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Note

Liquid chromatographic determination of carbohydrates with pulsed amperometric detection and a membrane reactor

JUN HAGINAKA*
Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68, Koshien Kyuban-cho, Nishinomiya, Hyogo 663 (Japan)
and
TETSUO NOMURA
Scientific Instrument Department, Abe Trading Co. Ltd., 6-32, 3-chome, Nakanoshima, Kita-ku, Osaka 530 (Japan)

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High-performance liquid chromatography (HPLC) has played an important rôle in recent advances in carbohydrate chemistry and biochemistry. The separation modes employed are based on gel permeation, borate-complex anion exchange and partition chromatography, while the detection methods are mainly based on refractivity and low-wavelength ultraviolet (UV) absorption, though a number of post-column derivatization methods have been developed[1]. However, refractive index and UV detection are insensitive and non-selective. Recently, the direct separation of carbohydrates by anion-exchange chromatography in combination with pulsed amperometric detection using a gold working electrode was developed by Rocklin and Pohl[2]. The method is highly sensitive for CHOH-bearing compounds such as carbohydrates and alcohols, but it has a disadvantage that the separation mode is limited to an anion-exchange column (OH^-) with a highly alkaline eluent. In order to extend the use of pulsed amperometry to the detection of CHOH-bearing compounds separated by various modes, we tried changing the pH of the eluent to alkaline values by using a membrane reactor. This paper deals with the determination of carbohydrates by pulsed amperometry in combination with a membrane reactor.

EXPERIMENTAL

Reagents and materials

Carbohydrates and glycerol were obtained from Nakarai Chemicals (Kyoto, Japan). Their stock solutions (1.0 mg/ml) were prepared in water and diluted in the eluent before injection onto a column. Methanol and acetonitrile of HPLC grade were obtained from Wako Pure Chemical Industries (Osaka, Japan). Water prepared by a Nanopure unit (Barnstead, Boston, MA, U.S.A.) was used for the preparation of the sample and the eluent.

A cation-exchange membrane (AMMS-1) was obtained from Dionex (Sunnyvale, CA, U.S.A.).

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Chromatography

The HPLC system comprised a Model 4000i pump and a pulsed amperometric detector (both from Dionex) equipped with a gold working electrode. The columns and eluents used were as follows: method A, a Sugar SP1010 column (30 cm × 8 mm I.D.) (Showa Denko, Tokyo, Japan), which is a cation-exchange column loaded with lead, and water at a flow-rate of 1.0 ml/min; method B, an RSpak DC-613 (15 cm × 6 mm I.D.) (Showa Denko), which is a cation-exchange column loaded with sodium, and water–acetonitrile–methanol (3:6:1, v/v/v) at a flow-rate of 0.8 ml/min. The columns were maintained at 80 and 50°C, respectively, in methods A and B using a TU-310 column oven (Japan Spectroscopic, Tokyo, Japan). Sodium hydroxide solutions (0.75 *M* for method A and 1.5 *M* for method B) were used as the eluent pH modifier, and delivered into the membrane reactor (AMMS-1) at a flow-rate of 1.0 ml/min. The three potentials applied, E_1, E_2 and E_3, were 200, 600 and -800 mV at duration times of 60, 60 and 240 ms, respectively, as reported previously[2]. A 50-μl portion of the sample was loaded onto a column.

RESULTS AND DISCUSSION

Detection

Hughes and Johnson[3,4] reported an HPLC method for the determination of carbohydrates which involved their separation on a cation-exchange resin loaded with calcium using water as an eluent, modification of the eluent pH to alkaline values by delivery of sodium hydroxide solution with a pump and detection by pulsed amperometry. However, the method has a disadvantage in that the detector noise caused by flow noise and mixing inhomogeneity prevents the sensitive detection of

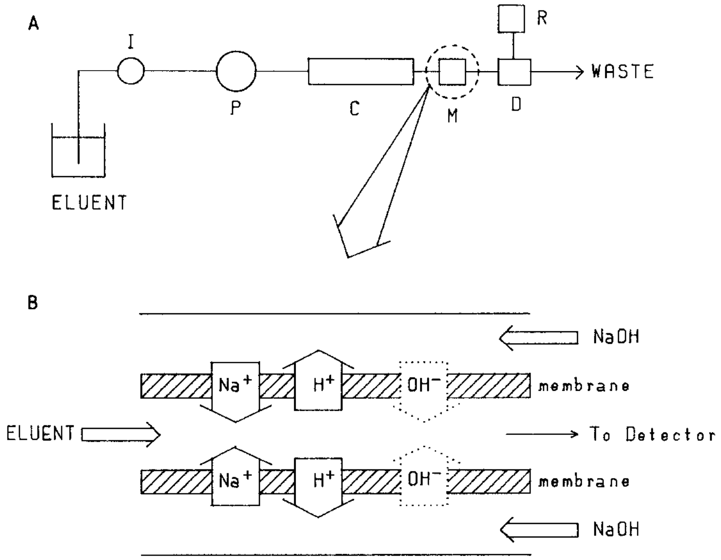
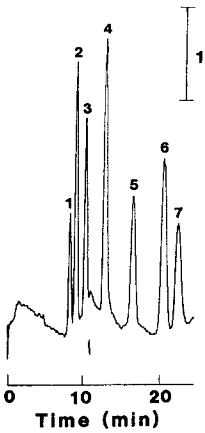


Fig. 1. Experimental set-up used (A) and the mechanism of eluent alkalization using a cation-exchange membrane and sodium hydroxide solution (B): I = injector; P = pump; C = column; M = membrane reactor; D = detector; R = recorder.



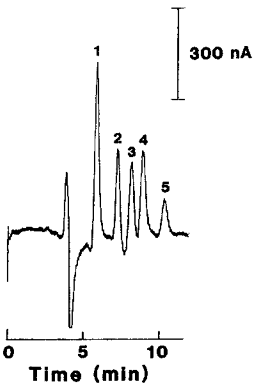


Fig. 2. Chromatogram of carbohydrates. Peaks: 1 = maltose; 2 = glucose; 3 = galactose; 4 = glycerol; 5 = mannitol; 6 = xylitol; 7 = sorbitol, each 2.0 μg/ml. HPLC conditions: column, Sugar SP1010 (30 cm × 8 mm I.D.); eluent, water; flow-rate, 1.0 ml/min; temperature, 80°C. Other conditions, see Experimental.

Fig. 3. Chromatogram of carbohydrates. Peaks: 1 = xylose; 2 = sucrose; 3 = maltose; 4 = lactose; 5 = maltotriose, each 5.0 μg/ml. HPLC conditions: column, RSpak DC-613 (15 cm × 6 mm I.D.); eluent, water–acetonitrile–methanol (3:6:1, v/v/v); flow-rate, 0.8 ml/min; temperature, 50°C. Other conditions, see Experimental.

carbohydrates. In previous papers, we reported that a hollow-fibre membrane can be used for postcolumn reactions (pH modification[5], degradation reaction[6,7] and derivatization[8]) with lower baseline noise and bandbroadening compared with the conventional postcolumn reaction using a pump(s) for delivering the reagent solution.

Fig. 1 illustrates the experimental set-up used in this study and the mechanism of eluent alkalization using a cation-exchange membrane and sodium hydroxide solution. As shown in Fig. 1B, the eluent is made alkaline by both exchange of sodium ion with hydrogen ion and permeation of hydroxide ion, which is a forbidden ion for a cation exchanger. Figs. 2 and 3 show chromatograms of carbohydrates separated on the cation-exchange columns using water and a mixture of water, acetonitrile and methanol, respectively, as eluents and detected by pulsed amperometry after modification of the eluent pH. These results reveal that the present method is useful for the detection of carbohydrates separated by using not only water but also a mixture of water and an organic modifier as an eluent. As shown in Fig. 3, in the presence of an organic modifier, pulsed amperometric detection is also possible. This suggests that the present method can be extended to the detection of various CHOH-bearing compounds separated by various modes.

Precision, linearity and detection limits

The precision of the present method is as follows: method A, 1.2–3.8% (*n* = 5) at each carbohydrate concentration of 2.0 μg/ml; method B, 3.2–5.0% (*n* = 5) at each carbohydrate concentration of 5.0 μg/ml. The calibration graphs of peak height *versus* concentration, over the ranges between 0.2 and 50 μg/ml (method A) and 0.5 and 25 μg/ml (method B), were linear with a correlation coefficient of $\geqslant 0.995$. The detection limits were *ca.* 10 pmol in method A and *ca.* 50 pmol in method B at a signal-to-noise ratio of 3.

We are now investigating the application of this method to the detection of carbohydrates using gradient elution and to the detection of CHOH-bearing compounds.

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