

# Effect of the number of sulpho groups on the electrophoretic mobility of sulphonated azo dyes

Jiří Gasparič\*, Antonie Sedmíková

*Department of Biophysics and Physical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203,  
501 65 Hradec Králové, Czech Republic*

## Abstract

Paper electrophoretic mobilities of azo dyes containing one to six sulpho groups were studied in alkaline (0.1 M NaOH) and acidic (30% acetic acid) media. The most significant contribution to the mobility is caused by the presence of the second and third sulpho groups in the original structure. The influence of the fifth and sixth sulpho groups is nearly negligible. The fact that isomers containing the same number of sulpho groups are separated indicates that further factors must be involved. The influence of the presence of the carboxy group and other structural factors was also studied.

## 1. Introduction

Most reports on the paper electrophoresis of water-soluble azo dyes (for reviews see refs. 1 and 2) have been concerned with practical separations of food colours and did not consider the chemical structures of the dyes. This paper deals with azo dyes, the solubility of which in water is caused by the presence of one or more sulpho groups in their molecules. Dyes with such structures are either acid dyes, used for dyeing of wool, foodstuffs or pharmaceuticals, or direct dyes, used for cotton dyeing or as acid–base indicators.

Four basic structures (A–D, see Tables 1–4)

were systematically substituted by one to six sulpho groups or modified in other desired ways in order to find factors that might influence their electrophoretic mobilities.

## 2. Experimental

### 2.1. Chemicals

The dyestuffs used within this work were either commercial products with known structures or model compounds prepared by known methods [3]. Their purity was examined by thin-layer chromatography on Silufol sheets, mostly using the mobile phase 1-propanol–ammonia (2:1) [4]. All other chemicals were of analytical-reagent grade.

\* Corresponding author.

## 2.2. Apparatus

High-voltage electrophoresis was carried out using a Camag HVE instrument and Whatman No. 2 paper ( $18 \times 40$  cm), which was connected with the electrodes by double paper bridges ( $6.5 \times 18.5$  cm). The operating voltage was 2500 V. Some preliminary experiments were performed using low-voltage electrophoresis. A laboratory made device of the free hanging paper strip in a moist chamber type was used. The distance of the electrodes was 30 cm, the voltage was 300 V and the field strength was  $10 \text{ V cm}^{-1}$ . Whatman No. 2 paper of 11.5 cm width was used and the operating time was 6 h.

## 3. Results and discussion

Azo dyes of four structural types (A–D, see Tables 1–4) were used to study some of the structural effects that could influence their electrophoretic mobilities. Our attention was directed to the influence of the number of sulpho groups, the contributions of the carboxy group, the hydroxy group and the electrophoretically inactive substituents and the effect of dimerizing the molecule.

All electrolytes mentioned in previous papers [1,2] were tested in preliminary experiments. Aqueous 0.1 M solutions of sodium or potassium hydroxide were found to be the most suitable. The mobilities of the dyes at lower concentrations of the electrolytes, *e.g.*, 0.05 M NaOH, were higher and the separations were excellent, but both the mobilities and the form of the spots were strongly affected by the amount of the dyes spotted. The addition of alcohols or glycols did not bring about any improvements. Good separations with well defined spots were also achieved using an organic buffer solution of pH 5.9 according to Savvin and co-workers [5–7] or simply 30% acetic acid. Therefore, 0.1 M sodium hydroxide solution was chosen for further work and the two acid electrolytes were used in some complementary experiments.

### 3.1. Effect of the sulpho group

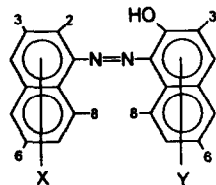
It has been stated by previous workers [1,8] that the electrophoretic mobility of sulphonated azo dyes is caused by the presence of the sulpho group and is increased with increase in the number of these groups. In fact, the dyes of the basic skeleton but without any sulpho group do not migrate at all (dye I) provided there were no other dissociable groups present. It can be seen from Table 1 and Fig. 1 that the mobility of the dyes increases with increasing number of sulpho groups. A nearly linear increase in mobility is caused by the introduction of the first, second, third and fourth sulpho groups; the fifth and sixth sulpho groups, however, do not bring about any remarkable change in mobility. The highest contribution to the mobility is caused by the second and/or third sulpho groups (see Fig. 2). Similar results can be seen in Fig. 3, where the mobilities are plotted against  $M/z$ , where  $M$  is the molecular mass of the anion and  $z$  the charge. Probably the anions of the dyes may be solvated in aqueous media to different extents and hence calculations based on the number of sulpho groups or molecular masses may not be exactly correct. The fact that isomeric dyes show some differences in mobilities indicates that the contribution of the sulpho group in different positions is not equivalent and that factors other than their number must be involved. It has been shown with anthraquinone dyes [9] that some isomers are separated by electrophoresis that could not have been distinguished by planar chromatography under common conditions.

Analogous results were obtained with type B and C dyes (Tables 2 and 3). The separation in acidic media is also determined by the number of sulpho groups. This means that the sulpho group is completely dissociated under these conditions (pH 1.5 and 5.9).

### 3.2. Effect of the carboxy group

The carboxy group is also dissociated in alkaline media (0.1 M NaOH) and its contribution to the mobility of the dyes is only slightly lower

Table 1  
Electrophoretic mobilities of type A azo dyes



No.	X	Y	$M^a$	$M/z^a$	$u \times 10^9$ ( $\text{m}^2 \text{V}^{-1} \text{s}^{-1}$ )
I	H	H	298.3		0
II	2-SO <sub>3</sub> H	—	377.4	377.4	2.31
III	4-SO <sub>3</sub> H	—	377.4	377.4	1.42
IV	—	6'-SO <sub>3</sub> H	377.4	377.4	1.60
V	4-SO <sub>3</sub> H	6'-SO <sub>3</sub> H	456.4	228.2	5.51
VI	3,6-(SO <sub>3</sub> H) <sub>2</sub>	—	456.4	228.2	4.09
VII	—	3',6'-(SO <sub>3</sub> H) <sub>2</sub>	456.4	228.2	3.91
VIII	4-SO <sub>3</sub> H	3',6'-(SO <sub>3</sub> H) <sub>2</sub>	535.5	178.5	7.64
IX	3,6-(SO <sub>3</sub> H) <sub>2</sub>	6'-SO <sub>3</sub> H	535.5	178.5	7.28
X	3,6,8-(SO <sub>3</sub> H) <sub>3</sub>	—	535.5	178.5	8.53
XI	—	3',6',8'-(SO <sub>3</sub> H) <sub>3</sub>	535.5	178.5	8.36
XII	3,6-(SO <sub>3</sub> H) <sub>2</sub>	3',6'-(SO <sub>3</sub> H) <sub>2</sub>	614.5	153.6	9.78
XIII	3,6,8-(SO <sub>3</sub> H) <sub>3</sub>	6'-SO <sub>3</sub> H	614.5	153.6	11.02
XIV	3,6-(SO <sub>3</sub> H) <sub>2</sub>	3',6',8'-(SO <sub>3</sub> H) <sub>3</sub>	692.6	138.5	11.56
XV	3,6,8-(SO <sub>3</sub> H) <sub>3</sub>	3',6'-(SO <sub>3</sub> H) <sub>2</sub>	692.6	138.5	10.49
XVI	3,6,8-(SO <sub>3</sub> H) <sub>3</sub>	3',6',8'-(SO <sub>3</sub> H) <sub>3</sub>	772.6	128.8	11.20

Whatman No. 2 paper, 0.1 M NaOH, 2500 V, 62.5 V cm<sup>-1</sup>, 15 min.

<sup>a</sup>  $M$  = molecular mass of the dye anion;  $z$  = charge number.

than that of the sulpho group. Thus, *e.g.*, the mobilities of the corresponding sulpho derivatives are slightly higher than those of the carboxy

derivatives: **XXVII** > **XXXIV**; **XXXI** > **XXXV**; **XXXIII** > **XXXVIII**. In acidic media (30% acetic acid) the carboxy group is hardly dissociated and therefore the mobilities of the carboxy deriva-

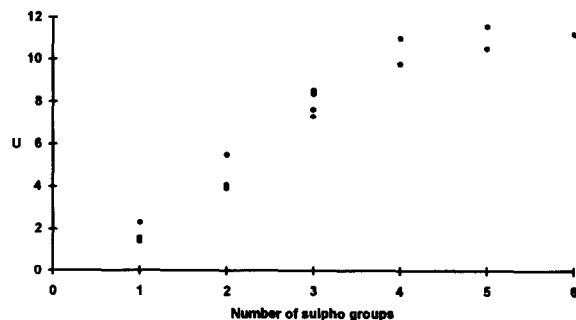


Fig. 1. Dependence of the electrophoretic mobilities ( $u \times 10^9$ ,  $\text{m}^2 \text{V}^{-1} \text{s}^{-1}$ ) of azo dyes on the number of sulpho groups (data from Table 1).

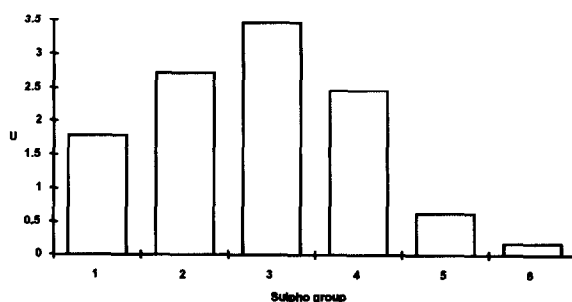


Fig. 2. Schematic presentation of the contributions of the first to sixth sulpho groups to the electrophoretic mobilities of azo dyes ( $\Delta u \times 10^9$ ,  $\text{m}^2 \text{V}^{-1} \text{s}^{-1}$ ; data from Table 1).

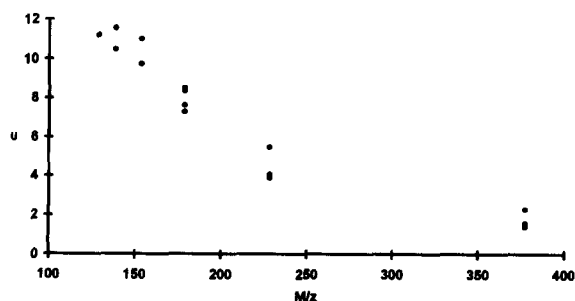


Fig. 3. Dependence on the electrophoretic mobilities ( $u \times 10^9$ ,  $\text{m}^2 \text{V}^{-1} \text{s}^{-1}$ ) of type A azo dyes on the ratio  $M/z$  ( $M$  = molecular mass,  $z$  = charge; data from Table 1).

tives are strongly decreased in comparison with the corresponding sulpho derivatives: the dyes containing a carboxy group have lost the charge and the carboxy group contributes negatively to the mobility by the increase in molecular mass of the dye and as an electrophoretically inactive substituent. Thus, comparison of the electrophoretic migration in alkaline and acidic media could permit the detection of the presence of a carboxy group in the molecule of a dye of

unknown structure similarly as reported for acidic anthraquinone dyes [9].

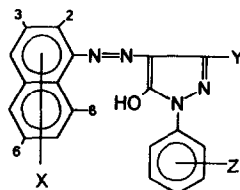
### 3.3. Effect of the hydroxy group

The hydroxy group contributes to the migration of the azo dyes only when it is dissociated under the experimental conditions used. This is the case with the pyrazolone dyes **XVII** and **XXVI**.

### 3.4. Effect of electrophoretically inactive substituents

Electrophoretically inactive substituents (compounds **XXXIX–XLIV**) increase the molecular mass of the dye, the charge remaining the same. In Fig. 4 the migration rate of such dyes is plotted against their molecular mass and the dependence obtained applies to compounds containing substituents of the same type; *c.f.*, compound **XLIV**. This is in accordance with the observations of Iijima and Sekido [10].

Table 2  
Electrophoretic mobilities of type B azo dyes

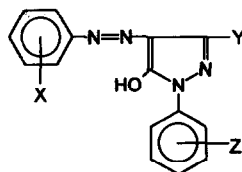


No.	X	Y	Z	$M^a$	$M/z^a$	$u \times 10^9$ ( $\text{m}^2 \text{V}^{-1} \text{s}^{-1}$ )
<b>XVII</b>	H	$\text{CH}_3$	H	328.4	—	0
<b>XVIII</b>	H	$\text{CH}_3$	3'- $\text{SO}_3\text{H}$	407.4	407.4	0.35
<b>XIX</b>	4- $\text{SO}_3\text{H}$	$\text{CH}_3$	4'- $\text{SO}_3\text{H}$	486.5	243.2	2.84
<b>XX</b>	4- $\text{SO}_3\text{H}$	$\text{CH}_3$	3'- $\text{SO}_3\text{H}$	486.5	243.2	3.91
<b>XXI</b>	3,6-( $\text{SO}_3\text{H}$ ) <sub>2</sub>	$\text{CH}_3$	3'- $\text{SO}_3\text{H}$	565.5	188.5	8.71
<b>XXII</b>	3,6,8-( $\text{SO}_3\text{H}$ ) <sub>3</sub>	$\text{CH}_3$	3'- $\text{SO}_3\text{H}$	644.5	161.1	11.02
<b>XXIII</b>	—	$\text{COOH}$	4'- $\text{SO}_3\text{H}$	436.4	218.2	3.56
<b>XXIV</b>	3,6-( $\text{SO}_3\text{H}$ ) <sub>2</sub>	$\text{COOH}$	4'- $\text{SO}_3\text{H}$	594.5	148.6	10.49
<b>XXV</b>	3,6,8-( $\text{SO}_3\text{H}$ ) <sub>3</sub>	$\text{COOH}$	4'- $\text{SO}_3\text{H}$	673.6	134.7	11.02

Whatman No. 2 paper, 0.1 M NaOH, 2500 V, 62.5  $\text{V cm}^{-1}$ , 15 min.

<sup>a</sup>  $M$  = molecular mass of the dye anion;  $z$  = charge number.

Table 3  
Electrophoretic mobilities of type C azo dyes



No.	X	Y	Z	$M^a$	$M/z^a$	$u \times 10^9$ ( $m^2 V^{-1} s^{-1}$ )
XXVI	H	CH <sub>3</sub>	H	278.3	—	1.78
XXVII	2-SO <sub>3</sub> H	CH <sub>3</sub>	H	357.4	357.4	4.98
XXVIII	3-SO <sub>3</sub> H	CH <sub>3</sub>	H	357.4	357.4	4.98
XXIX	4-SO <sub>3</sub> H	CH <sub>3</sub>	H	357.4	357.4	4.27
XXX	H	CH <sub>3</sub>	3'-SO <sub>3</sub> H	357.4	357.4	4.09
XXXI	4-SO <sub>3</sub> H	CH <sub>3</sub>	4'-SO <sub>3</sub> H	436.4	218.2	9.60
XXXII	2,5-(SO <sub>3</sub> H) <sub>2</sub>	CH <sub>3</sub>	H	436.4	218.2	8.89
XXXIII	2,5-(SO <sub>3</sub> H) <sub>2</sub>	CH <sub>3</sub>	3'-SO <sub>3</sub> H	515.5	171.8	11.20
XXXIV	2-COOH	CH <sub>3</sub>	H	321.3	—	3.38
XXXV	2-COOH	CH <sub>3</sub>	4'-SO <sub>3</sub> H	400.4	200.2	7.47
XXXVI	2-COOH	CH <sub>3</sub>	3'-SO <sub>3</sub> H	400.4	200.2	7.64
XXXVII	H	COOH	4'-SO <sub>3</sub> H	386.3	193.1	8.18
XXXVIII	2-SO <sub>3</sub> H	COOH	2'-SO <sub>3</sub> H	465.4	155.1	8.71
XXXIX	4-CH <sub>3</sub>	CH <sub>3</sub>	3'-SO <sub>3</sub> H	371.4	371.4	3.20
XL	4-Cl	CH <sub>3</sub>	3'-SO <sub>3</sub> H	391.8	391.8	1.96
XLI	2,4-Cl <sub>2</sub>	CH <sub>3</sub>	3'-SO <sub>3</sub> H	426.2	426.2	1.24
XLII	4-Br	CH <sub>3</sub>	3'-SO <sub>3</sub> H	436.2	436.2	1.16
XLIII	4-I	CH <sub>3</sub>	3'-SO <sub>3</sub> H	483.3	483.3	1.07
XLIV	4-C <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub>	3'-SO <sub>3</sub> H	433.5	433.5	0.36

Whatman No. 2 paper, 0.1 M NaOH, 2500 V, 62.5 V cm<sup>-1</sup>, 15 min.

<sup>a</sup>  $M$  = molecular mass of the dye anion;  $z$  = charge number.

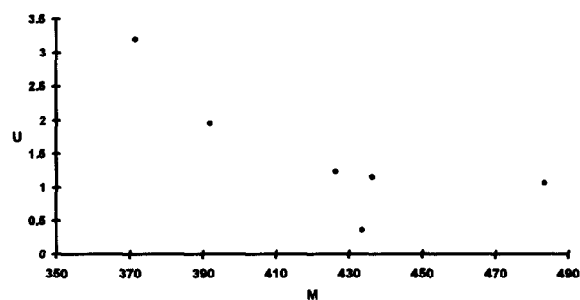
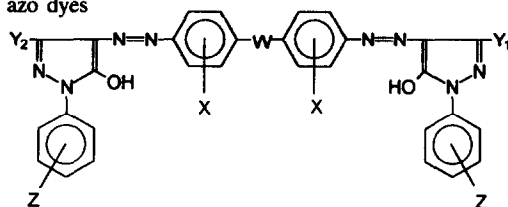


Fig. 4. Dependence of the electrophoretic mobilities ( $u \times 10^9$ ,  $m^2 V^{-1} s^{-1}$ ) of type C azo dyes with electrophoretically inactive substituents on their molecular masses (data from Table 3).

The behaviour of dyes which have a dimeric molecule (XLV–L; see Table 4) is also very interesting. Dyes derived from benzidine (XLV and XLVI) do not migrate at all, which is due to the adsorption of their planar molecules on cellulose (substantivity). Analogous dyes derived from 4,4'-diaminodiphenylmethane (L) migrate without problems. The interruption of the conjugation of their double bonds by the methylene group makes these dyes no longer planar, as they can rotate freely round the C–CH<sub>2</sub>–C bonds and are therefore no longer substantive. Dyes derived from benzidine-3,3'-disulphonic acid (XLVII–XLIX) migrate similarly to the corre-

Table 4

Electrophoretic mobilities of type D azo dyes



No.	X	W	Y <sub>1</sub> = Y <sub>2</sub>	Z	M <sup>a</sup>	u × 10 <sup>9</sup> (m <sup>2</sup> V <sup>-1</sup> s <sup>-1</sup> )
XLV	–	–	CH <sub>3</sub>	3'-SO <sub>3</sub> H	712.7	0
XLVI	–	–	COOH	4'-SO <sub>3</sub> H	770.7	0
XLVII	3-SO <sub>3</sub> H	–	CH <sub>3</sub>	–	712.7	3.02
XLVIII	3-SO <sub>3</sub> H	–	CH <sub>3</sub>	4'-SO <sub>3</sub> H	870.9	3.56
XLIX	3-SO <sub>3</sub> H	–	COOH	4'-SO <sub>3</sub> H	928.8	8.89
L	–	CHC <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub>	4'-SO <sub>3</sub> H	802.8	1.96

Whatman No. 2 paper, 0.1 M NaOH, 2500 V, 62.5 V cm<sup>-1</sup>, 15 min.<sup>a</sup> M = molecular mass of the dye anion.

sponding 4,4'-diaminodiphenylmethane derivatives (sulpho groups decrease the substantivity). Their mobilities are slightly lower than those of the half molecules with one sulpho group (cf., Table 3).

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