Note

High-performance liquid chromatographic determination of formic acid in cleavage reactions of carbohydrates

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Oxidative cleavage of carbohydrates by aqueous periodate yields formic acid. This reaction is used for the identification and quantitation of carbohydrates. For example, glucose gives 5 moles of formic acid according to eqn. 1.

$$Glucose + 5IO_4^- \rightarrow 5HCOOH + HCHO + 5IO_3^- \tag{1}$$

As the reaction usually takes place in quantitative yield, valuable information can often be obtained by measuring the consumption of periodate and/or the yield of formic acid as well as by identifying the carbonyl products.

Quantitative analysis of formic acid is usually carried out by means of chemical methods [mercury(II) chloride test, reduction to formaldehyde by zinc–hydrochloric acid][1,2], thin-layer chromatography[3], spectrophotometry[4], gas chromatography[5] and ion-exclusion chromatography[6].

We have developed a fast and accurate ion-exchange high-performance liquid chromatographic (HPLC) method, which has been proved successful for (i) the quantitation of monosaccharides; (ii) the determination of the reducing groups of polysaccharides; and (iii) the measurement of formic acid produced in the oxidation of polysaccharides by metal ions.

The method, whilst being quick and sensitive (lower limit of detection, 2 mg/l), avoids most of the disadvantages (long and complicated experimental procedures, necessity of clear solutions, derivatization, etc.) encountered with other techniques.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Wescan 260 ion analyser equipped with a Wescan 269-001 anion column and a Wescan 213A conductivity detector.

A 4 mM potassium hydrogen phthalate solution (pH 4.5) was used as eluent. The flow-rate was 2 ml/min.

Reagents and procedures

Formic acid and glucose, AnalaR reagents, and polygalacturonic acid (PGA) were obtained from J. T. Baker.

PGA was purified according to described methods[7].

The periodate oxidation of the polysaccharide was performed by suspending PGA (*ca.* 1.0 g) in an aqueous solution containing a 100% excess of potassium periodate.

Oxidation of PGA by $NaVO_3$ was carried out in aqueous suspension (pH *ca.* 4.5).

After being stood overnight at 5°C, the suspensions were dialysed (three times) against deionized water and the dialysates were brought to 200 ml and analysed for formic acid.

RESULTS AND DISCUSSION

Typical chromatograms of standard solutions of formic acid are shown in Fig. 1. Calibration curves were constructed by plotting the amount of formic acid against the height of the peak. Good results were obtained, as demonstrated by the correlation coefficient (0.997), calculated using a simple regression equation. Linearity was
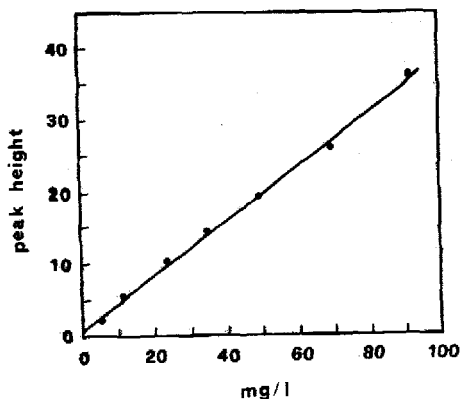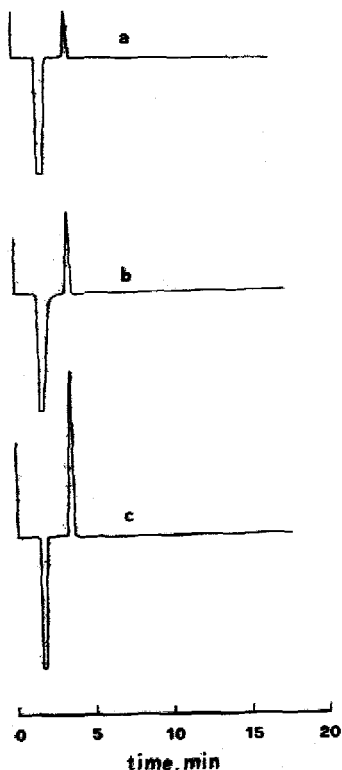

Fig. 1. HPLC chromatograms of standard aqueous solutions of formic acid: a, 25 mg/l; b, 45 mg/l; c, 90 mg/l.

Fig. 2. Calibration graph for the determination of formic acid in aqueous solution.

TABLE I

QUANTITATION OF GLUCOSE THROUGH PERIODATE REACTION AND HPLC DETERMINATION OF FORMIC ACID

Glucose concentration		Formic acid (mg/l)	
mg/l	mol/l	Calculated	Found
3.9	$0.22 \cdot 10^{-4}$	5.6	5
8.6	$0.48 \cdot 10^{-4}$	11.5	13
18.0	$1.00 \cdot 10^{-4}$	23.0	25
26.6	$1.48 \cdot 10^{-4}$	34.0	35
36.1	$2.00 \cdot 10^{-4}$	46.1	47
54.8	$3.04 \cdot 10^{-4}$	70.0	66
72.2	$4.01 \cdot 10^{-4}$	92.2	91

observed over the concentration range investigated (0–100 mg/l) (Fig. 2). The detection limit under the aforementioned experimental conditions, calculated as the concentration which gives a response equal to the noise of the detector, was 2 mg/l.

In order to check the effectiveness of the analytical method in the carbohydrate analysis, aqueous solutions of glucose, at varying concentrations, were prepared and submitted to periodate oxidation. The reaction mixtures were analysed for formic acid. The features of the chromatograms were as good as those obtained in calibration tests. Reliable quantitation of glucose, performed according to eqn. 1, was achieved in the concentration range 0–20 mg/l (see Table I).

The above method has been applied also in the case of a polysaccharide (polygalacturonic acid) for the quantitative determination of (i) the number of reducing end-units, through the periodate reaction, and (ii) the amount of formic acid formed upon oxidation by VO_3^-.

To find the best conditions for chromatographic analysis, in both cases it was necessary to separate formic acid from the polysaccharide. The suspensions were dialysed against deionized water and the dialysates were tested for formic acid.

The amount of formic acid in the solution obtained from the periodate reaction allowed us to measure the number of reducing end-units. The values obtained for two different samples ($0.87 \cdot 10^{-4}$ and $2.04 \cdot 10^{-4}$ mol per g of PGA on the basis of the theoretical yield of three molecules of formic acid per reducing end-unit compare well with those found using an independent method[8].

TABLE II

REPRESENTATIVE DATA CONCERNING THE INTERACTION OF PGA (1.0 g) WITH NaVO₃ SOLUTIONS

PGA sample with $2.04 \cdot 10^{-4}$ mol of reducing end-unit per g of polysaccharide.

VO_3^+ added (mol)	VO_3^+ remaining in solution* (mol)	Vanadium reduced (mol)	Formic acid formed (mol)
$9.44 \cdot 10^{-4}$	$1.60 \cdot 10^{-4}$	$7.84 \cdot 10^{-4}$	$1.80 \cdot 10^{-4}$
$18.94 \cdot 10^{-4}$	$11.06 \cdot 10^{-4}$	$7.88 \cdot 10^{-4}$	$1.75 \cdot 10^{-4}$

* Reduced vanadium is adsorbed on PGA and can be removed from solution.

In the case of the VO_3^- reduction, the chromatographic analysis showed the formation of 1 mole of formic acid per reducing end-unit of the polysaccharide. The stoichiometry of eqn. 2, supported by the analysis of the reduced vanadium, has thus been demonstrated[9] (see Table II).

$$-CH(OH)-CHO + 4VO_3^- + 12H^+ \rightarrow \qquad\qquad (2)$$

$$-COOH + 4VO^{2+} + HCOOH + 6H_2O$$
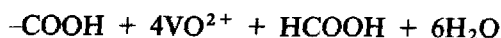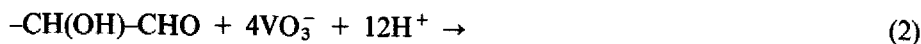
In conclusion, the present study has shown (i) that formic acid in aqueous solution can be reliably estimated by HPLC using anion-exchange columns; and (ii) that this method is applicable to the analysis of mono- and polysaccharides. Preliminary separation steps are required in the case of polysaccharides.

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