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Estimation of the charge density of arabic acid by potentiometry and dye binding

M.C. Vandevelde and J.C. Fenyo*

Laboratoire des Echanges Cellulaires, SCUEOR, UA 203 du CNRS, Faculté des Sciences et Techniques de l'Université de Rouen, F-76130 Mont-Saint-Aignan, France

SUMMARY

Potentiometric data of arabic acid in salt-free aqueous solutions can be interpreted by the Lifson-Katchalsky cell model. The best fit between experimental and theoretical titration curves is obtained using a spatial intercharge distance of 11 A. This value is corroborated by equilibrium dialysis binding of Ethidium Bromide. When compared with already published results, the apparent charge density of arabic acid is four to six times higher than if it was a linear polyelectrolyte.

INTRODUCTION

Acacia senegal gum is a complex natural highly branched polysaccharide, composed of neutral sugars, a molar ratio of 0.18 of D-glucuronic acid and some 4-O-methyl-D-glucuronic acid. Its detailed structure remains a subject of investigations (STREET and ANDERSON, 1983 ; CHURMS **et al**, 1983 ; CONNOLLY **et al**, 1987). **Acacia senegal** exhibits polyelectrolyte properties in aqueous solutions as shown by its neutralization curves (THOMAS and MURRAY, 1928 ; VEIS, 1953 ; SARKAR, 1974). More or less sophisticated models have been elaborated to explain the titration and binding experimental data of polyelectrolytes. VEIS (1953) used for **Acacia senegal** a spherical model with an underestimated radius of 80 A but the agreement between experimental and calculated titration was poor. Treatment of polyelectrolyte solutions based on local cylindrical symmetry such as the LIFSON-KATCHALSKY cell model (1954) and the MANNING's limiting laws (1969) has proved to be suitable for ionic polysaccharides such as substituted carboxymethylcellulose (RINAUDO, 1974) and arabates (YOMOTA **et al**, 1984). Usually, the two structural parameters needed in the theoretical expressions of the electrostatic potential in the vicinity of polyions : **a**, the minimum distance of approach to the axis by the counterions and **b**, the intercharge ionic distance, are easy to assign for linear polyelectrolytes, which is not the case for **Acacia senegal**. An other approach is to

* To whom offprint requests should be sent

measure some properties and to "calculate" the structural parameters which best fit experimental data. In this way, YOMOTA **et al** (1984) found a spatial intercharge distance of 6.6 Å for the D-glucuronic acid residues by measuring the molal osmotic coefficients and counterion activity coefficients of arabates using the Manning's theory, while **b** would be 35 Å if arabic acid were a linear polyelectrolyte. The same procedure had been previously used by KOHN **et al** (1979) for the acid polysaccharides of peach gum.
Other independent ways to estimate **b** can be found : sophisticated models have been thoroughly checked for the potentiometry of hyaluronic acid, the disaccharide repetitive unit which contains glucuronic acid (CLELAND **et al**, 1982) ; however, this approach seems unrealistic for **Acacia senegal**. For these reasons, the simple Lifson-Katchalsky cell model was applied. The difficulty is to assign a **b** value. Moreover, we have already demonstrated that the binding of some dyes as Ethidium Bromide is largely influenced by the charge density of linear polyions such as partly hydrolyzed polyacrylamides (FENYO **et al**, 1979 ; VANDEVELDE, 1986).
In this paper, we report potentiometric results and dye binding data of Ethidium Bromide which allowed us to estimate the intercharge distance of **Acacia senegal** gum.

EXPERIMENTAL

The origin and characteristics of **Acacia senegal** gum and partly hydrolyzed polyacrylamides have already been described (VANDEVELDE and FENYO, 1985 ; MULLER **et al**, 1979). Acid solutions were prepared by passing samples through a column containing Dowex 50 W X-8 (H^+ form, 20-50 mesh) ion-exchange resin. The solutions were titrated under nitrogen at 25°C with sodium hydroxide, added with a Gilmont S 3200 A microburette, using a Radiometer pHM 52 pHmeter equipped with a combined Metrohm EA 121 glass electrode.
pKa (Fig. 1) were calculated as usual by the formula : $pKa = pH + \log((1 - \alpha)/\alpha)$, α being the ionization degree of the polyelectrolyte. To compare theoretical and experimental data (Fig. 2) it was necessary to assign a pKo value. For each neutralization, a $pKo^{\alpha=0.5}$ was obtained by substracting from the experimental $pKa^{\alpha=0.5}$ the theoretical $\Delta pK^{\alpha=0.5}$:

$$\Delta pKo^{\alpha=0.5} = pKa^{\alpha=0.5} - \Delta pKo^{\alpha=0.5}.$$

All other experimental ΔpKa^{α} were calculated by this method (for more details, see FENYO **et al**, 1979).
Equilibrium dialysis measurements were performed with a DIANORM Diachema A.G. apparatus (Switzerland) as described previously (VANDEVELDE and FENYO, 1979 ; FENYO **et al**, 1979). After equilibria, Ethidium Bromide (from FLUKA) was evaluated in both half-cells by absorption spectroscopy. The bound fraction q is then calculated :

$$q = ((EtBr)_t - (EtBr)_f)/(EtBr)_t$$

$(EtBr)_t$ being the concentration in the half-cell which contains the polyelectrolyte and $(EtBr)_f$ in the half-cell without the polyelectrolyte.

RESULTS AND DISCUSSION

The concentration dependence of apparent pK, pKa, of arabic acid when neutralized by NaOH in salt-free aqueous solution is reported in Fig. 1. The quasi linear variations in pKa is significant of a low charge density polyion which does not have a saturation effect at high ionization.

To compare experimental and theoretical results, we used the procedure just described which implies coincidence between the two sets of data at half neutralization (FENYO **et al**, 1979). The best fit is observed (Fig. 2) for **b**, the intercharge ionic distance of 11 A, using **a** as 6 A (RINAUDO, 1974). The charge parameter λ is 0.65 for this **b** value . The limits of the procedure is shown by the slight variation of the $pKo^{\alpha=0.5}$ with concentrations : pKo 3.0 and 3.2 for Ca 4.5×10^{-2} or 9.75×10^{-3} eq/L respectively. However these values are close to pKo given for model compounds as glucuronic acid (3.23 ± 0.1) and hyaluronic acid (2.9 ± 0.1) (CLELAND **et al**, 1982), the behaviour of arabic acid being very similar to these fairly strong and non complexing models. In particular, salts lower the pH and pK cannot be calculated in $CaCl_2$ 1M as the self-dissociation is very high even at low neutralization. Circular dichroism and viscosity measurement of sodium arabate, calcium arabate and of an equivalent mixed salt confirm (VANDEVELDE, 1986) that divalent ions are only electrostatically bound as already demonstrated by YOMOTA **et al** (1984).

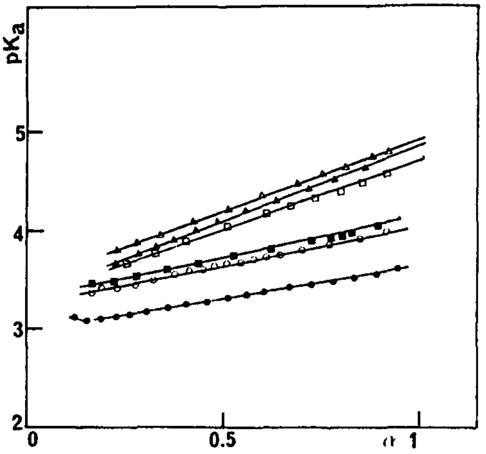

Fig.1. pKa of arabic acid

Ca : ● 4.5×10^{-2} ; ○ 1.4×10^{-2}
■ 9.75×10^{-3} ; □ 4.1×10^{-3}
▲ 3.2×10^{-3} ; △ 2.1×10^{-3}

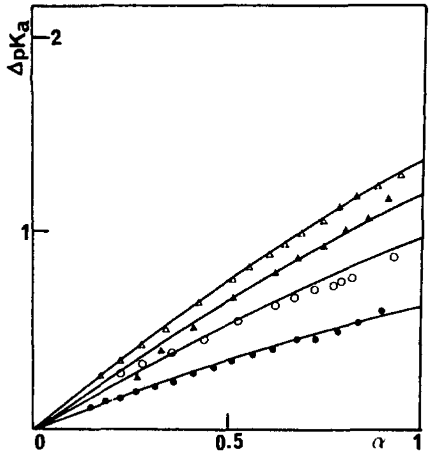

Fig.2. Calculated (full lines) and experimental (symbols) ΔpKa

Ca : ● 4.5×10^{-2} ; ○ 9.75×10^{-3}
▲ 4.1×10^{-3} ; △ 2.1×10^{-3}

The binding of cationic dyes such as Auramine O or Ethidium Bromide by polyanions is dependent on a number of parameters such as conformation of the polymer, polymer-on-dye ratio,

ionic strength and charge density (FENYO **et al**, 1979 ; FENYO **et al**, 1980 ; VANDEVELDE, 1986). In experimental conditions which favour binding (high polymer-on-dye ratio and moderate ionic strength), we have shown (Fig. 3) that the fixation of Ethidium Bromide on a series of partly hydrolyzed polyacrylamides, HPAM-X, evaluated by equilibrium dialysis fit well with an unic curve depending only on the charge parameter. Results obtained in the same experimental conditions with **Acacia senegal** are close to those of HPAM-22, the charge parameter being λ = 0.62. This is coherent with the **b** value deduced from potentiometric titrations. Nevertheless, it can be seen in Fig. 4 that the experimental results are comprised between HPAM-10 (λ = 0.28) and HPAM-35 (λ = 0.99).
To conclude, the discrepancy between our results and those of YOMOTA for the spatial intercharge distance of arabic acid can be moderated as :
1) it is well known that different theoretical models do not necessarily lead to the same results ;
2) YOMOTA used the molal osmotic coefficient for Na^+ which is a counterion dependent value, higher for example for Li^+ or $(C_4H_9)_4N^+$; moreover, the counterion activity coefficient of Na^+ sharply increases when concentration decreases under 0.01 eq/L ;
3) it has not been proved that the distribution of arabic acid is regular in the macromolecular core of **Acacia senegal**.
All these results can only be considered as statistical.

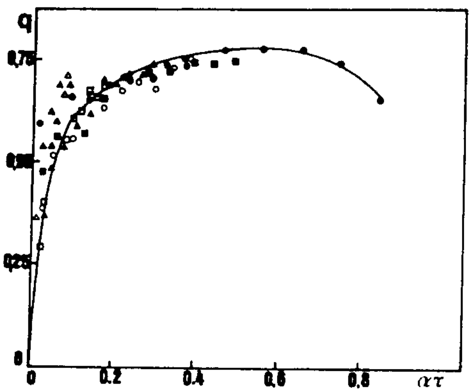

Fig.3. Fraction of bound dye q as a function of the charge parameter

P/D = 100, I = 2.10^{-3}M (KCI)

τ : △10 ; □22 ; ▲35 ; O45 ; ■60 ; ●100 (polyacrylic acid)

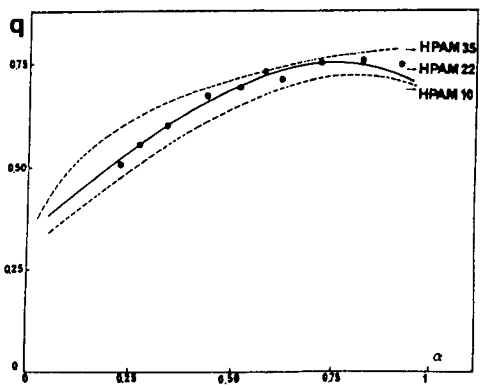

Fig.4. Fraction of bound dye q as a function of ionization

● arabic acid

It can thus be reasonably assumed that the charge density of arabic acid is about four to six times higher than that of a similar linear polyelectrolyte which contains the same amount of uronic acid.

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