most current models of HS treat both fulvic and humic acids as particles, even when the former have sizes in the order of 1 nm or even less.[59] The present model can be considered, specially in the case of FA, as an average statistical representation of the actual molecular behaviour.

From the point of view of environmental application, it is desirable to have a body of reliable model parameters allowing the prediction of metal binding (specially trace metals) by natural organic matter. In this work it is shown that the NICA-EPN model is able to predict adequately metal binding to HS; however, more experimental data is required to achieve an extensive model calibration. Also, the EPN model addresses another important property of HS, shrinking/swelling in response to changes in the binding state. In the application to poorly known environments, previous calibration may be difficult, time consuming, and even not feasible. A possible solution is the use of generic or average parameters, such as those of Milne et al.,[19] Gustafsson,[40] etc.; this approach is known to give predictions within 0.5 - 1 log units, which may not be appropriate in some circumstances. However, it has been recently shown[53,60] that by modification of just one parameter the agreement can be considerably improved, which opens the possibility that a set of generic parameters could be tailored for a specific environment with the adjustment of few, easily measurable parameters.

5. Conclusions

The following conclusions stem from the present work:

1. The NICA-EPN model describes well the proton and metal binding to HS over a wide range of pH and ionic strength values.

2. The electrostatic contribution to the Gibbs free energy of metal-humic interaction in the EPN model is lower than that predicted by the Donnan volume model.

3. The intrinsic mean binding constants for protons are close to independent estimations and to the range of common carboxylic acids.

4. The metal biding parameters are comparable with values for metal binding to monocarboxylic acids for low affinity sites and with chelate type complexes for high affinity sites.

5. In most cases studied, the pH dependence of the model electrostatic variables at high metal loading vanishes, indicating a metal-dominated electrostatic behaviour.

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APPENDIX A. Supporting online material

Supporting data associated with this article can be found in the online version.

FIGURE CAPTIONS

Fig. 1. Schematic representation of the Elastic Polyelectrolyte Network model: a fraction g_f of the total humic particle is modeled as a gel-like region in Donnan equilibrium with the bulk solution, defining a Donnan potential ψ_D which affects the ion activity inside the gel. The remainder fraction 1 - g_f is assumed to be in equilibrium with the bulk. The small circles represent negatively charged sites; small circles with a single plus sign represent bound H^+ ions; circles with two plus signs represent bound divalent metal cations. Solution ions are not represented for clarity.

Fig. 2. Metal binding curves to humic substances fitted to the NICA-EPN model: (a) Al(III) binding to Suwannee River fulvic acid (dataset FAl-01); (b) Ca(II) binding to PUHA; (c) Cu(II) binding to Eliot silt loam humic acid (HCu-08); (d) Cd(II) binding to purified peat humic acid (HCd-03 and 07); (e) Pb(II) binding to purified peat humic acid (HPb-05, 06 and 07). The symbols are experimental points and the lines are curves of the best fit to the NICA-EPN model.

Fig. 3. Competitive metal binding to PPHA (symbols) modelled with the NICA-EPN model (lines): (a) Cd binding in the presence of Ca(II) (approximately 10^{-3} M) at different pH values, in 0.1 M KNO_3; data from Ref. [61]; (b) Pb(II) binding in the absence and presence of Al(III), at pH = 4.5 and varying I; data from Ref. [48]; (c) Cd(II) binding in the absence and presence of Al(III) at different pH values, at I = 5 mM; data from Ref. [48].

Fig. 4. Electrostatic contribution to the Gibbs free energy of binding as predicted by the Donnan volume (DV) and EPN models for PPHA, as a function of the logarithm of the ionic strength for different humic charges; I is in mol L^{-1} and Q in mol kg^{-1}.

Fig. 5. Gel volume relative to the dry humic volume as a function of net humic charge for different ionic strengths, as predicted by the NICA-EPN model; I is in mol L^{-1} and Q in mol kg^{-1}.

Fig. 6. Donnan potential inside the Donnan volume (DV, dashed line) or the Donnan gel (EPN, solid line) for PPHA as a function of ionic strength for several humic charges (a) and as a function of charge for an ionic strength of 0.01 M (b).

Fig. 7. Humic gel charge density (a), Donnan potential (b) and relative humic gel volume (c) for Cu(II) binding to ESLHA, as a function of Cu activity.

Fig. 8. Donnan potential (a) and relative humic gel volume (b) for Pb(II) binding to FHA, as a function of Pb activity.

Fig. 9. Humic gel charge density (a) and relative humic gel volume (b) for Al(III) binding to SRFA, as a function of Al activity.

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