Inorg. Chem. **2004**, *43*, 2013–2017

Characterization and Purification of Supramolecular Metal Complexes Using Gel-Permeation Chromatography

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Received July 25, 2003

Gel-permeation chromatography (GPC) has been used to analyze transition-metal-based squares, triangles, and related supramolecular complexes. Using rhenium-containing molecular squares of different sizes, a linear calibration curve has been established, which was used for confirming the relative sizes of other assemblies. GPC can also discriminate cyclic trimers and tetramers of a dirhodium building block. Preparative GPC has been used to resolve macroscopic samples of a rhenium-based supramolecular mixture into pure syn and anti isomers. A mixture of "triangle" and "square" has also been successfully separated.

Introduction

Transition-metal-based directed assembly has emerged as a popular strategy for the synthesis of highly ordered, symmetrical supramolecular complexes and closely related materials.[1−6] Although synthesis via directed assembly is typically both facile and high yielding, the difficulty in structural characterization and purity analysis for the products has been highlighted in the recent literature.[1−4] Unfortunately, the usual methods of characterization [elemental analysis (EA), IR, ^{1}H NMR, MS, UV−Vis] for simple molecular compounds are often insufficient and can sometimes be misleading because of the high symmetry and compositional similarity of the possible products.

Size-exclusion chromatography, also known as gel-permeation chromatography (GPC), is an analytical technique in which the separation of components in a reaction mixture is based on size rather than chemical affinity, as in HPLC. As such, GPC has been extensively used in the determination of polymer purity, dispersity, and molecular weight,[7] as well as in the analysis of large biomolecules, such as proteins.[8] Additionally, larger-scale preparative GPC can be used for the separation and purification of samples containing multiple components.[9] The use of GPC for supramolecular characterization, in principle, can greatly facilitate the analysis of sample purity, provide a qualitative prediction of the molecular size and shape, and allow access to macroscopic amounts of pure materials via preparative methods. Recent examples of the utilization of GPC in supramolecular chemistry include the determination of the relative size and stability of several similar hydrogen-bonded supramolecular aggregates[10,11] and the relative size of organometallic dendrimers,[12,13] as well as general analyses of the purity for samples of polyporphyrins,[14,15] bridged metallocenes,[16] and oligomeric metal carbohydrates.[17] Herein, we report that GPC

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10.1021/ic034883h CCC: $27.50
Published on Web 02/24/2004

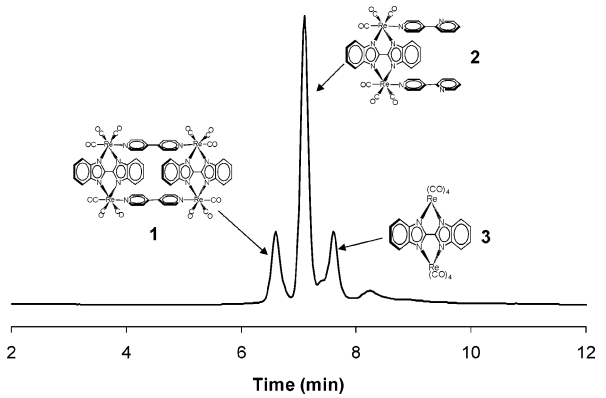

Figure 1. Analytical GPC trace of an artificial mixture of a molecular rectangle and subunits.

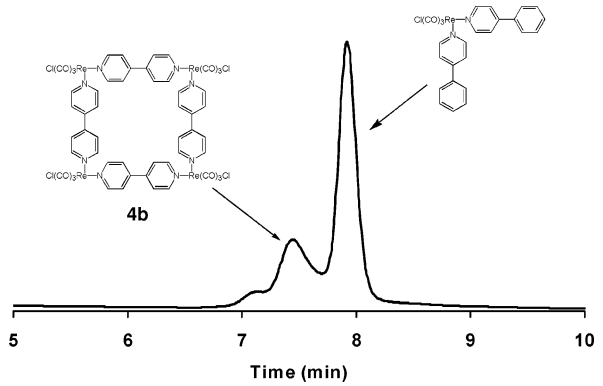

Figure 2. Analytical GPC trace showing the separation of an artificial mixture of **4b** and "corners".

can be used more diagnostically in the determination of size and purity of transition-metal-directed supramolecular assemblies (TMDSA), as well as in the deduction of byproducts in the syntheses of these materials. We show that it is possible to calibrate a GPC column for a specific set of related structures and then use the resulting calibration curve to determine the identity of a particular species in a mixture. We also show that preparatory GPC can be used in the purification of mixtures of TMDSA.

Results and Discussion

GPC Analysis of Artificial Reaction Mixtures. Our initial studies focused on ascertaining the feasibility of GPC to cleanly separate the multiple possible products of the transition-metal-directed-assembly reactions in addition to the separation of the product from starting materials. Thus, we examined the separation of an artificial mixture of the potential byproducts in a transition-metal-directed-assembly reaction. Complexes **2** and **3** are models of the edge and partially formed rectangle that could be obtained as byproducts in the directed assembly of molecular rectangle **1**[18] (Figure 1).

As shown in Figure 1, GPC could be used to separate the three components of this mixture from largest to smallest, as confirmed by comparison to individual GPC traces of the components. The polydispersity index (PDI) of each of the three peaks was 1.00, indicative of a single molecular composition per peak. Further, the recovery of the components is quantitative, indicating no decomposition on the GPC column.

An artificial mixture of 4,4′-bipyridine-based molecular square[19] **4b** and an analogous "corner" was also successfully separated (Figure 2).

GPC Analysis of *fac*-Re(CO)$_3$Cl-Based Molecular Assemblies. *fac*-Re(CO)$_3$Cl-based directed assembly of the molecular squares has been reported to yield multiple structures, i.e., squares and triangles, depending on the ligand

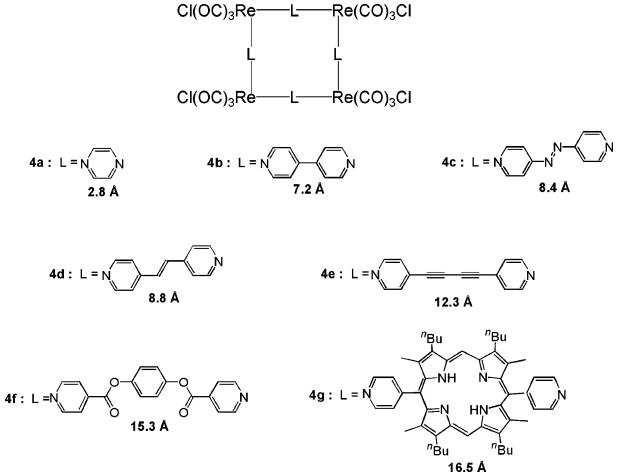

Figure 3. Series of rhenium-based molecular squares based on bridging ligands of variable length.

or solvent used in the synthesis.[20] To date, all such structures have been described as pure samples of either the square or triangular form. The GPC analysis of several large neutral assemblies of this kind[4,19,21,22] (Figure 3) demonstrated that this technique can be used to ascertain the purity of samples of supramolecular complexes and the number of components that exist in crude reaction mixtures (Figures 4 and 5, vide infra).

Calibration of a GPC Column for *fac*-Re(CO)$_3$Cl-Based Molecular Assemblies. Given the mechanism for analyte separation in GPC, one would anticipate that the retention times for a series of similar supramolecular complexes formed from ligands of varying length, such as those shown in Figure 3, would scale inversely with the size of the complex. If this were the case, then it would be possible to create a calibration curve for a particular GPC column and a series of structurally similar compounds that varied only in overall size. This calibration curve could subsequently be used to extract the relative size of components in an incompletely characterized sample, on the basis of the sample's retention time. Because the molecular squares are semirigid open-shell structures and not flexible linear coils,

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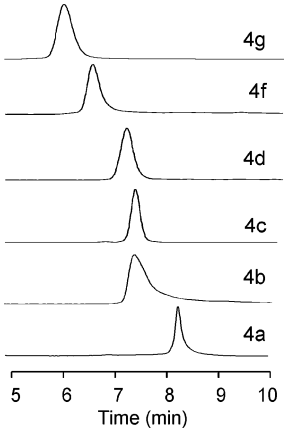

Figure 4. Analytical GPC traces of the crude reaction products formed in the syntheses of **4a**−**d**, **4f**,**g**.

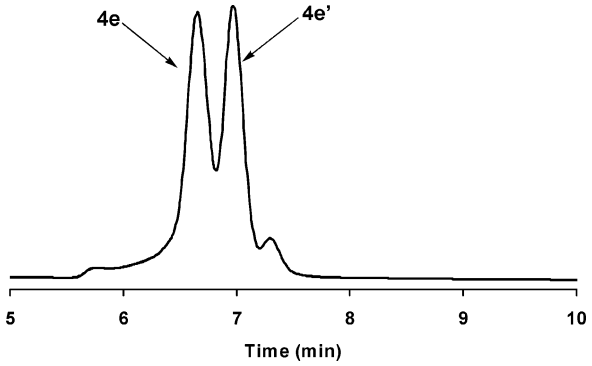

Figure 5. Analytical GPC trace of the crude reaction products formed in the synthesis of **4e**.

Table 1. Ligand Lengths, Analytical GPC Retention Times, and PDIs for *fac*-Re(CO)$_3$Cl-Based Molecular Assemblies

square	ligand length[a] (Å)	retention time (min)	PDI
4a	2.8	8.3	1.00
4b	7.2	7.4	1.04
4c	8.4	7.4	1.00
4d	8.8	7.2	1.00
4e, 4e′	12.3	6.8, 7.2	1.00, 1.00
4f	15.3	6.6	1.00
4g	16.5	6.2[b]	1.00

[a] Determined by Chem3D modeling. [b] 0.5% (v/v) triethylamine added to the CH_2Cl_2 eluent.

as is the case with most organic polymers, the typically used parameters for a polymer GPC calibration curve (log of molecular weight versus retention time) would not be appropriate for our calibration curve. Because the ligand length is the primary factor in determining the relative size and shape of most semirigid open-shell TMDSA, it should be a better parameter to plot against retention time in constructing a calibration curve.

Because squares **4a**, **4c**, **4d**, **4f**, and **4g** are pure complexes, as evidenced by a single, sharp peak in each of their GPC traces (Figure 4) and by small PDIs (Table 1), they form the basis (along with **4b**) for the calibration curve. The trace of **4b** shows a slight tailing, corresponding to a PDI of 1.04, which could arise from either a small amount of an impurity similar in size to **4b** or a slight retention/degradation of the square on the column. The calibration curve constructed

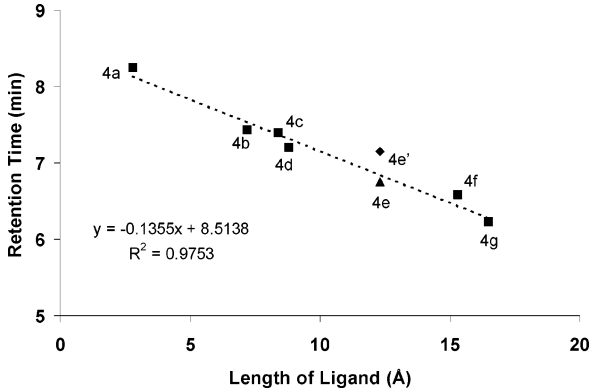

Figure 6. GPC retention time versus ligand length for a series of rhenium-based molecular squares.

(Figure 6) from the relative GPC retention times of **4a**−**d** and **4f**,**g** shows an excellent correlation to their respective ligand lengths (as modeled by Chem3D, Table 1).

A caveat is the secondary interactions between the analyte and the column packing material that can come into play when molecular squares with metalated or complexing bridging ligands are analyzed, resulting in either longer than expected retention times or irreversible binding to the column. For example, initially, the free-base porphyrin complex **4g** was completely retained on the column. Addition of 0.5% (v/v) of triethylamine to the CH_2Cl_2 eluent, however, evidently eliminated the secondary interactions responsible for immobilization. Column packings other than Phenomenex Phenogel, such as Waters Styragel, were also found to yield decreased secondary chemical interactions for complex **4g**.

The calibration curve can then be used to elucidate, at least in part, the nature of the product or products of a supramolecular synthesis. For the synthesis of square **4e**, two distinct peaks of equal intensity (**4e** and **4e′**) were observed for the crude product. Again, we interpret the data to mean that two closely related supramolecular complexes, possibly the square and a smaller species [partially formed square (vide supra) or triangle], were formed in the reaction. Complex **4e** is potentially the square (its retention time correlates better with the calibration curve), and complex **4e′** is potentially the smaller species. We note that these two components could not be differentiated by more conventional methods such as NMR, MS (the mass of a partially formed square or a triangle cannot be discriminated from that of a fragmented square), or EA. Unfortunately, the severely limited solubility of the [**4e** + **4e′**] mixture in common organic solvents has negated the possibility of further isolation, characterization, and stability studies of these species by preparative GPC.

Preparative GPC. A synthetic mixture of *fac*-Re(CO)$_3$Cl-molecular square **5** and triangle **6** was evaluated via GPC. The separation of the two components was carried out via semipreparative-scale GPC (Figure 7). A total of 18 mg of a 50:50 mixture of **5** and **6** was dissolved in 500 μL of CH_2Cl_2, and this solution was separated on a 100 Å column via a series of 10 μL injections. Fractions were collected via an automated fraction collector set to collect on the basis of peak height and width. Product-containing fractions were

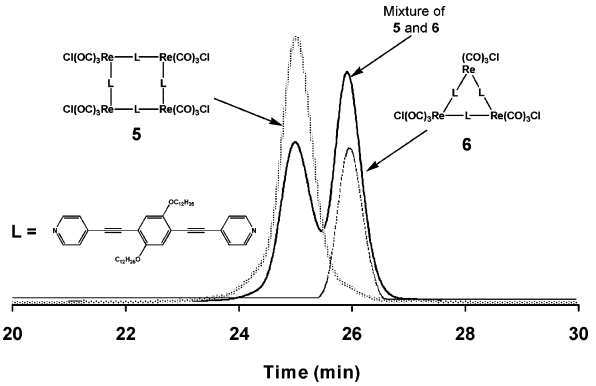

Figure 7. Semipreparative gel-permeation chromatogram of a $Re(CO)_3Cl$-based square/triangle mixture (dark trace). Chromatograms of the pure components reinjected on the same GPC column after isolation are shown in gray.

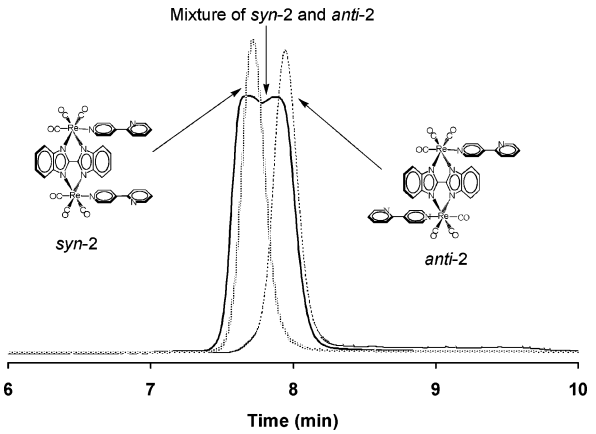

Figure 8. Analytical GPC traces of a crude mixture of *syn*- and *anti*-rhenium bis(benzimidazolate) complexes, and pure samples of each component obtained by preparative GPC.

collected and combined to yield 7.2 mg of pure **5** (PDI = 1.00), 5.1 mg of a mixture of **5** and **6**, and 5.7 mg of **6** (PDI = 1.00).

We further found that structural isomers can be reliably differentiated by analytical-scale GPC and separated by preparative GPC (Figure 8). The component isomers of a crude sample of **2** were easily identified by comparison with the retention times of pure samples (obtained via conventional chromatography). Because the isolation of the two isomers of **2** via conventional chromatography on silica is exceedingly tedious, we reasoned that automated preparative GPC could be used instead. A total of 30 mg of a 50:50 mixture of *syn*-**2** and *anti*-**2** were dissolved in 500 μL of CH_2Cl_2 and fully injected on a preparative GPC column. Again, fractions were collected via an automated fraction collector set to collect on the basis of peak height and width. Analysis of the collected fractions yielded 13.2 mg of *syn*-**2** (PDI = 1.00), 8.6 mg of *anti*-**2** (PDI = 1.00), and 8.2 mg of a mixture of the two.

GPC Analysis of Non-Rhenium-Containing Systems. To extend the use of GPC as a characterization tool for supramolecular assemblies beyond the rhenium-linked systems, we examined others based on dirhodium corner units.[6,23] The analysis of **7** by GPC proceeded smoothly, and a single peak corresponding to the dirhodium-based molecular square was observed at a retention time that is in the range of similarly sized rhenium squares. An interesting feature of the dirhodium system is that the ratio of dirhodium corner starting material to oxalate linker used in the preparation of the complex controls the final structure. At a 1:1 ratio, the triangle **8** is isolated, while in the presence of 10-fold excess oxalate, the square **7** is formed.[6,23] Independent synthesis and analysis of **7** and **8** by GPC indicated that these two structures had significantly different retention times. Here as well, the artificial mixtures of the two are cleanly separable via preparative GPC (Figure 9) to give isolated pure components that exhibit single peaks under analytical GPC conditions (PDI = 1.00 for each).

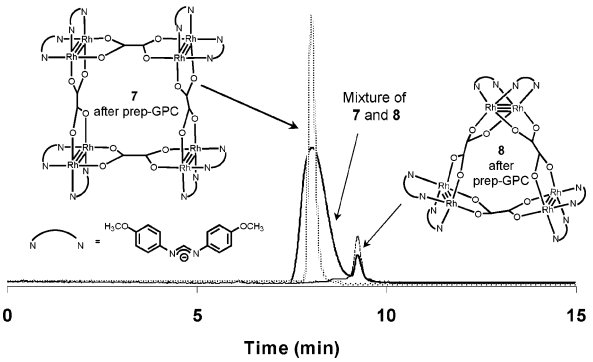

Figure 9. Preparative GPC trace of a **7**/**8** (square/triangle) mixture (black) and analytical GPC traces for the separated fractions (gray).

In summary, GPC has proven to be a useful tool in the determination of product structural purity and implementation of size- and shape-based product separation in supramolecular synthetic chemistry. It may be of greatest value in those cases where (a) NMR and MS are ambiguous, and the compounds have resisted crystallization or (b) closely similar, difficult to distinguish, product mixtures are obtained. Indeed, in many cases, preparative or semipreparative GPC can be used for the separation/purification of product mixtures from supramolecular syntheses.

Experimental Section

Materials and General Procedures. Molecular square complexes **4a**,**b**,**d**,[19] **4f**,[24] **4c**,**e**,[20,25] **4g**,[5] **7**, and **8**,[23] were synthesized according to the literature methods. Samples of **2** and **3**[18] were kindly provided by Mr. Peter Dinolfo of Northwestern University. HPLC-grade CH_2Cl_2 (EM Science), rhenium pentacarbonyl chloride (Strem Chemicals), and all other chemicals (Aldrich Chemical Co.) were purchased from commercial sources and used as received.

Equipment and General Procedures. All of the GPC experiments were carried out on an automated Agilent 1100 series HPLC equipped with a multiwavelength detector and a Gilson FC-204 fraction collector equipped with 240 15-mL test tubes. Samples were run in CH_2Cl_2 and monitored at 254 nm.

Analytical GPC analyses of the reaction mixtures were carried out using a Phenomenex Phenogel 100-Å column packed in CH_2Cl_2 with a 7.80-mm inner diameter and a 300-mm length (flow rate = 1 mL/min).

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Semipreparative GPC was carried out using a Phenomenex Phenogel 100-Å column packed in CH_2Cl_2 with a 7.80-mm inner diameter and a 300-mm length (for mixture of **5** and **6**, flow rate = 1 mL/min). Preparative GPC was carried out using a Waters Ultrastyragel 100-Å column packed in CH_2Cl_2 with a 19-mm inner diameter and a 300-mm length (for the isomer mixture of **2** and mixture of **7** and **8**, flow rate = 2 mL/min).

PDI values for molecular square samples were obtained through comparison with a calibration curve established from pure molecular standards.

Compounds 5 and 6. A mixture of $Re(CO)_5Cl$ (79 mg, 0.22 mmol) and the ligand, 1,4-bis-(4′-ethynylpyridyl)-2,5-bis(dodecyloxy)benzene[25] (142 mg, 0.22 mmol), in THF was refluxed under nitrogen for 2 days. The solvent volume was reduced to ∼5 mL under vacuum, and this concentrated solution was then added into a 500-mL round-bottom flask containing rapidly stirring hexanes (300 mL). The resulting yellow precipitate was collected by filtration and dried in air to give 180 mg (87%) of a 50:50 mixture of **5** and **6**. ^{1}H NMR (400 MHz, $CDCl_3$) for square **5** δ: 8.72 (d, 16 H, J = 6.4 Hz), 7.33 (d, 16 H, J = 6.0 Hz), 7.00 (s, 8 H), 4.00 (t, 16 H, J = 7.6 Hz), 1.86−1.81 (m, 16 H), 1.50−1.47 (m, 16 H), 1.37−1.24 (m, 128 H), 0.87 (t, 24 H, J = 6.8 Hz). MS−FAB: calcd for $[M - Cl]^+$ (m/z), 3782.5; found, 3782.1. Anal. Calcd for $C_{180}H_{240}Cl_4N_8O_{20}Re_4 \cdot 2CHCl_3$: C, 56.24; H, 6.01; N, 2.76. Found: C, 55.99; H, 5.71; N, 2.39. ^{1}H NMR (400 MHz, $CDCl_3$) for triangle **6** δ: 8.75 (d, 16 H, J = 5.6 Hz), 7.37 (d, 16 H, J = 6.0 Hz), 7.02 (s, 8 H), 4.02 (t, 16 H, J = 6.0 Hz), 1.86−1.81 (m, 16 H), 1.50−1.47 (m, 16 H), 1.37−1.24 (m, 128 H), 0.87 (t, 24 H, J = 6.8 Hz). MS−FAB: calcd for $[M - Cl]^+$ (m/z), 2828.1; found, 2827.9. Anal. Calcd for $C_{141}H_{180}Cl_3N_6O_{15}Re_3$: C, 59.13; H, 6.33; N, 2.93. Found: C, 59.36; H, 6.17; N, 2.81.

Acknowledgment. We gratefully acknowledge the financial support from the National Science Foundation and the Department of Energy through a grant administered by the Northwestern University Institute for Environmental Catalysis. We thank the Phenomenex Co. for the initial loan of the Phenogel column and Mr. Peter Dinefo for samples of **2** and **3**. S.T.N. additionally acknowledges support from Union Carbide, the Beckmann Foundation, the Packard Foundation, and the A. P. Sloan Foundation. C.R.G. is an NSERC PGS-A International Predoctoral Fellow.

IC034883H