

## The Osmotic Coefficients of the Sodium Form of Some Biopolymers

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### Synopsis

The osmotic coefficients  $\phi_{p,Na}$  of dilute solutions of the sodium form of some weakly acidic polymers are theoretically predicted in this work. Based on the measured value 0.73 of  $\gamma_{Na}$ , the activity coefficient of free  $Na^+$ , of the completely ionized humic acid (sodium salt) in a salt-free solution, the effective interligand distance  $b$  is calculated to be 11.34 Å by using Manning's counterion condensation theory [Manning, G. S. (1969) *J. Chem. Phys.* 51(3), 924]. The corresponding values of  $\gamma_{Na}$  (measured experimentally) and  $b$  for the completely ionized exopolymer of *Pseudomonas atlantica* are 0.624 and 7.57 Å when cultivated at a dilution rate  $D = 0.015 h^{-1}$ , 0.647 and 8.19 Å at  $D = 0.025 h^{-1}$ , and 0.613 and 7.29 Å at  $D = 0.06 h^{-1}$ . For alginic acid (in the completely ionized sodium form),  $\gamma_{Na} = 0.40$  and  $b = 4.71$  Å. The osmotic coefficients  $\phi_{p,Na}$  for the partially and the completely ionized polymers are then predicted with Manning's theory as well.

### INTRODUCTION

Many polymers of biological origins exhibit polyelectrolyte behavior in aqueous solutions. In our laboratory, attempts have been made to characterize the polyelectrolyte properties of the following weakly acidic polymers: humic acid; *Pseudomonas atlantica* exopolymers cultivated at dilution rates of  $D = 0.015, 0.025,$  and  $0.06 h^{-1}$ ; and alginic acid. For a dilute solution of the polymer, Manning's limiting laws<sup>1</sup> can be used to calculate the interligand distance  $b$  of the polymer from the measured activity coefficient  $\gamma_{Na}$  of the completely ionized Na form of the polymer in a salt-free solution:

$$\xi = -2 \ln \gamma_{Na} \quad \text{for } \xi < 1 \quad (1)$$

$$\xi = 0.607(\gamma_{Na})^{-1} \quad \text{for } \xi > 1 \quad (2)$$

where

$$\xi = \frac{e^2}{4\pi\epsilon_0\epsilon_r kT} \frac{\alpha}{b} \quad (\text{SI unit}) \quad (3)$$

In Eq. 3,  $e$  is the charge of a monovalent species ( $= 1.6 \times 10^{-19}$  C),  $\epsilon_0$  the permittivity in vacuo ( $= 8.854 \times 10^{-12}$  C<sup>2</sup>/J - m),  $\epsilon_r$  the relative permittiv-

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ity ( $= 78.5$  for water at  $25^\circ\text{C}$ ),  $k$  the Boltzmann constant ( $= 1.38 \times 10^{-23}$  J/K),  $\alpha$  the fraction of ligands that are ionized ( $\alpha = 1$  for a completely ionized polymer), and  $T$  the absolute temperature (in degrees Kelvin). According to Manning's theory, the critical value  $\xi = 1$  corresponds to the charge spacing  $(b/\alpha) = 7.135 \times 10^{-10}$  m (7.135 Å).

With the interligand distance  $b$  determined for a completely ionized polymer, the theoretical osmotic coefficient of the dilute solution of a partially ionized polymer ( $0 < \alpha < 1$ ) can be predicted with the following formulae:

$$\phi_{p,Na} = 1 - 0.5\xi \quad \text{for } \xi < 1 \quad (4)$$

$$\phi_{p,Na} = (2\xi)^{-1} \quad \text{for } \xi > 1 \quad (5)$$

## EXPERIMENTAL

### Biopolymer Samples

#### *Alginic Acid*

The acid form of alginic acid from Sigma (A-7003) was used as received.

#### *Exopolymer*

The bacterium *P. atlantica* T<sub>6</sub>C was cultivated in a BioFlo II chemostat fermentor (New Brunswick) at  $20^\circ\text{C}$  under three different dilution rates: 0.015, 0.025, and  $0.06 \text{ h}^{-1}$ . The medium composition and the method of biopolymer extraction are described elsewhere.<sup>2</sup> The final product in the powder form was stored in the desiccator.

#### *Pretreatment of Polymer Samples*

One-half gram of humic acid (sodium salt, H1675-2, Aldrich) was dissolved directly in 150 mL doubly distilled water. About 0.3, 0.1, and 0.3 g of exopolymer material obtained from culture of *P. atlantica* at dilution rates of 0.015, 0.025, and  $0.06 \text{ h}^{-1}$ , respectively, were added to 150 mL of a NaOH solution at pH 9.0 with mild agitation. (It took approximately 3 h for the exopolymer material to completely dissolve in NaOH.) The polymers were then converted to their protonated form by the addition of dilute HCl to achieve a pH of 2.5 (which is below the  $\text{pK}_a$  values of most weakly acidic, metal-binding functional groups). Another purpose of this acidification step was to remove any multivalent cations that might have existed in the original biopolymer sample. The solutions were then dialyzed exhaustively against doubly distilled water to remove cations and other low molecular weight material ( $< 12,000$  MW) associated with the polymer preparations. Each polymer solution was distributed into 5-mL volumes and stored at  $-80^\circ\text{C}$  prior to use. The three exopolymer samples at dilution rates of 0.015, 0.025, and  $0.060 \text{ h}^{-1}$  contained 1242, 421, and 324 g carbohydrate/mL of solution, respectively, as determined by the colorimetric method outlined in Dubois et al.<sup>3</sup>

Thawed polyethylene vial polymer sphere was (ISM-146-N) at neutral biopolymer micro glass and a pH/ by a 5-posit

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## Instrumentation and Apparatus

Thawed polymer solutions were transferred to an acid-washed polypropylene vial positioned in a water bath equilibrated to 25°C. A nitrogen atmosphere was maintained over the polymer solution. A sodium ion electrode (ISM-146-Na Microelectrode, Lazar Laboratory, Los Angeles, CA) calibrated at neutral pH was used to measure the free  $\text{Na}^+$  concentration in the biopolymer solution. The pH of the polymer solution was monitored with a micro glass combination electrode (AccupHast 13-639-280, Fisher Scientific) and a pH/ion meter (Accumet 825MP, Fisher Scientific), which was controlled by a 5-position electrode switch box (Model 753, Fisher Scientific).

## Titration Procedure

About 0.04 g of alginic acid powder was dissolved in 10 mL doubly distilled water. The volumes of the salt-free exopolymer from *P. atlantica* and humic acid were adjusted to 10 mL with doubly distilled water. The polymer samples were then titrated with 0.1M NaOH using a micropipetter (Oxford). During the course of titration, the pH values were plotted against the volume of NaOH solution added and the inflection point on the curve was taken as the end point of the titration. The concentration of free sodium ions at the end point can be measured with confidence using a sodium ion selective electrode, because the interference from hydrogen ion with the measurement electrode should disappear at neutral pH. At the end point of titration, the polymer was completely ionized and the moles of total sodium ions added (as NaOH) just balanced the negatively charged ionized ligands. Thus, the activity coefficient  $\gamma_{\text{Na}}$  at  $\alpha = 1.0$  can be calculated as the concentration of free sodium ions divided by the overall concentration of sodium ions (the moles of NaOH added to achieve the end point of titration divided by the volume of the solution at the end point).

## RESULTS AND DISCUSSION

The results of base titration of salt-free solutions of completely ionized polymers along with calculated values of  $\xi_{\alpha=1}$  and  $b$  are summarized in Table I. The theoretical values of  $\phi_{p,\text{Na}}$  for partially and completely ionized polymers calculated from Eqs. (4) or (5) are presented in Table II. The  $\xi$  values at  $\alpha = 1$  are smaller than the critical value 1.0 except in the case of alginic acid. Although the structures of humic acid and *P. atlantica* exopolymers are not fully understood, the results from this work indicate that their charge densities are smaller than that of alginic acid. The neutral carbohydrate residues on the exopolymer chain are likely responsible for the large distance between ionizable ligands.

The  $\xi$  value of partially ionized alginic acid exceeds the critical value 1.0 as  $\alpha > 0.66$ . Therefore, Eq. (5) was used to predict  $\phi_{p,\text{Na}}$  for  $\alpha > 0.66$ . The interligand distance  $b$  of alginic acid (4.71 Å) calculated by Manning's counterion condensation model agrees very well with the projected lengths of monomer units of alginic acid obtained by conformational energy calculations.<sup>4</sup>

Alternative predictions for the interligand distance  $b$  were calculated to be 12.12 Å (humic acid), 8.28, 8.93, and 7.98 Å (*P. atlantica* culture of  $D = 0.015$ ,

TABLE I  
 Results of Base Titration of Salt-Free Polymer Solutions

Polymer	mmoles NaOH Required	Overall Na <sup>+</sup> Concentration (M)	Free Na <sup>+</sup> Concentration (M)	$\gamma_{Na}$	$\xi_{\alpha=1}$	$b$ (Å)
Humic acid	0.0195	0.00195	0.00145	0.73	0.6294	11.34
<i>P. atlantica</i> exopolymer						
$D = 0.015 \text{ h}^{-1}$	0.0066	0.00066	0.000412	0.624	0.9432	7.57
$D = 0.025 \text{ h}^{-1}$	0.0024	0.00024	0.000156	0.647	0.8708	8.19
$D = 0.060 \text{ h}^{-1}$	0.0069	0.00069	0.000423	0.613	0.9788	7.29
Alginic acid	0.147	0.0147	0.00587	0.400	1.516	4.71

0.025, and 0.06 h<sup>-1</sup>, respectively), and 3.24 Å (alginic acid) (L. K. Jang, unpublished result), using the exact solution of the salt-free cylindrical Poisson-Boltzmann equation<sup>5</sup> and the cell model result that  $\gamma_{Na} = \phi_{p,Na}$ .<sup>6</sup> It can be noted that this approach gives  $b$  values of humic acid and *P. atlantica* polymers that are about 10% larger than those obtained by Manning's limiting laws. As for alginic acid, which has a much higher charge density, the  $b$  value is underestimated by 31.2%. Manning<sup>1</sup> has pointed out that  $\phi_p$  does not equal  $\gamma$ , although each can be interpreted as the fraction of "free" counterions in a salt-free solution of the polyelectrolyte. This difference arises because  $\phi_p$  and  $\gamma$  are given by different derivatives of the free energy of the polyelectrolyte solution used in Manning's theory. However, particular expression for the Poisson-Boltzmann approach, as reviewed, for example, by Katchalsky et al.<sup>6</sup> predicts that there is no difference between the osmotic coefficient and the single ion (counterion) activity coefficient for a salt-free solution.

 TABLE II  
 The Theoretical Values of Osmotic Coefficients  $\phi_{p,Na}$  of Dilute Polymer Solutions

Degree of Ionization	Polymer				
	Alginic Acid	Humic Acid	<i>P. atlantica</i> Exopolymer		
			$D = 0.015 \text{ h}^{-1}$	$D = 0.025 \text{ h}^{-1}$	$D = 0.06 \text{ h}^{-1}$
0	1.0	1.0	1.0	1.0	1.0
0.1	0.9242	0.9685	0.9528	0.9565	0.9511
0.2	0.8484	0.9371	0.9057	0.9127	0.9021
0.3	0.7726	0.9056	0.8585	0.8694	0.8532
0.4	0.6968	0.8741	0.8114	0.8258	0.8042
0.5	0.6210	0.8427	0.7642	0.7823	0.7553
0.6	0.5452	0.8112	0.7170	0.7388	0.7064
0.7	0.4711	0.7797	0.6699	0.6952	0.6574
0.8	0.4122	0.7482	0.6227	0.6517	0.6085
0.9	0.3665	0.7169	0.5756	0.6081	0.5595
1.0	0.3298	0.6853	0.5284	0.5646	0.5106

This work was supported by grants from the National Science Foundation (ECE-8521693 and CBT-8721943).

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Received July 11, 1988

Accepted October 12, 1988

$\lambda$	$b$ (Å)
294	11.34
432	7.57
708	8.19
788	7.29
116	4.71

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$$D = 0.06 \text{ h}^{-1}$$

- 1.0
- 0.9511
- 0.9021
- 0.8532
- 0.8042
- 0.7553
- 0.7064
- 0.6574
- 0.6085
- 0.5595
- 0.5106