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Sensitive Determination of Carbohydrates by Fluorimetric Method with Ce(IV) and Sodium Triphosphate

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A new simple and sensitive fluorimetric method for the determination of carbohydrates is described. The method is based on the reaction between carbohydrates and Ce(IV) in the presence of sulfuric acid. All the reductive carbohydrates can be detected indirectly by the fluorescence of Ce(III) produced. The addition of sodium triphate enhances the sensitivity of the method by more than 10-folds. Under optimum conditions, an excellent linear relationship was obtained between the fluorescence intensity and the concentration of carbohydrates. The limits of detection lie in the range of 9.3×10^{-10}–1.3×10^{-9} mol/L. As compared to the normal fluorimetric method, the proposed method is faster and more sensitive.

KEY WORDS: Carbohydrate; Ce; fluorescence; determination.

Carbohydrates are important bioactive matters and information molecules. They are not only central to the generation and storage of energy but also useful in the diagnosis of diseases and dynamic adjustment for the functions of protein. Hence establishing highly sensitive and effective methods for the quantitative analysis of carbohydrates is important for understanding their role in nature and in almost all the life process of higher animals. At present, the methods used are mainly based on spectroanalysis [1–3], electric detection [4,5], chemical sensor [6,7] and chromatography [8]. Among these methods, fluorimetry is important because of its sensitivity and selectivity. Since carbohydrates have neither chromophores nor the fluororophore group that can be sensitively detected using the usual methods, the choice of fluoresence probes is important. According to the literatures [9], there are two types of such probes: one is derivatizing organic agents that can condense with carbohydrates to produce fluorescent compounds, such as 1,2-di(4-methoxyphenyl)ethylenediamine (DME) [10], 1,2-phenylenediamine (PDM) [11], guanidine [12], arginine [13], 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) [14], 6-aminoquinoly-*N*-hydroxysuccinimidyl carbamate (AQC) [15] and 2-methyl-3-oxo-4-phenyl-2,3-dihydrafunan-2-yl acetate (PDFAc) [16]. Recently, our have reported two new reagents of this type which are 2,3-diaminonaphthalene [17] and urea. The other type of probes is metal ion, by whose fluorescence the carbohydrates can be determined indirectly. Kikutani and co-workers found that the fluorescence intensity of some metals in the lanthanide series (Eu, Tb, Dy) [18] could be enhanced on being heated with some carbohydrates. However, the detection limit was low, for example, that of glucose was only 10 μmol/L.

Studies show that Ce(IV) can be reduced by carbohydrates to Ce(III), which can emit natural fluorescence in sulfuric acid. The addition of sodium triphosphate enhanced the sensitivity of the system by more than 10-folds. The proposed method is simple, fast and sensitive. In addition, this method can be used to determine all the reductive carbohydrates with the detect limit of 10^{-9} mol/L with a good linear range of determination.

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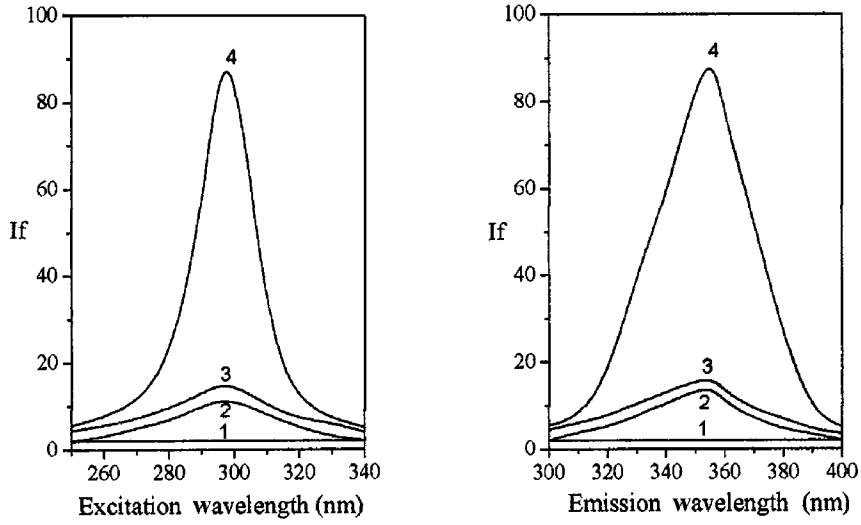

Fig. 1. Fluorescence spectra of Ce(IV)-carbohydrate systems. (a) Excitation spectra; (b) Emission spectra. 1, Ce^{4+}–H_2SO_4; 2, Glucose–H_2SO_4–$Na_5P_3O_{10}$; 3, Ce^{4+}–Glucose–H_2SO_4; 4, Ce^{4+}–Glucose–H_2SO_4–$Na_5P_3O_{10}$. Conditions: Glucose 1.00×10^{-5} mol/L; Ce^{4+} 8.00×10^{-5} mol/L; $Na_5P_3O_{10}$ 0.015 mol/L; H_2SO_4 0.05 mol/L; Heating time: 1 h.

EXPERIMENTAL

Ce(IV) standard solution (1.00×10^{-2} mol/L) was prepared by dissolving $Ce(SO_4)^{-}4H_2O$ in 100 mL of 1:1 (v:v) H_2SO_4 solution.

Sodium triphosphate solution (0.05 mol/L) was prepared by dissolving $Na_5P_3O_{10}$ in 100-mL deionized water.

Glucose, fructose, galactose, glucosamine hydrochloride and *N*-acetylglucosamine were all made by Detecting Department of Medicines and Bioproduct of China. Fucose and xylose were made by Medicine Company of China. α-Rhamnose was made by Chemical Reagents Company of Beijing. *L*-Sorbose was made by Beijing Fangcao Medicine and Chemo-technology Research Company. *D*-Arobinose was made by Medicine Store of Military Medical College of China. Solutions of the carbohydrates were prepared by dissolving them in distilled water to get a concentration of 1.00×10^{-2} mol/L. These solutions were stored at 0–4°C.

Sulfuric acid solution (1.0 mol/L) was prepared by dissolving 5.5-mL sulfuric acid in 100 mL of distilled water.

All the other reagents were of analytical reagent grade and distilled deionized water was used in this

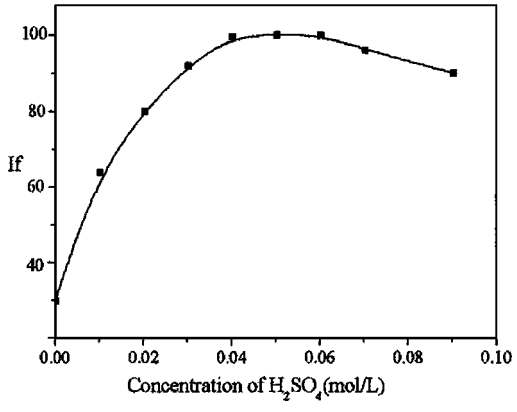

Fig. 2. The effect of the concentration of H_2SO_4. Conditions: Glucose 1.00×10^{-5} mol/L; Ce^{4+} 8.00×10^{-5} mol/L; $Na_5P_3O_{10}$ 0.015 mol/L; Heating time: 1 h.

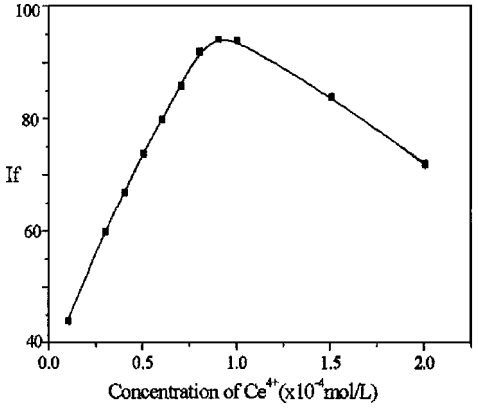

Fig. 3. The effect of the concentration of Ce (IV). Conditions: Glucose 1.00×10^{-5} mol/L; $Na_5P_3O_{10}$ 0.015 mol/L; H_2SO_4 0.05 mol/L; Heating time: 1 h.

Table I. The Linear Range and Detection Limit of Different Carbohydrates

Carbohydrates	Linear range(mol/L)	Correlation coefficient	Detection limit(mol/L)
Glucose	5.0×10^{-7}–5.0×10^{-5}	0.9926	4.5×10^{-9}
Frucose	5.0×10^{-7}–5.0×10^{-5}	0.9948	8.0×10^{-10}
D-Xylose	5.0×10^{-7}–5.0×10^{-5}	0.9909	9.3×10^{-10}
D-Galactose	1.0×10^{-7}–5.0×10^{-5}	0.9906	1.3×10^{-9}
D-Arabinose	3.0×10^{-7}–1.0×10^{-5}	0.9954	1.7×10^{-9}
α-Rhamnose	3.0×10^{-7}–1.0×10^{-5}	0.9972	2.7×10^{-9}
L-Sorbose	3.0×10^{-7}–1.0×10^{-5}	0.9987	2.2×10^{-9}
HCl-Glucosamine	—	—	—-
N-Acetylglucosamine	3.0×10^{-7}–1.0×10^{-5}	0.9927	1.9×10^{-9}

study. The fluorescence spectra and all fluorescence intensities were measured with a Hitachi Model 850 spectrofluorimeter.

Procedure

Solutions were added in the following order in a 25-mL test tube: glucose standard solution, 0.8 mL of Ce(IV) solution of 1.0×10^{-3} mol/L and 0.5 ml of H_2SO_4 of 1.0 mol/L. The mixture was diluted to 10 mL with water and then heated in a boiling water bath for 60 min. The mixture was cooled to room temperature, and its fluorescence intensity was measured in a 1-cm quartz cell with excitation and emission wavelengths of 297 and 355 nm, respectively. The excitation and emission band widths were both 10 nm. The wavelength scanning speed was set at 300 nm/min.

RESULTS AND DISCUSSION

The fluorescence spectra of the Ce(IV)–H_2SO_4, Ce(IV)–H_2SO_4–$Na_5P_3O_{10}$, Ce(IV)–glucose–H_2SO_4 and glucose–Ce–H_2SO_4–$Na_5P_3O_{10}$ systems are shown in Fig. 1. It can be seen from Fig. 1 that the luminescence spectra of (3) and (4) are similar, the wavelengths of the excitation and emission peaks are 338 and 405 nm, respectively, which is the intrinisic luminescence peak of Ce(III). In addition, the luminescence intensity of (4) is more than 10-folds that of (3). This indicates that $Na_5P_3O_{10}$ has greater enhancement for the luminescence of 2 Ce(III). In the experiment, the system was heated open to air and trace Ce(IV) could be reduced into Ce(III), which causes the very weak fluoresence illustrated by curve (2).

The effect of the concentration of H_2SO_4 was studied (see Fig. 2). The results showed that the fluorescence intensity of system reached the maximum and remained constant when the concentration of H_2SO_4 was 0.04–0.06 mol/L. So 0.05 mol/L was chosen for further experiments.

Under the given conditions, the fluorescence intensity of the system reached the maximum when the concentration of Ce(IV) was in the range of 5.0–10.0 $\times$ 10^{-5} mol/L (see Fig. 3). Therefore the concentration of

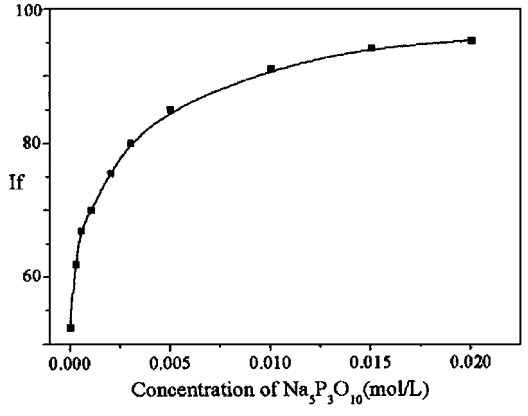

Fig. 4. The effect of the concentration of $Na_5P_3O_{10}$. Conditions: Glucose 1.00×10^{-5} mol/L; Ce^{4+} 8.00×10^{-5} mol/L; H_2SO_4 0.05 mol/L; Heating time: 1 h.

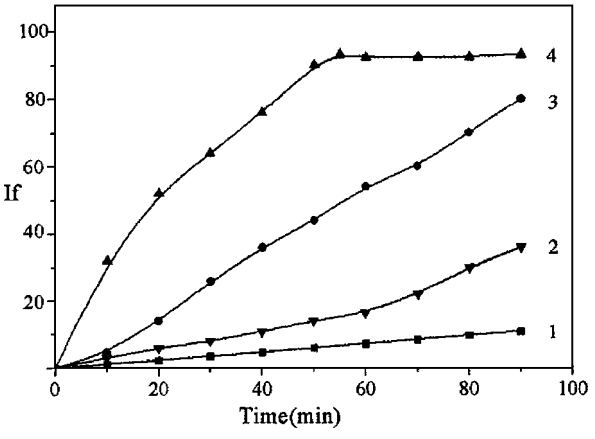

Fig. 5. The effects of different temperature and heating time. Conditions: Glucose 1.00×10^{-5} mol/L; Ce^{4+} 8.00×10^{-5} mol/L; $Na_5P_3O_{10}$ 0.015 mol/L; H_2SO_4 0.05 mol/L; Heating time: 1 h.

Table II. Recoveries of Cabonhydrates

Samples	Added(10^{-5} mol/l)	$x \pm s(n = 5)$	Recovery (%)
Glucose	1.00	0.985 ± 0.011	98.5
Frucose	1.00	0.986 ± 0.012	98.6
D-Xylose	1.00	0.974 ± 0.014	97.4
D-Galactose	1.00	0.973 ± 0.017	97.3

Ce(IV) solution was kept as 8.0×10^{-5} mol/L in further studies.

The effect of concentration of $Na_5P_3O_{10}$ was studied (see Fig. 4). The result showed that the fluorescence intensity increased with the increase of concentration of $Na_5P_3O_{10}$, until it reached 0.01 mol/L. In further experiments, 3 mL of $Na_5P_3O_{10}$ (0.05 mol/L) was used.

The reaction in the proposed method takes place in two steps. The first step is the oxidation reduction reaction between glucose and Ce(IV). Experiments were carried out to show the effects of different temperature and heating time. Studies were carried out at 40, 60, 75 and 100°C (see Fig. 5). Results showed that at some temperature, the reaction was very slow. At higher temperature, the luminescence develops more rapidly a plateau was reached after heating for 60 min at 100°C. Therefore, experiments were carried out in a water bath. Heating time of 60 min was found suitable.

The second step is the complexing reaction between Ce(III) and $Na_5P_3O_{10}$ [19]. For this step, experimental results showed that optimum temperature to be 10–50°C. Hence, the solution was cooled and fluorescence intensity was measured at room temperature.

Under the optimum conditions determined as above, a linear relationship (correlation coefficient ≈0.99) was obtained between the fluorescence intensity and the concentration of the studied carbohydrates. The limits of detection were of the order of 10^{-9} mol/L (S/N = 2) (Table I).

The standard addition method was used for determinations of glucose, fructose, D-xylose and galactose solutions. The recovery ratios were in the range of 97.3–98.5% (Table II). The proposed method was also used to determine the glucose in standardized 50% glucose injection solution (Jinan Mingshui Limin Pharmaceutical, China). In the proposed method the average found ($n = 5$) is 49.0% with standard deviation of ±0.54, while in the 2,3-diaminonaphthalene method [23] the average is 49.3% with standard deviation of ±1.58. It indicated that the proposed method was accurate and precise (Table III).

Table III. Determination of Glucose in Sample (Glucose Injection Solution)

	Average found (%)($n = 5$)	Standard deviation
2,3-Diaminonaphthalene method [23]	49.3	±1.58
The proposed method	49.0	±0.54

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REFERENCES

1. R. Zhang, Z. D. Zhang, G. Q. Lin, and Y. Hidaka (1993). High-performance liquid chromatographic determination of neutral and amino monosaccharides by ultraviolet and fluorescence detection of sugar 9-fluoroenylmethoxycarbonyl hydrazones and 9-fluoroenylmethoxycarbonyl amino sugars at picomole and subpicomole levels. *J. Chromatogr*. **646**, 45–52.
2. H. Y. Xu and Z. M. Zhao (1996). Spectrophotometic determination of total sugar in export nutritive liquor. *J. Jilin Univ. (Chin.)* **4**, 98–100.
3. L. W. Chang, R. F. Yao, Y. Chen, and J. Kong (1994). Determination of monosaccharides by capillary zone electrophoresis. *Fenxi Huaxue (Chin.)* **1**, 1–5.
4. G. G. Neuburger and D. C. Johnson (1987). Pulsed amperometric detection of carbohydrates at gold electrodes with a two-step potential waveform *Anal. Chem*. **59**(1), 150–154.
5. R. E. Reim and R. M. Van Effen (1986). Determination of carbohydrates by liquid chromatography with oxidation at a nickel(III) oxide electrode *Anal. Chem*. **58**(14), 3203–3207.
6. L. C. Clark and G. Lyons (1962). Electrode system for continuous monitoring in cardiovascular surgery, *Ann. N. Y. Acad. Sci*. **102**, 29–45.
7. M. S. Abdel-Latif and G. G. Guilbault (1988). Fiber-optic sensor for the determination of glucose using micellar enhanced chemiluminescene of the peroxyoxalate reaction *Anal. Chem*. **60**(24), 2671–2674.
8. S. Katz, W. W. Pitt, J. E. Mrochok Jr., and S. Dinsmore (1974). Sensitive fluorescence monitoring of carbohydrates eluted by a borate mobile phase from an anion-exchange column *J. Chromatogr*. **101**, 193–197.
9. F. Huang, X. L. Liu, G. J. Zhou, X. Wu, J. H. Yang (2000). The progress of the derivatization methods of carbohydrate. *Chem. Online (Chin.)* **63**, 00023.
10. Y. Umegae (1989). High performance liquid chromatography of bioactive substances using bifunctional fluorogenic reagents for derivatization *Anal. Sci*. **5**, 371–388.
11. J. C. Towne and J. E. Spikner (1963). Fluorimetric microdetermination of carbohydrates *Anal. Chem*. **35**(2), 211–214.
12. S. Yamauchi, N. Nimure, and T. Kinoshita (1993). Development of a high sensitive fluorescence reaction system for liquid chromatographic analysis of reducing carbohydrates *Analyst* **117**, 773–776.
13. H. Mikami and Y. Ishida (1983). Post-column fluorimetric detection of reducing sugars in high-performance liquid chromatography using arginine, *Bunseki Kagaku* **32**, E207–E210.
14. M. Stefansson and M. Novotny (1994). Separation of complex oligosaccharide mixtures by capillary electrophoresis in the open-tubular format *Anal. Chem*. **66**(7), 1134–1140.

15. J. Diaz, J. Ll. Lliberia, L. Comellas, and F. Broto-Puig (1996). Amino acid and amino sugar determination by derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate followed by high-performance liquid chromatography and fluorescence detection *J Chromatogr*. **719A**, 171–179.
16. P. Chen and M. V. Novotny (1997). 2-methyl-3-oxo-4-phenyl-2,3-dihydrofuran-2-yl acetate: A fluorogenic reagent for detection and analysis of primary amines *Anal. Chem.* **69**(14), 2806–2811.
17. J. H. Yang, X. H. Cao, F. Huang, X. Wu, and G. J. Zhou (2000). Fluorimetric determination of carbohydrates with 2,3-diaminonaphthalene *Chem. J. Internet* **2**(1):3.
18. N. Kikutani, H. Ichikawa, T. Tomomastsu, and H. Nakamura (1995). Unique enhanced fluorescence of some lanthanides induced by heating with reducing sugars *Anal. Sci*. **11**, 843–845.
19. A. Aycicek and R. Yeber (1996). Fluorimetric trace determination of cerium(III) with sodium triphosphate *Talanta* **43**, 1983–1988.