

Specific Interactions versus Counterion Condensation. 2. Theoretical Treatment within the Counterion Condensation Theory

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Polyuronates such as pectate and alginate are very well-known examples of biological polyelectrolytes undergoing, upon addition of divalent cations, an interchain association that acts as the junction of an eventually formed stable hydrogel. In the present paper, a thermodynamic model based on the counterion condensation theory has been developed to account for this cation-induced chain pairing of negatively charged polyelectrolytes. The strong interactions between cross-linking ions and uronate moieties in the specific binding site have been described in terms of chemical bonding, with complete charge annihilation between the two species. The chain-pairing process is depicted as progressively increasing with the concentration of cross-linking counterions and is thermodynamically defined by the fraction of each species. On these bases, the total Gibbs energy of the system has been expressed as the sum of the contributions of the Gibbs energy of the (single) chain stretches and of the (associated) dimers, weighted by their respective fractions $1 - \theta$ and θ . In addition, the model assumes that the condensed divalent counterions exhibit an affinity free-energy for the chain, $G_C^{\text{aff},0}$, and the junction, $G_D^{\text{aff},0}$, respectively. Moreover, a specific Gibbs energy of chemical bonding, $G^{\text{bond},0}$, has been introduced as the driving force for the formation of dimers. The model provides the mathematical formalism for calculating the fraction, θ , of chain dimers formed and the amount of ions condensed and bound onto the polyelectrolyte when two different types of counterions (of equal or different valence) are present. The effect of the parameter $G^{\text{bond},0}$ has been investigated and, in particular, its difference from $G_{C,D}^{\text{aff},0}$ was found to be crucial in determining the distribution of the ions into territorial condensation and chemical bonding, respectively. Finally, the effect of the variation of the molar ratio between cross-linking ions and uronic groups in the specific binding sites, σ_0 , was evaluated. In particular, a remarkable decrease in the amount of condensed counterions has been pointed out in the case of $\sigma_0 = 1/3$, with respect to the value of $\sigma_0 = 1/4$, characterizing the traditional “egg-box” structure, as a result of the drop of the charge density of the polyelectrolyte induced by complete charge annihilation.

1. Introduction

Solutions containing linear polyelectrolytes and counterions of the same or different valence are known to show different degrees of counterion association with the polyelectrolyte.^{1–4} The problem of a detailed description of the counterion–polyion interactions has drawn particular attention in the polyelectrolyte literature.^{1,4,5–7} The most widespread formalisms included the mean-field approaches of the Poisson–Boltzmann equation^{8–10} and the counterion condensation (CC) theory.^{11–15} The description of a system composed of a polyelectrolyte in the presence of mixtures of counterions has already been tackled within the framework of the CC theory,^{4,16–18} and a mathematical formalism over a range of experimental conditions has been proposed.¹⁹ Albeit crude, the CC theory has been applied to describe in detail the thermodynamics of the conformational transition of charged biopolymers²⁰ and has enabled the solution of some controversial cases.^{21,22} An important feature such as the biopolymer chain flexibility can efficiently be included in the formal treatment of this simplified theory, providing an accurate description of the experimental observations.²³ In addition, a recent reformulation of the CC theory²⁴ has led to the introduc-

tion into the mathematical formalism of an additional contribution to the total Gibbs energy of the system stemming from a specific “affinity” of the polyelectrolyte toward the counterion, which is beyond the purely electrostatic interaction. Further exploitation of the influence of condensed divalent counterions showing a preferential “affinity” for polyanions with respect to monovalent ones has been presented, and the results nicely compared with data from isothermal microcalorimetry experiments.²⁵

One should recall that the CC theory requires only the equilibrium concentration (or fraction per fixed charge) of the condensed counterions to be constant and equal to what is dictated by the geometry (i.e., linear charge density) and by the physical chemistry of the system (i.e., medium dielectric permittivity and temperature). Free and condensed counterions are in dynamic exchange equilibrium under the sole above condition. Moreover, the condensed counterions are supposed to move along and around the polyelectrolyte in fast chemical exchange between polymer-charged sites. “Specific affinity” interactions, if present, just contribute to change the thermodynamic potentials, but they still keep this process as a comparatively fast one on the time scale of orientational and translational motions of the polyelectrolyte chain.²⁶ By enabling polyelectrolyte–ion interactions to be affected by specific affinity, the “territorially” condensed counterions confined in

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the condensation volume have to be regarded as fundamental in determining the relative stability of different conformations of a biopolymer and in modulating the corresponding conformational transition.²⁷ It is important to realize that within the framework of the CC theory the counterions are loosely associated to the polyelectrolyte and that any specific affinity does not modify the expected fraction of condensed counterions in systems containing only one type of counterion. It accounts exclusively for an increase in the relative amount of the more affine territorially condensed counterions in mixed systems, without implying any permanent site association.²⁵ The condensation is a confinement of the counterions within a (condensation) volume in the immediate vicinity of the polyelectrolyte as a result of long-range polyelectrolytic interactions only:¹³ fluctuations of ions within the condensation volume and exchange with ions free in solution is totally allowed.

Therefore, the CC theory in its original formulation does not take into account short-range, site-specific binding processes that heavily depend on the peculiar features of the counterion and the ligand up to the level of “complete charge annihilation”. It should be strongly emphasized that in the following approach the term “complete charge annihilation” does not necessarily imply the formation of covalent bonds between the ion and the polymer charged groups (although it may). A very close contact between the cation and the polymer negative charges, in which they both keep their formal charge (e.g., “ionic bond” in the jargon of protein structure or “inner-sphere complex” in that of metal-ion complexes), is perfectly compatible with this definition. The “chemically bound” ions can be considered as “localized” in a specific site on the polyelectrolyte chain for a residence time much longer than both that of the “territorially condensed” ions (even in the presence of additional specific affinity interactions)²⁴ and that of the chemical exchange of the condensed ions with the free ions in solution.

One of the best-known and well-described examples of such strong site-specific bonding is ion-induced chain association (pairing) of some polyuronates. In fact, ionic polysaccharides such as pectate and alginate are characterized by the ability to bind divalent cations, such as calcium or barium, into interchain junctions that eventually lead to the formation of a wall-to-wall hydrogel. In these cases, calcium ions are “entrapped” between sequences of galacturonate (in the case of pectate) or guluronate (for alginate), and the interchain junctions are commonly described using the so-called “egg-box” model.^{28–30} In view of the strong binding of divalent cations in the junctions, the term “chemical bonding” seems to be appropriate to describe the interaction occurring between the cross-linking ion and the uronic moieties in the egg-box.

A first step toward the description of a strong binding between polyelectrolytes and ions has been successfully achieved by Porasso et al.³¹ for the case of linear (nonassociating) polymers by introducing the fraction of counterions that are chemically bound to the polyelectrolyte (with annihilation of the fraction $z_j \cdot \sigma$ of all polyelectrolyte-charged groups being $0 < \sigma < \sigma_0$). This implies that the fraction σ of counterions is statistically delocalized over a large number of specific sites, thus exhibiting a distribution similar to that of territorially condensed counterions. It is important, however, to mention that the model presented by Porasso et al.³¹ failed to realistically describe the ion-induced chain association of polyuronates.³²

As pointed out by Nordmeier,³³ territorial and site-specific binding usually occur simultaneously and, in most of the cases, experimental techniques are unable to identify the contribution of each mode. In this respect, it appeared particularly appealing

to develop, within the framework of the CC theory, a model enabling a general and realistic description of the ion-induced chain association, with particular attention to the dimeric association of polyuronates. In addition, this model should be able to provide the mathematical formalism required to theoretically discriminate (and calculate) the amount of territorially condensed versus chemically bound counterions. The main objective of the present paper is the development of a new theoretical model that accounts for a nonfractional charge annihilation. The mathematical formalism has been derived for a polyelectrolyte solution containing two species of counterions of valence z_i and z_j arising from the supporting and the cross-linking salt, respectively, and a progressively increasing chain pairing at each addition of cross-linking ion has been considered. Moreover, in the analytical expression of the total free energy of the system, Gibbs energies, stemming from an affinity of the cross-linking counterion toward the chain, $G_C^{\text{aff},0}$, and the junction, $G_D^{\text{aff},0}$, and a Gibbs free energy of chemical bonding, $G^{\text{bond},0}$, associated with the formation of the “egg-box” structures, have been introduced. To model the bonding interaction between cross-linking counterions and uronate moieties in the “egg-box” structures, complete charge annihilation between the two species has been assumed. At variance with previous results,³¹ a constant value of the stoichiometry molar ratio between the “chemically” bound counterions and the charged groups in the junction binding site, σ_0 , was used. Focusing on dimer formation, we explored the functional dependence of the fraction of territorially condensed and chemically bound counterions, as well as the fraction of dimer formed at each concentration of the cross-linking counterions, on the physico-chemical parameters of the solution.

The scope of this paper is to discuss the main features and implications of the devised model, while the agreement between the experimental results^{34–36} and the theoretical predictions will be assessed, in the case of calcium-induced dimer formation with polyuronates, in the forthcoming paper.³²

2. Theory

(A) Model and Background. In accordance with the CC theory, the polyuronate is described as an infinitely long, linear polyelectrolyte in solution with counterions of different valences. The structural charge per ionized group on the polyelectrolyte is $q_{\text{str},C}$ (assumed to be unitary), and the average structural intercharge distance projected onto the polyelectrolytic axis is equal to $b_{\text{str},C}$. The structural charge density is calculated as (eq 1)

$$\xi_{\text{str},C} = \frac{l_B}{b_{\text{str},C}} \quad (1)$$

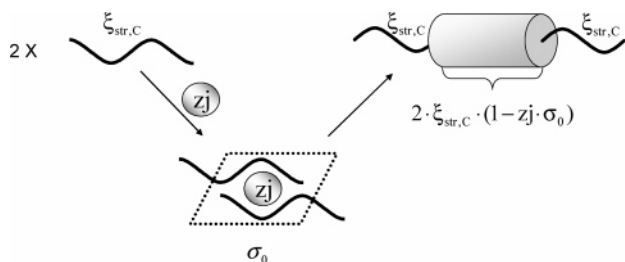
with (eq 2)

$$l_B = \frac{e^2}{\epsilon k_B T} \quad (2)$$

l_B is the Bjerrum length ($l_B = 7.135 \text{ \AA}$ for water at 298.15 K), e is the elementary charge, ϵ is the (bulk) relative permittivity of the medium, k_B is the Boltzmann's constant, and T is the absolute temperature.

The aim of the present model is to describe, in general terms, the association, in the presence of the proper cross-linking counterions, of n (single) chains (C) into an n -valent junction

Scheme 1. Schematic Description of the z_j -Valent Counterion-Induced Chain Dimerization, According to the Theoretical Model Devised in the Present Paper^a



^a See text for definitions of quantities.

($Y_{(n)}$; eq 3)



In the simplest case ($n = 2$), the junction is a dimer (D; eq 4)



In the more general case, however, we should consider a junction as formed by n chains and characterized by a structural charge density $\xi_{\text{str},Y_{(n)}}$ equal to (eq 5)

$$\xi_{\text{str},Y_{(n)}} = \rho n \xi_{\text{str},C} \quad (5)$$

Where ρ is a front factor that accounts for any conformational variation that, by occurring to the (single) chain in the pairing process, affects its interchange distance $b_{\text{str},C}$. Trivially, in the case of $\rho = 1$ (no conformational modification of interchange distance) and $n = 2$ (formation of dimers induced by the cross-linking counterions; eq 6)

$$\xi_{\text{str},Y_{(2)}} = 2\xi_{\text{str},C} \quad (6)$$

To model the “chemical bonding” between the cross-linking counterions and the negatively charged uronic groups on the polymer in the chain-pairing process, a complete charge annihilation between the charges of the ion and an equivalent number of oppositely charged groups in the binding sites has been assumed. Thus, the structural charge (per polymeric charged group) of the junction is reduced to (eq 7)

$$q_{\text{str},Y_{(n)}} = q_{\text{str},C}(1 - z_j\sigma_0) \quad (7)$$

where z_j is the valence of the cross-linking (“chemically bound”) ion and σ_0 is the molar ratio between the cross-linking ion and the uronic moieties in the binding site. From what was stated above, it follows that the effective charge density of the junction is reduced to (eq 8)

$$\xi_{\text{eff},Y_{(n)}} = \rho n \xi_{\text{str},C}(1 - z_j\sigma_0) \quad (8)$$

Similarly, the effective interchange distance in the junction is (eq 9)

$$b_{\text{eff},Y_{(n)}} = \frac{b_{\text{str},C}}{\rho n(1 - z_j\sigma_0)} \quad (9)$$

As an example, the calcium-induced chain dimerization of pectate (Scheme 1), as described in the “egg-box” model, is characterized by a value of $\sigma_0 = 1/4$ and by an effective charge density $\xi_{\text{eff},D} = \xi_{\text{str},C}$.

In an attempt to provide a general description of the ion-induced chain association process, the mathematical treatment has been derived, within the framework of the CC theory, in the presence of a mixture of two salts, where z_i and z_j are the valences of the counterion of the supporting electrolyte (in most cases, monovalent) and of the cross-linking counterion, respectively. Paoletti et al.¹⁹ have shown that the thermodynamic properties of mixed-valence counterion polyelectrolyte solutions can be analytically described by assuming that, in the condensation regime, both species of counterions are found in the condensation volume under all circumstances. This follows as a bare consequence of the entropy of mixing in the framework of the theory of regular solutions, which will predict values of the concentration of condensed species “j” and “i” correlated with the total concentration of each species in the system.¹⁹ In contrast, site specificity favors the bonding of one species over the other; in the present case, the z_j -valent cross-linking counterions will preferentially accumulate into the junctions, while preventing in this region the inclusion of z_i -valent ions. Focusing on the z_j -valent counterions, it is, therefore, important to realize that in the present model one can identify four different types of ions:

- Ions “condensed” on the (single) chain component.
- Ions “condensed” on the n -valent junction component.
- Ions “entrapped” in the n -valent junction structure. Any fluctuation of these ions is neglected, and they can be considered as chemically bound (i.e., with charge annihilation) into the specific cavity. The molar ratio between cross-linking ions and uronic moieties in the junction is equal to σ_0 .
- Ions “free” in solution.

Conversely, z_i -valent counterions, which originate from the supporting salt, have no cross-linking properties, and they will distribute just over types i, ii, and iv.

In the case of a polyelectrolyte characterized by a $\xi_{\text{str},C} > \xi_{\text{critical}}$ (as for polygalacturonate, where $b_{\text{str},C} \approx 4.43 \text{ \AA}$, i.e., shorter than l_B),³⁷ a fraction r_C of counterions is condensed onto the polyelectrolyte (single) chain (per polymeric charged group). Considering that under this condition both z_i - and z_j -valent counterions are present in the condensation volume,¹⁹ r_C can be rewritten as (eq 10)

$$r_C = r_C^i + r_C^j = r_C(x_C^i + x_C^j) \text{ (with } x_C^i + x_C^j = 1) \quad (10)$$

where r_C^i and r_C^j stand for the fraction of condensed counterions of valence z_i and z_j , respectively, on the (single) chain component of the system. The condensation of counterions leads to a reduction in the effective charge on each ionized site on the polyelectrolyte, $q_{\text{eff},C}$, with respect to the structural charge, $q_{\text{str},C}$ (eq 11)

$$q_{\text{eff},C} = q_{\text{str},C}(1 - z_i r_C^i - z_j r_C^j) \quad (11)$$

Similarly, a fraction $(1 - z_j\sigma_0)r_{Y_{(n)}}$ of counterions condenses onto the junction (per equivalent polymeric charged group; eq 12), as long as its effective charge density is higher than the critical value for counterion condensation,¹⁹ inducing a variation of the effective charge (eq 13)

$$(1 - z_j\sigma_0)r_{Y_{(n)}} = (1 - z_j\sigma_0)(r_{Y_{(n)}}^i + r_{Y_{(n)}}^j) = (1 - z_j\sigma_0)r_{Y_{(n)}}(x_{Y_{(n)}}^i + x_{Y_{(n)}}^j) \text{ (with } x_{Y_{(n)}}^i + x_{Y_{(n)}}^j = 1) \quad (12)$$

$$q_{\text{eff},Y_{(n)}} = (1 - z_j\sigma_0)q_{\text{str},C}(1 - z_i r_{Y_{(n)}}^i - z_j r_{Y_{(n)}}^j) \quad (13)$$

where $r_{Y(n)}^i$ and $r_{Y(n)}^j$ stand for the fraction of condensed counterions of valence z_i and z_j , respectively, on the n -valent junction component of the system.

(B) Mathematical Treatment. To provide a mathematical expression for calculating the total Gibbs energy of the system under analysis, it is important to formally devise a model that represents the best (achievable) approximation of the “real” ion-induced chain pairing. In this sense, a model based on an all-or-none process on the length scale of the accepted “cooperative junction unit” (say, about a dozen or more sugars on each pairing chain) would not be appropriate because polyuronates exhibit significant binding already at a very low molar ratio between the cross-linking counterion and the polysaccharide repeating units (R_j).^{35,36,38} Therefore, the junction formation is initiated by small amounts of the z_j -valent cross-linking counterion; the junction is then progressively elongated and completed by increasing the R_j ratio. In view of these considerations, we decided to describe the ion-induced chain pairing for polyuronates as a stepwise process where the equilibrium condition is constantly evolving from the “single” chain to the full length of the n -valent aggregate ($Y_{(n)}$) by increasing R_j . It follows that, at each concentration of the z_j -valent cross-linking counterion, the total excess molar Gibbs free energy of the system (per mole of fixed charge), \bar{G}^T , is determined by the contribution of both the chain and the aggregate ($Y_{(n)}$), each of which is weighted for the corresponding fractions ($1 - \theta$ and θ , respectively; eq 14). For the sake of simplicity, the analytical expression of the total molar Gibbs free energy of the model will be derived for the case of dimer formation (thus $n = 2$) by (single) chains that are not affected by any conformational variation of the interchange distance, that is, $\rho = 1$. Moreover, in accordance with the CC theory, end effects are neglected

$$\bar{G}^T = (1 - \theta)\bar{G}_C^T + \theta\bar{G}_D^T \quad (14)$$

The latter can be expressed, in terms of the reduced molar total Gibbs free energy, g^T , as (eq 15)

$$g^T = \frac{\bar{G}^T}{RT} = (1 - \theta)g_C^T + \theta g_D^T \quad (15)$$

In the present formalism, one should write the reduced Gibbs energy for both the single chain (C) and the dimer (D), $g_{C,D}^T$, as the sum of the ionic, the affinity, and the bonding contributions (eq 16)

$$g_{C,D}^T = g_{C,D}^{\text{ion}} + g_{C,D}^{\text{aff}} + g_D^{\text{bond}} \quad (16)$$

where the terms $g_{C,D}^{\text{aff}}$ and g_D^{bond} have been added to account for the affinity of the uronic acids toward type j (with respect to type i) condensed cations and for the chemically bound ones, respectively.²⁵

The full analytical derivation of the free-energy contributions in eq 16 is reported in Appendix A. It is, however, important to summarize that the mathematical description of the model involves the introduction of a set of parameters describing the intrinsic affinity and bonding processes. In particular, the (reduced) intrinsic Gibbs energy of affinity for the single chain and the dimer, $g_C^{\text{aff},0}$ and $g_D^{\text{aff},0}$, respectively, have been introduced and are both expressed per mole of condensed counterion. For the affinity terms (as well as for the others) the (reduced) intrinsic Gibbs energy can be factorized in two (reduced)

intrinsic thermodynamic terms of enthalpic (h_C and h_D) and entropic (s_C and s_D) origin. It trivially holds the following:

$$h_{C,D}^{\text{aff},0} = \frac{H_{C,D}^{\text{aff},0}}{RT} \quad s_{C,D}^{\text{aff},0} = \frac{S_{C,D}^{\text{aff},0}}{R} \quad \text{and} \quad g_{C,D}^{\text{aff},0} = h_{C,D}^{\text{aff},0} - s_{C,D}^{\text{aff},0}$$

Although the theoretical treatment has been accomplished with the separate identification of $g_C^{\text{aff},0}$ and $g_D^{\text{aff},0}$, for simplicity in the following calculations, we will assume that $g_C^{\text{aff},0} \equiv g_D^{\text{aff},0} \equiv g^{\text{aff},0}$. This assumption does limit the theory and allows us to reduce the number of parameters of the system. The affinity-driven interaction of the single chain and of the dimer with the j -type ion is expected to lead to a relevant variation in the volume of the solution as a consequence of the condensation ($\Delta V_C^{\text{aff},0}$ and $\Delta V_D^{\text{aff},0}$, respectively: $\Delta V_C^{\text{aff},0}$ is in mL per mole of condensed counterion). In addition, $g^{\text{bond},0}$ is the (reduced) intrinsic Gibbs energy variation (per mol of “chemically bound” counterion) associated with the formation of the egg-box complex between the uronic groups and the ion. This can be influenced or not by the presence of z_j -valent counterions already bound. In the latter case, $g^{\text{bond},0}$ is constant in all the R_j ($[M^{2+}]/[\text{Pol}]_{\text{ru}}$ ratio) range, while in the former, $g^{\text{bond},0}$ can be favored (becoming more negative by increasing R_j ; cooperativity) or disfavored (becoming more positive by increasing R_j ; anti-cooperativity) by previously bound ions. Also, $g^{\text{bond},0}$ can be factorized into an enthalpic ($h^{\text{bond},0}$) and an entropic ($s^{\text{bond},0}$) term associated with the chelation of the z_j -valent cross-linking counterion ($g^{\text{bond},0} = h^{\text{bond},0} - s^{\text{bond},0}$), which, in addition, can lead to a variation of the volume of the system ($\Delta V^{\text{bond},0}$, in mL per mol of chemically bound counterion).

Once the analytical expression of the reduced total Gibbs energy of the system (eq 15) is obtained as derived in the Appendix A, the contribution of each counterion species to the total condensed fraction and the fraction θ of dimers formed are calculated¹⁹ by minimizing g^T (eq 17)

$$\frac{\partial g^T}{\partial r_C} = 0; \quad \frac{\partial g^T}{\partial r_D} = 0; \quad \frac{\partial g^T}{\partial x_C^i} = 0; \quad \frac{\partial g^T}{\partial x_D^i} = 0; \quad \frac{\partial g^T}{\partial \theta} = 0 \quad (17)$$

As usual, in the CC theory,⁴ the limiting behavior at infinite dilution determines the value of the total fraction of condensed counterions on the single chain and on the dimer components of the system, r_C and r_D respectively.¹⁹ For the case of a mixture of z_i - and z_j -valent counterions, they read (eqs 18)

$$r_C = \frac{1}{[zix_C^i + zj(1 - x_C^i)]} \left[1 - \frac{1}{\xi_{\text{str},C}[zix_C^i + zj(1 - x_C^i)]} \right] \quad (18)$$

$$r_D = \frac{1}{zix_D^i + zj(1 - x_D^i)} \times \left[1 - \frac{1}{2\xi_{\text{str},C}(1 - zj\sigma_0)[zix_D^i + zj(1 - x_D^i)]} \right]$$

The overall result is a system of seven equations in seven unknowns (r_C , x_C^i , r_D , x_D^i , V_C , V_D , θ) to be solved at each concentration of the cross-linking z_j -valent counterion, that is, at each value of R_j .¹⁹ The average fractions (per equivalent polymeric charged group) of z_j -valent counterions condensed on the chain and on the dimer, C^j and D^j , respectively, and chemically bound, σ , can be straightforwardly calculated at each

value of θ (eqs 19–21)

$$C^j = (1 - \theta)r_C(1 - x_C^i) \quad (19)$$

$$D^j = \theta(1 - z_j\sigma_0)r_D(1 - x_D^i) \quad (20)$$

$$\sigma = \theta\sigma_0 \quad (21)$$

3. Results and Discussion

The application of the model has been carried out on the specific and well-known case of pectate, where the homopolymer composition ($\alpha - 1 \rightarrow 4$ linked galacturonate units) greatly simplifies the description of the model. In this respect, in the following calculation we will assume that the (single) chain is characterized by the geometrical features of this polyuronate ($b_{\text{str,C}} \approx 4.43 \text{ \AA}$; $\xi_{\text{str,C}} = 1.61$) and that the addition of a cross-linking salt (such as $\text{Ca}(\text{ClO}_4)_2$, with Ca^{2+} as the z_j -valent counterion) in the presence of a monovalent supporting 1:1 salt (NaClO_4 , with Na^+ as the z_i -valent counterion) induces dimer formation. The first assumption is not, in itself, a strong limitation; the two known helices (2/1 and 3/1) of polygalacturonate display a scarcely significant difference in their repeating unit advance and then, in their interchange distance.³⁹

In relation to what is reported above and for the sake of simplicity, we have assumed that the intrinsic Gibbs energies of affinity of the z_j -valent counterion for the chain and dimer toward the divalent cation, that is, $g_C^{\text{aff},0}$ and $g_D^{\text{aff},0}$, respectively, are equal. In practice, it corresponds to confining the strong interactions in the inner part of the dimer (the “interior of the egg-box”), assuming that the external parts are not too different from the sides of the isolated chain. The front factor ρ is set to equal 1. This assumption is based on nonmarginal evidence collected in this laboratory;^{40,41} a more detailed discussion is deferred to the forthcoming paper.³² Finally, in the analysis of the effect of the different parameters introduced in the model on condensation and bonding of counterions, we will focus on the divalent species because it is by far the most important one involved in the formation of dimers.

The model presented in the previous section will be analyzed in the presence of a constant concentration of supporting salt, that is, constant R_i , and only the effect of the concentration of divalent cations, that is, varying R_j , will be computed. It is important to stress that in the present paper particular attention is focused on the initial bonding of the divalent cations, thus, of the formation of dimers, and, therefore, a R_j range from about 0.015 to about 0.25 has been selected.

It is beyond the scope of the present paper to explore the effect of the variation of the charge density of the (single) chain $\xi_{\text{str,C}}$ (by changing pH or by varying the degree of carboxylic group esterification) or of the concentration of the supporting 1:1 electrolyte.

(A) Effect of $g^{\text{bond},0}$. As reported in the previous section, the minimization process performed on the thermodynamic function of the (reduced) total Gibbs energy, g^T , of the model leads to a system of seven equations with seven unknowns that, once solved for each value of the molar ratio between the cross-linking divalent cation added and the polymer-charged groups (R_j), provides the fraction of divalent counterions condensed on each component of the system, that is, C^j and D^j for the (single) chain and the dimer, respectively, and the fraction of formed dimer, θ . Moreover, also the amount of chemically bound counterions, σ , is straightforwardly computed.

Considering that $g^{\text{bond},0}$ represents the (reduced) intrinsic Gibbs energy associated with the formation of the “egg-box”

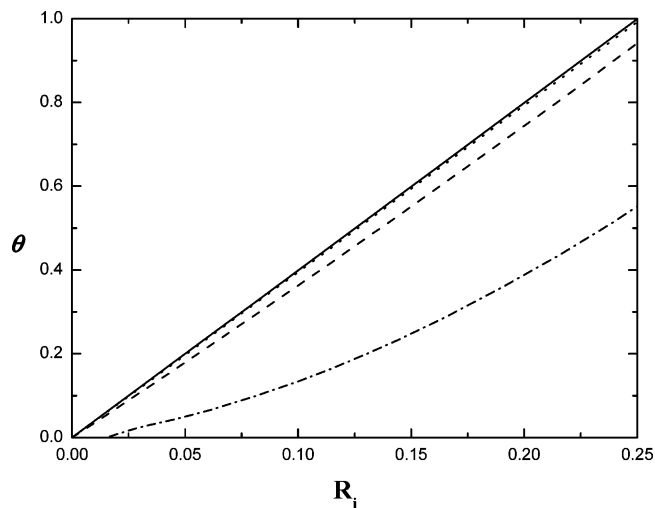


Figure 1. Dependence of the fraction of dimers formed θ from R_j in the cases $g^{\text{bond},0} = 0$ (—), $g^{\text{bond},0} = -2$ (---), $g^{\text{bond},0} = -4$ (···), and $g^{\text{bond},0} = -6$ (- · -). In all cases, $g_C^{\text{aff},0} = g_D^{\text{aff},0} = -4$. Here and in all following calculations, $C_p = 3.5 \times 10^{-3}$ mono mol/L and univalent salt [1:1 electrolyte] = 0.05 M.

structure, it is easily recognized that its absolute value will affect the fraction of dimer formed, θ . Therefore, the latter was computed for R_j spanning from 0.015 to 0.25, holding $g^{\text{bond},0}$ constant and equal to 0, -2, -4, and -6, respectively. In all these cases, the (reduced) intrinsic Gibbs energies of affinity of the chain and the dimer toward the divalent cation were assumed to be equal, namely, $g_C^{\text{aff},0} = g_D^{\text{aff},0} = -4$ (which would correspond to a molar free energy of affinity per mole of condensed ion $\approx -2.2 \text{ kcal mol}^{-1}$). The results, reported in Figure 1, show the marked tendency of the model to give rise to a higher amount of dimers upon raising the (reduced) free energy of binding, $g^{\text{bond},0}$, toward more negative values. In fact, by setting the latter as constant and equal to 0, the fraction θ of dimer reaches the value of ≈ 0.6 with $R_j \rightarrow 0.25$. In contrast, a decrease of the intrinsic Gibbs energy of bonding to -2 produces a more rapid increase of θ with R_j : it means that a higher amount of dimer is formed for the same amount of calcium added to the polyelectrolyte. An additional decrease of $g^{\text{bond},0}$ (to -4 and -6, respectively) has basically no noticeable effect. The conclusion that can be drawn from these data is that the fraction θ of dimer formed at each value of R_j is a consequence of the competition between two processes involving calcium ions: condensation and bonding. Although both processes remove divalent cations from the solution, the difference in Gibbs energy between them induces a prevailing effect of one process over the other.

This conclusion is supported by the analysis of Figure 2, which reports the dependence on R_j of the total amount of nonfree calcium ions, that is, the sum of the chemically bound (σ) and territorially condensed (C^j and D^j) ones. It should be noted that, in contrast with the results obtained for θ , the variation of $g^{\text{bond},0}$ has a negligible effect on the amount on nonfree calcium ions. Therefore, one should conclude that the present model removes from the solution, regardless of $g^{\text{bond},0}$, nearly the same amount of calcium ions. They are then redistributed between territorial condensation and chemical binding, according to the intrinsic Gibbs energies characterizing the two processes, that is, $g_{C,D}^{\text{aff},0}$ and $g^{\text{bond},0}$.

To clarify this point, it might be helpful to analyze Figure 3a–d, where the fractions of bound (σ) and condensed (C^j and D^j) divalent counterions are reported separately. By using the constant value of 0 for $g^{\text{bond},0}$ (Figure 3a), the fraction of

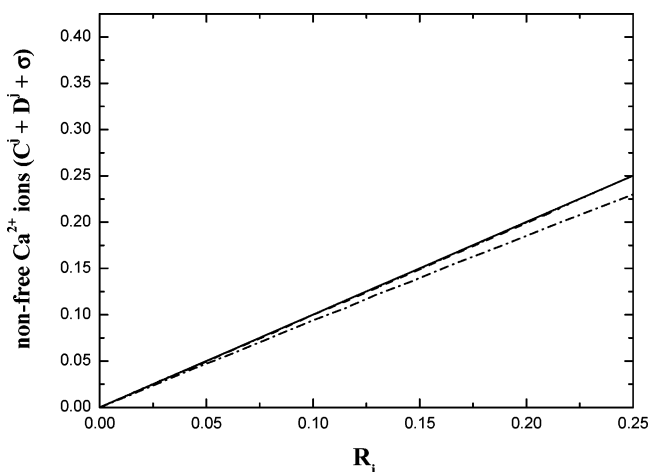


Figure 2. Dependence of the total fraction in solution of nonfree calcium ions from R_j . $g_C^{\text{aff},0} = g_D^{\text{aff},0} = -4$; symbols and conditions as in Figure 1.

condensed counterions is higher than that of the bound ones for low values of R_j . Only with additional calcium, that is, increasing R_j , the bound counterions prevail over the condensed ones. A completely different profile is displayed in Figure 3b, where a constant value of -2 was set for $g^{\text{bond},0}$. In this case, nearly all the available divalent ions are chemically bound into dimer structures: site-specific binding is, therefore, favored in the competition with the territorial condensation, whose contribution is negligible. A more uneven distribution of divalent counterions is found when $g^{\text{bond},0}$ is set to -4 and -6 , respectively, as reported in Figure 3c,d.

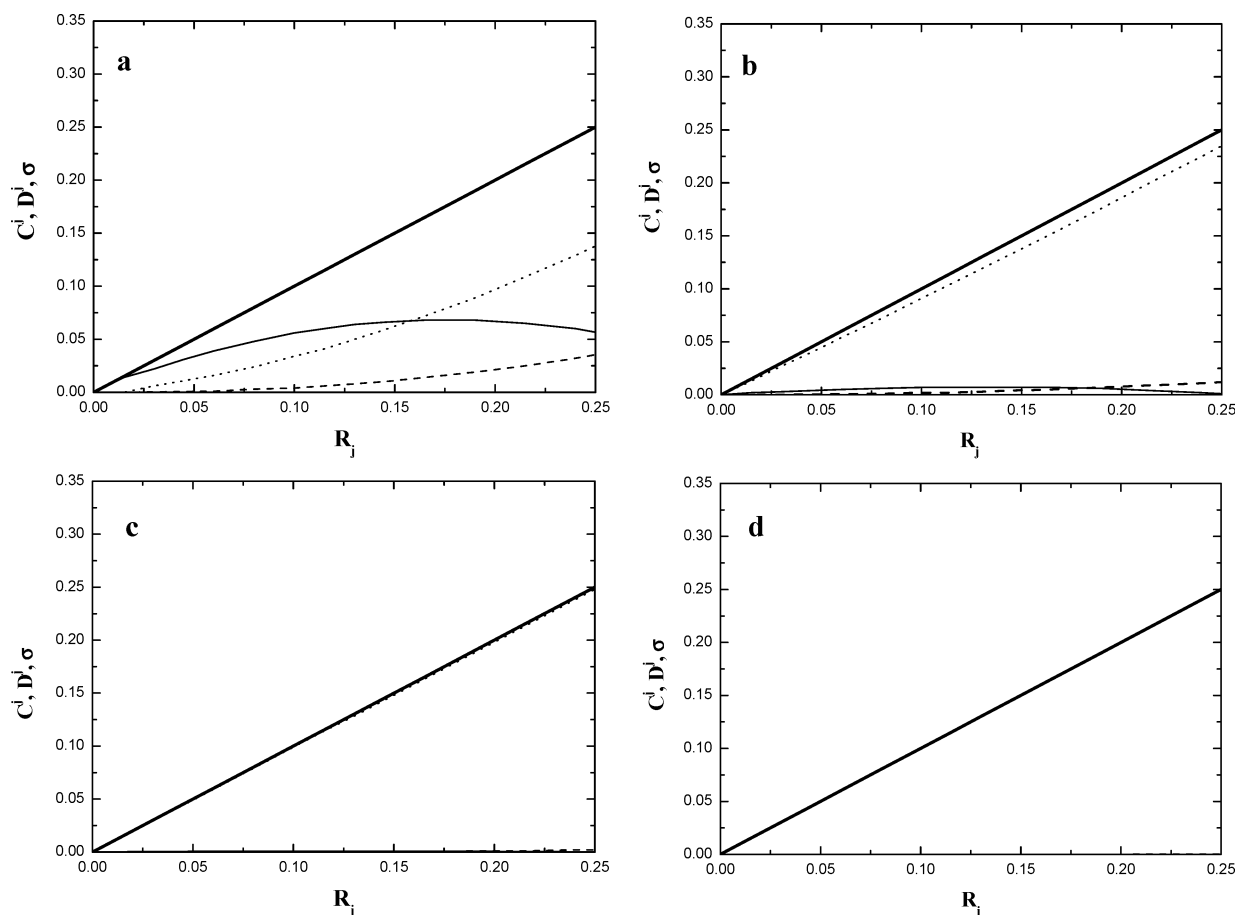


Figure 3. Fraction of territorially condensed counterions on the single chain (C^I , —) and dimer (D^I , —) and fraction of chemically bound counterions (σ , ...) in the cases (a) $g^{\text{bond},0} = 0$, (b) $g^{\text{bond},0} = -2$, (c) $g^{\text{bond},0} = -4$, and (d) $g^{\text{bond},0} = -6$. $g_C^{\text{aff},0} = g_D^{\text{aff},0} = -4$; conditions as in Figure 1. The bold line represents the $R_j = R_j$ correlation.

A further remark in Figures 1 and 3a should be added. In fact, the bonding of calcium ions in “egg-box” structures while keeping $g^{\text{bond},0}$ constant and equal to 0 might appear unrealistic, particularly in view of the much more favorable Gibbs energy of affinity ($g_{C,D}^{\text{aff},0} = -4$). However, it should be reminded that, in the present model, chemical bonding is accompanied by charge annihilation which, by reducing the structural charge on the ionized groups of the polyelectrolyte, leads to a net gain in the Gibbs energy of the polyelectrolyte solution. Thus, despite that $g^{\text{bond},0} = 0$, the bonding of calcium in dimers is energetically driven due to the reduction of the “polyelectrolytic nature” of the overall system.

Once the fractions of condensed ions of different valences, the condensation volumes (V_C and V_D for the single chain and the dimer, respectively), and the fraction θ of egg-box structures formed are calculated, the total Gibbs energy of the system, \bar{G}^T , can be computed (Figure 4) using eq 14. In all the cases analyzed, an almost linear dependence of \bar{G}^T from R_j was obtained, but marked differences on the gradient were detected. In particular, by decreasing the value of $g^{\text{bond},0}$ from 0 to -6 , a constant increase in the $\bar{G}^T - R_j$ gradient, stemming from the higher (negative) contribution of the chemically bound divalent cations, is shown.

In the above-reported results, the calculations were performed by assuming a constant value of $g^{\text{bond},0}$ over the R_j range considered, implying that the introduction of any divalent cation in egg-box structures is not affected by the presence of already-formed dimers. However, this represents an oversimplification of the ion-induced chain pairing process, where the bonding of an additional calcium ion might be favored or not by previously

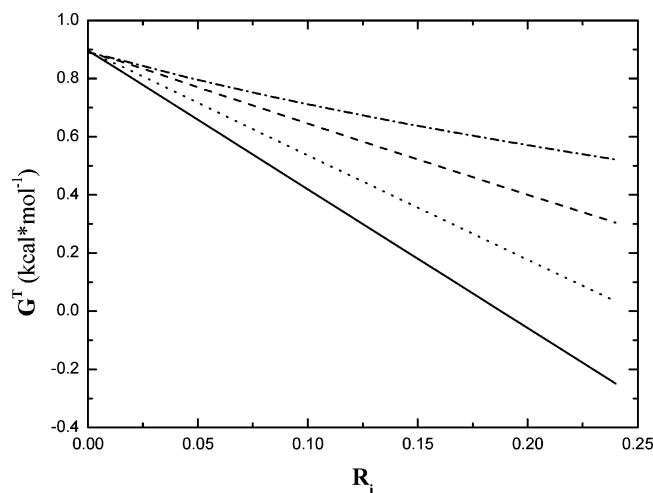


Figure 4. Total Gibbs energy of the system, G^T , for the cases $g^{\text{bond},0} = 0$ (— · —), $g^{\text{bond},0} = -2$ (---), $g^{\text{bond},0} = -4$ (— · — · —), and $g^{\text{bond},0} = -6$ (—). $g^{\text{aff},0}_C = g^{\text{aff},0}_D = -4$; conditions as in Figure 1.

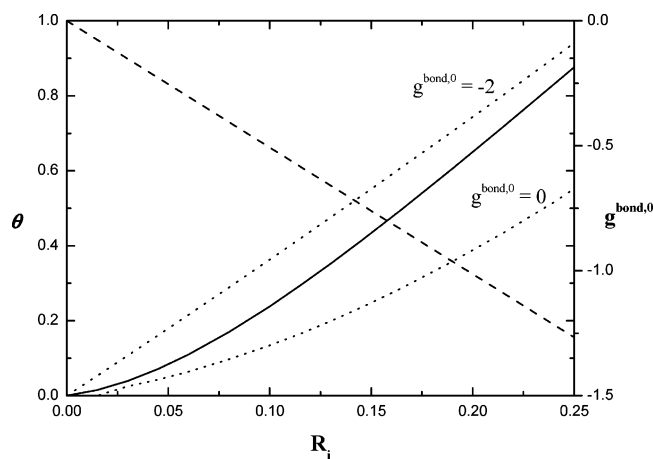


Figure 5. Dependence of the fraction of dimers formed, θ , from R_j (—, scale reported on left-hand side), with a varying value of $g^{\text{bond},0}$ (---, scale reported on the right-hand side) in the case of positive cooperativity. The behavior of θ for a constant value of $g^{\text{bond},0}$ equal to 0 and -2 , respectively, is reported (···).

formed dimers. The former case can be described as cooperativity, while the latter represents an anti-cooperativity.

To simulate a cooperativity in calcium bonding by the model developed in the theory section, $g^{\text{bond},0}$ was varied linearly with R_j . In particular, a value of the intrinsic Gibbs energy of binding increasing from 0 ($R_j \rightarrow 0$) to almost -1.3 ($R_j \rightarrow 0.25$) was used, and the fraction θ of dimers formed was computed for each value of R_j . The results obtained from the calculations (Figure 5) show the interesting behavior of θ , which, for low values of calcium added, parallels the trend exhibited in the case of $g^{\text{bond},0} = 0$ and, for higher R_j , evolves toward the case of $g^{\text{bond},0} = -2$. This is in line with what is expected for cooperativity, where the bonding of additional calcium in egg-box structures is energetically favored by the presence of previously formed dimers, thus, their complete filling is enhanced.

The case of anti-cooperativity in calcium bonding was modeled by considering a linear variation of $g^{\text{bond},0}$ from a value of -4 ($R_j \rightarrow 0$) to approximately -0.5 ($R_j \rightarrow 0.25$; Figure 6). It is interesting to note that the fraction of dimers formed, θ , shows a Langmuir-type curve with a progressive leveling-off for the higher values of R_j , if compared to the behavior calculated with a constant $g^{\text{bond},0}$ of -4 . In this latter case, the

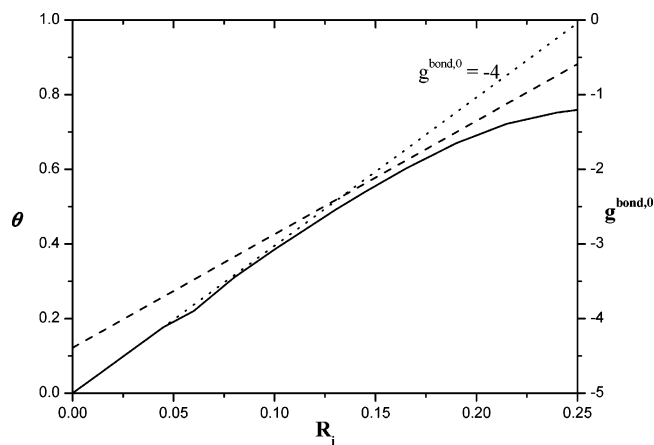


Figure 6. Dependence of the fraction of dimers formed, θ , from R_j (—, scale reported on left-hand side), with a varying value of $g^{\text{bond},0}$ (---, scale reported on the right-hand side) in the case of negative cooperativity. The behavior of θ for a constant value of $g^{\text{bond},0}$ equal to -4 is reported (···).

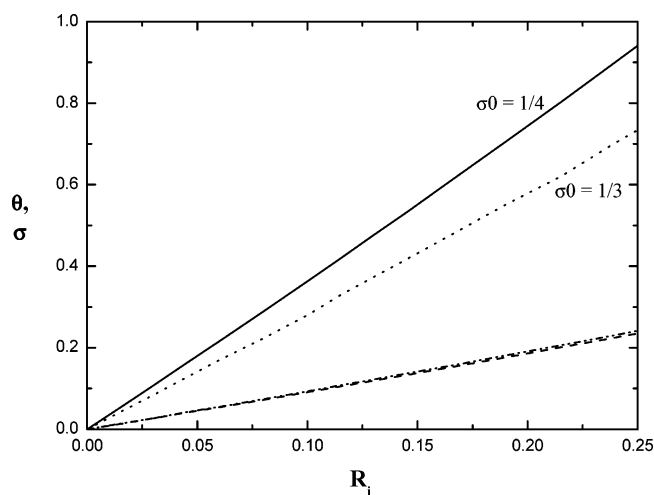


Figure 7. Fraction of dimers formed, θ , in the case of $\sigma_0 = 1/4$ (—) and $\sigma_0 = 1/3$ (···), and the fraction of chemically bound counterions σ in the case of $\sigma_0 = 1/4$ (— · —) and $\sigma_0 = 1/3$ (---). $g^{\text{bond},0} = -2$. $g^{\text{aff},0}_C = g^{\text{aff},0}_D = -4$; conditions as in Figure 1.

additional binding of calcium ions in egg-box structures is energetically nonfavored by the lowering of the (reduced) intrinsic Gibbs energy of binding, and the complete filling of all the possible sites is hampered.

(B) Effect of σ_0 . One of the parameters introduced in the theory section is the molar ratio between the bound divalent counterions and the polymer-charged units in the specific binding sites, that is, σ_0 . To propose a wide application and achieve a more detailed understanding of the model devised in the present paper, the effect of the variation of σ_0 on the amount of bound (σ) and condensed (C^+ and D^+) calcium ions, as well as on the total Gibbs energy, \bar{G}^T , of the system was investigated. The variation of σ_0 is intended to cover variability in the structural parameter (helical repeat) and in the geometry of the resulting pairing chains.

The system of seven equations with seven unknowns, resulting from the minimization procedure, has been solved in the R_j range spanning from 0.015 to 0.25, with σ_0 equal to $1/4$ and $1/3$, respectively. Figure 7 reports the fraction θ of dimers formed at each value of R_j for the two cases mentioned above. It can be noted that $\theta_{1/3}$, that is, θ calculated in the case of $\sigma_0 = 1/3$, is always lower than $\theta_{1/4}$, leading to the conclusion that the higher the molar ratio between bound counterions and

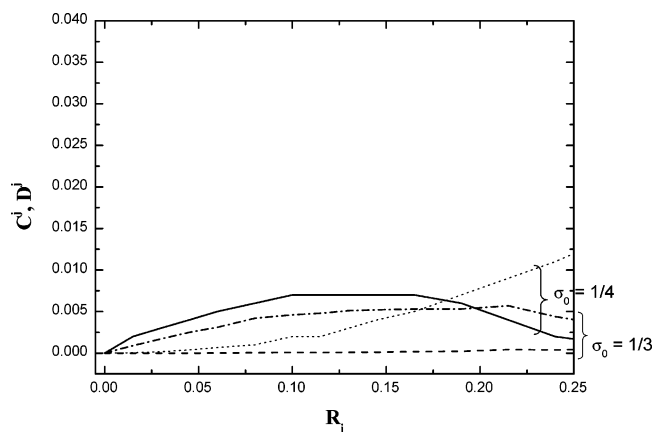


Figure 8. Fraction of territorially condensed counterions on the single chain (C) and on the dimer (D) at equilibrium in the case of $\sigma_0 = 1/4$ (—, ...) and $\sigma_0 = 1/3$ (---, - · -), respectively. $g_{\text{bond},0}^{\text{bond},0} = -2$. $g_{\text{C},0}^{\text{aff},0} = g_{\text{D},0}^{\text{aff},0} = -4$; conditions as in Figure 1.

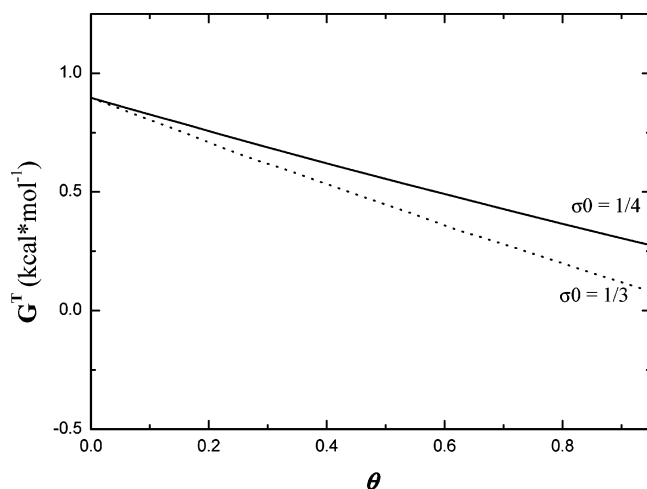


Figure 9. Calculated total Gibbs energy of the system, \bar{G}^T , in the case of $\sigma_0 = 1/4$ (—) and $\sigma_0 = 1/3$ (···). $g_{\text{bond},0}^{\text{bond},0} = -2$. $g_{\text{C},0}^{\text{aff},0} = g_{\text{D},0}^{\text{aff},0} = -4$; conditions as in Figure 1.

polymer-charged groups, the lower the fraction of egg-box structures formed. However, in the same figure, it is shown that the fraction of bound counterions σ ($\sigma = \sigma_0 \theta$) is approximately the same in both cases. Thus, by using a value of σ_0 equal to $1/3$, the equilibrium condition of the system is reached, for each R_j value, by forming less dimer structures, in which the divalent counterions are more densely packed than those obtained in the case of $\sigma_0 = 1/4$.

The increase of σ_0 has also the non-negligible effect of reducing the effective charge density of the dimer component of the system at the equilibrium (eq 8); in the case of polygalacturonate, $\xi_{\text{eff},D}$ drops from 1.61 ($\sigma_0 = 1/4$) to 1.06 ($\sigma_0 = 1/3$). Two main consequences can be directly correlated to this charge-density reduction. The first concerns the effect of the variation of σ_0 on the fraction of divalent counterions condensed on the polyelectrolyte. In fact, as reported in Figure 8, a net decrease of D is experienced upon increasing σ_0 from $1/4$ to $1/3$ as a result of the relatively low charge density of the dimer in the latter case. The second consequence of the reduction of $\xi_{\text{eff},D}$ is detected upon calculating the total Gibbs energy, \bar{G}^T , of the system (Figure 9). In particular, when σ_0 is equal to $1/3$, the total Gibbs energy calcium-added (R_j) gradient was found to be higher than the one calculated in the case of $\sigma_0 = 1/4$. This effect can be directly traced back to the charge annihilation

between the divalent counterions and the negatively charged uronic groups in the specific binding sites that enhance the stability of the system by reducing its polyelectrolytic nature.

4. Conclusions

In the present paper, the CC theory was exploited to develop a thermodynamic model accounting for ion-induced chain pairing of a negatively charged polyelectrolyte, with particular attention given to the case of calcium-induced chain dimerization in polygalacturonate. The following achievements seem noteworthy.

(1) The consistency of the model has been tested upon varying one of the key parameters introduced into the mathematical description of the ion-induced chain pairing process, that is, the (reduced) Gibbs energy of bonding of z_j -valent counterions in “egg-box” structures, $g_{\text{bond},0}^{\text{bond},0}$.

(2) The analysis revealed the correlation existing between $g_{\text{bond},0}^{\text{bond},0}$ and $g_{\text{aff},0}^{\text{aff},0}$, that is, the (reduced) Gibbs energy of affinity; the difference between these two values determines the uneven distribution of z_j -valent ions between territorial condensation and chemical bonding. The possibility of simulating the ion-induced chain pairing by assuming a constant or varying value of $g_{\text{bond},0}^{\text{bond},0}$ demonstrates the wide applicability of the model developed in the present paper. In particular, it is noteworthy that the model can reasonably simulate cooperativity or anti-cooperativity in the ion-induced chain pairing process and predict the fraction of dimers formed, θ .

(3) The variation of the molar ratio between the cross-linking ions and the uronic moieties in the specific binding site, that is, σ_0 , led to non-negligible effects both on counterion condensation and on the fraction of dimers formed at each concentration of divalent counterion, as well as on the total Gibbs energy of the system, due to the net reduction of the effective charge density on the dimers. This result might reveal an appealing application of the model in the discrimination of junctions characterized by different σ_0 values.

In conclusion, the present paper provides a general description of the ion-induced chain pairing, proposing a model based on the CC theory that includes the possibility of a chemical bonding of counterions. The application of this model to a sample case, that is, the calcium-induced dimer formation in polygalacturonate, will be addressed in the forthcoming paper.³²

Appendix A

(A1) The ionic part of eq 16, that is, $g_{\text{C,D}}^{\text{ion}}$, is factorized¹⁹ into the sum of the purely electrostatic, $g_{\text{C,D}}^{\text{el}}$, and the entropic (energy of ion mixing), $g_{\text{C,D}}^{\text{mix}}$, contributions to the total reduced Gibbs energy (per equivalent polymeric-charged group; eq 22)

$$g_{\text{C,D}}^{\text{ion}} = g_{\text{C,D}}^{\text{el}} + g_{\text{C,D}}^{\text{mix}} \quad (22)$$

where (eqs 23)

$$\begin{aligned} g_{\text{C}}^{\text{el}} &= -\xi_{\text{str,C}} [1 - r_{\text{C}}(z_j - x_{\text{C}}^j(z_j - z_i))]^2 \ln(1 - e^{-k b_{\text{str,C}}}) \\ g_{\text{D}}^{\text{el}} &= -2\xi_{\text{str,C}} (1 - z_j \sigma_0)^2 [1 - r_{\text{D}}(z_j - x_{\text{D}}^j(z_j - z_i))]^2 \times \\ &\quad \ln(1 - e^{-k(b_{\text{str,C}}/[2(1 - z_j \sigma_0)])}) \end{aligned} \quad (23)$$

where k^{-1} is the Debye length.

The contribution of the entropy of mixing, that is, $g_{\text{C,D}}^{\text{mix}}$, of the different mobile ionic species (counterions of valence z_i CDV

and z_j that are either free to move in the bulk solution or condense onto the polyelectrolyte chain, similions, and solvent molecules) is given by eq 24

$$g_{C,D}^{\text{mix}} = g_{C,D}^{\text{cond},i} + g_{C,D}^{\text{cond},j} + g_{C,D}^{\text{free},i} + g_{C,D}^{\text{free},j} + g_{C,D}^{\text{simil}} + g_{C,D}^{\text{solv}} \quad (24)$$

The first two terms correspond to the entropic contribution (per equivalent polymeric-charged group) of the condensed counterions of both valences z_i and z_j (eqs 25) on single chain and dimer

$$\begin{aligned} g_C^{\text{cond},i} &= r_C x_C^i \ln \left(\frac{r_C x_C^i}{(R_i + 1) V_C C_p} \right) \\ g_C^{\text{cond},j} &= r_C (1 - x_C^i) \ln \left(\frac{r_C (1 - x_C^i)}{R_j V_C C_p} \right) \\ g_D^{\text{cond},i} &= (1 - z_j \sigma_0) r_D x_D^i \ln \left(\frac{(1 - z_j \sigma_0) r_D x_D^i}{(R_i + 1) V_D C_p} \right) \\ g_D^{\text{cond},j} &= (1 - z_j \sigma_0) r_D (1 - x_D^i) \ln \left(\frac{(1 - z_j \sigma_0) r_D (1 - x_D^i)}{R_j V_D C_p} \right) \end{aligned} \quad (25)$$

where R_i and R_j are the molar ratios between the supporting and cross-linking salts and the polymer repeating units, respectively. C_p is the (molar) concentration of polyelectrolyte. V_C and V_D are the condensation (molar) volumes of the single chain and dimer, respectively.

On calculating the contribution to the entropy of mixing (per equivalent polymeric-charged group) of the free z_i - and z_j -valent counterions for the single chain and the dimer components of the system, that is, $g_{C,D}^{\text{free},i}$ and $g_{C,D}^{\text{free},j}$, one should take into account all the counterions already territorially condensed or chemically bound at each stage of the equilibrium transformation from the (single) chain to the dimer (eqs 26)

$$\begin{aligned} g_C^{\text{free},i} &= [(R_i + 1) - (1 - \theta) r_C x_C^i - \theta r_D x_D^i] \times \\ &\quad \ln \left(\frac{[(R_i + 1) - (1 - \theta) r_C x_C^i - \theta r_D x_D^i]}{(R_i + 1)(1 - V_C C_p)} \right) \\ g_C^{\text{free},j} &= [R_j - (1 - \theta) r_C (1 - x_C^i) - \theta r_D (1 - x_D^i) - \theta \sigma_0] \times \\ &\quad \ln \left(\frac{[R_j - (1 - \theta) r_C (1 - x_C^i) - \theta r_D (1 - x_D^i) - \theta \sigma_0]}{R_j (1 - V_C C_p)} \right) \\ g_D^{\text{free},i} &= [(R_i + 1) - (1 - \theta) r_D x_D^i - \theta r_D x_D^i] \times \\ &\quad \ln \left(\frac{[(R_i + 1) - (1 - \theta) r_D x_D^i - \theta r_D x_D^i]}{(R_i + 1)(1 - V_D C_p)} \right) \\ g_D^{\text{free},j} &= [R_j - (1 - \theta) r_D (1 - x_D^i) - \theta r_D (1 - x_D^i) - \theta \sigma_0] \times \\ &\quad \ln \left(\frac{[R_j - (1 - \theta) r_D (1 - x_D^i) - \theta r_D (1 - x_D^i) - \theta \sigma_0]}{R_j (1 - V_D C_p)} \right) \end{aligned} \quad (26)$$

The contribution of the similions and the solvent molecules to $g_{C,D}^{\text{mix}}$ for the single chain and dimer can be written as follows

(eqs 27)

$$\begin{aligned} g_C^{\text{simil}} &= (R_i + z_j R_j) \ln \left[\frac{1}{(1 - V_C C_p)} \right] & g_C^{\text{solv}} &= r_C \\ g_D^{\text{simil}} &= (R_i + z_j R_j) \ln \left[\frac{1}{(1 - V_D C_p)} \right] & g_D^{\text{solv}} &= (1 - z_j \sigma_0) r_D + \sigma_0 \end{aligned} \quad (27)$$

(A2) Two additional (reduced) free energy terms related to the intrinsic thermodynamic parameters $g_C^{\text{aff},0}$ and $g_D^{\text{aff},0}$ (i.e., the (reduced) intrinsic Gibbs energy of affinity for the single chain and the dimer expressed per mole of condensed counterion) have to be added, accounting for the affinity toward the z_j -valent counterion (eqs 28)

$$\begin{aligned} g_C^{\text{aff}} &= r_C (1 - x_C^i) g_C^{\text{aff},0} \\ g_D^{\text{aff}} &= (1 - z_j \sigma_0) r_D (1 - x_D^i) g_D^{\text{aff},0} \end{aligned} \quad (28)$$

(A3) Finally, the chemical bonding of z_j -valent counterions in the dimer structures is accompanied by a (reduced) Gibbs energy variation expressed as g_D^{bond} (eq 29)

$$g_D^{\text{bond}} = \sigma_0 g_D^{\text{bond},0} \quad (29)$$

where $g_D^{\text{bond},0}$ is the (reduced) intrinsic Gibbs energy variation (per mol of “chemically bound” counterion) associated with the formation of the egg-box complex between the uronic groups and the ion.

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