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Short communication

Development of the isotachophoretic method for determination of aldonic acids produced after alkaline oxidation of hexoses with palladium(II) chloride

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Abstract

An isotachophoretic method was developed and used for separation, identification and determination of aldonic acids formed by oxidation of aldoses with Pd(II) chloride in alkaline solution. The optimal conditions for separation and determination were found, and aldonic acids were investigated in the reaction mixture after the alkaline oxidation of glucose and mannose with Pd(II) chloride at different pH values. When the oxidation was carried out at pH 11.66, four acids were found: a mixture of epimeric six-carbon acids: mannonic and gluconic, with mannonic predominant (major product), and arabinonic and glyceric acids as minor products. But, when the oxidation was carried out at pH 9.00, only mannonic acid was determined showing 99% from the starting aldose, whether glucose or mannose.

Keywords: Aldonic acids; Palladium chloride; Glucose; Mannose; Mannonic acid; Gluconic acid; Arabinonic acid; Glyceric acid

1. Introduction

Aldonic acids are formed by alkaline oxidation, degradation and redox disproportion of poly-, oligo- and monosaccharides. Polyhydroxy acids derived from carbohydrates have been analyzed by paper chromatography, TLC, GC, HPLC and GC–MS [1–5]. But none of these techniques can completely resolve the complex mixture of organic acids produced by alkaline treatment of carbohydrates and therefore additional information was obtained by applying a combination of ion-exchange chromatography and GC–MS [6,7].

Isotachophoretic techniques are widely used for the separation and determination of a great number of components, such as organic and inorganic acids [8], and anionic products from the oxidation of sugars such as aldonic [9] and saccharinic acids [6]. It possesses some advantages over chromatographic methods, especially the minimum consumption of sample, and direct analysis without preliminary sample modification or derivatisation.

The procedure was developed for the determination of reductive sugars with palladium(II) chloride [10–12], based on reduction of $PdCl_2$ and oxidation of carbohydrates. The oxidation of carbohydrates with palladium(II) chloride in an alkaline medium probably gives aldonic acids, but no information about the structure of the product obtained was known.

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The aim of this paper was the development of an isotachophoretic method for separation, identification and quantification of aldonic acids and to use this method to analyse products of alkaline oxidation of aldoses (glucose and mannose) with $PdCl_2$.

2. Experimental

The oxidation of aldoses (glucose and mannose) was carried out using $PdCl_2$ in alkaline solution. The reaction between aldose 0.6 mg/ml (5 ml) and $PdCl_2$ 1 mg/ml (5 ml) prepared with $KNaC_4H_4O_6$ (sodiumpotassiumtartrate; 3 mg/ml) and Na_2SO_3 6 mg/ml (2 ml) in alkaline solution NaOH 15 mg/ml (2 ml; pH 11.66) was carried out at 70°C for 50 min, and with NaOH 15 mg/ml (0.5 ml; pH 9.00) at 70°C for 90 min, respectively. The samples were then evaporated to dryness under reduced pressure and dissolved in 5 ml of water.

Oxidation products were analyzed by a capillary column analyser LKB Bromma 2147 with UV and conductivity detector. The mixture of standard solutions and samples of 3 μl were injected into the tachophoretic column (200×0.3 mm I.D.) by Hamilton syringe (1–5 μl). The leading electrolyte was 1×10^{-2} *M* HCl altered to pH 3.00 with β-alanine and 0.2% Triton X, and the terminal electrolyte was 5.6×10^{-3} *M* sodium acetate. The degradation products were analysed at a driving current of 225 μA reduced to 25 μA during detection.

The standard solutions were made to a concentration of 1 mg/ml from D-mannono-γ-lactone and D-arabinono-δ-lactone, and from D-gluconic acid sodium salt and DL-glyceric acid calcium salt made as lactones (using 10 drops of acetic acid), and diluting with water to concentrations of 2.5, 5.0, 7.5 and 10 μg/ml.

3. Results and discussion

Isotachophoretic analysis could be used mainly for separation and identification and then for quantification of aldonic acids produced after oxidation of glucose and mannose with $PdCl_2$ in alkaline solution.

The identification of aldonic acids was performed on the basis of the measurement and comparison of the characteristic values of the relative step heights (r.s.h.) of the sample and of the relevant standards, under the already established conditions for separation of saccharinic acids [6]. The individual acids were then quantitated by the use of calibration curves made separately from the standard mixture for every standard respectively. These curves were evaluated statistically by linear regression, and the parameters are given in Table 1. Each calibration curve has been calculated from 10 measurements. From the results it follows that the dependencies of the zone length vs. concentration are linear. Relative deviations were within the range ±1–5%.

The contents of aldonic acids were determined in samples after the oxidation of glucose and mannose with $PdCl_2$ at pH 11.66 and pH 9.00, respectively.

When the oxido–reductive reaction between hexoses and $PdCl_2$ was carried out at pH 11.66, four acids were identified as products after oxidation of aldoses, i.e. mannonic, gluconic, arabinonic and glyceric (Fig. 1b,c).

The results from their quantitative determination showed that epimeric six-carbon acids, mannonic and gluconic, were produced as the major product, 61.9%, but arabinonic five-carbon acid and glyceric three-carbon acid were also detected in lower quantities, 26.9% and 6.4%, respectively. The important fact was that mannonic acid was formed in considerably higher concentration, 75.6%, than gluconic acid, 24.4%, whatever the starting aldose (Table 2).

When the oxidation reaction of glucose and mannose with $PdCl_2$ was carried out at pH 9.00, only one aldonic acid was identified and determined, compared with the isotachophoregram of the standard compounds, and that was mannonic acid in a con-

Table 1
Parameters of the calibration line $y=a+bx$

Acid	Parameter		
	a	*b*	*r*
Mannonic	-3.3×10^{-2}	5.07×10^{-1}	0.9999
Gluconic	-4.3×10^{-2}	4.1×10^{-1}	0.9989
Arabinonic	9.9×10^{-3}	4.57×10^{-1}	0.9999
Glyceric	6.5×10^{-3}	4.43×10^{-1}	0.9999

a=Intercept (mm); *b*=slope (mm/mg); *r*=correlation coefficient.

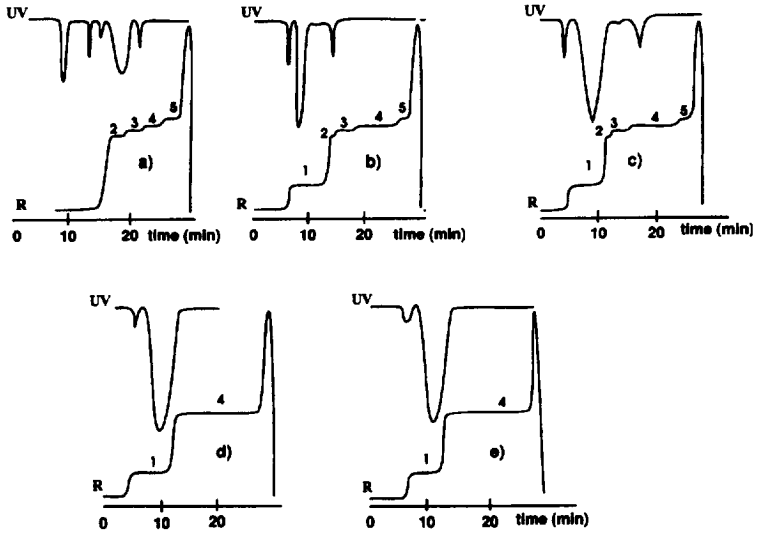

Fig. 1. Isotachophoregram of the aldonic acids. (a) Mixture of standard compounds; (b) mixture of acids produced after oxidation of glucose at pH 11.66; (c) mixture of acids produced after oxidation of mannose at pH 11.66; (d) mixture of acids produced after oxidation of glucose at pH 9.00; (e) mixture of acids produced after oxidation of mannose at pH 9.00. 1=$KNaC_4H_4O_6$; 2=glyceric acid; 3=arabinonic acid; 4=mannonic acid; 5=gluconic acid.

centration of 98.87% from the starting aldose, either glucose or mannose (Fig. 1d,e, Table 2).

These results were the same as previously obtained with the autoanalysing system (Technicon Autoanalyser), used primarily for separation and identification of the aldonic acids. After the oxidation of glucose with $PdCl_2$ in alkaline medium, the separation of the obtained acids was done using ion-exchange analysis, on ion-exchange resin (Dowex AG 1×8 in acetate form). Sample lines in the system were connected to the base of the anion-exchange column with an analysis system for spectrophotometric identification of the formed aldonic acids. The spectrophotometric analysis was carried out using a cysteine–sulphuric acid reagent, and measuring of the absorbance of the chromophore at 420 nm [13].

The aldonic acids were determined on the base analysis of the standards under the same conditions at which individual peaks were identified (Fig. 2). Structural information concerning the compounds analysed was obtained from the spectra acquired by ion-exchange spectrophotometric analysis, and by comparison of the relative retention coefficients of reference compounds.

The results from the quality and quantity isotachophoretic analysis showed that when the reaction was carried out at a higher pH value primarily an oxidation reaction of reductive carbohydrates was carried out, but degradation was present as well,

Table 2
Results from the isotachophoretic determination of aldonic acids

Sample oxidized	Determined acid (mg/ml)			
	Mannonic	Gluconic	Arabinonic	Glyceric
Glucose, pH 11.66	0.2810	0.0906	0.1616	0.0384
Mannose, pH 11.66	0.3000	0.0986	0.1816	0.0402
Glucose, pH 9.00	0.6220	–	–	–
Mannose, pH 9.00	0.6300	–	–	–

Starting concentration of aldose, 0.6 mg/ml; starting concentration of $PdCl_2$, 1 mg/ml.

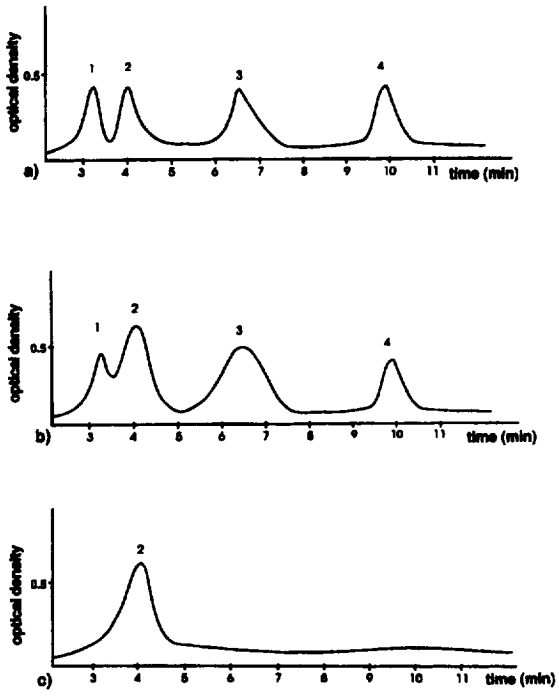

Fig. 2. Automated ion-exchange analysis of aldonic acids, starting concentration of aldose 0.6 mg/ml. (a) Standards; (b) mixture of products after oxidation of glucose with $PdCl_2$ at pH 11.66; (c) mixture of products after oxidation of glucose with $PdCl_2$ at pH 9.00. 1, Gluconic acid; 2, mannonic acid; 3, arabinonic acid; 4, glyceric acid.

secondarily, giving five- and three-carbon acids in lower concentrations. Such degradation apparently contributed to the complex products observed when food samples were oxidized by other ions at high pH [14–17].

But, when the oxidation of aldoses with Pd(II) chloride was carried out at pH 9.00 (lower value) only direct oxidation of investigated aldoses into six-carbon aldonic acid (mannonic acid) was observed, probably because of the weaker alkaline medium.

4. Conclusion

The developed isotachophoretic method was simple and rapid, as well as accurate and sensitive, with direct analysis of samples without prior treatment. The oxidation of glucose and mannose with Pd(II) chloride in alkaline solution at pH 11.66 gave four acids, with a mixture of epimeric six-carbon acids as the major products. The same reaction at pH 9.00 gave only mannonic acid. The acids obtained after the oxidation of investigated aldoses depended on the pH value of the reaction mixture.

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