ELSEVIER

Journal of Chromatography A, 887 (2000) 287–297

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Capillary electrochromatography with novel stationary phases IV. Retention behavior of glycosphingolipids on porous and non-porous octadecyl sulfonated silica

Minquan Zhang[a], Gary K. Ostrander[b], Ziad El Rassi[a,*]

[a]*Department of Chemistry, Oklahoma State University, Stillwater, OK 74078-3071, USA*

[b]*Department of Biology and Department of Pathology, Johns Hopkins University, Baltimore, MD 21218, USA*

**Abstract**

In this investigation, capillary electrochromatography (CEC) with a novel stationary phase proved useful for the separation of neutral and acidic glycosphingolipids (GSLs). Four different gangliosides, namely $G_{M1a}$, $G_{D1a}$, $G_{D1b}$ and $G_{T1b}$, served as the acidic GSLs model solutes. The following four GSLs: galactosylceramide (GalCer), lactosylceramide (LacCer), globotriaosylceramide ($Gb_3$Cer) and globotetraosylceramide ($Gb_4$Cer) served as the typical neutral GSLs. The stationary phase, octadecyl sulfonated silica (ODSS), consisted of octadecyl functions bonded to a negatively charged layer containing sulfonic acid groups. Porous and non-porous ODSS stationary phases were examined. The retention behavior of the acidic and neutral GSLs was examined over a wide range of elution conditions, including the nature of the electrolyte and organic modifier and the pH of the mobile phase. The porous ODSS stationary phase yielded the separation of the four different gangliosides using a hydro-organic eluent of moderate eluent strength whereas the non-porous ODSS stationary phase permitted the separation of the four neutral GSLs with a mobile phase of relatively high eluent strength. 

*Keywords:* Stationary phases, CEC; Retention behaviour; Silica, octadecyl sulfonated; Electrchromatography; Glycosphingolipids; Lipids

## 1. Introduction

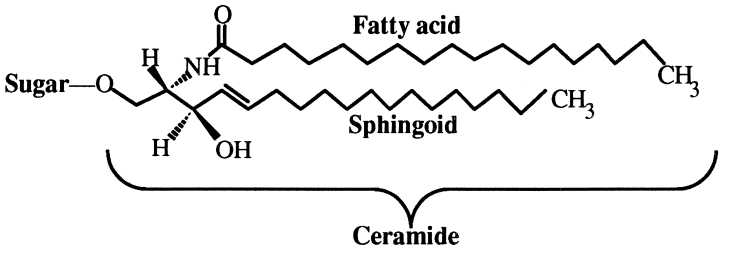
Glycosphingolipids (GSLs) are amphiphilic molecules composed of a lipophilic tail and a carbohydrate polar head group [1]. The lipophilic moiety, which is a ceramide, consists of a long chain base (sphingoid) substituted at the amino group by a fatty acid, see Fig. 1. The carbohydrate head group can vary in size from a monosaccharide to large branched structures composed of >20 monosaccharide units [2].

GSLs are found in nearly all vertebrate cells as well as in non-vertebrates such as insects and molluscs, and even plants and microorganisms [1]. In animal cells, GSLs exist mainly as components of cell surface membrane whereby their hydrophobic tail is embedded in the lipid bilayer while the sugar polar head group extends to the outside. Thus, GSLs serve as cell surface markers that can provide a sensitive indicator of the development status of a given cell. GSLs exhibit several biological properties [3] acting, among other things, as immunogens.

*Corresponding author. Tel.: +1-405-744-5931; fax: +1-405-744-6007.

*E-mail address:* zelrassi@biochem.okstate.edu (Z. El Rassi)

0021-9673/00/$ – see front matter 
PII: S0021-9673(99)01248-0




| Structure | Abbreviation | Type of GSL |
|---|---|---|
| Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer<br>3<br>↑<br>2αNeuAc | $G_{M1a}$ | Ganglioside of the ganglio series |
| Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer<br>3 3<br>↑ ↑<br>2αNeuAc 2αNeuAc | $G_{D1a}$ | Ganglioside of the ganglio series |
| Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer<br>3<br>↑<br>2αNeuAc8←2αNeuAc | $G_{D1b}$ | Ganglioside of the ganglio series |
| Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer<br>3 3<br>↑ ↑<br>2αNeuAc 2αNeuAc8←2αNeuAc | $G_{T1b}$ | Ganglioside of the ganglio series |
| Galβ1→1Cer | GalCer | Gala series |
| Galβ1→4Glcβ1→1Cer | LacCer | Lacto series |
| Galα1→4Galβ1→4Glcβ1→1Cer | $Gb_3Cer$ | Globo series |
| GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer | $Gb_4Cer$ | Globo series |

Abbreviations used: Cer, ceramide; Glc, glucose; Gal, galactose; GalNac, N-acetylgalactosamine; NeuAc, N-acetylneuraminic acid.

Fig. 1. Structures, abbreviations and types of the GSLs used in this study.

GSLs can also serve as cell receptors for bacteria toxins and possibly for bacteria and viruses. Although the biological properties of GSLs are primarily linked to their carbohydrate head group, the lipophilic moiety appears essential in strengthening the biological properties such as antigenicity, selective glycosylation, and possibly the organization and orientation of the carbohydrate chains [1]. Thus, analytical methods for the separation and determination of intact GSLs are of utmost importance.

There are two major classes of GSLs, neutral and acidic GSLs. Neutral GSLs include typically the mollu series; arthro series; gala, isogala and neogala series; ganglio series; lactosyl and glucosyl-ceramide series; globo and isoglobo series; and lacto and neolacto series. In addition, the fucolipids including the fucosylated GSLs of the globo, ganglio and lacto series are among the neutral GSLs. The gangliosides (or sialylated GSLs) derived from either the ganglio, lacto or globo series, the phospho-GSLs and the sulfated GSLs (sulfatides) belong to the class of acidic GSLs. As can be seen in Fig. 1, four acidic GSLs, namely gangliosides derived from the ganglio series and four neutral GSLs of three different series were used as model solutes in our studies.

Traditionally, thin layer chromatography [4,5], low pressure liquid chromatography [6] and high-performance liquid chromatography (HPLC) [7–9] have been the techniques of choice for the isolation and analysis of GSLs. Very recently, capillary electrophoresis (CE) has proved useful for the analysis of GSLs [10–12]. Thus far, and to the best of our knowledge capillary electrochromatography (CEC) has not been introduced yet to the analysis of GSLs despite the need for complementary techniques of unique selectivity such as CEC. As CE, CEC should be suitable for the analysis of GSLs, which are often only available in minute amounts.

Very recently, we introduced a novel silica-based stationary phase for CEC with a relatively strong electroosmotic flow (EOF) [13–15]. This stationary phase consists of a relatively hydrophilic and charged sublayer covalently attached to the silica support and a non-polar top layer of octadecyl functions chemically bonded to the sublayer. The charge in the sublayer is ensured by the permanent ionization of a strong sulfonic acid group providing a strong EOF. The stationary phase, ODSS [15], proved to be useful for the separations of some nucleosides and their bases [14], some small and large nucleotides [13]. In the present study, we report the application of the ODSS stationary phase to the separation of GSLs over a wide range of elution conditions.

## 2. Experimental

### 2.1. Materials

Nucleosil silica, with an average particle diameter of 5 μm, an average pore diameter of 120 Å and a specific surface area of 200 $m^2/g$, was obtained from Alltech Associates Inc. (Deerfield, IL, USA). Non-porous silica having 2 μm mean particle diameter with a specific surface area of less than 1 $m^2/g$ was synthesized in-house according to well established procedures [16,17]. HPLC grade acetonitrile and isopropanol were from Baxter (McGaw Park, IL, USA). HPLC grade methanol and sodium borate were obtained from EM Science (Gibbstown, NJ, USA). Sodium phosphate monobasic was obtained from Mallinckrodt (Paris, KY, USA). Monosialoganglioside ($G_{M1a}$), disialogangliosides ($G_{D1a}$, $G_{D1b}$) and trisialoganglioside ($G_{T1b}$) were obtained from Matreya (Pleasant Gap, PA, USA), and are depicted in Fig. 1. Galactosylceramide (GalCer), lactosylceramide (LacCer), globotriaosylceramide ($Gb_3Cer$) and globotetraosylceramide ($Gb_4Cer$) were isolated and purified in our laboratory (G.K. Ostrander), and are illustrated in Fig. 1. The fused-silica capillary columns (100 μm I.D.×360 μm O.D.) were from Polymicro Technologies (Phoenix, AZ, USA).

### 2.2. Instruments

The instrument for CEC was a P/ACE 5010 capillary electrophoresis system from Beckman Instrument Inc. (Fullerton, CA, USA) equipped with a UV detector and a data handling system comprised of an IBM personal computer and P/ACE software. A Shandon column packer from Keystone Scientific (Bellefonte, PA, USA) was used for capillary column packing.

### 2.3. Stationary phases

The 5 μm porous ODSS and 2 μm non-porous ODSS stationary phases were made in a three-step process described previously [13–15] using 5 μm Nucleosil silica, and 2 μm in-house synthesized silica, respectively. The stationary phases comprised a sulfonated, relatively hydrophilic sublayer and an octadecyl retentive top layer.

### 2.4. Methods and procedures

#### 2.4.1. Capillary column packing

The capillary column preparation was described in details in recent works from our laboratories [13–15,18]. Briefly, fused-silica capillaries (360 μm O.D.×100 μm I.D.) were packed with the ODSS stationary phases using the pressure slurry packing technique. Acetone was used to prepare the suspension of the ODSS stationary phase, and isopropanol was used as the packing solvent. A moderately strong sintered porous frit was made at the outlet of the fused-silica capillary by first tapping the capillary end into bare 5 μm silica moistened with deionized water, a step that filled a 0.5–1.0 mm segment at the tip of the capillary, and second by heating the capillary tip over a Bunsen burner for about 1 min. Then the capillary column was packed with ODSS stationary phase, followed by flushing the column with deionized water for about 30–60 min, and cutting it to the desired length. The inlet retaining frit was made in the same way as the outlet frit. At last, the column was washed with acetonitrile and mounted to a Beckman capillary cartridge. In our work, whole packed capillary columns were produced with a detection window at 6.5 cm from the outlet end of the column, which was made before packing by burning off the polyimide coating of the capillary with a thermal wire stripper. This column configuration gives an even conductance along the capillary axis and a stable EOF profile, resulting in much reduced bubble formation.

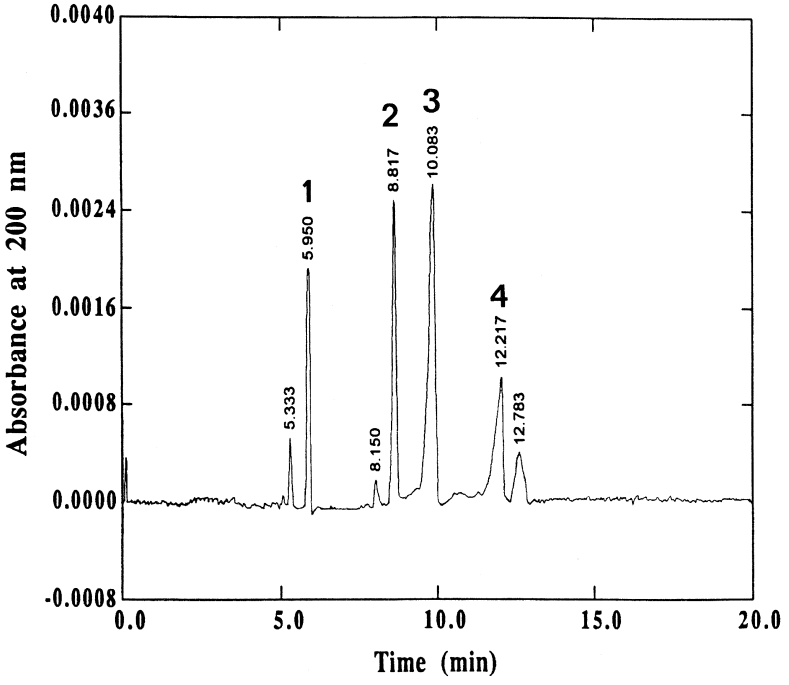



Fig. 2. Electrochromatogram of four gangliosides obtained on porous ODSS stationary phase. Capillary column: packed with 5 μm ODSS stationary phase, 20.5/27 cm×100 μm I.D.; mobile phase: hydro-organic eluent containing 10 m*M* sodium borate and composed of acetonitrile–methanol–aqueous sodium borate, pH 9.40, 30:50:20 (v/v); running voltage: 20 kV; electrokinetic injection: 1 kV for 2 s; solute order: (1) $G_{M1a}$, (2) $G_{D1a}$, (3) $G_{D1b}$, (4) $G_{T1b}$.

*2.4.2. Capillary electrochromatographic preconditioning*

Before the CEC runs, the packed capillary column mounted into the capillary cartridge was equilibrated with acetonitrile at 2 kV for a period of 40 min, followed by equilibration with a running mobile phase of chosen composition at 4 kV for about 30–60 min until the detector output was constant.

## 3. Results and discussion

### *3.1. CEC of Gangliosides*

#### *3.1.1. Complexing mobile phase*

In aqueous solutions, gangliosides form stable micelles with critical micellar concentration (CMC) in the range of $10^{-10}$–$10^{-8}$ *M* [19,20]. This fact requires mobile phase conditions in CEC that ensure the separation of the gangliosides as monomers. Reducing the sample concentration to below the CMC poses a detection problem because the CMC is far below the limit of detection of gangliosides in the UV. In CE, the problem of self-aggregation of gangliosides was circumvented by incorporating in the running electrolyte organic solvents or additives such as acetonitrile or cyclodextrins [11,12].

Fig. 2 shows the isocratic separation of the four gangliosides obtained on the 5 μm porous ODSS stationary phase with a hydro-organic mobile phase containing 10 m*M* sodium borate and composed of sodium borate buffer, pH 9.40–methanol–acetonitrile, 20:50:30 (v/v). This represents the optimum separation conditions as far as the pH, borate concentration and organic modifier content are concerned. The tetrahydroxyborate ion, which is the predominant ionic species at alkaline pH, is known to complex with the vicinal diols of sugars [21], thus

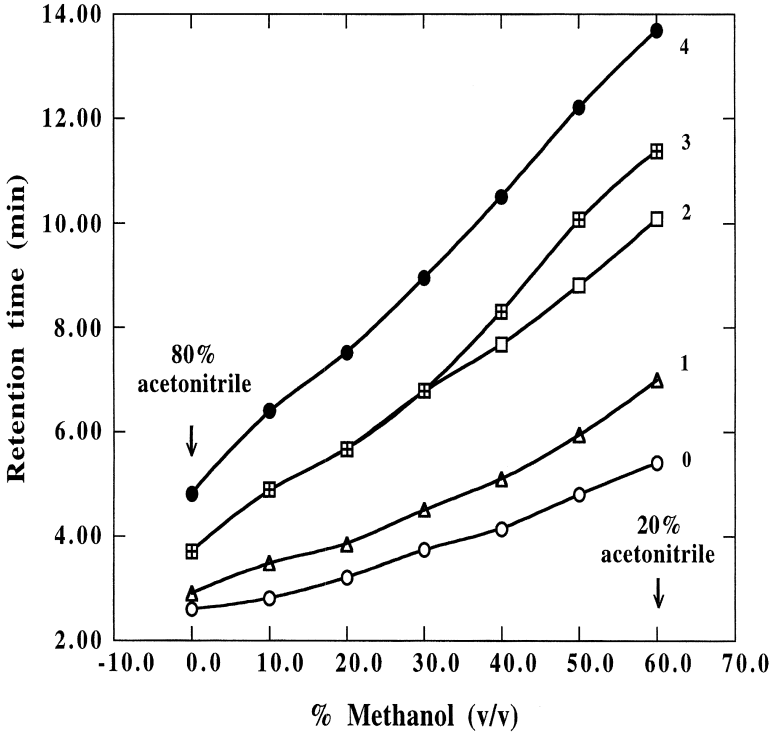



Fig. 3. Effect of the organic modifier on the retention time of gangliosides. Mobile phase, hydro-organic eluent containing 10 m*M* sodium borate and composed of organic modifier–aqueous sodium borate, 80:20 (v/v). The amount of methanol was increased from 0 to 60% while that of acetonitrile was decreased from 80 to 20% (v/v). Other conditions as in Fig. 2. Curves: 0, EOF marker: (1) $G_{M1a}$, (2) $G_{D1a}$, (3) $G_{D1b}$, (4) $G_{T1b}$.

modifying the ionic character of the sugar head group of the gangliosides. While acetonitrile was used to allow the elution of the gangliosides and to eliminate the aggregation of the gangliosides, methanol was added to the mobile phase to primarily adjust the selectivity of the system, and also to bring about elution. When only acetonitrile was added to the mobile phase, the gangliosides could not be fully separated, and only three different peaks were detected. $G_{D1a}$ and $G_{D1b}$ co-eluted over a wide range of acetonitrile concentration in the mobile phase (for up to 90%). The nature of the organic modifier had significant influence on the electrochromatographic behavior of gangliosides. As shown in Fig. 3, successful separation of the mixture of gangliosides depended to a large extent on the methanol content of the mobile phase. In this figure, the mobile phase consisted of 20% aqueous sodium borate and a total of 80% methanol and acetonitrile. A 30% (v/v) acetonitrile content seemed to be the optimum amount as far as the retention time and the disaggregation of gangliosides are concerned and in turn the peak sharpness, see Fig. 2. Increasing the methanol content from 0 to 60% (v/v) resulted in a three-fold increase in the retention time of all the gangliosides. The eluent strength of methanol is weaker than that of acetonitrile, and also hydro-organic mobile phases containing methanol are more viscous than those containing acetonitrile [22]. Only when the methanol content is increased to 30–40%, can the isomers $G_{D1a}$ and $G_{D1b}$ be separated. When the methanol content reached 50%, an optimal separation of the gangliosides was obtained.

From Fig. 2, one can see that besides the main four gangliosides, which were well separated within 7.5 min, each of the commercial gangliosides yielded one or more small peaks in the capillary electrochromatogram. This may reflect a microheterogeneity in the ceramide portion of the gangliosides. To get enough resolution for all the gangliosides and

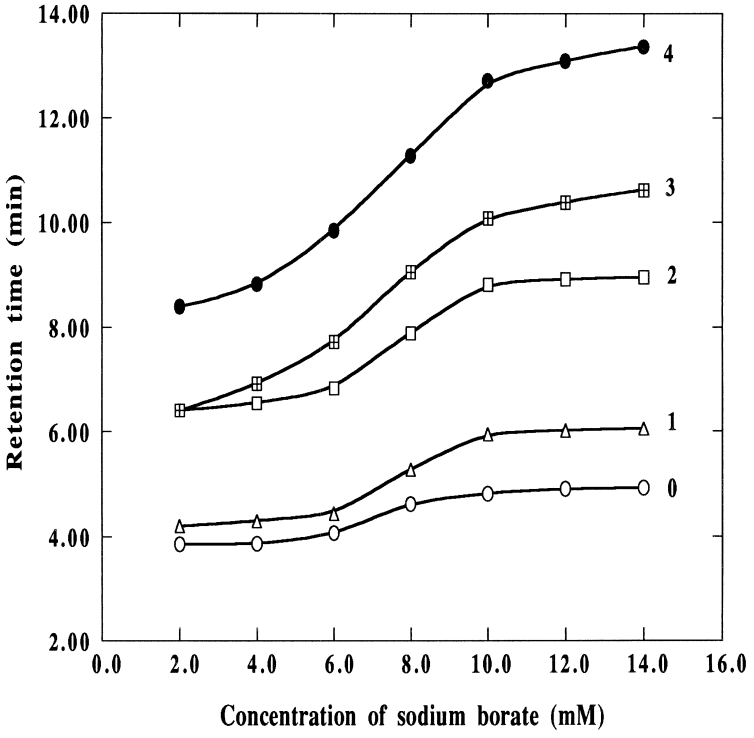



Fig. 4. Effect of the concentration of sodium borate on the retention time of gangliosides and the EOF marker. The system peak produced by injecting pure acetonitrile was taken as the EOF marker. Other conditions as in Fig. 2. Curves: 0, EOF marker: (1) $G_{M1a}$, (2) $G_{D1a}$: (3) $G_{D1b}$, (4) $G_{T1b}$.

their associated small peaks, 10 m*M* sodium borate in the mobile phase was needed. The microheterogeneity was not observed at a borate concentration of 8 m*M* or less. The lipophilic ceramide portion shows microheterogeneity with regard to the sphingoid and the fatty acid composition [23]. In fact, it has been reported that with increasing sialic acid content brain gangliosides show an increasing proportion of $C_{20:1}$ eicosasphing-4-enine over $C_{18:1}$ eicosasphing-4-enine. Microheterogeneity in gangliosides can also be attributed to the sialic acid moiety. Although the predominant species is *N*-acetylneuraminic acid (NeuAc) as the sialic acid moiety, brain gangliosides may also contain *N*,*O*-diacetylneuraminic acid and trace amounts of *N*-glycolylneuraminic acid [23].

Fig. 4 shows the effect of borate concentration on the retention time of gangliosides. The retention time of the gangliosides and that of the EOF marker increased as the borate concentration increased. Increasing the ionic strength of the running electrolyte decreased the EOF while increased the amount of ganglioside–borate complex. The net result was an increase in the migration time of the gangliosides. At a borate concentration of lower than 2 m*M*, the separation of isomers $G_{D1a}$ and $G_{D1b}$ was not possible. When the concentration reached 6 m*M*, the four gangliosides $G_{M1a}$, $G_{D1a}$, $G_{D1b}$ and $G_{T1b}$ were well separated.

The migration times of all gangliosides decreased as the pH of the borate-based mobile phase increased from 8.0 to 10.0. This is due to the stronger complexation with borate at higher pH values. In reversed-phase chromatography, increasing the charge on the solute decreases its retention. This may indicate that the retention of the gangliosides on the ODSS stationary phase is a more significant factor in controlling the migration of the gangliosides than their electrophoretic mobility. It should be noted that in the pH range 8.0–10.0, the EOF did not change significantly, and, consequently, its effect on solute migration could be neglected. The optimal pH for maximum resolution of all the gangliosides was found to be 9.4. Increasing the pH further did not decrease the migration time substantially. In contrast, decreasing the pH of the mobile phase from 9.4 to 8.0 not only increased the retention time but also

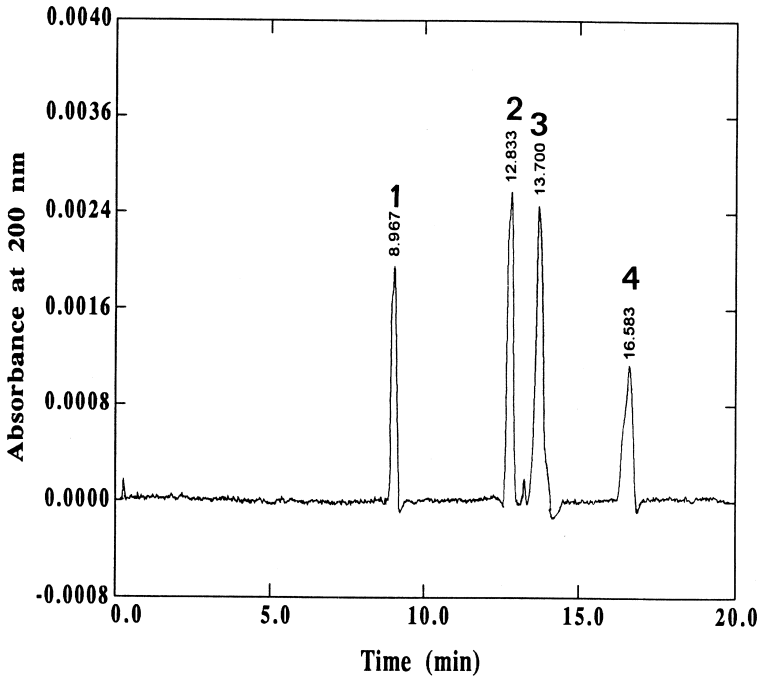



Fig. 5. Eectrochromatogram of gangliosides obtained on porous ODSS stationary phase. Mobile phase as in as Fig. 2 but containing 10 m*M* potassium phosphate, pH 6.00. Other conditions as in Fig. 2. Solute order: (1) $G_{M1a}$, (2) $G_{D1a}$, (3) $G_{D1b}$, (4) $G_{T1b}$.

broadened the elution peaks, which resulted in the poor resolution of gangliosides.

### *3.1.2. Non-complexing mobile phase*

In order to better understand the electrochromatographic behavior of the gangliosides, and to provide other useful mobile phases for their separation, potassium phosphate was also used as the electrolyte. For comparison with the sodium borate-based mobile phase, the experiments were performed under otherwise identical conditions. As can be seen in Fig. 5, the four gangliosides were all separated. However, the retention times increased by 1.5-fold, and the gangliosides did not yield small peaks in the electrochromatogram, which are believed to arise from the microheterogeneity of the gangliosides.

The pH value of the running buffer was also found to influence the electrochromatographic behavior of gangliosides in this kind of buffer system. Increasing the pH of the running buffer resulted in the decrease of the retention time of the gangliosides. The retention time of all gangliosides decreased first drastically and then slowly as the pH increased first from 5.0 to 7.0 and then from 7.0 to 10.0. This behavior parallels that of the EOF. In other words, the EOF increased from 0.5 to 0.8 mm/s (60% increase) as the pH increased from 5.0 to 7.0, while the EOF increased from 0.8 mm/s to 1.1 mm/s when going from pH 7.0 to 10.0 (an increase of 37%).

## *3.2. CEC of Neutral GSLs*

### *3.2.1. Non-complexing mobile phase*

Similarly to gangliosides, the neutral GSLs could not be separated as monomers in aqueous media. Unlike the gangliosides, the neutral GSLs could not be eluted from the porous ODSS stationary phase over a wide range of mobile phase composition. Going to non-porous ODSS, the phase ratio was reduced by a factor greater than 40, a condition that allowed the elution and separation of the neutral GSLs.

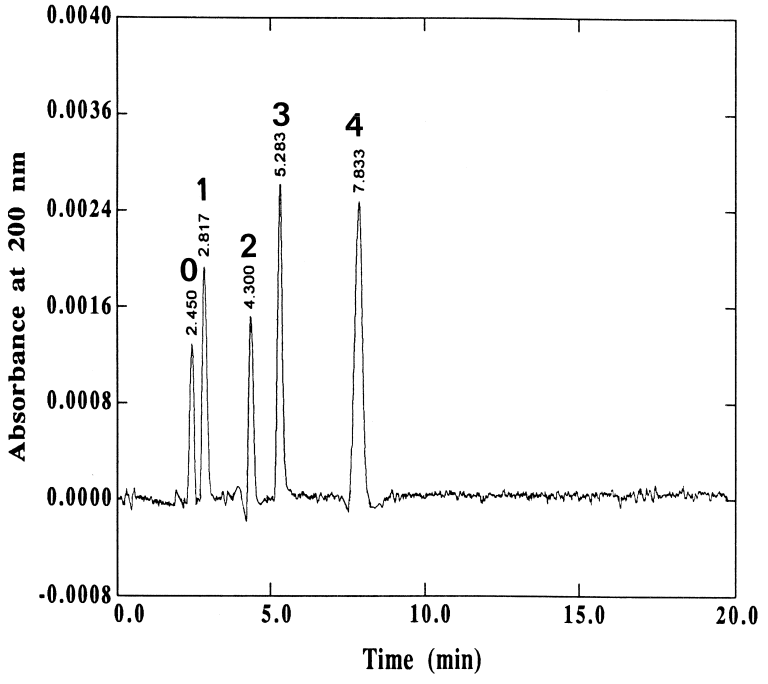



Fig. 6. Electrochromatogram of some neutral GSLs obtained on non-porous ODSS stationary phase. Capillary column: packed with 2 μm ODSS stationary phase, 20.5/27 cm×100 μm I.D.; mobile phase: hydro-organic eluent containing 2 m*M* ammonium phosphate and composed of tetrahydrofuran–aqueous ammonium phosphate, pH 7.00, 80:20 (v/v); running voltage: 20 kV; electrokinetic injection: 1 kV for 2 s; peak order: 0, tetrahydrofuran as a EOF marker: (1) GalCer, (2) LacCer, (3) $Gb_3Cer$, (4) $Gb_4Cer$.

Neither methanol nor acetonitrile allowed the solubilization of the GSLs, which excluded the use of a mobile phase containing methanol and/or acetonitrile. On the other hand, tetrahydrofuran (THF) was a good solvent for the neutral GSLs. Fig. 6 shows the separation of the four neutral GSLs with a mobile phase consisting of 80% (v/v) THF and 20% (v/v) aqueous solution containing 2 m*M* ammonium phosphate, pH 7.0. These are the optimum conditions for the separation of the four GSLs, namely, GalCer, LacCer, $Gb_3Cer$ and $Gb_4Cer$, and was achieved in about 8 min.

The amount of ammonium phosphate added to the mobile phase influenced the retention time, see Fig. 7. The increase in retention time upon increasing the ammonium phosphate concentration is primarily the result of decreasing the EOF velocity. As expected, in the ammonium phosphate concentration range studied, the retention factor, $k'$, of the neutral GSLs remained about the same. The observed insignificant fluctuations in the $k'$ values of the neutral GSLs were within the experimental errors (results not shown).

Since the four neutral GSLs were not soluble at all in aqueous solutions, it was imperative to investigate the pure organic mobile phase. Under the identical conditions described in the preceding section, but substituting the 20% aqueous with 20% (v/v) methanol and replacing ammonium phosphate with ammonium acetate, resulted in increasing the migration time of the four neutral solutes, see Fig. 8. Ammonium acetate was used instead of ammonium phosphate because the latter is not soluble in the non-aqueous mobile phase. When the methanol content of the mobile phase was increased from 20 to 50% while that of THF was decreased from 80 to 50%, the retention time increased simply because (i) methanol is a weaker eluent than THF [22] and (ii) the magnitude of the EOF decreased with increasing the methanol content of the mobile phase, due to the fact that methanol is more viscous that THF [22].

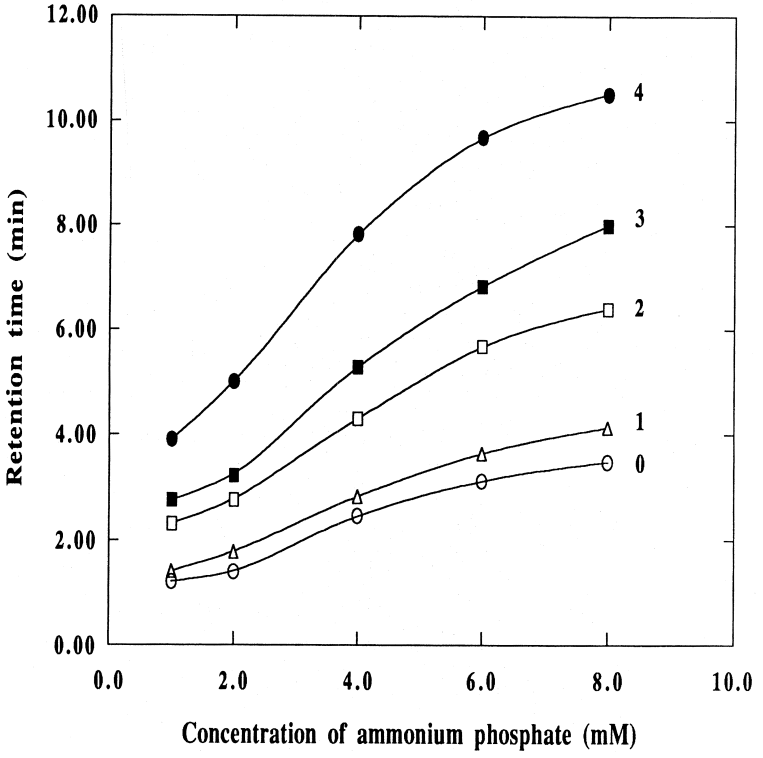



Fig. 7. Effect of the concentration of ammonium phosphate on the retention time of some GSLs and the EOF marker on the non-porous ODSS column. Other conditions as in Fig. 6. Curves: 0; system peak as the EOF marker: (1) GalCer, (2) LacCer, (3) $Gb_3Cer$, (4) $Gb_4Cer$.

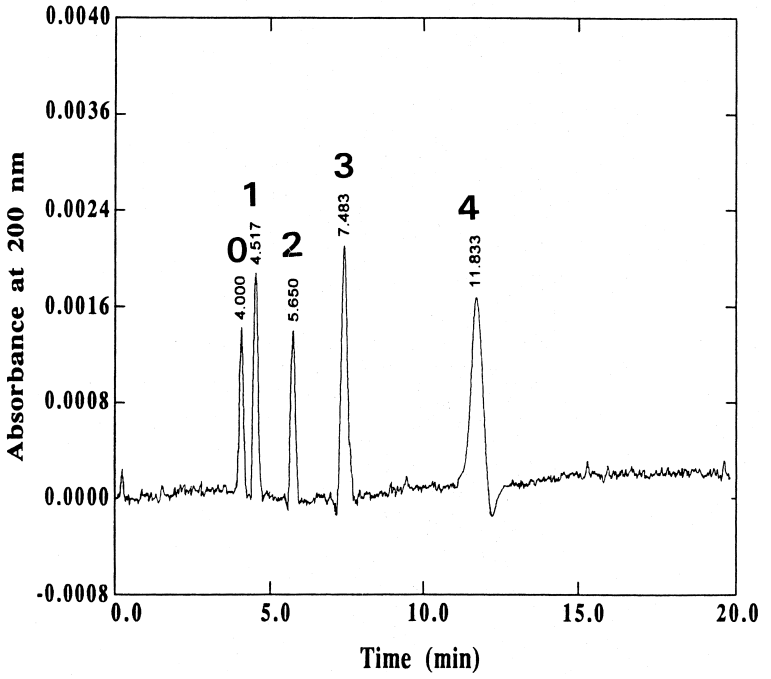



Fig. 8. Electrochromatogram of some GSLs obtained on non-porous ODSS stationary phase. Non-aqueous mobile phase, containing 2 m*M* ammonium acetate and composed of tetrahydrofuran–methanol 80:20 (v/v). Other conditions as in Fig. 6. Peak order: 0; tetrahydrofuran as an EOF marker: (1) GalCer, (2) LacCer, (3) $Gb_3Cer$, (4) $Gb_4Cer$.

*3.2.2. Complexing mobile phase*

Fig. 9 shows the separation of the four neutral GSLs in the presence of a complexing mobile phase. In this case, the ammonium phosphate was replaced by sodium borate and the pH was raised from 7.0 to 9.0 to ensure the complexation of borate with the sugar head group of the GSLs. As can be seen in Fig. 9, the four GSLs were well separated but the retention time increased by a factor of 1.24 while the retention time span for the four neutral GSLs decreased by a factor of 0.65. With a mobile phase of 80% (v/v) THF and 20% (v/v) aqueous, substituting ammonium phosphate with sodium borate decreased the EOF from 1.39 to 0.57 mm/s.

## 4. Conclusions

The ODSS stationary phase proved very suitable for the separation of acidic and neutral GSLs. Optimization of the chromatographic conditions, such as the type of electrolyte and its concentration, the pH of the mobile phase, the type of organic modifier and its amount, etc., resulted in successful separation of various GSLs. The CEC separation of the neutral GSLs required a non-porous ODSS stationary phase and a mobile phase of high eluent strength while the CEC separation of the gangliosides was readily achieved on a porous ODSS stationary phase with a mobile phase of moderate eluent strength.

## Acknowledgements

ZER gratefully acknowledge the financial support by the Cooperation State Research, Education and Extension Service, US Department of Agriculture under Agreement No. 96-35201-3342. GKO was funded by an NIH grant No. CA54590.

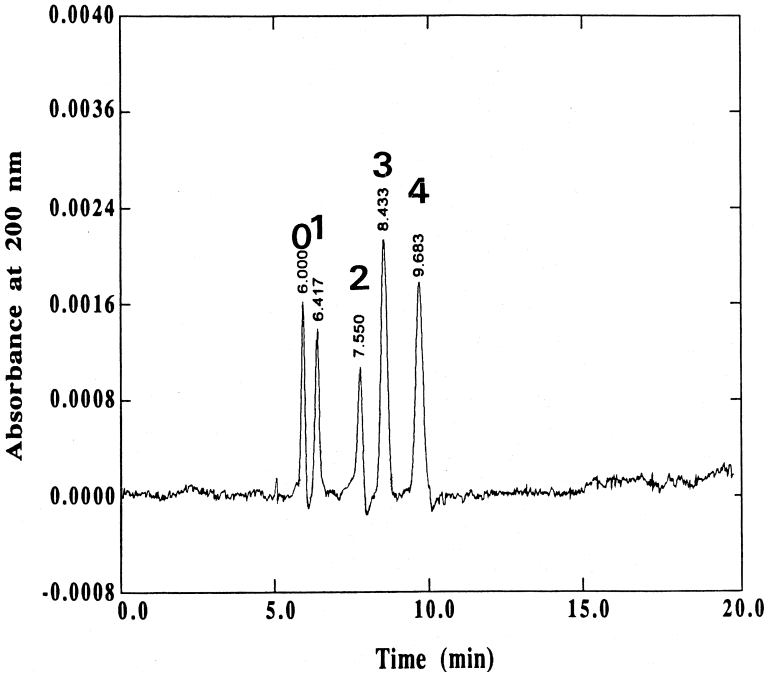



Fig. 9. Electrochromatogram of some glyco-sphingolipids obtained on non-porous ODSS stationary phase. Mobile phase: hydro-organic eluent containing 2 m*M* sodium borate and composed of tetrahydrofuran–aqueous sodium borate, pH 9.00, 80:20 (v/v); other condition as in Fig. 6; peak order: 0; tetrahydrofuran as an EOF marker: (1) GalCer, (2) LacCer, (3) $Gb_3$Cer, (4) $Gb_4$Cer.

## References

[1] A. Makita, N. Taniguchi, in: H. Weigandt (Ed.), Glycolipids, Elsevier, Amsterdam, 1985, p. 1.
[2] C.L.M. Stults, C.C. Sweeley, B.A. Macher, Methods Enzymol. 179 (1989) 167.
[3] R.M. Bell, A.H. Merrill, Y.A. Hannun, Functions and breakdown products, in: R.M. Bell, A.H. Merrill, Y.A. Hannun (Eds.), Sphingolipids, Academic Press, Inc, San Diego, 1993.
[4] L. Svennerholm, Neurochem. 12 (1963) 613.
[5] R.L. Schnaar, L.K. Needham, Methods Enzymol. 230 (1994) 371.
[6] R.L. Schnaar, Methods Enzymol. 230 (1994) 348.
[7] T. Traylar, D. Koontz, E. Hogan, J. Chromatogr. 272 (1983) 9.
[8] M.D. Ullman, R.H. McCluer, J. Lipid Res. 26 (1985) 501.
[9] A. Suziki, S.K. Kundu, D.M. Marcus, J. Lipid Res. 21 (1980) 473.
[10] Y. Liu, K.-F.J. Chan, Electrophoresis 12 (1991) 402.
[11] Y. Mechref, G.K. Ostrander, Z. El Rassi, J. Chromatogr. A 695 (1995) 83.
[12] Y.S. Yoo, Y.S. Kim, G.-J. Jhon, J. Paark, J. Chromatogr. A 652 (1993) 431.
[13] M. Zhang, C. Yang, Z. El Rassi, Anal. Chem. 71 (1999) 3277.
[14] M. Zhang, Z. El Rassi, Electrophoresis 20 (1999) 31.
[15] M. Zhang, Z. El Rassi, Electrophoresis 19 (1998) 2068.
[16] J. Yu, Z. El Rassi, J. Liq. Chrom. Rel. Technol. 20 (1997) 183.
[17] J. Yu, Z. El Rassi, J. Liq. Chromatogr. 16 (1993) 2931.
[18] C. Yang, Z. El Rassi, Electrophoresis 19 (1998) 2061.
[19] H. Nakalayashi, M. Iwamori, Y. Naga, J. Biochem. 96 (1984) 977.
[20] B. Ulrich-Bott, H. Wiegandt, J. Lip. Res. 25 (1984) 1233.
[21] Z. El Rassi, W. Nashabeh, in: Z. El Rassi (Ed.), Carbohydrate Analysis: High-Performance Liquid Chromatography and Capillary Electrophoresis, Elsevier, Amsterdam, 1995, p. 267.
[22] W.R. Melander, C. Horváth, in: Cs. Horváth (Ed.), High-Performance Liquid Chromatography, Academic Press, New York, 1980, p. 113.
[23] H. Wiegandt, in: H. Wiegandt (Ed.), Glycolipids, Elsevier, Amsterdam, 1985, p. 199.