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Paper Ionophoresis of Aldehydes and Ketones in the Presence of Hydrogen Sulphite

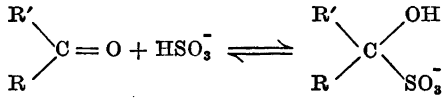
OLOF THEANDER

Organisk-kemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, and Träkemiska Avdelningen, Svenska Träforskningsinstitutet, Stockholm, Sweden

A number of aldehydes and ketones of different types have been studied by paper ionophoresis in hydrogen sulphite buffers of different concentrations and pH. The method is useful for the separation and characterisation of many carbonyl compounds.

Sugars have been studied by paper ionophoresis, generally in borate buffers [1], but electrophoretic methods for the separation of other aldehydes and ketones have not yet been developed. In a recent paper, Frahn and Mills [2] describe the ionophoretic behaviour of some sugars and glycols in aqueous solutions of borate, tungstate, vanadate, molybdate, arsenite, basic lead acetate and sodium hydroxide.

In the present publication a method is described for paper ionophoresis of aldehydes and ketones after conversion to the hydroxysulphonic acids. The ionophoresis is carried out in hydrogen sulphite buffers. The formation by aldehydes and many ketones of hydroxysulphonates, which can often be obtained in a crystalline state and are easily decomposed by alkali and acids, is well known.



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The present method was worked out for fractionation and characterisation of a number of carbonyl compounds obtained from the oxidation of methyl glucosides [3]. Some other carbohydrates, and some aromatic aldehydes and other carbonyl compounds have also been investigated by this method.

Hydroxysulphonic acids are strong acids and can be considered as completely dissociated in the buffers used. The rate of migration of a carbonyl compound in a sulphite buffer should therefore depend largely upon the migration velocity of the ion and on the equilibrium I, assuming of course that the equilibrium is set up sufficiently fast that the carbonyl compound and its

sulphite adduct move as one substance. By using a low concentration of hydrogen sulphite, it is obviously possible to decrease the migration velocity of substances which give an adduct with a high dissociation constant, so that they separate better from substances with low dissociation constants. The equilibrium constants and the velocity coefficients are dependent on the temperature.

In a sugar solution there is an equilibrium between the different forms, the α and β pyranosides and furanosides. The interconversion of these forms, which is, of course, influenced by pH, temperature and other factors, probably takes place through the intermediate formation of the open aldehyde or keto forms of the sugar. The equilibrium constants for the hydrogen sulphite adducts of different sugars have been determined by Sundman [4]. He found that in each of the groups: glucose and the disaccharides with glucose at the reducing end, the remaining hexoses and pentoses, the methylpentoses and the uronic acids, the equilibrium constants were fairly closely related to the mutarotation constants. The higher the mutarotation constant the lower the equilibrium constant. The fractionation of sugars on ion exchange columns in the hydrogen sulphite form has been studied by Samuelsson [5].

Since the completion of this work a paper by Frahn and Mills [6] has been published describing the ionophoresis of some sugars and oligosaccharides in hydrogen sulphite solution. Under the conditions used (0.4 M unbuffered hydrogen sulphite at room temperature) the equilibrium between the sugar and the adduct was only slowly established. Each sugar gave two spots with streaking between, one representing the uncharged sugar with no migration and the other, which became quite faint after prolonged runs, the hydroxysulphonate ion. The distances between these spots were roughly equal for isomeric sugars but decreased with the molecular weight. This makes it possible to determine the chain length of lower oligosaccharides.

The addition of hydrogen sulphite to carbonyl compounds other than carbohydrates has been studied very little. Smith and Nichols [7] studied the addition to methylbenzaldehydes. The analogous cyanhydrin reaction has been more thoroughly investigated [8–11].

As an index of migration for the ions of hydrogen sulphite carbonyl compounds the term M_v is suggested, where for any substance

$$M_v = \frac{\text{true distance of migration of the substance}}{\text{true distance of migration of vanillin}}$$

Vanillin is chosen as reference substance since it migrates reasonably rapidly. The phenolic group is not ionized under the standard conditions used. The movement due to electro-endosmotic flow is corrected for by using methyl-β-glucoside as a reference compound.

Table 1 shows the influence of pH on the separation and shows for comparison the migration rates in some buffers not containing hydrogen sulphite. Sundman [4] worked with pH 4.7 as standard, and this pH has also been chosen here as standard. The concentration of hydrogen sulphite ion has a flat maximum at about pH 5 (the dissociation constants are $K_1 = 1.66 \times 10^{-2}$ and $K_2 = 1.02 \times 10^{-7}$). A better separation can be obtained in some cases at

higher pH values (*e. g.* between 6-aldehydo- and 3-keto-methyl-β-glucopyranoside) or by using pH 4.7 and a lower concentration of hydrogen sulphite. (Table 2). It is interesting to note that a good separation is obtained between glucuronic acid and galacturonic acid, but that the reversed order of migration is obtained in the absence of hydrogen sulphite. A disadvantage of using a higher pH is the increasing ionization of phenolic groups (*e. g.* in vanillin and formylvanillin). Unbuffered hydrogen sulphite was also tried but was found, to be less satisfactory. Freshly made it had a pH of 3.8 at 0.1 M concentration but after a few runs the pH was below 3.0.

Table 2 shows migration rates for some substances in hydrogen sulphite buffers at pH 4.7 and varying concentrations. It is evident that the migration velocity increases with increasing concentration. In agreement with the argument above, the separation is improved by decreasing the concentration of hydrogen sulphite. This is also shown in Table 4 for some aromatic aldehydes and oxidized glucosides. A disadvantage of using an electrolyte of too low a concentration is the tendency to tail and the lack of reproducibility after the solvent has been used for a few runs.

The M_v values at room temperature for some aldehydes and ketones in the buffer chosen as standard, 0.1 M hydrogen sulphite buffered to pH 4.7, are presented in Table 3. These values are reproducible to within ± 0.02 units or less. The spots obtained were round and sharp. As the unsubstituted sugars gave more or less long streaking spots under standard conditions, due to the relatively slow equilibrium between sugar and adduct they were also run at 60 °C (Table 5). At this temperature they gave single, concentrated spots, but the M_v values were approximately the same as those obtained at room temperature. The M_v values are in good qualitative agreement with the equilibrium constants of sugar hydrogen sulphite adducts estimated by Sundman [4]. It is obvious that for characterising and separating sugars the method is a good complement to the borate electrophoresis method and to paper chromatography.

It will be noticed that methyl β-3-ketoglucoside has a higher affinity for hydrogen sulphite than methyl α-3-ketoglucoside. The M_v values for the oxidized glucosides in the series are comparable with the M_v values of aldehydo-pentacetylglucose and *n*-heptanal and the aldehydo-glucoside is more reactive than the keto glucosides. On the other hand the difference in affinity between keto-glucosides and ketoses is remarkable. The separation of oxidized glucosides is improved by using a lower concentration of hydrogen sulphite (Table 3) or a higher temperature [3].

It is interesting to compare periodate oxidized methyl-β-glucopyranoside with periodate oxidized methyl-β-xylopyranoside. The first gave two spots of approximately the same intensity, with streaking between, and M_v values of about 1.0 and 1.5, respectively. The other gave only one spot with an M_v value of about 1.6. Those spots might indicate one (M_v 1.0) and two (M_v 1.5; 1.6) carbonyl groups available, respectively, for the formation of hydrogen sulphite adducts *.

* *Added in proof:* These results are confirmed by recent investigations of periodate oxidized methyl glycosides (Smith, F. *et al. J. Am. Chem. Soc.* **79** (1957) 691, and Mester, L. and Móczár, E. *Chemistry & Industry* **1957** 761).

The expected contribution to the M_v value when a new carbonyl group is introduced can be seen by comparison between the M_v values of *cyclo*hexanone and *cyclo*hexane-1:3-dione. In the group of substituted benzaldehydes investigated there is in some cases no separation at all and in others a good separation, particularly with low concentration of hydrogen sulphite. Lapworth and Manske [8] have investigated the effect of different substituents on the addition of hydrogen cyanide to substituted benzaldehyde. For *ortho*-, *meta*-, and *para*-hydroxy and *para*methoxy substituted benzaldehydes the M_v values and the equilibrium constants of the corresponding cyanhydrins were parallel. An exception was found in the *ortho*- and *para*-hydroxybenzaldehydes at 20 °C. At 60 °C, however, the order of their M_v values was reversed. There was no appreciable improvement in the separation of the aromatic aldehydes when higher temperatures were used during the runs. The distance of migration of the aromatic aldehydes over the same potential gradient and time was 10—20 % greater at 60 °C than at room temperature.

Barker, Bourne and Theander [12] used a borate containing eluant for the carbon column chromatography of some sugars which were difficult to separate by ordinary carbon column chromatography, but showed different electrophoretic mobilities in borate buffers. The analogous use of sulphite in the eluant is obvious and a mixture of methyl-β-glucoside and methyl β-2-ketoglucoside has recently been separated by that method [3].

Table 1. The migration of some aldehydes and ketones in electrolytes of different pH (Whatman No. 3, room temperature, 14.8 V/cm, 3—5 h; the migration of galacturonic acid is taken as 1.00).

	In the presence of hydrogen sulphite				In the absence of hydrogen sulphite		
pH	4.7	6.0	7.0	9.4	4.7	6.0	7.0
Xylose	0.24	0.21	0.17	0	0	0	0
Aldehydo-penta acetylglucose	0.60	0.63	0.64	0	0	0	0
Methyl β-D-3-keto-glucopyranoside	0.62	0.65	0.67	0	0	0	0
Methyl β-D-6-aldehydo-glucopyranoside	0.65	0.78	0.81	0.24	0	0	0
Glucuronic acid	0.67	0.82	0.83	1.11	0 1.13	0 1.06	0 1.06
Galacturonic acid	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2-Ketogluconic acid	0.80	0.88	0.90	1.16	1.19	1.13	1.12
4-Hydroxy-3-methoxybenz-aldehyde (Vanillin)	0.71	0.77	0.90	0.75	0	0.08	0.29
3-Aldehydo-4-hydroxy-5-methoxybenzaldehyde (Formylvanillin)	1.07	1.21	1.33	0.89	0.23	0.84	0.88

Table 2. The migration (in cm) of some aldehydes and ketones in hydrogen sulphite buffers of various molarity and pH 4.7. (Whatman No. 3, room temperature, 12 V/cm for 5 h).

Molarity	0.01	0.05	0.10	0.20
Glucose	0	0.4	0.6	1.5
Xylose	2.5	4.6	6.1	12.4
Methyl β-D-3-keto-glucopyranoside	8.2	14.4	15.1	17.0
Methyl β-D-6-aldehydo-glucopyranoside	15.5	16.0	16.4	17.4
Galacturonic acid	21.1	23.8	25.0	27.7
2,4-Dihydroxy-benzaldehyde	6.2	12.0	14.2	16.9
Vanillin	16.3	17.0	17.5	19.3
Formylvanillin	26.2	26.8	27.1	28.7

Table 3. M_v values of some aldehydes and ketones. (0.1 M hydrogen sulphite, pH 4.7, Whatman No. 3, room temperature, 13—18.5 V/cm for 3—5 h.)

	M_v
Aldehydo-pentacetylglucose	0.86
Methyl α-D-3-ketoglucopyranoside	0.50
Methyl β-D-2-ketoglucopyranoside	0.88
Methyl β-D-3-ketoglucopyranoside	0.85
Methyl β-D-6-aldehydoglucopyranoside	0.92
Glucuronic acid	0.95
Galacturonic acid	1.43
2-Ketogluconic acid	1.13
5-Ketogluconic acid	1.02
Periodate oxidized methyl-β-glucopyranoside	1.02 1.48
Periodate oxidized methyl-β-xylopyranoside	1.62
n-Heptanal	0.98
Diacetyl	0.69
Citral	1.36
Cinnamic aldehyde	1.18
*Cyclo*hexanone	1.14
*Cyclo*hexane-1:3-dione	1.78
Furfural	1.29
ω-Hydroxymethyl furfural	1.07
Benzaldehyde	1.16
o-Hydroxybenzaldehyde (Salicylaldehyde)	0.95
m-Hydroxybenzaldehyde	1.16
3,4-Dimethoxybenzaldehyde (Veratraldehyde)	1.03
3,4,5-Trimethoxybenzaldehyde	1.01
4-Hydroxy-3-methoxybenzaldehyde (**Vanillin**)	**1.00**
2-Hydroxy-3-methoxybenzaldehyde (Orthovanillin)	1.10
4-Hydroxy-3,5-dimethoxybenzaldehyde (Syringaldehyde)	0.95
2,4-Dihydroxybenzaldehyde	0.82
3,4-Dihydroxybenzaldehyde (Protocatechualdehyde)	1.00
2,6-Dihydroxy-4-methylbenzaldehyde (Atranol)	0.74
3-Aldehydo-4-hydroxy-5-methoxybenzaldehyde (Formylvanillin)	1.53

Table 4. M_v values of some aromatic aldehydes and oxidized glucosides in 0.01 M hydrogen sulphite pH 4.7. (Whatman No. 3, room temperature, 12 V/cm for 5 h)

	M_v
Atranol	0.14
2,4-Dihydroxybenzaldehyde	0.38
o-Hydroxybenzaldehyde	0.91
Syringaldehyde	0.94
Vanillin	**1.00**
Protocatechualdehyde	1.02
3,4,5-Trimethoxybenzaldehyde	1.03
Veratraldehyde	1.04
Orthovanillin	1.10
p-Hydroxybenzaldehyde	1.12
Anisaldehyde	1.14
m-Hydroxybenzaldehyde	1.28
Benzaldehyde	1.31
Formylvanillin	1.61
Methyl α-D-3-ketoglucopyranoside	0.10
Methyl β-D-2-ketoglucopyranoside	0.55
Methyl β-D-3-ketoglucopyranoside	0.49
Methyl β-D-6-aldehydoglucopyranoside	0.95

EXPERIMENTAL

The apparatus and technique of the paper ionophoresis are essentially the same as described by Foster [13]. The ionophoresis was usually run at room temperature on Whatman No. 3 paper, but in some cases the temperature was held at 60 °C with a thermostat circulating warm water through the condensing block, which supports the glass plates and paper. The compounds were located by the use of UV light, dinitrophenylhydrazine, silver nitrate-sodium ethoxide or anisidine hydrochloride. Methyl-β-glucoside used to indicate the endosmotic effect, was placed on the margins together with vanillin, the standard reference substance. The latter, which was also put on the middle of the starting line, was located under UV, the margin strips were cut out, methyl-β-glucoside was located with the silver reagent, and the main paper was treated with a suitable reagent. The potential gradient used varied between 12 and 18.5 V/cm; the time between 3 and 5 h. The substances to be investigated were generally applied as 1 % solutions to the dry paper. The paper was dipped and the potential gradient was applied about 5 min after the electrolyte had risen from both sides up to the starting line.

Electrolytes. The 0.1 M hydrogen sulphite solution at pH 4.7 used as standard electrolyte contained per litre, 9.5 g of dry sodium *pyro*sulphite, 8.8 g of sodium acetate trihydrate and acetic acid to a pH of 4.7 (about 2.3 ml). The buffers of pH 6 and 7 contained the same concentrations of hydrogen sulphite and were buffered with potassium dihydrogen phosphate (0.05 M) and sodium hydroxide, the electrolyte of pH 9.4 was 0.1 M sodium sulphite. The electrolytes of varying hydrogen sulphite concentration, 0.01 – 0.2 M, all contained the same amount of sodium acetate as the standard hydrogen sulphite electrolyte (0.1 M) and were adjusted to pH 4.7 with acetic acid. The non-sulphite buffers of pH 6 and 7, respectively, contained 0.05 M potassium hydrogen phosphate adjusted to correct pH with sodium hydroxide and the electrolyte of pH 4.7 contained 0.1 M sodium acetate adjusted to correct pH with acetic acid.

The periodate oxidation of the glucosides was carried out with 0.03 M sodium periodate at 4 °C for 20 h in darkness. The aldehydo and ketoglucosides were obtained by chromate

Table 5. M_v values of some sugars at 60 °C and dissociation constants (K) of the hydrogen sulphite adducts at 19 °C (Sundman [4]). (0.1 M hydrogen sulphite, pH 4.7, Whatman No. 3, 13–18.5 V/cm for 3 h).

	M_v	K
Xylose	0.32	0.069
Arabinose	0.36	0.041
Ribose	0.40	0.028
Lyxose	0.55	0.019
Fucose	0.08	0.200
Rhamnose	0.13	0.127
Glucose	0.05	0.587
Allose	0.08	—
Galactose	0.19	0.104
Mannose	0.22	0.066
Altrose	0.32	—
Fructose, Sorbose, Tagatose, Allulose	0.00	—

oxidation of methyl-β-glucopyranoside [14] and of the 6-tritylethers of methyl-α-glucopyranoside and methyl-β-glucopyranoside [3].

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