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Interaction of 9-Aminoacridinium and 10-Methylacridinium Cations with Polyelectrolytes, with Emphasis on the Bound-dye Spectra[1)]

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Visible and UV absorption spectra of two dyes, 9-aminoacridinium (AA) and 10-methylacridinium (MeA) cations, were measured at 25 °C in the presence of three different polyelectrolytes—sodium salts of poly(*p*-styrenesulfonate) (NaPSS), polyphosphate (NaPP), and polyacrylate (NaPA)—for polymer residue-to-dye ratios from zero to near unity. The optical titration curves of AA–polyanion systems showed a slightly sigmoidal curvature. The method of extended principal component analysis was applied to the experimental spectra of the AA–NaPSS, –NaPP, –NaPA and MeA–NaPSS systems. Only two absorbing component dyes, *i.e.*, bound and unbound, were verified to exist in each system. The equilibrium constant of the binding between dye and polyanion, the pure spectrum of bound dye, and the adjustable parameter α, by which the sigmoidal titration curve is characterized, were determined. In each of the above systems, the bound-dye spectrum behaves in opposition to the common notion of "metachromasy," showing a bathochromic shift without any new band. The spectra of MeA changed only moderately by the addition of NaPSS. The ultrafiltration of the MeA–NaPP and –NaPA solutions confirmed that most of the MeA cations remain unbound.

Although the physicochemical basis of metachromasy still remains to be clarified becasue of its extraordinary complexity, it has become apparent that the metachromatic behavior of a cationic dye depends on the combination between its chemical structure and the charged functional group and conformation of the associated polyelectrolyte.[2—4)] The metachromasy of acridine dyes is known to be most marked in the presence of charged polyanions of various types when the dyes possess the exonuclear amino or disubstituted amino groups at the 3- and 6-positions.[5)] Indeed, the symmetric 3,6-substituted aminoacridines and their derivatives such as Proflavine (PF),[6)] Trypaflavine (TF),[2—4,7)] and Acridine Orange (AO)[6,8)] have been most extensively documented. Their interactions with both natural and synthetic polyanions have been studied theoretically and experimentally from two points of view: metachromatic changes of absorption spectra[8)] and binding reactions.[10)]

By means of the extended principal component analysis (the EPCA procedure), the interactions between TF, one of the most typical 3,6-diaminoacridines, and various polyanions were recently studied to extract the bound-dye spectrum out of a family of experimental spectra.[2)] The result is surprising in that each unmasked spectrum of bound TF always shows two broad but distinct bands whose maxima are located on both the longer- and shorter-wavelength sides of the original peak of TF without polymer. This finding requires some modifications to be made in the concept of metachromasy, which has been defined as the hypsochromic and hypochromic changes in color.[11)] The complexity of spectral changes of the dye–polymer systems is mainly associated with the elusive behavior of the visible absorption spectra of the above dyes, which belong to the C_{2v} point symmetry group and should, therefore, have two orthogonally polarized transition moments. A recent work on the film dichroism of those dyes ascertained beyond doubt that both the short-axis polarized band (the ^1L_a band) and the long-axis polarized band (the ^1L_b band) are involved in the seemingly simple absorption band.[12,13)]

It is now clear that use of some acridine dyes optically simpler than TF will help to understand the physical basis of metachromasy and, possibly, the binding mechanism. Both 9-aminoacridinium (AA) and 10-methylacridinium (MeA) cations are suited to this purpose. Each absorption spectrum shows two unequivocally assigned bands (the ^1L_a and ^1L_b bands) which are widely separated, so that the observed spectral changes may be related to each transition.[12—14)] Furthermore, both AA and MeA have been shown to bind to DNA and other polyelectrolytes.[15—21)] In this work, the observed absorption spectra of these dyes in the presence of three different polyanions were treated by the EPCA procedure to extract the spectra of AA and MeA bound to the polymers; the polymer residue-to-dye mixing ratios from zero to near unity were investigated. The optical titration data were analyzed to evaluate the apparent equilibrium constant and the molar fraction of the bound dye species.

Experimental

Materials. The three polymer and two dye samples used in this work are sodium poly(*p*-styrenesulfonate) (NaPSS, the degree of polymerization DP=1360),[2)] sodium polyphosphate (NaPP, DP=154),[2)] sodium polyacrylate (NaPA, DP=7700),[2)] 9-aminoacridinium chloride (denoted simply as AA),[4,20)] and 10-methylacridinium chloride (denoted as MeA).[12,20,21)]

Preparations of Sample Solutions. The dye concentration was determined by the use of the molar absorption coefficients, ε in $\mathrm{M^{-1}\,cm^{-1}}$ ($1\ \mathrm{M}=1\ \mathrm{mol\,dm^{-3}}$), 10500 at 401 nm for AA and 21000 at 357 nm for MeA. The absorption of the dye–polymer solution was expressed with the apparent ε of the dye present in the solution, unless otherwise stated. The dye–polymer solution contained neither extra neutral salts nor buffer salts to avoid any side effects.[7)] A concentrated polymer solution (*ca.* 2 mM) was delivered dropwise to a dye solution (7 or 14 ml) in an optical cell (either 2 cm or 5 cm in path length) through a microburet (20 μl in each portion). The mixture was stirred for a few minutes before measuring each absorption spectrum. The molar mixing ratio of polymer residues to dye (P/D) was thus varied from zero to slightly over unity without appreciable volume increase. The volume correction for the observed absorbance was always made according to the procedure given in Ref. 2.

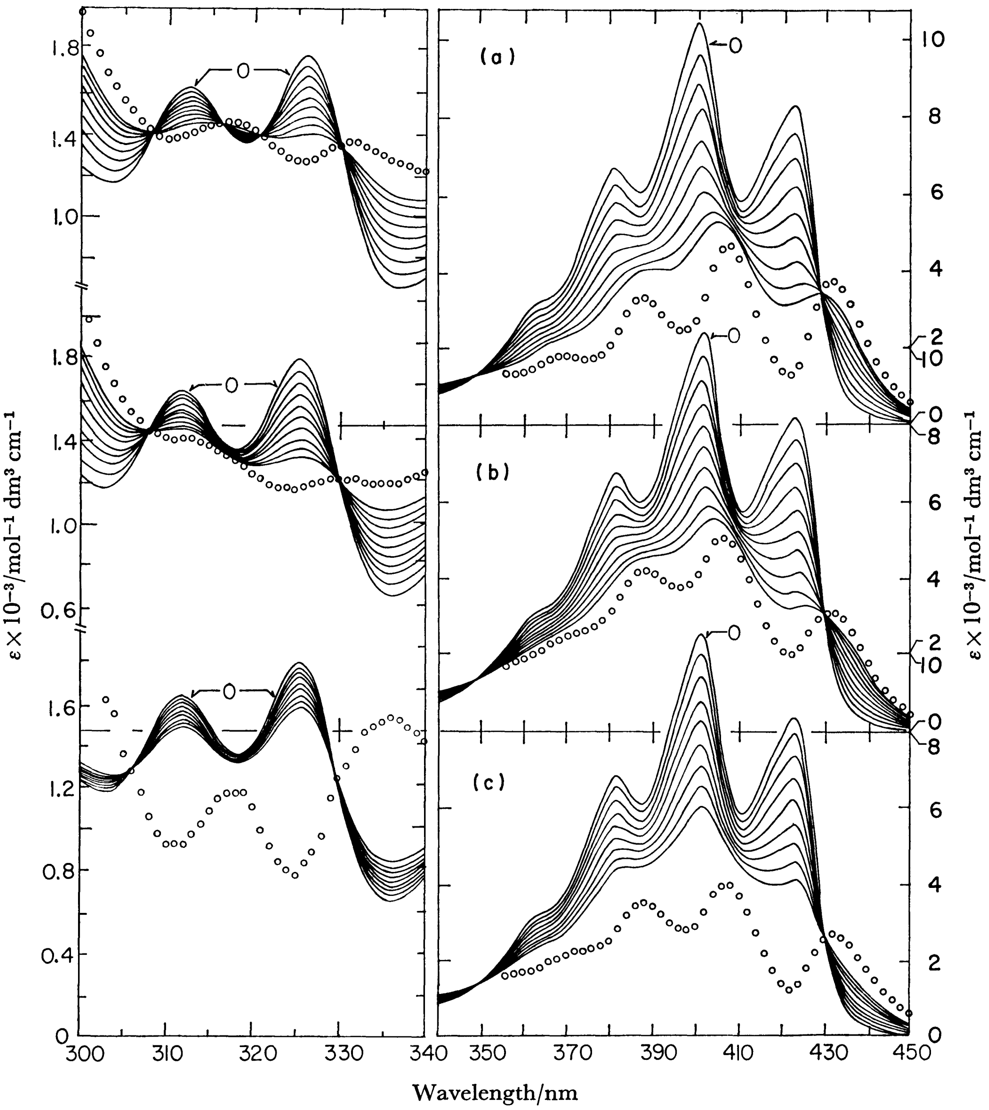

Fig. 1. The observed visible and UV absorption spectra of AA in the presence of (a) NaPSS, (b) NaPP, and (c) NaPA in the *P*/*D* range between 0 and nearly unity as specified below, together with the corresponding bound-dye spectra (circles).
The dye concentrations in mol/dm^3 are: (a) 3.83×10^{-5}, (b) 4.43×10^{-5}, and (c) 3.78×10^{-5}. The pH values of the dye-polyanion systems are in the range of 6—7. In each family of spectra, the *P*/*D* values are in the following order which corresponds to the decrease in absorptions at 401 and 326 nm: (a) 0, 0.122, 0.243, 0.365, 0.486, 0.608, 0.730, 0.851, 0.973; (b) 0, 0.113, 0.226, 0.339, 0.451, 0.564, 0.677, 0.789, 0.902, 1.015; (c) 0, 0.160, 0.320, 0.481, 0.641, 0.801, 0.961, 1.120, 1.280. The isosbestic points in nm are: (a) 429, 349, 330, 320.6, 316, 308.3; (b) 429, 351, 330, 308; (c) 430, 351, 330, 306.

Ultrafiltration. Filtration experiments were performed, whenever needed, to detect the presence of unbound dye in the dye–polymer solutions in a manner similar to a previous case.[7]

Measurements. The absorption spectra were measured at 25 °C in the same manner as before.[2–4] The 5 cm and 2 cm cells were used for the visible and UV regions, respectively.

Data Analysis by the EPCA Procedure. The EPCA method utilized in this work has been described in detail elsewhere.[2–4,22] Since the number of the absorbing components involved in the present dye–polymer system was determined to be two (*vide post*), a generalized empirical expression (Eq. 1) was employed to describe the binding equilibrium between a dye D and a dye–polymer complex PD*:

$$K = \frac{[\mathrm{DP}^*]}{[\mathrm{D}][\mathrm{P}]^{\alpha}}, \qquad (1)$$

where the notations have the same meaning as before.[2] The α is an empirical, adjustable parameter.

Results and Discussion

Visible and UV Absorption Spectra of the AA–Polymer Systems. The observed visible and UV spectra of AA in the presence of NaPSS, NaPP, and NaPA are shown over a *P*/*D* range of 0—1 in Figs. 1(a)—(c). The longest-wavelength band of AA in the 450—350 nm region is the short-axis polarized transition (the

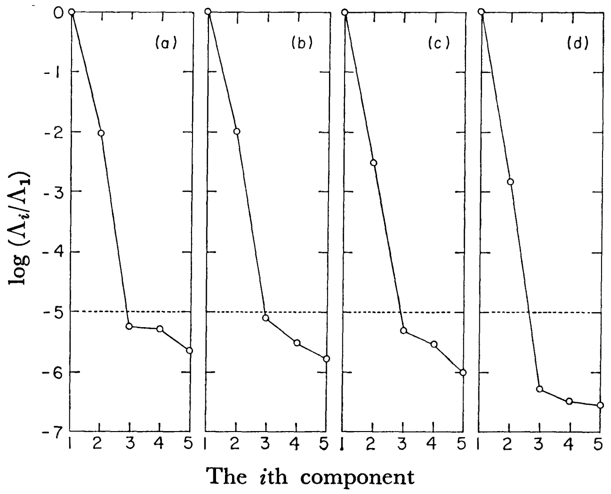

Fig. 2. Variations in the eigenvalue Λ_i ($i=1$, 5) with the *i*th absorbing component for three AA–polyanion systems [(a) NaPSS, (b) NaPP, and (c) NaPA] and for the MeA–NaPSS systems (d).

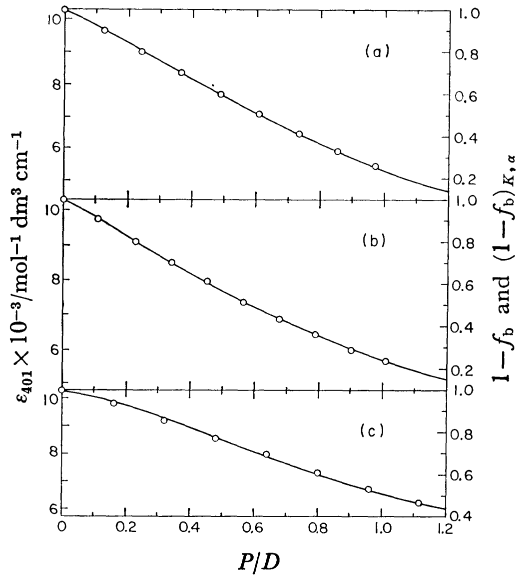

Fig. 3. Optical titration data of three AA–polyanion systems [(a) NaPSS, (b) NaPP, and (c) NaPA] and the fraction of bound AA.

The titration data (open circles) are plotted in terms of ε_{401} (left ordinate) against P/D. The fractions of unbound dye are also plotted against P/D (right ordinate). They were calculated by two different methods; $(1-f_\mathrm{b})_{K,\alpha}$ from the values of K and α according to Eq. 2 of Ref. 3 (it is drawn with the solid curve) and $(1-f_\mathrm{b})$ directly from the EPCA procedure with the aid of Eq. 4 of Ref. 3 (it is actually undistinguishable with the circle).

$^1\mathrm{L}_\mathrm{a}$ band), *i.e.*, the axis is in the direction bisecting the 10-nitrogen and 9-carbon atoms of the acridine nucleus, while the short-wavelength band in the 340–300 nm region is the long-axis polarized band (the $^1\mathrm{L}_\mathrm{b}$ band), *i.e.*, the axis is perpendicular to the short axis in the molecular plane.[14] The $^1\mathrm{L}_\mathrm{a}$ band of AA with a vibrational structure and the $^1\mathrm{L}_\mathrm{b}$ band both decreased batho- and hypochromically by the dropwise addition of any of the three different polyanions. The peaks and shoulders of these bands were shifted toward the long wavelengths, but all fine structure was clearly retained. These spectral changes are contrary to the previous results for the 3,6-disubstituted acridine dye-polymer systems for which the hypso- and hypochromic effects were noted in a P/D range below unity.[2,3,6,8–10,23]

In each family of the spectra in Fig. 1, the isosbestic points (*cf.* the caption of Fig. 1) exist for both the $^1\mathrm{L}_\mathrm{a}$ and $^1\mathrm{L}_\mathrm{b}$ bands. The existence of these isosbestic points strongly indicates that two dye species, *i.e.*, unbound and bound, are present in each dye–polymer system.[2,3] Comparison of these points between three AA–polymer systems also indicates that the spectral profiles of AA bound to three polyanions are similar to one another, in spite of the marked differences in their ionized monomer units and molecular weights. In order to ascertain the number of absorbing species in each system, the PCA procedure was applied to the data spectra given in Fig. 1.

The Number of Absorbing Species in the AA–Polyanion Systems. In each system, the data matrix $\boldsymbol{D}$ was constructed with a set of absorbances at 48 selected wavelengths, at 2 nm intervals, between 356 nm and 450 nm and with a set of ten or nine experimentally obtained absorption spectra. The variation of the *i*th eigenvalue, Λ_i, with the *i*th component is shown in Fig. 2, where the ratios of Λ_i to Λ_1 are plotted on a logarithmic scale against components. It is clear from Figs. 2(a)—(c) that a large gap in eigenvalues exists between the second and third components ($i=2$ and 3) for each AA–polyanion system. In addition, the ratio of the eigenvalue of the third component Λ_3 to that of the first component is at most 1×10^{-5} (the dotted lines), which does not fulfill the criterion previously set for a genuine component.[2,22] These results can be taken as evidence that there are indeed two light-absorbing species in each system, *i.e.*, bound and unbound AA.

Optical Titration Curves and the Fraction of Bound AA. The optical titration curves of the AA–polyanion systems are shown in Fig. 3, where the apparent ε values at 401 nm, the peak position of free AA, were plotted against P/D. The ε values decrease with a slightly sigmoidal curvature with the increase in P/D, as was already noted for other dye–polymer systems.[2,3] To reproduce these titration data, the EPCA procedure was applied to the visible absorption spectra of each system shown in Fig. 1 with the aid of Eq. 1. The values of the parameter α and the equilibrium constant K were evaluated and are given in Table 1. The α values are larger than unity, as is expected from the sigmoidal curvature of the titration data.[2,3] The K' value at $P/D=1$ is a measure which can be compared with the commonly used equilibrium constant for a binding reaction, *viz.*, $K'=[\mathrm{DP^*}]([\mathrm{D}]\cdot[\mathrm{P}])^{-1}=K[\mathrm{P}]^{\alpha-1}$. The K' values are of the same order of magnitude as those obtained for other acridine dye–polyanion systems, indicative of a high binding ability of AA.[2]

The molar fraction of bound dye, f_b, is defined as the ratio of the concentration of bound dye to the

TABLE 1. EMPIRICAL PARAMETER, α, AND EQUILIBRIUM CONSTANTS, K AND K', AT 25 °C FOR THE DYE–POLYANION SYSTEMS

Polymers	Dyes					
	AA			MeA		
	α	K a)	K' b)	α	K a)	K' b)
NaPSS	1.5	1.1×10^8	3.4×10^5	1.5	8.2×10^6	0.31×10^5
NaPP	1.2	3.0×10^6	3.0×10^5	—	—	—
NaPA	1.7	9.2×10^7	0.50×10^5	—	—	—

a) The dimension is $[\mathrm{dm^3\,mol^{-1}}]^\alpha$. b) $K'=[\mathrm{DP^*}]/([\mathrm{D}]\cdot[\mathrm{P}])=K[\mathrm{P}]^{\alpha-1}$ at $P/D=1$.

TABLE 2. BAND PEAKS AND SEPARATIONS BETWEEN SUCCESSIVE PEAKS IN THE SPECTRA OF FREE AND BOUND DYES

Free and Bound Dyes	Bands									
	^1L_a Subbands							^1L_b d) Subbands		
	1		2		3		4	1		2
AA a)	422.5 b)		400.5		381		362	326		312.5
		1.30 c)		1.28		1.37			1.33	
AA-NaPSS	432 b)		408		388		369	331		317.5
		1.36 c)		1.26		1.33			1.29	
AA-NaPP	431.5 b)		406		387.5		370	331		313.5
		1.45 c)		1.18		1.22			1.69	
AA-NaPA	432 b)		407		388		372.5	335		318
		1.42 c)		1.20		1.08			1.60	
MeA a)	440 b)		415		397		—	357		342
		1.37 c)		1.09		—			1.23	
MeA-NaPSS	451 b)		425		405		—	362.5		346
		1.36 c)		1.16					1.31	

a) Free dye as the reference. b) The values in this row are the peak wavelength expressed in nm. c) The values in this row are the separation of the successive subbands expressed in (wave number) $\times10^{-3}$ (cm^{-1}). d) The positions of this band are only apparent, because of the overlap by an intense band in the shorter wavelength region.

sum total of the concentrations of bound and free dyes in solution. The values of $(1-f_b)$ were calculated by two different methods, which are described in detail in Ref. 3 (*cf.* Eqs. 2 and 4 given therein). The calculated $(1-f_b)$ curves are shown for three AA–polyanion systems in Fig. 3. It is clear from Fig. 3 that AA binds to these polyanions by 50% or more at a P/D of unity in spite that AA is rather simple and small in molecular size as compared with TF.2)

Visible and UV Spectra of Bound AA. A major advantage of the EPCA method is the simultaneous determination of the bound-dye spectrum and the equilibrium constant for a given dye–polymer system.2–4,22) The pure spectra of bound AA in three AA–polyanion systems, extracted from the observed absorption spectra in the visible region, are shown by open circles in Figs. 1(a)—(c). Since the changes in the observed UV spectra of the same systems are not large enough for the EPCA method to be applied with high accuracy, the bound-AA spectra in the UV region were calculated by the use of the value of f_b, which was obtained from the binding curve $(1-f_b)$, and the observed ε_λ at a given wavelength λ. The molar absorption coefficient of the bound dye, ε^b_λ, at the same wavelength can be expressed as

$$\varepsilon^b_\lambda = \varepsilon^f_\lambda + \frac{\varepsilon_\lambda - \varepsilon^f_\lambda}{f_b}, \tag{2}$$

where ε^f_λ is the molar absorption coefficient of the unbound dye at λ.

The unmasked bound-AA spectra reveal a number of interesting points. First, they all show a large bathochromic, but not hypsochromic, shift. As the spectral data in Table 2 indicate, the ^1L_a bands are shifted by 10 nm toward the red. Secondly, both ^1L_a and ^1L_b bands of the bound spectra retain the spectral profile of the original AA as regards the vibrational structure and the separation of successive subbands. In particular, no new band appears in the bound-AA spectra. Thirdly, the ^1L_a and ^1L_b bands of the bound spectra are both hypochromic, *i.e.*, the intensity of AA is decreased tremendously by its binding to a polymer site. The first and second features are in contrast with the definition of metachromasy11) and also with the observed metachromatic behavior of the 3,6-diaminoacridine and its derivatives which

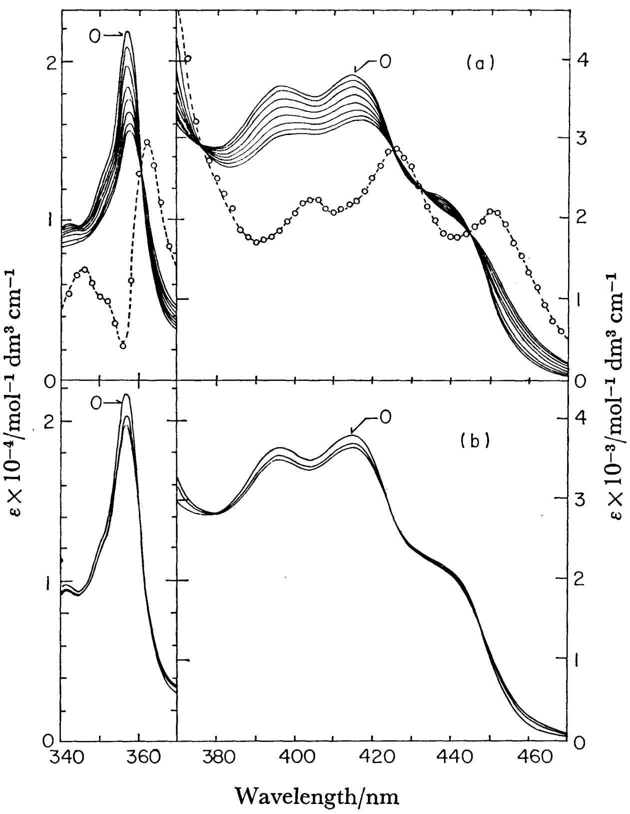

Fig. 4. The observed visible and UV absorption spectra of MeA in the presence of (a) NaPSS and (b) NaPP in the P/D range between 0 and nearly unity as specified below, together with the corresponding bound-dye spectrum (circles).

The dye concentrations are 2.20×10^{-5} mol dm^{-3} for both (a) and (b). The isosbestic points in nm are 445, 430.5, 426, 376.5, 360.5 in (a). The P/D values are in the following order which corresponds to the decrease in absorptions at 357 and 415 nm: (a) 0, 0.171, 0.342, 0.513, 0.685, 0.856, 1.027, 1.20.

are bound to synthetic polyelectrolytes in the low P/D range.[2,3,24]

Visible and UV Absorption Spectra of the MeA–Polyanion Systems. The observed visible and UV absorption spectra of MeA in the presence of NaPSS and NaPP are shown in Figs. 4(a) and (b), respectively. The absorption band of MeA in the 470—370 nm region is the short-axis polarized transition (^1L_a), while the band in the 370—340 nm region is the ^1L_b band.[12,13] In contrast with the cases of AA, the spectra of MeA changed only moderately by the dropwise addition of NaPSS and only silghtly with NaPP. It should be noted that the spectra of the MeA–NaPA system changed less than 10% in the P/D range between 0 and near 2. This trend is peculiar in the sense that all three polymers can be bound to AA in very favorable proportions and that the chemical structure of MeA does not seem to differ from that of AA. Thus, it appears that the observed discrepancy should be accounted for by some other reasons.

The ultrafiltration of both the MeA–NaPP and –NaPA solutions at a P/D value near 3 was performed. The filtrated solutions all showed the absorption spectrum characteristic of MeA free from either NaPP or NaPA. This result thus leads to the conclusion that most of the MeA cations in the NaPP or NaPA solution remain unbound and are filterable through a semipermeable membrane. Under considerations of the degree of polymerization of these polymers (see Experimental), this conclusion can not be explained by the size (or chain length) effect. Hence, it is most reasonable to attribute the difference in binding abilities to the geometry and the chemical properties of the ionized binding site of the polymer (sulfonate *versus* phosphate and carboxylate residues).

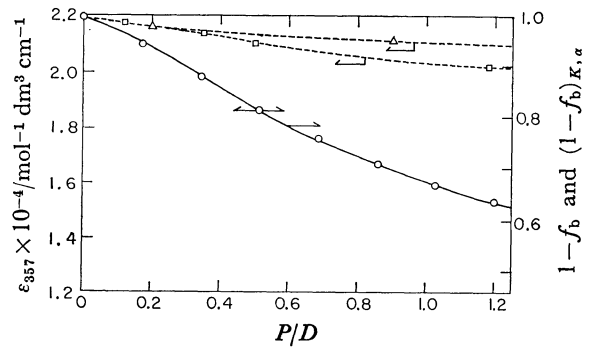

Fig. 5. Optical titration and the fraction of bound MeA.

The titration data (open symbols) are plotted with ε_{357} (left ordinate) against P/D.

(○) NaPSS, (□) NaPP, (△) NaPA. The fraction of unbound MeA was calculated only for the MeA–NaPSS system in the same manner as noted in Fig. 3.

The experimental spectra of MeA–NaPSS system are, however, sufficient to reveal the spectral behavior of the bound MeA. As the P/D values increase, the intensity of both the ^1L_a and ^1L_b bands of MeA decreases with a concomitant shift toward the red, similarly to the case of the AA–NaPSS system. The result of the PCA treatment is shown in Fig. 2(d), where a large gap exists between the second and third components. Hence, the MeA–NaPSS system is indeed verified to contain two absorbing species, bound and unbound MeA.

Optical Titration, Fraction, and Spectrum of MeA Bound to NaPSS. The optical titration data of the MeA–polyanion systems are shown in Fig. 5, where the apparent ε values at 357 nm, a peak wavelength of free MeA, were plotted against P/D. The ε values of MeA in the presence of NaPSS decrease sigmoidally, which is in accord with an α value larger than unity. The fractions of MeA bound to NaPSS, $(1-f_b)_{K,\alpha}$, were calculated with the values of α and K given in Table 1. It is clear from Fig. 5 that more than 30% of MeA are bound to NaPSS at $P/D=1$ and that the calculated curve agrees well with the observed ε values. This latter result assures us that the EPCA procedure was applied properly. As are shown in Fig. 5, the changes in ε values with P/D are very small for the MeA–NaPP and –NaPA systems; for these, therefore, the α and K values could not be evaluated by the EPCA method.

The visible spectrum of MeA bound to NaPSS was extracted from the observed spectra, while the UV spectrum of the bound MeA was calculated with Eq.

2 from the f_b values in Fig. 5. The bound spectrum of MeA (circles in Fig. 4) is again, as in the case of bound AA, bathochromic and hypochromic relative to the unbound MeA. The optical data are given in Table 2, which shows that the separations of the successive peaks of the visible band (^1L_a) remain nearly constant for each of the unbound and bound MeA species. Thus, it can be concluded that the bound MeA retains the spectral features of unbound MeA itself showing neither a new "metachromasy band" nor metachromatic behavior, *viz.*, the blue shift of the absorption bands, in the presence of NaPSS.

Closing Remarks on Bound-dye Spectra and Binding Ability of AA and MeA. The bound-AA and -MeA spectra should result from the dyes which are bound to polymer sites to a maximal extent, since they are extracted in the P/D range between 0 and near unity. Yet, the AA and MeA spectra bound to various synthetic polyanions show the bathochromic and hypochromic changes, *i.e.*, both the short-axis and long-axis polarized bands shift toward the red rather than the blue. This trend is evidently opposite to the previous results obtained for 3,6-diaminoacridines, TF[2] and AO,[23] and contradicts the widely prevailing notion of metachromasy, *i.e.*, the shift of the visible absorption maximum toward the blue with a concomitant decrease in its intensity.[11] The spectrum of MeA bound to NaPSS (Fig. 4) is surprisingly close to that of MeA bound to DNA, in spite of a vast difference in their primary and secondary structures.[21] Hence, the bound-AA and -MeA spectra are rather insensitive to the structure of the ionizable groups of polymers and their overall conformations. The apparent equilibrium constants are in the order of NaPSS>NaPP>NaPA for AA, and NaPSS>NaPP≈NaPA for MeA. As regards the dye species, the fraction of bound AA is about equal to that of previously reported TF[2] at a P/D value of unity, but much higher than that of MeA, which is the simplest of all acridine dyes. The binding reactions between both AA and MeA and three polyanions seem to resemble the previous cases in which TF and Crystal Violet were employed,[2,3] although spectral changes of the former differ from those of the latter. Hence, it may be inferred that the binding mechanism formally expressed by Eq. 1 prevails in many dye–polymer combinations.

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