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Note

The use of a liquid scintillation counter for the colorimetric determination of carbohydrates

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Noble *et al.*[1] have shown that colorimetric analyses can be carried out on a liquid scintillation counter. A far wider range of concentrations can be measured by this technique than is possible with the same colorimetric analysis and a conventional light spectrophotometer. For example, the amount of inorganic phosphorus that can be measured by the method of Chen *et al.*[2] with a visible spectrophotometer is in the range 0–8 μg, whereas the range with a scintillation counter is 0–0.4 mg. The need for time-consuming dilutions, with their inherent sources of error, is largely eliminated.

The method involves use of a sealed, glass, miniature vial containing $\sim 10^5$ d.p.m. of either ^{14}C or ^{3}H in a conventional scintillation medium, which is inserted into the centre of a conventional scintillation vial. Quantification is achieved through attenuation of photon detection (net count rate) of the sealed standard when coloured reagents are introduced into the annular space between the two vials. The colorimetric method used for the carbohydrate analysis was that of Lever[3], in which reducing sugars react with *p*-hydroxybenzohydrazide to give a yellow chromophore that is assayed at 410 nm. The relative response of individual reducing sugars (D-glucose = 100) by this method was reported to range from 62 for D-xylose and D-galactose to 112 for maltose. The presence of protein, as bovine serum albumin, at a concentration of 2 mg.ml^{-1} increased the apparent D-glucose content by 3%, and concentrations of 100 mg.ml^{-1} increased the value by 13%.

Fig. 1 shows the net counts per minute from the miniature liquid scintillation standards against the concentration of D-glucose for both a ^{14}C standard and a ^{3}H standard. Also included is a plot of absorbance at 410 nm for the same standards. If the normally accepted range of 0.0–0.8 absorbance unit is used for quantifying the amount of reducing sugar present, the range for D-glucose concentrations per sample is less than 60 μg. Using the scintillation method with the ^{14}C probe, amounts of D-glucose well in excess of 1 mg can be accurately measured, and samples containing 3 mg can be assayed with reasonable accuracy. When the scale was expanded to cover a range of D-glucose concentrations up to 200 μg, although the ^{14}C and ^{3}H curves

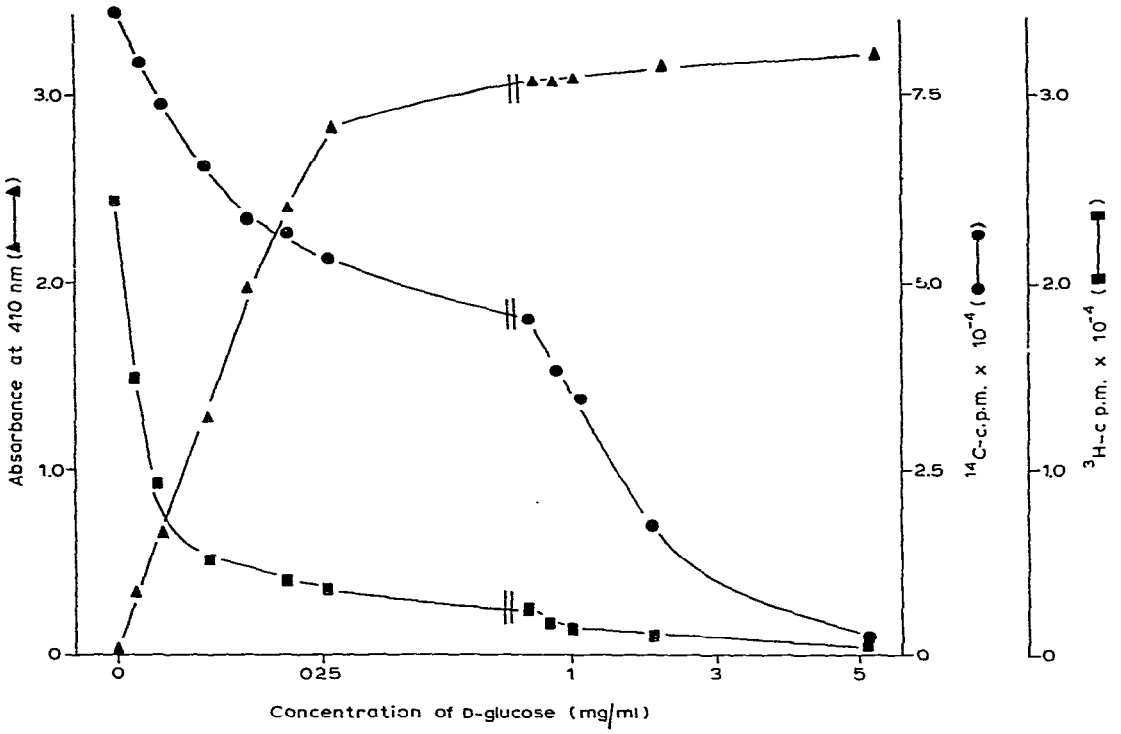

Fig. 1. Concentration of D-glucose against absorbance at 410 nm, c.p.m. for the miniature vial containing ^{14}C, and c.p.m. for the miniature vial containing ^{3}H, using the Lever method.

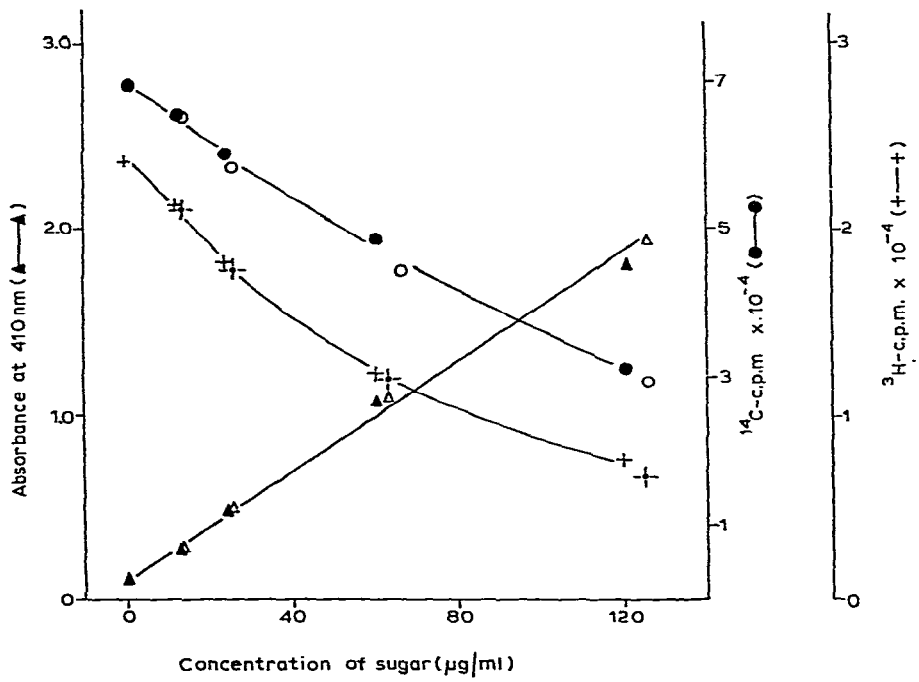

Fig. 2. Concentration of sugar against absorbance at 410 nm, c.p.m. for the miniature vial containing ^{14}C, and c.p.m. for the miniature vial containing ^{3}H, using the Lever method. Closed symbols are for D-glucose and open symbols are for D-xylose.

were not linear (whereas that for the spectrophotometer was), the accuracy for determination was equally good from the ^{14}C or ^{3}H curves. This is shown in Fig. 2, which also shows a plot for D-xylose. On a weight basis, the D-xylose curves follow the D-glucose curves, although, if molarities are used, the D-xylose curve is ~15% lower. The difference is not nearly as great as that reported by Lever[3]. When other monosaccharides were assayed on a weight basis, they all appeared to fit one general curve.

When protein, as bovine serum albumin, was added to the original solution at both 50 and 500 $\mu g.ml^{-1}$, a considerable decrease in absorbance was noted with both concentrations, but there was no significant difference between the two levels of protein. Similarly, the ^{14}C and ^{3}H counts were higher in the presence of protein. This observation is shown in Table I. The concentration of protein therefore affects the measurement of D-glucose by this method, and the amount measured is dependent on the protein concentration. If the approximate protein concentration is known, suitable calibration curves can be drawn. However, in our hands, the presence of protein had a considerable effect on both the spectrophotometric and scintillation method. There is no reason why a deproteinisation step should not be added if maximum sensitivity is required from biological fluids. Such reagents as trichloroacetic acid do not affect the scintillation method.

TABLE I

ABSORBANCE AT 410 nm, ^{14}C-C.P.M., AND ^{3}H-C.P.M. FOR D-GLUCOSE CONCENTRATIONS UP TO 1 $mg.ml^{-1}$ ON ADDITION OF BOVINE SERUM ALBUMIN AT 50 AND 500 $\mu g.ml^{-1}$.

Protein ($\mu g.ml^{-1}$)	D-*Glucose* ($\mu g.ml^{-1}$)	*Absorbance at 410 nm*	^{14}C-*c.p.m.* ($\times 10^{-4}$)	^{3}H-*c.p.m.* ($\times 10^{-4}$)
0	0	0.056	8.63	3.36
	105	0.636	7.51	2.02
	210	1.074	6.93	1.59
	526	2.497	5.58	1.02
	1051	3.200	4.62	0.67
50	0	0.067	8.58	3.40
	105	0.230	8.31	2.90
	210	0.341	8.14	2.61
	526	0.780	7.45	2.04
	1051	1.352	6.66	1.53
500	0	0.066	8.66	3.48
	105	0.208	8.39	2.98
	210	0.316	8.15	2.77
	526	0.710	7.51	2.07
	1051	1.250	6.93	1.65

The scintillation method was also tried with the Somogyi reducing-sugar method[4], and the results are shown in Fig. 3. The reagents themselves cause excessive

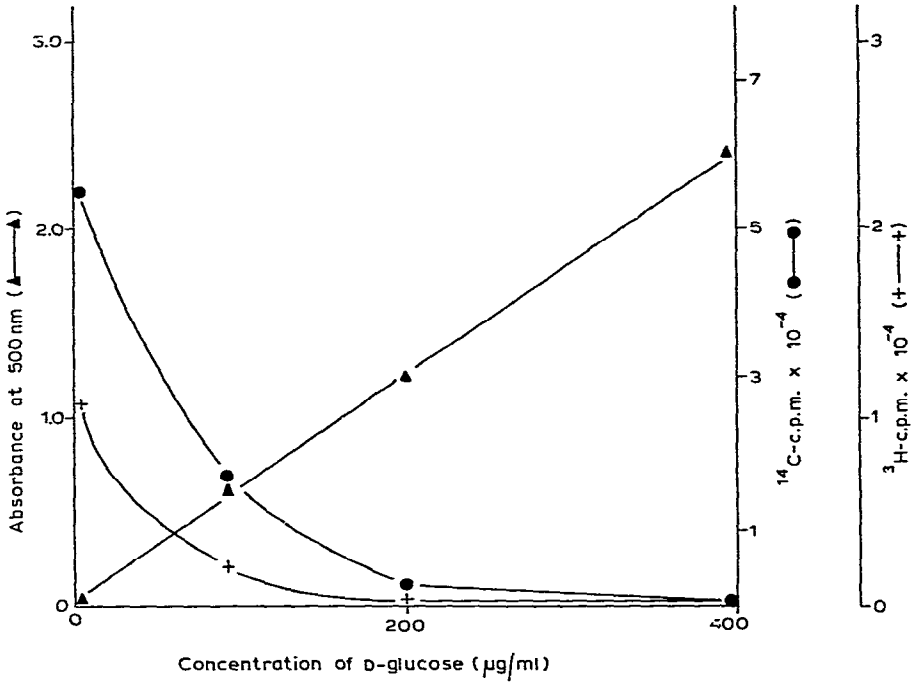

Fig. 3. Concentration of D-glucose against absorbance at 500 nm, c.p.m. for the miniature vial containing ^{14}C, and c.p.m. for the miniature vial containing ^{3}H, using the Somogyi method.

quenching, and the blue colour that develops also causes strong quenching. Therefore, this method is unsuitable for use with a scintillation counter.

There are many assays for reducing sugars and the following guidelines could be used to consider the potential of the scintillation method for a particular assay. If the reagents involved cause strong quenching themselves, the method will only be suitable if this quenching is constant over the range to be measured. Colorimetric reactions producing a colour in the violet end of the visible spectrum will be more sensitive than those generating a colour at the red end of the spectrum. This does not rule out reactions of the latter type, since their accuracy can be increased by increasing the gross counts in the standard.

There is no chemical reason that prevents use of any of the total carbohydrate assays involving strong mineral acid. However, since the sample is counted in a very inaccessible area of the scintillation counter used, any spillage of the acid would cause serious damage to the instrument. A scintillation counter of a different design may be more amenable.

EXPERIMENTAL

Scintillation counting. — The basic procedure has been adequately described by Noble *et al.*[1]. All counting was carried out on a Packard 2425 scintillation spectro-

meter with factory settings for ^{3}H and the following settings for ^{14}C; gain, 7%; upper window, 1000; lower window, 25.

Reducing-sugar determinations. — The method of Lever[3] was used except that the sugar standard (1.0 ml) was mixed with the *p*-hydroxybenzohydrazide (5.0 ml). The determinations were carried out in triplicate and were read at 410 nm with a Gilford Model 240 spectrophotometer. The method of Somogyi[4] was used as described.

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