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Metachromatic behavior of methylorange in the presence of ionenes

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Abstract: The addition of different ionenes, polycations with defined structure and charge distances, to methylorange **1** in very dilute solutions of water resulted in an observable shift of the absorption maxima from 464 nm to shorter wavelengths. The extent of this so-called metachromasy effect was found to be dependent on the nature of the hydrophobic unit of the polymer backbone. The comparison of UV/VIS spectra's of these solutions with the absorption of a film of a model substance – a dication with methylorange as counteranion – lead to the conclusion that ionenes undergo an exchange of the counteranions when added to a dilute solution of methylorange. Precipitation is slowed down, but the absorption is equivalent with solid product. The new absorption spectra of methylorange can be explained by the interaction of the quaternary nitrogen with the dye anion.

Key words: Aromatic and aliphatic ionenes – metachromasy – absorption – UV - VIS spectroscopy

1. Introduction

Since the discovery of metachromasy in the 19th century many research groups have studied this phenomena [1, 2]. Although metachromasy was originally observed on staining tissue sections with several dyes, this effect could also be shown by these dyes in an aqueous solution in the presence of synthetic polyelectrolytes of opposite charge. The most commonly used systems comprised cationic dyes and anionic polyelectrolytes. However, despite the fact that Schubert and Hamerman [3] stated that all known chromotropes are anionic, several anionic dye/cationic polyelectrolyte systems have been reported. Spectral changes as bathochromic or hypsochromic shifts were reported for dyes such as Congo Red, and Eosin. Gummow and Roberts [4, 5] examined especially the effect of C.I. Acid Orange and C.I. Acid Red on modified chitosan as a cationic polyelectrolyte.

Several authors examined the hyposochromic shift of the absorption maxima of the anionic dye methylorange **1** in the presence of cationic polyelectrolytes. Two models are discussed in the published literature. One reason for the observed effect of **1** in the presence of polyelectrolytes is the formation of dimers and higher aggregates of the dye at adjacent sites on the polyelectrolyte [6]. Dye ions are preferably bound at a site adjacent to an already occupied site. This preference has been attributed to the decrease in energy arising from dye/dye interactions, and these interactions have been suggested as the driving force behind the dye-ion binding [7]. Takagishi et al. described that more dye molecules are bound to the polyelectrolyte by increasing the size of hydrophobic parts R_1 of **2**, or by increasing the chain length of the dialkylamino groups of **1** [8, 9].

A second hypothesis describes the phenomena of metachromasy by a mechanism that does not involve aggregation of dye ions bound to the polyelectrolyte [10]. In aqueous solutions of polyelectrolytes the counterions are not distributed uniformly, but enriched in the electrostatic domain of the polyelectrolyte. The addition of dyes results in a high concentration of these molecules in this sphere and an aggregation of the dye ions. This aggregation lowers the extent of the hydrophobic surfaces of the dye ions and water. In addition, it results in an increase of the entropy of the system. Typical absorption maxima of **1** are determined between 358 and 377 nm when metachromasy has reached its full extent. This report describes a systematical investigation to clarify the correlation between charge density, chain flexibility, hydrophobic part, and water solubility of aliphatic and aromatic ionene polyelectrolytes and the shift of the absorption band of **1**.

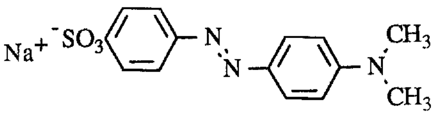
2. Experimental

A spectrometer from Perkin-Elmer Lambda 3 and Lambda 9 was used for recording of absorption spectra. The maxima was determined in a 10 mm cuvette in the range between 300 and 600 nm with a speed of 120 nm/min.

The used ionene polyelectrolytes are those described in an earlier publication [11]. Methylorange **1** was recrystallized two times and dissolved in bidistilled water at a concentration of 10^{-4} mol/l.

To 1 l of bidistilled water with 10^{-4} mol of **1** a solution of the desired polyelectrolyte was added in steps of 1 ml. The concentration of the polyelectrolyte in bidistilled water was calculated to compensate after the addition of 10 ml of the theoretical amount of opposite charges in 1 l of **1**. After each step the absorption spectrum was recorded and the maximum and the shift calculated.

2.1 *Formulae of substances*



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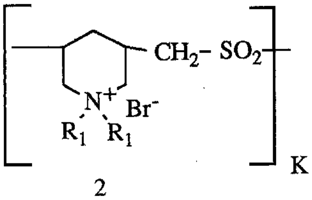

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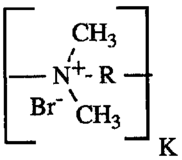
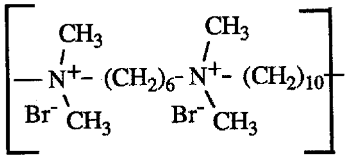


R =

$-(CH_2)_3-$	3
$-(CH_2)_4-$	4
$-(CH_2)_5-$	5
$-(CH_2)_6-$	6
$-(CH_2)_8-$	7
$-(CH_2)_{10}-$	8



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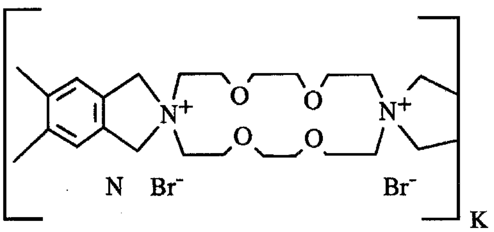

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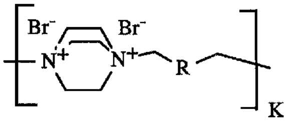
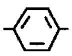


R =	$-(CH_2)-$	$-(CH_2)_2-$	$-(CH_2)_3-$	$-(CH_2)_4-$	$-(CH_2)_6-$	$-(CH_2)_8-$
	11	12	13	14	15	16



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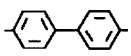

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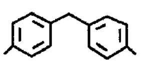

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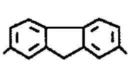

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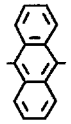

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3. Results and discussion

3.1. *Metachromasy induced by ionenes with dabco units* 11–21

The use of diazabicyclo [2.2.2] octane (dabco) as part of the polymer backbone enabled us to synthesize polycations with a defined charge distance and a sufficient solubility in water. Ionene with aromatic units, but without dabco are not watersoluble [11]. As a result we could investigate ionene with variable flexible aromatic units.

Figure 1 describes the absorption spectra of **1** at pH 1 and pH 14. The addition of HCl results in a change of color from orange to red. The reason for this behavior is the protonation of the diazo group [12]. All metachromasy experiments were carried out at pH 6 where the spectrum is identical with that of pH 14.

The addition of different amounts of **16** to **1** results in a hypsochromic shift of the absorption maxima from 464 to 383 nm (Fig. 2). The maximum of this shift is achieved at a polymer ionene/dye ratio of 1.15 (Fig. 3). Addition of more ionene **16** provides no further change in the absorption spectra. At approximately 400 nm an isosbestic point can be observed. This point can be regarded as a hint of a linear correlation between two species existing in the solution. This species could be free **1** and ionene-bound **1** existing in an equilibrium which depends on changes of the ionene concentration.

A comparison of the maxima of the metachromasy induced shift of **1** in the presence of the ionenes **11** to **16** is illustrated in Fig. 4a, and the effect of ionenes **17** to **21** on **1** can be compared in Fig. 4b.

Each of the ionene polymers employed showed a characteristic effect of the absorption spectra of **1**. This could be demonstrated by repeating several of the experiments. Even the addition of two ionene into the same solution of **1**, as for example, done with **16** and **15** (metachromasy maxima are found at 383 and 363 nm) has resulted in a maximum of 372 nm which is between the maxima of the ionenes. Based on this experience it can be said that shifts induced by the ionene **11** to **21** seem to depend on the nature of the backbone. But no statements about the effect of molecular weight and molecular weight distribution can be made, because of the lack of analytical methods for molecular weight determination.

3.2. *Metachromasy induced by ionenes* 3–10

Aliphatic ionenes are well known and can easily be synthesized according to the literature [11]. With this group of polycations the charge distance of the nitrogen cations on the polymer chain is identical in both directions. But the ionenes **11**–**21** exhibit alternating charge distances. If proximity of charges on the backbone would play an important role, an effect should be seen using

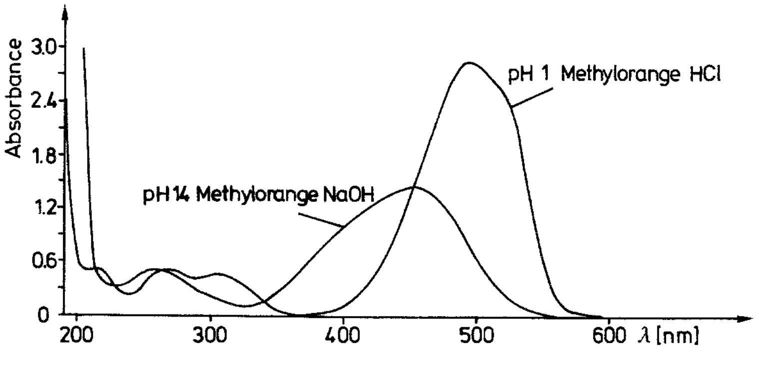

Fig. 1. Absorption spectra of **1** in water at a concentration of 10^{-4} mol/l at different pH

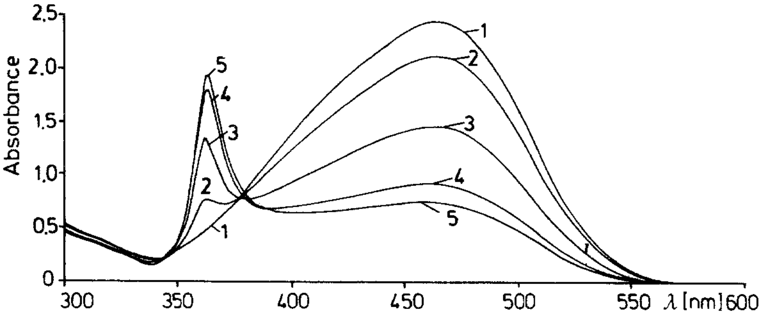

Fig. 2. Effect of metachromasy of **1** [10^{-4} mol/l] by addition of ionene **16** in water at pH 6; Curves (P/D ratio): 1 (0), 2 (0.2), 3 (0.6), 4 (1.0), 5 (1.2); P/D: ratio of polyelectrolyte/dye based on the concentration of the opposite charges

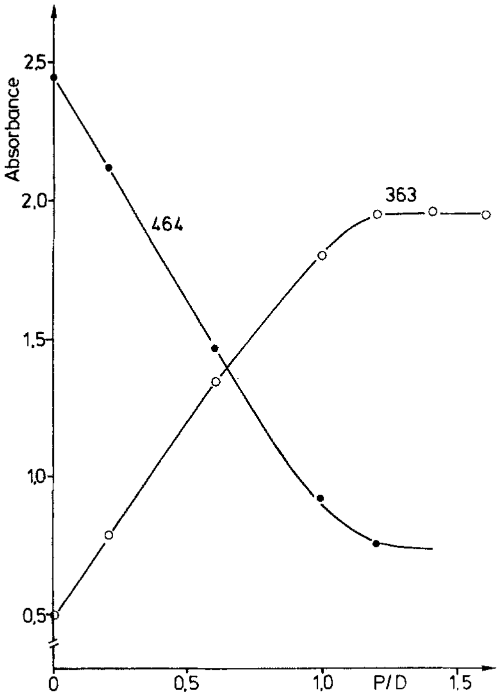

Fig. 3. Comparison of the linear decrease of the absorption at 464 nm (●) and increase of the metachromasy induced new absorption maximum at 383 nm (○) of **1** [10^{-4} mol/l] during addition of ionene **16** (see Fig. 2)

these types of polymers. Additionally, we examined ionene **10**, an ionene with a combination of very hydrophobic, very hydrophillic units, rather rigid and flexible units.

Table 1. Ratio polymer (P)/dye (D) of different ionenes in a 10^{-4} mol/l solution of **1** where metachromasy reaches its maximum absorbance; the ratio is based on calculated numbers of opposite charges

Ionene	P/D
3	1.0
4	1.0
5	1.1
6	1.5
7	2.0
8	2.0
9	1.5
10	2.0

Whereas the shape of the absorption band in the presence of **10** is comparable to those achieved in the presence of the aromatic dabco ionenes **17–21**, the new maxima of **1** in the presence of aliphatic ionenes **3–9** is less obvious. Additionally, we observed that increasing the hydrophobic parts of the ionenes an increasingly higher excess of polymer is necessary to reach the maximum of the absorbance of the metachromasy shift (Table 1).

3.3. *Comparison of metachromasy effects of ionenes 3–21*

The listing of the observed new absorption maximum illustrates that increasing the amount of flexible hydrophobic parts (ionenes **11** to **16**) results in an increased maxima of the shifts. The incorporation of a short or flexible aromatic unit (ionenes **17** and **19**) gives shifts quite similar as those in the presence of the aliphatic ionenes **11–16**.

Incorporation of more rigid and expansive aromatic units by synthesizing ionenes **17**, **18**, and **21** results in shorter shifts. For metachromasy induced from ionenes with dabco units an increase of the maximum can be observed with larger areas of aliphatic units and higher flexibility of the aromatic units. A correlation between a systematic increase of the hydrophobic part and the rigidity

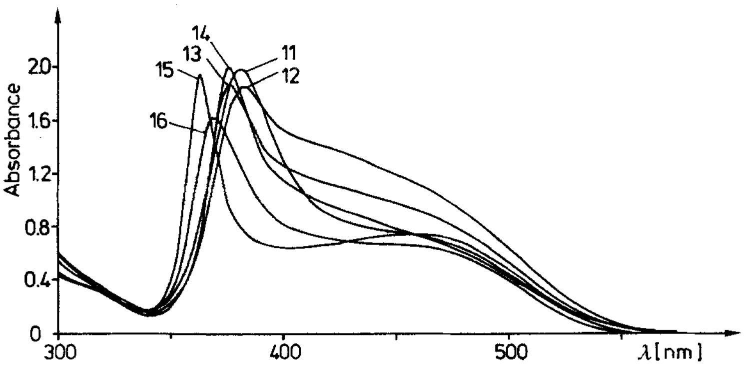

Fig. 4a. Comparison of the absorption spectra of ionenes **11–16** induced metachromasy of **1** [10^{-4} mol/l] when it has reached its ultimate absorbance

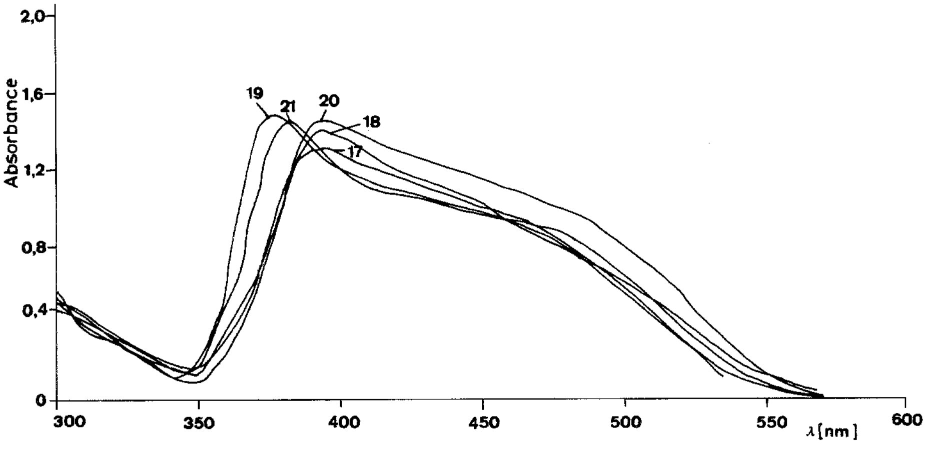

Fig. 4b. Comparison of the absorption spectra of ionenes **17–21** induced metachromasy of **1** [10^{-4} mol/l] when it has reached its ultimate absorbance

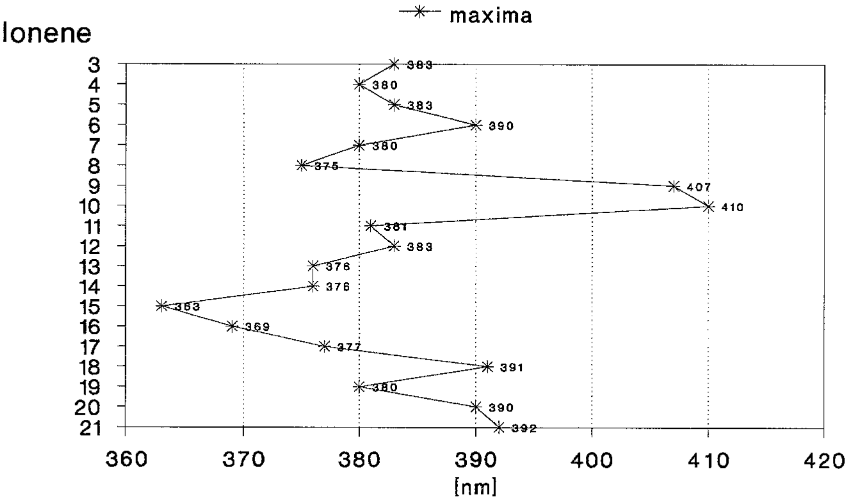

Fig. 5. Listing of the new absorption maxima of the examined ionene at full extent of the metachromasy effect

of these units and a decrease of the water solubility could not be found.

The aliphatic ionene **3**–**8** exhibit almost the same shift with the exception of **6** and **8**. The maxima of the shift were usually lower or higher than those of **3**, **4**, **5**, and **7**.

Metachromasy induced by polyelectrolytes **9** and **10** resulted only in a small hypsochromic shift.

The typical shifts of the ionenes used are summarized in Fig. 6.

All prepared solutions of **1** in the presence of different ionenes showed a small precipitate after 1 to 3 days. The absorption spectra of the resulting clear solution was identical with that of methylorange **1**, but with a lower absorbance of the band.

In a further experiment a solution was filtered in which the metachromasy effect reached the maximum absorbance of the shifted band (Fig. 7, curve 1). The resulting spectrum (Fig. 7, curve 2) was found to be identical with that of **1** (Fig. 1), but with much lower intensity of the absorbance band.

The addition of the model substance **22**, a dication, to **1** leads to an absorption spectra very similar to that with the polycations, but with lower absorbance (Fig. 8, curve 1). At the same time precipitation can be observed. After 20 min only the spectrum of **1** with a very low absorbance was found. Curve 2 describes the absorption spectrum of a film of **22** precipitated with **1** (this corresponds to an anion exchange of the bromide anion with the methylorange anion) and spin-coated in vacuum out of a solution of water/DMF. This spectrum is almost identical with those of **1** in the presence of polyelectrolytes.

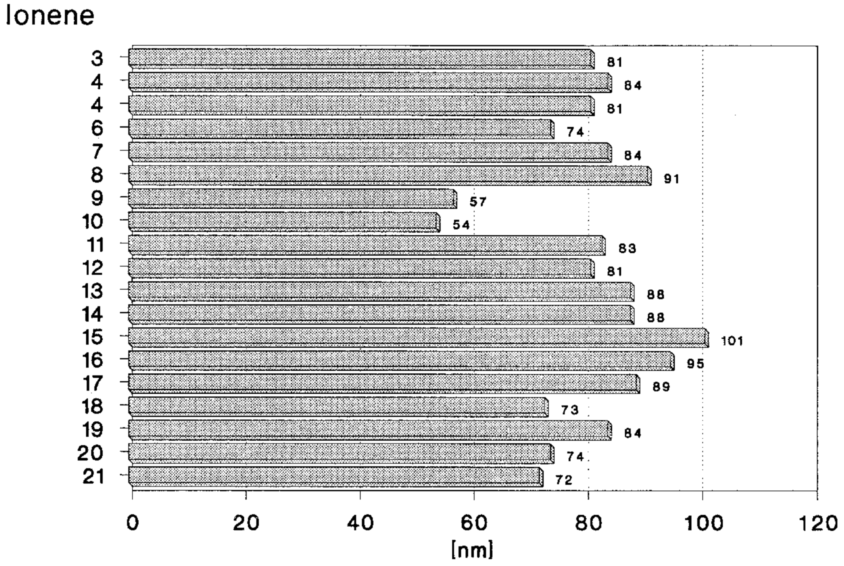

Fig. 6. Listing of the hypsochromic shifts of the absorption maxima of ionenes **3–21**

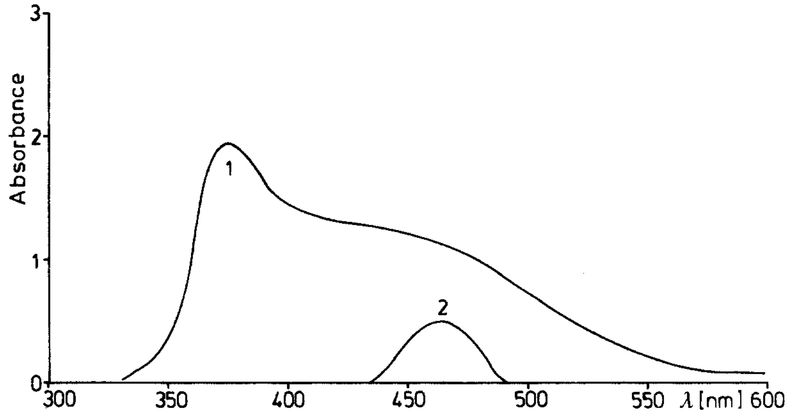

Fig. 7. Curve 1 – solution of **16** and **1** at maximum extent of metachromasy; curve 2 – filtrated solution

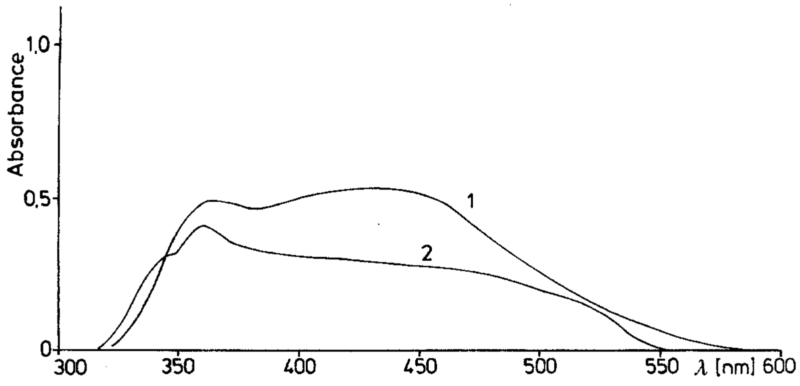

Fig. 8. Curve 1 – solution of **22** and **1**; curve 2 – spincoated film of a precipitate of **22** and **1**

4. Conclusions

The identity of the absorption spectra's of the fill of **22** and the ionenes induced metachromasy of **1** may explain the source for the hypsochromic shift of 1.

Metachromasy effects can only be observed at a concentration range of **1** close to 10^{-4} mol/l. At higher concentrations direct precipitation becomes visible and at a lower concentration no shift can be detected. The addition of the ionenes to **1** induces an enrichment of the dye anions in the sphere of the dissociated polycations. The polycation with the as anion-bound dye precipitates very slowly. The rate of sedimentation seems to depend on the nature of the polymer backbone. A fast precipitation results in an impoverishment of the absorbance at 360 nm and a virtual small shift of the absorption maximum. This happens, for example, with the ionenes **9**, **10**, **18**, **20**, and **21**. Ionenes which precipitate much more slowly display a stronger and sharper shift of the absorbance. This can be observed with ionenes **15** or **16**.

With ionenes **4–8** a second effect can be described. Decreasing the charge density of the polymer chain a larger excess of polymer has to be added.

On the one hand, the availability of the quaternary nitrogen cations seems to play a role. Assuming that the dissociation of the polymers in such diluted systems is almost 100%, the conformation of the polymer backbone could affect the accessibility for dye ions. On the other hand, it seems not to be imperative that dye ions have to be very close to each other to generate this effect.

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