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POTENTIOMETRIC TITRATION METHOD FOR THE DETERMINATION OF URONIC ACID ANHYDRIDE CONTENT OF ACIDIC POLYSACCHARIDES

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Authentic samples of polysaccharides having uronic acid anhydride contents, ranging from approximately 10 to 98 per cent, as well as a sample of monomer, i.e. galacturonic acid, have been analysed by potentiometric titration method and the results are compared with other standard methods. It is concluded that this simple and rapid method works very satisfactorily.

Key words: Potentiometric titration, Determination of uronic acid.

INTRODUCTION

Two analytical methods are mainly used for the determination of uronic acid anhydride contents of acidic polysaccharides. The first is Anderson's decarboxylation method [1] which consists of heating the polysaccharide sample with 19 % hydrochloric acid and estimating the amount of evolved carbon dioxide. The other is the carbazole method [2] consisting of colorimetric determination of acidic sugar residues in the polysaccharide by employing colour reaction among uronic acid units, sulphuric acid and carbazole, under strictly controlled experimental conditions. Besides the use of these two methods, some investigators [2-3] have estimated the percentage of galacturonic acid units in the pectic acids by potentiometric titration with sodium hydroxide. However, the later method has neither been standardised nor investigated in depth for general application.

In course of his investigations on acidic polysaccharides the author found potentiometric titration method very rapid and efficient and hence investigated the details of this analytical procedure with authentic substances, and compared the results with the other two methods, so that it may be used as a standard method.

EXPERIMENTAL

Potentiometric titrations were carried out with a Pye Unicam (Model 292, Mk 2) pH meter. The instrument was checked and adjusted, if necessary, with a standard buffer solution (pH 7.00, Corning scientific instruments). Unless otherwise stated, the titre used was 0.1 N sodium hydroxide solution free of carbon dioxide and titrations were done in a dropwise manner (particularly near the end point), at room temperature. The pH was noted after the addition of every 0.1 ml. sodium hydroxide solution and near the end point, after every 0.05 ml. of the alkali.

The analytical procedure was repeated with the following authentic materials, which were also analysed for uronic acid or uronic acid anhydride contents by decarboxylation [1] and carbazole [2] methods.

Uronic acid content of galacturonic acid. Sample of galacturonic acid monohydrate (citrus origin; BDH) (100.0 mg) was disssolved in de-ionized water (25 ml.) and titrated with 0.1N sodium hydroxide solution. The end point was established from the graph (Fig. 1 a and b) and the percentage of uronic acid was determined from the following equation:

$$\% \text{ of uronic acid} \quad \frac{212 \times N \times V}{1000 \times W} \times 100 \qquad (1)$$

where w g of acid requires V ml. of sodium hydroxide of normality N for complete neutralization.

The titration was repeated several times and the results showed reproducibility within the range of ± 0.2 %. The results were compared with those obtained by decarboxylation [1] and carbazole [2] methods and there was a close agreement among the results obtained by the three methods.

Uronic acid anhydride (uaa) content of degraded galacturonan. A sample of pure galacturonan was obtained by hydrolysis of apple pectin (BDH; 250 grade) by the following procedure: The pectin (7.5 g) was hydrolysed in N-sulphuric acid (200 ml.) on a boiling water bath for 18 hrs. After cooling the solution, degraded galacturonan (3.5 g) was precipitated with acetone (1 vol.) removed at centrifuge and washed with acetone: water (1 : 1) till the

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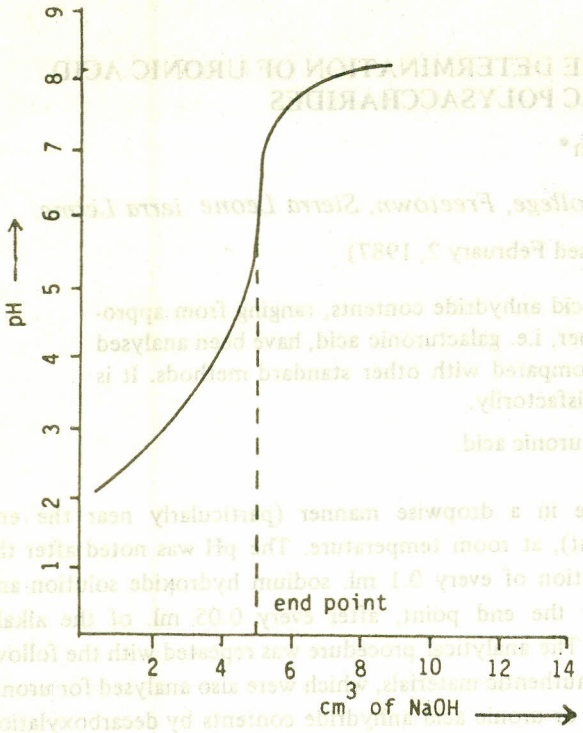

Fig. 1 (a). Titration curve of D-galacturonic acid NaOH.

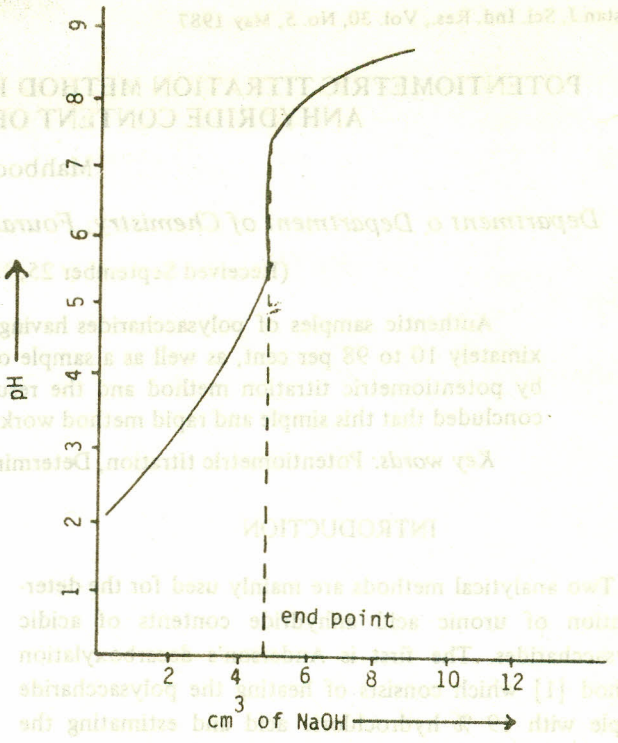

Fig. 2 (a). Titration curve of galacturonan NaOH.

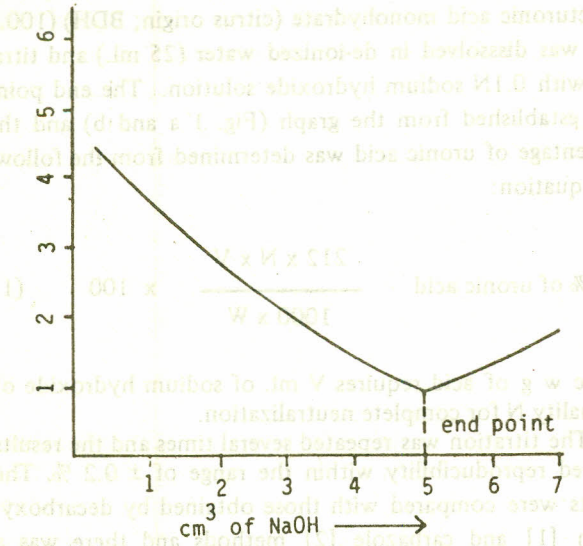

Fig. 1 (b). Differential curve pH/vol. of NaOH vol. of NaOH.

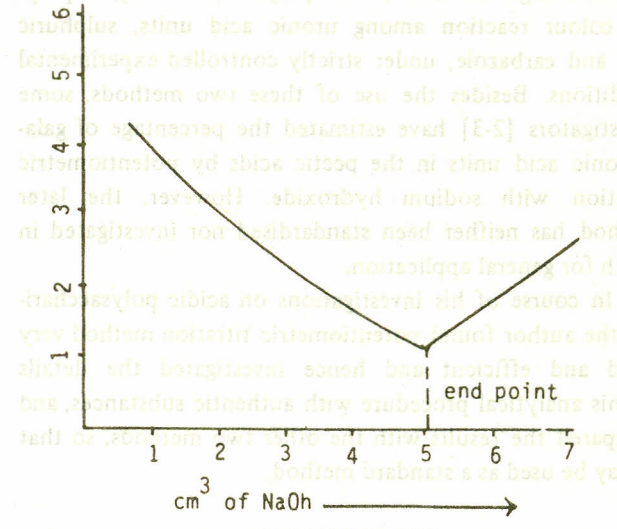

Fig. 2 (b). Differential curve pH/vol. of NaOH vol. of NaOH.

washings were free of acid and finally with acetone and ether.

A small portion of degraded galacturonan (20 mg) was further hydrolysed in N-sulphuric acid (10 ml.) on a boiling water bath for 8 hrs. On cooling the solution, sulphuric acid was neutralized with barium hydroxide and barium carbonate, insoluble inorganic salts were removed at centrifuge, the supernatant solution was de-ionised with Amberlit resin IR – 120(H) and concentrated to a syrup. The paper chromatographic examination [6] of the syrup in solvent systems: butanol: ethanol: water (4:1:5; upper layer) and butanol: ethanoic acid: water (4:1:6; upper layer), showed the presence of galacturonic acid and galacturonobiose and absence of any neutral sugar which indicates that the isolated galacturonan consists of galacturonic acid units only.

A sample of pistol dried galacturonan (100.0 mg) was dispersed in water (30 ml.) and the solution was titrated with 0.1N sodium hydroxide in the same way as described

earlier. The end point was established from the graph (Fig. 2 a and b). The uaa content was determined from the following equation:

$$\text{uaa content} = \frac{176 \times N \times V}{1000 \times W} \times 100\ \% \qquad (2)$$

where V ml. of sodium hydroxide of normality N is required to neutralize w g of the polysaccharide. The titration was repeated several times taking varying amounts of the polysaccharide and reproducible results (± 0.2 %) were obtained. Uronic acid anhydride content of the same sample was determined by decarboxylation (1) and carbazole (2) methods and the same results were obtained. The titration was repeated at 10°, 5° and 0° and also with sodium hydroxide of low strength (0.05 and 0.01N), but no change in the results was observed. All the results showed that the percentage of uronic acid anhydride in the sample of galacturonan was 97.9 – 98.3.

The uaa content of pectic acid obtained by saponification of apple pectin. Pectic acid was obtained from apple pectin (BDH; 250 grade) by the following procedure (7): Sodium hydroxide (1N) was added dropwise with stirring to pectin (2.5 g) in water (250 ml.) at 0° until pH 12 was reached. The solution was kept at 0° for 2 hrs. while the pH was maintained at 12. Aqueous 18 % hydrochloric acid was then added dropwise with stirring to the clear solution. When precipitation of the polysaccharide was complete the pectic acid gel was squeezed through a linen cloth and washed successively with ethanol – water (1:1; containing 5 ml of hydrochloric acid per 1), ethanol-water (3:2), ethanol and ether. The isolated pectic acid (1.8 g) was pistol dried and its uaa content was determined by decarboxylation (1) and carbazole (2) methods. The sample of pectic acid (200.0 mg) was dissolved in de-ionised water (40 ml) and titrated with sodium hydroxide (0.1 N) in the same manner as described before. The end point was established from the graph and uaa content was calculated from equation (2). The titration was repeated 5 times. All the results obtained from this method and the other two methods (1, 2) showed that the uaa content of the sample was 78.5 - 79.3 %. The titration was carried out with sodium hydroxide of low concentration (0.05N and 0.01N) and also at low temperatures (10°, 5°) but no changes in the results were observed.

The uaa contents of four other samples of acidic polysaccharides were determined by three methods and the results are shown in Table 1.

RESULTS AND DISCUSSION

For the detailed study of this analytical procedure, uronic acid content of D-galacturonic acid was determined by potentiometric titration with standard sodium hydro-

Table 1. Uaa contents of acidic polysaccharides samples

S. No.	Acidic Polysaccharide samples obtained by saponification(7) of pectins)	Carbazole method(2) %	Anderson's method(1) %	Potentiometric titration method %
1.	Pectic acid from American variety OH-1 of sunflower heads (extracted with ammonium oxalate (8)	75.4	75.0	75.2-75.6
2.	Pectic acid from Russian variety of sunflower heads (extracted with ammonium oxalate) (8)	66.8	66.0	66.0-66.4
3.	Acidic polysaccharide fraction* from gundar gum (9)	12.9	12.6	12.5-12.8
4.	Acidic polysaccharide fraction* from maklai gum (9)	10.8	10.5	10.4-10.8

*These acidic polysaccharide fractions were obtained by ion-exchange chromatography on diethylaminoethyl cellulose (Neukom's method) (10) of the polysaccharides extracted with water from the respective gums.

xide solution. The results are reproducible and confirmed by decarboxylation and carbazole methods. All the results show that the uronic acid content of the sample is 99.5 ± 0.02 %. The titration method is both simple and rapid.

The uronic acid anhydride contents of a pure galacturonan sample devoid of any neutral sugar, deesterified apple pectic acid and four samples of acidic polysaccharides containing a wide range of uaa contents (10.6 to 75.4 5) were determined by this method and the results were compared with those obtained by decarboxylation (1) and carbazole (2) methods. In all cases reproducible results (± 0.4 %) were obtained, when the titration was several times repeated. In view of alkaline degradation [11] of the acidic polysaccharides, the titration was carried out at low temperature and also with sodium hydroxide of low concentration to see whether it effects the end point, but no marked variations in all the results were observed. The titration curves (Fig. 1 a,b and 2 a,b) represent the titration of a weak acid with a strong base from which the end point can very easily be determined.

These observations clearly show that potentiometric titration method is a satisfactory analytical method for the determination of uronic acid anhydride content. The method may be applied to the polysaccharides having a wide range of uaa content. It is comparatively less time consuming and straight forward.

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