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ISOTACHOPHORETIC ANALYSIS OF THE ANIONIC PRODUCTS FORMED BY THE HOMOGENEOUS OXIDATION OF SUGAR

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SUMMARY

The choice of new buffer systems makes possible the analysis of acids that have only a small difference in mobility. Some analyses are shown and some conditions are given for the separation of the acids formed by the wet-oxidation of sugar.

INTRODUCTION

The homogeneous oxidation of sugar by oxygen in aqueous alkaline solutions has been extensively studied[1], because some of the products obtained are important for industrial purposes. Various kinds of reactors and catalysts have been tested[1]. To study the mechanism and kinetics, a complete analysis of the mixture finally obtained is required. The possibility of regulating the rate of oxidation needs a relatively rapid and reproducible method.

Gas–liquid chromatography (GLC) has proved to be a useful analytical tool in this field[2]. For acids with a low molecular weight, however, the analysis is difficult. The reproducibility is poor because the sample pre-treatment is rather complicated.

Isotachophoretic analyses of such mixtures are shown in this paper to be a useful analytical technique for the study of the anionic components of the mixtures finally obtained[3].

A combination of GLC and isotachophoresis gives a good analysis of all the products formed by the oxidation.

EXPERIMENTAL

The theory of isotachophoresis has been dealt with by several authors[4,5].

A current-stabilized power supply is used and the electrode compartments are connected to each other by a capillary filled with the leading electrolyte and have a sample tap. When a voltage is applied, the ionic substances are separated into consecutive zones, if the steady state has been reached. The concentrations of the electrolytes in these zones are adjusted to the concentration of the leading electrolyte zone[5].

For anion analyses, the capillary is filled with a very mobile anion, faster moving than any in the sample, and a cation with buffering capacity.

The cathode compartment is filled with an acid with a low effective mobility. The mobility of this anion in the system is less than that of any of the sample ions.

The sample is introduced between the leading electrolyte and the terminating electrolyte.

The analyses discussed here were carried out in an apparatus described elsewhere[6]. A four-way tap was used for introduction of the sample.

The anode compartment was separated from the capillary tube by a flat membrane of cellulose acetate, so that hydrodynamic flow was prevented and electro-endosmosis was reduced to negligible proportions.

The capillary tube (PTFE) had an inside diameter of 0.45 mm and an outside diameter of 0.72 mm, and the length was 1 m. Two thermocouples were used as detectors, made of copper and constantan wires of thickness 15 μm. The thermocouples were mounted on the capillary with an elastic cement at distances of 40 cm and 70 cm from the sample tap. The signals derived from these thermocouples were amplified by a knick-amplifier, type A, and recorded by a Hitachi–Perkin-Elmer recorder, type 196. The peaks were derived by differentiating the signals electronically[7].

A current-stabilized power supply with a maximum potential of 22 kV was applied, and the high tension was made by the Greinacher principle[8].

RESULTS AND DISCUSSION

Fig. 1 shows an electropherogram of the separation of seven important polyoxy-acids, normally to be found in the reaction mixtures mentioned below. The conditions for this analysis are given.

Without sample pretreatment, but only diluting with leading electrolyte from time to time, analyses with these polyoxy-acids were carried out over several pH ranges because the reproducibility of the analyses and the quantitative aspects were to be studied.

At neutral pH or higher, the acids considered are very mobile because they are fully dissociated. Low tensions are therefore adequate because the resistance is low. The difference in mobility, however, does not vary much, which means that longer capillaries and hence higher potentials must be used or that counterflow of electrolyte is necessary for separation of all the zones. In spite of this, some stable mixed zones will be formed, for instance between oxalic and tartronic acid.

Working at lower pH (3–4) means that the effective mobility of the acids is lowered. The difference in the pK values of the acids is more important in this case than is the difference in mobility. The potential gradient will also increase if a reasonable time for analysis (1 h) is allowed, because the resistance of the system is much higher than that at neutral pH.

The contribution of the hydrogen ions to the conductivity is decreased by using the chosen concentration of the electrolytes of 0.012 M.

It must be borne in mind that the pH of the zones following the leading electrolyte is increasing.

The experiments at neutral pH were carried out with imidazole (0.02 M) and

Fig. 1. Isotachopherogram of the separation of some acids. 1 = Chloride (0.0012 N); 2 = oxalic acid (0.0003 M); 3 = tartronic acid (0.00125 M); 4 = formic acid (0.00145 M); 5 = 2-keto-1-gulonic acid (0.00125 M); 6 = glycolic acid (0.00125 M); 7 = gluconic acid (0.00125 M); 8 = levulinic acid (0.00175 M); 9 = pivalinic acid (0.01 M). The buffer used was α-naphthylamine (0.01 M).

hydrochloric acid (0.01M) as electrolyte, and α-naphthylamine (0.01M) and hydrochloric acid (0.012M) were used at pH 2.85.

The sample tap had a volume of about 70 μl. The current was stabilized at 50 μA for the experiments at low pH and 80 μA for the experiments carried out at neutral pH. The conductivity of the anions at low pH is such, compared with the conductivity at neutral pH, that a great increase in temperature will result in the development of gas bubbles when working at 80 μA. However, the potential applied will be 22 kV at the end of the analysis at low pH, whereas 10 kV is normal for the end of the analysis at neutral pH.

To analyse the anionic products formed by the degradation of sugars in alkaline solutions by oxygen, first a reference mixture was made. Fig. 1 shows an electropherogram of the possible acids obtained by the oxidation of a sugar. The analysis was carried out at pH 2.85. A full separation without mixed zones could be obtained. An electropherogram of the analysis of this mixture at pH 6.95 is not given because it is rather complicated owing to the occurrence of many mixed zones between the real acid zones.

Table I gives more information about the possible acids in the reaction products. The effective mobilities, the zone concentrations and the zone pH were calculated with a computer. The equations suitable for this program have been given[5]. The data for pK values and mobilities were taken from the literature[9–12]. Some were determined from graphs, as shown in Figs. 2 and 3. Because the differences in pK values in the literature are often high, for some acids these values were determined from titration

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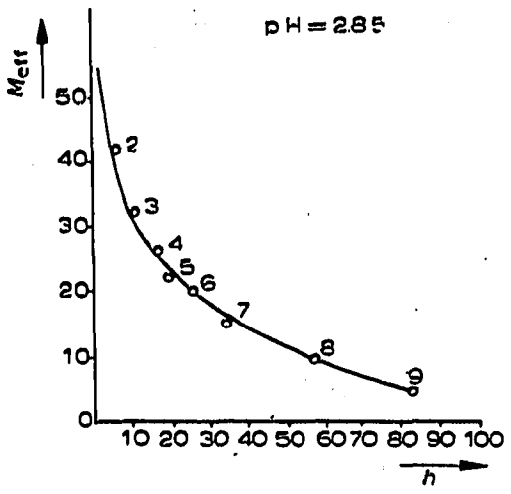
TABLE I

CALCULATED AND EXPERIMENTAL DATA FOR POSSIBLE PRODUCTS FORMED BY THE OXIDATION OF A REFERENCE SUGAR

Conditions: at pH 6.95, imidazole concentration = 0.02 M, hydrochloric acid = 0.01 M, pK = 6.95, mobility = 30 cm²/V sec; at pH 2.85, α-naphthylamine concentration = 0.01 M, hydrochloric acid = 0.012 M, pK = 3.92, mobility = 16 cm²/V sec. Symbols: h = step height in electropherogram; $m_{eff.}$ = effective mobility (cm²/V sec); pH_{zone} = calculated pH of the zone; c_{zone} = concentration of ionic species (mole/l); k_1, k_2 = dissociation constants of first and second steps, respectively; m = mobility (cm²/V sec).

Zones	*Products*	pK_1	pK_2	m_1	m_2	*Neutral pH (6.95)*				*Low pH (2.85)*			
						h	$m_{eff.}$	pH_{zone}	c_{zone}	h	pH_{zone}	$m_{eff.}$	c_{zone}
1	Chloride	−10		78.2		0	78.2	6.95	0.01	0	78.2	2.85	0.012
2	Oxalic acid	1.23	4.19	40.2	73	4.0	72.9	6.96	0.0049	6.5	42.1	3.09	0.0092
3	Tartronic acid	2.34	4.74	36	65	6.6	64.8	6.96	0.0048	10.5	32.6	3.20	0.0096
4	Formic acid	3.75		56.6		14.5	56.6	6.99	0.009	16.0	26.3	3.69	0.0139
5	2-Keto-1-gulonic acid[a]	3.25		35		58.4	35.0	7.08	0.0074	19.5	22.3	3.49	0.0108
6	Glyconic acid	3.83		45		29.7	45.0	7.02	0.0086	25.5	20.2	3.74	0.0128
7	Glycolic acid[a]	3.92		36		56.0	36.0	7.06	0.0077	34.7	15.5	3.80	0.0117
8	Levulinic acid	4.59		38		46.0	37.9	7.05	0.0079	57.3	9.9	4.13	0.0126
9	Pivalinic acid	5.4		38.1		46.8	37.3	7.08	0.0077	83.0	4.7	4.55	0.0129

[a] No suitable terminator is known at present for these compounds in neutral systems.



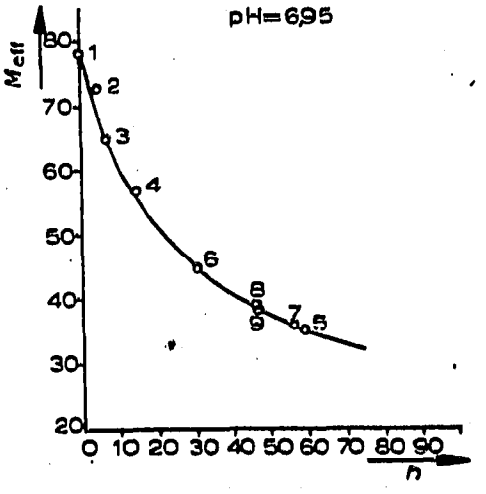


Fig. 2. The relation between the effective mobility of some acids at pH 2.85 and the step heights found in the isotachopherogram. The numbers on the curve refer to the zone numbers given in Table I.

Fig. 3. The relation between the effective mobility of some acids at pH 6.95 and the step heights found in the isotachopherogram. The numbers on the curve refer to the zone numbers given in Table I.

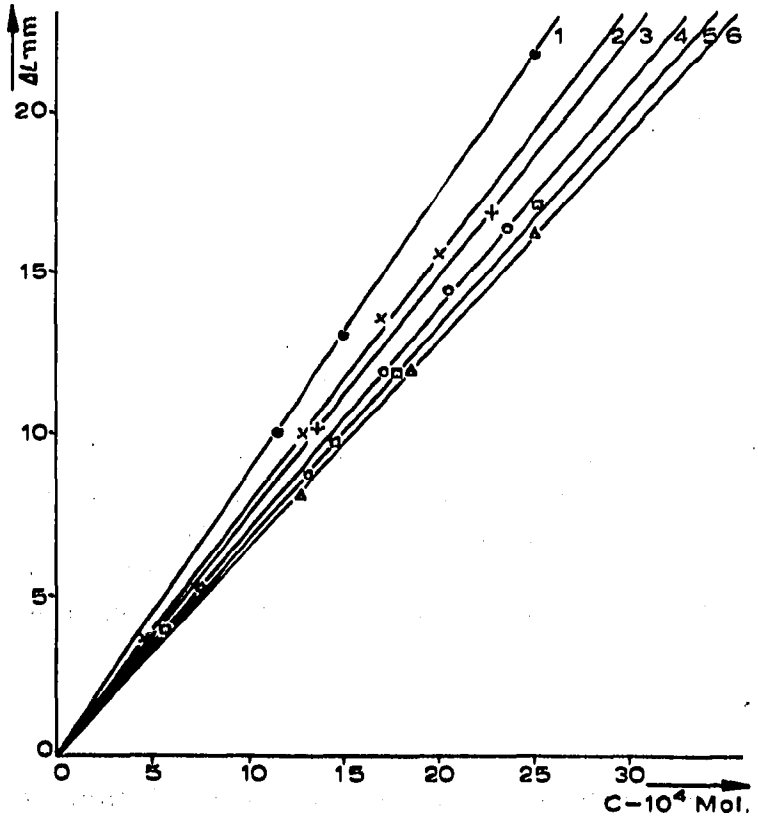


Fig. 4. The distance between two successive peaks of the isotachopherogram as a function of the concentration of a component in the sample mixture. 1 = 2-Keto-1-gulonic acid; 2 = tartronic acid; 3 = formic acid; 4 = levulinic acid; 5 = gluconic acid; 6 = glycolic acid.

curves measured with a Philips digital pH meter, type PW 9408. If the pK value was measured, the mobility was estimated from the curve given in Fig. 2.

The standard curve was derived from experiments with compounds for which the data in the literature agreed with our measurements. Because these experiments were carried out at neutral pH, the compounds were chosen such that they were fully dissociated, as are the acids of the reaction products. These estimated mobilities were used for the calculation of the effective mobilities at pH 2.85.

Again a plot of effective mobility against the step height was made. The smooth curve obtained indicates that the values for the mobilities estimated were correct. Table I shows that between oxalic acid and tartronic acid a mixed zone can be expected at neutral pH, and this was also found experimentally. The pHs of the zones are equal at neutral pH and the difference in mobility is small, whereas the difference at low pH is much greater and the pH of the consecutive zone is increasing.

Samples obtained from the catalytic oxidation of sorbose (7 h) and the partial catalytic oxidation of fructose at pH 11 (8 h) were analysed. The oxidation was carried out in an apparatus as described elsewhere[1].

Isotachophoretically the mixtures appeared to contain:

Sorbose oxidation	*Anionic components (%)*
oxalic acid	10.3
tartronic acid	9.2
formic acid	7.1
2-keto-1-gulonic acid	31.0
glycolic and glycerinic acids	42.4

Fructose oxidation	*Anionic components (%)*
formic acid	20.5
glycolic acid	52.0
gluconic acid	18.9
arabonic acid	8.6

The main component of interest, 2-keto-2-gulonic acid, was easy to determine and hence no further investigations were made as to whether glycolic or glycerinic acid or both were present. To investigate the presence of these acids, a different pH range must be chosen. Calculations of the effective mobility as a function of the pH shows that imidazole would be a suitable buffer for the separation of these two acids. The graph in Fig. 4 was used for quantitative determination. The relation between the distance between two successive peaks and the concentration of a component in the sample mixture is given. The average was taken from three measurements. The volume of the sample introduction is constant.

CONCLUSIONS

Isotachophoretic analysis of the ionic products formed by the oxidation of sugars gives a rapid and reproducible result. Because no sample pretreatment is needed, isotachophoresis can be used to follow and to regulate the oxidation. Together with GLC a complete analysis can be made of all the products formed.

REFERENCES

1 H. G. J. DE WILT, *Thesis*, University of Technology, Eindhoven, The Netherlands, 1970.
2 L. A. T. VERHAAR AND H. G. J. DE WILT, *J. Chromatogr.*, 41 (1969) 168.
3 W. J. M. KONZ, *Graduation Report*, University of Technology, Eindhoven, The Netherlands, 1971.
4 A. J. P. MARTIN AND F. M. EVERAERTS, *Proc. Roy. Soc. (London), Ser. A*, 316 (1970) 493.
5 F. M. EVERAERTS AND R. J. ROUTS, *J. Chromatogr.*, 58 (1971) 181.
6 J. L. BECKERS AND F. M. EVERAERTS, *J. Chromatogr.*, 51 (1970) 339.
7 F. M. EVERAERTS AND TH. P. E. M. VERHEGGEN, *J. Chromatogr.*, 53 (1970) 315.
8 H. P. J. BREKOO AND A. VERHOEFF, *Philips Tech. Rev.*, 23 (1961) 354.
9 *Handbook of Chemistry and Physics*, Chemical Rubber Publ. Co, 1968–1969, p. 49.
10 LANDOLT-BÖRNSTEIN, *Zahlenwerte und Funktionen*, 6 Aufl., Bd. II, Teil 7, Springer-Verlag, Berlin, 1960.
11 *International Critical Tables of Numerical Data, Physics, Chemistry and Technology*, Vol. VI, McGraw-Hill, New York and London, 1933.
12 G. KORTÜM, W. VOGEL AND K. ANDRUSSOW, *Dissociationskonstanten Organischer Säuren in Wässriger Lösung*, Butterworths, London, 1961.