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Note

Influence of the cellulose content of cellulose gels on the electrophoretic mobility

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It is generally accepted that the relationship between electrophoretic mobility, u , apparent charge, z , and molecular mass, M , may be represented by

$$u = azM^{-b} \quad (1)$$

where a and b are constants. Hais¹ found $b = 1$ for derivatives of hydroxycoumarin. From the mobilities of metal complexes and aromatic acids, Jokl² derived the equation

$$u = 14.7zM^{-\frac{1}{2}} - 0.29 \quad (2)$$

Offord³, on the basis of an extensive study of peptides, found $b = 2/3$. An investigation of electrophoretic mobilities on cellulose gel strips⁴ resulted in $b = 1/2$ for mercapto acids and 0.85 for trithiocarbonates. The experiments were always carried out in the presence of a standard, because only corrected or relative values of mobility can be introduced into the equation. On the other hand, the interaction between the support media and migrating substances was not taken into account.

In order to explain the contribution of the sieving effect and adsorption on cellulose gel, it is necessary to determine the electrophoretic mobility at different cellulose concentrations. The mobility in polyacrylamide gel is given by the well known Ferguson equation⁵:

$$\log u = \log u_0 - kc \quad (3)$$

where u_0 is the electrophoretic mobility at zero concentration, k the retardation coefficient and c the gel concentration. It has been found, however, that in cellulose gel the results better fit the equation

$$u = u_0/(1 + kr) \quad (4)$$

where r is the ratio of cellulose to water content and k is a constant that may be called the apparent partition coefficient.

EXPERIMENTAL

The preparation of cellulose gel strips was carried out as described previously⁶ using viscose at different dilutions. Samples of 100 g of viscose (11.6% cellulose content) were diluted one after the other with 5, 20, 50, 90, 150 and 200 ml of water containing 2, 4, 6, 8, 10 and 12 g of ethylenediamine, respectively. Sheets of glass-fibre paper were immersed in the viscose solution, pressed between two glass plates and set aside for 1 day. The strips thus obtained were washed with water, 3% (w/w) hydrochloric acid, 10% (w/w) sodium sulphite and 0.001 *M* Na₂EDTA and stored in 50% (v/v) ethylene glycol. Part of each viscose solution was placed in a test-tube and, after solidification, washed with water. The cellulose content was then determined by drying to constant weight at 105°C. The ratios of cellulose to water content (*r*) in the prepared strips were 0.175, 0.159, 0.135, 0.124, 0.105 and 0.080, respectively.

Buffer of pH 6.7 consisted of triethanolamine (20 g/l)–boric acid (40 g/l)–Na₂EDTA (2 g/l)–H₂EDTA (0.5 g/l)–ethylene glycol (50 ml/l). Buffer of pH 11.6 was prepared by equilibrating 200 ml of octane and 15 ml of triethylamine with 800 ml of 5% (v/v) ethylene glycol. Aqueous phase was used as a buffer and the organic phase as a cooling bath.

The electrophoresis was performed as described previously⁶ on vertical strips at 18°C and a voltage gradient of 40 V/cm. The electroosmotic flow was determined by means of ethylenethiourea, visualized with mercurated fluorescein⁴.

RESULTS AND DISCUSSION

According to eqn. 4 the reciprocal of mobility should bear a straight-line relationship to the ratio *r*:

$$\frac{1}{u} = \frac{1}{u_0} + \frac{kr}{u_0} \quad (5)$$

By plotting $1/u$ versus *r* it is possible to determine graphically, as demonstrated in Fig. 1, both u_0 and *k*. The values determined are summarized in Table I. The relationship between $\log(u_0/z)$ and $\log M$ is shown in Fig. 2.

The results indicate that the carboxylic acids (1–11, Table I) have reduced mobility compared with sulphonic acids (12–26). Comparison of the mobilities of 3-mercaptopropionic acid and 2-mercaptoethanosulphonic acid, methyl red and methyl orange and phenol red and phenolphthalein leads to the conclusion that the real charge of a sulphonic group must be higher than that of a carboxylic group in spite of their equal formal charges.

The best approximation relating the molecular mass to the mobility at zero cellulose concentration can be expressed for carboxylic acids by

$$u_0 = (330 \pm 10)zM^{-\frac{2}{3}} \quad (6)$$

and for sulphonic acids by

$$u_0 = (463 \pm 29)zM^{-\frac{2}{3}} \quad (7)$$

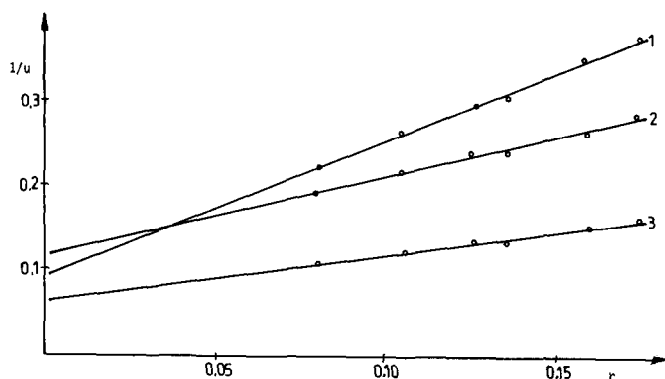


Fig. 1. Relationship between reciprocal values of electrophoretic mobility, kV min cm^{-2} , and the ratio r of cellulose to water content in cellulose gel. 1 = Methyl orange (pH 6.7); 2 = phenol red (pH 6.7); 3 = phenol red (pH 11.6).

TABLE I

ELECTROPHORETIC MOBILITY, u_0 ($\text{cm}^2 \text{min}^{-1} \text{kV}^{-1}$), AT ZERO GEL CONCENTRATION, APPARENT CHARGE, z , AND APPARENT PARTITION COEFFICIENT, k

No.	Ionic species	pH	z	u_0	k
1	3-Mercaptopropionic acid	6.7	1	16.0	4.3
2	Thiobenzoic acid	6.7	1	12.4	4.9
3	Thiomalic acid	6.7	2	20.8	5.5
4	Mercaptionicotinic acid	6.7	1	11.0	4.7
5	Thiosalicylic acid	6.7	1	11.8	5.0
6	Acetylcysteine	6.7	1	10.8	4.9
7	Captopril	6.7	1	9.8	6.5
8	Methyl red	11.6	1	7.1	7.9
9	Glutathione	6.7	1	7.5	6.2
10	Fluorescein	6.7	2	13.4	13.2
11	Phenolphthalein	11.6	2	12.5	6.0
12	2-Mercaptoethanesulphonic acid	6.7	1	18.0	6.3
13	Naphthionic acid	6.7	1	11.8	9.3
14	2-Hydroxynaphthionic acid	6.7	1	11.1	9.2
15	Methyl orange	11.6	1	11.2	19
16	Methyl orange	6.7	1	10.6	17
17	Alizarin red	6.7	2	20.0	16
18	7-Iodo-8-hydroxyquinoline-5-sulphonic acid	11.6	2	17.2	9.6
19	Phenol red	6.7	1	8.3	7.6
20	Phenol red	11.6	2	15.9	8.8
21	Chlorophenol red	11.6	2	15.4	11.0
22	Thymol blue	6.7	1	7.5	9.3
23	Thymol blue	11.6	2	13.2	10.0
24	Thorin	11.6	3	21.3	9.7
25	Bromothymol blue	11.6	2	15.4	12.3
26	Bromophenol blue	6.7	2	14.3	12.9

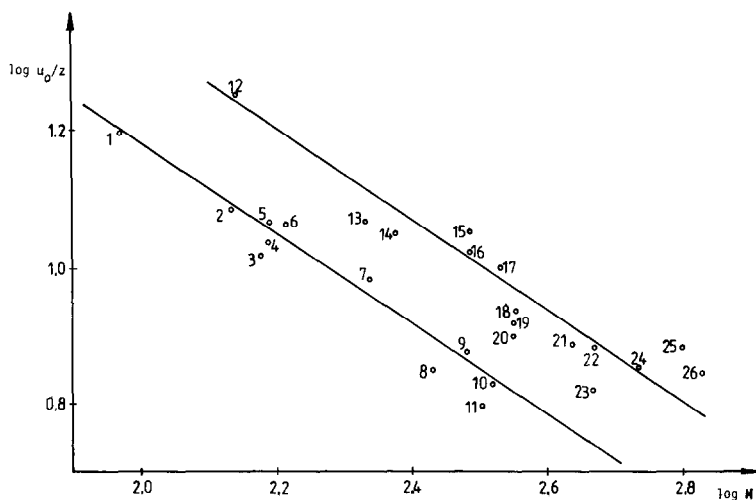


Fig. 2. Relationship between the logarithm of electrophoretic mobility, u_0 , at zero gel concentration divided by the apparent charge, z , and the logarithm of molecular weight, M . The numbers correspond to those in Table I.

The results are in general agreement with those presented by Offord³, who also found that the sulphonic group in cysteic acid enhances the mobility more than a carboxylic one.

The constant k in eqn. 4 represents the resistance resulting from interaction between the cellulose chains and a moving substance. It follows from Table I that the k values for sulphonic acids are higher than those for carboxylic acids.

Apart from a few compounds, such as fluorescein, methyl orange and methyl red, there is a distinct increase in k values with increasing molecular mass, indicating a sieving effect. For a reliable determination of molecular weight on basis of k values, it is obviously necessary to differentiate between a sieving effect and adsorption. An approach involving comparison of k and R_F values (Whatman 2 paper) was not satisfactory, with the exception of methyl orange, the high k value of which is in accordance with a low R_F value of 0.30. The other substances listed in Table I have R_F values between 0.6 and 0.98 but do not show any clear relationship with k values.

Let us now consider the influence of cellulose content on electrophoretic resolution expressed by difference in mobilities:

$$u_2 - u_1 = \frac{u_{02}}{1 + k_2 r} - \frac{u_{01}}{1 + k_1 r} \quad (8)$$

The resolution is equal to zero when $u_2 = u_1$, and then $r = r_0$:

$$r_0 = \frac{u_{02} - u_{01}}{k_2 u_{01} - k_1 u_{02}} \quad (9)$$

For example, the point of intersection of the mobilities of methyl orange and phenol red at pH 6.7 is 0.038.

The function $u_2 - u_1 = f(r)$ passes the maximum at $r = r_{\max}$:

$$r_{\max} = \frac{(k_2 u_{02})^{0.5} - (k_1 u_{01})^{0.5}}{k_2 (k_1 u_{01})^{0.5} - k_1 (k_2 u_{02})^{0.5}} \quad (10)$$

For example, for methyl orange and phenol red $r_{\max} = 0.17$ and for captopril and acetylcysteine $r_{\max} = 0.087$.

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