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ABSORPTION SPECTRA OF CARBOHYDRATES AND RELATED COMPOUNDS IN H_2SO_4

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The possibility of modifying the Dreywood anthrone method for spectrophotometric determination of carbohydrates in order to increase the accuracy and reproducibility of the results was examined. The classical method and a developed modification were compared. The relative uncertainty in a determination by the modified method was less than 4%.

Key words: Dreywood method, modification, carbohydrates, anthrone.

Sulfuric acid is widely used to analyze organic compounds. It gives colored products with carbohydrates in the presence of several phenols, in particular, a solution of anthrone in H_2SO_4 gives stable colored compounds [1]. In the classical method (CM), hydroxymethylfurfurol formed upon dehydration of monosaccharides reacts with anthrone in conc. H_2SO_4 to give bluish-green condensation products with absorption maxima at 520-625 nm [2]. The CM was used to develop several quantitative methods for determining monosaccharides [3, 4]. A drawback of these is the magnitude of the relative uncertainty and technical difficulties in working with concentrated mineral acids. Table 1 gives results from a metrological analysis of the CM using certain monosaccharides as examples. The large uncertainty is probably due to the viscosity of conc. H_2SO_4, which causes the colored products to be unevenly distributed in the sample volume.

We examined the possibility of thinning the samples with less viscous and more available solvents such as ethanol and water for phytochemical analysis in order to eliminate these drawbacks. Use of the latter is excluded because of the poor solubility of anthrone in aqueous H_2SO_4 solutions at concentrations less than 70%. Therefore, we used ethanol. The sample was diluted with 95% ethanol after the colored complex was formed.

The studied compounds were representatives of the pentose and hexose series, oligosaccharides, and polymeric compounds. The viscosities of the resulting solutions were satisfactory and facilitated even mixing of the reaction mixture. However, yellow solutions formed instead of the expected bluish-green ones. A check of the effect of temperature on the color of the formed complex showed that it affected only the intensity of the color of the final solution. Carrying out the reaction at 20-100°C gave identical results. After dilution with ethanol, yellow solutions were obtained.

Absorption spectra of the reaction products exhibited a characteristic band at 405-411 nm. Unreacted anthrone in the mixtures caused absorptions in the range 320-400 nm. This could be avoided by using an anthrone solution as a control. This improved the shape of the spectra and shifted the maxima to 428-431 nm (Fig. 1). The characteristic absorption spectrum was the same for all studied compounds except for galacturonic acid, the principal peak of which was located at 424 nm. The optical density was linear for 0.2-0.7 relative units for solutions with concentrations 5-35 μg/mL (Fig. 2). The exception was arabinose, for which the range was 25-120 μg/mL.

The time required to form the colored complex at 100°C was 10 min. Adding a cooling step after adding conc. H_2SO_4 is inadvisable because this has no effect on the intensity of the color of the final complex. The optical density of solution B after adding alcohol was stable for 10-12 h. The products from reaction of anthrone and glucose, fructose, galactose, arabinose, and galacturonic acid indicated that they react in an equimolar ratio.

Table 1 lists the metrological properties of the modified method.

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TABLE 1. Metrological Properties of the Classical (CM) and Developed Methods (DM) [n = 11, P = 95%, t(p,f) = 2.23]

Property*	Glc		Frc		GalUA	
	CM	DM	CM	DM	CM	DM
$\bar{x}$, %	98.24	99.16	99.03	99.27	92.67	92.18
S^2	146.85	12.04	128.21	9.09	164.99	23.07
S_x	3.65	1.05	3.41	0.91	3.87	1.45
±Δx, %	8.13	2.33	7.61	2.03	8.63	3.23
E, %	8.27	2.35	7.68	2.04	9.31	3.50

*$\bar{x}$, %, is the average mean; S^2, the standard deviation; S_x, the mean square deviation; ±Δx, %, the absolute uncertainty of the arithmetic mean; E, %, the relative uncertainty. Glc is glucose; Frc, fructose; GalUA, galacturonic acid.

TABLE 2. Spectral Properties of Carbohydrates and Related Compounds

Sample No.	Compound*	λ_{max}, nm		$E_{1cm}^{1\%}$	Linear regression equation (calibration curve)**
		Ethanol	Anthrone		
1	Glc	371, 392, 411	380 pl, 429	358	D = 0.0348462·c + 0.0110
2	Frc	370, 392, 414	381 pl, 431	423	D = 0.0407998·c + 0.0152
3	Gal	371, 393, 411	382 pl, 428	224	D = 0.0220681·c + 0.0051
4	Ara	372, 392, 410	380, 428	67	D = 0.0060162·c + 0.0426
5	GalUA	369, 393, 405	383, 424	214	D = 0.0190649·c + 0.0426
6	Mant	371, 392, 411	379, 431	-	-
7	Arat	371, 392, 411	381, 431	-	-
8	Suc	371, 391, 410	380 pl, 431	-	-
9	Lac	371, 390, 411	380 pl, 428	-	-
10	Raf	370, 390, 411	381 pl, 429	-	-
11	Stc	370, 392, 411	380 pl, 431	-	-
12	Inu	370, 391, 410	381 pl, 431	-	-

*Glc is glucose; Frc, fructose; Gal, galactose; Ara, arabinose; GalUA, galacturonic acid, Mant, mannite; Arat, arabite; Suc, saccharose; Lac, lactose; Raf, raffinose; Stc, starch; Inu, inulin. **D is optical density; *c*, carbohydrate concentration (μg/mL).

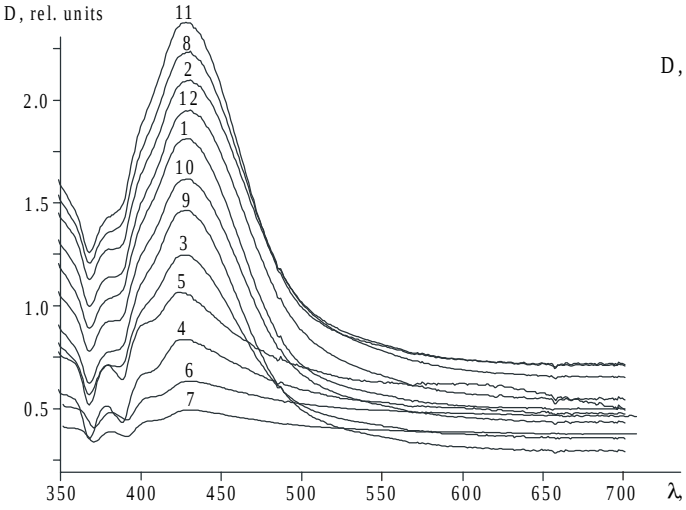

Fig. 1

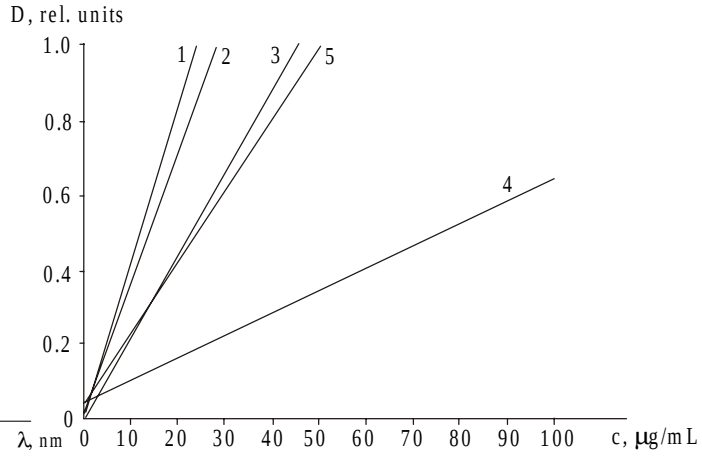

Fig. 2

Fig. 1. Absorption spectra of carbohydrates in H_2SO_4 (control, 95% ethanol; for numbering, see Table 2).

Fig. 2. Calibration curves (control, 95% ethanol; for numbering, see Table 2).

EXPERIMENTAL

Glucose (Roquette, ≥99%), fructose, arabinose, galactose, arabite, mannite, raffinose (Acros Organics, ≥99%), D-(+)-galacturonic acid (Fluka BioChemica, ≥93%), starch, inulin (Merck), saccharose, lactose, and anthrone (Reakhim) were used as standards. Absorption spectra were recorded on Cecil CE 2011 and Agilent 8453E UV-Vis spectrophotometers in quartz cuvettes with a 10-mm pathlength (Table 2).

Preparation of Carbohydrate Solutions. Compounds (100 mg, accurately weighed) were placed in a 100-mL volumetric flasks and dissolved in purified water (FS 42-2619-89). The volume was adjusted to the mark with the same solvent (Solution A). Solution A (1 mL) was placed in a 10-mL tube, treated with anthrone in conc. H_2SO_4 (4 mL, 0.2%), and heated on a boiling-water bath for 10 min. The solution was cooled and placed in a 25-mL volumetric flask. The volume was adjusted to the mark with 95% ethanol (Solution B).

Calibration curves were constructed and isomolar series were made up as before [5]. Results were processed using the recommended metrology [6]. The instrumental uncertainty of the spectrophotometer (K_{instr}) was calculated using potassium bichromate [7]. Regression analysis was performed using the Advanced Grapher ver. 2.07 program set (Alentum Software Inc.).

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