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Selective determination of D-sorbitol and D-mannitol in foodstuffs by ion chromatography with polarized photometric detection

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Abstract

A highly selective detection system for D-sorbitol and D-mannitol is described. Although these alditols have many chiral carbons, they show almost no optical rotation. However, in the presence of molybdate in a medium of low acidity, they form anionic complexes with molybdate that have remarkably large specific rotations. The detection system consists of an anion-exchange column using a molybdate solution as the eluent and a polarized photometric detector, which is a non-modulated polarimeter of our own design. With this system, alditols can be identified in foodstuffs after simple pretreatment.

Keywords: Polarized photometric detection; Detection, LC; Food analysis; Sorbitol; Mannitol; Sugar alcohols

1. Introduction

Sugar alcohols, also called alditols, are the reduced forms of the parent aldoses. Although they are inferior to the parent sugars in sweetness, they have recently attracted interest as food additives because of various advantages, e.g., low cariogenicity arising from the lack of formation of insoluble glucan by microbes such as *Streptococcus mutans*, low calories and high thermostability. Many alditols are currently approved for dietary use in Japan. Of these, D-sorbitol accounts for the majority of consumption because it is inexpensively manufactured from glucose as the raw material and has a good ability to resist moisture loss and to infiltrate into the food tissues compared with other alditols [1]. In contrast, D-mannitol is widespread in nature and is also used as a food additive. However, the actual amount of these alditols that are consumed is not clearly known because the gas chromatographic (GC) method that is commonly used for their determination requires the conversion of the alditols to suitably volatile derivatives [2,3].

A normal-phase partition method using an amino-bonded high-performance liquid chromatography (HPLC) column has been successfully applied to sugar analysis [4]. However, a problem with this method is that the elution of alditols overlaps with that of monosaccharides. In recent years, ion chromatography (IC) with pulsed amperometric detection (PAD) has been receiving increasing attention for sugar analysis [5,6]. Most sugars have pK_a values in the range of 12–13. With highly alkaline eluents, such as a sodium hydroxide solution, sugars may be separated on ion-exchange columns. Furthermore, PAD can detect ionized sugars with high sensitivity.

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The retention times of alditols in this system are reduced compared with those of the parent aldoses, because they have no anomeric hydroxyl group, which is the most acidic type of hydroxyl group, so this system is free of interference from other sugars. The analysis of alditols in foods by using a high capacity ion-exchange column specifically designed for this purpose has been reported [7], but the question of the late-eluting sugars, which exist in rather high quantities in food products, now arises.

Although D-sorbitol and D-mannitol are optically active compounds with many chiral carbons, their flexible structures in a medium result in almost no optical rotation. It is known that the formation of a molybdate complex of sorbitol or mannitol makes their structures rigid and increases their optical rotation [8]. This will increase the possibility of selective determination of these alditols. We proposed a novel chiroptical detection method, polarized photometric detection (PPD), by incorporating polarizers and/or retarders into a conventional photometric detector [9–12]. In this work, an attempt was made to establish a simple method for determining sorbitol and mannitol in foodstuffs by IC–PPD.

2. Experimental

The IC system consisted of a Shimadzu (Kyoto, Japan) LC-10AD pump, a Rheodyne (Cotati, CA, USA) 7125 injector, a Tosoh (Tokyo, Japan) CO-8011 column oven and a Shimadzu SPD-10AV UV–visible photometric detector.

Measurements of optical rotation were performed by a Jasco (Tokyo, Japan) DIP-181 polarimeter.

HPLC-grade acetonitrile, chemical grade cetyltrimethylammonium (CTA) bromide and other guaranteed grade reagents were purchased from Wako (Osaka, Japan). The molybdate solution used for equilibrating the analytical column was prepared as follows: A 3.63-g amount of sodium molybdate dihydrate was dissolved in about 40 ml of deionized water; 2.1 ml of concentrated nitric acid were added and the volume was made up to 50 ml with deionized water. The solution was then passed through a 0.45-μm membrane filter and diluted tenfold.

A dynamically coated column was prepared using a TPR-100 column (15 cm×4.6 mm I.D., Supelco, Bellefonte, PA, USA). This column was coated with CTA as follows: More than 50 ml of 25 m*M* CTA–acetonitrile (3:1, v/v) was passed through the column; the column was thoroughly washed with deionized water and was then pre-equilibrated with the molybdate solution before use. Analysis was performed with an eluent containing 2 m*M* sodium molybdate and 50 m*M* nitric acid (pH 1.4) at a flow-rate of 0.8 ml/min. The column oven was set at 40°C. PPD was performed by inserting the split-type flow cell assembly, on which Nippon Polaroid (Tokyo, Japan) HN32 polarizers were placed, into the photometric detector. For the sample cell, the plane of polarization of the polarizers on the transmitting light side with respect to that on the incident light side was set at 65° to the left when facing the light source, and for the reference cell, it was set at 55° to the right [13]. The detection wavelength was 600 nm.

Commercially available food products were used for the experiment. Samples were pretreated as follows: A sample (1–5 g) was cut into small pieces, placed in a beaker, and homogenized in about 30 ml of deionized water. The homogenate was diluted to 50 ml with deionized water and then ultrafiltered through a Tosoh Ultracent-30.

3. Results and discussion

3.1. Influence of molybdate concentration on the optical rotation of alditols

The effect of the pH of the medium on the optical rotation of the alditol–molybdate complex was examined. The optical rotation of alditol in a molybdate solution was very small under alkaline conditions. However, it steeply increased at pH values below 5 and reached a maximum at around pH 1.5. The relationship between optical rotation and the alditol concentration in the acidic 0.2 *M* molybdate solution is shown in Fig. 1. The ordinate indicates the relative optical rotations in relation to that of a 1% sorbitol solution. These values increased on addition of the alditols and reached a plateau in the presence of more than 2% alditols. This concentration suggests that the molar ratio of alditols to molybdate is equivalent to 0.5, and it corresponds to

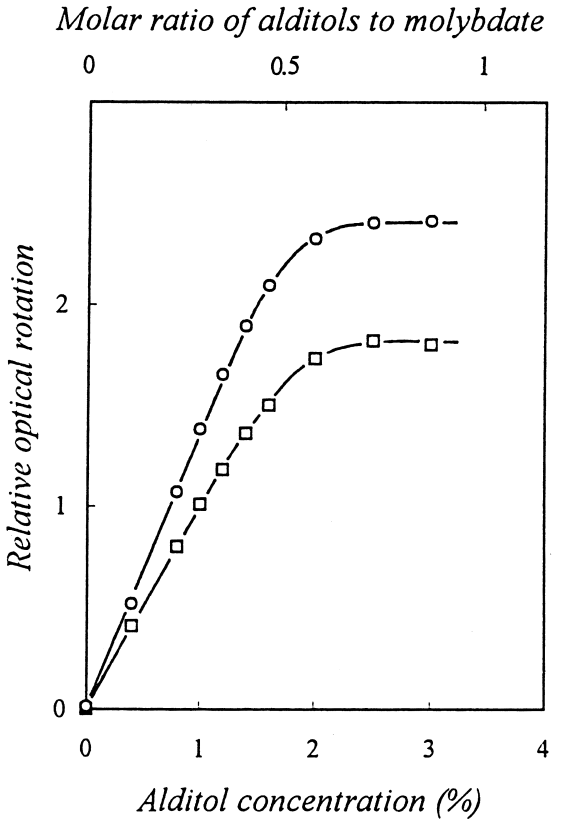

Fig. 1. Influence of concentration on the relative optical rotation of alditol at 589 nm. Symbols: ○, mannitol; □, sorbitol.

a structure of these complexes consisting of two moles molybdate per one mole of alditol [8]. Molybdate is self-polymerized in a high acidity medium. The fact that sugars with a ring structure do not form molybdate complexes and that the optical rotation of an alditol–molybdate complex in a low acidity medium is not large suggests that the optical rotation of an alditol emerges from the fixation of its flexible structure by inclusion into the molybdate polymer. Thus, molybdate reagent is very suitable for the polarimetric detection of alditols.

3.2. Selection of the separation mode

It is convenient to use the anionic property of alditols produced by the presence of molybdate for their separation with a conventional liquid chromatography (LC) system. Unfortunately, molybdate forms an insoluble complex with ammonium salts, so the ion-pair partition mode was not considered further. In the ion-exchange mode, the ion-exchange capacity of a typical column for IC was too small to retain the complexes, because polyvalent condensed molybdate acts as an eluting agent.Therefore, an ion-exchange column possessing a suitable ion-exchange capacity was prepared by coating the quaternary ammonium salts on the reversed-phase column. Three ammonium salts were compared as possible ion-exchangers. These comprised CTA bromide, 1-phenethyl-2-picolinium bromide and cetylpyridinium chloride. Of these, CTA provided the best peak shapes for alditols. CTA was coated onto four kind of columns, i.e., carbon, polymer resin, ODS and octadecyl bonded polymer (ODP) resin. The carbon column did not efficiently adsorb CTA. Although the other three provided suitable ion-exchange capacities, sorbitol and mannitol were inseparable on the polymer resin column. Coating with CTA could be expected to deteriorate the ODS column. Thus, the TPR-100 column, which was packed with ODP resin and exhibited an excellent resistance to alkalis and acids, was chosen for the analysis of alditols. This column, when coated with CTA, was robust enough to be used continuously for a few days. However, it is necessary to wash the CTA out of the column after use.

3.3. Optimization of the eluent

The influence of the molybdate concentration in the eluent on the retention of the alditols by the column was investigated. Fig. 2 shows the elution volumes of alditols and sucrose, where the eluent pH was kept at 2. The retention of sugars was independent of the molybdate concentration and the other mono- and disaccharides eluted in almost the same region as sucrose. For alditols, it can be seen that the elution volumes increase with decreasing molybdate concentration. Since their peak shapes broadened when the molybdate concentration was 1 m*M*, 2 m*M* molybdate was chosen as an eluent for subsequent experiments.

The optical rotations of the complexes have maxima at around pH 1.5. Fortunately, the TPR-100 column is designed to withstand a highly acidic mobile phase. In order to optimize the eluent pH, we

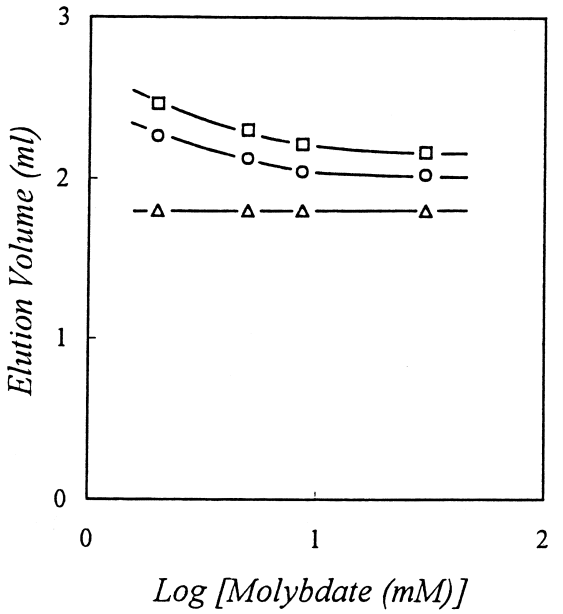

Fig. 2. Elution volumes of sorbitol (□), mannitol (○) and sucrose (△) as a function of molybdate eluent concentration (m*M*) at pH 2.0.

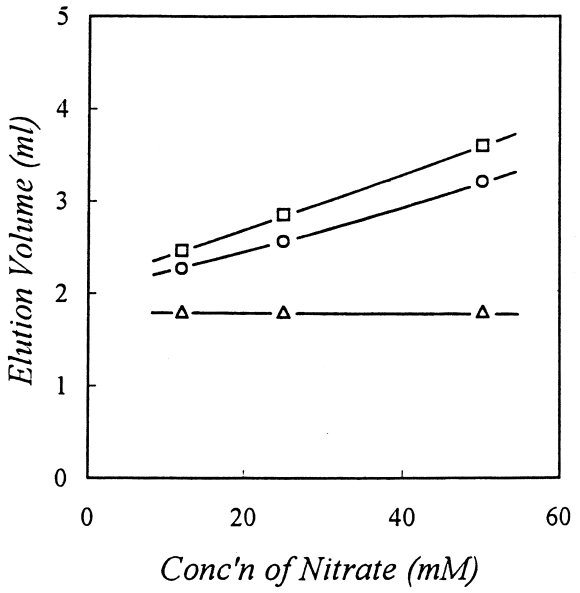

Fig. 3. Elution volumes of sorbitol, mannitol and sucrose as a function of nitrate concentration in an eluent containing 2 m*M* molybdate. Symbols are the same as in Fig. 2.

tested the concentrations of nitric acid added to the eluent. The results, shown in Fig. 3, indicate that the separation between sorbitol and mannitol becomes satisfactory as the eluent pH is lowered. Thus, an eluent containing 50 m*M* nitric acid and 2 m*M* molybdate (pH 1.4) was chosen for the analysis described below (Section 3.4).

3.4. Application to an actual sample

Although there are a considerable number of alditols other than sorbitol and mannitol in food products, many of them, such as xylitol and erythritol, are of the *meso*-form. PPD gives almost no signals for such optically inactive compounds. Consequently, this analytical system promises a highly selective quantitation for sorbitol and mannitol. Fig. 4a shows the chromatogram of a mixture prepared from sucrose and alditol standard. A detection wavelength of 600 nm was chosen due to the stability of light source and the higher values of specific rotation of the complexes. Both alditols were well-separated from each other. Their peak areas increased linearly with the amount injected on the column, up to 100 μg.

Samples were extracted with deionized water and ultrafiltered prior to IC injection. Fig. 4b shows an example of a chromatogram of a food sample. In this food extract, it seemed that sorbitol was used as a food additive and mannitol was carried over from tangleweed, in which it is one of the principal ingredients. No interference was observed when applying this technique to other food products. The limits of detection using a 10-μl injection of sample solution extracted with ten volumes of water were

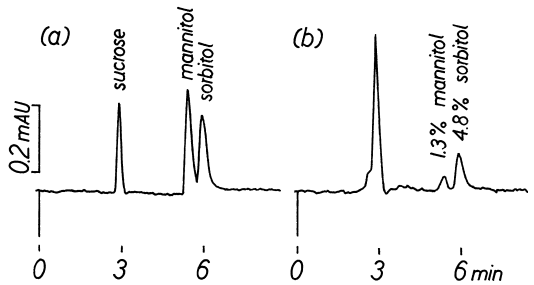

Fig. 4. Chromatograms obtained on injection of 5 μl of standard solution containing 1% each of alditols and sucrose (a) and "tsukudani" of tangleweed (kombu) extract (b).

0.1% (m/m) for sorbitol and 0.05% (m/m) for mannitol.

4. Conclusion

An attempt was made to establish a quantitative system for the determination of sorbitol and mannitol in foodstuff by PPD. These alditols showed large optical rotations on complexation with molybdate. Anion-exchange chromatography using an acidic molybdate solution as an eluent was used. An ODP column coated with CTA gave excellent separation between sorbitol and mannitol and was stable during prolonged use. The proposed system was highly selective for the separation and detection of sorbitol and mannitol, and required no laborious treatment of the sample. The important advantage of the PPD to note is that it can be easily constructed from an ordinary photometric detector. Thus, the proposed method is readily applicable to the conventional HPLC system.

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