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Linear polymers applied as pseudo-phases in capillary zone electrophoresis of azo compounds used as textile dyes

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Abstract

Nine synthetic organic dyes, including seven azo compounds, used as colouring matter for textiles were separated as anions by capillary zone electrophoresis. As most of the solutes are sulfonic acids, the separation could not be effected by varying the pH of the buffer solution. Therefore, two methods were applied to adjust the electrophoretic mobility in a specific way: complexation by bis-tris-propane and interaction with linear polymers added to the buffer and acting as pseudo-phases. A buffer system containing polyethylene glycol and polyvinylpyrrolidone permits the separation of all analytes. Retention of the dyes caused by the polymeric additives was related to the solute's structure. It was demonstrated by cluster analysis that the relative decrease in the electrophoretic mobility of the dyes correlates with the number of benzoaromatic rings in the solute molecules.

1. Introduction

After the first publication on azo compounds by Griess in 1858, extensive research started. In 1870, Kekulé discovered the coupling reaction of diazotized arylamines with phenols. Orange II (introduced by Roussin in 1876) belonged to the first acidic azo colorants, which dye wool directly and cotton by means of mordants. Acid 88 (synthesized by Caro in 1877) represented progress because of its deeper colour due to an additional aromatic ring [1].

Various techniques for the analysis of dyestuffs have been described [2,3]. Whenever the analytical question is the identification of known dyestuffs and not the structure elucidation of new compounds, separation methods are superior to those based on spectrometry, because most often the samples consist of blends and/or mixtures with matrix material which interfere with the analytical information. Paper chromatography [4] and thin-layer chromatography [5,6] were the most frequently used techniques for the analysis of organic dyestuffs and still have merits. However, in the meantime new methods have been developed which have great advantages, especially for the compounds of interest. HPLC offers the possibility of applying reproducible solvent gradients and coupling the separation technique on-line with a diode-array spectrophotometer [7]. For acidic dyes, reversed phases (RP) are recommended. They provide some kind of structure–retention relationship, e.g., referring to the number of sulfuric acid groups or to the size of the aromatic skeleton [7].

On the other hand, this method often involves problems arising from the very small retention of

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tri- and higher sulfonated compounds, which are fairly common. In those cases ion-pair RP chromatography [8] or ion-exchange chromatography [9] could be applied. However, these methods do not allow one to derive easily structure–retention relationships, which are sometimes useful when there is a lack of reference substances.

Capillary zone electrophoresis (CZE) should be an efficient separation and identification method for the ionic analytes under consideration. In free solution the solutes are separated according to their effective mobilities. For weak acids or bases this property is most dominantly influenced by the pH of the buffer, which enables one to adjust the degree of dissociation of the analytes. This strategy will not succeed, however, for the analytes investigated in this work, because they are mainly strong electrolytes (sulfonic acids). In this case, other strategies must be developed.

One method is commonly used for this purpose: the application of micelles formed in the background electrolyte. This technique, termed micellar electrokinetic capillary chromatography (MECC), has been applied in this context for the separation of some synthetic dyes [10,11]. Another strategy was used in previous work for the separation of diastereometric derivatives of enantiomers [12–14] and also for the improvement of the separation of cationic synthetic dyes [15], namely the use of polymeric additives. These polymers seem to be able to introduce lipophilic interactions with the solutes, and are thus also termed pseudo-stationary phases, in analogy with RP chromatography. Polyethylene glycol (PE) and polyvinylpyrrolidone (PVP) were added to the buffer to adjust separation selectivity in this work.

The impact of the additive on electrophoretic migration was evaluated by cluster analysis [16–18] in order to obtain a better understanding of which analyte property is responsible for the retention of the solute on addition of the polymer. This chemometric method was applied in previous work on capillary electrophoresis to characterize the similarity of electrolyte systems [19–21] and that of solutes [22,23].

2. Experimental

2.1. Chemicals and reagents

The chemicals used for the preparation of the buffer were of analytical-reagent grade (Merck, Darmstadt, Germany). PVP (25; $M_r \sim 25\,000$, Serva, Heidelberg, Germany) and PEG (M_r $2 \cdot 10^6$, Serva) were added to the buffer solution without further purification. Water was doubly distilled before use. The buffer solution was filtered through a membrane filter of 0.2-μm pore size. The dyes and 1,3-bis[tris(hydroxymethyl)methylamino]propane [bis-tris-propane (BTP), 99%+ purity] were purchased from Aldrich (Steinheim, Germany). The dyes were dissolved in water at a concentration of 40 ppm.

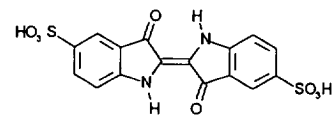
2.2. Apparatus

CZE was carried out with a P/ACE System 2100 (Beckman, Palo Alto, CA, USA) equipped with a UV absorbance detector with fixed wavelength (214 nm). The separation capillary was made from fused silica (75 μm I.D., total length 0.269 m, effective length 0.202 m; Supelco, Bellefonte, CA, USA). The temperature was held constant at 25°C. Polyacrylamide-coated capillaries were prepared according to the procedure described by Kilar and Hjerten [24] and modified by Schützner et al. [12]. The conditions of the analysis are described in Table 1.

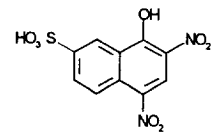
3. Results and discussion

3.1. Influence of a counter ion with complexing ability

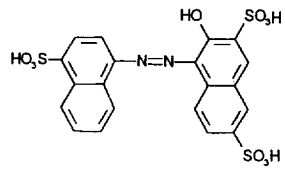
The dyes under discussion (see Fig. 1) can form anions under the appropriate pH conditions. When the pH is not too low, the amino groups on the aromatic ring in compounds D5 and D9 will not be protonated, because the pK_a of such substituted anilines is about 5. In contrast to acidic conditions, at neutral pH the



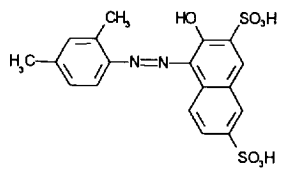
D1
Natural Blue 2



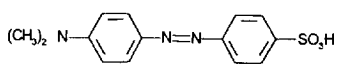
D2
Acid Yellow 1, Naphthol Yellow



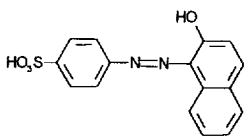
D3
Amaranth, Acid Red 27



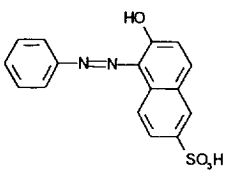
D4
Ponceau 2R, Acid Red 26
Xylidine



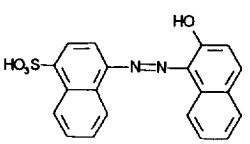
D5
Methyl Orange, Acid orange 52



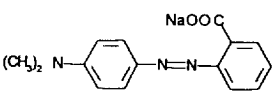
D6
Orange II, Acid orange7



D7
Crocein orange G, Acid orange 12



D8
Acid Red 88



D9
Methyl Red, Acid Red 2

Fig. 1. Structural formulae, abbreviations and trivial names of the organic dyes investigated.

Table 1
Buffers, additives and separation conditions for the electrophoresis of the organic dyes shown in Fig. 1

System	Buffer	Additive	pH	Voltage	Capillary
I	5 mmol/l NaH_2PO_4–NaOH	None	7.0	−20	Coated
II	5 mmol/l NaH_2PO_4–NaOH	0.5% PVP	7.0	−10	Coated
III	5 mmol/l NaH_2PO_4–NaOH	1.0% PVP	7.0	−10	Coated
IV	10 mmol/l BTP–HCl	None	6.5	−20	Coated
V	10 mmol/l BTP–HCl	0.25% PEG	6.5	−10	Coated
VI	10 mmol/l BTP–HCl	0.5% PEG	6.5	−10	Coated
VII	10 mmol/l BTP–HCl	1.0% PEG	6.5	−10	Coated
VIII	10 mmol/l HCl–Ethanolamine	None	10.0	+20	Uncoated
IX	10 mmol/l HCl–Ethanolamine	0.1% PEG	10.0	+10	Uncoated
X	10 mmol/l HCl–ethanolamine	0.25% PEG	10.0	+10	Uncoated
XI	10 mmol/l BTP–HCl	0.5% PEG + 0.01% PVP	6.5	−10	Coated
XII	10 mmol/l BTP–HCl	0.5% PEG + 0.05% PVP	6.5	−10	Coated
XIII	10 mmol/l BTP–HCl	0.1% PVP	6.5	−10	Coated

BTP, bis-tris-propane.

compounds will not form zwitterions, but will migrate electrophoretically as anions owing to the dissociation of the strongly acidic sulfonic (dyes D1–D8) or the weakly acidic carboxylic (dye D9) groups.

However, it can be seen from Fig. 2 that a usual buffer with a neutral pH does not exhibit a sufficiently high selectivity for full separation. A separation according to a difference in pK values would not be efficient for the case of sulfonic acids (they have negative pK values). Some compounds have phenolic groups with pK_a values around 10, but it will be shown below that at high pH co-migration of most solutes is observed. Hence it must be assumed that varying the pH will not improve resolution.

For the oligosulfonic acids, the selectivity can probably be enhanced by using a counter ion with complexing ability, e.g., a higher charged metal cation (Ca, Mg, Cu, Al) or a bivalent organic base such as BTP. This complexing compound has recently been applied to resolve inositol phosphates by capillary isotachophoresis [25,26]. Separation in such a buffer is shown in Fig. 3. In comparison with phosphate buffer (with sodium as counter ion, Fig. 2), a significant improvement in the separation is observed, but also in this case some compounds are not separated and co-migrate. It can also be seen that in this system Acid Red 88 (D8) is separated into two different components (indicated as D8A and D8B).

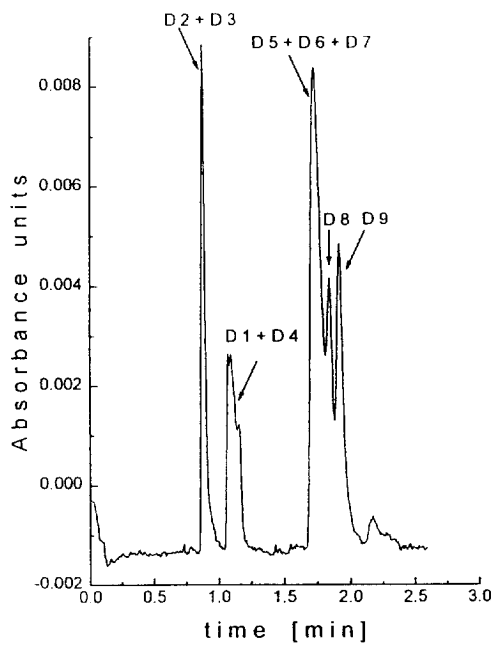


Fig. 2. Electropherogram of the dyes in a coated capillary at pH 7.0 without additive. Numbers as in Fig. 1; separation system I from Table 1 with sodium phosphate as buffer.

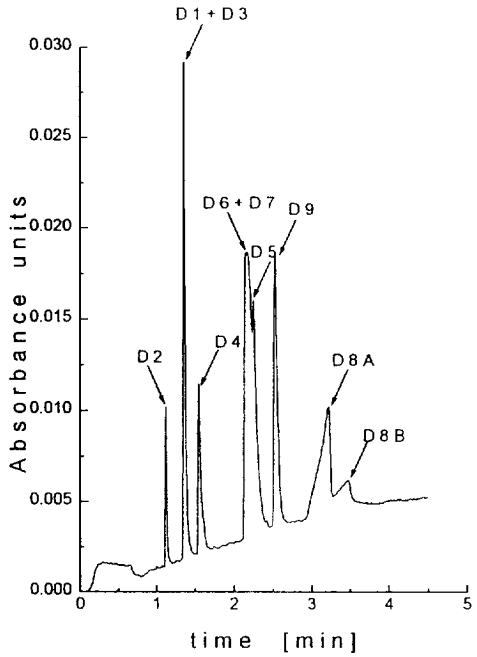


Fig. 3. Electropherogram of the dyes in a coated capillary at pH 6.5 with BTP as counter ion with complexing ability. Numbers as in Fig. 1; separation system IV in Table 1.

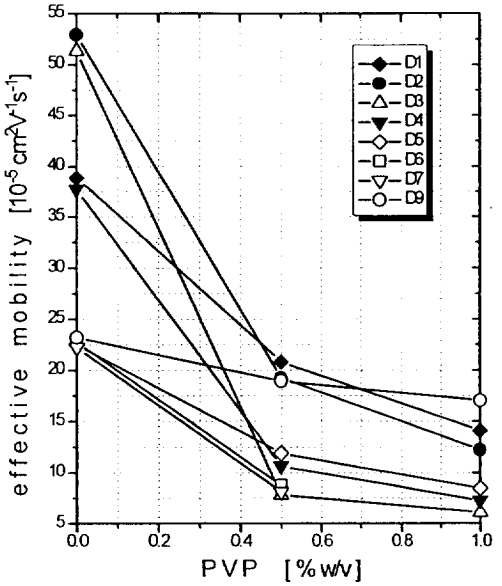


Fig. 4. Influence of the concentration of PVP on the effective mobility of the dyes in sodium phosphate buffer at pH 7.0. The mobilities of dyes D6 and D7 are less than $5 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} at 1% PVP and are therefore not indicated in the plot at this concentration. This is also the case for the dye D8 at a PVP concentration of 0.5% and higher. Numbers as in Fig. 1; separation systems I, II and III in Table 1.

3.2. *Effect of linear polymers added as pseudo-phases*

It was pointed out above that variation of the pH will not affect the separability of most of the analytes under consideration. For this reason, another approach was selected to enhance the selectivity, namely the use of linear polymers added as pseudo-phases to the background buffer. PVP and PEG were chosen as polymers. In Fig. 4, the influence of the concentration of PVP added to the phosphate buffer on the effective mobilities of the separands is depicted. It is apparent that PVP is a very efficient pseudo-phase for the dyes, because even 0.5% of the polymer reduces the effective mobilities by at least a factor of two and, moreover, it changes the migration sequence in a number of cases. The strongest affinity of PVP was found for Acid Red 88 (D8); 0.5% of PVP decreased the effective mobility of this compound to less than $5 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} (this limiting value is given by the time of measurement, which was chosen as 20 min maximum). Such a low mobility was also found for Orange II and Crocein Orange G (D6 and D7) at a PVP concentration of 1%.

PEG was found to have less influence than PVP on the selectivity. The effect of the addition of PEG on the effective mobilities was investigated in buffers based on BTP, because it was expected that with the complexing counter ion even smaller changes in selectivities caused by the polymer would suffice to enhance the separation. The result of this investigation is depicted in Fig. 5. It can be seen that all mobilities decrease slightly with increasing concentration of PEG, and also some changes in the migration order are found. In Fig. 6 only a partial improvement of the separation is visible compared with the buffer without PEG (Fig. 3).

The influence of addition of PEG to the buffer on the mobilities of the dyes was investigated

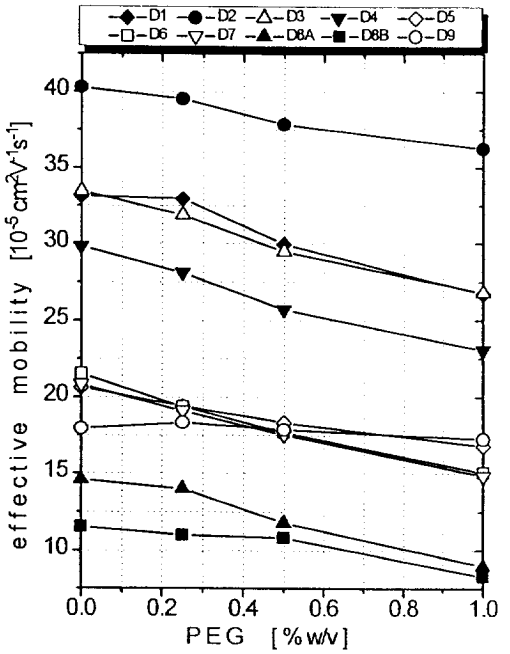


Fig. 5. Effect of the concentration of PEG on the effective mobilities of the dyes at pH 6.5 with BTP as complexing counter ion. Number as in Fig. 1; separation systems IV, V, VI and VII in Table 1.

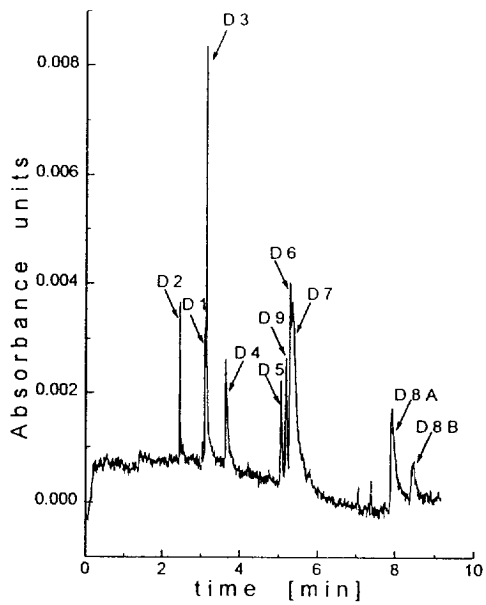


Fig. 6. Electropherogram of the dyes in a coated capillary at pH 6.5 with BTP as complexing counter ion and PEG as stationary pseudo-phase. Numbers as in Fig. 1; concentration of PEG, 0.5% (w/v) (separation system VI in Table 1).

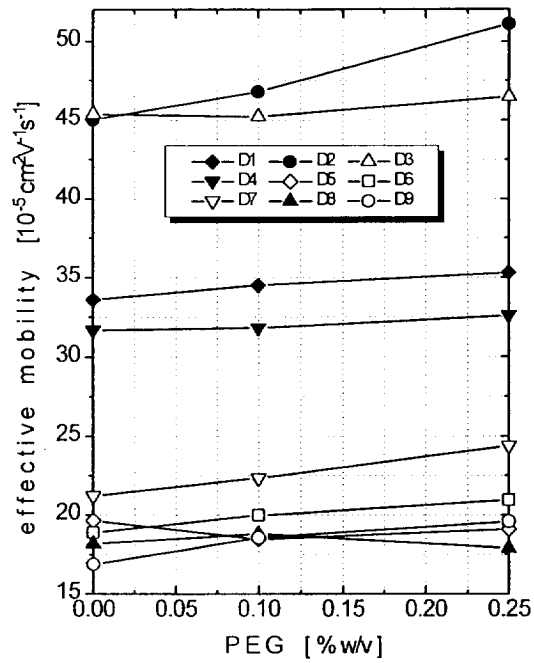


Fig. 7. Influence of PEG on the effective mobility of the dyes at pH 10. Numbers as in Fig. 1; separation systems VIII, IX and X in Table 1.

also at high pH (10.0), as mentioned above. The result of this investigation is shown in Fig. 7. It can be seen that for a number of solutes the measured effective mobilities increase slightly with increasing PEG concentration, an unexpected effect, which can be possibly related to inconstancy of the temperature inside the capillary. Nevertheless, it can be concluded that a concentration of even 0.1% leads to a significant improvement in the separation. In Figs. 8 and 9 two systems are shown for comparison, one without PEG and the other with 0.1% of PEG. It can be seen that D1–D4 and D2–D3 become fully separated on addition of PEG, and also D6–D7 show baseline separation. However, three dyes (D5, D8 and D9) still co-migrate, because the selectivity is not sufficient using only PEG as a pseudo-phase.

For further optimization of the separation, the selective reduction of the effective mobility of dyes D3 and D5 by PVP was utilized. For this reason the migration behaviour of the solutes in

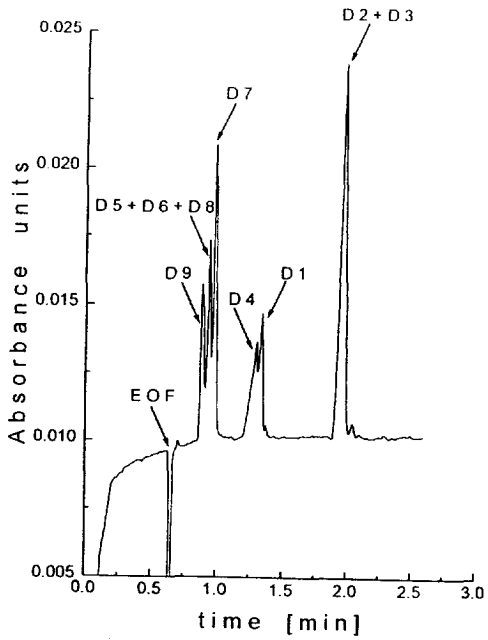


Fig. 8. Electropherogram of the dyes in an uncoated capillary at pH 10.0. Numbers as in Fig. 1; separation system VIII in Table 1.

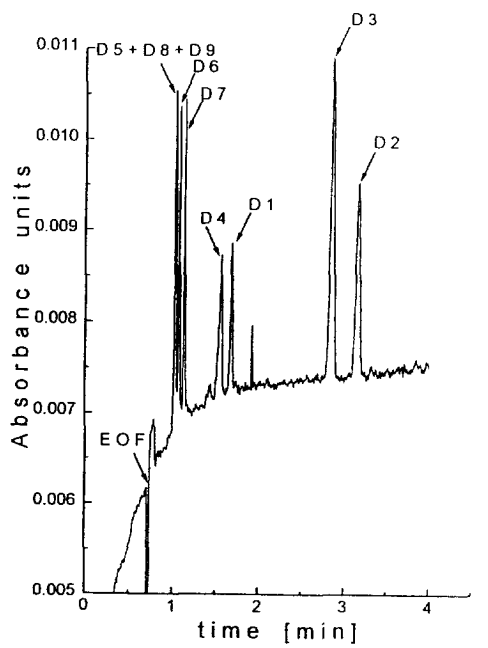


Fig. 9. Electropherogram of the dyes at pH 10.0 in the presence of PEG (0.1%, w/v). Numbers as in Fig. 1; separation system IX in Table 1.

the buffer based on BTP with 0.5% of PEG was influenced by PVP added at very low concentration.

The results of this investigation are depicted in Fig. 10. The addition of 0.05% of PVP led to the separation of all compounds. Even such a low concentration of PVP leads to baseline drift, which was not observed in the buffer without PVP. This baseline drift was, on the other hand, well reproducible and could be compensated for easily by the use of the computer software by subtraction of the signal from the blank run (injection of water).

An electropherogram after such a subtraction is shown in Fig. 11. All compounds were separated in this mixed system. It should be mentioned, however, that Acid Red 88 (D8) migrates with an effective mobility of less than $5 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}, which leads not only to a long analysis time but also causes several problems with detection in this system, because the peak of this component is very broad under these conditions and can hardly be distinguished

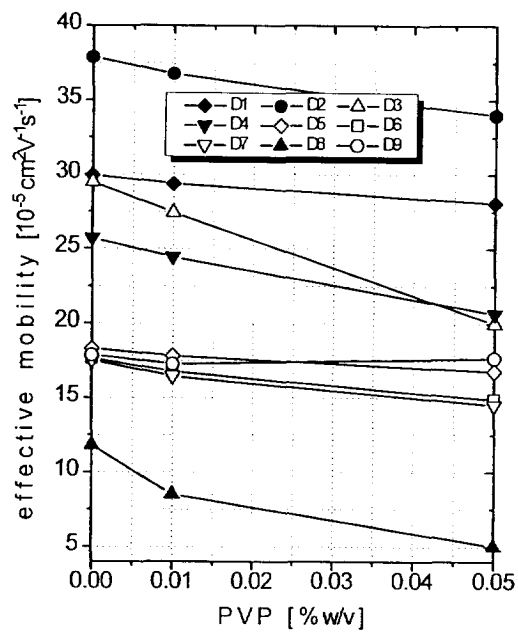


Fig. 10. Effect of the concentration of PVP on the effective mobility of the dyes in the separation system at pH 6.5 (BTP–HCl, 0.5% PEG). Numbers as in Fig. 1; separation systems VI, XI and XII in Table 1.

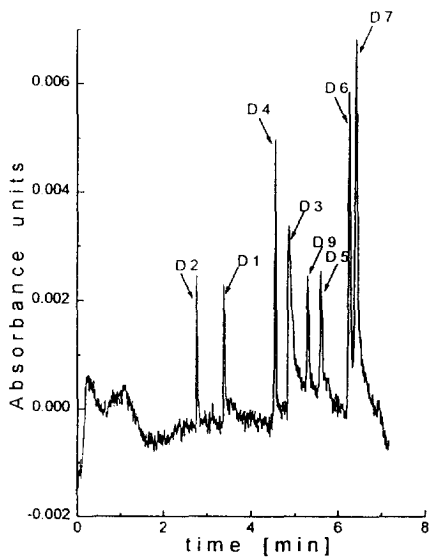


Fig. 11. Optimized electropherogram of the dyes in a coated capillary with a buffer at pH 6.5 with BTP as counter ion, 0.5% (w/v) PEG and 0.05% (w/v) PVP. Electropherogram of a blank run was subtracted. Numbers as in Fig. 1; separation system XII in Table 1.

from the noisy baseline, especially at low concentrations. If this component must be identified in the sample, the use of another separation system, IV in Table I, is therefore recommended, which allows the determination of Acid Red 88 in a much shorter time, within less than 4 min (see Fig. 3).

3.3. Relationship between solute structure and retention caused by the polymeric additive

The number of solutes studied is too small to allow a full interpretation of the relationship between the structure of the separands and the extent of interaction with the polymer. Some conclusions can be drawn, however, when the retarding effect of the polymer (PVP is considered because of its much stronger effect compared with PEG) on the dyes is expressed as the relative change in the solute mobility, as given in Table 2. It seems that within given buffer systems the relative retardation follows the number of benzoaromatic rings in the dye molecules. The solutes with two benzoaromatic rings exhibit the smallest retardation effect. Within this group, dye D9 is affected only to a minor extent by the polymer. Interestingly, D9 is the only component with a carboxylic function, in contrast to the other eight solutes, which are sulfonic acids. The dyes with four benzoaromatic rings, on the other hand, show the most pronounced retardation of all the compounds under consideration.

For better visualization, the azo-sulfonates are grouped by a sequential, hierarchical cluster analysis based on the average linkage algorithm, taking the solutes as the taxonomic units, and the relative mobility data from Table 2 as the features for constructing the corresponding simi-

Table 2
Effect of the polymer network on the retention of the solutes

Dye substance	No. of benzoaromatic rings	$\frac{u^{(I)}-u^{(II)}}{u^{(I)}}\cdot 100$	$\frac{u^{(I)}-u^{(III)}}{u^{(I)}}\cdot 100$	$\frac{u^{(IV)}-u^{(XIII)}}{u^{(IV)}}\cdot 100$	$\frac{u^{(VI)}-u^{(XI)}}{u^{(VI)}}\cdot 100$	$\frac{u^{(VI)}-u^{(XII)}}{u^{(VI)}}\cdot 100$
D1	2	49	65	17	1.9	6.2
D2	2	59	75	21	2.9	10.2
D5	2	46	62	21	2.7	8.2
D9	2	15	25	4	3	1
D4	3	73	82	36	4.9	20
D6	3	62	>82	36	5	16
D7	3	64	>82	36	5.9	17
D3	4	82	87	48	7	32
D8	4	>80	>80	>69	28	57

The relative retention (%) is given by the difference between the effective mobility, u, of the solute in buffer without polymer to that with polymer, related to the former. Numbers of dye substances as in Fig. 1; buffers (indices of the mobilities) as in Table 1.

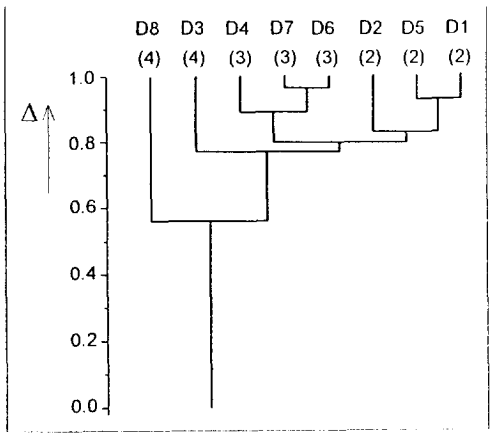


Fig. 12. Dendrogram as the result of the cluster analysis, demonstrating the relationship between the retarding influence of the polymeric additives and the structure of the dyes. Numbers of dyes as in Fig. 1. Numbers of benzoaromatic rings are given in the parentheses. Δ = normalized Euclidian distance.

larity matrix. The measure of (dis)similarity is the Euclidian distance between the taxonomic units in the five-dimensional vector space (five is the number of electrolyte systems).

The resulting dendrogram is shown in Fig. 12. The solutes with the same number of benzoaromatic rings are grouped in the subclusters formed. This result supports the assumption that the lipophilic interaction between the solutes and the polymeric additive is responsible for retardation.

4. Conclusions

It is possible to identify the dyes under discussion by CZE using different linear polymers as pseudo-phases to enhance the separation selectivity. The mobility of the solutes is not related to the viscosity, which is not surprising because Walden's rule (which states that the product of ionic conductivity and viscosity coefficient is a constant for different solvent systems) is not obeyed in many cases. This phenomenon can be related to the fact that the viscosity is a measure of the bulk phase, which clearly will be influenced when linear polymers are present in the solution. The charged dye molecules, on the other hand, moving through the solvent encounter the local viscosity of the environment, which may be affected by the polymer to a much smaller extent; the latter, not the former, determines the electrophoretic migration properties of the analyte. The extent of interaction of the dye with the polymer and thus its retardation increase with increasing number of benzoaromatic rings in the solute molecule.

From the practical point of view, it can be concluded that the identification of synthetic dyes used as textile colorants is possible using a system with BTP as a buffering and complexing ion at pH 6.5, with 0.5% of PEG and 0.01% of PVP. For better detectibility of Acid Red 88 and to avoid too long run times, the identification of this single analyte can be performed in the same buffer without a polymeric additive in less than 4 min.

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