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V. Crescenzi[a]; F. Quadrifoglio[a]; V. Vitagliano[a]
[a] CENTRO NAZIONALE DI CHIMICA DELLE MACROMOLECOLE (CNR) SEZIONE III ISTITUTO CHIMICO UNIVERSITÀ DI NAPOLI, NAPLES, ITALY

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Interaction of Crystal Violet and Polyanions in Aqueous Solution

V. CRESCENZI, F. QUADRIFOGLIO, and
V. VITAGLIANO
CENTRO NAZIONALE DI CHIMICA DELLE MACROMOLECOLE (CNR) SEZIONE III
ISTITUTO CHIMICO
UNIVERSITÀ DI NAPOLI
NAPLES, ITALY

Summary

The effect of several polyelectrolytes on the absorption spectrum of crystal violet (CV) has been studied over a wide range of pH. The following polymers were used: isotactic poly(methacrylic acid), PMA_i; conventional poly(methacrylic acid), PMA_c; a methacrylic acid (80%)-styrene (20%) copolymer, PMAS; and poly(styrene sulfonic acid), PSSA. Distinctly different effects were observed in the four cases. This has been interpreted in terms of a dependence of the degree and mode of binding of cationic dye molecules onto the polyacids on the hydrophobicity of the macroions.

The results of some measurements of the kinetics of CV fading at pH values between 9.7 and 10.3 are in qualitative agreement with the spectral indications.

Detailed studies of the interaction of cationic dyes with a number of polyacids in aqueous solution have yielded many interesting results. These results were shown to provide useful information on the distribution of charges along polymeric chains as well as on the conformation of these chains in dilute solution (*1*–*6*).

We wish to report here the results of an investigation carried out in this laboratory on the interaction of crystal violet, a cationic dye, with three polyelectrolytes, poly(methacrylic acid), a methacrylic acid-styrene (4:1) copolymer, and poly(styrene sulfonic acid). Isotactic and conventional samples of poly(methacrylic acid) were used.

Spectrophotometric measurements on crystal violet solutions in the presence of the polyelectrolytes, for a wide range of pH values, have revealed that the three polyanions studied exhibit quite different binding properties toward the cationic dye. Particularly interesting is the difference in dye binding which has also been found for isotactic and conventional (or syndiotactic) PMA. The differences in the binding of the cationic crystal violet cannot be attributed only to differences in charge density of the polyelectrolytes. It is presumed that hydrophobic interactions between dye molecules and the macroions chain, of particular relevance in the cases of the methacrylic acid-styrene copolymer and of poly(styrene sulfonic acid), would account for the observed effects.

At pH values greater than 9, crystal violet $(CV)^+$ undergoes the well-characterized reaction (*7,8*):

$$(\mathrm{CV})^+ + \mathrm{OH}^- \rightarrow \mathrm{carbinol}$$

As expected, binding of the dye onto polyanions results in an inhibition of the reaction (*9*).

Data on the rate of fading of crystal violet in alkaline solution in the presence of the polyelectrolytes are here shown to correlate with spectrophotometric dye binding results.

EXPERIMENTAL

Materials

BDH standard stain crystal violet was used without further purification. Isotactic poly(methacrylic acid) ($\bar{M}_v = 250.000$), conventional poly(methacrylic acid) ($\bar{M}_v = 260.000$), and styrene-methacrylic acid copolymer (1:4) were synthetized and purified in our laboratory. Preparation and characterization techniques have been described elsewhere (*10*). Poly(styrene sulfonic acid) was obtained by sulfonation of poly(styrene) (Bios product, $\bar{M}_v = 160.000$), as already reported (*11*). The degree of sulfonation was 0.88.

Spectrophotometry

Spectrophotometric measurements were carried out in the visible region recording the absorption spectra of CV ($\sim 10^{-5}$ *M*) in the presence of 2×10^{-3} *N* polyelectrolyte and 6×10^{-3} *N* NaCl (ratio polymer to dye P/D = 200). The degree of neutralization of the polyelectrolyte was varied by adding with a microsyringe proper amounts of concentrated NaOH directly into the cell. A Beckman DK2 spectrophotometer with a thermostated cell holder (25°C) was used.

Kinetics

Some kinetic measurements were made to compare the fading of buffered crystal violet in the absence and in the presence of the polyelectrolytes. All runs were carried out in phosphate buffer (ionic strength $= 6 \times 10^{-3}$). The polyelectrolyte concentration was 2×10^{-3}, the initial dye concentration 2×10^{-5}. The kinetics of the fading process were followed at 25 ± 0.1°C with a Beckman DU spectrophotometer, following the optical density of the solution at 590 mμ. The pH of the solutions was measured in the spectrophotometric cell before and after the runs. The accepted changes in pH were below 0.05 unit. The range of pH explored was between 9.7 and 10.3. First-order kinetics were observed in all cases.

RESULTS

In Fig. 1 the spectrum of CV in water for three dye concentrations is reported. The concentration dependence of the crystal violet spectrum is typical of that exhibited by a number of dyes (*2,12–15*). In analogy with the interpretation of the spectra of other dyes, it is here supposed that crystal violet absorption at 590 mμ (α-band) is due to the monomeric form of the dye, while the band with maximum at 540 to 545 mμ (β-band), which develops in more concentrated solutions, is attributed to aggregate CV (dimers). Our data also suggest that dimerization of CV in water is much less extensive than that occurring in solutions of other dyes.* Absorption spectra of crystal violet in aqueous solution in the presence of PMA_c, PMA_i, PMAS, and PSSA, respectively, for various degrees of neutralization of the polyacids, are reported in Figs. 2 to 5. Considering the whole set of spectra in Figs. 1 to 5, it appears that binding of CV to polyanions may be at least qualitatively treated in a way similar to that used in the interpretation of the binding of other dyes—in terms of monomeric and aggregated bound dye (*2,16,17*). A comparison of the spectra also clearly suggests that

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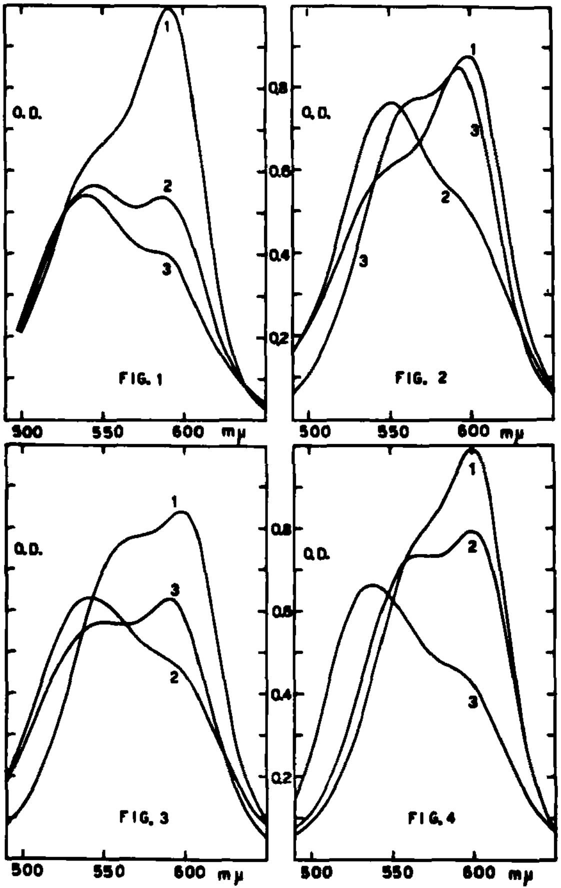
* This is probably to be attributed to the nonplanar character of CV molecules, which might impede efficient stacking of the dye molecules (25).

interaction of CV with PMA and PMAS must be distinctly different in nature and extent from that which is established with PSSA. The latter case therefore will be briefly discussed separately.



FIGS. 1–4. Figure 1 shows the crystal violet visible spectrum in water. Dye concentration (moles/liter): curve 1, 1×10^{-5}; curve 2, 8×10^{-4}; curve 3, 2×10^{-3}. Curves 2 and 3 are only indicative, the spectra having been recorded on a thin film of the solutions held between two microscope slides. Figures 2 to 4 show the crystal violet spectrum in poly(methacrylic acids) solutions for different degrees of neutralization of the polyacids. Concentrations: dye, 1×10^{-5} mole/liter; polyacid, 2×10^{-3} *N*; NaCl, 6×10^{-3} *N*: curve 1, $\alpha = 0.05$; curve 2, $\alpha = 0.50$; curve 3, $\alpha = 0.90$. Fig. 2, conventional poly(methacrylic acid); Fig. 3, isotactic poly(methacrylic acid); Fig. 4, methacrylic acid–styrene copolymer (4:1).

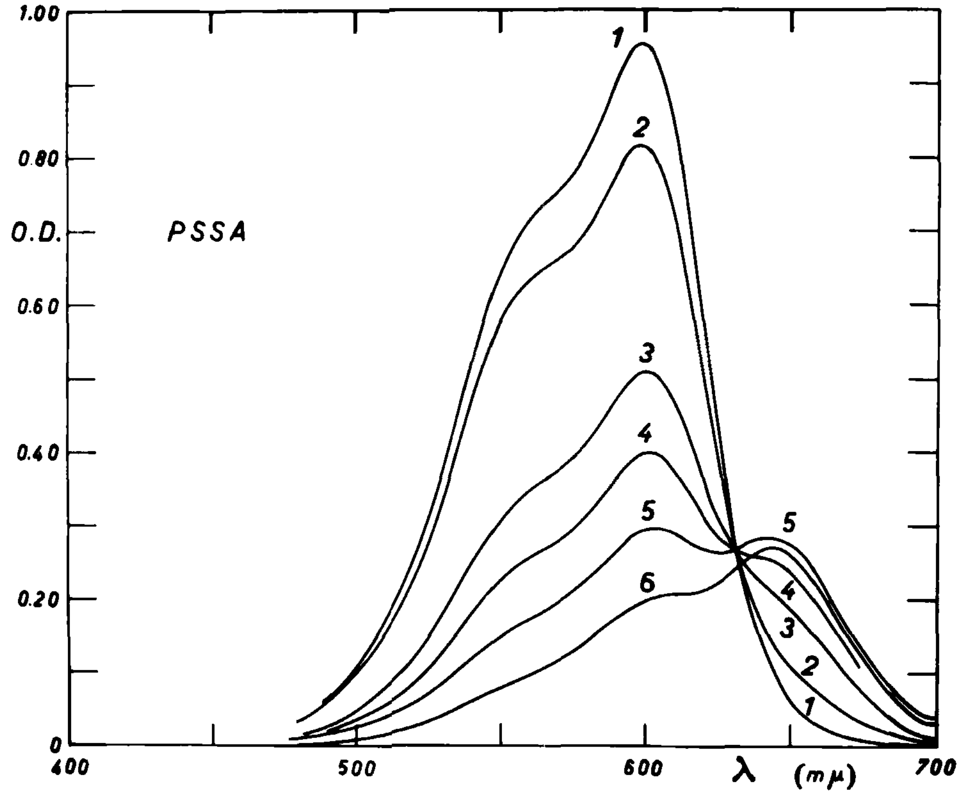


FIG. 5. Dependence of crystal violet spectra in poly(styrene sulfonic acid) solutions upon the degree of neutralization, α, of the polyacid. Concentrations: dye, 1×10^{-5} mole/liter; polyacid, 2×10^{-3} N; NaCl, 6×10^{-3} N: 1, $\alpha > 1$; 2, $\alpha = 0.90$; 3, $\alpha = 0.80$; 4, $\alpha = 0.70$; 5, $\alpha = 0.50$; 6, $\alpha = 0.10$.

Interaction of Crystal Violet with PMA_c, PMA_i, and PMAS

At very low degrees of neutralization of the three polyacids CV exhibits an absorption band similar to that in dilute water solution (α-band) but with a red shift of the maximum by about 10 mμ. In analogy with the behavior of other dyes (*2,6,17*), we may assume that this band is attributable to dye molecules associated in monomeric form with the polymeric coils. In the case of PMAS there is some evidence (Fig. 6) of a hyperchromic effect similar to that observed for acridine orange bound to PMA at very low degrees of neutralization (*6*).

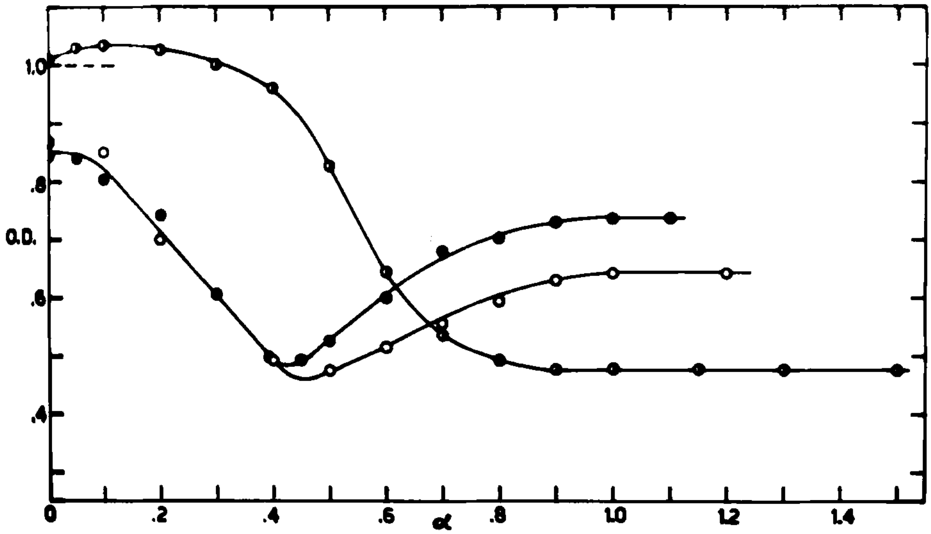


FIG. 6. Dependence of the absorbance at 590 mμ (maximum absorption of monomeric bound dye) of crystal violet in polyacids solutions upon the degree of neutralization, α, of the polyelectrolyte. Same experimental conditions as in Figs. 2 to 4. ◑, Methacrylic acid–styrene copolymer (4:1); ●, conventional poly(methacrylic acid); ○, isotactic poly(methacrylic acid). Dye concentration, 1×10^{-5} mole/liter. To correct slight differences in concentration among the three sets of data, all optical densities have been normalized, using Beer's law, to the same value (1.0, marked on the ordinate) for the optical density of free CV.

Increasing polyacid neutralization, in the range $\alpha = 0.1$ to 0.3 for PMA_c and PMA_i and in the range $\alpha = 0.4$ to 0.7 for PMAS, the macroions undergo a cooperative transition from compact coiled structures to open, more solvated conformations (*18,19*). Simultaneously, a very strong change is observed to take place in the absorption spectrum of the dye, consisting in a flattening of the band and a blue shift of the maximum by about 40 to 50 mμ. This new absorption band is similar to that of many metachromatic dyes in the presence of polyelectrolytes and has been interpreted as being due to a dimeric form of bound dye molecules (or, more generally, to aggregate dye molecules stacked along the macroion chains) (*2,16,17*).

With a further increase on neutralization ($\alpha > 0.5$ to 0.6) for PMA_c and PMA_i, dye molecules are released from the polyions and set free in solution.* This can be seen in the spectra by the appearance of the free dye absorption band. These features are more easily seen in Fig. 6, in which the optical density at a wavelength corresponding to the maximum of monomeric dye is plotted as a function of the degree of neutralization for PMA_c, PMA_i, and PMAS. The results indicate that the release of CV by the polyelectrolytes, at α-values greater than 0.5, is more limited for PMA_i than for PMA_c and that in the case of PMAS no release would take place even beyond full neutralization. This effect, which is contrary to what would have been expected in terms of simple charge-density considerations, emphasizes the importance of nonpolar interactions (*1*,*6*) between dye molecules and vinyl chains in aqueous solution. On this basis one can also suggest that isotactic PMA is more hydrophobic than its conventional counterpart.

The amount of dye released in PMA solution is furthermore noticeably increased by increasing the ionic strength. This is illustrated in Fig. 7 for the case of PMA_c. This fact is attributable to a screening effect on the fixed charges of macroions exerted by the added salt.

Interaction of Crystal Violet with PSSA

The spectra reported in Fig. 5 indicate that interaction of CV with PSSA chains is decidedly stronger than with the other polyelectrolytes considered here. This is consistent with the high charge density on PSSA macroions at all α-values and, in particular, with their highly hydrophobic character.

At low degrees of neutralization of PSSA, the pH is low enough to promote second ionization of CV (*20*) and thus to permit observation of the spectrum of the CV^{2+} ion. By increasing α the equilibrium $CV^{2+} \rightleftharpoons CV^+$ is shifted to the right, and the dye spectrum accordingly changes. It is interesting to note that at high α-values only dye bound in the monomeric state can be observed, as clearly shown by the spectra of Fig. 5. This may be taken as an indication

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* An independent proof of the existence of free dye molecules at pH > 7 has been obtained by dialyzing two samples of PMA_c + CV (50 ml) in phosphate buffer at pH 5 and 7.6 against (250 ml) buffer of the same pH (ionic strength 6×10^{-3}). After 3 days no dye was present in the buffer at pH 5. In the buffer at pH 7.6 the dye concentration was approximately one-half the concentration inside the dialysis bag (absence of polymer leakage was controlled) (inside conc. $\sim 10^{-5}$ *M*).

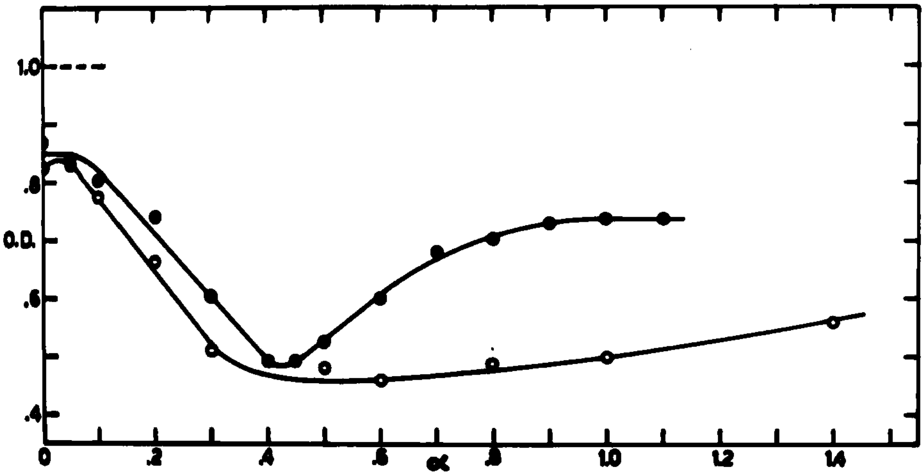

FIG. 7. Dependence of the absorption at 590 mμ of crystal violet in conventional poly(methacrylic acid) solution upon the degree of neutralization, α, of the polyelectrolyte. ○, 2×10^{-3} *N* polyacid; ●, 2×10^{-3} *N* polyacid, 6×10^{-3} *N* NaCl. Dye concentration, 1×10^{-5} mole/liter.

of the tendency of dye molecules to stack together with the phenyl rings along the PSSA chain. In contrast, dye-dye stacking is a common phenomenon in the case of other vinyl polyelectrolytes of high charge density (see also the spectra of Figs. 2 to 4).

Fading of Crystal Violet in Polyelectrolyte Solutions

It has been clearly indicated that a polymer may affect the reaction of two small molecules in aqueous solution by forming a complex with one of the reagents and thus altering its reactivity (*9*,*21*). A very interesting example illustrating this principle is the change in reactivity of triphenylmethane dyes with reducing agents brought about by the association of the cationic dyes with polyacids (*22*,*23*).

Another typical reaction which triphenylmethane dyes undergo in aqueous solution is the carbinol formation or fading reaction (*7*,*8*). The course of CV fading, $CV^+ + OH^- \rightarrow$ carbinol, in aqueous solutions has been studied in the past and the effect of added salts (*8*) or added colloidal electrolytes on the reaction kinetics has been throughly investigated (*24*). In the light of previous evidence (*21*) about the capability of macroions to affect the reaction of two small oppositely charged ions by forming a complex with one of the reagents and by repelling the other (inhibition), it was expected, in addition, that the kinetics of the hydroxyl-ion catalyzed fading of CV^+ ions would be inhibited in polyacid solutions.

Considering that the apparent pK of a polymeric acid in the fully charged form at low ionic strength is about 4 units higher than for monomeric analogs, the OH^- concentration in the neighborhood of the chain should be about 10^4 times lower than in the bulk of the polyacid solution. Thus the contribution of the bound CV to the over-all rate of fading would be expected to be negligible, if any CV is free.

Therefore, kinetics data and the extent of binding should, in principle, correlate. Some experimental data on the fading of CV in polyacid solutions are reported in Table 1. The results are the average of several runs carried out within the pH range 9.7 to 10.3.

The small number and the limited accuracy of the kinetic data reported here do not allow a quantitative correlation between inhibition of dye fading and extent of dye binding. It is interesting, however, to show how the above-mentioned correlation is clearly, although qualitatively, indicated by our data. Let K^0 and K be the second-order rate constants for CV fading in buffer solution and in polyacid buffered solutions, respectively. For reasons explained above, bound CV^+ ions may be considered as unreactive, while the specific rate constant for the fading of free CV^+ ions would be K^0.

TABLE 1

Rate Data at 25°C for the Reaction $CV^+ + OH^- \rightarrow$ Carbinol in Buffer Solution and in Polyelectrolyte Buffered Solutions

Solution	Unimolecular rate/[OH^-] (min^{-1}/mole)[a]
Buffer[b]	21 ± 0.9
PMA$_c$[c]	9.1 ± 0.8
PMA$_i$	6.5 ± 1.2
PMAS	2.4 ± 1.2
PSSA[d]	0

[a] In all cases first-order rate constants were found to be proportional to [OH^-] within the limits of experimental error.

[b] Phosphate buffer; approximate ionic strength 6×10^{-3}.

[c] 2×10^{-3} eq/liter of polyelectrolyte + buffer as in [b].

[d] No appreciable fading even after 24 hr.

Experimental values of K and K^0 might then be related in the following simple way: $K/K^0 = C_f/C_t$, where C_f and C_t are the concentrations of free and total CV. Assuming, from the data of Fig. 6, an optical density of 0.4 for CV in the bound form and an optical density of 1.0 for free CV^+ ions, the ratio C_f/C_t would then be 0.48 and 0.32 in PMA_c and in PMA_i solutions, respectively (at the pH of the kinetic runs). Correct magnitudes for the observed ratio of the kinetic constants are predicted by these figures. In fact, K/K^0 values, as derived from the data of Table 1, are 0.43 and 0.31 in the two cases considered. Analogous calculations in the case of PMAS are meaningless because of the low amount of free dye, which makes very critical the choice of the optical density value for bound dye. For PSSA the lack of reaction in 24 hr proves that the rate constant is at least 100 times slower than in buffer.

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Zusammenfassung

Der Einfluss verschiedener Elektrolyte auf das Absorptionsspektrum von Kristallviolett (CV) wurde über einen weiten pH-Bereich untersucht. Die folgenden Polymere wurden benutzt: isotaktische Polymethacrylsäure (PMA$_i$), handelsübliche Polymethacrylsäure (PMA$_c$), Methacrylsäure (80%)-Styrol (20%)-Copolymerisat (PMAS), und Polystyrolsulfonsäre (PSSA). Deutlich verschiedene Effekte wurden in diesen vier Fällen beobachtet. Die Interpretation dieser Effekte basierte auf der Abhängigkeit des Bindungsgrades und der Bindungsart des kationischen Farbstoffmoleküls mit der Polysäure von der Hydrophobizität des Makroions.

Die Resultate einiger Messungen über die Kinetik des Ausbleichens des CV in pH-Bereichen von 9.7 bis 10.3 sind in qualitativer Übereinstimmung mit den spektralen Befunden.

Résumé

On a étudié l'effet des plusieurs électrolytes sur le spectre d'absorption du violet crystallisé (VC), dans un large intervalle du pH. On a employé les polymères suivants; l'acide polyméthacrylique isotactique, APM$_i$; l'acide polyméthacrylique ordinaire, APM$_c$; un copolymère d'acide méthacrylique (80%) et de styrène (20%), PAMS; et l'acide polystryènesulfonique, APPS. Des effets nettement différents sont observés dans ces quatre cas. Ce phenomène est interpreté sous forme d'une relation entre le degré et le mode d'attachement des molécules de la teinture aux polyacides et la nature hydrophobe des macroions.

Les résultats de quelques mesures descinetiques de la disparition progressive du VC aux valeurs de pH comprises entre 9.7 et 10.3 sont en accord qualitatif avec les indications spectrales.