methods

Recovery of gangliosides from aqueous solutions on styrene-divinylbenzene copolymer columns[1]

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Abstract **An investigation was made using a styrene-divinylbenzene copolymer as a solid phase sorbent to recover gangliosides from aqueous solutions. A comparison with octadecyl-bonded (C18) silica gel showed that the general procedure used to purify gangliosides on C18 silica gel could be used with the copolymer. The yield of gangliosides depended on various parameters such as the composition of the conditioning solution, the salt concentration of the loading solution, and the amount of applied gangliosides per gram of copolymer. In optimal conditions, the recovery of gangliosides and other lipids present in the upper phases of partition was higher than 95%. Using radiolabeled gangliosides, it was found that gangliosides present in serum-containing medium could also be quantitatively recovered on copolymer, provided the medium was diluted with an equal volume of methanol prior to its application onto the column. The major advantage of the copolymer is its high stability in acidic or alkaline conditions that allows multiple cycles of cleaning and reconditioning of the sorbent without alteration of its chromatographic properties.**—Popa, I., C. Vlad, J. Bodennec, and J. Portoukalian. **Recovery of gangliosides from aqueous solutions on styrene-divinylbenzene copolymer columns.** ***J. Lipid Res.*** **2002.** 43: **1335–1340.**

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Solid phase extraction (SPE) is a method for the concentration and purification of analytes from solutions by adsorption onto a solid-phase cartridge followed by elution of analytes with an appropriate solvent (1–2). Retention and elution depend on interactions of the analyte with the solid and liquid phases. The mechanisms of retention include normal and reversed-phase and ion-exchange modes. The SPE procedure begins by conditioning the sorbent in order to remove impurities and moisten the surface functional groups. The sample is then loaded onto the SPE sorbent and the column is rinsed to remove the components that have not been adsorbed.

In order to purify gangliosides from tissues, the classical method (3) involves a lipid extraction with chloroform-methanol followed by a partition yielding a ganglioside-enriched aqueous phase. The recovery of the gangliosides from the aqueous phase can be achieved by reverse-phase chromatography on octadecyl-bonded (C18) silica gel (4). When a total ganglioside fraction is separated into individual species by anion-exchange column chromatography on DEAE-Sephadex (5) or MonoQ column (6). Using increasing concentrations of salts in methanol, the eluting solvent must be desalted, and C18 silica gel is well suited for this purpose.

In the present study, the purification of gangliosides was carried out on columns of a resin made of small, non-ionic, highly crosslinked styrene-divinylbenzene copolymer beads. Copolymers have not been used so far to purify gangliosides, although it has been reported that glycolipids can be adsorbed on the surface of polystyrene beads (7–8).

In order to define the optimal conditions of ganglioside recovery after adsorption on the sorbent, several parameters were studied, such as the conditioning cycle, the composition of the solution used to apply gangliosides on the column, the composition of the elution solution, the salt concentration, the dilution of applied radioactive gangliosides, and the nature of the sorbent.

MATERIALS AND METHODS

Total gangliosides were purified from bovine brain and separated according to their ionic charge by ion-exchange chromatography on DEAE-Sephadex (5). Individual species were isolated by HPLC on silica gel LiChrospher Si100 (5 μm) columns using a Hitachi L-6200 apparatus (Merck, Paris, France) with a

Abbreviations: C18 silica gel, octadecylsilyl-bonded silica gel; SPE, solid phase extraction; TUP, theoretical upper phase of partition.

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linear gradient of isopropanol-hexane-water from 55:33:12 to 55:29:16 (v/v/v). Gangliosides were assayed by the periodate-resorcinol method (9). GM3 was prepared from pure GM1 by lactonisation followed by acidic treatment according to Mauri et al. (10). AsialoGM1 was obtained by mild acid treatment of GM1 with 1 N formic acid at 80°C, 1 h (evaporation and purification by HPLC as described above).

GM3 and GT1b were labeled, as described by Rebbaa and Portoukalian (11), by *N*-deacetylation and reacetylation with [^{3}H]acetic anhydride (specific activity 20 Ci/mmol; Isobio, Fleurus, Belgium). AsialoGM1 was labeled with the same method using [1-^{14}C]acetic anhydride (specific activity 60 mCi/mmole; Isobio, Fleurus, Belgium). GM1 was labeled with tritiated sodium borohydride (specific activity 15 mCi/mmole; Isotopchim, Peyruis, France) using palladium acetate as a catalyst according to Schwarzmann (12). Phosphatidylcholine (dioleoyl-1-^{14}C) (specific activity 100 mCi/mmole) and [4-^{14}C]cholesterol (specific activity 50 mCi/mmole) were from Isobio, Fleurus, Belgium.

The resin made of small, nonionic, highly crosslinked styrene-divinylbenzene copolymer beads, with a particle size of 80–160 microns, a surface area of 620 m^2/g, and a mean pore size of 100–315 Å was produced at the Institute of Macromolecular Chemistry Petru Poni, Iassy 6600, Romania (13).

Before applying the gangliosides, the copolymer was preconditioned in various conditions. To optimize the results, the following conditioning procedures were tested: water; methanol-water, 1:1 (v/v); aqueous 0.015 M NaCl; methanol-0.015 M NaCl, 1:1 (v/v); and aqueous 0.15 M NaCl.

The gangliosides were applied at a flow rate of 3 ml per min on the columns in one of the following solutions: water; aqueous 0.015 M NaCl; aqueous 0.15 M NaCl; methanol; methanol-water, 1:1 (v/v); methanol-0.015 M NaCl, 80:20 (v/v); methanol-0.015 M NaCl, 60:40 (v/v); methanol-0.015 M NaCl, 40:60 (v/v); or theoretical upper phase of partition (methanol-aqueous 0.015 M NaCl-chloroform, 48:47:3, by volume).

After loading the gangliosides, the columns were washed with distilled water that was collected and evaporated to see to what extent the washing of non-lipidic contaminants could result in the loss of some gangliosides. The gangliosides were then eluted in methanol and chloroform-methanol, 1:1 (v/v). The pooled eluates were dried under nitrogen and analyzed by thin-layer chromatography (TLC). The fractions were spotted onto HPTLC silica gel 60 plates (Merck, Darmstadt, Germany) developed in three different solvent systems: solvent A [chloroform-methanol-0.2% aqueous calcium chloride, 60:35:8 (by volume)]; solvent B [chloroform-methanol-water-28% ammonia, 60:35:7:1 (by volume)]; solvent C [methyl acetate-N-propanol-chloroform-methanol-0.25% aqueous KCl, 25:20:20:20:17 (by volume)] (14). The gangliosides were visualized with the resorcinol-HCl spray reagent specific for sialic acid (15). The patterns of gangliosides separated by TLC on HPTLC silica gel 60 plates were determined by scanning densitometry using a dual-wavelength Chromatoscan CS-930 (Shimadzu, Kyoto, Japan) set at 630 nm.

The distribution of gangliosides was determined both in the filtrate (i.e., the unbound fraction passing through the column after sample application), the aqueous washing, and the lipid fraction eluted with methanol and methanol-chloroform, 1:1 (v/v).

The influence of the ratio methanol-water and of the salt concentration (aqueous 0.01 M to 0.15 M NaCl) on the elution of gangliosides in the different fractions was evaluated.

The gangliosides were applied on copolymer columns in a theoretical upper phase of partition (TUP) made of chloroform-methanol-aqueous 0.15 M NaCl, 3:48:47 (by volume). The fractions were then collected as above described.

The recovery of different radioactive lipids from 5 ml TUP was tested on 8 ml columns prepared with either copolymer or C18 silica gel. The amount of lipids was, respectively, 5 μg [^{3}H]GM3 (specific activity 5,000 dpm/μg), 5 μg [^{3}H]GM1 (specific activity 15,000 dpm/μg), 5 μg [^{3}H]GT1b (specific activity 20,000 dpm/μg), 5 μg [^{14}C]phosphatidylcholine (specific activity of 8,000 dpm/μg), 5 μg [^{14}C]asialoGM1 (specific activity 5,000 dpm/μg), and 5 μg [^{14}C]cholesterol (specific activity 5,000 dpm/μg). The samples were eluted as described above, and 5% of each collected sample was evaporated in a vial, taken up in 1 ml methanol, and mixed with 5 ml of scintillation fluid (Beckman, Paris, France). The radioactivity was counted in a β-counter (Packard, Paris, France). Each condition was assessed with triplicate samples.

In order to determine the maximal capacity of the copolymer sorbent, samples of 5 μg (75,000 dpm) of [^{3}H]GM1 were mixed with a range of 0.1 to 7.5 mg of polysialogangliosides (total gangliosides minus monosialogangliosides) and applied on 1 ml columns as described above.

To determine whether gangliosides adsorbed on proteins can be recovered, a study was made with [^{3}H]GM1 taken up in increasing volumes of culture medium RPMI 1640 containing 10% fetal calf serum. The ganglioside-containing medium was kept for 1 hour at room temperature then applied on 1 ml and 2 ml columns packed with copolymer or with C18 silica gel.

RESULTS AND DISCUSSION

Before applying the gangliosides, the copolymer columns (1 ml vol) were conditioned with various solutions as detailed in **Table 1** and the proportions of retained gangliosides from a sample of 0.2 mg of tritiated GM1 were determined. The presence of both salts and methanol in the conditioning solution seems necessary to ensure a good retention of the gangliosides upon application and the best result was obtained with methanol-0.15 M NaCl, 1:1 (v/v). After loading gangliosides onto the column, the copolymer was washed with distilled water to remove salts and any other non-lipidic contaminants.

The distribution of radioactive GM1 in the eluted fractions was studied with gangliosides loaded in one of the following solutions: water; aqueous 0.15 M NaCl; methanol; water-methanol 1:1 (v/v); aqueous 0.015 M NaCl-methanol 20:80 (v/v), 40:60 (v/v), 50:50 (v/v), or 60:40 (v/v); and a TUP made of aqueous 0.015 M NaCl-methanol-chloroform, 47:48:3 (v/v/v). In **Table 2**, the results based on the surface areas of the peaks obtained by scanning densitometry of HPTLC plates are compared. When the copolymer column was loaded with gangliosides taken up in water, only a few percent of the gangliosides were retained onto the column, as already reported using C18 silica gel (4). An aqueous solution with salts, even at a concentration as low as 0.015 M, strikingly increased the retention rate on the copolymer, but part of the gangliosides were then washed out with water. The presence of methanol in the loading solution enhanced the retention when methanol was added up to a maximum of 50%. Above this proportion, a significant loss of gangliosides occurred. However, once loaded onto the column in an appropriate solution such as TUP, the retained gangliosides were not displaced by washing with water.

By assessing the influence of the ratio water-methanol

TABLE 1. Influence of the solution used for conditioning 1 ml copolymer columns before applying 0.2 mg tritiated GM1 in 1 ml TUP and washing with water

Conditioning Solution	Gangliosides Retained
	%
Water	28.4 ± 4.7
Methanol-water, 1:1 (v/v)	43.6 ± 5.5
Aqueous 0.015 M NaCl	52.4 ± 4.4
Methanol-0.015 M NaCl, 1:1 (v/v)	89.6 ± 4.1
Aqueous 0.15 M NaCl	63.9 ± 3.8
Methanol-0.15 M NaCl, 1:1 (v/v)	91.2 ± 4.3

Data are from five different experiments.
Results are mean ± SD.

and of the salts, the best recovery (above 95%) was obtained by applying the gangliosides in a solution of methanol-0.01 M NaCl, 40:60 (v/v). A slightly lower percentage of recovery was obtained when the gangliosides were applied in a theoretical upper phase of partition.

Table 3 shows that all lipids in aqueous solutions are adsorbed on the copolymer as well as on C18 silica gel. The presence of other lipids besides gangliosides in upper phases of partition is well known (4) and a solvent system selectively eluting gangliosides from the columns would be of interest. In our study, several solvent systems were tried to elute gangliosides separately from other lipids loaded on the columns, but none of these solvents gave satisfactory results.

In order to determine the maximal capacity of the copolymer sorbent, samples of 5 μg (25,000 dpm) of [^{3}H]GM1 were mixed with 0.1 mg to 15 mg of GM1 or other lipids and applied on 1 ml copolymer columns as described above. As shown in **Table 4**, the recovery of [^{3}H]GM1 was in the range of 85–95% with up to 5 mg lipids applied to the 1 ml copolymer column. The recovery decreased sharply above the 5 mg that seems to be the saturation level for 1 ml of copolymer loaded with gangliosides. The very low recovery above this amount can be explained by the competition for adsorption of lipids such as gangliosides when a steric hindrance occurs as a result of the diffusional barriers on the path to the active sites (16).

TABLE 2. Influence of the loading solution on the recovery of tritiated GM1 from a 1 ml copolymer column

Loading Solution	Distribution of Gangliosides		
	Filtrate	Washing	Eluate
Water	39.1%	59.9%	1.0%
Aqueous 0.015 M NaCl	2.6%	12.1%	85.3%
Aqueous 0.15 M NaCl	1.0%	11.4%	87.6%
Methanol	82.5%	5.7%	11.8%
Methanol-water, 1:1 (v/v)	17.0%	1.6%	81.4%
Methanol-0.015 M NaCl, 80:20 (v/v)	60.8%	19.7%	19.5%
Methanol-0.015 M NaCl, 60:40 (v/v)	18.2%	18.9%	62.9%
Methanol-0.015 M NaCl, 40:60 (v/v)	2.3%	1.0%	96.6%
TUP: methanol-0.015 M NaCl-chloroform, 48:47:3 (by volume)	6.0%	3.5%	90.5%

Data are from one representative experiment for each solution. The variations between 10 different experiments were below 5%.

TABLE 3. Recovery of radioactive lipids taken up in 1 ml TUP

Radioactive Lipids	Distribution of Radioactivity	Sorbent	
		Copolymer	C18 Silica Gel
^{3}H-GM3	in filtrate	3.5%	5.4%
	in washing	2.9%	3.1%
	in eluate	93.6%	91.5%
^{3}H-GM1	in filtrate	4.8%	8.9%
	in washing	2.6%	2.7%
	in eluate	92.6%	88.4%
^{3}H-GT1b	in filtrate	5.2%	9.7%
	in washing	4.0%	4.2%
	in eluate	90.8%	86.1%
^{14}C-asialoGM1	in filtrate	2.7%	3.6%
	in washing	2.8%	2.5%
	in eluate	94.5%	93.9%
^{14}C-phosphatidylcholine	in filtrate	6.0%	6.8%
	in washing	3.8%	3.5%
	in eluate	90.2%	89.7%
^{14}C-cholesterol	in filtrate	2.5%	4.4%
	in washing	1.7%	2.2%
	in eluate	95.8%	93.4%

Methanol-0.015 M aqueous NaCl-chloroform, 48:47:3 (by volume) and applied onto 1 ml copolymer and C18 silica gel columns.
Data are from one representative experiment. Values are percentages of the total radioactivity recovered per column. Variations between eight different experiments were below 5%.

The pattern of gangliosides eluted by the solvents was similar to that of the ganglioside solution applied to the columns. **Figure 1** shows that no degradation of GT1b ganglioside occurred when the ganglioside was taken up in phosphate-buffered saline, pH 7.4, and recovered on copolymer and C18 silica gel.

Gangliosides are usually purified from a lipid mixture by partition in a biphasic system obtained by mixing chloroform-methanol-aqueous 0.015 M NaCl in a ratio of 2:1:0.5 (by volume) that gives, after centrifugation, an upper phase made of chloroform-methanol-aqueous 0.015 M NaCl, 3:48:47 (by volume). Bovine brain polysialogangliosides were taken up in a TUP and applied on copolymer and C18 silica columns. The filtrate was collected at the bottom of

TABLE 4. Recovery of [^{3}H]GM1 (10 μg) with various lipids taken up in 10 ml theoretical upper phase and applied onto 1 ml copolymer columns

Added Lipids	Amount of Added Lipids	Recovery of [^{3}H]GM1 from 1 ml Copolymer Column
GM3	1 mg	92.4%
	2.5 mg	88.7%
	5 mg	84.4%
	7.5 mg	17.1%
Sphingomyelin	1 mg	91.4%
	2.5 mg	88.6%
	5 mg	82.5%
	7.5 mg	18.3%
Cholesterol	1 mg	90.6%
	2.5 mg	85.2%
	5 mg	81.9%
	7.5 mg	14.5%

Data are from one representative experiment. Values are percentages of the total radioactivity recovered per column. Variations between eight different experiments were below 5%.

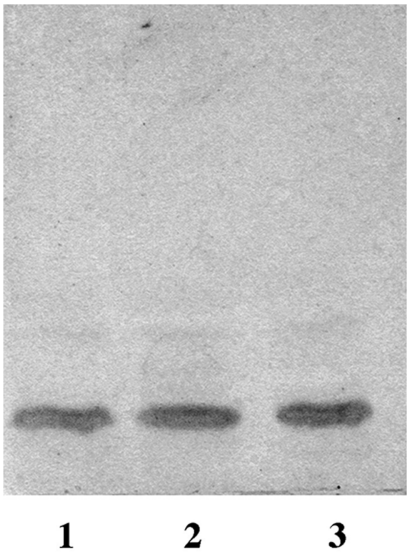

Fig. 1. HPTLC of 10 μg GT1b taken up in 5 ml of PBS pH 7.4 and applied to 1 ml copolymer and C18 silica gel columns and recovered as described in Materials and Methods. *1*) Standard GT1b; *2*) GT1b recovered from the copolymer column; *3*) GT1b recovered from the C18 silica gel column. Solvent system A (two successive migrations). Visualization with resorcinol-HCl spray reagent.

the column, then the column with adsorbed gangliosides was washed with deionized water to elute non-lipidic impurities such as salts and free sugars, then the gangliosides were eluted with methanol and methanol-chloroform, 1:1 (v/v). The HPTLC pattern of a mixture of di- and trisialogangliosides of bovine brain, recovered respectively from a copolymer column and from a C18 silica gel column, was apparently unchanged using in three different solvent systems to migrate the gangliosides (**Fig. 2**). No significant alteration in the distribution of the gangliosides processed on either solid phase sorbent could be seen by scanning densitometry (not shown) of plates on which up to 10 μg of ganglioside-bound sialic acid were spotted.

The recovery of very small amounts (below 3 μg) of gangliosides from C18 silica gel columns is usually lower than the recovery with milligram amounts applied, and it would be of interest to have an alternative method. One microgram of radioactive GM1 was applied in 1 ml of methanol-0.01 M NaCl 40:60 (v/v) on 1 ml columns of copolymer and C18 silica gel. Out of five separate experiments, the respective ranges of recovery of GM1 were 91% to 98% with copolymer and 68% to 76% with C18 silica gel.

As is already known, gangliosides can be recovered from aqueous solutions by absorption and concentration on C18 silica gel that has hydrophobic binding sites only at the surface of the material (4), or by gel filtration of the micellar gangliosides on G-25 Sephadex (2). The copolymer sorbent presents active binding sites inside the pores along with a large surface on which the active sites are exposed. In order to verify which type of behavior prevails with gangliosides on copolymer columns, experiments to recover gangliosides were carried out using a minimal (2 ml) and a maximal (20 ml) volume of TUP to take up 10 μg of tritiated GM1. With both dilutions, the recovery was similar to that given for TUP in Table 2. These results suggest that the copolymer acts as a gel filtration matrix as well as a concentration matrix. This may be due to the porous structure of the copolymer, which allows gangliosides to diffuse into the pores before being retained by hydrophobic bonds, whereas on C18 silica gel, the gangliosides on the bead surface are only adsorbed through hydrophobic forces.

The method was applied to the purification of ganglio-

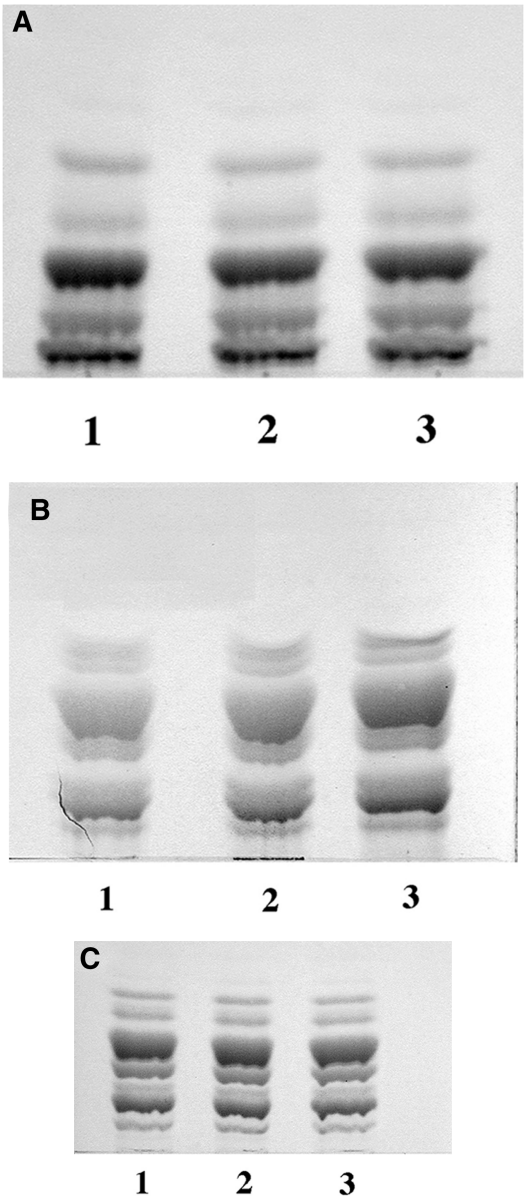

Fig. 2. HPTLC of bovine brain polysialogangliosides (total gangliosides minus monosialo fraction) taken up in theoretical upper phase of partition (TUP), applied to 1 ml copolymer and C18 silica gel columns, and recovered as described in Materials and Methods. Lane 1: Bovine brain polysialoganglioside fraction (total gangliosides minus monosialoganglioside fraction) before chromatography; lane 2: fraction recovered from the copolymer column; lane 3: fraction recovered from the C18 silica gel column. A: Developed in solvent system A. B: Developed in solvent sytem B. C: Developed in solvent sytem C. Visualization with resorcinol-HCl spray reagent.

sides from different sources with good results, comparatively, with the earlier technique using C18 silica gel. **Figure 3** shows the gangliosides of human melanoma tumors (17) and from rainbow trout liver (18) after Folch's extraction of lipids and recovery of gangliosides from the upper phases of partition. The amounts of melanoma gangliosides recovered from the upper phase of partition using copolymer and C18 silica gel columns were exactly the same (5.2 μg of lipid-bound sialic acid per mg protein) and the amount of gangliosides purified from the rainbow trout liver were also the same (1.1 μg of lipid-bound sialic acid per mg protein). The patterns of gangliosides of both tissues migrated on HPTLC plates were found to be similar using either sorbent (Fig. 3), with GM3 and GD3 as major gangliosides of human melanoma tumors (17) and *O*-acetylGD3 as the major ganglioside of the rainbow trout liver (18).

One possible application of the present method is the recovery of shed gangliosides from a culture medium. Handling large volumes of aqueous medium makes the purification of shed gangliosides quite difficult. Shedding of gangliosides can be studied with cells in culture and a method to recover the shed gangliosides directly from the culture medium would be of interest. Although gangliosides are shed under several forms, a large proportion of these gangliosides is shed the monomeric form (19), and these monomers become rapidly associated with proteins in any physiological medium. Purified gangliosides have been shown to bind to serum lipoproteins (20), but it is not known whether the recovery of lipoprotein-associated exogenously added gangliosides requires a complete denaturation of the lipoproteins. Therefore, a comparison was made in the present study between the styrene-divinylbenzene copolymer and C18 silica gel (4) to define their capacity to recover gangliosides adsorbed to the proteins of culture medium. [^{3}H]GM1 ganglioside (15,000 dpm) was taken up in culture medium and left at 37°C for 24 h before applying the solution to 1 ml columns of copolymer and C18 silica gel. In preliminary experiments, the recovery of labeled GM1 directly from the aqueous medium was low and hardly reached one-third of the applied sample. However, diluting the culture medium with an equal volume of methanol resulted in a much better yield of gangliosides on copolymer as well as on C18 silica gel. The addition of 1 vol methanol at room temperature to a culture medium containing 10% fetal calf serum does not precipitate the proteins, and there is no need to filter the sample before passing on the columns. The results in **Table 5** show that 1 ml columns can be overloaded by the many components of the culture medium. These components probably adsorb onto C18 silica gel and polymer, resulting in a competition with gangliosides for binding on both types of sorbents. In case of such overloading, gangliosides are only loosely bound to the sorbent and a significant proportion of gangliosides are lost in the filtrates and the washings. With 2 ml columns, the recovery was satisfactory with C18 silica gel and copolymer as well. After reusing many times, the styrene-divinylbenzene copolymer still gave reproducible results whereas the loading capacity of C18 silica gel decreased rapidly when this sorbent was re-used.

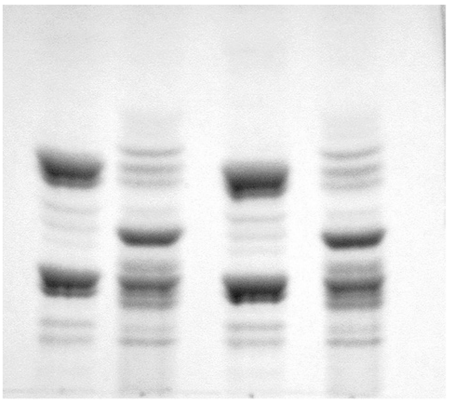

Fig. 3. Recovery of gangliosides of human melanoma tumors and rainbow trout livers after application of the respective upper phases of partition on 1 ml copolymer and C18 silica gel columns. Lanes 1 and 3: Ten micrograms of total gangliosides of human melanoma tumors and rainbow trout livers purified on copolymer columns. Lanes 2 and 4: Ten micrograms of total gangliosides of human melanoma tumors and rainbow trout livers purified on C18 silica gel columns. Solvent system A and visualization with rersorcinol-HCl spray reagent.

The copolymer sorbent showed a distinct advantage when used to purify gangliosides taken up in culture medium. The lipid-binding capacity of C18 silica gel and copolymer columns decreases rapidly upon passing culture medium (diluted with 1 vol methanol) and this is probably due to the adsorption of non-lipidic hydrophobic components present in the culture medium that are not subsequently eluted by solvents. These hydrophobic compounds tend to accumulate on the sorbent and alter the capacity to bind lipids. In one typical experiment, after passing 5 ml and 10 ml of the medium solution on 1 g columns, the GM1-binding capacity decreased respectively by 46% and 94% with C18 silica gel, and by 34% and 89% with the copolymer. Ninhydrin-positive compounds could not be detected in the eluate (not shown), suggesting that no peptide that is present in the loading solution and binds to the columns can be eluted by the solvents subsequently used to recover the gangliosides. Removal of such

TABLE 5. Recovery of [^{3}H]GM1 (10 μg) taken up in 4 ml of culture medium and kept 1 h before dilution with 1 vol methanol and application onto copolymer and C18 silica gel columns

	Radioactivity in Fractions			
	1 ml Columns		2 ml Columns	
Fractions	Copolymer	C18 Silica	Copolymer	C18 Silica
Filtrate	37.7%	27.8%	4.5%	8.2%
Washing	10.5%	9.4%	1.5%	2.7%
Eluate	51.4%	62.8%	94.0%	89.1%

Data are from one representative experiment. Values are percentages of the total radioactivity recovered per column. Variations between eight different experiments were below 5%.

non-lipidic hydrophobic compounds from C18 silica gel is very difficult because of strong alkaline or acidic treatment result in an extensive alteration of the sorbent (1). In contrast, the copolymer could be easily cleaned and regenerated with either glacial acetic acid or with 1 N NaOH to remove all contaminants adsorbed on the polymer. The columns were then sequentially washed with several cycles of water and PBS (pH 7.4) to neutralize any remaining trace of acid or alkali, and the sorbent was finally reconditioned with aqueous 0.15 M NaCl-methanol, 1:1 (v/v). The regenerating process did not significantly change the properties of the polymer sorbent. A study on the stability of the copolymer showed that after 20 consecutive cycles of cleaning and reconditioning, the ganglioside-binding capacity of the sorbent was still at 94% of the initial value.

In conclusion, a convenient method to purify gangliosides from aqueous solutions was developed using a sorbent made of styrene-divinylbenzene copolymer. On a large scale (i.e., above 5 mg of gangliosides) or with small quantities (10 μg), gangliosides can be applied to the copolymer column and recovered with a high yield. The major advantage in using the copolymer over the C18 silica gel is the possibility of reconditioning the copolymer many times to remove any kind of contaminant, and the sorbent still gives satifactory results after up to 2 years of intense utilization. Thus, although a commercially available copolymer sorbent will probably be more expensive than C18 silica gel, the high number of possible re-utilizations should greatly reduce the operating cost of using this copolymer as a suitable sorbent to purify gangliosides.

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