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# Polyaromatic-Anion Behavior of Different Polyelectrolytes Containing Benzenecarboxylate Units

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*Received: February 24, 2010; Revised Manuscript Received: April 23, 2010*

The polyaromatic-ion behavior of poly(sodium *N*-maleoyl-2-aminobenzoate-*co*-sodium acrylate) in a comonomer composition 1:2, poly(sodium *N*-maleoyl-4-aminobenzoate-*co*-sodium acrylate) in a comonomer composition 1:2, and poly(sodium *N*-maleoyl-4-aminobenzoate-*co*-*N,N*-dimethylacrylamide) in a comonomer composition 1:1 is studied. The copolymers undergo short-range aromatic–aromatic interactions with methylene blue and rhodamine B, as a consequence of which their UV–vis monomer band is shifted to lower energies. As a result of their polyaromatic-anion behavior, methylene blue is easily dispersed on the polymeric domains. Moreover, the  $pK_a$  of rhodamine B is shifted from 3.2 to 4.5–5 in the presence of these copolymers. This behavior is also observed in the presence poly(sodium 4-styrenesulfonate), but not in the presence of poly(sodium vinyl sulfonate), which are taken as reference polymers. Compared with poly(sodium 4-styrenesulfonate), a lower resistance to the cleaving effect of added NaCl on the interaction with methylene blue is found. A different influence on the reduction of 2,3,5-triphenyl-2*H*-tetrazolium chloride with ascorbic acid was found for the different polyelectrolytes.

## 1. Introduction

Polyelectrolytes are suitable building blocks that can lead to nanoscale structures by means of their association with complementary charged polyelectrolytes,<sup>1–3</sup> multivalent counterions,<sup>4,5</sup> surfactants,<sup>6–8</sup> or low-molecular-weight molecules susceptible to undergo self-association such as liquid crystals<sup>9</sup> and dyes.<sup>10</sup> The standard theory regarding the interactions between polyelectrolytes and their counterions is based on long-range electrostatic interactions and described by the counterion condensation theory of G. S. Manning.<sup>11–14</sup> According to Manning's theory, a higher concentration of hydrated counterions is found around the polymer chains; these counterions are able to move on the polymer surface, so that the interaction is considered non-site-specific. Long-range electrostatic interactions may be considered primary interactions between polyelectrolytes and their counterions.

However, when additional secondary short-range interactions such as hydrogen bonding, coordination binding, or aromatic–aromatic interactions are held, the general picture for the polyelectrolyte–counterion interaction may change dramatically, and efforts to afford a suitable theory for the polyelectrolyte/counterion interaction are found in the literature.<sup>14–17</sup> An interesting work of Hao and Harvey arises as an extension of Manning's original condensation theory and introduces the free energy of a counterion bound on a single binding site.<sup>14</sup> It is by means of these secondary interactions that supramolecular assemblies based on charged molecules can be achieved with controlled structure and geometry. Nanoscale architectures with

different functionalities may thus be designed under the so-called ionic self-assembly (ISA).<sup>18–35</sup> In this context, ISA is usually accompanied by a cooperative binding mechanism,<sup>33,36</sup> so that the molecules bind preferably adjacent to each other: the first bonds stimulate further binding which propagates toward the final self-assembled structures. The cooperativity of the ionic binding process is of major importance in ISA and the reason for the simplicity of synthesis, structural perfection, and stability of related nanoassemblies. In particular, charged dye molecules may produce aggregates by self-stacking due to aromatic–aromatic interactions with defined shape and regular mutual overlap interactions.<sup>37</sup> Their interesting luminescent and electronic properties have implications on the important applications of materials containing this kind of molecule. The cooperative binding of charged dyes on polyelectrolytes is already a classic theme and has been used in polyelectrolyte analytics.<sup>10,31–35,38–45</sup> This is the case of porphyrins<sup>10,38–42</sup> and xanthene dyes such as rhodamine B (RB)<sup>43</sup> and methylene blue (MB).<sup>44,45</sup>

Among secondary interactions, short-range aromatic–aromatic interactions<sup>46–52</sup> arise as an important tool to control the structure and properties of supramolecular assemblies. They are one of the principal noncovalent forces governing molecular recognition and biomolecular structure. They are important in the stabilization of DNA and its association with intercalators.<sup>52–55</sup> They also play an important role in protein stabilization<sup>56–59</sup> and protein functionality, as in enzymes,<sup>60,61</sup> trans-membrane channels,<sup>62,63</sup> etc. The major contribution to aromatic–aromatic interactions arises from van der Waals interactions, including solvophobic effects, while short-range electrostatic interactions<sup>47</sup> and dispersion forces<sup>48–50</sup> determine the geometry of the interaction. On the basis of aromatic–aromatic interactions, we have recently described that polyelectrolytes containing aromatic rings may undergo short-range aromatic–aromatic interactions with aromatic counterions.<sup>43,44,64–71</sup> These interactions are

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particularly strong when the polyelectrolyte charge is supported on the polymeric aromatic ring and may constitute the major forces driving the interaction with the counterions. Since these polyelectrolytes present a different behavior regarding the binding of aromatic counterions, we call them “polyaromatic ions” (anions, cations, or zwitterions). We point out as relevant the dispersant ability of polymers such as poly(sodium 4-styrenesulfonate) (PSS) for counterions such as xanthenes dyes<sup>43,44</sup> or poly(4-vinylpyridine) (P4VP) for counterions such as sulfonated porphyrins<sup>71,72</sup> as well as the resistance of the interaction to the cleaving effect of added NaCl. These two characteristics define the typical polyaromatic-ion behavior by opposition to a typical polyelectrolyte behavior characterized by the induction of self-aggregation of dyes and a high sensitivity to the cleaving effect of the interaction produced by the addition of NaCl, since long-range electrostatic interactions are predominant in the dye–polyelectrolyte mutual interaction. These findings open new possibilities for the formation of nanostructures under the scope of ISA. By means of these short-range interactions, site-specific binding between the counterion and the polymeric aromatic functional groups is held, and hydrophobic ion pairs are formed; these ion pairs tend to aggregate depending on the polyelectrolyte/counterion ratio, a fact that may be crucial for the behavior, structure, and properties of the systems. Under appropriate conditions the self-stacking tendency of aromatic counterions such as charged dyes may be overcome by the polymers, thus inhibiting the cooperative binding tendency of the counterions and showing a high dispersant ability. The dispersant ability of polyaromatic ions may be of potential use in controlling counterion properties such as redox, luminescent, and acid–base properties and may serve to monitor the importance of secondary aromatic–aromatic interactions between the polyelectrolyte and the counterions.

Not all the polyelectrolytes bearing aromatic groups behave as polyaromatic ions, and some polymers present an intermediate behavior between typical polyaromatic ions such as PSS or P4VP and analogous typical polyelectrolytes such as poly(sodium vinyl sulfonate) (PVS) or poly(allylamine) (PALA).<sup>68–71</sup> The aim of this paper is to analyze the polyaromatic-anion character of some polyelectrolytes bearing aromatic groups such as poly(sodium *N*-maleoyl-2-aminobenzoate-*co*-sodium acrylate) in a comonomer composition 1:2 (P(NM2AB<sub>1</sub>-*co*-AA<sub>2</sub>)), poly(sodium *N*-maleoyl-4-aminobenzoate-*co*-sodium acrylate) in a comonomer composition 1:2 (P(NM4AB<sub>1</sub>-*co*-AA<sub>2</sub>)), and poly(sodium *N*-maleoyl-2-aminobenzoate-*co*-*N,N*-dimethylacrylamide) in a comonomer composition 1:1 (P(NM4AB<sub>1</sub>-*co*-NNDMAAm<sub>1</sub>)) and compare their behavior with commercially available PSS and PVS. By means of very simple experiments, we will discuss the ability of these polymers to disperse MB upon interaction in solution, to change the *pK<sub>a</sub>* of RB, and to protect 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) from reduction with ascorbic acid (ASC).

## 2. Experimental Section

**2.1. Reagents.** PSS (Aldrich), PVS (Aldrich), MB (Synth), RB (Sigma), TTC (TCI), and ASC (Merck) were commercially available. The synthesis of P(NM2AB<sub>1</sub>-*co*-AA<sub>2</sub>), P(NM4AB<sub>1</sub>-*co*-AA<sub>2</sub>), and P(NM4AB<sub>1</sub>-*co*-NNDMAAm<sub>1</sub>) will be described elsewhere. Briefly, maleimide monomers were obtained by reaction of maleic anhydride in THF with 2- or 4-aminobenzoic acid for 1 h at room temperature to yield a maleamic acid followed by cyclation of the maleamic acid by reaction with acetic anhydride for 1 h at 80 °C, obtaining the final *N*-maleoyl derivative. The copolymer synthesis was carried out using

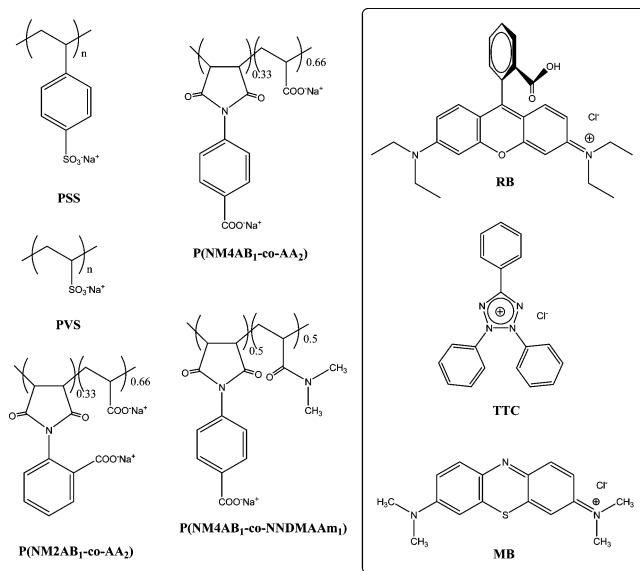


Figure 1. Molecular structures.

benzoyl peroxide as initiator at a relative concentration of 0.5 mol % for 22 h at 85 °C. The monomer feed concentration was maleimide:acrylic = 1:2 and maleimide:acrylamide = 1:1. The copolymers were purified by diafiltration over a diafiltration membrane of 10 000 g mol<sup>−1</sup> and then freeze-dried. Solutions of these reactants were made in deionized distilled water. The structures of the different polyelectrolytes and aromatic counterions are shown in Figure 1. The pH was adjusted with minimum amounts of NaOH and HCl. NaCl (Scharlau) was used to adjust the ionic strength.

**2.2. Equipment.** Distilled water was deionized in a Simplicity Millipore deionizer. The pH was controlled on a UltraBasic Denver Instrument pH meter. UV–vis measurements were performed in a Helios  $\gamma$  spectrophotometer.

**2.3. Procedures.** Conventional and well-known procedures have been followed. Particular experimental conditions are provided in the figure captions. Quartz vessels of 1 cm of optical path length have been used for UV–vis analyses. The polymer concentration is given in moles of sulfonate or benzenecarboxylate units per liter in all experiments. The reduction of TTC in the presence of ASC was followed by UV–vis spectroscopy by successively adding into a quartz vessel (1 cm path length) 1 mL of a  $2 \times 10^{-3}$  M TTC aqueous solution, 1 mL of a  $2 \times 10^{-2}$  M polyelectrolyte solution at pH 7, 0.5 mL of a  $4 \times 10^{-2}$  M ASC solution, and 0.5 mL of a 0.5 M NaOH solution. After shaking for 2 s, the reactions were followed by UV–vis spectroscopy at 524 nm. As the magnitude to be observed is the rate of appearance of the reduction product of TTC (the corresponding red formazan), the experimental absorbances are plotted after subtracting the ordinate at the origin for clarity. This ordinate at the origin appears as a consequence of the delay between the mixing of the reactants and the beginning of the spectrophotometric measurements ( $t = 0$ ). Differences in temperature result in different kinetics for the reaction, so, in order to ensure comparable experimental conditions, all experiments were done at room temperature within an hour. At least two repetitions of all experiments were done under comparable conditions with good reproducibility (differences in the experimental values for the rate of appearance of the corresponding formazan within 2%).

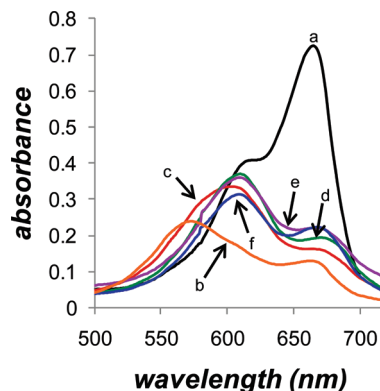
### 3. Results and Discussion

**3.1. Different Polyelectrolytes.** The copolymers under study in this work consisted of maleic acid derivatives copolymerized with vinyl monomers, as can be seen in Figure 1. The concentration of the copolymers is given in moles of benzenecarboxylate units per liter (i.e., in moles of maleic derivatives per liter), and in order to compare between the different polyelectrolytes, the same concentration is kept in a series of experiments, including PSS and PVS, so that the apparent concentration of charged aromatic groups is constant. That makes that the presence of vinylic moieties in the copolymers can be regarded as the inclusion of additional groups on idealized poly(sodium *N*-maleoylamino benzoate) homopolymers. Because of the tendency of maleic acid derivatives to produce alternate copolymers, it can be assumed that the maleimide units are inserted between two vinylic residues. In this sense, a different linear aromatic density, defined as the amount of aromatic groups per length unit of the polymer chain, is found for the different polymers in the order PSS > P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) > P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) = P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>). It has been already shown that the linear aromatic density of polyaromatic anions is important on their behavior.<sup>68–71</sup> On the other hand, P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) presents the lowest linear charge density, since the vinylic residue is not charged, so that it can be considered that this polymer is the most hydrophobic among the copolymers shown in this study. On the other hand, the acrylate units in P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) and P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) should produce hydrophilic extended conformations for these copolymers due to their negative charges. Finally, P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) and P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) differentiate each other on the position of the carboxylate group. It can be then considered that the carboxylate unit in P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) is sterically hindered due to its ortho-position with respect to the maleimide ring, while the one in P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) is less sterically hindered due to its para-position.

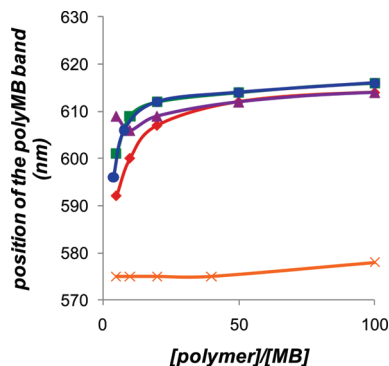
**3.2. Dispersion of MB.** MB self-aggregation in water can be followed by UV–vis.<sup>73–75</sup> A UV–vis band corresponding to monomeric MB appears at 665 nm with a shoulder at around 615 nm. By increasing the extent of dimerization based on sandwich-like H-type contacts, the band at 615 nm increases at the expense of the monomer band, so that it is called the dimer band. When higher-order aggregates based on H-type contacts are formed, such as supramolecular polymerization of MB on negatively charged surfaces, a broad band appears around 575 nm, which we called the polyMB band. The position of the maximum of this band is variable and, according to the exciton theory, considered to be related to the extent and size of the aggregates.

Typical polyelectrolytes induce MB cooperative self-aggregation on their surface, as is clearly seen by the appearance of the polyMB band. It can be seen in Figure 2 the spectra of 10<sup>−5</sup> M MB in the presence of 10 times excess of the polyelectrolytes. At the concentration used in the experiments, MB does not aggregate as reflected by the corresponding spectrum which shows monomeric MB. In the case of PVS, the polyMB band clearly appears at 575 nm, indicating the induction of higher-order aggregates. The position of the polyMB band is an indicative of the dispersant ability of the polymers since smaller aggregates should be found as this property becomes more important. Thus, the polymers containing aromatic moieties also induce aggregation of MB at this concentration, but the aggregates are of lower order, since the polyMB band is shifted toward the dimer band.

Dispersion of MB on the polymeric domain strongly depends on the structure of the polymers. While hydrophilic polymers



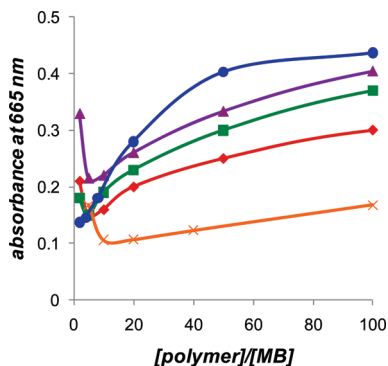
**Figure 2.** UV–vis spectra of  $1 \times 10^{-5}$  M MB solutions at pH 7.0 in the absence of any polyelectrolyte (a) and in the presence of  $1 \times 10^{-4}$  MPVS (b), P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) (c), P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) (d), P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) (e), and PSS (f).



**Figure 3.** Position of the local maximum corresponding to MB aggregates as a function of the polymer concentration relative to MB concentration at pH 7.0 in presence of (x) PVS, (◆) P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>), (■) P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>), (▲) P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>), and (●) PSS.

induce MB aggregation, hydrophobicity and flexibility are also factors that contribute to the stabilization of MB in its monomeric form.<sup>44</sup> However, the presence of charged aromatic moieties has revealed to be of major importance in order to achieve completely dispersed molecules, as is the case of PSS for xanthenes dyes such as RB<sup>43</sup> and MB<sup>44</sup> and P4VP for anionic porphyrins.<sup>71,72</sup> On the other hand, we have recently shown that copolymers of sodium 4-styrenesulfonate with maleic acid progressively lose their ability to disperse MB as the amount of maleic acid increases. This is due to the decrease on the linear aromatic density, while the linear charge density does not change much or increase, the decrease on the hydrophobic character of the copolymers, and a probable more rigid and extended conformation. Dispersion of MB not only is a function of the nature of the polyelectrolyte but also depends of the total MB concentration and on the relative polyelectrolyte to dye ratio. By increasing the MB absolute concentration, the self-stacking of the dye becomes more probable; by increasing the polymer to dye ratio, the dispersion tendency due to the increasing excess of polymer competes with the cooperative character of the MB self-stacking, so that dispersion may be held. It is shown in Figure 3 the position of the polyMB band as a function of the polymer concentration for a fixed MB absolute concentration. It can be clearly seen that PVS does not produce a significant change on the state of aggregation of MB by increasing the polymer concentration, reflecting the cooperative character of the self-stacking of the dye on the polymer environment. Concerning the polyelectrolytes bearing charged aromatic groups, the polyMB band is shifted to lower energies, reaching



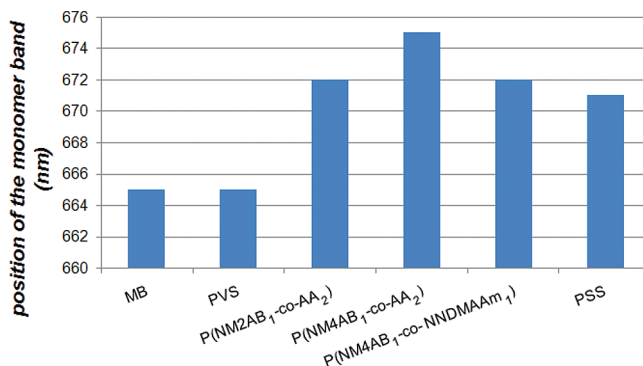


**Figure 4.** Absorbance at 665 nm as a function of the polymer concentration relative to MB concentration at pH 7.0 in presence of (x) PVS, (◆) P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>), (■) P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>), (▲) P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>), and (●) PSS.

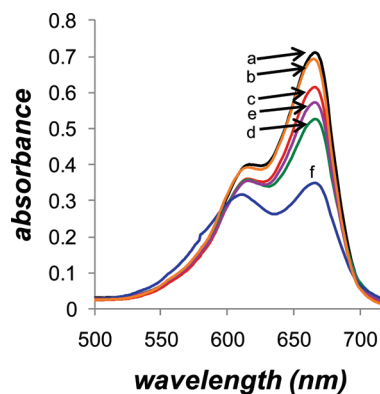
a plateau at 616 nm at increasing polyelectrolyte concentration, indicating the formation of dimers. PSS and P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) present a similar and high dispersant ability, since the dimer band is reached at low excess of the polyelectrolyte. In the case of P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) a high dispersant ability is noticed at very low concentrations of the polyelectrolyte probably due to a lower aggregation associated with steric hindrance of the dimethylacrylamide residues and/or more hydrophobicity provided by this polymer. Moreover, as the dimethylacrylamide functional groups are not charged, the linear charge density of this polymer is lower, so that the cooperative self-binding of the dye is expected to be decreased. Small differences are found between P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) and P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>). In the case of P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>), the binding to the negatively charged carboxylate groups by means of short-range interactions may be sterically hindered as a consequence of the its ortho-position, so that the highly charged system may present a higher typical polyelectrolyte behavior.

Another effect resulting from the presence of secondary aromatic–aromatic interactions, and related to the ability of the polymers to disperse the dyes, is the increase on the monomer band by increasing the polymer concentration. The absorbance at 665 nm is plotted in Figure 4 versus the polymer concentration. It can be seen that the monomer band increases sharply in the case of PSS achieving the highest values at a moderate excess of the polymer. The increase on the monomer band with the polymer concentration is not so sharp in the case of P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>), a fact that may be related to the lower linear aromatic density of this polymer with respect to PSS, while the linear charge density remains similar; this may produce a more extended conformation for this polymer and a consequent higher facility for the dyes to cooperatively self-stack. The higher hydrophobicity of P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) enhances the appearance of monomeric MB, while steric hindrance of the charged aromatic groups of P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) and its relatively high linear charge density partially minimize MB dispersion in the form of monomers. A low concentration of monomeric MB is found in the presence of PVS, corresponding to its typical polyelectrolyte behavior.

On the other hand, it has been shown that presence of short-range aromatic–aromatic interactions between xanthene dyes and polymeric charged aromatic groups produce a shifting of about 8 nm of the monomer band to lower energies as a result of the interaction of the transition moment of the dye with the stacked surrounding molecules.<sup>43,44</sup> This is also the case for the polyelectrolytes containing charged aromatic groups presented in this study, as can be seen in Figure 5, where the position of



**Figure 5.** Position of the maximum of absorbance (nm) of the band corresponding to monomeric MB for a  $1 \times 10^{-5}$  M MB solution in absence (MB) and in presence of 100 times the different polyelectrolytes.

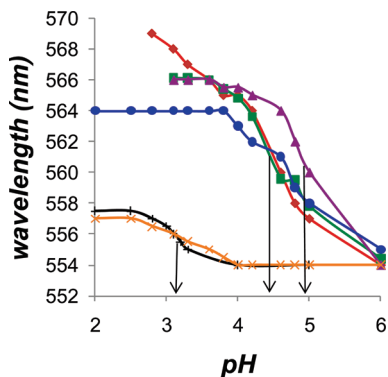


**Figure 6.** UV-vis spectra of  $1 \times 10^{-5}$  M MB solutions at pH 7.0 and in the presence of 0.1 M NaCl, in the absence of any polyelectrolyte (a), and in the presence of  $1 \times 10^{-4}$  M PVS (b), P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) (c), P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) (d), P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) (e), and PSS (f).

the monomer band is plotted for every polymer which is in excess of 100 times. The monomer band is shifted from 665 nm in the absence of any polymer to 671–685 nm in the presence of the polyelectrolytes, except in the case of PVS, which does not bear aromatic rings.

Finally, short-range aromatic–aromatic interactions are more resistant to the cleaving effect produced upon addition on NaCl. It can be seen in Figure 6 that in the presence of 0.1 M NaCl the MB aggregation is practically cleaved in the case of all the polyelectrolytes, except in the case of PSS. This is reflecting the importance of the linear aromatic density on the stabilization of the ion pairs formed. These ion pairs should be stabilized in hydrophobic environments. The presence of acrylate moieties inserted between the maleimide groups may produce more extended conformations and a higher tendency for the polymers to behave as typical polyelectrolytes. A certain resistance to the cleaving effect of added salt is found in the order P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) > P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) > P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>), consistent with a decreasing probability to undergo short-range interactions in the same order.

In summary, P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>), P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>), and P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) present a polyaromatic-anion behavior revealed by a high dispersant ability of MB. Their presence in excess of 100 times produces a shift of the MB monomer band to lower energies as a consequence of short-range aromatic–aromatic interactions. However, the lower intensity of the monomer band and the lack of resistance to the cleaving effect of added NaCl with respect to PSS may indicate the importance of the linear

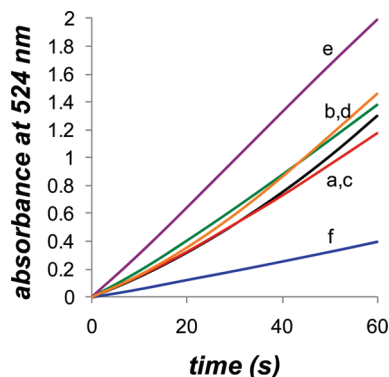


**Figure 7.** Position of the maximum of absorbance of  $1 \times 10^{-5}$  M RB solutions as a function of the pH in the absence of any polyelectrolyte (+) and in presence of  $6 \times 10^{-4}$  M of PVS (x), P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) (◆), P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) (■), P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) (▲), and PSS (●).

aromatic density on the polyaromatic-anion behavior, so that the polymers may retain some of the typical polyelectrolyte behavior.

**3.3. Change on RB  $pK_a$ .** Upon interaction with polyelectrolytes, changes on the  $pK_a$  of polyvalent organic molecules such as porphyrins<sup>39,71,72,76</sup> or chlorpheniramine maleate<sup>67</sup> have been observed. This change on the  $pK_a$  is due to the stabilization of the charges of the low-molecular-weight molecules by the complementary charges of the polymer. For typical polyelectrolytes, it is necessary that primary electrostatic long-range interactions between the polyelectrolyte and the acid–base sensitive molecule take place, so that an additional charge in the low-molecular-weight molecule could be stabilized on the environment of the polymer. However, as RB is a zwitterionic molecule at pH above 3.2, long-range electrostatic interactions are not held at these conditions. So, in order to observe a change on its  $pK_a$ , secondary interactions must be present. RB has a planar, positively charged xanthene group and, perpendicular to it, a benzene carboxylate group. Its negative charge is stabilized by the positive charge of the xanthene group, as the carboxylate is orientated toward it, a fact that explains its low  $pK_a$ . Upon interaction with polyanions by means of secondary interactions, the negative charges of the polymer stabilize the positive charge of the xanthene group, and thus, the carboxylate group becomes more prone to protonate.<sup>43,66</sup> Moreover, if short-range interactions such as aromatic–aromatic interactions are held between the dye and polymeric complementary charged aromatic groups, ion pairs may be formed once the dye protonates, a situation that can be stabilized in hydrophobic environments.

The change on the  $pK_a$  of RB can be followed by UV–vis spectroscopy, since the protonated and unprotonated species absorb at different wavelengths (557 and 554 nm, respectively). The maximum of absorbance of RB has been plotted as a function of the pH in Figure 7. Note that, as in the case of MB, the band of the protonated, positively charged RB is shifted to lower energies in the presence of the polyelectrolytes containing aromatic moieties revealing the occurrence of short-range aromatic–aromatic interactions between the dye and the polymers. It can be also seen that all the mentioned polyelectrolytes change the  $pK_a$  of RB. PSS, P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>), and P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) produce a shift of the  $pK_a$  from 3.2 to around 4.5. On the other hand, a higher shift is found for P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) probably due to the higher hydrophobicity of this polymer, in the presence of which the  $pK_a$  of RB is changed



**Figure 8.** Absorbance at 524 nm as an indicative of TTC reduction vs time in the absence of any polyelectrolyte (a) and in the presence of PVS (b), P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) (c), P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) (d), P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) (e), and PSS (f).

to around 5. On the contrary, PVS does not produce either a shift on the protonated RB band or any change on the  $pK_a$  of the dye.

Interestingly, the polymers bearing the weak carboxylic acid are sensitive to the pH, so that at low pH these polymers may precipitate. Coprecipitation of RB is achieved by lowering the pH from pH 3, so that this dye may serve as marker. To show this, solutions of the complexes containing polyelectrolytes at a concentration of  $3 \times 10^{-4}$  M and RB at a concentration of  $10^{-5}$  M were acidified to pH 2.5 and filtered, and the concentration of RB in the supernatants was analyzed. The decrease on the concentration of RB exceeded 1 order of magnitude. The same results are found for MB.

In summary, regarding the change on the  $pK_a$  of RB, P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>), P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>), and P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) present a polyaromatic-anion behavior, similarly to PSS, producing a shift of the  $pK_a$  from 3.2 to 4.5–5.

**3.4. Protection of TTC from Reduction.** TTC can be reduced to the corresponding formazan both chemically by reaction with ASC and electrochemically. As it has been demonstrated before, PSS can be used to decrease the rate of reduction of TTC, and this protection effect is due to the existence of short-range aromatic–aromatic interactions.<sup>65,68,69</sup> Besides, it has also been shown the importance of the linear aromatic density on this protection effect: as the linear aromatic density decreases, the protection ability decreases.<sup>68,69</sup>

It can be seen in Figure 8 the absorbance at 524 nm as a function of time, which indicates the appearance of the 1,3,5-triphenylformazan, as a measure of the reduction rate of TTC by reaction with ASC. Although PSS clearly protects TTC from reduction with ASC, as can be seen by the lower rate of appearance of the formazan, the other polyelectrolytes do not show the same effect on TTC reduction. The lack of protection associated with the polyelectrolytes containing aromatic rings P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) and P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) may be attributed to a more open conformation due to their lower linear aromatic density in relation to their high linear charge density. The hydrophobic P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>), which presents a low linear charge density and low linear aromatic density, may highlight the discussion whether protection is due to confinement of TTC in a hydrophobic environment or to the possibility to undergo double stacking with the polymeric aromatic rings.<sup>69</sup> The latter hypothesis is reinforced, since the hydrophobicity of this polymer seems to impact more efficiently the stabilization of the hydrophobic formazan, and instead of protecting TTC from reduction, it accelerates it.

In summary, contrarily to PSS, P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>), P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>), and P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) do not protect TTC from reduction with ASC, while P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) accelerates it.

**3.5. Polyaromatic-Anion Behavior.** Aromatic–aromatic interactions between aromatic counterions and polymeric aromatic groups are responsible for the special properties that polyaromatic anions in conjunction with aromatic species present. These interactions have a short-range character, which define the behavior of the system when these interactions are predominant.<sup>13</sup> The intensity of aromatic–aromatic interactions between low-molecular-weight molecules is related to their hydrophobicity, electrostatic effects, steric effects, and polarizability of the interacting molecules.<sup>46–51</sup> Besides, the polymer effect is revealed by the influence of the linear aromatic density on the overall interactions shown by copolymers of maleic acid and styrenesulfonate.<sup>68–70</sup> The more compact conformation of PSS with respect to the corresponding copolymers showed to enhance the polyaromatic-anion behavior stabilizing the ion pairs formed with molecules such as MB or TTC. As a consequence of this, under an excess of PSS, MB molecules can be found as monomers stacked on benzenesulfonate moieties, and TTC rate of reduction with ASC is decreased 1 order of magnitude. Upon insertion of maleate units between styrenesulfonate groups, the corresponding copolymers show a decreasing linear aromatic density, while the linear charge density remains equal or increases. A more extended conformation for these copolymers is expected, and <sup>1</sup>H NMR analyses showed a decrease on the self-stacking of the aromatic moieties.<sup>69,70</sup> As a consequence of this, self-aggregation of MB is not completely avoided on the copolymer surface, and the protection of TTC from reduction with ASC is not so efficient. In the case of the copolymers presented in this work, P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) and P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) present a lower linear aromatic density compared to PSS and its studied copolymers<sup>68–70</sup> and a comparable linear charge density. Both structures quench the formation of large aggregates of MB based on H-type contacts, and aggregates are found in the form of dimers (Figure 3). However, the isolation of the dye in form of monomers stacked on the benzenesulfonate groups is less efficient in the case of the copolymers presented here than in the case of PSS (Figure 4), which confirms the importance of more compact, hydrophobic structures. The lack of protection of TTC from reduction with ASC in the presence of the copolymers presented here points in the same direction.

On the other hand, P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) presents a linear aromatic density intermediate between PSS and P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) and P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>) but presents the lower linear charge density, which provides a higher hydrophobicity. Upon interaction with MB, the formation of large aggregates of MB based on H-type contacts is also quenched (Figure 3), and isolation of the dye in form of monomers stacked on the benzenecarboxylate groups is achieved to a higher extent than in the case of the other copolymers (Figure 4). Moreover, the reduction of TTC with ASC is accelerated in the presence of this polymer, probably due to stabilization of the resulting hydrophobic formazan (Figure 8).

The position of the carboxylate group in the maleic derivatives shown in this work also has an influence on the occurrence of the aromatic–aromatic interactions and a higher excess of P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>), which has the carboxylate group in the ortho position, is needed to achieve the same extent of quenching of MB large aggregates as well as the same amounts of

monomeric dyes stacked on benzenecarboxylate groups, as obtained for P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>), which has the carboxylate group in a para-position.

Finally, as a signature of the occurrence of short-range aromatic–aromatic interactions all the copolymers, as well as PSS, produce a shift of the MB and RB monomer bands to lower energies (Figure 5) and a shift of the pK<sub>a</sub> of RB, a fact that is not seen in the presence of PVS (Figure 7).

#### 4. Conclusions

The copolymers P(NM4AB<sub>1</sub>-co-AA<sub>2</sub>), P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>), and P(NM2AB<sub>1</sub>-co-AA<sub>2</sub>) present a polyaromatic-anion behavior revealed by a high dispersant ability of MB and the ability to change the pK<sub>a</sub> of RB from 3.2 to 4.5–5. In the presence of excess of the copolymers the UV–vis monomer band of both cationic dyes is shifted to lower energies as a consequence of short-range aromatic–aromatic interactions with the polymers. Coprecipitation of these dyes is held at acid pHs. However, compared to PSS, the lower linear aromatic density of these copolymers produces a lower resistance to the cleaving effect of added NaCl on the interaction with MB and the lack of protection of TTC from reduction with ASC. The high hydrophobicity of P(NM4AB<sub>1</sub>-co-NNDMAAm<sub>1</sub>) associated with its relative lower linear charge density produces a catalytic effect on the reduction of TTC with ASC.

**Acknowledgment.** The authors thank Fondecyt (Grant No. 1090341, Chile) and the Global COE program “Practical Chemical Wisdom” at Waseda University from MEXT, Japan, for financial support.

#### References and Notes

- (1) Decher, G. *Science* **1997**, *277*, 1232.
- (2) Zhao, Y.; Tanaka, M.; Kinoshita, T.; Higuchi, M.; Tan, T. *Biomacromolecules* **2009**, *8*, 2764.
- (3) Park, M.-K.; Onishi, K.; Locklin, J.; Caruso, F.; Advincula, R. *Langmuir* **2003**, *19*, 8550.
- (4) Grenha, A.; Seijo, B.; Remuñán-López, C. *Eur. J. Pharm. Sci.* **2005**, *25*, 427.
- (5) Willerich, I.; Gröhn, F. *Chem.—Eur. J.* **2008**, *14*, 9112.
- (6) Kogej, K.; Evmenenko, G.; Theunissen, E.; Škerjanc, J.; Berghmans, H.; Reynaers, H.; Bras, W. *Macromol. Rapid Commun.* **2000**, *21*, 1226.
- (7) Kogej, K.; Evmenenko, G.; Theunissen, E.; Berghmans, H.; Reynaers, H. *Langmuir* **2001**, *17*, 3175.
- (8) Antonietti, M.; Conrad, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1869.
- (9) Thünemann, A. F.; Ruppelt, D.; Ito, S.; Müllen, K. *J. Mater. Chem.* **1999**, *9*, 1055.
- (10) Ruthard, C.; Maskos, M.; Kolb, U.; Gröhn, F. *Macromolecules* **2009**, *42*, 830.
- (11) Manning, G. S. *Q. Rev. Biophys.* **1978**, *11*, 179.
- (12) Manning, G. S. *J. Phys. Chem.* **1984**, *88*, 6654.
- (13) Nordmeier, E. *Macromol. Chem. Phys.* **1995**, *196*, 1321.
- (14) Hao, M. H.; Harvey, S. C. *Macromolecules* **1992**, *25*, 2200.
- (15) Gregor, H. P.; Gregor, J. M. *J. Chem. Phys.* **1977**, *66*, 1934.
- (16) Winkler, R. G.; Gold, M.; Reineker, P. *Phys. Rev. Lett.* **1998**, *80*, 3731.
- (17) Pack, G. R.; Klein, B. J. *Biopolymers* **1984**, *23*, 2801.
- (18) Faul, C. F. J.; Antonietti, M. *Adv. Mater.* **2003**, *15*, 673.
- (19) Gröhn, F. *Macromol. Chem. Phys.* **2008**, *209*, 2295.
- (20) Willerich, I.; Gröhn, F. *Chem.—Eur. J.* **2008**, *14*, 9112.
- (21) Gröhn, F.; Klein, K.; Brand, S. *Chem.—Eur. J.* **2008**, *14*, 6866.
- (22) Ruthard, C.; Maskos, M.; Kolb, U.; Gröhn, F. *Macromolecules* **2009**, *42*, 830.
- (23) Yildiz, Ü. H.; Koynov, K.; Gröhn, F. *Macromol. Chem. Phys.* **2009**, *210*, 1678.
- (24) Kogej, K.; Evmenenko, G.; Theunissen, E.; Škerjanc, J.; Berghmans, H.; Reynaers, H.; Bras, W. *Macromol. Rapid Commun.* **2000**, *21*, 1226.
- (25) Kogej, K.; Evmenenko, G.; Theunissen, E.; Berghmans, H.; Reynaers, H. *Langmuir* **2001**, *17*, 3175.
- (26) Gohy, J. F.; Antoun, S.; Sobry, R.; van der Bossche, G.; Jerome, R. *Macromol. Chem. Phys.* **2000**, *201*, 31.



- (27) Thünemann, A. F.; Ruppelt, D.; Burger, C.; Müllen, K. *J. Mater. Chem.* **2000**, *10*, 1325.
- (28) De Santis, S.; Ladogana, D.; Diociaiuti, M.; Masci, G. *Macromolecules* **2010**, DOI: 10.1021/ma9026542.
- (29) Antonietti, M.; Conrad, J.; Thünemann, A. *Macromolecules* **1994**, *27*, 6007.
- (30) Willerich, I.; Ritter, H.; Gröhn, F. *J. Phys. Chem. B* **2009**, *113*, 3339.
- (31) Würthner, F.; Thalacker, C.; Diele, S.; Tschierske, C. *Chem.—Eur. J.* **2001**, *7*, 2245.
- (32) Imae, T.; Gagel, L.; Tunich, C.; Platz, G.; Iwamoto, T.; Funayama, K. *Langmuir* **1998**, *14*, 2197.
- (33) Faul, C. F. J.; Antonietti, M. *Chem.—Eur. J.* **2002**, *8*, 2764.
- (34) von Berlepsch, H.; Böttcher, C.; Ouart, A.; Burger, C.; Dähne, S.; Kirstein, S. *J. Phys. Chem. B* **2000**, *104*, 5255.
- (35) Horn, D. *Prog. Colloid Polym. Sci.* **1978**, *65*, 251.
- (36) Schwartz, G.; Klose, S.; Balthasar, W. *Eur. J. Biochem.* **1970**, *12*, 454.
- (37) Chen, Z.; Lohr, A.; Saha-Möller, C. R.; Würthner, F. *Chem. Soc. Rev.* **2009**, *38*, 564.
- (38) Egawa, Y.; Hayashida, R.; Anzai, J. *Langmuir* **2007**, *23*, 13146.
- (39) Kubát, P.; Lang, K.; Janda, P.; Anzenbacher, P. *Langmuir* **2005**, *21*, 9714.
- (40) Lauceri, R.; Campagna, T.; Raudino, A.; Purello, R. *Inorg. Chim. Acta* **2001**, *317*, 282.
- (41) Synytsya, A.; Synytsya, A.; Blafková, P.; Ederová, J.; Spěvaček, J.; Slepíčka, P.; Král, V.; Volka, K. *Biomacromolecules* **2009**, *10*, 1067.
- (42) Van Patten, P. G.; Shreve, A. P.; Donohoe, R. J. *J. Phys. Chem. B* **2000**, *104*, 5986.
- (43) Moreno-Villoslada, I.; González, F.; Arias, L.; Villatoro, J. M.; Ugarte, R.; Hess, S.; Nishide, H. *Dyes Pigm.* **2009**, *82*, 401.
- (44) Moreno-Villoslada, I.; Torres, C.; González, F.; Nishide, H. *Macromol. Chem. Phys.* **2009**, *210*, 1167.
- (45) Soedjak, H. S. *Anal. Chem.* **1994**, *66*, 4514.
- (46) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525.
- (47) Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210.
- (48) Lee, E. C.; Kim, D.; Jurečka, P.; Tarakeshwar, P.; Hobza, P.; Kim, K. S. *J. Phys. Chem. A* **2007**, *111*, 3446.
- (49) Paliwal, S.; Geib, S.; Wilcox, C. S. *J. Am. Chem. Soc.* **1994**, *116*, 4497.
- (50) Kim, E.; Paliwal, S.; Wilcox, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 11192.
- (51) Mignon, P.; Loverix, S.; De Proft, F.; Geerlings, P. *J. Phys. Chem. A* **2004**, *108*, 6038.
- (52) Mignon, P.; Loverix, S.; Steyaert, J.; Geerlings, P. *Nucleic Acids Res.* **2005**, *33*, 1779.
- (53) Marzilli, L. G.; Pethö, G.; Mengfen, L.; Kim, M. S.; Dixon, D. W. *J. Am. Chem. Soc.* **1992**, *114*, 7575.
- (54) McKnight, R. E.; Zhang, J.; Dixon, D. W. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 401.
- (55) Martin, J. N.; Muñoz, E. M.; Schwergold, C.; Souard, F.; Asensio, J. L.; Jiménez-Barbero, J.; Cañada, J.; Vicent, C. *J. Am. Chem. Soc.* **2005**, *127*, 9518.
- (56) Tatko, Ch. D.; Waters, M. L. *Protein Sci.* **2003**, *12*, 2443.
- (57) Bodkin, M. J.; Goodfellow, J. M. *Protein Sci.* **1995**, *4*, 603.
- (58) Bhattacharyya, R.; Samanta, U.; Chakrabarti, P. *Protein Eng.* **2002**, *15*, 91.
- (59) Ranganathan, D.; Haridas, V.; Gilardi, R.; Karle, I. L. *J. Am. Chem. Soc.* **1998**, *120*, 10793.
- (60) Versées, W.; Loverix, S.; Vandemeulebroucke, A.; Geerlings, P.; Steyaert, J. *J. Mol. Biol.* **2004**, *338*, 1.
- (61) Greenblatt, H. M.; Dvir, H.; Silman, I.; Sussman, J. L. *J. Mol. Neurosci.* **2003**, *20*, 369.
- (62) Li, H. L.; Galue, A.; Meadows, L.; Ragsdale, D. S. *Mol. Pharmacol.* **1999**, *55*, 134.
- (63) Li, J.; Lester, H. A. *Chem.—Eur. J.* **2005**, *11*, 6525.
- (64) Moreno-Villoslada, I.; González, R.; Hess, S.; Rivas, B. L.; Shibue, T.; Nishide, H. *J. Phys. Chem. B* **2006**, *110*, 21576.
- (65) Moreno-Villoslada, I.; González, F.; Rivera, L.; Hess, S.; Rivas, B. L.; Shibue, T.; Nishide, H. *J. Phys. Chem. B* **2007**, *111*, 6146.
- (66) Moreno-Villoslada, I.; Jofré, M.; Miranda, V.; González, R.; Sotelo, T.; Hess, S.; Rivas, B. L. *J. Phys. Chem. B* **2006**, *110*, 11809.
- (67) Moreno-Villoslada, I.; González, F.; Rivas, B. L.; Shibue, T.; Nishide, H. *Polymer* **2007**, *48*, 799.
- (68) Moreno-Villoslada, I.; Soto, M.; González, F.; Hess, S.; Takemura, I.; Oyaizu, K.; Nishide, H. *J. Phys. Chem. B* **2008**, *112*, 5350.
- (69) Moreno-Villoslada, I.; González, F.; Soto, M.; Nishide, H. *J. Phys. Chem. B* **2008**, *112*, 11244.
- (70) Moreno-Villoslada, I.; Torres-Gallegos, C.; Araya-Hermosilla, R.; Nishide, H. *J. Phys. Chem. B* **2010**, *114*, 4151.
- (71) Moreno-Villoslada, I.; Murakami, T.; Nishide, H. *Biomacromolecules* **2009**, *10*, 3341.
- (72) Zhao, L.; Ma, R.; Li, J.; Li, Y.; An, Y.; Shi, L. *Biomacromolecules* **2009**, *10*, 3343.
- (73) Michaelis, L.; Granick, S. *J. Am. Chem. Soc.* **1945**, *67*, 1212.
- (74) Spencer, W.; Sutter, J. R. *J. Phys. Chem.* **1979**, *83*, 1573.
- (75) Valdés-Aguilera, O.; Neckers, D. C. *Acc. Chem. Res.* **1989**, *22*, 171.
- (76) Gandini, S. C. M.; Yushmanov, V. E.; Borissevitch, I. E.; Tabak, M. *Langmuir* **1999**, *15*, 6233.