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QUANTITATIVE DETERMINATION OF CARBOHYDRATES IN CELLULOSIC MATERIALS BY GAS–LIQUID CHROMATOGRAPHY

AUTOMATIC INTEGRATION OF ALDITOL ACETATE PEAK AREAS

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SUMMARY

Automatic integration, based on the voltage-to-frequency conversion principle, has been systematically studied to improve further the gas chromatographic method which was developed earlier for the quantitative analysis of wood and pulp carbohydrates. Mixtures containing various amounts of alditol acetates (L-arabinitol, D-xylitol, D-mannitol, D-galactitol, and D-glucitol) were injected into the gas chromatograph and the output data were then evaluated by integrator. The precision of the analysis was found to be good even in the case of some overlapping of the peaks. However, for reaching high accuracy, excellent chromatographic separation is a prerequisite.

INTRODUCTION

Gas chromatography of monosaccharides as fully acetylated alditol derivatives is a very useful procedure which can be applied to the quantitative determination of carbohydrates in wood and pulp after total hydrolysis. In our earlier work[1] the peak area measurements were made manually, *i.e.* by weighing the cut chromatograms. In the present work automatic integration of the peak areas was studied in order to improve further the reliability of the method as well as to minimise the time required for the analysis.

Several integrator types are commercially available for the evaluation of gas chromatograms. However, the instruments based on the electronic voltage-to-frequency conversion technique are considered most reliable[2]. Such integrators have been earlier studied by JOHNSON *et al.*[3] with respect to the reproducibility and linear dynamic range of integration. Mixtures consisting of acrylonitrile, *n*-propanol, and dioxane were used. Also, BAUMANN AND TAO[4] studied the effect of slope sensitivity, filtering and baseline correction rate on accuracy.

Integrators based on the voltage-to-frequency conversion principle were used also in the present work. In addition to the reproducibility of integration, factors influencing the analytical accuracy were subjected to a systematic study. The mixtures

used consisted of fully acetylated pentitols (L-arabinitol, D-xylitol) and hexitols (D-glucitol, D-mannitol, D-galactitol).

EXPERIMENTAL

A Perkin-Elmer gas chromatograph Model 900 equipped with dual columns and flame ionisation detector, was used. The 1/8 in. stainless-steel columns (2 m) were filled with packing material (2.3 g) consisting of a mixture of 2.5% Silicone oil XF-1150 and 2% ethylene-glycol succinate on 100–120 mesh Chromosorb W-H.P. The plate number, measured from the mannitol hexaacetate peak, was about 2100.

Nitrogen was used as the carrier gas (30 ml/min). The gas flow rates to the detectors were adjusted to 55 ml/min for the hydrogen and 300 ml/min for the oxygen–nitrogen gas mixture (20% O_2, 80% N_2). The injection port was maintained at 300°, the detectors at 220°, and the columns at 175°.

For the measurements of the peak areas an Infotronics integrator Model CRS-100 was mainly used with following adjustments: baseline tracking up 6 μV/min, down 200 μV/min; slope sensitivity, 2; peak rate, 3 sec/peak; input noise rejection, 3; shoulder printout off; digital filtering, 2000 counts; false trip reject on; count rate, 1000 counts/mV.

Some experiments were made using a Hewlett-Packard integrator, Model 3370 A. The adjustments were the following: noise suppression max.; slope sensitivity up 0.01 mV/min, down 0.01 mV/min; peak Σ level, 1000 mV; shoulder control, front off, rear 1000 mV; baseline reset delay 0, during the third peak the switch was turned to ∞ position for the rest of the run.

Various mixtures containing accurately weighed amounts (10–100 mg) of alditol acetates, prepared as described earlier[1], were dissolved in pyridine (1–2 ml). The amount injected was *ca.* 0.5 μl.

RESULTS AND DISCUSSION

The five alditol acetates were not all separated quite completely in the column

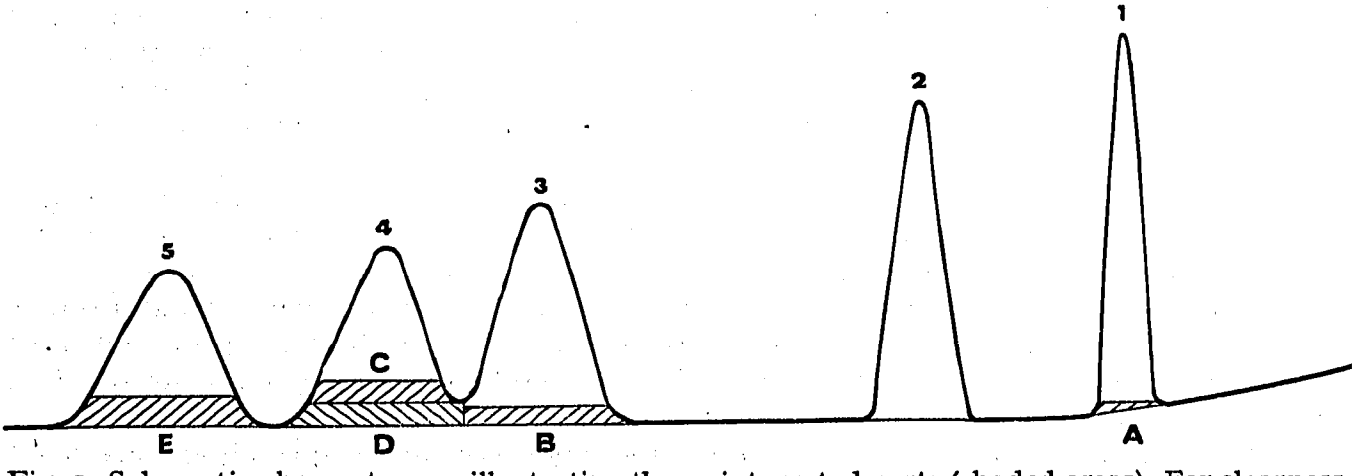

Fig. 1. Schematic chromatogram illustrating the unintegrated parts (shaded areas). For clearness, the errors have been exaggerated. 1 = Arabinitol pentaacetate; 2 = Xylitol pentaacetate; 3 = Mannitol hexaacetate; 4 = Galactitol hexaacetate; 5 = Glucitol hexaacetate.

used. As appears from Fig. 1, mannitol and galactitol hexaacetate peaks overlap to a certain extent. Therefore, when all five alditol acetates are present, the resulting chromatogram is "non-ideal". Three five-component mixtures containing varying proportions of alditol acetates were introduced into the gas chromatograph and the injection of each mixture was repeated ten times. The alditol acetate composition was then calculated on the basis of the pulses counted by the integrator. The results obtained with the Infotronics integrator are summarised in Table I.

As can be seen the precision of the analysis is comparatively good but the accuracy is unsatisfactory. This is obviously connected with several factors, *cf.* Fig. 1. One source of error is the fact that the peak corresponding to arabinitol pentaacetate

TABLE I

ANALYSIS OF ALDITOL ACETATE MIXTURES—INFOTRONICS INTEGRATOR

Alditol acetate	*Calc.*[a] *y*	*Found*[b] *x*	*S.D.*	*Range*
Arabinitol	6.58	6.97	0.16	0.53
Xylitol	8.89	9.24	0.17	0.56
Mannitol	6.53	7.21	0.08	0.28
Galactitol	6.53	6.43	0.08	0.31
Glucitol	71.48	70.15	0.25	0.17
Arabinitol	10.05	10.38	0.11	0.36
Xylitol	10.25	10.39	0.34	0.39
Mannitol	10.21	10.72	0.19	0.16
Galactitol	19.89	19.75	0.16	0.20
Glucitol	49.60	48.76	0.42	0.48
Arabinitol	19.21	19.65	0.24	0.74
Xylitol	16.95	16.99	0.17	0.46
Mannitol	23.08	23.06	0.26	0.97
Galactitol	20.63	20.45	0.11	0.28
Glucitol	20.12	19.85	0.24	0.74

[a] The percent composition of the alditol acetate mixture.
[b] These figures refer to the average percent composition calculated on the basis of 10 runs.

is very close to the solvent (pyridine) front. The integrator automatically follows the baseline until integration begins; integration is referred to the level reached at the beginning of a peak. The baseline is continuously sloping during the integration of the arabinitol peak, and hence, the shaded area A in Fig. 1 remains unintegrated. Further, the last three peaks are relatively gently sloping. The voltage change from the detectors, *i.e.* the slope of the chromatogram primarily determines the starting point of integration. Because of the insufficiently fast change, a certain rise in the baseline level occurs. Therefore, the areas marked with B, C, and E in Fig. 1 remain unintegrated. This could be avoided by using the highest slope sensitivity value (position 1 in Infotronics CRS-100 integrator). However, this high sensitivity caused vagueness in the termination of integration and hence was not advantageous. Finally, peaks Nos. 3 and 4 (mannitol and galactitol hexaacetates) are not separated completely. Since the peaks are relatively gently sloping there is sufficient time for the

TABLE II

ANALYSIS OF ALDITOL ACETATE MIXTURES—HEWLETT-PACKARD INTEGRATOR

Alditol acetate	*Calc.*[a] *y*	*Found*[b] *x*	*S.D.*	*Range*
Arabinitol	6.74	7.50	0.11	0.31
Xylitol	7.09	7.55	0.08	0.22
Mannitol	7.11	8.03	0.04	0.14
Galactitol	8.04	8.04	0.17	0.63
Glucitol	71.02	68.87	0.24	0.65
Arabinitol	10.40	10.68	0.11	0.35
Xylitol	9.94	10.18	0.29	0.81
Mannitol	10.62	10.88	0.11	0.39
Galactitol	20.16	19.71	0.20	0.64
Glucitol	48.89	48.57	0.43	1.14
Arabinitol	20.01	20.27	0.45	1.71
Xylitol	19.70	19.92	0.29	0.88
Mannitol	20.05	19.68	0.20	0.57
Galactitol	20.01	19.84	0.17	0.49
Glucitol	20.22	20.29	0.43	1.28

[a] The percent composition of the alditol acetate mixture.
[b] These figures refer to the average percent composition calculated on the basis of 10 runs.

integrator to change the baseline level after trailing edge of peak No. 3. The area marked with D is therefore not integrated.

Additional experiments were made using the Hewlett-Packard integrator. This instrument makes it possible to adjust the positive and negative slope detector sensitivities separately. However, the most sensitive positions (0.01 mV/min) gave the best results. Similar chromatographic conditions as in previous runs were used although the column characteristics were somewhat different. As can be seen from Table II, the precision and accuracy do not significantly differ from the previous runs.

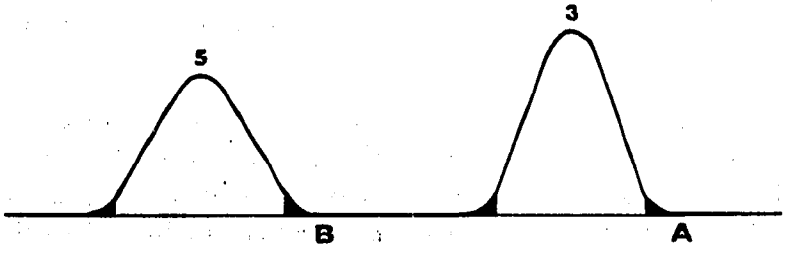

Fig. 2. Schematic chromatogram illustrating the unintegrated parts (shaded areas). For clearness, the errors have been exaggerated. 3 = Mannitol hexaacetate; 5 = Glucitol hexaacetate.

To approach as ideal chromatographic conditions as possible mixtures containing only three components (xylitol, mannitol, and glucitol) were studied. In addition, baseline rise during peaks 3 and 5 (mannitol and glucitol) was prevented manually so as to correspond to the levels marked by A and B in Fig. 2. The results from these runs are reproduced in Table III. It appears that both the precision and the accuracy are fairly good.

TABLE III

ANALYSIS OF ALDITOL ACETATE MIXTURES—INFOTRONICS INTEGRATOR

Alditol acetate	*Calc.*[a] *y*	*Found*[b] *x*	*S.D.*	*Range*
Xylitol	5.01	4.88	0.08	0.30
Mannitol	10.10	10.11	0.08	0.26
Glucitol	84.89	85.01	0.13	0.39
Xylitol	9.90	9.81	0.17	0.47
Mannitol	83.29	83.35	0.16	0.43
Glucitol	6.81	6.85	0.15	0.46
Xylitol	19.93	19.96	0.14	0.48
Mannitol	30.32	30.39	0.05	0.16
Glucitol	49.75	49.65	0.13	0.35
Xylitol	32.78	33.05	0.22	0.58
Mannitol	33.30	33.18	0.12	0.45
Glucitol	33.92	33.78	0.14	0.38
Xylitol	33.63	34.04	0.28	0.81
Mannitol	33.08	32.93	0.14	0.33
Glucitol	33.29	33.03	0.16	0.48
Xylitol	40.36	40.41	0.11	0.26
Mannitol	49.79	49.74	0.07	0.20
Glucitol	9.85	9.86	0.06	0.15
Xylitol	70.12	70.36	0.08	0.24
Mannitol	5.42	5.37	0.04	0.14
Glucitol	24.46	24.27	0.06	0.20

[a] The percent composition of the alditol acetate mixture.
[b] These figures refer to the average percent composition calculated on the basis of 10 runs.

The figures in Table IV were further examined comparing the found values (x) with the calculated values (y). For this purpose the constants of the best line ($y = a + bx$) and the scatter were determined. When comparing a and s_a values in different cases it can be concluded that for 95% probability limit the constant a does not significantly differ from zero, which means that there is no systematic error. It can

TABLE IV

ESTIMATION OF ANALYTICAL ACCURACY[a]

Alditol acetate	*Intercept*		*Slope*	
	a	s_a[b]	b	s_b[b]
Xylitol	0.07	0.12	0.994	0.003
Mannitol	0.06	0.08	0.999	0.002
Glucitol	0.12	0.10	0.999	0.002

[a] These figures were calculated on the basis of data in Table III and they refer to the best line ($y = a + bx$) within the corresponding concentration range of each alditol acetate.
[b] Standard deviation.

be concluded that constant b does not significantly deviate from 1.000. This value which corresponds to the "response factor" is identical for the three studied alditol acetates.

Finally, on the basis of the above results it is obvious that the studied electronic integrators are very suitable for the analysis of alditol acetate mixtures. However, for high analytical accuracy a sufficient chromatographic separation is a prerequisite. Work is now in progress for further improvement of the column packing material.

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