Titration in Canonical and Grand-Canonical Ensembles

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ABSTRACT: We discuss problems associated with the notion of pH in heterogeneous systems. For homogeneous systems, standardization protocols lead to a well-defined quantity, which, although different from Sørensen's original idea of pH, is well reproducible and has become accepted as the measure of the "hydrogen potential". On the other hand, for heterogeneous systems, pH defined in terms of the chemical part of the electrochemical activity is thermodynamically inconsistent and runs afoul of the Gibbs−Guggenheim principle that forbids splitting of the electrochemical potential into separate chemical and electrostatic parts, since only the sum of two has any thermodynamic meaning. The problem is particularly relevant for modern simulation methods which involve charge regulation of proteins, polyelectrolytes, nanoparticles, colloidal suspensions, and so forth. In this paper, we show that titration isotherms calculated using semigrand canonical simulations can be very different from the ones obtained using canonical reactive Monte Carlo simulations.
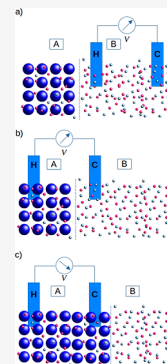
INTRODUCTION

The concept of pH was first introduced by Sørensen[1] in 1909. The original definition referred to pH as $-\log_{10}[c_{H^+}/c^{\ominus}]$, where $c^{\ominus} = 1$ M is the standard concentration. Since in practice pH is measured using electrodes, Sørensen later redefined pH in terms of activity of hydronium ions, pH $= -\log_{10}[a_+/c^{\ominus}]$, which was thought to be related to the electromotive force (EMF) measured by the system of electrodes through the Nernst equation. Later, Linderstrøm-Lang recognized that the experimental procedure used to measure pH did not lead exactly to pH $= -\log_{10}[c_{H^+}/c^{\ominus}]$, nor to pH $= -\log_{10}[a_+/c^{\ominus}]$, but to some other quantity which due to the convenience, became widely accepted as the measure of the hydrogen potential.[2] The problem with a direct measurement of pH is that the separation of the electrochemical potential into chemical and electric potentials is purely arbitrary since only the sum of two has any physical meaning. The Gibbs−Guggenheim principle states that the difference of electrostatic potential between two points located in regions of different chemical composition cannot be measured.[3,4] As early as 1899, Gibbs wrote in a letter:[5] "Again, the consideration of the electrical potential in the electrolyte, and especially the consideration of the difference of potential in the electrolyte and electrode, involves the consideration of quantities of which we have no apparent means of physical measurement, while the difference of potential in pieces of metal of the same kind attached to the electrodes is exactly one of the things which we can and do measure". In 1929, Guggenheim[6] formalized the observation of Gibbs by stating that "the decomposition of the electrochemical potential into the sum of a chemical term μ and an electrical term $e\psi$ is quite arbitrary and without physical significance. In other words, the chemical potential, or the activity of a single ion, and the electric potential

difference between two points in different media are conceptions without any physical significance."[7]

The confusion between exactly what can and is being measured has led to a proliferation of "local" pH measurements in soft matter and biophysics literature. The problem has become particularly acute since the modern simulation methods employed to study charge regulation of protein and polyelectrolyte solutions often rely on constant pH (cpH) algorithms, which are intrinsically semigrand canonical.[8−10] In such a procedure, pH is specified inside a reservoir of acid and salt, and the protonation state of a protein, polyelectrolyte, or colloidal suspension is calculated using a suitably constructed Monte Carlo algorithm that must respect the detailed balance. Since only microions are exchanged between the simulation box and the reservoir, the two must be at different electrostatic potentials. For an experimental system in which a colloidal suspension is separated from an external reservoir of acid and salt, this is known as the Donnan potential. Traditionally, pH is defined in terms of the chemical part of the electrochemical potential. However, since the Gibbs-Guggenheim principle forbids us from breaking up the electrochemical potential into separate electrostatic and chemical contributions, such a definition appears to be thermodynamically unacceptable.

In practice, pH is measured using EMF between a glass or hydrogen electrode and a saturated calomel (reference)

electrode. Consider a colloidal suspension separated from a reservoir by a semipermeable membrane that allows free movement of ions but restricts colloidal particles to system's interior (see Figure 1). If the calomel reference electrode is placed in the reservoir and the EMF is measured between it and the hydrogen electrode, one finds constant EMF independent of the position of the hydrogen electrode, either in the reservoir or in the system's interior; see Figure 1 panels (a) and (b). This is a

Figure 1. Colloidal crystal separated from a reservoir of acid and salt by a semipermeable membrane. Panels (a), (b), and (c) show the different locations of the (reference) calomel (C) electrode and the hydrogen (H) electrode. Note that EMF readings in panels (a) and (b) are the same, while in panel (c), it is different.

clear indication of a constant electrochemical potential of hydronium ions across both the system and the reservoir. On the other hand, if the two electrodes are placed inside the colloidal suspension, the EMF will depend on the distance of the reference electrode from the membrane; see Figure 1 panels (c). Clearly, such measurement would result in a thermodynamically ill-defined "local" pH. Such experimental measurements were performed by Teorell et al.[11] more than 85 years ago. Already at that time, he noted the difficulties with the usual definition of pH when applied to heterogeneous systems. One can argue that if both electrodes are placed deep into the system, far away from the membrane, the resulting EMF will stabilize and will allow us to define the system pH, which will be different from that of the reservoir. This is correct, but it does not resolve the underlying problem arising from the violation of the Gibbs−Guggenheim principle.[11] For example, consider now a colloidal suspension in a gravitational field.[12−14] Because of finite buoyant mass, the colloidal column will become progressively rarefied with the height: characteristic gravitational length of colloidal particles is between micrometers and millimeters. On the other hand, on the experimental length scale, ionic buoyant mass is negligible. Therefore, the top part of the suspension will be composed of a pure acid-salt electrolyte with a well-defined pH, since according to the Gibbs-Guggenheim principle, this is the region of uniform chemical composition in which one can measure the electro-static potential difference between two points. In the present case, the gravitational field plays the role of a membrane that establishes the inhomogeneity of the suspension. This results in a height-dependent Donnan potential $\varphi_D(z)$ along the column, which in turn leads to different ionic concentrations at each z. Nevertheless, if we place our reference electrode in the top (colloid-free) portion of the suspension, we will get exactly the same EMF (and consequently the same pH) independent of the placement of the hydrogen electrode inside the colloidal column. On the other hand, if the reference electrode is moved into the colloid-dense region, each different position will lead to different EMFs and, consequently, a different pH. One might argue that if both hydrogen and calomel electrodes are placed at exactly the same height z, the pH obtained using such measurements will have some physical meaning. Such proposition, however, once again seems untenable in view of the Gibbs-Guggenheim principle, since only the full electro-chemical potential has any thermodynamic meaning. The confusion in the literature is such that in a paper published some years back Brezinski wrote: "the uncertainty regarding interpretation of pH readings for colloids has led to the opinion that the pH value of neither the sediment nor the supernatant is very meaningful or useful for characterizing colloids".[15] Based on the preceding discussion, such a view seems overly pessimistic. While pH in the homogeneous supernatant of a suspension is well-defined thermodynamically, in the interior of a highly inhomogeneous suspension, it runs afoul of the Gibbs-Guggenheim principle. On the other hand, from a purely theoretical perspective, knowledge of pH in the inhomogeneous part of the suspension is completely irrelevant. Specification of pH and salt concentration in the homogeneous reservoir (supernatant) should be sufficient to calculate the state of protonation of colloidal particles and their density profile, both of which are easily accessible to experimental measurements. In theory—or simulation—one could even calculate the hydronium density profile inside an inhomogeneous suspension; however, there is no clear connection between this local density of
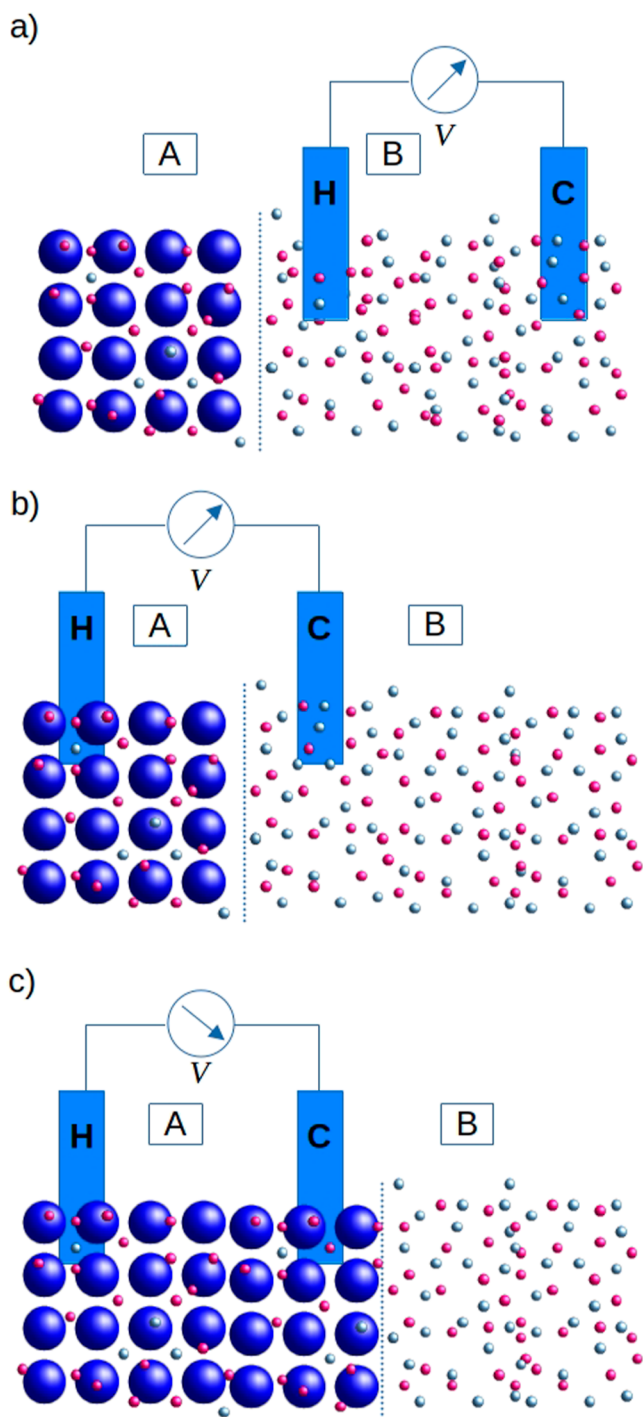
hydronium ions and the extra-thermodynamic quantity such as the "local" pH of an inhomogeneous suspension.[14]

When performing classical charge regulation simulations, one has two options−either a semigrand canonical constant pH (cpH) simulation in which the system is placed in contact with an implicit reservoir or acid and salt,[8,9] or a canonical simulation in which a fixed number of polyelectrolytes, protons, ions, and water molecules are placed inside a simulation box.[16,17] The two approaches are very different, requiring distinct implementations of the Monte Carlo algorithm to take into account protonation/deprotonation moves.[17] When performing cpH simulations, the insertion of a proton into the system is accompanied by a simultaneous insertion of an anion to preserve the overall charge neutrality. On the other hand, in a canonical simulation, a proton is transferred from a hydronium molecule inside the simulation box to a polyelectrolyte monomer, so that the charge neutrality is always preserved. This requires a completely different implementation of the MC algorithm. Furthermore, in a canonical simulation, pH is not an input parameter and can only be calculated a posteriori after the system has equilibrated. The consistency between the two simulation methods can be tested a posterior. For example, we can run a cpH simulation for a given pH and salt concentration in the reservoir. This will provide us with the average number of protonated groups on polyelectrolytes as well as with the average number of ions of each type inside the simulation cell. We can then isolate the system from the reservoir (canonical ensemble), keeping exactly the same number of ions inside the simulation cell as the averages obtained in the cpH simulation. We then strip all of the associated protons from polyelectrolyte and place them randomly (in the form of hydronium ions) together with the other ions into the simulation cell. We then run a canonical reactive MC algorithm. Equivalence between ensembles then requires that we obtain exactly the same number of protonated groups as was previously found using cpH simulation. This is precisely what is observed, showing consistency of the two simulation methods.[17]

The cpH simulations start with a specified value of pH_{gc} and the salt concentration inside the reservoir. On the other hand, in canonical simulations, pH_c has to be determined a posterior using the Widom insertion method. If we define pH in the semigrand canonical system in terms of the total electrochemical potential, corresponding to keeping the calomel electrode inside the reservoir while the hydrogen electrode is "placed" into the simulation cell, then the system pH_{sys} will be the same as of the reservoir pH_{gc}, and will, in general, be different from pH_c in the canonical system. On the other hand, if we disregard the Gibbs−Guggenheim principle and separate the Donnan potential from the rest of the electrostatic potential, then the pH inside the system will be different from pH_{gc} and the *same* as canonical pH_c. This situation corresponds to "placing" both hydrogen and the reference electrode inside the simulation cell of a semigrand canonical system. In practice, a calculation of the electrochemical potential in a canonical simulation is quite complicated, in particular if the pH is large, since the simulation box will have only very few hydronium ions, resulting in very poor statistics. This led to the popularization of the thermodynamically poorly defined "local" pH $(\mathbf{r}) = -\log_{10}[c_H^+(r)/c^{\ominus}]$.[18] To avoid these difficulties and clearly demonstrate the effect of ensembles on titration isotherms, in this paper we will use a recently developed theory, which was shown to be in excellent agreement with the explicit ion cpH simulations.[19]

THEORY

Semi-Grand Canonical Titration Theory. To explore the difference between canonical and grand canonical titration, we will use a cell model first introduced by Lifson and Katchalsky, and Marcus[20,21] to study polyelectrolyte and colloidal systems of finite volume fractions. The model consists of a colloidal particle of radius $a = 60$ Å placed at the center $r = 0$ of a spherical cell of radius R, which is determined by the volume fraction of the colloidal suspension $\eta_c = a^3/R^3$. The cell is assumed to be in contact with a reservoir of acid and 1:1 salt at concentrations c_a and c_s, respectively. All ions are treated as hard spheres of diameter $d = 4$ Å with a point charge located at the center. The nanoparticle has $Z = 600$ carboxylic groups of $pK_a = 5.4$, uniformly distributed over its surface. Ref [19] showed that the average number of deprotonated groups of a colloidal particle is given by

$$Z_{eff} = \frac{Z}{1 + 10^{-pH_{gc} + pK_a} e^{-\beta(q\varphi_0 + \varphi_{disc} - \mu_{sol})}} \tag{1}$$

where q is the proton charge. The pH in the reservoir is determined by $pH_{gc} = -\log_{10}[a_H^+/c^{\ominus}]$, with the activity of hydronium ions in the reservoir $a_H^+ = c_H^+ \exp(\beta\mu_{ex})$, where $\mu_{ex} = \mu_{CS} + \mu_{MSA}$ is the excess chemical potential. The nonideality effects due to Coulomb interactions are taken into account at the mean spherical approximation (MSA) level, while the hard core contribution is calculated using the Carnahan−Starling equation of state[22−31]

$$\beta\mu_{MSA} = \frac{\lambda_B(\sqrt{1 + 2\kappa d} - \kappa d - 1)}{d^2\kappa}, \quad \beta\mu_{CS} = \frac{8\eta - 9\eta^2 + 3\eta^3}{(1 - \eta)^3} \tag{2}$$

where $\eta = \frac{\pi d^3}{3}c_t$, $c_t = c_s + c_a$ is the total concentration of salt and acid, $\lambda_B = q^2/\epsilon_w k_B T = 7.2$ Å is the Bjerrum length, and $\kappa = \sqrt{8\pi\lambda_B c_t}$ is the inverse Debye length. The surface groups are characterized by $pK_a = -\log_{10}[K_a/c^{\ominus}]$, where K_a is the acid dissociation constant of surface groups and φ_0 is the mean-field electrostatic potential at the surface titration sites. The ion concentration inside the cell, for $r \geq a + d/2$, is given by the Boltzmann distribution

$$\rho_i(\mathbf{r}) = c_i e^{-\beta q_i \varphi(\mathbf{r})} \tag{3}$$

where c_i is the concentration of ions of type i in the reservoir. The mean field potential, $\varphi(r)$, satisfies the Poisson−Boltzmann equation for $r \geq a + d/2$

$$\nabla^2\varphi(r) = \frac{8\pi q}{\epsilon_w}(c_a + c_s) \sinh[\beta q\varphi(r)] \tag{4}$$

and Poisson equations for $a < r < a + d/2$. The discreteness of surface sites is taken into account self-consistently using the electrostatic potential[19]

$$\beta\varphi_{disc} = -\frac{\lambda_B M Z_{eff}}{a\sqrt{Z}} \tag{5}$$

where M is the Madelung constant of the two-dimensional one-component plasma in a hexagonal crystal state.[19,32] Finally, μ_{sol} is the electrostatic solvation-free energy of an isolated charged site

$$\beta\mu_{sol} = \frac{\lambda_B}{2}\int_0^\infty \frac{k - \sqrt{\kappa^2 + k^2}}{k + \sqrt{\kappa^2 + k^2}} e^{-kd} \, dk \tag{6}$$

Solving numerically the nonlinear PB equation with the boundary condition of vanishing electric field at the cell boundary (charge neutrality) and colloidal charge determined self-consistently by eq 1, we obtain the number of protonated groups for a given pH_{gc}. Note that at the surface of the cell there is a jump in the electrostatic potential: the reservoir is taken to be at zero potential, while at the cell boundary, $r = R$, the electrostatic potential is calculated to have a finite value φ_D. This is the Donnan potential of a suspension that is in contact with a reservoir of acid and salt through a semipermeable membrane. The titration curves for a fixed concentration of 1:1 salt inside the reservoir are presented by dashed red lines in Figures 1 and 2 as a function of pH in the reservoir.
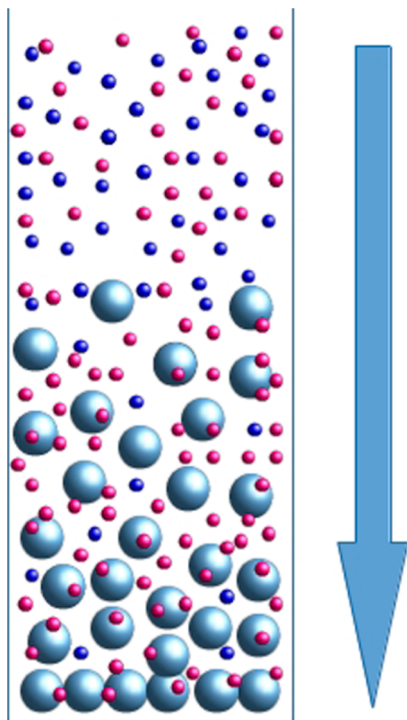

Figure 2. Colloidal suspension in a gravitational field. The top portion is a homogeneous, colloid-free electrolyte solution, where the pH has a well-defined thermodynamic meaning.

Canonical Titration Theory. Suppose we run a cpH simulation, from which we calculate the average number of deprotonated groups Z_{eff}, the average number of free hydronium ions, and the average number of sodium and chloride ions inside the cell. We then isolated the cell from the reservoir (canonical ensemble), keeping exactly this number of free ions inside the cell and fixing the colloidal charge at $Q = -qZ_{eff}$. Since the cell is no longer connected with the external reservoir, there is no Donnan potential at the cell boundary, and the electrostatic potential must be continuous between inside and outside the cell. Since outside the cell $\phi = 0$, we conclude that $\phi(R) = 0$.

At the level of approximation of the present theory, the distribution of hydronium ions inside the cell of a canonical system is given by

$$\rho_{H^+}(\mathbf{r}) = \frac{N_{H^+}e^{-\beta q\phi(\mathbf{r})}}{4\pi \int_{a+d/2}^{R} e^{-\beta q\phi(\mathbf{r})}r^2 \, dr} \tag{7}$$

where N_{H^+} is the number of free hydronium ions inside the cell and $\phi(r)$ is the mean field electrostatic potential.

The electrochemical potential of hydroniums inside the cell is

$$\beta\mu_c = \ln[\rho_{H^+}(\mathbf{r})] + \beta q\phi(\mathbf{r}) + \beta\mu_{ex} \tag{8}$$

where μ_{ex} is the excess chemical potential due to the electrostatic and steric interactions between the ions, which at the level of the present theory we take to be constant and equivalent to $\mu_{CS} + \mu_{MSA}$ in the reservoir. Clearly, the fact that the system became disconnected from the reservoir after equilibration does not affect the distribution of hydronium ions inside the cell, which must remain exactly the same as before. The only difference is that the canonical electrostatic potential is shifted from its grand canonical value by the Donnan potential, $\phi(r) = \varphi(r) - \varphi_D$, which does not affect the distribution given by eq 7. Therefore, the hydronium density profile, eq 7, can also be written in terms of the acid concentration c_a in the original reservoir (see eq 3) and the Donnan potential

$$\rho_{H^+}(\mathbf{r}) = c_a e^{-\beta q\phi(\mathbf{r}) - \beta q\varphi_D} \tag{9}$$

Substituting this expression into eq 8, we obtain the relation between canonical and semigrand canonical electrochemical potentials

$$\mu_c = \mu_{gc} - q\varphi_D \tag{10}$$

The activity of hydronium ions inside an isolated suspension is then $a_{H^+} = \exp[\beta\mu_c]/c^{\ominus}$, so that canonical and semigrand canonical pH are found to be related by

$$pH_c = pH_{gc} + \frac{\beta q\varphi_D}{\ln 10} \tag{11}$$

■ RESULTS AND DISCUSSION

In Figures 3 and 4, we present the titration isotherms for colloidal suspensions of various volume fractions and salt concentrations. The red dashed curves correspond to systems in which colloidal particles are separated from the acid-salt reservoir of pH_{gc} and c_s by a semipermeable membrane. On the other hand, the black solid curves correspond to titrations performed in isolated colloidal suspensions containing a fixed salt concentration c_s, as indicated in the figures. To calculate these canonical titration curves, the concentration of salt in the reservoir is adjusted to get the desired concentration of salt inside the system while solving the PB equation with the boundary conditions of vanishing electric field at $r = R$ and the nanoparticle charge determined by eq 1. The pH_c is then obtained using eq 11. We see that for suspensions of high volume fractions and low salt content, the canonical titration curves are very different from their semigrand canonical counterparts. If the salt content of the suspension increases or if the volume fraction of colloidal particles decreases, we see that the difference between titration isotherms vanishes. This explains why these problems were not previously observed in the cpH simulations used to study biologically relevant proteins. Such simulations are usually conducted at physiological concentrations of electrolyte when the difference between canonical and grand canonical pH vanishes.

■ CONCLUSIONS

After 115 years, the measure of "hydrogen potential" still causes conceptual and practical difficulties. The original idea of Sørensen was to relate the pH directly to the concentration of
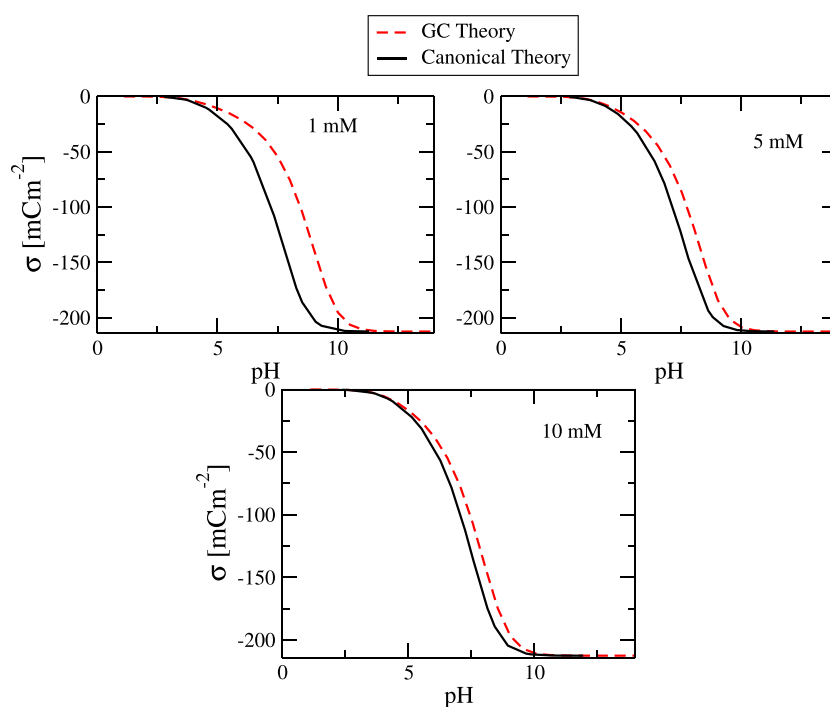
Figure 3. Titration isotherms for canonical and semigrand canonical ensembles. For an open system, the pH refers to the pH in the reservoir, while for a closed system, it is for the interior of the suspension. Similarly, for an open system, c_s refers to the salt content in the reservoir, while for a closed system, it is the salt concentration inside the system. At higher salt concentrations, the difference between canonical and semigrand canonical titration curves vanishes. Colloidal volume fraction is $\eta = 11\%$ in all cases. Colloidal surface charge density σ is measured in millicoulombs per m^2.
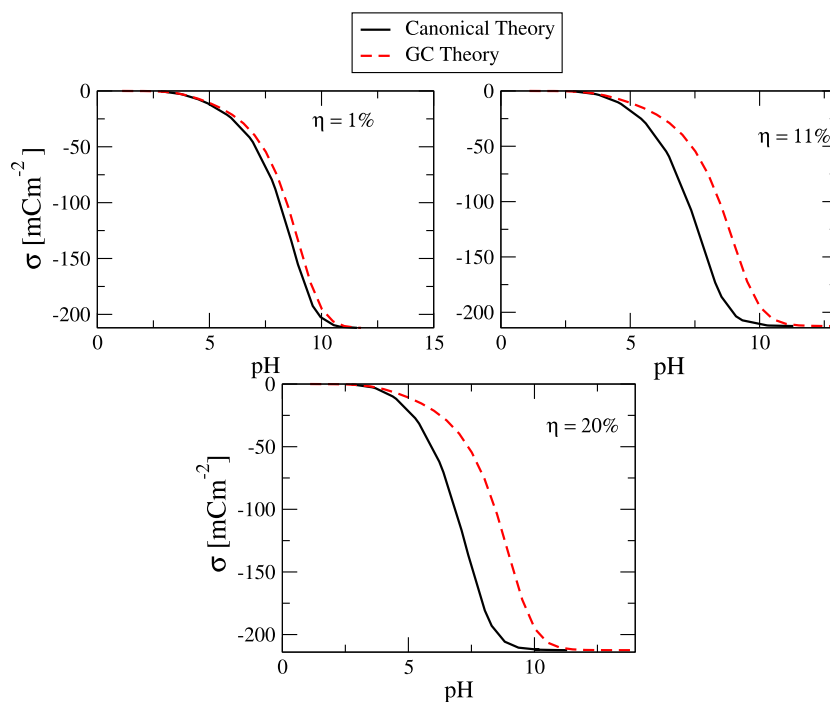

Figure 4. Titration isotherms for canonical and semigrand-canonical systems of different colloidal volume fractions η. Salt concentration is $c_s = 1$ mM. For dilute suspensions (low colloidal volume fraction), the difference between ensembles vanishes. Colloidal surface charge density σ is measured in millicoulombs per m^2.

hydronium ions. The complexity of measuring local concentrations of protons led him to later redefine pH in terms of the activity of hydronium ions. This, however, resulted in a whole new set of difficulties since the measurements of individual activity coefficients are not possible by any electrochemical means. What is in fact being measured using hydrogen and calomel electrodes is neither activity nor concentration of hydronium ions, but some other quantity.[33] Nevertheless, due to the standardization of such measurements, they have become well accepted by the scientific community.[2] For homogeneous

single-phase systems, the situation, therefore, appears to be reasonably well understood. The difficulties arise when one tries to extend such measurements to heterogeneous systems, such as colloidal suspensions in gravitational fields or even colloidal lattices in which translational symmetry is broken and the electrostatic potential is a strongly inhomogeneous function of position. The Gibbs−Guggenheim principle forbids us from splitting the electrochemical potential into separate chemical and electrostatic parts since only the sum of two has any thermodynamic meaning.[a] This suggests that the correct definition of activity should involve the full electrochemical potential $a_{H^+} = \exp[\beta\mu]/c^\ominus$, where $\mu = \mu_{chem}(\mathbf{r}) + q\varphi(\mathbf{r})$. Although both the chemical potential $\mu_{chem}(\mathbf{r})$ and the total electrostatic potential $\varphi(\mathbf{r})$ are local functions of position, their sum is constant throughout an inhomogeneous system in which protons are in equilibrium. Such definition, however, would make the activity of protons and pH in heterogeneous systems with Donnan equilibrium the same on both sides of a semipermeable membrane transparent to H^+, even though the concentrations of hydronium ions on the two sides of such a membrane are different. The price of such definition would, therefore, be to move the notion of pH even farther from Sørensen's original idea of measuring local hydronium concentration. The gain, however, would be to make pH a true thermodynamic variable directly related to the electrochemical potential. The current state of affairs seems to be untenable for heterogeneous systems in which the local electrostatic potential is a strongly inhomogeneous function of position. It is unclear what thermodynamically relevant information can be extract from pH measurements that are based on standard protocols, in which the position of both reference calomel and hydrogen electrodes is changed throughout the measurements. The notion of "local" activity lacks any thermodynamic meaning and cannot be measured. If such measurements are attempted, the results will be "accidently determined" by the sample preparation and electrode positioning, as was already stressed by Guggenheim almost 100 years ago.[14] This explains the confused state of affairs in colloidal science exemplified by the so-called "suspension effect" in which the pH measured in a charged sediment is found to be very different from that of the supernatant.[15] In the present paper, we suggest that for strongly inhomogeneous systems, only pH_{gc} in the homogeneous part has any significance. If one wants to study the thermodynamics—and statistical mechanics—of inhomogeneous suspensions—such as, for example, density profiles of colloidal particles in a gravitational field—only pH in the supernatant can be used as an input in any theoretical investigations, the pH in the sediment will be "accidental".[14] Specification of pH_{gc} in the "reservoir" avoids the difficulties associated with splitting the electrochemical potential into separate chemical and electrostatic contributions. A corollary of this is that titration curves in open systems that are in contact with a reservoir, plotted as a function of pH_{gc}, can be significantly shifted from titration curves calculated for closed (canonical) systems of exactly the same volume fraction and electrolyte concentration, as is demonstrated in the present paper.

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Notes
The authors declare no competing financial interest.

■ ADDITIONAL NOTE

[a]For reasons why it is not possible to uniquely define the "mean" electrostatic potential, see the discussion of N. Bjerrum in ref 33.

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