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Limitations of some common indicators of electroendosmosis in paper electrophoresis (including interactions between carbohydrates and inorganic phosphate)

Many compounds have been recommended as non-migrating indicators of electroendosmotic flow in paper electrophoresis[1] but it is probable that most do not remain electrically neutral or unadsorbed by the paper support under all experimental conditions. Neutral carbohydrates, for example, are often employed as indicators, but polyols and especially reducing sugars cannot be used in highly alkaline electrolytes[2]. They also form charged complexes in electrolytes containing a variety of inorganic anions[1-3] while other indicators such as *s*-trinitrobenzene[4] and N-β-hydroxyethyl-2,4-dinitroaniline[5] are reported to be partially adsorbed by cellulose in aqueous medium.

The validity of some neutral compounds which have been proposed as indicators of electroendosmotic flow has now been tested in a selection of common electrolytes spanning a wide range of pH. It is shown that inorganic phosphate can be added to the list of anions which form charged complexes with carbohydrates.

Experimental

Materials. The indicators (listed in Table I) were either laboratory preparations or commercial samples of analytical grade. Caffeine was prepared for use as a saturated aqueous solution, others as 0.1–0.2 *M* solutions.

Electrolytes. Electrolyte solutions (Table I) were each about 0.1 *M* with respect to the anion except the borate buffer (pH 9.2), which was 0.05 *M* borax, and Tris buffer (pH 7), which was prepared from tris(hydroxymethyl)aminomethane as described by GOMORI[6].

Apparatus and procedure. The enclosed-strip paper electrophoresis apparatus and procedure are described in detail elsewhere[2,7]. Whatman No. 4 paper was used and the indicator solutions applied in line across the middle of paper strips by means of a platinum loop delivering approx. 0.5 μl.

Detection of indicators. Satisfactory procedures for detecting most indicators are given in the references included in Table I. Hydrogen peroxide was detected after electrophoresis in 0.1 *N* NaOH if papers were heated in the oven (100°) for only 1–2 min before spraying them, while still wet with electrolyte, with chromium trioxide–permanganate–sulphuric acid[2] or ferric chloride (0.2 %)–potassium ferricyanide (0.4 %) or alkaline silver nitrate[8]. Hydrogen peroxide is more stable in less alkaline media and survived on papers impregnated with other electrolytes if they were gently heated until not quite dry. Alkaline silver nitrate was also used for detecting urea on all papers.

Results and discussion

The apparent mobilities of the indicators (in mm/h/kV of applied potential) in twelve electrolytes are given in Table I. Positive values represent anionic mobilities, negative values cationic mobilities.

Electroendosmosis was negligible in 0.1 *N* HCl but in all other electrolytes the

TABLE I

MOBILITIES OF SOME INDICATORS OF ELECTROENDOSMOTIC FLOW IN VARIOUS ELECTROLYTES

Mobilities are expressed in mm/h/kV of applied potential. Positive values signify anionic mobilities; negative values, cationic mobilities.

Indicator	*Ref.*	*0.1 N HCl*	*Formic acid–sodium formate (pH 2.0)*	*Formic acid–ammonium formate (pH 3.6)*	*Acetic acid–sodium acetate (pH 4.6)*	*Sodium perchlorate (pH 6)*	*Tris buffer (pH 7.0)*	*Sodium bicarbonate (pH 8.2)*	*Phosphate buffer (pH 8.2)*	*Sodium carbonate–bicarbonate (pH 9.2)*	*Borate buffer (pH 9.2)*	*Sodium carbonate (pH 10.2)*	*0.1 N NaOH*
2,3,4,6-Tetra-O-methyl-D-glucose	17	0	0	0	0	1	0	0	0	0	0	0	34
2,3,6-Tri-O-methyl-D-glucose	2	0	0	0	0	1	0	0	1	0	0	0	32
D-Glucose	18	0	0	0	0	0	0	1	6	3	81	6	39
Glycerol	19	0	0	0	0	0	0	0	2	0	41	2	0
Caffeine	2,9	−9	−1	0	2	13	2	2	2	2	2	2	1
Urea	20	−16	−2	0	1	1	1	2	2	2	2	2	1
Hydrogen peroxide	8	0	0	0	1	0	1	7	7	12	67	14	118

flow was toward the cathode so that indicators which were partially adsorbed by the paper support during electrophoresis appeared to behave as anions. This was shown to be the reason for the apparent anionic mobilities of caffeine and urea in most electrolytes used in this and earlier work[2]. Mixtures of glucose with caffeine or urea were applied to papers through which water or electrolyte solutions were allowed to flow by capillary attraction. Glucose was found, in every case, to travel faster and to separate completely, within 2 h, from caffeine and urea, showing that the latter compounds are reversibly adsorbed by wet cellulose.

The cationic mobilities of caffeine and urea on electrophoresis in strongly acid electrolytes, on the other hand, reflect their weakly basic character. CRESTFIELD AND ALLEN[9] warned that caffeine may be used as a valid indicator of electroendosmosis only above about pH 3. The same restriction applies to urea but it appears occasionally to have been used with electrolytes of lower pH (ref. 5). In the sodium perchlorate electrolyte, caffeine has appreciable anionic mobility. It is known that caffeine and sodium perchorate form a crystalline addition compound in equimolecular ratio[10], and it is possible that an equilibrium mixture containing the corresponding complex anion forms spontaneously in aqueous solution.

The electrophoretic behaviour of reducing sugars in 0.1 *N* NaOH has already been reported[2] but values for the anionic mobilities of glucose and the methylated glucoses are included in Table I to emphasise their magnitude by comparison with other interactions described here. The ionisation of glucose appears to begin at a relatively low pH and to increase progressively in carbonate electrolytes of increasing pH. The mobilities of glucose and glycerol in borate buffer (pH 9.2) are also included for purposes of comparison.

It was shown, as described above that, with respect to glucose, caffeine is preferentially adsorbed by paper impregnated with phosphate buffer (pH 8.2). The greater anionic mobility of glucose on electrophoresis in phosphate buffer must therefore be due to the formation of a charged phosphate complex of glucose. Interactions between carbohydrates and phosphate are evidently quite general as the following mobilities of other reducing sugars and some polyols in the phosphate buffer indicate: galactose (5), mannose (5), rhamnose (4), xylose (6), arabinose (4), ribose (4), sorbose (6), fructose (5), maltose (7), melibiose (7), cellobiose (6), lactose (5), sucrose (7), raffinose (7), mannitol (7), dulcitol (7), sorbitol (6) and *myo*-inositol (6). The interactions are also evident, though less strongly, in phosphate electrolytes at pH 7.0 and 9.2, but in 0.1 *M* sodium dihydrogen phosphate (pH 4.5) all carbohydrates tested moved with the electroendosmotic flow. The anionic displacements of carbohydrates in phosphate electrolytes above pH 7 are not large but they are easily reproducible using the enclosed-strip method of paper electrophoresis. They are probably less easily detected using methods which do not afford the same control over temperature and moisture content of the paper strip and this may account for the effect being overlooked in previous work[11].

Hydrogen peroxide also has appreciable anionic mobility in phosphate buffer (pH 8.2), which is probably due to the formation of a perphosphate ion as a component of an equilibrium mixture. Similarly, the spontaneous formation of perborate[12] and percarbonate[13] anions probably explains the electrophoretic behaviour of hydrogen peroxide in the borate and carbonate electrolytes at or below pH 9.2. At higher pH values its anionic mobility may be reinforced by the presence of peroxide ions. Al-

though the use of peroxide as an indicator of electroendosmosis in neutral and acid electrolytes appears to be justified, it evidently cannot be used even in mildly alkaline media containing borates, carbonates or phosphates[14].

The dissociation constant of hydrogen peroxide, pK_a (25°) = 11.6 (ref. 15), indicates that in 0.1 *N* NaOH it should be almost completely ionised, and, in contradiction to an earlier report[16], it was found to be highly mobile as an anion in the sodium hydroxide electrolyte.

Of the indicators compared in this study the methylated glucoses provide the most accurate measure of electroendosmotic flow in all but one or two electrolytes but, of the more common compounds, caffeine and urea are generally useful for work not requiring a high order of precision. Before selecting any one indicator for use with different electrolytes it is suggested, as a precaution against errors due to unexpected interactions, that several different indicators be compared simultaneously under the new experimental conditions.

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1 H. WALDMANN-MEYER, *Chromatog. Rev.*, 5 (1963) 1.
2 J. L. FRAHN AND J. A. MILLS, *Australian J. Chem.*, 12 (1959) 65.
3 H. WEIGEL, *Advan. Carbohydrate Chem.*, 18 (1963) 61.
4 J. T. EDWARD AND R. CRAWFORD, *J. Chromatog.*, 1 (1958) 449.
5 D. WALDRON-EDWARD, *J. Chromatog.*, 20 (1965) 556.
6 G. GOMORI, in S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in Enzymology*, Vol. I, Academic Press, New York, 1955, p. 144.
7 J. L. FRAHN AND J. A. MILLS, *Australian J. Chem.*, 17 (1964) 256.
8 J. L. ENGELKE, H. H. STRAIN AND S. E. WOOD, *Anal. Chem.*, 26 (1954) 1864.
9 A. M. CRESTFIELD AND F. W. ALLEN, *Anal. Chem.*, 27 (1955) 422.
10 *Beilsteins Handbuch der Organischen Chemie*, 4. Aufl., 1. Ergänzungswerk, Bd. XXVI (Springer-Verlag, Berlin, 1938, p. 137.
11 R. CONSDEN AND W. M. STANIER, *Nature*, 169 (1952) 783.
12 I. R. WILSON, *Australian J. Chem.*, 13 (1960) 582.
13 T. P. FIRSOVA, A. N. MOLODKINA, T. G. MOROZOVA AND I. V. AKSENOVA, *Khim. Perekisnykh Soedin., Akad. Nauk SSSR, Inst. Obshch. i Neorgan. Khim.*, (1963) 119; *C.A.*, 60 (1964) 15408b.
14 J. L. ENGELKE AND H. H. STRAIN, *Anal. Chem.*, 26 (1954) 1872.
15 K. YU. SALNIS, K. P. MISHCHENKO AND I. E. FLIS, *Zh. Neorgan. Khim.*, 2 (1957) 1985; *C.A.*, 52 (1958) 13401d.
16 G. GRASSINI AND M. LEDERER, *J. Chromatog.*, 2 (1959) 326.
17 A. B. FOSTER, *Chem. Ind. (London)*, (1952) 828.
18 J. G. FEINBERG AND I. SMITH, *Chromatography and Electrophoresis on Paper*, Shandon Scientific Co., London, 1962, pp. 78, 107.
19 E. J. BOURNE, D. H. HUTSON AND H. WEIGEL, *J. Chem. Soc.*, (1960) 4252.
20 R. WEBER, *Helv. Chim. Acta*, 34 (1951) 2031.

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