
**Separation Science and Technology**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

# Studies on the Extractability of Gangliosides with Various Solvents

Mehran Vajdi[a]
[a] QUALITY ASSURANCE DEPARTMENT MILES INCORPORATED, TARRYTOWN, NEW YORK

**To cite this Article** Vajdi, Mehran(1994) 'Studies on the Extractability of Gangliosides with Various Solvents', Separation Science and Technology, 29: 15, 2067 — 2072
**To link to this Article: DOI:** 10.1080/01496399408002190
**URL:** http://dx.doi.org/10.1080/01496399408002190

SEPARATION SCIENCE AND TECHNOLOGY, 29(15), pp. 2067–2072, 1994

TECHNICAL NOTE

# Studies on the Extractability of Gangliosides with Various Solvents

MEHRAN VAJDI
QUALITY ASSURANCE DEPARTMENT
MILES INCORPORATED
511 BENEDICT AVENUE
TARRYTOWN, NEW YORK 10591



## ABSTRACT

Studies on the extactability of gangliosides with various solvents have indicated a new behavioral feature of gangliosides concerning nonpolar solvents. It was shown, contrary to previous understandings, that gangliosides have an affinity for hexane as a nonpolar solvent. Among the three solvents studied, the highest recovery of gangliosides was indicated with hexane compared to methylene chloride and chloroform. It is believed that the nonpolar alkyl chain of hexane and various alkyl chains of the fatty acids on gangliosides exhibit a mutual affinity, leading to enhanced extractability. Recoveries of gangliosides were also enhanced by the addition of TEA to the extracting solvent. It appears that positive and negative forces of attractions on both molecules are factors which play a role on the recovery of gangliosides during extraction.

## INTRODUCTION

Gangliosides, a group of complex, acidic sialoglycosphingolipids, are widely distributed in mammalian tissues. Brain is a unique organ characterized by an exceedingly high concentration of gangliosides compared to the extraneural organs or tissues, and also by the higher molecular diversity and complexity of gangliosides (1, 2). The significance and role of gangliosides in neuritogenesis, regeneration, and potential clinical applications have been studied by various investigators. An intensity of research in gangliosides was observed in the 1980s, and it continues to be significant and interesting (3).

Extraction, separation, and recovery of gangliosides from brain has gone through several modes and stages of developments. The most common method of extraction and separation involves the Folch procedure. Gangliosides have been considered polar in nature and have been found in the methanol/water fraction of the Folch two-phase partition. The experiments reported in this paper were carried out to study the extractability and behavior of gangliosides in various solvents.

## EXPERIMENTAL

Bovine brain and porcine omentum tissues were cryoground to form a homogeneous tissue powder for extraction purposes. Several methods of extraction were performed on the tissue powders in order to compare the extractability behavior of gangliosides in different solvents.

Experiment 1 involved extraction of the tissue powder using three different solvents (hexane, chloroform, methylene chloride). For each 100 g of the brain powder, 1 liter of the solvent was used. Following adequate mixing for 30 minutes, the mixture was filtered and the recovered solvent was evaporated to dryness. Both fractions (dried solvent and residue) were subjected to the classical Folch procedure and cleaned according to an in-house developed process. Reversed-phase C18 column chromatography was used to remove salt and proteinaceous materials. Gangliosides were removed from the column with methanol, evaporated, and weighed. The above experiments were carried out six times for each solvent to obtain adequate representative data for comparative purposes.

Experiment 2 was performed on bovine brain powder using 10 times the volume of chloroform/methanol (2:1) followed by filtration and drying of the solvent fraction. 100 g of the material from this recovery was used for each extraction. The lipid extract was partitioned after adequate mixing by using the Folch ratios of the three solvent mixtures (hexane/methanol/water, chloroform/methanol/water, and methylene chloride/methanol/water). The final biphase was separated, and each fraction was subjected to the Folch procedure and C18 column chromatography for the recovery of gangliosides. Four replicates were performed on each solvent system.

Experiment 3 involved extraction of gangliosides from the cryoground porcine omentum powder. The first section of this experiment was performed on 100 g of the porcine omentum powder similar to that of Experiment 1 involving hexane and residue fractions. The second section was similar to Experiment 2 involving H/M/W partition of 100 g of the extracted lipids.

Experiment 4 was designed to compare the effect of triethylamine (TEA) on the extractability of gangliosides with ethanol. The first section of this experiment involved acetone drying of 100 g of the bovine brain powder followed by filtration and extraction using 1 L ethanol at 60°C for 5 minutes. The mixture after extraction was filtered, and the solvent was evaporated to dryness for the Folch procedure. The second section of this experiment involved the addition of 0.4 mL TEA to 1 L ethanol for the extraction process. Gangliosides extracted from both experiments were weighed and taken up in chloroform/methanol (1 : 1) and applied to HPTLC silica plates by the Camag Linomat IV applicator. The developed solvent was composed of chloroform/methanol/0.2% aqueous $CaCl_2$ (55/45/10). The plates were sprayed with sulfuric acid solution and scanned with the Camag scanning densitometer using the white light reflectance for measurements.



## RESULTS

Recoveries of gangliosides in Experiment 1 indicated that gangliosides were present in greater quantities in hexane than in either chloroform or methylene chloride. Gangliosides recoveries in hexane, chloroform, and methylene chloride were 0.036, 0.016, and 0.004 g, respectively. Standard deviations of recoveries for the six replicates in the above experiments were less than $\pm 0.004$. Total recoveries (solvent and residue) of ganglioside were 0.12, 0.13, and 0.11 g for the above experiments.

Results of Experiment 2 with the three solvent partitioning systems (hexane/methanol/water, chloroform/methanol/water, and methylene chloride/methanol/water) indicated a similar trend. Gangliosides had a greater affinity for hexane than chloroform or methylene chloride. Recoveries of gangliosides from hexane, chloroform, and methylene chloride were 0.12, 0.07, and 0.03 g, respectively. Standard deviations of recoveries for the four replicates were less than $\pm 0.01$ for the above solvents. Total recoveries of gangliosides (upper and lower phases) from the above experiments were 0.15, 0.16, and 0.14 g, respectively. An interesting observation made here was the greater recovery of gangliosides in hexane from the partitioning system than by direct extraction. It should be noted that in Experiment 2 (partitioning) the lipids were already extracted with chloroform/methanol and removed from the tissue; therefore, their mobility in the solvent partition would be enhanced and greater compared to that of Experiment 1. In addition, co-transfer of the gangliosides with other lipids into the hexane upper phase may account for the greater presence of the gangliosides in the hexane fraction. Similar higher recoveries were

also experienced for chloroform and methylene chloride extractions in the Experiment 2 studies.

Recoveries of the gangliosides from the omentum tissue using various solvents is somewhat different in that omentum, unlike brain, is mainly composed of nonpolar lipids of a triglyceride nature. Extraction and recovery of gangliosides are therefore influenced by the compositional nature of the tissue.

As observed in Experiment 3, the greater portion of the gangliosides were found in the hexane fraction during the direct extraction procedure. It appears that during the extraction of the nonpolar lipids (triglycerides) with hexane, gangliosides were also removed with the fat transfer. Recoveries of gangliosides in hexane from the direct extraction and partitioning were 0.006 and 0.004 g, respectively. Standard deviations of recoveries for the four replicates were less than $\pm 0.002$ for the above experiments. The total recovery of gangliosides from the omentum tissue was 0.014 g for both extractions.

We feel that the affinity of gangliosides for hexane is based on the attractions of the nonpolar segments of the molecules to the solvent. A similar situation may also exist with regard to the non-polar nature of the



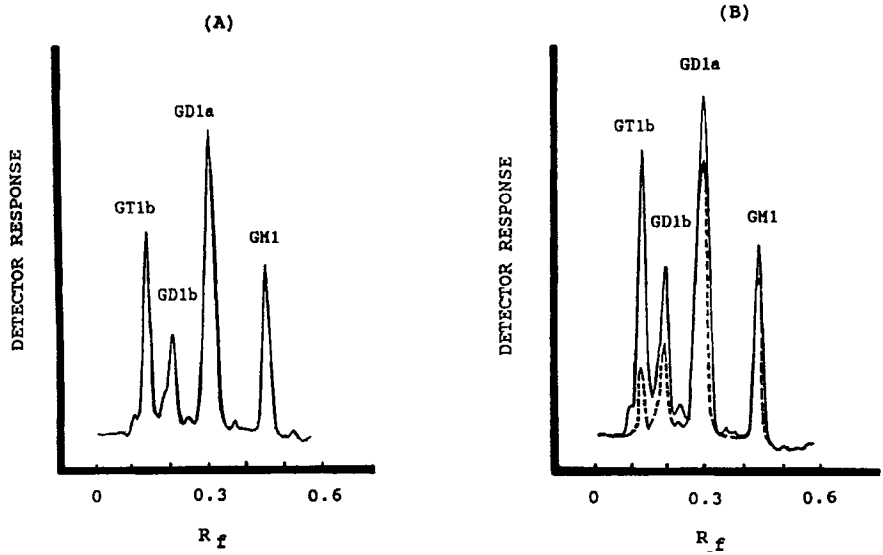


FIG. 1 HPTLC and densitometric evaluations of gangliosides extracted with various solvents. (A): Folch method. (B): Ethanol (dashed line), ethanol and TEA (solid line). GM1: Monosialogangliosides. GD1a, GD1b: Disialogangliosides. GT1b: Trisialogangliosides.

fat, hexane, and the fatty acid moieties of gangliosides. The absorption behavior of gangliosides on the C18 reverse-phase resin during column chromatography also suggests the same rationale. Therefore, based on the above observations and experience, it is interesting to see that gangliosides considered polar in nature could also have affinity for hexane as a solvent. During the recovery studies it was realized that the gangliosides content of the bovine brain tissue is approximately 10 times the amount of gangliosides found in the porcine omentum tissue. The recovery of pure gangliosides from bovine brain was found to be 1.4 g/kg of tissue by the Folch method of extraction.

Results of Experiment 4 showed that certain compounds such as amines can enhance the recovery of gangliosides from tissue. The amount of gangliosides obtained from 100 g of bovine brain powder using ethanol was found to be 0.096 g. The recovery of gangliosides with TEA in ethanol showed a higher yield of 0.128 g/100 g of the tissue powder.

A typical HPTLC chromatogram of gangliosides from the classical Folch procedure is shown in Fig. 1A. $R_f$ values of 0.12, 0.21, 0.30, and 0.46 were observed for GT1b, GD1b, GD1a and GM1, respectively. HPTLC analysis of the recovered gangliosides from ethanol (dashed line) and ethanol containing TEA (solid line) are superimposed in Fig. 1B. It was shown that enhancement of recovery of individual gangliosides was proportional to the number of the sialic acids (negative charge) on the molecules. As shown in Fig. 1B, the effect of TEA was more pronounced for GT1b than for GD1a in the gangliosides mixture.



## CONCLUSION

Studies on the extractability of gangliosides with various solvents showed a feature of gangliosides contrary to the previous understanding of their polar behavior. Comparative analysis of several solvents indicated that gangliosides also have an affinity for hexane as a nonpolar solvent. The highest recovery during the several modes of extraction was realized with hexane followed by chloroform and methylene chloride. A similar behavior has been also experienced with C18 reverse-phase chromatography of gangliosides where they have been selectively retained on the column. It is believed that the nonpolar nature of hexane (C6), the reverse phase (C18), fats (fatty acids), and gangliosides (fatty acids moiety) share and exhibit a natural and mutual affinity which have been demonstrated and experienced during various aspects of this study.

It was also shown that the addition of TEA to ethanol enhances the recovery of gangliosides from bovine brain tissue. In this connection, it

appears that positive and negative forces on the molecules are the factors responsible for enhancement during extraction.

## ACKNOWLEDGMENT

This work was supported by the Angio-Medical Corporation.

## REFERENCES

1. Y. Nagai and M. Iwamori, *Mol. Cell Biochem.*, *29*, 81 (1980).
2. Y. Nagai and M. Iwamori. *Adv. Exp. Med. Biol.*, *174*, 135 (1984).
3. R. W. Ledeen, R. K. Yu, M. Rapport, and K. Suzuki, *Ibid.*, *174*, 407 (1984).

*Received by editor January 24, 1994*
*Revised March 4, 1994*