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# Fluorometric Analysis of Biological Materials. I. A Fluorophotometric Determination of Carbohydrates Using Taurine and Borate

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Taurine was found to react with carbohydrates by heating at neutral pH giving intense fluorescence. The development of fluorescence was proved to be greatly enhanced by addition of borate in the reaction mixture. This reaction was specific for the reducing sugars having C-2 hydroxyl or C-2 amino groups. The fluorescence intensity of oligosaccharides decreased with the increase in the degree of polymerization of monosaccharide units. Application of these findings provided a rapid, sensitive and reproducible assay method for carbohydrates. Standard curve for glucose was linear in the range of final concentration of 0.1 to 10.0 μg/ml of the sample and the coefficient of variation of 6.0 μg/ml of glucose was 1.4%.

**Keywords**——fluorometry, sugars taurine; sugars, fluorometry taurine; fluorescence, sugars determination; sugars, determination fluorescence; taurine, sugars fluorometry; carbohydrate, fluorometry taurine; carbohydrate, reaction taurine borate; fluorescence, carbohydrate taurine; microanalysis, carbohydrate; sugars, microanalysis

Microanalysis of carbohydrates has been of invariable biochemical value not only in the assay of individual sugars but also in the elucidation of oligo- and poly saccharide structure. In addition, there is a growing demand for the assay of congenital metabolic diseases. Fluorometry seems to be a promising tool for these purposes and a number of assays utilizing fluorescence reaction have been reported.[2-5] However, these methods employed strongly acidic conditions. Recently, Honda and co-workers[6] reported a fluorometric assay of reducing carbohydrates employing ethylenediamine under a slightly alkaline pH. However, prolonged reaction time was required in their method.

In order to overcome these shortcomings of the conventional methods, we have devised a novel method for the fluorometry of reducing carbohydrates employing taurine and borate buffer.

## Materials and Methods

Carbohydrates were purchased from Wako Pure Chemical Co., Ltd. Amylooligosaccharide fractoin (α-1, 4-glucan consisted of 12 glucose units, amylodextrin-12) was prepared from corn starch according to the method previously reported.[7] Taurine was obtained from Nakarai Chemical Co., Ltd. $KH_2PO_4$–$Na_2B_4O_7$ buffer, 0.08 M pH 7.4, was prepared by dissolving 7.6 g of $KH_2PO_4$ and 8.6 g $Na_2B_4O_7$ in redistilled water to make 1 l. Taurine reagent was prepared by dissolving 67 mmol (8.4 g) of taurine in this buffer to make 1 l.

**Assay Procedure**——Each 1.0 ml of a sample solution is pipetted in a screw-capped test tubes and to the sample is added 3.0 ml of taurine reagent. Thin walled test tubes of equal size should be used in order to secure uniform heating of the samples. The test tube is then capped and heated in a boiling water bath for 30 min. After cooling the tube in running water, the fluorescence intensity of the mixture is measured

1) Location: *Shirokane, Minato-Ku, Tokyo 108, Japan.*

2) J.E. Spikner and J.C. Towne, *Anal. Chem.*, 34, 1468 (1962); *idem, ibid.*, **35**, 211 (1963).
3) C.G. Rodgers, C.W. Chambers, and N.A. Clarke, *Anal. Chem.*, **38**, 1851 (1966).
4) T. Momose and Y. Ohkura, *Chem. Pharm. Bull.* (Tokyo), **6**, 412 (1958); *idem, ibid.*, **7**, 31 (1959).
5) S. Nakano, H. Taniguchi, T. Furuhashi, and K. Mikoshiba, *Yakugaku Zasshi*, **93**, 350 (1973).
6) S. Honda, K. Kakimoto, K. Sudo, K. Kakehi, and K. Takiura, *Analytica Chimica Acta*, **70**, 133 (1974).
7) S. Kikumoto, N. Nimura, Y. Hiraga, and T. Kinoshita, *Carbohydrate Res.*, in press.

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at excitation wavelength of 357 nm and emission wavelength of 440 nm with a Shimazu RF-510 Spectrofluorophotometer.

## Results

Excitation and emission spectra of glucose reacted with taurine at pH 7.4 are demonstrated in Fig. 1. Excitation and emission maxima were at 357 nm and 440 nm, respectively.

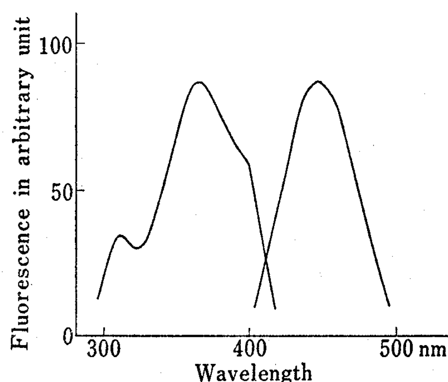


Fig. 1. Excitation and Emission Spectra of Glucose

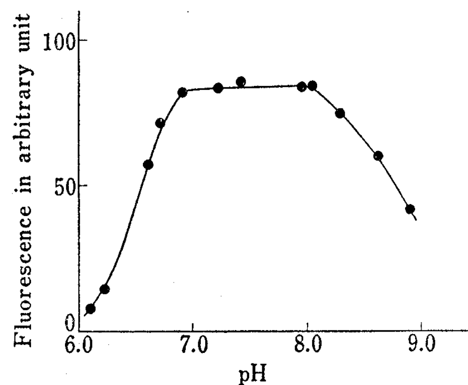


Fig. 2. pH Profile of Fluorescence Intensity of 0.6 μg/ml of Glucose

The fluorescence intensity was found to be profoundly influenced by pH, and, in addition, by the components of the buffer used. Among a variety of buffers examined, $KH_2PO_4$-$Na_2B_4O_7$ buffer gave the most intense fluorescence. Figure 2 displays the pH profile of the fluorescence intensity of glucose in this buffer. Since the optimum pH ranges from 7.0 to 8.0, pH 7.4 was adopted in the standard procedure.

Figure 3 exhibits the effect of the concentration of taurine on the fluorescence intensity. Maximum fluorescence was observed in the range of 65 μmol/ml and 75 μmol/ml with respect to the concentration of taurine in the taurine reagent. Consequently, 67 μmol/ml of taurine solution in the buffer was employed.

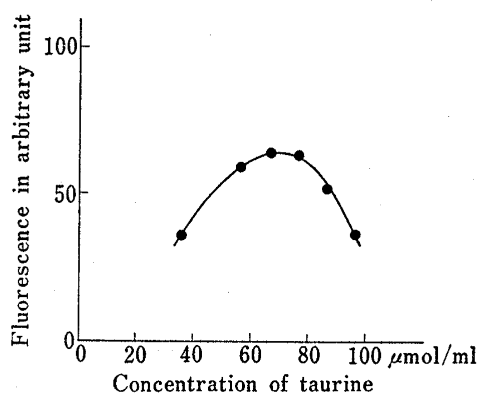


Fig. 3. Effect of the Concentration of Taurine on the Fluorescence Intensity of 0.6 μg/ml of Glucose

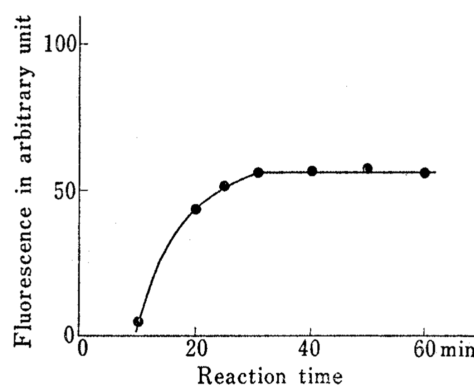


Fig. 4. Effect of Reaction Time on the Fluorescence Intensity of 1 μg/ml of Glucose

Figure 4 indicates that the fluorescence intensity reached a plateau after 30 min of the reaction. Accordingly, a reaction time of 30 min was adopted in the standard procedure. The fluorescence developed was stable for at least 17 hr at room temperature.

The standard curve of glucose was linear in the range of final concentration of 0.1 to 10.0 μg/ml. The coefficient of variation of 6.0 μg/ml of glucose was 1.4% ($n$=7).

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Fluorescence intensity of various carbohydrates is listed in Table I. Reducing monosaccharides showed the most intense fluorescence. Glucosamine and glucuronic acid fluoresced more intensely than glucose. Glyceraldehyde yielded about a half fluorescence in comparison with glucose. Fluorescence of maltose and maltotriose was about a half and one third of glucose, respectively. Amylodextrin-12 showed only slight fluorescence, and no fluorescence was observed for amylose. On the other hand, sorbitol, sucrose, methyl-α-D-glucoside, and 2-deoxy-D-ribose showed no fluorescence, and N-acetylglucosamine gave poor fluorescence.

Table I. The Fluorescence Intensity of Various Carbohydrates

| Carbohydrate | Relative fluorescence intensity | Carbohydrate | Relative fluorescence intensity |
|---|---|---|---|
| D-Glucose | 100 | D-Glucuronic acid | 162 |
| DL-Glyceraldehyde | 46 | Maltose | 44 |
| D-Arabinose | 67 | Cellobiose | 65 |
| D-Ribose | 70 | Lactose | 25 |
| D-Xylose | 69 | Maltotriose | 32 |
| D-Galactose | 86 | Nägeli amylodextrin | 1 |
| D-Mannose | 115 | 2-Deoxy-D-ribose | 0 |
| D-Fructose | 105 | Sucrose | 0 |
| L-Sorbose | 177 | D-Solbitol | 0 |
| L-Rhamnose | 107 | Amylose | 0 |
| D-Glucosamine HCl | 130 | Methyl α-D-glucoside | 0 |
| N-Acetyl-D-glucosamine | 29 | | |

Relative fluorescence intensity of glucose in the presence of several compounds are listed in Table II. Ethanol, phenol, uric acid, acetaldehyde, glycerine, arginine, did not interfere with the reaction when the same amount of these compounds with glucose was added to the sample. However, addition of a large excess amount of these compounds showed some interferences. Formaldehyde was found to affect the assay. Sialic acid did not affect the reaction.

Table II. Relative Fluorescence Intensity of Glucose (10 μg/ml) in the Presence of Several Compounds

| Compound | Amount of compound added (μg/ml) | Recovery (%) |
|---|---|---|
| None | — | 100 |
| Ethanol | 10 | 100 |
| | 100 | 118 |
| Phenol | 10 | 99 |
| | 100 | 101 |
| | 1000 | 127 |
| Uric acid | 10 | 102 |
| | 100 | 119 |
| Formaldehyde | 10 | 159 |
| Acetaldehyde | 10 | 103 |
| | 100 | 137 |
| Glycerine | 10 | 98 |
| | 100 | 142 |
| L-Arginine | 10 | 100 |
| | 100 | 109 |
| Sialic acid | 10 | 100 |
| | 100 | 102 |

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## Discussion

Reaction of reducing carbohydrates with taurine gave intense fluorescence in a buffer solution containing borate. Only poor fluorescence was observed when borate is eliminated from the reagent. On the other hand, Honda and co-workers[6] reported that phosphate buffer yieled the most intense fluorescence among a variety of buffers examined for the reaction of sugars with ethylenediamine. These facts suggest that there is a significant difference between the reaction mechanism for taurine and that for ethylenediamine. Borate may fix the sterically favorable structure of carbohydrates in the reaction with taurine.

A number of methods have been devised for the fluorometry of reducing carbohydrates. Spikner and Towne[2] applied *o*-phenylenediamine–50% sulfuric acid method for this purpose. Methods using resorcinol–sulfuric acid,[3] a highly sensitive reagent for reducing sugars, and 5-hydroxytetralone-sulfuric acid,[4] a specific reagent for hexose, were also reported. However, these methods require strongly acidic reaction conditions which may cause unfavorable side reactions and inconvenience in handling the reagent. Although Honda and co-workers[6] have found a fluorescence reaction of carbohydrates with ethylenediamine in a phosphate buffer, pH 8.0, this reaction required 3 hr for its completion. On the contrary, the present method afforded the maximum fluorescence in 30 min at neutral pH. Moreover, both taurine reagent and the fluorescence developed from sugars were sufficiently stable.

Data on the fluorescence intensity of various sugars indicate that reducing group and C-2 hydroxyl group are essential for the reaction. Glucosides, sugar alcohol, 2-deoxysugar yielded no fluorescence. The fluorescence of oligosaccharides decreased with the increase in the degree of polymerization of monosaccharide units.

Pentoses showed less intense fluorescence than hexoses, whereas rhamnose, a methyl pentose, gave similar intensity as hexoses. The length of carbon chain of monosaccharides may affect the reaction. However, further study should be made for the elucidation of the reaction mechanism.

The standard curve was linear in the wide range of glucose concentration, and the reaction was found to be highly reproducible.

The present method provides a simple, rapid and sensitive tool for the fluorometry of carbohydrates. Since the reaction conditions are mild, this procedure appears to be suitable for the detection of sugars in chromatography.

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