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Addressing the electrostatic component of protons binding to aquatic nanoparticles beyond the Non-Ideal Competitive Adsorption (NICA)-Donnan level: Theory and application to analysis of proton titration data for humic matter

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► To cite this version:

José Paulo Pinheiro, Elise Rotureau, Jérôme F.L. Duval. Addressing the electrostatic component of protons binding to aquatic nanoparticles beyond the Non-Ideal Competitive Adsorption (NICA)-Donnan level: Theory and application to analysis of proton titration data for humic matter. *Journal of Colloid and Interface Science*, 2021, 583, pp.642-651. 10.1016/j.jcis.2020.09.059 . hal-02989151v1

HAL Id: hal-02989151

<https://hal.univ-lorraine.fr/hal-02989151v1>

Submitted on 16 Nov 2020 (v1), last revised 29 Sep 2021 (v2)

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1 Graphical Abstract

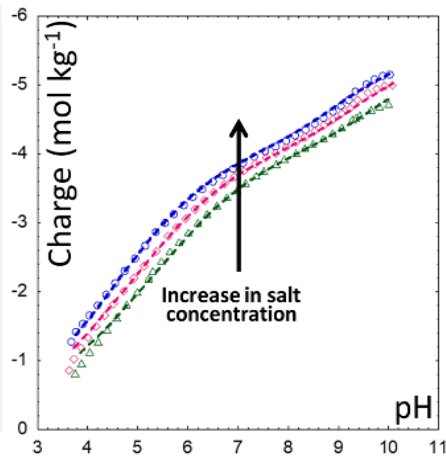
Soft Poisson-Boltzmann Evaluation of Proton Titration for Nanoparticles (SPBT)

Theoretical dependence vs. pH and [salt]
Ion-permeable (soft) particle representation

Experimental data

Module for optimization of SPBT parameters (PEST)

Matching theory to proton titration and affinity spectra data



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18 **Addressing the electrostatic component of protons binding to aquatic**
19 **nanoparticles beyond the Non-Ideal Competitive Adsorption (NICA)-**
20 **Donnan level: theory and application to analysis of proton titration data for**
21 **humic matter.**

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50 **Abstract**

51 *Hypothesis.*

52 Charge descriptors of aquatic nanoparticles (NPs) are evaluated from proton titration curves measured at
53 different salt concentrations and routinely analysed by the Non-Ideal Competitive Adsorption-Donnan
54 (NICAD) model. This model, however, suffers from approximations regarding particle electrostatics,
55 which may bias particle charge estimation. Implementation of Poisson-Boltzmann (PB) theory within
56 consistent treatment of NPs protolytic data is expected to address NICAD shortcomings.

57 *Experiments.*

58 An alternative to NICAD is elaborated on the basis of nonlinearized PB equation for soft particle
59 electrostatics to properly unravel the electrostatic and chemical components of proton binding to NPs. A
60 numerical package is developed for automated analysis of proton titration curves *and* proton affinity
61 spectra at different salt concentrations. The performance of the method is illustrated for humic matter
62 nanoparticles with different charge and size, and compared to that of NICAD.

63 *Findings.*

64 Unlike NICAD, PB-based treatment successfully reproduces particle charge dependence on pH for
65 practical salt concentrations from the thin to thick electric double layer limit. Donnan representation in
66 NICAD leads to moderate to dramatic misestimations of proton affinity and binding heterogeneity
67 depending on particle size to Debye layer thickness ratio. Interpretation of NPs protolytic properties with
68 PB theory further avoids adjustment of the ‘particle Donnan volume’ empirically introduced in NICAD.

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71 **Keywords.** Poisson-Boltzmann theory, NICA-Donnan model, Particle electrostatics, Proton titration
72 curves, Nanoparticles, Humic matter.

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85 1. INTRODUCTION

86 Natural organic matter (NOM), the most important source of organic carbon on earth, is ubiquitous
87 in soils and in aquatic ecosystems [1]. NOM refers to a broad spectrum of heterogeneous mixtures of
88 macromolecules, nanoparticles and/or colloidal aggregates that differ in terms of physical and chemical
89 properties including size, molecular weight, composition and charge [2]. The humic substances (HS),
90 which represent the major NOM fraction, may be differentiated according to their solubility: the insoluble
91 fraction, the humin, exists only in soils, whereas soluble fulvic and humic acids (hereafter denoted as FA
92 and HA, respectively) are found in soils and natural waters [3]. Solubility of FA and HA further differs
93 with respect to pH, with FA being fully soluble even under extreme acid conditions, and HA being soluble
94 solely in weakly acidic and alkaline solutions [4]. Both FA and HA colloids are defined by nanometric
95 dimensions with radius of *ca.* 1 nm and 2 to 10 nm [5], respectively. Accordingly, they are now generically
96 termed as humic nanoparticles (HNPs in short) in literature.

97 The charge carried by HNPs strongly affects the mobility and the speciation of trace metal ions (M)
98 in aquatic media. Indeed, it dramatically impacts on (i) the stability of HNPs-M complexes [6],[7]. (ii) the
99 kinetics of the processes driving the formation and the dissociation of these complexes in solution [8],[9],
100 and (iii) their lability, *i.e.* the extent to which complexes dissociate on the time scale of their diffusion
101 toward M-consuming microorganisms [10],[11],[12]. The resulting connection between HNPs charge and
102 metal bioavailability in aqueous environment motivates the need to address the electrostatic properties of
103 HNPs at the proper quantitative level. The magnitude of the charge carried by HNPs inherently depends
104 on solution pH and on background electrolyte concentration, both affecting the dissociation degree of the
105 carboxylic and phenolic groups distributed throughout the HNPs volume [13]. In turn, the evaluation of
106 HNPs charge properties from *e.g.* proton titration data analysis necessarily asks for an adequate
107 differentiation between the chemical (intrinsic) and electrostatic components of the affinity of the protons
108 for the HNPs carboxylic and phenolic sites [14]. Successful unravelling of these contributions is obviously
109 tied to the robustness of the models in integrating the relevant electric double layer properties of HNPs,
110 or, equivalently, the electrostatic potential distribution that is operational at the HNPs/solution interface.

111 So far, proton binding to HNPs has been mainly addressed from applications of the Windermere
112 Humic Aqueous Model (WHAM) and of the Non-Ideal Competitive Adsorption (NICA) model developed
113 in the 90's [15-18]. For the sake of conciseness, we refer the reader to Koopal *et al.* [19],[20] and Town
114 *et al.* [7] for review and critical review of these modelling approaches. In their first attempt to integrate
115 electrostatics within NICA formalism, de Wit *et al.* [21] interpreted proton titration data measured on
116 various humic substances at different solution ionic strengths by means of the Poisson-Boltzmann (PB)
117 theory applied to hard particles, *i.e.* particles impermeable to ions from the background electrolyte.
118 Although such a PB-based modelling of humics electrostatic effects was reported to reproduce
119 'reasonably well' experimental titration data [21], a simpler but empirical electrostatic representation was
120 proposed where the potential is *a priori* considered constant within the whole particle body *and* in a

121 surrounding solution volume adjusted to match proton titration data [13]. In its initial version, this
122 ‘Donnan-like’ electrostatic model took the form of an equation involving 2 parameters describing
123 empirically the dependence of a ‘Donnan volume’ on solution ionic strength [22], replaced later by an
124 equation involving a single adjustable parameter [15]. It is this last version of the ‘Donnan-like
125 representation’ that is integrated in the NICA-Donnan (NICAD) model and, except for some differences
126 detailed elsewhere [7], in the WHAM model.

127 Even though NICA-Donnan modelling is largely adopted in metal speciation and proton binding
128 studies involving HNPs [16] or other types of nanoparticles [19], there are severe inconsistencies in its
129 account of particle electrostatics, as recently detailed by *e.g.* Town *et al.* [7]. Briefly, the spatial
130 distribution of the electrostatic potential at the HNPs/solution interface adopted in NICAD violates the
131 fundamental Poisson-Boltzmann formalism, a founding element of the classical DLVO theory [23]. Indeed,
132 in the limit where particle radius well exceeds the electric Debye layer thickness in solution (a situation
133 reached at sufficiently large electrolyte concentrations), the rigorous solution of the PB equation takes the
134 form of a step-like potential profile where the potential is essentially constant inside the particle volume
135 (this is the so-called Donnan potential) *and* zero outside: this is the *true* Donnan situation [24],[25],[26].
136 Accordingly, provided that medium salinity and particle size guarantee a correct application of the
137 Donnan representation in line with the inequality $\kappa r_p \gg 1$ with r_p the particle radius and $1/\kappa$ the Debye
138 layer thickness, the Donnan volume cannot exceed the physical volume of the particles [7],[26]. This
139 requirement is not respected by the NICAD model applied to HNPs [27]. In addition, within the NICAD
140 modelling framework, the Donnan electrostatic picture is adopted regardless of the size of the particles
141 considered and of the background electrolyte concentration, *i.e.* irrespectively of the value taken by κr_p .
142 This implies that the Donnan representation is basically considered valid over the whole spectrum of
143 electric double layer regimes, from the thick to the thin double layer extremes corresponding to $\kappa r_p \ll 1$
144 and $\kappa r_p \gg 1$, respectively. Obviously, this assumption is incorrect, recalling that a Donnan potential
145 profile is strictly operational under the only condition $\kappa r_p \gg 1$ [7]. Given a radius of say 5 nm for HA
146 particles, it is estimated that $\kappa r_p \sim 1.7$ to 5 in the range 10 mM to 100 mM electrolyte concentrations,
147 respectively, this salinity range being commonly considered for HA proton titration experiments [28].
148 Therefore, under conditions where the electrostatic component is largest (*i.e.* at low κr_p) or, equivalently,
149 when screening of particle electrostatics is least significant, the condition underlying the applicability of
150 a Donnan potential representation is not respected, which makes inappropriate the use of Donnan-based
151 modelling framework for HNPs [7].

152 The above shortcomings of the Donnan electrostatic model applied to HNPs are not new. Already in
153 1999, Avena *et al.* [27] explicitly recognized that “application of a simple Donnan model will only give
154 results that are comparable to those obtained with the [PB] impermeable sphere model if the Donnan
155 volumes used are larger than the specific volumes of the hydrated humics”. In 2007, aware of the empirical
156 nature of the adjustment of the ‘particle Donnan volume’ in NICAD, Companys *et al.* [29] proposed a

157 formulation of the Donnan volume on the basis of analytical solutions of Poisson-Boltzmann equation.
158 The approach, however, excludes salinity conditions of practical interest where Donnan representation
159 for HNPs is not applicable and it does not integrate the whole PB-based spatial distribution of the
160 electrostatic potential. In 2005, Duval *et al.* [30] reported an electrokinetic analysis of HNPs particles
161 over a large range of pH and electrolyte concentration conditions on the basis of an electro-hydrodynamic
162 theory developed for the generic case of soft particles (*i.e.* permeable to ions from background electrolyte)
163 [31] whose electrostatic properties were tackled at the non-linear PB level. The authors thereby
164 demonstrated the inapplicability of the hard particles-representation for HNPs that display, instead, the
165 characteristic electrokinetic signature of soft particles whose intraparticulate potential approaches the
166 Donnan potential at sufficiently large κr_p . It is emphasized that the hard sphere-representation proposed
167 earlier for HNPs [21] is not compatible with the establishment of a Donnan potential in the particle body:
168 this potential follows indeed from the compensation of the *intraparticulate* HNPs structural charges by
169 electrolyte ions that have been transferred from bulk solution to particle volume by conduction-diffusion
170 transport. Finally, Saito *et al.* [32] revealed dramatic (sometimes exceeding 100 %) discrepancies between
171 average HA particle potentials as determined by NICAD and that measured from fluorescence quenching.
172 Instead, they evidenced better agreement with average potentials estimated on the basis of ion permeable-
173 sphere model and with adopting an electrostatic particle representation that integrates the extraparticulate
174 electric double layer contribution.

175 In view of the above elements, a sound physicochemical modelling of HNPs protolytic properties
176 where particle electrostatics is tackled at the proper non-linear Poisson-Boltzmann level for soft spheres
177 is still lacking in literature. In this work, we report such a formalism and we elaborate a numerical
178 procedure able to handle automatically, consistently and simultaneously, the recovery of proton titration
179 curves measured on HNPs of given size at different solution ionic strengths. These results are
180 systematically compared to those published using empirical NICAD model, thereby clearly identifying
181 the cases where application of NICAD is erroneous or approximate. The numerical package designed in
182 this work is available on demand.

183

184 **2. MATERIALS**

185 Polydisperse HNPs samples may form aggregates depending on pH, medium salinity and nature of
186 the cations in solution [33]. In turn, the evaluation of HNPs radius (r_p) and molar mass (M_w) from proton
187 titration data modelling remains uncertain as the reliability of the obtained results necessarily depends on
188 the way particle electrostatics is pictured, recalling that rigorous PB-based potential distribution at
189 HNPs/solution interface intrinsically depends on particle size. To provide a clear comparison -free of the
190 above uncertainties- between HNPs-protons binding properties derived following our approach and those
191 obtained from NICAD, the analysis below is based on protons titration data sets collected on HNPs for
192 which particle radius is measured by independent techniques and for which NICAD-based fitting
193 parameters are explicitly provided in literature. Using such particle size estimation, we evaluated M_w of

194 the HNPs after exploiting M_w data reported for HNPs of composition similar than those considered in this
 195 work. As detailed below, this procedure constrains the modelling of protolytic titration data *versus* pH
 196 and electrolyte concentration and makes transparent the inherent electrostatic limits of NICAD model. In
 197 contrast, it advocates for the recourse to more rigorous soft PB theoretical framework. In line with the
 198 above elements, we selected five proton titration data sets published for HNPs with known particle radius,
 199 namely: the Ribeirão Preto Humic acid (FPHA)[34], Suwanee River Fulvic Acid (SRFA)[28], Laurentian
 200 Fulvic acid (LFA) [35] and the fulvic (Hf:FA) and humic acid (Hf:HA) from Sierra de Buio, Spain [36].
 201 A detailed description of these HNPs, of their size measurement and of the estimation of their molar mass
 202 (**Table 1**) is provided in **Supporting Material (SM, section A** therein).

203

204 3. THEORY

205 The evaluation of HNP charge and protolytic properties is commonly carried out from the analysis of
 206 protons titration experiments performed in 1:1 electrolyte whose concentration is varied in the range 1
 207 mM to 100 mM. The strategy thus consists in using the proton as a probe to explore the electrostatic-
 208 dependent charging behaviour of HNPs. In turn, pending proper theoretical treatment, titration data
 209 provide the desired relevant electrostatic descriptors and charge of HNPs as a function of pH and
 210 electrolyte concentration.

211 *3.1. Electrostatic and chemical descriptors of HNPs.* The total charge Q^0 carried by a single HNP and
 212 accessible from titration experiments may be expressed as the integral over the intraparticulate volume of
 213 the density $\rho_0^{(s)}$ (in C m⁻³) of net charges stemming from the dissociation of the structural carboxylic and
 214 phenolic groups distributed throughout the HNPs volume:

$$215 \quad Q^0(\text{pH}, c_{\text{elec}}^\infty) = \frac{4\pi}{FV_p} \int_0^{r_p} \rho_0^{(s)}(r, \text{pH}, c_{\text{elec}}^\infty) r^2 dr \quad (1)$$

216 where $V_p = 4\pi r_p^3/3$ is the volume of an individual HNP (m³), r is the radial coordinate with the particle
 217 centre positioned at $r=0$, r_p is the particle radius, F is the Faraday and Q^0 is expressed as a concentration
 218 of monovalent charges per unit HNPs volume (mol m⁻³). Eq 1 makes explicit that Q^0 and $\rho_0^{(s)}$ both depend
 219 on solution pH and on the background electrolyte concentration denoted as c_{elec}^∞ . The charge density $\rho_0^{(s)}$
 220 is determined by the local concentration of protons within the HNP body which itself depends on the
 221 potential profile $\psi(r)$. For the sake of convenience, we introduce the dimensionless local electrostatic
 222 potential defined by $y(r) = zF\psi(r)/RT$ where z is the valence of the $z:z$ background electrolyte ($z=1$
 223 under commonly adopted titration experiment conditions). The radial dependence of $y(r)$ is governed by
 224 the Poisson-Boltzmann (PB) equation where contributions from *both* (immobile) structural charges
 225 carried by HNPs *and* (mobile) ions from the background electrolyte are accounted for. After
 226 straightforward arrangements, the dimensionless PB equation applicable to soft HNPs particles reads
 227 as[37][‡]

[‡] There is a typo error in eq 3 of ref. 37 where a minus sign is missing in front of the charge density term.

$$228 \quad \frac{d^2 y(\tilde{r})}{d\tilde{r}^2} + \frac{2}{\tilde{r}} \frac{dy(\tilde{r})}{d\tilde{r}} - \sinh[y(\tilde{r})] = - \frac{\rho_0^{(s)}(\tilde{r}, \text{pH}, c_{\text{elec}}^\infty)}{2Fz c_{\text{elec}}^\infty} \quad (2)$$

229 where \tilde{r} is the dimensionless radial position defined by $\tilde{r} = \kappa r$ with κ the reciprocal Debye length given
 230 by

$$231 \quad \kappa = \sqrt{2F^2 z^2 c_{\text{elec}}^\infty / (RT \epsilon_0 \epsilon_r)} \quad (3)$$

232 with ϵ_0 the dielectric permittivity of vacuum and ϵ_r the relative dielectric permittivity of the medium. Eq
 233 3 tacitly implies that the contribution of the protons and hydroxyl ions to the total solution ionic strength
 234 is not significant, which is legitimate under the pH and 1:1 electrolyte concentration conditions adopted
 235 in this work (and in many reports from literature [28]). Relaxing this condition is straightforward pending

236 replacement (i) of eq 3 by $\kappa = \sqrt{\sum_{j=1}^N F^2 z_j^2 c_j^\infty / (RT \epsilon_0 \epsilon_r)}$ where the index j runs over all types of ions

237 with bulk concentration c_j^∞ present in solution, including H^+ and OH^- , and (ii) modification of the sinh
 238 term in eq 2 along the lines given elsewhere [38]. In addition, the current version of the program makes
 239 it possible the analysis of measurements performed in 2:1 electrolytes based on a proper transformation
 240 of eq 2 along the lines detailed in [6] (see eqs S1-S2 therein). Application of the formalism to other
 241 mixtures of electrolytes is further possible pending differentiation in eq 2 between the various Boltzmann
 242 terms associated with the respective contributions of the mobile ions to the overall charge of the extra-
 243 and intra-particulate electric double layers. Last, the program allows, if required, the extraction of the
 244 Boltzmann factor for proton accumulation (or that of any ions present in the electrolyte) within the overall
 245 body of the particle. This is done by spatial integration of the relevant local Boltzmann factor, as detailed
 246 in [6]. Also, if required, the Boltzmann factor pertaining to ion accumulation in the extraparticulate double
 247 layer can be easily retrieved upon proper setting of the limits of the corresponding spatial integration.

248 The boundary conditions associated to eq 1 are provided by

$$249 \quad y(\tilde{r} \rightarrow \infty) = 0 ; \left. \frac{dy(\tilde{r})}{d\tilde{r}} \right|_{\tilde{r}=0} = 0 \quad (4a,b)$$

250 where eq 4a reflects electroneutrality condition in bulk electrolyte solution and eq 4b results from
 251 symmetry of the potential profile at the particle centre. For the sake of demonstration, the developments
 252 below are illustrated for HNPs particle but the formalism remains valid for any particle type pending
 253 implementation of the relevant (Dirichlet- or Neumann-type of) electrostatic boundaries [39] and
 254 consideration of the proper number of structural charges type (set to 2 for HNPs) [40].

255 Under given pH and solution ionic strength conditions, the characteristic potential distribution $y(\tilde{r})$
 256 derived from eqs 1-2 takes the form of a bell shape with a maximum reached at the particle center, and it
 257 decreases gradually to zero value at few $1/\kappa$ distances far from the particle surface located at $r = r_p$ [37].
 258 In the limit $\kappa r_p \gg 1$, the electrostatic Donnan situation is achieved with $y(r \leq r_p) = y_D$ and $y(r > r_p) =$
 259 0 where y_D is the dimensionless Donnan potential [7]. It is emphasized that the local pH inside the HNPs
 260 volume depends on position r and thus differs from the solution pH to an extent that depends on the
 261 particle charge, or equivalently, on the magnitude of the intraparticulate potential.

262 The raw data Q obtained from proton titration experiments are classically expressed in equivalent
 263 concentration of monovalent charges per mass of titrated HNPs (*i.e.* Q is in mol kg⁻¹). Q is then related to
 264 the volume charge density Q^0 (mol m⁻³) of a single HNP particle (eq 1) according to

$$265 \quad Q = Q^0 V_p N_A / M_W = Q^0 v_p \quad (5)$$

266 where N_A is the Avogadro number, M_W (kg mol⁻¹) is the molar mass of the considered HNP material, and
 267 $v_p = V_p N_A / M_W$ is the specific volume of HNP (in m³ kg⁻¹). The conversion of Q^0 to a charge expressed
 268 in standard experimental unit (mol kg⁻¹) therefore requires knowledge of the particle radius r_p and of the
 269 molecular weight M_W , or of the only specific volume v_p . This conversion is systematically required
 270 because PB-based electrostatics of particles does not depend on the total amount of charges carried by the
 271 particles but, instead, by their volume density of charges.

272 The structural charges carried by HNPs originate from the (de)protonation of carboxylic and phenolic
 273 groups. Due to the intrinsic chemical heterogeneity of HNP material, equilibrium protonation constants
 274 are classically modelled as continuous distributions [41] involving mean protonation constants denoted
 275 as \bar{K}_{a1} and \bar{K}_{a2} for the carboxylic and phenolic groups, respectively, and the distribution width m_{H1} and
 276 m_{H2} . The later reflects the chemical heterogeneity of the carboxylic and phenolic binding sites throughout
 277 HNPs due to differences in their molecular environments. The limits $m_{H1}=m_{H2}=1$ apply to ideal situations
 278 where HNP composition is homogeneous. The local structural charge density, $\rho_0^{(s)}(r, \text{pH}, c_{\text{elec}}^\infty)$ involved
 279 in eqs 1-2 is then provided by

$$280 \quad \rho_0^{(s)}(r, \text{pH}, c_{\text{elec}}^\infty) = \frac{\rho_{\text{maxH1}}^{(s)}}{1+10^{m_{H1}(\text{p}\bar{K}_{a1}-\text{pH})}\exp[-m_{H1}y(\bar{r})]} + \frac{\rho_{\text{maxH2}}^{(s)}}{1+10^{m_{H2}(\text{p}\bar{K}_{a2}-\text{pH})}\exp[-m_{H2}y(\bar{r})]} \quad (6)$$

281 where $\rho_{\text{maxH1}}^{(s)}$ and $\rho_{\text{maxH2}}^{(s)}$ are the maximum densities of structural charges (mol m⁻³) achieved under pH
 282 conditions where all carboxylic and phenolic sites are fully deprotonated, respectively. $\rho_{\text{maxH1}}^{(s)}$ and
 283 $\rho_{\text{maxH2}}^{(s)}$ may be transformed in mol kg⁻¹, by multiplication by v_p , resulting in Q_{maxH1} and Q_{maxH2} ,
 284 respectively. The set of eqs 1-6 fully determines the dependence of the searched charge Q on pH and ionic
 285 strength. A Fortran program (named **SPBT** for **S**oft **P**oisson **B**oltzmann-based **T**itration) was developed
 286 to compute eqs 1-6. In particular, the solution of eq 2 was derived according to the numerical strategy
 287 employed elsewhere [42] using Fortran COLSYS subroutine [43] which approximates the solution
 288 through spline-collocation at Gaussian nodes and selects mesh subdivision following an auto-adaptative
 289 strategy. This algorithm has already been proven extremely efficient for evaluation of *e.g.* complex soft
 290 particle electrokinetics [44] and soft macrosurface electrohydrodynamics [45]. The one-component NICA
 291 equation commonly adopted for proton titration analysis is a Langmuir-Freundlich equation that involves,
 292 similarly to eq 6, the protolytic parameters $\rho_{\text{maxH1}}^{(s)}, \rho_{\text{maxH2}}^{(s)}$ (or Q_{maxH1} and Q_{maxH2}), $\bar{K}_{a1}, \bar{K}_{a2}, m_{H1}$ and
 293 m_{H2} (see details of NICAD modelling in **SM, section B**). The electrostatic component of this NICA
 294 equation is tackled *via* a Donnan-like representation of the potential profile inside the particle body *and*
 295 in its aqueous surrounding, as commented in the Introduction section and further detailed in **SM, section**
 296 **B**.

297 3.2. *Fitting proton titration data to theory: strategy.* The theoretical framework provided by eqs 1-6
 298 involves 8 parameters that must be consistently adjusted to reproduce proton titration data collected at
 299 different solution ionic strengths, namely r_p , M_w , $\rho_{\max H1}^{(s)}$, $\rho_{\max H2}^{(s)}$ (or $Q_{\max H1}$ and $Q_{\max H2}$), \bar{K}_{a1} , \bar{K}_{a2} ,
 300 m_{H1} and m_{H2} , all of them being independent of the solution ionic strength provided that particles do not
 301 significantly swell with varying pH or c_{elec}^∞ , which is the case for *e.g.* FA and HA (see related discussion
 302 in the next section) [7],[46]. As the range of r_p and M_w values is known (**Table 1**), this number basically
 303 reduces to 6, which is consistent with the conclusion by Lenoir and Manceau [47] who evidenced, *via*
 304 Principal Component Analysis (PCA) of proton titration data collected on a large series of fulvic and
 305 humic samples, that only 6 independent parameters could be adjusted to reproduce experiments with least-
 306 square minimization. The analysis of proton titration data using the current version of NICAD requires
 307 one additional parameter to be adjusted. This parameter, termed b , connects empirically the ‘particle
 308 Donnan volume’ V_D (erroneously allowed to exceed V_p) where the potential is considered *a priori*
 309 constant, to the logarithm of the solution ionic strength, $\log I$. Lenoir *et al.* [47] argued that using NICAD,
 310 “the total covariance of the system cannot be reduced by measuring titration data at various ionic strengths
 311 because $\log I$ is correlated to $\log V_D$ ”. This observation mirrors the failure of NICAD in establishing at
 312 the correct PB level the connection between potential profiles at the HNPs/solution interface and medium
 313 salinity. Janot *et al.* [35] compared unconstrained and constrained proton procedures for titration data
 314 fitting with use of NICAD. They concluded that different sets of model parameters derived with different
 315 procedures could lead to similar quality of data fit, thereby questioning again the physical relevance of
 316 the obtained model parameters. Another important aspect is that the lack of obvious inflection points in
 317 the proton titration curves implies that the quality of the theoretical recovery of the titration data,
 318 regardless of the funding modelling approximations, may depend on the initial estimates of the adjusted
 319 parameters, which in turn conditions the performance of the algorithm to converge to a solution with
 320 minimisation of residuals [48]. Accordingly, to obtain more reliable information on HNPs charge and
 321 electrostatic properties from confrontation between experiments and theory, the fitting procedure we
 322 adopt, detailed in the next section, considers *simultaneously* the reconstruction of the proton titration data
 323 (Q vs. pH) and that of its first derivative with respect to pH (dQ/dpH vs. pH), the so-called proton affinity
 324 spectrum.

325 3.3. *Procedure for proton titration data reconstruction.* The parameters r_p , M_w , $Q_{\max H1}$, $Q_{\max H2}$, \bar{K}_{a1} ,
 326 \bar{K}_{a2} , m_{H1} and m_{H2} were optimized to reproduce the raw proton titration data and the associated affinity
 327 spectra by coupling our home-made Fortran program SPBT with **PEST**, an open source software for
 328 adjustment and estimation [49] of parameters by confrontation of theoretical models to experimental data.
 329 The minimization procedure adopted in PEST for fitting theory to data is based on Gauss-Marquardt-
 330 Levenberg algorithm which minimizes the weighted sum Σ of squared differences between model-
 331 generated data (supplied here by SPBT) and corresponding experimental measurements. **Figure S1** in **SM**
 332 (**section C**) summarizes the key steps of the proton titration data treatment by the coupled SPBT-PEST

333 codes, which includes the estimations of sound initial guessed values for the chemical parameters $Q_{\max H1}$,
 334 $Q_{\max H2}$, m_{H1} , m_{H2} , \bar{K}_{a1} and \bar{K}_{a2} (**SM, Figure S2, section D**) as well as refinement of measured r_p and M_w .
 335 Optimization of r_p and M_w at one side and $Q_{\max H1}$, $Q_{\max H2}$, m_{H1} , m_{H2} , \bar{K}_{a1} and \bar{K}_{a2} at the other is
 336 performed according to a two-step process detailed in **Figure S1**. The reader is referred to **SM (section**
 337 **C)** for exhaustive details of the procedure driving the iterative dialog between PEST (in charge of the
 338 generating parameters and confronting experimental prediction to experimental data) with SPBT that
 339 computes (Q vs. pH and I) and (dQ/dpH vs. pH and I) data for given set of parameters.

340 In the next section, we discuss the performance of SPBT-PEST in reproducing the Q and dQ/dpH vs.
 341 pH data measured at different background electrolyte concentrations for the HNPs samples listed in **Table**
 342 **1**. We further compare the corresponding sets of optimized parameters with those derived by NICAD-
 343 based modelling and reported in literature for FPHA [34], SRFA [28], LFA [35], Hf:FA [36] and Hf:HA
 344 [36] particles.

345

346 4. RESULTS AND DISCUSSION

347 4.1. Comparison between HNPs specific volume derived from SPBT-PEST and Donnan 348 volume estimated from NICAD modelling

349 As detailed in the theoretical section, the particle radius r_p and molecular weight M_w can be used to
 350 compute the particle specific volume v_p ($\text{m}^3 \text{kg}^{-1}$). **Table 1** collects r_p , M_w and v_p obtained from SPBT-
 351 PEST analysis of the proton titration data measured at different solution ionic strengths for the various
 352 HNPs of interest. For the sake of comparison, **Table 1** further reports the NICAD-based Donnan Volume
 353 V_D (also expressed in $\text{m}^3 \text{kg}^{-1}$) estimated for the various HNPs types considered on the basis of NICAD
 354 model parameters published in literature.

355 **Table 1:** Comparison between HNPs specific volumes v_p derived in this work and model-generated Donnan volume
 356 V_D computed from the parameters reported in literature for NICAD modelling. Eq S3 and eq S4 given in **SM** specify
 357 the way V_D was estimated, *i.e.* either *via* the two-parameter (α , β) dependent or the one-parameter (b) dependent
 358 NICAD empirical equations that relates V_D to the logarithm of ionic strength. Initial estimates (superscript i in the
 359 Table) and refined values (superscript ii) of particle size r_p and molecular weight M_w by SPBT-PEST are further
 360 indicated. Numbers between brackets correspond to κr_p values, recalling that Donnan potential-representation is
 361 strictly applicable for $\kappa r_p \gg 1$.
 362

HNPs type	r_p ($\times 10^{-9}$ m)	M_w (kg mol $^{-1}$)	v_p ($\text{m}^3 \text{kg}^{-1}$)	Donnan fitting parameters	Model-generated Donnan volume V_D ($\text{m}^3 \text{kg}^{-1}$)		
				α, β for eq. S3 or b for eq. S4	100 mM	10 mM	1 mM
FPHA [34]	3.1 to 5.9* ⁱ 5.0 ⁱⁱ	19.2 to 23 ⁱ 23 ⁱⁱ	0.0137	$\alpha=0.60\pm 0.32$ $\beta=-0.27\pm 0.05$	0.0074 (5.20)	0.0138 (1.64)	0.0257 (0.52)
SRFA [28]	0.6 to 1.05 ⁱ 0.6 ⁱⁱ	0.5 to 1.5 ⁱ 0.545 ⁱⁱ	0.0010	$b=0.87$	0.0055 (0.60)	0.0407 (0.20)	0.3020 (0.06)

LFA [35]	1.9 to 3.6 ⁱ	5.5 to 11 ⁱ	0.0118	$b=0.35 \pm 0.02$	0.0004 (3.74)	0.0009 (1.18)	0.0019 (0.37)
	3.6 ⁱⁱ	10 ⁱⁱ		$\alpha=0.93 \pm 0.20$ $\beta=-0.27 \pm 0.05$	0.0158 (3.74)	0.0295 (1.18)	0.0550 (0.37)
Hf:FA [36]	0.75 to 1.0 ⁱ 1.0 ⁱⁱ	0.5 to 1.5 ⁱ 0.9 ⁱⁱ	0.0028	$b=0.43$	0.0007 (1.04)	0.0019 (0.33)	0.0052 (0.10)
Hf:HA [36]	0.75 to 1.0 ⁱ 1.0 ⁱⁱ	1.0 to 3.0 ⁱ 1.8 ⁱⁱ	0.0014	$b=0.34$	0.0005 (1.04)	0.0010 (0.33)	0.0023 (0.10)

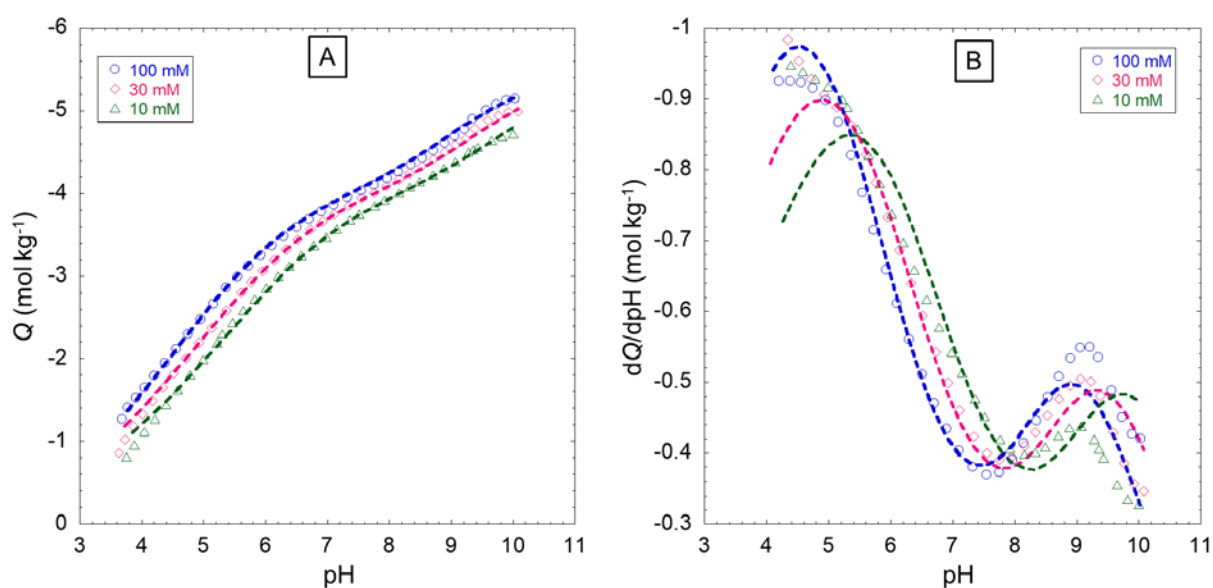
363 *Determined in this work by Scanned Stripping Chronopotentiometry (SSCP), see details in **SM (section A)**, therein).

364 The Donnan volume V_D significantly increases with decreasing solution ionic strength I . This
365 dependence of V_D on I , systematically observed with NICAD modelling regardless of sample types [19],
366 has been motivated by viscosity measurements and argued to stem from particle swelling [50]. However,
367 as discussed by Town *et al.*, [7] this justification is questionable because it ignores the influence of primary
368 and secondary electroviscous effects that originate from particle electric double layer interactions and
369 interparticulate electrohydrodynamic interactions. These effects, in turn, can lead to substantial variations
370 in the viscosity of particle dispersions with pH and ionic strength even under *constant* particle size
371 conditions [51],[52],[53]. Besides, may the particle size (and thus V_D) increases significantly with
372 decreasing I , one would anticipate a strong dependence of V_D with pH, similarly to that observed for *e.g.*
373 responsive PNIPAM particles [54], essentially because the driving electrostatic force for particle swelling
374 depends on particle charge and thus on both I and pH. In contradiction to this anticipation, V_D is
375 considered constant with pH in NICAD modelling. Last, for both HA and FA of soil and aquatic origins,
376 particle size measurements performed with different techniques [7],[55],[56] show that there is no
377 significant ionic strength- nor pH- dependent swelling/contraction of the particles in aqueous dispersions
378 (the argument excludes *extreme* pH and I conditions where particles aggregation may take place). These
379 elements highlight the empirical *ad hoc* fitting/calibration of V_D for every salt concentration tested. In
380 addition, for many of the situations considered in **Table 1**, V_D exceeds v_p . This basically comes to position
381 particle structural charges within a volume that exceeds the physical volume of the particle, which is
382 inconsistent from an electrostatic point of view. Within the PB framework adopted in SPBT-PEST
383 analysis of proton titration data and proton affinity spectrum, the HNPs specific volume v_p is independent
384 of solution pH and ionic strength and, accordingly, it does not suffer from the inconsistencies of V_D
385 estimates listed above. Last, it is clear that for most of the salinity and particle size conditions examined,
386 the $\kappa r_p \gg 1$ condition required for rigorous application of a Donnan representation of the electrostatic
387 potential distribution at HNPs/solution interface is not satisfied (see **Table 1**), which further questions the
388 physical meaning of the NICAD framework applied under such conditions.

389
390 **4.2. Performance of SPBT-PEST in recovering proton titration and affinity spectrum data.**

391 **Figures 1** and **2** report the SPBT-PEST theoretical analyses of the proton titration and affinity
 392 spectrum data measured for FPFA and SRFA at different NaNO_3 electrolyte concentrations, respectively,
 393 and the corresponding adopted model parameter values are listed in **Table 2** together with their statistical
 394 dispersion evaluated by PEST. For the sake of conciseness, reconstruction of data pertaining to LFA,
 395 Hf:FA and Hf:HA are given in **SM (section E, Figures S3-S5)** and corresponding model parameters are
 396 provided in **Table 2**.

397 **Figure 1A** shows a good agreement between titration experiments and SPBT-based theory for FPFA
 398 particles. In particular, the analysis reproduces with accuracy the electrostatic component of the proton
 399 binding to the particles, which is materialized by the correct positioning of the theoretical curves with
 400 varying background electrolyte concentration. This successful match between theory and experiments
 401 does not suffer from *ad hoc* adjustment of particle size and it intrinsically integrates the way
 402 intraparticulate *and* extraparticulate electrostatic potential distributions are modified with varying particle
 403 size and electrolyte concentration, features that are not shared by the empirical NICAD modelling
 404 framework. It is further emphasized that data treatment on the basis of SPB-PEST is significantly more
 405 constrained than that performed with using NICAD as the former, unlike the latter, considers simultaneous
 406 analysis of proton titration *and* affinity spectrum.



407 **Figure 1:** Proton titration curves for FPFA (A) and corresponding proton affinity spectra (B) collected at three
 408 NaNO_3 electrolyte concentrations: 10 mM (Δ), 30 mM (\diamond) and 100 mM (\circ). Symbols: measurements. Dotted lines:
 409 SPBT-PEST modelling results. Model parameters are listed in **Tables 1-2**. The charge is expressed in moles of
 410 equivalent charges per kg of FPFA material.
 411

412 **Table 2:** SPBT-PEST optimized values of the chemical parameters involved in the Langmuir-Freundlich equation
 413 (eq 6) for the various HNPs types considered in this work. Indicated dispersions of the parameters correspond to the
 414 95% confidence interval provided by PEST. The parameters are retrieved from SPBT-PEST and NICAD modelling
 415 of the proton titration curves measured at different salt reconstructions and reported in **Figures S6-S10** for the five
 416 HNPs samples of interest. SPBT-PEST and NICAD theoretical reconstructions of these proton titration data *versus*
 417 pH and salt concentration are also provided in **Figures S6-S10**.
 418
 419

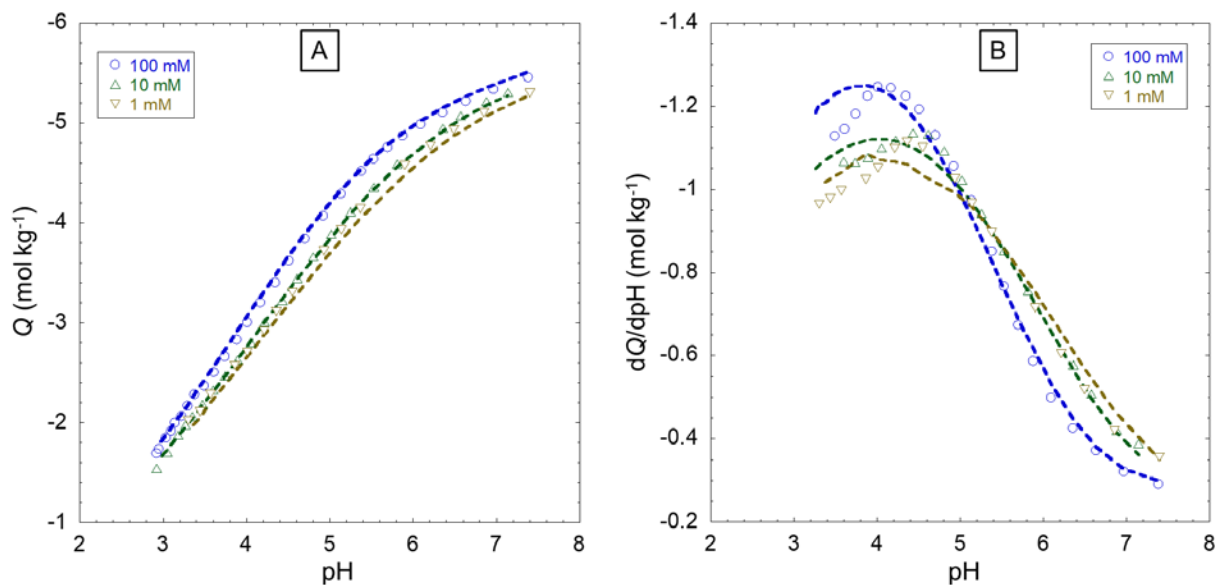
HNPs type	Model	$Q_{\max H1}$ (mol kg ⁻¹)	$\log \bar{K}_{a1}$	m_{H1}	$Q_{\max H2}$ (mol kg ⁻¹)	$\log \bar{K}_{a2}$	m_{H2}
FPHA	SPBT-PEST	4.08 ± 0.02	4.26 ± 0.02	0.46 ± 0.01	1.39 ± 0.07	8.57 ± 0.09	0.59 ± 0.03
	NICAD/eq.S3 [34]	3.18 ± 0.92	3.65 ± 0.23	0.66	3.02 ± 2.4	8.07 ± 0.77	0.29
SRFA	SPBT-PEST	5.47 ± 0.06	3.20 ± 0.02	0.52 ± 0.01	1.0 ± 0.4	7.8 ± 0.2	0.5 ± 0.2
	NICAD/eq.S4 [28]	5.55	3.01	0.42	NA*	NA*	NA*
LFA	SPBT-PEST	3.79 ± 0.12	3.94 ± 0.03	0.52 ± 0.02	4.94 ± 0.10	8.5 ± 0.2	0.27 ± 0.01
	NICAD/eq.S4 [35]	2.8 ± 1.2	2.5 ± 0.2	0.62 ± 0.17	7.4 ± 2.5	7.4 ± 0.7	0.18 ± 0.06
	NICAD/eq.S3 [35]	5.1 ± 0.0	4.1 ± 0.1	0.41 ± 0.01	2.8 ± 0.0	8.6 ± 0.1	0.46 ± 0.02
Hf:FA	SPBT-PEST	4.35 ± 0.09	3.81 ± 0.01	0.91 ± 0.02	1.75 ± 0.06	7.1 ± 0.3	0.33 ± 0.01
	NICAD/eq.S4 [36]	5.0±0.01	2.29±0.01	0.44	0.94 ±0.03	6.85±0.02	0.59
Hf:HA	SPBT-PEST	3.00 ± 0.05	4.40 ± 0.02	0.83 ± 0.03	1.3 ± 0.2	7.5 ± 0.2	0.49 ± 0.05
	NICAD/eq.S4 [36]	3.44± 0.02	3.35 ± 0.01	0.46	0.64 ± 0.04	6.68 ± 0.03	0.88

420 NA (not available)*: NICAD fit was carried out only for site 1 type (*i.e.* carboxylic) due to the experimental data
421 that cover a reduced range of pH values (see Fig. 2)

422
423 **Figure 1B** shows some discrepancies between SPBT-PEST outcome and proton affinity spectrum data
424 especially at low and high pH values. In the low pH range, suspensions of HNPs are indeed more prone
425 to aggregation due to significant lowering of the charge carried by the particles [57], while pH
426 measurements by glass membrane electrodes become unstable at sufficiently high pH due to unbalanced
427 competition between protons and sodium ions from background electrolyte solution [58]. In addition to
428 these unavoidable experimental difficulties, the estimation of dQ/dpH may be subject to significant error
429 as tiny deviations of the dependence of Q on pH compared to a smooth trajectory, may lead to significant
430 changes in estimated dQ/dpH .

431 The titration data collected from literature for SRFA particles relate to pH lower than 7.5 (**Figure 2A**).
432 Accordingly, the chemical parameter values retrieved for the phenolic sites should be considered with
433 caution as their dissociation is not significant in the pH range examined, which is supported by the absence
434 of a second peak in the affinity spectrum (**Figure 2B**). The experimental titration data at 1 and 10 mM
435 NaNO₃ are anomalously superimposed for this HNPs sample type, which may be explained by an
436 inaccurate positioning of the experimental curves by Milne *et al.* [28]. The SRFA titration curve is marked
437 by a steep increase of the particle charge with increasing pH from 3 to 6, similarly to that observed for a
438 weak acid: this feature is consistent with the low $\log \bar{K}_{a1}$ value of 3.2 derived for the carboxylic groups.

439 The lack of data at higher pH values is reflected by the large errors made in the estimation of the optimised
 440 parameters pertaining to the phenolic sites, particularly for $Q_{\max H_2}$ and m_{H_2} (**Table 2**). The variations of
 441 the proton affinity spectrum at low pH are better defined for the sub-nanometric SRFA particles than
 442 those measured for the larger FPFA particles (**Figure 1B**), which supports that particles aggregation -
 443 facilitated with increasing particle size [57]- likely affects data quality in this pH range.



444 **Figure 2:** Proton titration curves for SRFA (A) and corresponding proton affinity spectra (B) collected at three
 445 NaNO₃ electrolyte concentrations (indicated). Symbols: measurements. Dotted lines: SPBT-PEST modelling results.
 446 Model parameters are given in **Tables 1-2**. The charge is expressed in moles of equivalent charges per kg of SRFA
 447 material.
 448
 449

450 The experimental results collected for LFA particles are of good quality up to pH 8 (**SM, Figure S3**)
 451 but some suspicious changes in the measurements can be observed at larger pH, especially at 30 mM ionic
 452 strength, which is particularly evidenced by inspection of the proton affinity spectra. The SPBT-PEST
 453 reconstruction of the LFA proton titration data is satisfactory for all electrolyte concentrations conditions
 454 examined, which contrasts with the theoretical quality of the corresponding proton affinity spectra. As
 455 mentioned above, this is so because the computation of the derivative dQ/dpH is very sensitive to changes
 456 of Q with pH, even very small. The results derived for Hf:FA (**SM, Figure S4**) and Hf:HA (**SM, Figure**
 457 **S5**) are quite similar, with the noticeable result that chemical heterogeneity viewed by the prism of m_{H_1} is
 458 modest for both samples (**Table 2**). A difference is that carboxylic sites fraction is more significant in
 459 Hf:FA and associated $\log \bar{K}_{a1}$ is significantly lower than that for Hf:HA. Inspection of **Figures S4-S5**
 460 reveals that SPBT-based theory slightly overestimates experiments at low electrolyte concentrations (10
 461 mM and 5 mM). Whereas it is difficult to provide firm explanations of such differences for Hf:FA and
 462 Hf:HA samples, possible origins of the mismatch between experiments and theory (mismatch that is more
 463 pronounced with NICAD-based modelling, see **SM, section F**) can be advanced: the effectiveness of the
 464 protocol for separating the sub-nanometric fulvic and humic fractions in these samples, an approximate

465 positioning of the experimental titration data at 5 and 10 mM, or the occurrence of physico-chemical
466 reactions affecting the structure of the particles at sufficiently low medium salinity.

467

468 **4.3. Comparison between protolytic chemical parameters of HNPs retrieved by SPBT- 469 PEST and NICAD**

470 As discussed above, the Donnan-like representation considered within NICAD modelling does not
471 provide a consistent picture of the soft HNPs electrostatic properties. It is then of importance to address
472 how the inconsistency of this unconstrained, empirical modelling approach is reflected in the NICAD-
473 derived protolytic chemical parameters. For that purpose, **Table 2** collect $Q_{\max H1}$, $Q_{\max H2}$, m_{H1} , m_{H2} ,
474 \bar{K}_{a1} and \bar{K}_{a2} derived by NICAD and provided in the literature for the samples of interest, to be compared
475 with the values obtained by SPBT-PEST. In addition, for the sake of completeness we report in **Figures**
476 **S6-S10 (SM, section F)** the NICAD-based fitting of the proton titration curves for all HNPs listed in
477 **Table 1** together with those obtained by SPBT-PEST. Briefly, data reconstruction quality by NICAD for
478 the largest FPFA and LFA HNPs considered in this work is quite comparable for all examined ionic
479 strengths to that achieved by SPBT-PEST, whereas it is significantly worse for all other HNPs defined by
480 lower r_p . This conforms qualitatively with the fact that the NICAD assumption of a constant
481 intraparticulate potential is best verified for large particles, more prone to satisfy the criterion $\kappa r_p \gg 1$
482 under given medium salinity conditions. Surprisingly, NICAD manages to provide a reasonable estimates
483 of proton titration data for FPFA and LFA at low ionic strengths where Donnan criterion is not satisfied
484 (see **Table 1**): it does so, however, at the cost of unrealistic adjustment of V_D already invoked by Town *et*
485 *al.* [7] and Lenoir *et al.* [48] Further comparison between $\bar{K}_{a1,2}$ derived by NICAD and SPBT-PEST
486 (**Table 2**) shows important differences, especially for carboxylic groups for which this difference may
487 amount to more than an order magnitude. This difference is most important for Hf:FA and Hf:HA samples
488 for which NICAD modelling is inappropriate (**SM, Figures S9-S10**), and it remains marginal for SRFA
489 and LFA despite of the corresponding poor NICAD-based titration data reconstruction (**SM, Figure S7**)
490 and/or the inapplicability of the Donnan criterion over the whole range of solution ionic strength
491 considered (**Table 1**). For Hf:FA, the $\log \bar{K}_{a1}$ (2.29 ± 0.01) obtained by NICAD is further anomalously low
492 for carboxylic acid and it compares to the first proton dissociation constant of a stronger acid like
493 phosphoric acid ($pK_a=2.12$) [59]. As a matter of fact, this suspicious $\log \bar{K}_{a1}$ value just mirrors the attempt
494 of NICAD to balance its physically erroneous description of particle electrostatics *via* artificial adjustment
495 of V_D . The reader is referred to **Table 1** and **Table 2** to appreciate the over- or under-estimation of the
496 quantities $Q_{\max H1}$, $Q_{\max H2}$, m_{H1} and m_{H2} by NICAD as compared to those derived by SPBT-PEST. It is
497 worth mentioning that huge differences of these quantities (some approaching or exceeding 100%, see
498 **Table 2** for LFA data) are obtained by NICAD depending on the nature of the V_D - $\log I$ relationship
499 adopted (either one or two-parameters dependent relation, see eqs S3 and S4 in **SM**, respectively). This
500 observation, further supported by the large differences in V_D (see **Table 1** for LFA) simply reflects, again,

501 the phenomenological nature of the NICAD fitting exercise that is based on an electrostatic representation
502 unsupported by the particle size-dependent intra- and extra-particulate spatial distributions governed by
503 PB formalism for soft particles. Even with application of NICAD model under conditions where its
504 founding electrostatic approximation is not verified, proton titration data reconstruction can be more or
505 less ‘satisfactory’: this underpins the empirical nature of NICAD-based fitting of such data.
506 Unsurprisingly, adopting eq S3 provides better reconstruction of proton titration data than with using eq
507 S4 simply because the degree of parameters adjustment offered to the user is larger with the former
508 equation.

509

510 **5. CONCLUSIONS**

511 Since the 90’s when the first versions of the Non-Ideal Competitive Adsorption-Donnan model
512 (NICAD) were developed [15-18], the community of environmental physical chemists has largely
513 employed the NICAD model to estimate the chemical charge carried by aquatic nanoparticles from proton
514 titration data measured at different salt concentrations [15-18,21,22,28,34-36,40,48,60-62]. Accurate
515 knowledge of the charge of these particles is fundamental as *e.g.* it largely contributes to define the fate,
516 speciation and bioavailability of charged contaminants like metals in the aqueous compartments of
517 ecosystems. In particular, there is a large amount of literature data reporting analysis by NICA-Donnan
518 or related models, of the protolytic properties of humic matter nanoparticles (HNPs) commonly viewed
519 as model surrogates for organic matter particles in aquatic environments or soils [18,19,21,22,28,34-
520 36,47,48,50,60-62]. For the sake of illustration, ecotoxicologists often employ HNPs as model systems to
521 analyse the speciation of hazardous metals in dispersions of charged colloids and the implications thereof
522 in terms of identification of the toxic metal forms towards living organisms (*e.g.* plants, bacteria, or algae)
523 [63].

524 Despite of its popularity, NICA-Donnan suffers from severe approximations on the way particle
525 electrostatics is implemented, recalling that estimation of the charge carried by any colloidal particles
526 necessarily requires a proper formulation of the electrostatic component of the binding of the charge-
527 determining ions (protons) to the ionogenic sites distributed at the particle surface and/or in the particle
528 body volume. Namely, NICAD assumes the applicability of Donnan electrostatic representation for
529 charged (nano)particles regardless of the ratio between particle size and thickness of the operational
530 electric Debye layer thickness [7]. This approximation necessarily fails for HNPs with radius 1 nm to 10
531 nm and practical salt concentrations in the range 1 mM to 100 mM. A consequence is that NICAD
532 requires, for proton titration data fitting, the adjustment of a ‘particle Donnan volume’ that (i) exceeds the
533 physical size of the particles and (ii) systematically increases with decreasing salt concentration, even in
534 the absence of significant particle swelling [7,28,34-36]. Items (i) and (ii) are inconsistent from an
535 electrostatic point of view and simply reflect the inapplicable electrostatic description given in NICAD
536 for particles whose size does not significantly exceed the Debye layer thickness [7]. In line with these

537 observations, force is to recognize that a sound alternative to NICAD modelling for NPs charge evaluation
538 is urgently needed.

539 Accordingly, in this work we elaborate a new approach for the analysis of NPs proton titration data
540 where Poisson-Boltzmann theory for soft particle electrostatics is consistently implemented. The particle
541 charge is evaluated from proper spatial integration of the local particle charge density defined by an
542 isotherm reflecting the chemical binding of charge determining protons to ionogenic sites located at the
543 particle surface and within the particle body volume, and corrected by local electrostatics retrieved from
544 numerical solution to the nonlinear Poisson-Boltzmann equation valid for weakly to highly charged
545 particles. Unlike NICAD, this framework is used to generate proton titration curves with a consistent
546 account of particle size-dependent potential profile over the whole spectrum of Debye layer extension,
547 *i.e.* from the thin to the thick double layer regime. A complete numerical package (that we make available
548 for the community) combining a home-made FORTRAN program (named SPBT) for PB-based
549 computations of particle electrostatics and charge, and a free module (called PEST) for optimized
550 adjustment of the relevant chemical and electrostatic model parameters, is developed. The combined
551 SPBT-PEST programs allow for (i) the automated and constrained reconstruction of measured proton
552 titration curves *and* associated proton affinity spectra collected at different salt concentrations, and (ii)
553 the evaluation of the relevant particle electrostatic and protolytic features.

554 The whole methodology is successfully tested for a range of HNPs particles whose titration data and
555 size measurements are available in literature, and the performance of SPBT-PEST duo is further explicitly
556 compared with that of NICAD. In detail, unlike NICAD [36], SPBT-PEST successfully reproduces the
557 dependence of measured particle charge on pH and salt concentration for all HNPs tested, including those
558 for which Donnan representation is not applicable. For HNPs whose size legitimates the applicability of
559 Donnan potential profile, NICAD succeeds in reproducing the experimental data *albeit* at the cost of a
560 ‘particle Donnan volume’ that is adjusted to values that exceed the physical volume of the particles, which
561 is physically impossible [7,28,34,35]. In addition, SPBT-PEST properly recovers proton titration data for
562 HNPs with maintaining a constant particle radius for all salt concentrations tested, which contrasts with
563 the empirical adjustment of the NICAD ‘particle Donnan volume’ found to systemically increase with
564 decreasing salt concentration [28,34-36]. We find that the chemical parameters pertaining to proton
565 binding and proton affinity distribution, as retrieved from data modelling by SPBT-PEST and NICAD,
566 may largely differ, especially so for the smallest HNPs where Donnan representation is not appropriate.
567 This finding simply highlights the empirical electrostatic foundation of NICAD model and implications
568 thereof in terms of particle charge estimation. NICAD may succeed to reproduce, quantitatively,
569 experimental proton titration data even for particles whose size forbids *a priori* its application. It does so
570 thanks to the adjustment of the ‘particle Donnan volume’, which artificially counterbalances the
571 electrostatic deficiency of the NICAD model to achieve data fitting. As a support of this element, it is
572 found that both the Donnan volume and the key chemical parameters involved in the model can vary by
573 100% depending on the reported form of the empirical NICAD equation that defines the dependence of

574 ‘particle Donnan volume’ on medium salt concentration. In contrast, SPBT-PEST does not require the
575 adjustment of a ‘particle Donnan volume’ for fitting proton titration curves, recalling that this volume,
576 when relevant, necessarily identifies with the physical volume of the particle and the electrostatic potential
577 therein (the Donnan potential) is independent of position. The Donnan electrostatic potential
578 representation at the particle/solution interface is nothing else than the solution of the PB equation in the
579 limit of thin double layers. As such, SPBT-PEST bypasses the necessity by NICAD to recourse to a
580 questionable over-parameterisation of particle electrostatics to reproduce experimental data. Unlike
581 NICAD [22,28,25], the PB-based alternative we propose integrates consistently the intimate connection
582 between particle double layer potential, particle size and background electrolyte concentration.

583 We believe that the here-highlighted shortcomings of NICAD in its phenomenological representation
584 of particle electrostatics should be of concern for the community as NICAD, together with WHAM that
585 suffers from similar electrostatic approximations, constitutes a widely employed basis for computing *e.g.*
586 metal-to-humic thermodynamic complexation or addressing metal bioavailability in relation to toxicity
587 effects assessment. Unlike NICAD and WHAM, the PB theoretical framework developed in this study
588 can be extended to account *explicitly* for the presence of multivalent ions in solution [6], an option that is
589 particularly interesting for analysing particle charging behaviour in natural waters rich in divalent cations.
590 In addition, depending on the nature of particles considered, inhomogeneous particle structure
591 composition and gradients in dielectric permittivity between particle body and extraparticulate medium
592 can be easily implemented within the PB equation along the lines described elsewhere [39],[64]. Such
593 particle structure and electrostatic refinements may be valuable provided that independent measurements
594 targeting these aspects (*e.g.* diffusion neutron scattering and dielectric spectroscopy) are available to avoid
595 the adjustment of an ill-defined set of parameters when reconstructing particle proton titration curves.

596
597 **Supporting material.** **A.** Detailed description of the HNPs considered in this work, of their size
598 measurement and of the estimation of their molar mass. **B.** Details of the NICA-Donnan modelling
599 framework. **C.** Details of here-developed SPBT-PEST treatment of proton titration data and associated
600 proton affinity spectra. **D.** Evaluation of the initial estimates of the chemical parameters involved in eq 6.
601 **E.** Performance of SPBT-PEST in recovering HNPs proton titration and affinity spectra data. **F.**
602 Comparison between SPBT-PEST and NICAD reconstructions of proton titration data. Additional details
603 on the procedure outlined in **E** with step-by-step treated examples are provided in the form of a SPBT-
604 PEST user manual that is available on request together with the SPBT executable file.

605
606 This research did not receive any specific grant from funding agencies in the public, commercial, or not-
607 for-profit sectors.

608
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797 **Figure captions.**

798 **Figure 1:** Proton titration curves for FPHA (A) and corresponding proton affinity spectra (B) collected at
 799 three NaNO₃ electrolyte concentrations: 10 mM (Δ), 30 mM (◇) and 100 mM (o). Symbols: measurements.
 800 Dotted lines: SPBT-PEST modelling results. Adopted model parameters are listed in **Tables 1-2**. The
 801 charge is expressed in moles of equivalent charges per kg of FPHA material.

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803 **Figure 2:** Proton titration curves for SRFA (A) and corresponding proton affinity spectra (B) collected at three
 804 NaNO₃ electrolyte concentrations (indicated). Symbols: measurements. Dotted lines: SPBT-PEST modelling results.
 805 Adopted model parameters are given in **Tables 1-2**. The charge is expressed in moles of equivalent charges per kg
 806 of SRFA material.

807 **Tables.**

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809 **Table 1:** Comparison between HNPs specific volumes v_p derived in this work and model-generated Donnan volume
 810 V_D computed from the parameters reported in literature for NICAD modelling. Eq S3 and eq S4 given in **SM** specify
 811 the way V_D was estimated, *i.e.* either *via* the two-parameter (α , β) dependent or the one-parameter (b) dependent
 812 NICAD empirical equations that relates V_D to the logarithm of ionic strength. Initial estimates (superscript i in the
 813 Table) and refined values (superscript ii) of particle size r_p and molecular weight M_w by SPBT-PEST are further

815 indicated. Numbers between brackets correspond to κr_p values, recalling that Donnan potential-representation is
 816 strictly applicable for $\kappa r_p \gg 1$.
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HNPs type	r_p ($\times 10^{-9}$ m)	M_w (kg mol $^{-1}$)	v_p (m 3 kg $^{-1}$)	Donnan fitting parameters	Model-generated Donnan volume V_D (m 3 kg $^{-1}$)		
				α, β for eq. S3 or b for eq. S4	100 mM	10 mM	1 mM
FPHA [34]	3.1 to 5.9* ⁱ 5.0 ⁱⁱ	19.2 to 23 ⁱ 23 ⁱⁱ	0.0137	$\alpha=0.60\pm 0.32$ $\beta=-0.27\pm 0.05$	0.0074 (5.20)	0.0138 (1.64)	0.0257 (0.52)
SRFA [28]	0.6 to 1.05 ⁱ 0.6 ⁱⁱ	0.5 to 1.5 ⁱ 0.545 ⁱⁱ	0.0010	$b=0.87$	0.0055 (0.60)	0.0407 (0.20)	0.3020 (0.06)
LFA [35]	1.9 to 3.6 ⁱ 3.6 ⁱⁱ	5.5 to 11 ⁱ 10 ⁱⁱ	0.0118	$b=0.35 \pm 0.02$	0.0004 (3.74)	0.0009 (1.18)	0.0019 (0.37)
				$\alpha=0.93\pm 0.20$ $\beta=-0.27\pm 0.05$	0.0158 (3.74)	0.0295 (1.18)	0.0550 (0.37)
Hf:FA [36]	0.75 to 1.0 ⁱ 1.0 ⁱⁱ	0.5 to 1.5 ⁱ 0.9 ⁱⁱ	0.0028	$b=0.43$	0.0007 (1.04)	0.0019 (0.33)	0.0052 (0.10)
Hf:HA [36]	0.75 to 1.0 ⁱ 1.0 ⁱⁱ	1.0 to 3.0 ⁱ 1.8 ⁱⁱ	0.0014	$b=0.34$	0.0005 (1.04)	0.0010 (0.33)	0.0023 (0.10)

818 *Determined in this work by Scanned Stripping Chronopotentiometry (SSCP), see details in **SM (section A, therein)**.

819 **Table 2:** SPBT-PEST optimized values of the chemical parameters involved in the Langmuir-Freundlich equation
 820 (eq 6) for the various HNPs types considered in this work. Indicated dispersions of the parameters correspond to the
 821 95% confidence interval provided by PEST. The parameters are retrieved from SPBT-PEST and NICAD modelling
 822 of the proton titration curves measured at different salt reconstructions and reported in **Figures S6-S10** for the five
 823 HNPs samples of interest. SPBT-PEST and NICAD theoretical reconstructions of these proton titration data *versus*
 824 pH and salt concentration are also provided in **Figures S6-S10**.
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HNPs type	Model	$Q_{\max H1}$ (mol kg $^{-1}$)	$\log \bar{K}_{a1}$	m_{H1}	$Q_{\max H2}$ (mol kg $^{-1}$)	$\log \bar{K}_{a2}$	m_{H2}
FPHA	SPBT-PEST	4.08 ± 0.02	4.26 ± 0.02	0.46 ± 0.01	1.39 ± 0.07	8.57 ± 0.09	0.59 ± 0.03
	NICAD/eq.S3 [34]	3.18 ± 0.92	3.65 ± 0.23	0.66	3.02 ± 2.4	8.07 ± 0.77	0.29
SRFA	SPBT-PEST	5.47 ± 0.06	3.20 ± 0.02	0.52 ± 0.01	1.0 ± 0.4	7.8 ± 0.2	0.5 ± 0.2
	NICAD/eq.S4 [28]	5.55	3.01	0.42	NA*	NA*	NA*
LFA	SPBT-PEST	3.79 ± 0.12	3.94 ± 0.03	0.52 ± 0.02	4.94 ± 0.10	8.5 ± 0.2	0.27 ± 0.01
	NICAD/eq.S4 [35]	2.8 ± 1.2	2.5 ± 0.2	0.62 ± 0.17	7.4 ± 2.5	7.4 ± 0.7	0.18 ± 0.06
	NICAD/eq.S3 [35]	5.1 ± 0.0	4.1 ± 0.1	0.41 ± 0.01	2.8 ± 0.0	8.6 ± 0.1	0.46 ± 0.02

Hf:FA	SPBT-PEST	4.35 ± 0.09	3.81 ± 0.01	0.91 ± 0.02	1.75 ± 0.06	7.1 ± 0.3	0.33 ± 0.01
	NICAD/eq.S4 [36]	5.0 ± 0.01	2.29 ± 0.01	0.44	0.94 ± 0.03	6.85 ± 0.02	0.59
Hf:HA	SPBT-PEST	3.00 ± 0.05	4.40 ± 0.02	0.83 ± 0.03	1.3 ± 0.2	7.5 ± 0.2	0.49 ± 0.05
	NICAD/eq.S4 [36]	3.44 ± 0.02	3.35 ± 0.01	0.46	0.64 ± 0.04	6.68 ± 0.03	0.88

826 NA (not available)*: NICAD fit was carried out only for site 1 type (*i.e.* carboxylic) due to the experimental data
827 covering a reduced pH range (see Fig. 2)

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SUPPORTING MATERIAL

Addressing the electrostatic component of protons binding to aquatic nanoparticles beyond the Non-Ideal Competitive Adsorption (NICA)-Donnan level: theory and application to analysis of proton titration data for humic matter.

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This supporting material contains 12 pages, 6 equations, 18 references and 10 figures. It is organized as follows:

- A.** Detailed description of the HNPs considered in this work, of their size measurement and of the estimation of their molar mass.
- B.** Details of the NICA-Donnan modelling framework.
- C.** Details of here-developed SPBT-PEST treatment of proton titration data and associated proton affinity spectra.
- D.** Evaluation of the initial estimates of the chemical parameters involved in eq 6.
- E.** Performance of SPBT-PEST in recovering HNPs proton titration and affinity spectra data.
- F.** Comparison between SPBT-PEST and NICAD reconstructions of proton titration data.

894 **A. Detailed description of the HNPs considered in this work, of their size measurement**
895 **and of the estimation of their molar mass.**

896 **A1. FPFA particle type.** FPFA sample was extracted from a peat sample collected in the Mogi river
897 region of Ribeirão Preto, São Paulo State, Brazil [1], following the procedure recommended by IHSS for
898 extraction and purification of organic matter [2]. Particle diffusion coefficient was measured by Scanning
899 stripping chronopotentiometry (SSCP, see details below). The quadruplicate measurements yielded values
900 of 5.2, 4.1, 5.9 and $4.7 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$. Using Stokes-Einstein equation to convert diffusion coefficient into
901 particle radius r_p , we obtained $r_p = 4.5 \text{ nm}$ and a 95% confidence interval of 3.1 to 5.9 nm. The FPFA
902 sample was extracted from a tropical peat and measured particle size shows that it is a relatively large
903 HNP material. In the literature, the closest sample we found is PPHA, for which molar masses of 23 kg
904 mol^{-1} has been measured by equilibrium UV scanning ultracentrifugation [3]. Perminova *et al.* [4] reported
905 number (M_n) and weight (M_w) average molecular weights of 6.4 and 19.2 kg mol^{-1} , respectively, by size
906 exclusion chromatography on 8 peat humic substances samples.

907 *Determination of FPFA diffusion coefficient by SSCP.* Diffusion coefficient was determined using Cd(II)
908 as a probe according to the procedure detailed by Pinheiro *et al.* [5] The experiments were carried out
909 using an Ecochemie μ Autolab III and a PGStat 12 in conjunction with a Metrohm 663 VA stand
910 (Metrohm, Switzerland). The setup was controlled by the GPES 4.9 software from EcoChemie, the
911 Netherlands. A three electrodes configuration was used, with a Hg thin film plated onto a rotating glassy
912 carbon disk (2 mm diameter, Metrohm) serving as a working electrode, a GC rod counter electrode, and
913 an Ag/AgCl reference electrode from World Precision Instruments DRIFEF-5 (electrolyte leakage $<$
914 $8 \times 10^{-4} \mu\text{L h}^{-1}$). SSCP experiments were carried out in 20 mL of 10 mM NaNO_3 solution with a Cd(II)
915 concentration in the range 6 to $10 \times 10^{-7} \text{ mol l}^{-1}$ and a FPFA concentration in the range 8 to $10 \times 10^{-5} \text{ kg l}^{-1}$.
916 The adopted experimental conditions were: deposition time (t_d) 45 s, oxidizing current (I_s) $2 \times 10^{-6} \text{ A}$
917 applied until the potential reached -0.300 V with respect to Ag/AgCl reference electrode, rotation speed
918 1000 rpm. All solutions were purged for 15 min at the beginning of every experiment and for 20 s after
919 each measurement (assisted by mechanical stirring of the rotating electrode). Measurements were
920 performed for a range of deposition potentials, from the foot to the plateau of the SSCP wave, *i.e.* from -
921 0.850 to -0.600 V for Cd(II).

922 **A2. SRFA particle type.** The Suwanee river fulvic acid (SRFA) is one of the International Humic
923 Substances Society (IHSS) reference materials and probably the most studied HNP sample type. In 2001,
924 Hosse and Wilkinson [6] measured SRFA diffusion coefficient by Fluorescence correlation spectroscopy
925 (FCS) under different pH and ionic strength conditions and reported values between 2.1 and 2.6×10^{-10}
926 $\text{m}^2 \text{ s}^{-1}$, which correspond to particle radius in the range 0.85 to 1.05 nm. De Wit *et al.* [7] reported a value
927 of 0.6 nm, placing this material in the low end of the 1-2 nm range commonly reported for fulvic acids.
928 Molar masses of $0.829 \text{ kg mol}^{-1}$ and $0.545 \text{ kg mol}^{-1}$ were reported for SRFA by Aiken *et al.* [8] and De

929 Wit *et al.* [7], respectively, while Her *et al.* [9] reported value of 1.116 kg mol⁻¹ obtained by HPSEC/DOC
930 and 1.385 kg mol⁻¹ by HPSEC/UVA measurements.

931 **A3. LFA particle type.** LFA is a soil fulvic acid, extracted and purified from a podzol obtained in the
932 Laurentian forest preserve of Laval University, Quebec, Canada [10]. The diffusion coefficients measured
933 by voltammetry for the LFA range from 6×10^{-11} to 1.2×10^{-10} m² s⁻¹ at 10 mM ionic strength, corresponding
934 to particle radius from 1.9 to 3.6 nm [11]. The LFA was extracted from a forest soil and its size is larger
935 than that of SRFA. Shinozuka *et al.* [12] measured M_n of 9.7 and 11 kg mol⁻¹ for Inogashira and Elliot
936 soil fulvic acids by MALDI-TOF MS, while Perminova *et al.* [4] reported M_n of 5.5 kg mol⁻¹ and M_w of
937 10.8 kg mol⁻¹ averaged over 9 different samples.

938 **A4. Hf:FA and Hf:HA particle types.** Hf:FA and Hf:HA are the fulvic and humic acids extracted from
939 the upper horizon (0-60 cm) of an ombrotrophic peat bog in Sierra del Buio, Northwest Spain. Pinheiro
940 *et al.* [5] reported similar diffusion coefficients derived by FCS and SSCP, for both particle types with
941 values in the range $2.3-2.8 \times 10^{-10}$ m² s⁻¹ at 10 and 100 mM ionic strength, which corresponds to r_p between
942 0.75 and 1.0 nm. This small particle size may be due to the fact that this material was extracted from the
943 soil upper horizon where humification process is still recent. According to the authors [13], the collected
944 sample contained reddish-brown fibric materials, while in the deeper horizons plant detritus was fully
945 decomposed [13]. Hf:FA and Hf:HA particle radius indicates that they are a peculiar sample. Such a small
946 radius is not compatible with large molar masses, thus we defined a low limit of 0.5 kg mol⁻¹ for the FA
947 and 1.0 kg mol⁻¹ for the HA and fixed the higher limit at 1.5 and 3 kg mol⁻¹ for the FA and HA samples,
948 respectively in line with the M_w reported for small fulvic and humic NPs [7].

949

950 **B. Details of the NICA-Donnan modelling framework.**

951 NICA-Donnan model assumes *a priori* that the potential within the whole particle volume is constant
952 (and takes the Donnan value ψ_D), and it further considers the accumulation of the only counterions within
953 this volume and it neglects that of the coions that is however operational at sufficiently low particle charge
954 and/or large electrolyte concentrations. In this model, cations accumulate inside the volume of (negatively
955 charged) HNPs particles according to the Boltzmann accumulation factor

$$956 a_{i,D} = a_i \exp(-z_i F \psi_D / RT) \quad (S1)$$

957 where a_i and z_i are the bulk activity and valence of cation i , respectively, F is the Faraday constant, R the
958 gas constant and T is the temperature. Within the Donnan representation, the structural charge Q (in
959 mol/kg of HNP material) located in the HNPs is neutralized by the cations, and the Donnan volume V_D
960 (which identifies with the specific hydrodynamic volume of the particle in m³/kg of HNP material) is
961 defined by the electroneutrality equation:

$$962 Q/V_D + \sum_i z_i (a_{i,D} - a_i) = 0 \quad (S2)$$

963 Benedetti *et al.* [14] proposed an empirical relationship between V_D and the solution ionic strength (I) for
964 HNPs:

965 $\log V_D = \alpha + \beta \log I$ (S3)

966 where α and β are parameters adjusted to match proton titration data and they depend on the nature of the
 967 HNP considered. The authors proposed later a ‘simplification’ of eq S3 with the following one-parameter
 968 dependent correlation between V_D and $\log I$ [15]

969 $\log V_D = b(1 - \log I) - 1$ (S4)

970 Regarding the chemical component of the NICA equation,[16] it takes the form of a continuous equation
 971 that describes the competitive binding of cations to the HNP:

972
$$Q_{i,T} = \left(\frac{n_i Q_{\max H1}}{n_{H1}} \right) \frac{(\tilde{K}_{i1} a_{i,D})^{n_{i1}}}{\sum_i (\tilde{K}_{i1} a_{i,D})^{n_{i1}}} \frac{(\sum_i (\tilde{K}_{i1} a_{i,D})^{n_{i1}})^{p_1}}{1 + (\sum_i (\tilde{K}_{i1} a_{i,D})^{n_{i1}})^{p_1}} +$$

 973
$$\left(\frac{n_i Q_{\max H2}}{n_{H2}} \right) \frac{(\tilde{K}_{i2} a_{i,D})^{n_{i2}}}{\sum_i (\tilde{K}_{i2} a_{i,D})^{n_{i2}}} \frac{(\sum_i (\tilde{K}_{i2} a_{i,D})^{n_{i2}})^{p_2}}{1 + (\sum_i (\tilde{K}_{i2} a_{i,D})^{n_{i2}})^{p_2}}$$
 (S5)

974 where $Q_{i,T}$ is the total amount of species i bound to HNPs (mol kg⁻¹), \tilde{K}_{i1} and \tilde{K}_{i2} are the corresponding
 975 complexation parameters, n_{i1} and n_{i2} are the stoichiometric parameters relative to the binding of species i
 976 to the carboxylic (index 1) and phenolic (index 2) groups, respectively, $Q_{\max H1}$ and $Q_{\max H2}$ refer to the
 977 concentrations of carboxylic and phenolic groups, and p_1 and p_2 correspond to the intrinsic heterogeneity
 978 parameters of the carboxylic and phenolic binding sites. For the situation met in protolytic titrations
 979 experiments performed in inert NaNO₃, it can be legitimately assumed that the monovalent cations do not
 980 compete with the hydrogen ion [17], and eq S5 then reduces to

981
$$Q_{H,T} = Q_{\max H1} \frac{(\tilde{K}_{H1} a_{H,D})^{p_1 n_{H1}}}{1 + (\tilde{K}_{H1} a_{H,D})^{p_1 n_{H1}}} + Q_{\max H2} \frac{(\tilde{K}_{H2} a_{H,D})^{p_2 n_{H2}}}{1 + (\tilde{K}_{H2} a_{H,D})^{p_2 n_{H2}}}$$
 (S6)

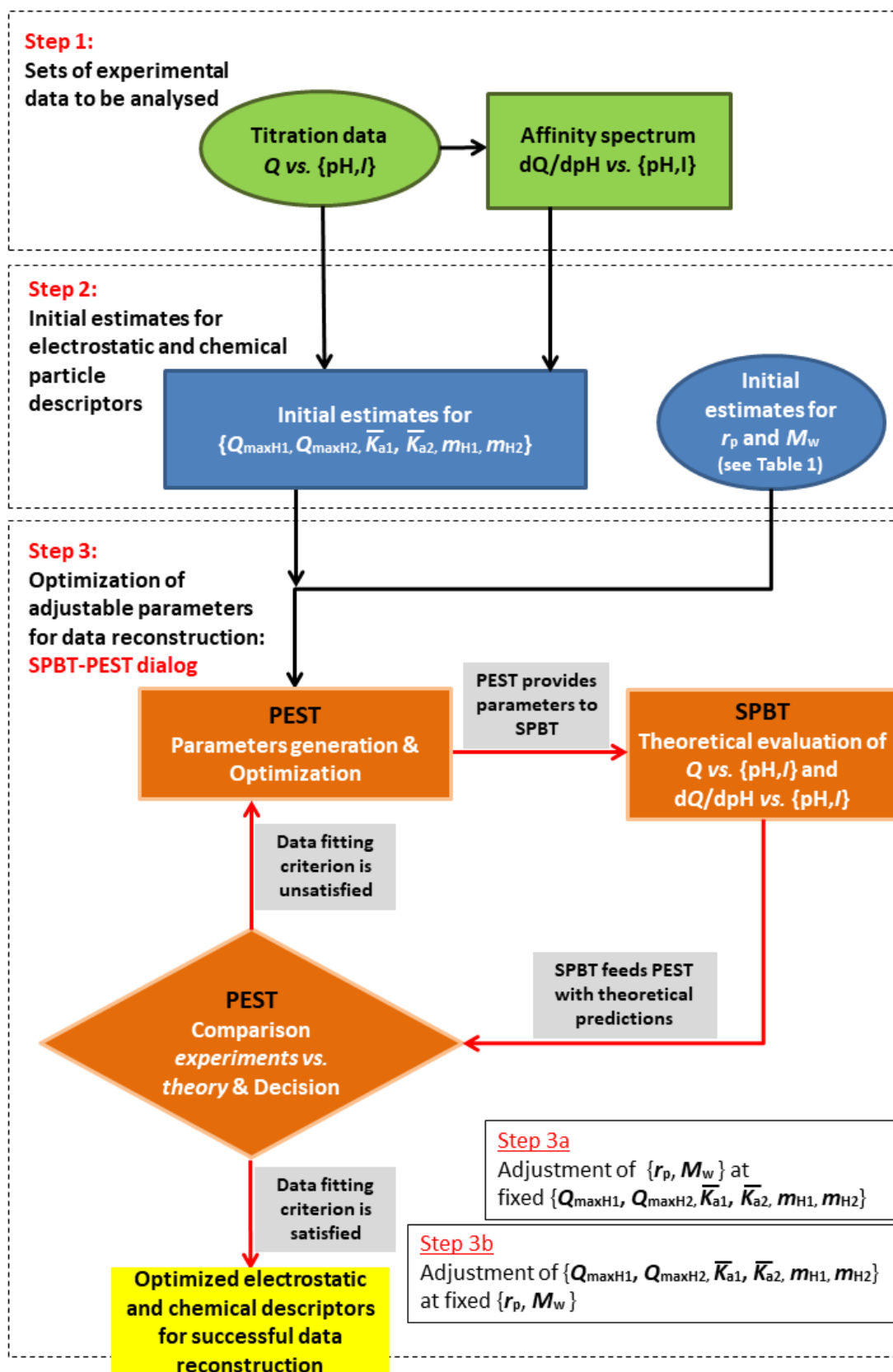
982 Eq S6 used to perform the reported NICAD analysis of the titration data collected for the samples of
 983 interest in this work is identical to eq 6 with the equivalences $m_{H1} = n_{H1} p_1$ and $m_{H2} = n_{H2} p_2$.

984

985 **C. Details of here-developed SPBT-PEST treatment of proton titration data and** 986 **associated proton affinity spectra.**

987 This section describes in detail the optimization of the model parameters r_p , M_w , $\rho_{\max H1}^{(s)}$, $\rho_{\max H2}^{(s)}$ (or
 988 $Q_{\max H1}$, $Q_{\max H2}$), \bar{K}_{a1} , \bar{K}_{a2} , m_{H1} and m_{H2} to reproduce the raw proton titration data and the associated
 989 affinity spectra by coupling SPBT with PEST. The optimization procedure is further summarized in the
 990 flowchart displayed in **Figure S1** and briefly discussed below.

991 In the **first step** of the data treatment, an *input data* file containing the measured HNPs charge Q (in
 992 mol kg⁻¹) vs. pH at given solution ionic strength I and the associated proton affinity spectrum (d Q /dpH vs.
 993 pH) is created. This spectrum was computed using Matlab™ *via* cubic spline smoothing of the
 994 experimental Q vs. pH data and subsequent derivation of the splined data with respect to pH.



995 **Figure S1:** Flowchart detailing the procedure adopted for the reconstruction of proton titration data and proton
 996 binding affinity spectra, and for the evaluation of the chemical and electrostatic properties of HNPs. See details in
 997 the text.
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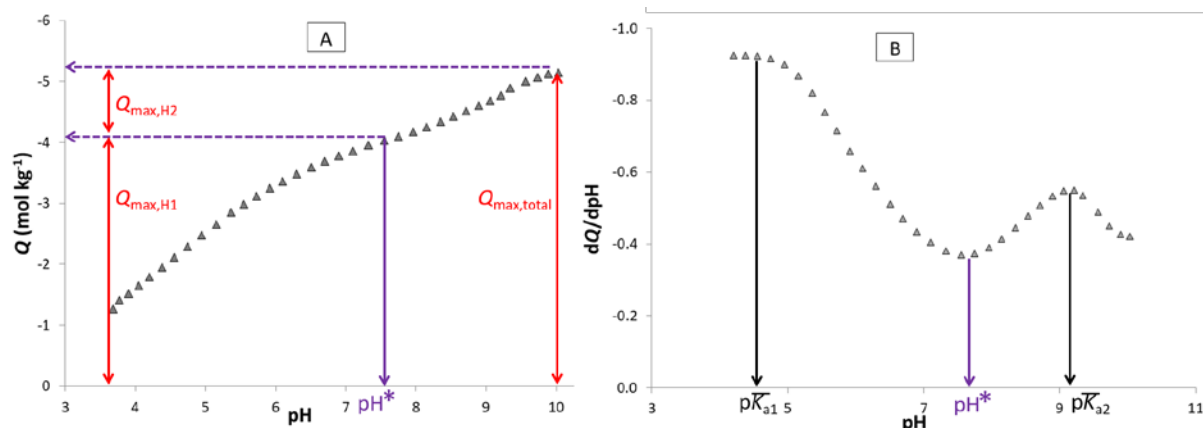
999 The **second step** consists in establishing initial estimates for all parameters. As far as r_p and M_w are
1000 concerned, their respective estimates are listed in **Table 1** whereas those for $Q_{\max H1}$, $Q_{\max H2}$, m_{H1} , m_{H2} ,
1001 \bar{K}_{a1} and \bar{K}_{a2} were evaluated from the proton titration curve Q vs. pH and the corresponding affinity
1002 spectrum dQ/dpH vs. pH measured at the largest electrolyte concentration (so as to minimize particle
1003 electrostatics contribution) along the lines detailed in **SM (section D, Figure S2)**.

1004 The back and forth communication between SPBT and PEST to achieve theoretical reconstruction of
1005 titration data takes place in a third step. First (step 3a in **Figure S1**), the *parameter template* file instructs
1006 PEST to fit r_p and M_w with the parameters $Q_{\max H1}$, $Q_{\max H2}$, m_{H1} , m_{H2} , \bar{K}_{a1} and \bar{K}_{a2} fixed to their respective
1007 initial estimates. PEST then creates the *input parameter* file and it calls for execution of SPBT that
1008 subsequently returns an *output* file containing the Q and dQ/dpH versus pH and I data generated for the
1009 set of parameters values given in the *parameter* file. PEST then reads this *output* file, computes the
1010 weighted sum Σ of squared differences between model-generated values and experimental data.
1011 Subsequently, PEST uses a Gauss-Marquardt-Levenberg algorithm to generate an updated set of values
1012 for the adjustable parameters (those provided in the *parameter template* file), and it informs SPBT to re-
1013 evaluate Q and dQ/dpH versus pH and I with this new set of parameters. PEST then reads the
1014 corresponding *output* file and compares the newly computed Σ with that obtained in the previous iteration.
1015 The SPBT-PEST programs duo iterates this procedure until the relative parameter variation is smaller
1016 than a prescribed value after execution of a number of iterations specified by the user. It is stressed in
1017 PEST manual [18] that a relative parameters variation lower than 1% after 3 successive iterations is a
1018 good compromise between accuracy and running time, and these criteria were therefore adopted in our
1019 procedure. Once this parameters optimization criterion is satisfied, the updated values of r_p and M_w are
1020 given by PEST in a *result* file that further contains the Q and dQ/dpH vs. pH and I theoretically generated
1021 by SPBT. In step 3b of **Figure S1**, SPBT-PEST duo resumes the full procedure detailed in step 3a *albeit*
1022 with now considering r_p and M_w as non-adjustable parameters with values given by those obtained at the
1023 end of step 3a, and with adjusting $Q_{\max H1}$, $Q_{\max H2}$, m_{H1} , m_{H2} , \bar{K}_{a1} and \bar{K}_{a2} to achieve Q and dQ/dpH vs.
1024 pH and I data fitting. This is done *via* modification of the *parameter template* file that indicates to PEST
1025 which parameters it should now adjust and which ones should be maintained constant all along the iterated
1026 fitting procedure. Further details on the above procedure with step-by-step treated examples are provided
1027 in the form of a SPBT-PEST fitting manual that is available on request together with the SPBT executable
1028 file.

1029 1030 **D. Evaluation of the initial estimates of the chemical parameters involved in eq 6.**

1031 An example of the procedure followed to evaluate initial estimates of the chemical parameters
1032 involved in eq 6 is provided in **Figure S2** for FPHA for which proton titration data were published by
1033 Botero *et al.* [1] The raw data are presented in terms of charge equivalents per kg of humic matter *versus*
1034 pH. Titration data collected at large background electrolyte concentration (100 mM NaNO₃ in **Figure S2**)

1035 are selected to minimize as much as possible the contribution of electrostatics to proton binding and thus
 1036 to provide best initial estimates of the only chemical terms involved in eq 6.
 1037



1038 **Figure S2:** Illustration of the procedure followed for evaluation of the initial estimates of the chemical parameters
 1039 involved in eq 6 from inspection of (A) proton titration data and (B) corresponding proton affinity spectrum. The
 1040 data (symbols) refer to FPFA humic acids in 100 mM NaNO₃.
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1043 *Initial estimates of Q_{maxH1} , Q_{maxH2} .* The pH at which all carboxylic acids are titrated corresponds roughly
 1044 to the onset of a plateau establishment for the particle charge Q with increasing pH. The clear location of
 1045 this plateau is here necessarily impaired by the ongoing dissociation of the phenolic groups. For the lack
 1046 of better, initial estimate of Q_{maxH1} is therefore read at the pH value marking a clear transition between
 1047 dissociation features of carboxylic and phenolic HNP sites, *i.e.* at the pH value where the affinity spectrum
 1048 displays a minimum with increasing pH (**Figure S2B**). Then, estimate for Q_{maxH2} can be obtained by
 1049 simple difference between the maximum charge $Q_{max,total}$ and Q_{maxH1} defined in **Figure S2**.

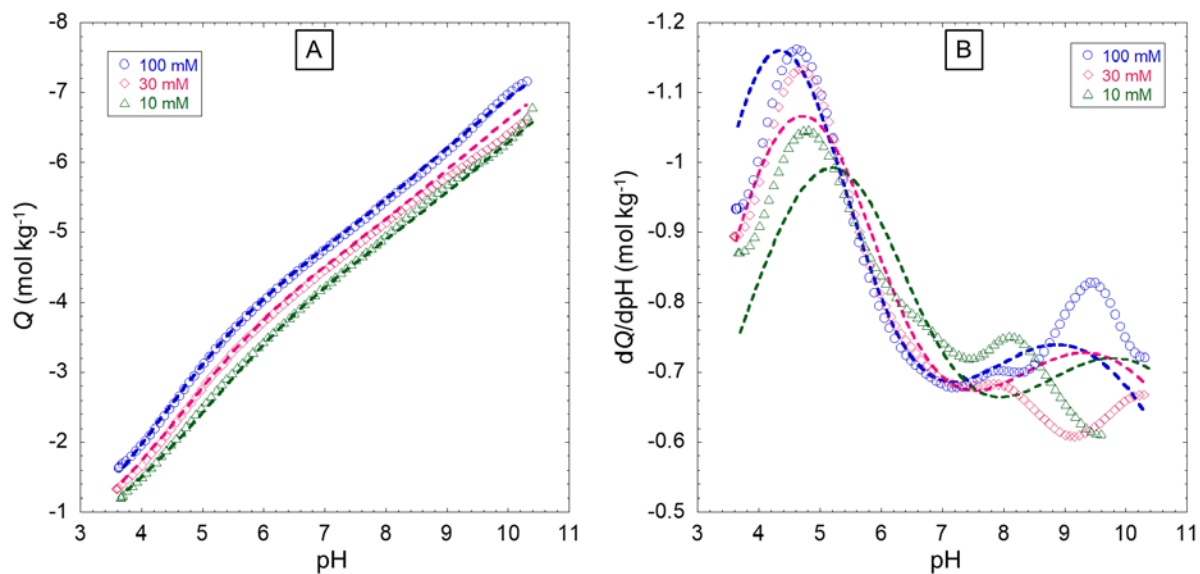
1050 *Initial estimates of pK_{a1} and pK_{a2} .* The two maxima in the proton affinity (**Figure S2B**) spectrum provides
 1051 the desired initial estimates of pK_{a1} and pK_{a2} .

1052 *Initial estimates of m_{H1} and m_{H2} .* The parameters m_{H1} and m_{H2} correspond roughly to the half-height widths
 1053 of the peaks detected for the carboxylic and phenolic groups in the proton affinity spectrum. A difficulty
 1054 for their evaluation is that experimental affinity spectrum is generally incomplete in terms of the covered
 1055 pH range (especially at low and high pH values), which explains why initial guessed values for m_{H1} and
 1056 m_{H2} are commonly set to 0.5. For situations where optimized m_{H1} and m_{H2} significantly differ from 0.5,
 1057 we restarted the overall SPBT-PEST fitting procedure with choosing as initial guessed values of m_{H1} and
 1058 m_{H2} those obtained from the previous optimization scheme performed with adopting 0.5 as initial
 1059 estimates.

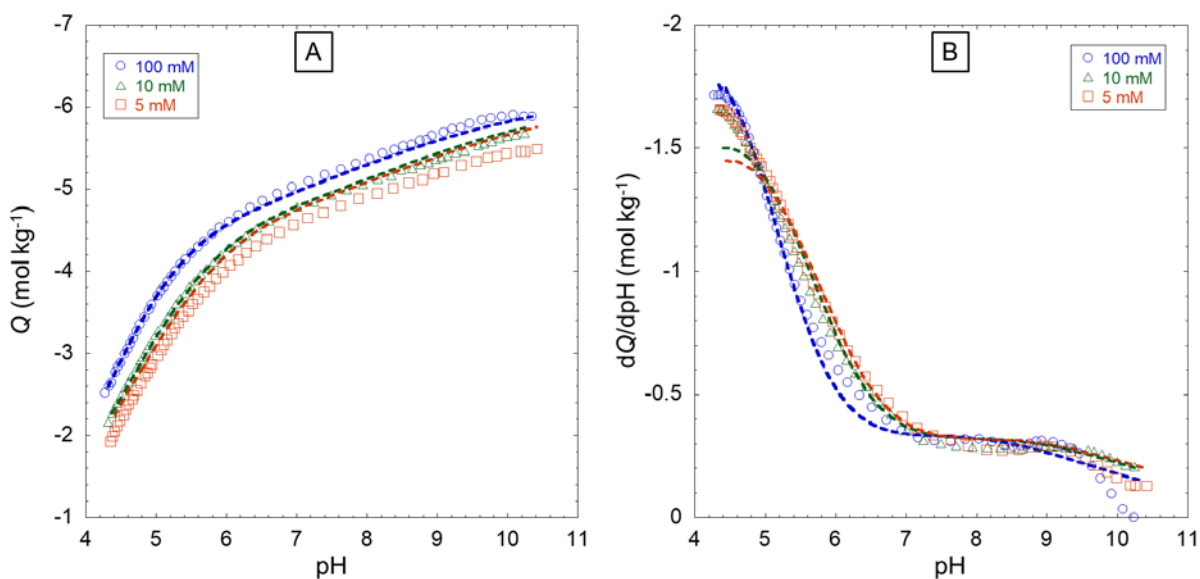
1061 **E. Performance of SPBT-PEST in recovering HNPs proton titration and affinity spectra** 1062 **data.**

1063 The model parameters adopted for SPBT-PEST analysis of the charge and electrostatic descriptors
 1064 of LFA, Hf:FA and Hf:HA are collected in **Tables 1-2** and we provide below in **Figure S3** (LFA),

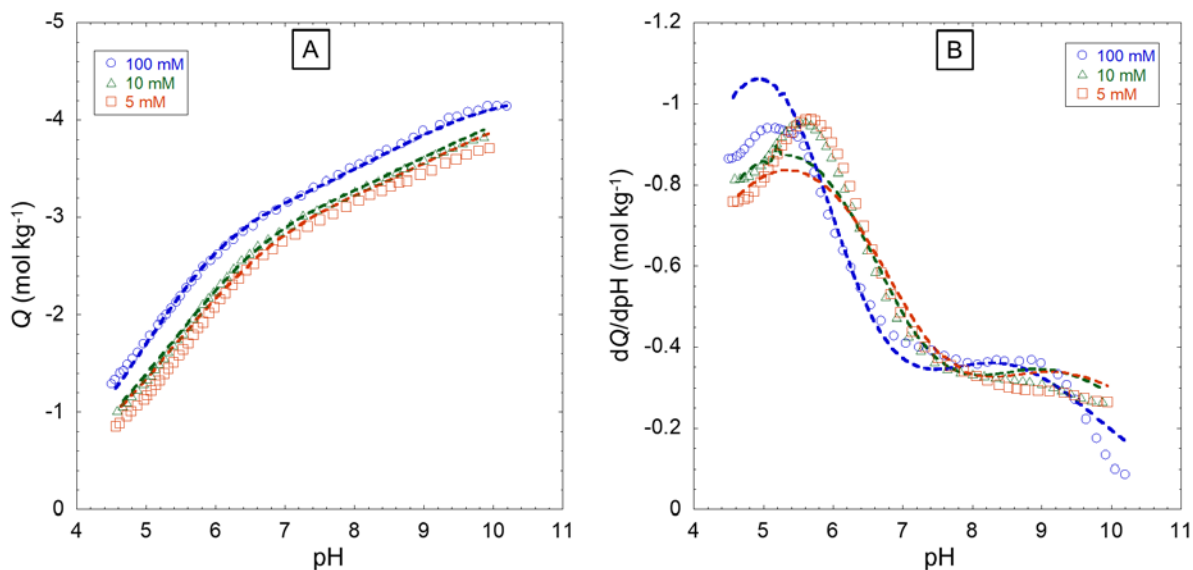
1065 **Figure S4 (Hf:FA) and Figure S5 (Hf:HA)** the explicit confrontation between theoretical results and
1066 measured proton titration and corresponding affinity spectra.



1067 **Figure S3:** Proton titration curves for LFA (A) and corresponding proton affinity spectra (B) collected at three
1068 NaNO₃ electrolyte concentrations: 10 mM (Δ), 30 mM (\diamond) and 100 mM (\circ). Symbols: measurements. Dotted lines:
1069 SPBT-PEST modelling results. Model parameters are listed in **Tables 1-2**. The charge is expressed in moles of
1070 equivalent charges per kg of LFA material.



1071
1072 **Figure S4:** Proton titration curves for Hf:FA (A) and corresponding proton affinity spectra (B) collected at three
1073 NaNO₃ electrolyte concentrations (indicated). Symbols: measurements. Dotted lines: SPBT-PEST modelling results.
1074 Model parameters are listed in **Tables 1-2**. The charge is expressed in moles of equivalent charges per kg of Hf:FA
1075 material.



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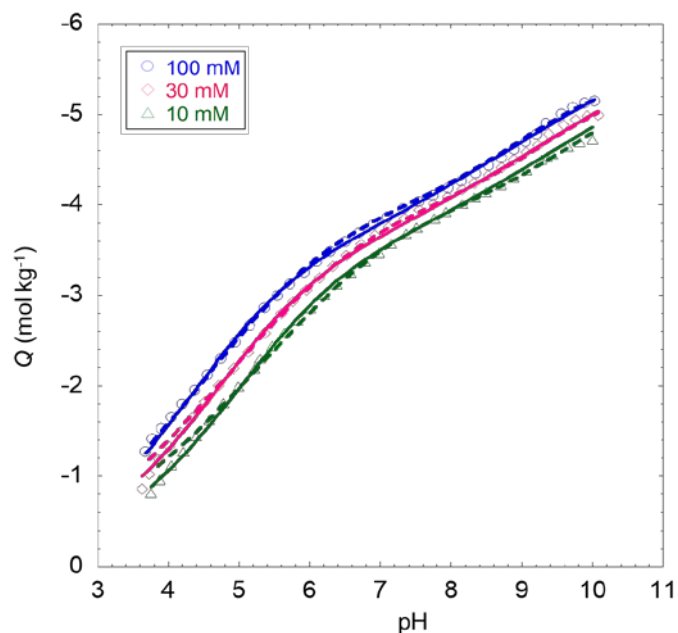
1077 **Figure S5:** Proton titration curves for Hf:HA (A) and corresponding proton affinity spectra (B) collected at three
 1078 NaNO₃ electrolyte concentrations (indicated) Symbols: measurements. Dotted lines: SPBT-PEST modelling results.
 1079 Model parameters are listed in **Tables 1-2**. The charge is expressed in moles of equivalent charges per kg of Hf:HA
 1080 material.

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1082 **F. Comparison between SPBT-PEST and NICAD reconstructions of proton titration** 1083 **data.**

1084 For the sake of comparison, **Figures S6-S10** collect the experimental Q vs. pH and I data for the
 1085 different HNPs samples of interest together with their theoretical fitting realized by NICAD and SPBT-
 1086 PEST.

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1099 **Figure S6:** Proton titration curves for FPHA collected at three NaNO₃ electrolyte concentrations (indicated).
 1100 Symbols: measurements. Dotted lines: SPBT-PEST modelling. Full lines: NICAD modelling. Model parameters are
 1101 listed in **Tables 1-2**. The charge is expressed in moles of equivalent charges per kg of FPHA material.

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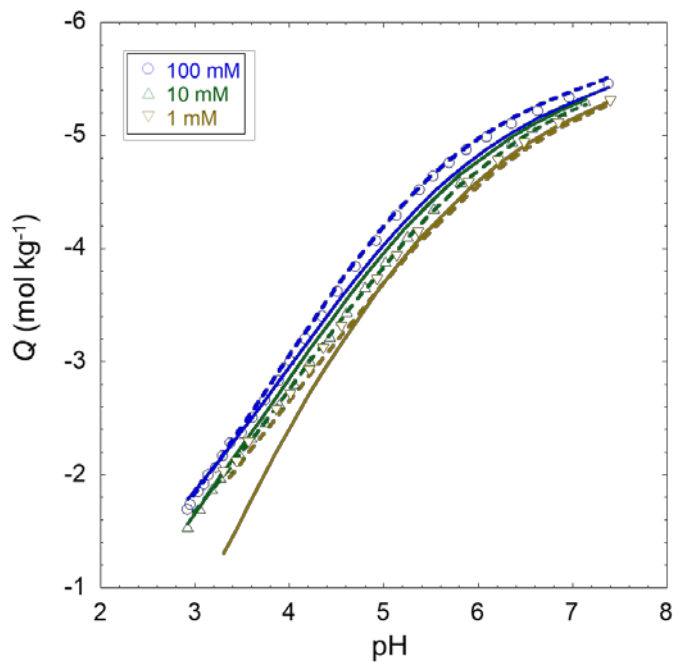


Figure S7: Proton titration curves for SRFA collected at three NaNO_3 electrolyte concentrations (indicated). Symbols: measurements. Dotted lines: SPBT-PEST modelling. Full lines: NICAD modelling. Model parameters are listed in **Tables 1-2**. The charge is expressed in moles of equivalent charges per kg of SRFA material.

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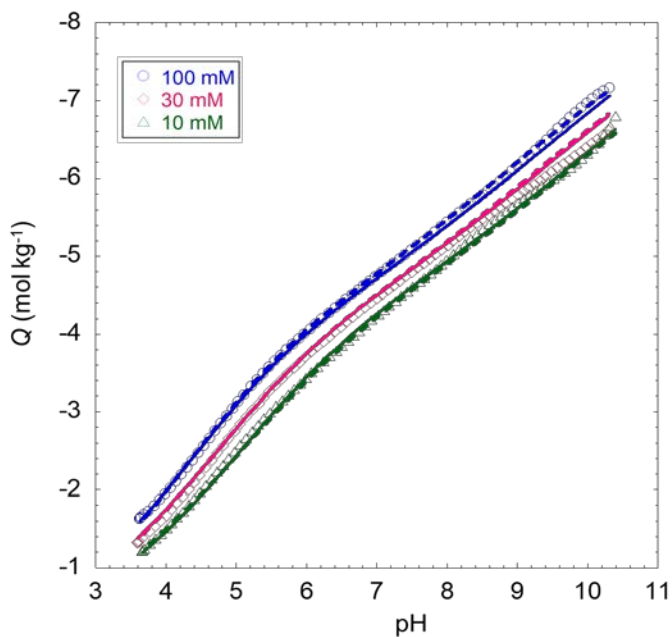


Figure S8: Proton titration curves for LFA collected at three NaNO_3 electrolyte concentrations (indicated). Symbols: measurements. Dotted lines: SPBT-PEST modelling. Full lines: NICAD modelling. Model parameters are listed in **Tables 1-2**. The charge is expressed in moles of equivalent charges per kg of LFA material.

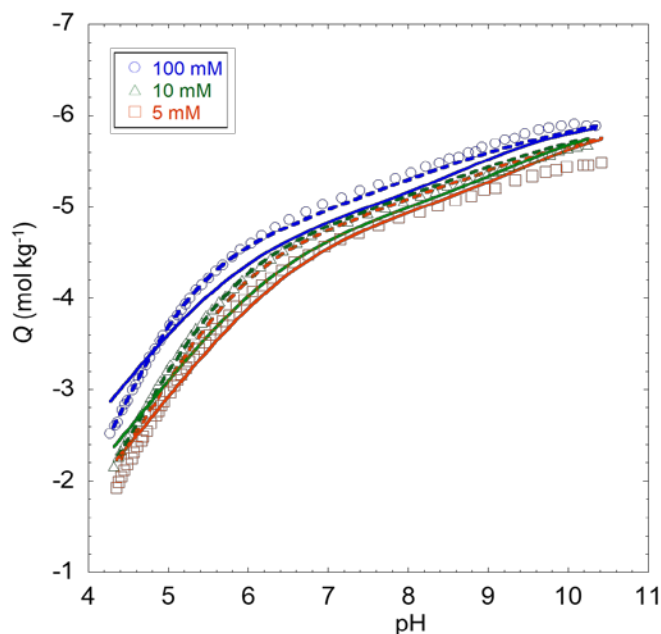
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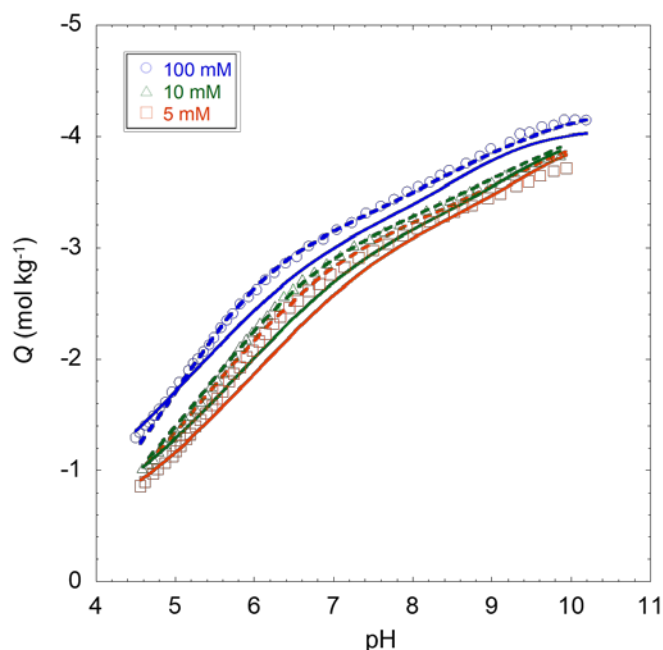
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1156 **Figure S9:** Proton titration curves for Hf:FA collected at three NaNO₃ electrolyte concentrations (indicated).
1157 Symbols: measurements. Dotted lines: SPBT-PEST modelling. Full lines: NICAD modelling. Model parameters are
1158 listed in **Tables 1-2**. The charge is expressed in moles of equivalent charges per kg of Hf:FA material.

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1170 **Figure S10:** Proton titration curves for Hf:HA collected at three NaNO₃ electrolyte concentrations (indicated).
1171 Symbols: measurements. Dotted lines: SPBT-PEST modelling. Full lines: NICAD modelling. Model parameters are
1172 listed in **Tables 1-2**. The charge is expressed in moles of equivalent charges per kg of Hf:HA material.

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