QUANTIFICATION OF POLYSACCHARIDE CONTENTS WITH THE CHEMICAL OXYGEN DEMAND INDEX (COD)

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The quantification of carbohydrate contents through measurements of chemical oxygen demand index (COD) is demonstrated on three polysaccharides: pectin, sodium alginate and xanthan. Strong, linear relationships are obtained between polysaccharide content and the COD, with a close match between measured values and theoretical estimates. The COD method offers some advantages, and may complement the traditional phenol–sulfuric acid and anthrone–sulfuric acid assays in carbohydrate content measurements.

Keywords: carbohydrates, polysaccharides, quantification

INTRODUCTION

Widely used methods to quantify carbohydrate content (including that of oligo- and polysaccharides) are the phenol– and anthrone–sulfuric acid assays.[1] The operational principle in both is the degradation of carbohydrates under the action of heat in strong acids to yield furan derivatives, which condense with added phenol or 9(10*H*)-Anthracenone (anthrone) to yield colored compounds. The quantification is through spectrophotometric measurements of the developed color, as it is linearly proportional to the carbohydrate content. The reactions are non-stoichiometric, so calibration plots are required. Ideally, the plots are constructed from measurements on known amounts of the carbohydrate under analysis. For mixtures, the plots may be constructed with individual carbohydrates in the same proportions as in the analyte, if known.[2] In non-ideal circumstances, calibration plots are constructed with D-glucose and the results are quantified in glucose equivalents.

This paper focuses on polysaccharide quantification through measurements of the Chemical Oxygen Demand index (COD). The index is a measure of the amount of oxidant consumed in the digestion of an organic material expressed in mass equivalents of oxygen, and is widely used in standard tests of wastewater quality. The applicability of the method is demonstrated with the standard potassium dichromate procedure on three polysaccharides: pectin, sodium alginate and xanthan gum. Advantages of the COD over the phenol– and anthrone–sulfuric acid assays are discussed. Further, the paper highlights options that obviate shortcomings in the standard COD procedure, of long digestion times, use of toxic and corrosive reagents, limited tolerance to chlorine, and problems with digestion of nitrogenous organic substances.

EXPERIMENTAL

Materials

Pectin from citrus peel (product number P9135), xanthan gum (product number G1253), and sodium alginate (product number A2158) in the form of solid powders were purchased from Sigma-Aldrich® and used as received. Representative structures of the polysaccharides are shown in Figure 1. All other reagents were of analytical grade. Deionized water was used in the formulation of solutions.

Procedures

The polysaccharide powders were first conditioned for a minimum of 24 h in standard atmosphere of 20 ± 2 °C and 65 ± 2% RH. Stock solutions of 0.30% w/w concentration were prepared by dispersing requisite

amounts of the conditioned solids in deionized water, leaving them to stand overnight, and stirring for 30 min. Test solutions of 0.02-0.10% w/w polysaccharide were prepared from the stock solutions by dilution with deionized water. All exhibited density in the range 1.013 ± 0.001 g/ml.

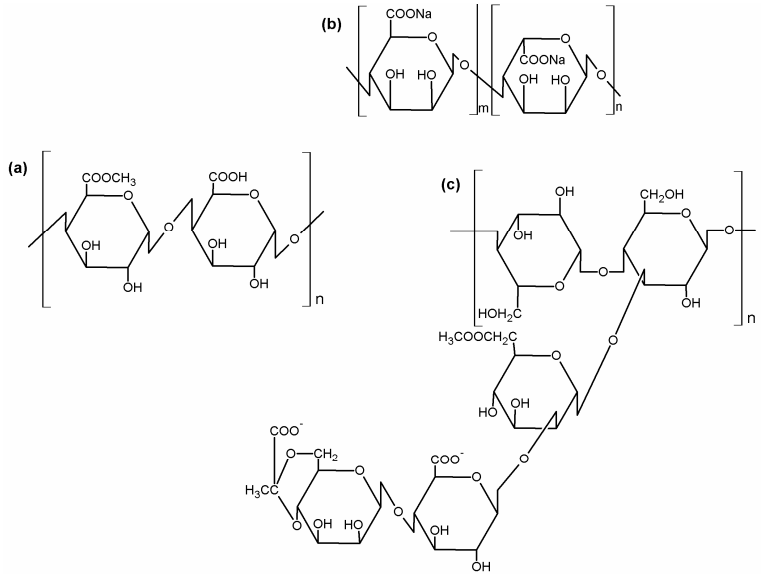

Figure 1: Representative structures of the repeat unit in (a) pectin, (b) sodium alginate, and (c) xanthan

The COD was measured with the photometric detection procedure of the small-scale sealed-tube method ISO 15705:2002.[3] The reagent mixture as described in the standard – 0.5 ml of 0.1 mol/l $K_2Cr_2O_7$, 0.2 ml of 1.35 mol/l $HgSO_4$ in dilute H_2SO_4, and 2.5 ml of 0.038 mol/l Ag_2SO_4 in concentrated H_2SO_4 – was prepared in the laboratory, in capped vials of 16 mm diameter.

For COD measurements, 2 ml polysaccharide test solutions were added to the reagent vials, capped, and refluxed at 150 ± 2 °C for 2 h. The vials were allowed to cool to ambient temperature, and then placed in a photometer to measure absorbance of the digested samples at 585 nm. A sample of deionized water was included in each measurement set as blank.

The absorbance values were converted to COD (mg O_2/l) units with the aid of a calibration plot from measurements on standard solutions of potassium hydrogen phthalate. A linear relationship was obtained of slope $4.43 \pm 0.15 \times 10^{-4}$ absorbance units per unit COD over the range 0-1000 mg O_2/l from three independent determinations (R^2 = 0.9969, standard error = 7.54×10^{-3}). The intercept was of negligible significance.

To determine moisture content in the conditioned polysaccharides, about 1.0 g of accurately weighed specimens were dried in an oven at 105 °C for 4 h, allowed to cool to ambient temperature in a P_2O_5 desiccator, and reweighed. The moisture content was calculated from the difference in mass between the conditioned and dried specimens as a percentage of the initial, conditioned mass.

RESULTS AND DISCUSSION

The COD measured on the test solutions of the three polysaccharides, corrected for blank, are shown in Figure 2. The values increased linearly with polysaccharide contents up to 1.0 g/l concentration. The 'g/l' concentrations were derived from the original % w/w units with the solution densities. Ordinary least squares linear regressions with the spreadsheet software Microsoft® Office Excel yielded good fits, with R^2 ranging between 0.9897-0.9990 and standard errors of 8.82-28.66. The intercept was non-significant for pectin; but small, statistically significant intercepts were obtained for sodium alginate and xanthan, of 14.75 and 16.26 mg O_2/l COD, respectively, at 0 g/l polysaccharide. These are attributed to measurement errors of indeterminate origin, and deemed negligible. The slopes from the linear regressions are shown in Table 1, under 'Measured COD'.

Assuming complete oxidation to CO_2, H_2O and Na_2O, the theoretical oxygen demand (ThOD) of an organic substance with the molecular formula $C_cH_hO_oNa_{na}$ of molecular weight '*MW*' may be estimated with the following equation:[4]

$$ThOD = \frac{16 \cdot \left[2c + \frac{1}{2}h + \frac{1}{2}na - o \right]}{MW} \qquad (1)$$

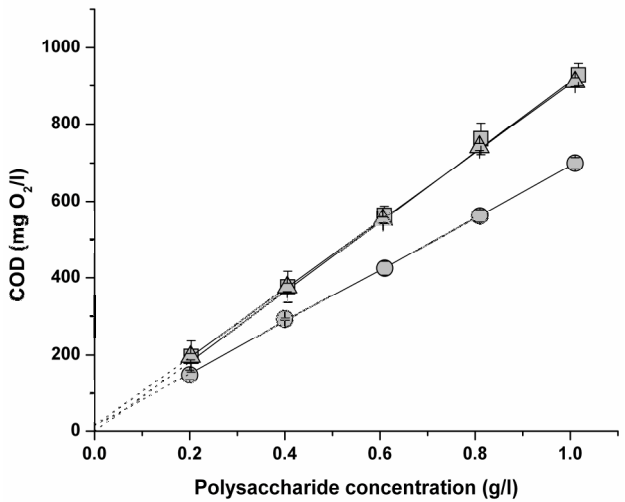

Figure 2: COD measured on 2 ml test solutions of pectin (□), sodium alginate (○), and xanthan (△). Results are the mean of three independent determinations. The error bars show 95% confidence intervals, and the lines show the linear regressions

Table 1
Measured and calculated values for different polysaccharides

Polysaccharide (PS)	Formula unit[a]	ThOD (mg O_2/g PS)	Measured COD (mg O_2/g PS)	COD/ThOD (%)	Moisture content (%)
Pectin	$C_{4.3}H_6O_4$	1000	910.15 ± (55.59)[b]	91	11.1
Sodium alginate	$C_6H_7O_6Na_1$	808	676.15 ± 20.72	84	14.6
Xanthan	$C_7H_{10.2}O_{5.8}$	1138	887.56 ± 17.21	78	14.1

[a] Derived from representative structures of the repeat units shown in Figure 1; [b] 95% confidence intervals

The ThOD of the three polysaccharides calculated with formula units and weights derived from the representative structures in Figure 1, are shown in Table 1. The measured COD were lower than the corresponding ThOD by 9-22%. Adjustingthe measured COD for moisture content in the polysaccharides (see Table 1) yielded average values of 1023.79, 791.74 and 1033.25 mg O_2/g PS for pectin, sodium alginate and xanthan respectively, which are 91-102% of the corresponding ThOD.

The close correlation of measured COD indices with the ThOD provides a good basis for application of the method to quantify polysaccharide amounts in solutions. The advantages include the wider range of calibration of up to 1 g/l polysaccharide as compared to the upper limit of 0.1 g/l in the phenol–sulfuric acid assay.[1] The reagent mixes are stable for up to a year, and the test is robust against excess digestion temperature and time. These are significant challenges in the anthrone–sulfuric acid assay.[5,6]

There is a limit on tolerance to chlorides of 1 g/l in the standard method used in this work, but there exist options to significantly increase chloride tolerance.[7,8] There also exist options to reduce digestion time from 2 h down to 10-15 min by employing mixed acids (H_3PO_4 and H_2SO_4) or microwave irradiation.[9,10] Good correlations between ThOD and COD have been demonstrated for carbohydrates in tests with the permanganate method (COD_{Mn}), as well as in the emerging method of photocatalytic oxidation.[11] These methods offer the advantages of reducing or even eliminating the use of toxic and corrosive reagents. Options for automated measurements are commercially available for the dichromate and permanganate methods. The photocatalytic method offers the capability of on-line measurements.[12] It is also highly effective with nitrogenous compounds.[13]

CONCLUSION

As with the phenol– and anthrone–sulfuric acid assays, the COD method works best for quantifications of individual polysaccharides. With mixtures, total contents may be quantified if the proportions of individual polysaccharides in the analyte are known. Otherwise, the polysaccharide contents may be quantified in COD units, or converted to glucose equivalents from the ThOD of 1067 mg O_2/g glucose. The standard procedures of isolating polysaccharide(s) for assays[2] will also be required for the COD method. It should be noted that contamination of samples with other organic matter will lead to errors, which in many cases, may be minimized by the use of appropriate blanks or control samples.

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