DETERMINATION OF THE AMOUNT OF URONIC ACIDS AND SULFATE GROUPS IN POLYSACCHARIDES BY A GAS-CHROMATOGRAPHIC METHOD

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We have previously proposed a method for determining uronic acids (UAs) in various polysaccharides (PSs) which is based on decarboxylation with 57% hydriodic acid and the recording of the carbon dioxide liberated by means of gas chromatography [1]. In the present paper we report a broadening of the possibilities of this method by replacing the hydriodic acid by a mixture of hydriodic and hydrochloric acids with the addition of sodium hypophosphite reducing mixture.

We have established that at an elevated temperature the reducing mixture quantitatively decarboxylates UAs and reduces the sulfate group (SO_4^{2-}) to hydrogen sulfide, which enables these components to be determined on one sample of the substance being analyzed. The reaction was performed in a closed cell consisting of a box with two side-arms attached to the gas system of the chromatograph. After the end of the reaction, the reaction products were driven into the chromatographic column and separated, and their presence was recorded by means of a thermal conductivity detector.

A preliminary testing of a number of reducing mixtures most frequently used for the determination of SO_4^{2-} in organic and biological materials [2-7] showed that they were equivalent in relation to their decarboxylating and reducing properties. However, in the final gas-chromatographic analysis a mixture of 57% HI (100 ml), concentrated HCl (60 ml), and H_2O (40 ml) with the addition of 30 g of NaH_2PO_2 had some advantage: it is simple to prepare, stable on storage, and in contrast to other reducing mixtures, liberates insignificant amounts of phosphine on reaction with the material under investigation.

The contribution from neutral monosaccharides to the error of the determination for UAs using the reducing mixture is the same as when using 57% HI alone, and does not exceed 1%.

A trial of the completeness of the reduction of SO_4^{2-} groups of PSs showed that reaction in a closed cell leads to somewhat low results in comparison with the determination of sulfate in a flow-through system providing for the elimination of the H_2S from the reaction mixture as it is formed [6]. For inorganic sulfate (K_2SO_4) identical results were obtained in the two systems. Subsequent observations of these noncorrespondences led to the conclusion that the prolonged contact of H_2S and of the products of the reaction of PSs with mineral acids in a closed system leads to some loss in H_2S; it becomes more considerable in the presence of monosaccharides.

It was also established that the rates of the reduction and the decarboxylation reactions differ considerably; in the first 10 min about 90% of the SO_4^{2-} groups present in the PSs are reduced, while the complete decarboxylation of the UAs requires a longer time and a higher temperature. These differences enable the difficulties mentioned to be eliminated by performing the reaction in two stages. After each stage the reaction products are driven out into the chromatographic column and are recorded by the detector.

The losses of H_2S due to oxidation in the absorption column that is used to purify the CO_2 and H_2S before chromatography can be eliminated by filling this column with silica gel impregnated with sodium hypophosphite. This prevents the oxidation of the accompanying HI to elementary iodine by atmospheric oxygen in the purging of the reaction system.

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TABLE 1. Results of Determination of the Amounts of Uronic Acids (UAs) and Sulfate Groups in Polysaccharides (%)

Polysaccharide	Combined determination		Separate determination	
	UAs	SO_4^{2-}	UAs	SO_4^{2-}
Heparin, sodium salt	14,00	43,56	13,98	41,12
Chondroitin sulfate, sodium salt	23,65	15,26	23,93	14,79
Dextran sulfate, sodium salt	—	49,07	—	47,20

The results of determinations of UAs and SO_4^{2-} in PSs by the proposed method have been compared with the results obtained by the separate determination of the UAs by decarboxylation with 57% HI followed by gas chromatography [1] and of SO_4^{2-} by combustion in a flask with oxygen [8] (Table 1).

The range of determinable amounts, for which the signals of the detector was linear was 0.1-1.5 mg of UAs, and 0.1-1.0 mg of SO_4^{2-}.

The mean square error at n = 5 was 2% relative for UAs and 3.5% relative for SO_4^{2-}.

EXPERIMENTAL

Materials and Reagents. All the PSs were commercial samples. The reducing mixture was prepared by mixing 100 ml of freshly distilled 57% HI, 60 ml of concentrated HCl, 30 g of NaH_2PO_2, and 40 ml of water. The mixture was boiled in a flask with a reflux condenser in a current of argon for 6 h, stored in the same flask over the precipitate in a place protected from light, and used within two months. The KSK silica gels (0.5-1.0 mm) of the absorption column were prepared by the deposition of a saturated solution of sodium hypophosphite (5% of NaH_2PO_2 on the weight of the silica gel) followed by activation at 120°C for 6 h.

The apparatus has been described previously [1]. The chromatographic column (250 × 0.3 cm) contained Polysorb 1.

Method. The reducing mixture (1 ml) was poured into the reaction vessel and a light glass boat with a weighed amount of PS (1-3 mg) was carefully placed on its surface. After the purging of the reaction system had been completed, the boat was capsized by shaking the vessel. The first stage of the reaction was carried out at 130-135°C for 10 min and the second stage at 140-145°C for 30 min. After each stage, the reaction products were chromatographed at room temperature; the detector current was 125 mA, sensitivity 8, rate of flow of helium 50 ml/min. The amounts of the components determined were calculated from the overall integral parameters of the CO_2 and H_2S peaks with retention times of 2 and 8 min, respectively. Calibration was carried out with individual samples of glucuronolactone (0.1-1.5 mg) and potassium sulfate (0.2-2.0 mg).

SUMMARY

A new method is proposed for the simultaneous gas-chromatographic determination of the amounts of uronic acids and sulfate groups in polysaccharides.

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