[Chem. Pharm. Bull. 27(5)1268—1271(1979)]

UDC 543.83.06 : 543.544.2.061

Simultaneous Fluorometric and Colorimetric Detection of Carbohydrates on Silica Gel Plates using *o*-Aminobenzenesulfonic Acid

Fumio Iinuma,[1a] Yayoi Hiraga, Toshio Kinoshita,[1b] and Mitsuo Watanabe,[1a]

Faculty of Pharmaceutical Sciences, Teikyo University[1a] and School of Pharmaceutical Sciences, Kitasato University[1b]

(Received November 24, 1978)

Rapid, simple and sensitive detection of carbohydrates on thin layer plates was achieved by utilizing *o*-aminobenzenesulfonic acid–H_3PO_4 reagent, which was found to be stable in solution. This method facilitated the simultaneous fluorometric and colorimetric detection of carbohydrates with excellent sensitivity. This method was also applied for the determination of sugars using a scanning fluorophotometer.

Keywords——carbohydrates; thin-layer chromatography; *o*-aminobenzenesulfonic acid; fluoretric detection; colorimetric detection

Various reagents have been reported for the detection of carbohydrates on chromatograms[2–11] since carbohydrates have no absorption in the range measured by usual ultraviolet (UV) or fluorescence detectors.[12] However, colorimetric detections generally show poor sensitivity and the fluorometric methods are susceptible to quenching by co-existing materials or excessive sample concentrations. Simultaneous colorimetric and fluorometric detection is preferable for the unambiguous location of carbohydrates.

The authors found that *o*-aminobenzensulfonic acid (*o*-ABS) gives intense color and fluorescence on reaction with mono- and oligosaccharides and applied this finding to the detection of carbohydrates on thin-layer chrometograms.

Materials and Methods

Carbohydrates used were of reagent grade. *o*-ABS was purchased from Wako Pure Chemical Co., Ltd. and recrystallized from water. Silica gel precoated thin layer plates without fluorescence indicator were obtained from Merk Co.

***o*-ABS Reagent**——Two grams of *o*-ABS was dissolved in aqueous 4% (w/v) H_3PO_4 solution to make 100 ml.

Assay Procedure——One microliter of a sugar solution was applied to a silica gel plate and the plate was developed using *n*-propanol–acetone–water (5 : 3 : 1) as a solvent. The plate was sprayed with 20 μl/cm^2 of ABS reagent and heated at 120° for 5 minutes. The spots were detected by irradiation at 365 nm using a Super-Light, model LS-DI, Nikko Seiki Works Co. The fluorescence intensity was measured with a Densitron FR-1 scanning fluorometer, Jookoo Sangyo Co., Ltd.

1) Location: *a*) *Sagamiko, Kanagawa 199-01, Japan*; *b*) *Shirokane, Minato-ku, Tokyo 108, Japan.*
2) T. Nakai, M. Koyota, and H. Demura, *J. Chromatog.*, **50**, 338 (1970).
3) B.H. Howard, *Biochemical Journal*, **67**, 643 (1957).
4) R. Bird and R.H. Hopkins, *Biochemical Journal*, **56**, 86 (1954).
5) J.L. Garraway and S. Cook, *J. Chromatog.*, **46**, 134 (1970).
6) E. Chargaff, C. Levine, and C. Green, *J. Biol. Chem.*, **175**, 67 (1948).
7) G. Zweig and J. Sherma, "Handbook of Chromatography," Vol. 2, Chemical Rubber Co. Ohio, 1972 p. 125.
8) G. Saglietto and G. Mantovani, *Z. Zucherind.*, **20**, 17 (1970).
9) E. Sawicki, D.R. Engel, W.C. Elbut, and K. Gerlach, *Talanta*, **15**, 803 (1968).
10) S. Honda, K. Kakimoto, K. Kakehi, and K. Takiura, *Anal. Chim. Acta*, **64**, 310 (1973).
11) G. Avigad, *J. Chromatog.*, **139**, 343 (1977).
12) T. Kato and T. Kinoshita, *Chem. Pharm. Bull.* (Tokyo), **26**, 1291 (1978).

Results and Discussion

Aniline-phosphoric acid reagent[3-5] has been employed for the colorimetric detection of carbohydrates, and this method simultaneously facilitated the fluorometric detection. Since this reagent is unstable to oxidation, several aromatic amines have been examined as spray reagents for the fluorometric detection of sugars, but free amines are somewhat unstable to autoxidation and most naphthylamino derivatives are known to be carcinogenic.[13] *o*-ABS reagent is stable for at least 2 weeks and *o*-ABS exhibited a lower background fluorescence than its *m*- or *p*-isomers. The stability of *o*-ABS may be partly due to protonation of the amino group by zwitterion formation, which makes the *o*-isomer more resistant to autoxidation than the *m*- and *p*-isomers. Acidic solvents were found to enhance the fluorescence intensity of the reaction product between carbohydrates and *o*-ABS, and H_3PO_4 was superior to other acids tested, as summarized in Table I. Although pyridine was also useful as a solvent, phosphoric acid was adopted because it is non-volatile.

Table I. Fluorescence Intensity of 0.5 nmol of Glucose reacted with *o*-ABS in Various Solvents

	Relative fluorescence intensity
2% ABS in 2% H_3BO_3	55
2% ABS in 4% HPO_3	80
2% ABS in 4% H_3PO_4	100
5% ABS in pyridine	94

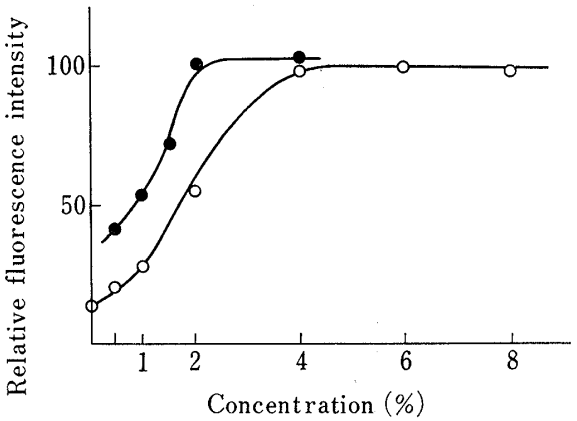

Fig. 1. Effects of *o*-ABS Concentration (●) and H_3PO_4 Concentration (○) in the Presence of 2% *o*-ABS on the Fuorescence Intensity of 0.5 nmol of Glucose

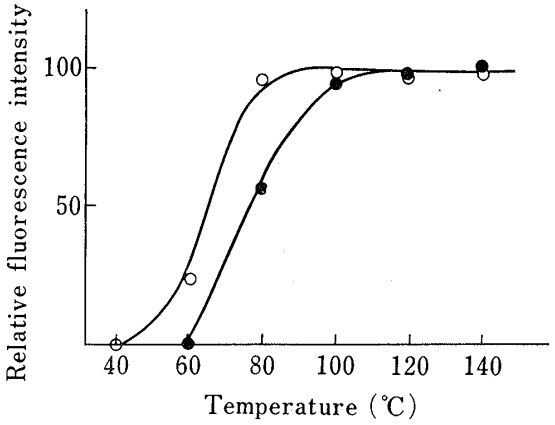

Fig. 2. Effect of the Reaction Temperature on the Fluorescence Intensity of 0.5 nmol of Glucose

The thin-layer plates were heated for 5 min (●) and 10 min (○).

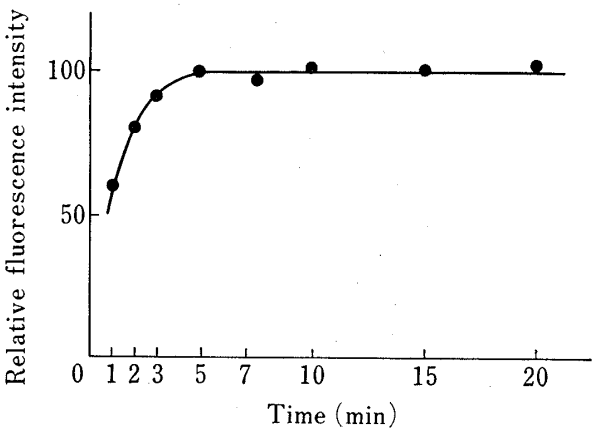

Fig. 3. Effect of Reaction Time on the Fluorescence Intensity of 0.5 nmol of Glucose

The thin-layer plate was heated at 120°.

13) R.A.M. Case, M.E. Hosker, D.B. McDonald, and J.T. Pearson, *Brit. J. Ind. Med.*, **11**, 75 (1954).

Figure 1 shows the effects of *o*-ABS concentration and H_3PO_4 concentration on the fluorescence intensity. Although the solubility of *o*-ABS in 4% (w/v) H_3PO_4 was 2% at room temperature, *o*-ABS at a concentration of 4% was prepared by heating the mixture and the resulting supersaturated solution was sprayed. The fluorescence intensity reached a plateau at 2% (w/v) *o*-ABS and 4% (w/v) H_3PO_4 and these concentrations were adopted in the standard procedure.

As shown in Fig. 2, maximum fluorescence intensity was observed when the thin layer plate was heated at 80° for 10 min or at 120° for 5 min in an electric oven. The latter condition was used in the standard procedure in order to minimize the detection time. The fluorescence was not affected when the heating was prolonged for up to 20 min at 120,° as indicated in Fig. 3. The fluorescence and the color are stable for 3 hr at room temperature when the plate is covered with a glass plate and stored in the dark.

Table II. Limits of Detection for Carbohydrates

Carbohydrate	Limit of detection (nmol) by fluorescence	Limit of detection (nmol) by color (tint)
D-Glucose	0.05	1.0 (yellow-brown)
D-Mannose	0.05	1.0 (yellow-brown)
D-Galactose	0.1	1.0 (yellow-brown)
L-Rhamnose	0.1	1.0 (yellow-brown)
Lactose	0.05	1.0 (yellow-brown)
D-Glucosamine·HCl	0.05	1.0 (yellow-brown)
Sucrose	0.05	1.0 (yellow-brown)
Maltose	0.05	1.0 (yellow-brown)
Maltotriose	0.05	1.0 (yellow-brown)
Maltotetraose	0.05	1.0 (yellow-brown)
D-Raffinose	0.05	1.0 (yellow-brown)
Stachyose	0.05	1.0 (yellow-brown)
Maltopentaose	0.05	1.0 (yellow-brown)
D-Ribose	0.5	1.0 (orange-brown)
D-Xylose	0.5	1.0 (orange-brown)
D-Arabinose	0.5	1.0 (orange-brown)
D-Fructose	0.5	1.0 (orange-brown)
L-Sorbose	0.5	1.0 (orange-brown)
D-Glucuronolactone	0.5	1.0 (dark-red)
D-Galacturonic acid	0.5	1.0 (dark-red)
N-Acetylglucosamine	5.0	5.0 (green)
N-Acetylmannosamine	5.0	5.0 (green)
DL-Glyceraldehyde	N.D.[a]	50 (yellow)
L-Ascorbic acid	N.D.	50 (brown)
α-Methyl-D-glucoside	N.D.	N.D.
α-Methyl-D-mannoside	N.D.	N.D.
D-Mannitol	N.D.	N.D.
D-Sorbitol	N.D.	N.D.

a) N.D.= not detectable at 50 nmol.

Table II lists the limits of detection of the sugars tested. The sensitivity as fluorometirc detection for aldohexoses was higher than those for pentose, ketose, N-acetylhexosamines and uronic acids. Colorimetric detection was rather sensitive. The colors are also shown in this table. Table III implies that the present method is also suitable for the detection of oligosaccharides. Aldoses and oligosaccharides which readily generate reducing groups on hydrolysis, such as raffinose or stachyose, gave intense fluorescence. On the other hand, methyl glycosides and sugar alcohols were not detectable by this method, indicating that the reducing group of sugars plays an important role in the development of both color and fluores-

cence. DL-Glyceraldehyde and L-ascorbic acid did not give fluorescence. Nevertheless, these compounds could be detected by the color reaction.

The present method was also applicable to the determination of sugars on thin-layer plates utilizing a scanning fluorophotometer. The standard plot for glucose showed a linear relationship in the range of 0.1 to 0.5 nmol/spot and passed through the origin. The relative fluorescence intensities of 0.5 nmol each of glucose, galactose, mannose, maltose, maltotriose measured by this assay procedure were 100, 56, 73, 96 and 77, respectively.

Acknowledgements The authors would like to express their gratitude to Miss Motoko Ejima for her skilful assistance. They also wish to thank Wako Pure Chemical Co. for generous gifts of maltotriose, maltotetraose and maltopentaose.

[Chem. Pharm. Bull. 27(5)1271—1273(1979)]

UDC 547.672.04 : 547.438.1.04

Anodic Phosphonylation of Anthracene

HIDENOBU OHMORI, SHIRO NAKAI, and MASAICHIRO MASUI

Faculty of Pharmaceutical Sciences, Osaka University[1)]

(Received December 14, 1978)

Controlled potential electrolysis of anthracene at a glassy carbon anode in acetonitrile containing an excess of triethylphosphite and subsequent treatment of the product with sodium iodide resulted in the formation of 9,10-bis(diethylphosphonyl)-9,10-dihydroanthracene. Anodic phosphonylation was also performed on 4′-methoxybenzanilide to give 2′-diethylphosphonyl-4′-methoxybenzanilide.

Keywords——anodic oxidation; controlled potential electrolysis; anodic phosphonylation; anthracene; triethylphosphonium ion; 4′-methoxybenzanilide

Previously we have reported the anodic arylation of trialkylphosphites,[2)] in which nucleophilic attack of alkylbenzene on an anodically generated trialkylphosphite cation radical results in the formation of a trialkoxy arylphoshponium ion (eq. 1): the latter was converted to the corresponding dialkyl arylphosphonate on treatment with sodium iodide.

$$(RO)_3P + ArH \xrightarrow[MeCN,\ NaClO_4,\ K_2CO_3]{\text{glassy carbon anode}} (RO)_3\overset{+}{P}\text{–}Ar + 2e \qquad (1)$$

Since the oxidation of trialkylphosphite takes place at fairly high positive potentials (>1.5 V *vs* S.C.E. in MeCN), the aromatic compounds available were limited. If nucleophilic attack of trialkylphosphites on a cation radical, a cation, or a dication derived from aromatic cmpounds can be achieved, the applicability of this process will be enhanced. Anodic phosphonylation would be a new addition to the list of anodic substitution reactions.[3)] We selected anthracene and triethylphosphite as typical substrates and examined the possibility of such a reaction. Phosphonylation was also examined in the case of 4′-methoxybenzanilide for which anodic pyridination has been reported.[4)]

1) Location: *133-1 Yamadakami, Suita, Osaka.*
2) H. Ohmori, S. Nakai, and M. Masui, *J. Chem. Soc. Perkin I*, accepted.
3) L. Eberson and K. Nyberg, *Tetrahebron*, **32**, 2185 (1976).
4) S. Ikenoya, M. Masui, H. Ohmori, and H. Sayo, *J. Chem. Soc. Perkin II*, **1974**, 571.