

## A TECHNIQUE FOR THE DETERMINATION OF FORMALDEHYDE PRODUCED DURING THE PERIODATE OXIDATION OF POLYSACCHARIDES

S.M. HEGAZI

*National Research Centre, Dokki, Cairo, Egypt*

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**Abstract.** A clarifying method is given for destroying the periodate and iodate from the reaction mixture during the polysaccharides oxidation, to allow accurate measurement of the formaldehyde produced by the chromotropic reagent. Lead acetate and potassium oxalate solutions lead to a successful determination of the formaldehyde. A modified technique has also been developed for the determination of formaldehyde with the phenylhydrazine hydrochloride and potassium ferricyanide solutions.

The primary alcohol group, found in the sugars and polysaccharides, produces formaldehyde on periodate oxidation.<sup>2</sup>

Lampert and Neish<sup>3</sup> used the chromotropic acid reagent for determining the formaldehyde colorimetrically after removing the periodate and iodate ions from the reaction mixture. The periodate ions were first eliminated by using arsenite,<sup>4</sup> barium carbonate,<sup>5</sup> sodium sulfite,<sup>6</sup> or 2,3-butandiol.<sup>7</sup>

Direct determination of the formaldehyde, produced during the polysaccharides oxidation after destroying the excess of periodate ions, was unsuccessful. Interfering discoloration was encountered which did not allow accurate measurement of the colour developed by the chromotropic reagent. Clarifying procedures were necessary to overcome such a problem.

In the present work a technique has been developed for clarifying the periodate ions in the reaction mixture during the polysaccharides oxidation. This technique allows accurate measurement of the formaldehyde by the chromotropic reagent. A highly standardized colour reagent has also been prepared for the determination of formaldehyde. This reagent can be easily prepared daily and it eliminates the necessity of heating the mixture for a long time for developing the colour. This reagent is phenylhydrazine hydrochloride plus a small amount of concentrated hydrochloric acid.

### Experimental

#### *Periodate Oxidation of the Polysaccharide*

Potato-starch (1.5-2.0g) was mixed with periodate solution (0.12M) in a volumetric flask. The reaction mixture was kept at 0-3°C and a blank experiment was carried out under the same conditions. At intervals, aliquots were taken for the determination of formic acid, periodate consumption and formaldehyde.

(a) *Determination of Formaldehyde using the Chromotropic Acid Reagent.* The quantity of formaldehyde was determined by the chromotropic acid method<sup>3</sup> after removing or destroying the excess periodate ions as follow: Formaldehyde periodate mixture (20 ml) containing 200 µg of formaldehyde, was treated with lead acetate (8 ml 20% solution) and the excess lead

acetate was precipitated with potassium oxalate solution (10 ml, 20%). The precipitate was removed by centrifugation and the clear solution and the washings were collected in a volumetric flask (50 ml) followed by the addition of sulphuric acid (1 ml, 10N) whence the iodine was liberated. A few drops of sodium bisulphite (10%, freshly prepared) were added till iodine disappeared and finally the volume was adjusted to the mark with water. Aliquots (1 ml) were taken and 10 ml chromotropic acid reagent was added. The tubes were heated in a boiling water-bath for 30 min and the violet colour developed was measured at 580 nm. Lead acetate (10%) and potassium hydrogen phosphate or sodium sulphate was added for clarification, as shown in the Table 1.

(b) *Determination of Formaldehyde by Phenylhydrazine Ferricyanide Reagent.* Starch solution (20 ml) containing formaldehyde and periodate mixture was transferred to a volumetric flask (50 ml), and then acidified with sulphuric acid (1 ml, 10N). Sodium arsenite solution (5 ml, 1M) was added to the flask for destroying periodate ions and the volume was adjusted with water after 5-10 min. Aliquots (5 ml) were taken in a test tube and 0.5 ml phenylhydrazine hydrochloride was added. After 10 min in the dark, 0.2 ml potassium ferricyanide solution was added. The tubes were then placed in an ice-bath for 4-5 min before the addition of 2 ml concd HCl. After 12 min optical density was measured at 530 nm. The effect of the clarifying treatments given in A, and the use of phenylhydrazine hydrochloride reagent, and the recovery of formaldehyde was carried out on a known formaldehyde solution. The results are given in Tables 1 and 2.

#### *Preparation of the Phenylhydrazine Hydrochloride Reagent*

1. The reagents were prepared daily by dissolving 338 mg phenylhydrazine hydrochloride in 20 ml distilled water, followed by addition of 2 ml concd HCl and distilled water up to the volume of 25 ml.
2. Potassium ferricyanide 4.5 g, 0.137 moles was dissolved in water and made up to 100 ml.
3. Concentrated HCl.

TABLE 1. RECOVERY OF FORMALDEHYDE AFTER CLARIFICATION.

Treatment	Formaldehyde $\mu\text{g}$		Recovery(%)
	Actual	Found	
Periodate	4	3.9	97.5
Reaction mixture(10 ml) + lead acetate (10 ml, 20%) + potassium (10 ml), oxalate (8 ml, 20%)	8	7.78	97.2
	10	10.00	100.0
	12	11.90	99.1
Reaction mixture (10 ml) + lead acetate (10 ml, 10%) + potassium hydrogen phosphate (8 ml, 10%)	4	3.60	90.0
	8	7.30	91.1
	10	9.50	95.0
	12	11.20	93.0
Reaction mixture (10 ml) + lead acetate (10 ml, 10%) + $\text{Na}_2\text{SO}_4$ (8 ml, 10%)	4	4.50	103.0
	8	7.00	26.0
	10	9.30	93.0
	12	11.00	91.70

TABLE 2. RECOVERY OF FORMALDEHYDE DETERMINED BY THE PHENYLHYDRAZINE FERRICYANIDE REAGENT.

Sample No.	Formaldehyde concentration ( $\mu\text{g}$ )		Recovery (%)
	Actual	Found	
1	2.0	1.85	92.5
2	3.5	3.30	97.0
3	4.9	4.60	94.0
4	8.8	7.60	97.4
5	9.1	9.00	99.0

### Results and Discussion

Periodic acid and its salts are widely applied in the structural studies of simple sugars, their derivatives, oligosaccharides and polysaccharides.<sup>1,2</sup> The formaldehyde released during the periodate oxidation of the polysaccharides was determined by the chromotropic acid reagent given by Lampert and Neish.<sup>3</sup>

In the study of the mucilages polysaccharides isolated from the tubers, the direct estimation of formaldehyde produced during the periodate oxidation with the chromotropic acid reagent was unsuccessful. Interfering discoloration was encountered which did not allow accurate measurement of the colour produced with the chromotropic reagent. Clarifying procedures were necessary to overcome such a problem. In the present work lead acetate, potassium hydrogen phosphate and sodium sulphate solutions were used as shown in Table 1.

Table 1 shows that lead acetate and potassium oxalate treatment give the best results in removing the periodate ions, and that this treatment is quite adequate since the recovery of formaldehyde was more than 97%. The colour reaction, which was given in the glyceride determination by Jover<sup>8</sup> and modification by Randurup,<sup>9</sup> was adopted for determining the formaldehyde contents produced during the periodate oxidation of polysaccharides. Formaldehyde can be directly determined in presence of low concentrations of periodate (0.05–0.007M) by this reagent. But the high concentrations of the periodate do not give accurate measurement since the iodine liberated interferes with the colour. Sodium arsenite solution (1M) was found suitable for eliminating the periodate ions (Table 2).

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