CHROMATOGRAPHY AND PURIFICATION

OF

ENDOHEDRAL METALLOFULLERENES

by

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Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Chemistry

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December 1995

Blacksburg, Virginia

Key words: Metallofullerenes, Chromatography, Isolation, HPLC-EPR
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(ABSTRACT)

At the conception of this research, a separation methodology for obtaining purified metallofullerene \([A_m \circ C_{2n}]: m = \# \text{ of metal atoms}, A, \text{ and } C_{2n} = \# \text{ of carbons in the surrounding cage}\) samples was not yet developed. Isolation of these metal-encapsulated fullerenes was strongly desired for characterization of their physical and chemical properties. Predicted applications for these novel species include their use as possible superconductors, catalysts, and non-linear optical devices. However, initial purification efforts have been hindered by several difficulties. These factors include a low abundance (<1%) in the raw extract, uncertain stability in aerobic environments, co-elution of \(A_m \circ C_{2n}\) with empty-cage fullerenes, and the need for selective chromatographic detection.

In this research, these difficulties have been overcome with the development of a continuous-flow, on-line HPLC-EPR apparatus. Advantages include a selective, non-invasive detector with chromatographic separations being performed in a controlled anaerobic environment. This on-line approach permits the selective detection of only those metallofullerenes with an odd-number of encapsulated atoms. The ability to continually monitor separations of these paramagnetic species ultimately permits the optimization of chromatographic parameters. The methodology developed from this on-line HPLC-EPR approach has ultimately resulted in purified empty-cage (C\(_{60}\), C\(_{70}...C_{90}\)) and metallofullerene samples (Sc\(_2\)@C\(_{74}\), Sc\(_2\)@C\(_{76}\), Sc\(_2\)@C\(_{78}\), Sc\(_2\)@C\(_{80}\), Sc\(_2\)@C\(_{82}\), Sc\(_2\)@C\(_{84}\) - two isomers, Sc\(_4\)@C\(_{86}\), Sc\(_4\)@C\(_{86}\), Sc\(_4\)@C\(_{90}\), Sc\(_4\)@C\(_{92}\), Sc\(_4\)@C\(_{94}\), La\(_2\)@C\(_{72}\), Er\(_2\)@C\(_{82}\), Er\(_2\)@C\(_{82}\) - two isomers, and Er\(_2\)@C\(_{92}\)).
DEDICATION

This dissertation is dedicated to my dying father and my beautiful children, Jordan and Cole. I love them with every "fiber of my being." This work is also dedicated to the woman I love and think about, literally, every day of my life. Although I made the mistake of a lifetime and pushed her out of my life, she is - and always will be - my inspiration, my "kindred spirit", and my "Anne of Green Gables." Lord willing, I pray that someday we will be together again - only this time forever.
ACKNOWLEDGEMENTS

I would like to express my gratitude toward many individuals who have contributed to this research. I acknowledge Dr. Harry Dorn, my research advisor, with whom many enlightening metallofullerene discussions took place. His advice and wisdom (sometimes followed) was an asset throughout my graduate studies.

From our research group, Paul Burbank, an undergraduate chemistry major, has been a vital link to the success of this project. From the automated system to the manual collection of fractions, his assistance is the most appreciated. Others who have contributed are Ziqi Sun and John Bailey.

From the Biochemistry department, a hearty acknowledgement is extended toward Kim Harich for his mass spectral services. His rapid turnaround of samples and mass spectra is very much appreciated. Kim did many favors for us throughout this research, and his "extra mile" approach is not forgotten.

From Analytical Services, Tom Glass provided insight into the computer programming of the HPLC-EPR apparatus. From the Glass Shop, several glass flow cells and hundreds of EPR tubes were cheerfully and promptly made by this competent staff. From the Electronics Shop, Larry Jackson and Jim Coulter repaired our EPR spectrometer when necessary. Finally, acknowledgement is extended to Don
Bethune, Mattanjah de Vries, Bob Johnson, and others at their research facility in California (IBM, Almaden) who provided financial support, arc-vaporized metallofullerene soot (raw extract), and mass spectral characterization of selected samples. This relationship between our Virginia Tech research group and IBM was the epitome of a symbiotic relationship at its finest - the way science should be done.
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CHAPTER 1: INTRODUCTION

1.1 Metallofullerenes

Historically, graphite and diamond were the only known allotropes of carbon. Recently, however, a third form of carbon has been discovered.\textsuperscript{1} Specifically, the fullerenes\textsuperscript{1-133} \((\text{C}_{2n}, \ n \geq 30)\) consist of fused, five- and six-membered rings in an all-carbon framework to form a class of hollow spherical/ovoid species. Following their discovery, numerous experiments exploring the reactivity\textsuperscript{91-112} and characterization\textsuperscript{33-37,113-133} (e.g. EPR\textsuperscript{113-129}, NMR\textsuperscript{33-37,130-133}) of fullerenes quickly ensued. The structures of several purified fullerenes (e.g. \(\text{C}_{60}\), \(\text{C}_{70}\)) have only recently been elucidated.\textsuperscript{33-37}

The encapsulation of a metal atom(s) inside these vacant empty-cage fullerenes has produced an intriguing class of compounds\textsuperscript{134-211} whose chemical and structural properties remain uncertain. These metallofullerenes\textsuperscript{134-211} \((\text{A}_{m}\text{C}_{2n}; \ A=\text{Sc, Y, La}; \ m=1-3; \ n=30-60)\) are of interest due to their predicted applications as superconductors,\textsuperscript{160} catalysts,\textsuperscript{175} non-linear optical devices,\textsuperscript{175} and in biological systems.\textsuperscript{2-4}

However, their successful isolation and characterization were initially unsuccessful due to their low abundance\textsuperscript{134,144-146,155,162} (<1\% of raw extract) and
uncertain stability in aerobic environments.\cite{137,174} Efforts to separate these $A_m@C_{2n}$ species from empty-cage fullerenes have focused primarily on chromatographic methods. Production from the usual electric arc-burning synthesis yields at least 30 to 50 distinct $A_m@C_{2n}$ and fullerene species with unique variations in the number of carbons, the number of structural isomers, and the number of encapsulated metal atoms. For these reasons, one can readily envision the difficulty of developing a successful separation protocol which would be selective enough to yield a purified $A_m@C_{2n}$ species starting from this complex mixture.

1.2 Detection

Since those $A_m@C_{2n}$ species with an odd number of encapsulated atoms ($m = 1,3$) are "EPR active,"\cite{137,140,142,143,145-147,151,152,156,158,160,164,166,169,173,174,176,177,179,181,182} the implementation of an on-line HPLC-EPR approach would permit selective monitoring of these compounds. Since conventional chromatographic detection (e.g. UV) is typically unable to distinguish between $A_m@C_{2n}$ and empty-cage fullerenes, the necessity for more selective detection would be beneficial. This becomes especially important as chromatographic conditions and selection of stationary phases are optimized. With an on-line EPR approach, the eluting metallofullerenes can be
separated in a controlled anaerobic environment, and any air-sensitive $A_m@C_{2n}$ compounds would not be compromised. Furthermore, accurate chromatographic retention times for these EPR active metallofullerenes can be readily established by observation of the on-line HPLC-EPR spectra as a function of time.

This ability to continually monitor EPR active metallofullerene species has permitted the development of successful purification methodologies in our laboratory. Briefly, the EPR active metallofullerenes, Sc@$C_{82}$, Sc$_3@$C$_{82}$, Y@$C_{82}$, and La@$C_{82}$, have been selectively monitored with on-line HPLC-EPR detection$^{174}$ for an initial separation of the metallofullerene fraction from the abundant empty-cage fullerenes utilizing a combination of polystyrene columns. This preparative "clean-up" procedure has been followed by HPLC-EPR detection of these species employing a more selective column [e.g. $\pi$-acidic stationary phase$^3$ (Buckyclutcher)] for their final stages of purification. With this methodology, the concomitant isolation of both empty-cage fullerenes and diamagnetic metallofullerenes (e.g. Sc$_2@$C$_{2n}$) has also been achieved in this research.

1.3 Research Problem

When this research began, the field of metallofullerene science was in its early
stages. There was no methodology to obtain isolated metallofullerene samples. Instead, there was a complex and intimidating mixture of at least 30 to 50 unique fullerene and metallofullerene species in a typical initial raw extract. There were no columns available that were manufactured specifically for fullerene separations. In fact, it was debatable whether or not chromatographic methods could succeed at all in providing purified metallofullerene samples. It was already difficult to obtain milligram quantities of the empty-cage C$_{60}$ and C$_{70}$ fullerenes, which were highly abundant species (>90%) in the raw extract.

1.4 Research Objectives

The primary objective of this research was to develop a methodology to obtain a purified metallofullerene sample(s). Since it was an arbitrary decision regarding which metallofullerene to isolate, the initial decisions focused on the paramagnetic A$_m$@C$_{2n}$ species. The logic being that an on-line EPR approach would be selective for only one or two compounds whereas the majority of species (C$_{2n}$ and A$_2$@C$_{2n}$ - diamagnetic) present in the initial raw extract would not be detected. Since the HPLC-EPR approach had not yet been extended toward metallofullerene separations, a secondary objective was to demonstrate its feasibility and potential impact on
metallofullerene separations. In this regard, the HPLC-EPR approach would later become invaluable in developing a successful purification methodology.
CHAPTER 2: HISTORICAL BACKGROUND

2.1 Empty-cage Fullerenes (C_{28})

2.1.1 Discovery

Named after Buckminster Fuller (famous architect of the geodesic dome), the presence of empty-cage fullerenes was first proposed in 1985 by Kroto et al.\textsuperscript{1} According to landmark mass spectral data,\textsuperscript{1} a series of peaks separated by 24 mass units was obtained. A striking feature of this mass spectrum was a dominant peak at m/z = 720 that was noticeably more intense relative to other surrounding peaks. An assignment to a highly symmetrical icosahedral empty-cage C_{60} species was made, and the field of fullerene science was born. At this stage, however, production of the fullerene species was limited to the microgram to nanogram level.

It was not until 1990 that Krätschmer et al.\textsuperscript{6} developed a method to produce fullerenes in *macroscopic* quantities. In this procedure, graphite rods were "burned" in an electric-arc synthesis.\textsuperscript{6} Although some of the resulting soot was insoluble material, a significant portion of the extract contained C_{60}, C_{70}, C_{84}, etc. in milligram quantities.
2.12 Structure

The structure for any of the fullerenes was not elucidated until the early 1990's. In general, the fullerenes$^{1-133}$ consist of fused five- and six-membered rings in a hollow spherical/ovoid all-carbon framework as demonstrated below.

\[ C_{60} \quad C_{70} \]

According to the "isolated pentagon rule",$^{212-214}$ two pentagon rings can not be located adjacent to each other. Instead, pentagons are bordered by hexagon rings to preserve this isolated pentagon rule. Using this rule, one of the smallest possible structures that can be formed is the $C_{60}$ fullerene. This species was predicted to possess a truncated icosahedral symmetry. If this highly symmetrical assignment was correct,
then its $^{13}$C NMR spectrum would be predicted to have only one signal - despite having sixty carbon atoms in its structure.

2.13 Chromatography and Isolation

The initial attempts to obtain purified fullerene samples have focused on chromatographic methods.$^{33-36}$ In 1990, Taylor$^{33}$ and Ajie$^{34}$ successfully isolated C$_{60}$ by alumina chromatography. Since obtaining macroscopic amounts (mg level) of C$_{60}$ was difficult in these early stages of development, it was fortunate that only one NMR signal was predicted. Had there been 20 to 60 expected signals, this early NMR experiment likely would have failed at this time. Nevertheless, in 1990 Taylor,$^{33}$ Johnson,$^{34a}$ and Ajie$^{34b}$ successfully obtained a $^{13}$C NMR spectrum for C$_{60}$. Experimentally, the spectrum contained only one signal (142.68 ppm) and was consistent with earlier theoretical predictions. With these data, the highly symmetrical icosahedral structure was confirmed. Few molecules are known to possess this unusual type of symmetry.

Other empty-cage fullerenes have subsequently been isolated.$^{33,34}$ The C$_{70}$ species was also chromatographically isolated in 1990 by Taylor$^{33}$ and Ajie.$^{34}$ Subsequent $^{13}$C NMR characterization revealed that C$_{70}$ possessed five distinct signals corresponding
to a \( D_{5h} \) cage symmetry. Shortly thereafter, the empty-cage \( C_{76} \) species (\( D_2 \) symmetry) was isolated and characterized.\(^7\)\(^8\)

Early isolation and NMR studies\(^7\)\(^8\)\(^11\)\(^12\) on \( C_{78} \) and higher mass fullerenes have been (and still are to some extent) hampered due to the existence of structural isomers of various cage symmetries. For example, the \( C_{78} \) fullerene actually possesses two isomers of \( C_{2v} \) and \( D_3 \) symmetries.\(^8\)\(^14\) Each of these cage symmetries would correspond to different NMR spectra. Typically, the smaller cage sizes possess a higher symmetry and fewer NMR lines. However, purified \( C_{78} \) samples had to be obtained before any NMR characterization could be performed. Chromatographically, finding a stationary phase that was selective enough to distinguish between these two \( C_{78} \) isomers would be extremely difficult. Nevertheless in 1991, Diederich et al.\(^8\) successfully isolated these two \( C_{78} \) isomers. Subsequent \(^{13}\)C NMR data confirmed the previously theoretical symmetries - (\( D_3 \); 13 lines) and (\( C_{2v} \); 21 lines).

For the fullerene species (e.g. \( C_{60} \), \( C_{70} \), \( C_{76} \), \( C_{78} \), \( C_{84} \) etc.), increasing the number of carbon atoms in the outer-cage generally results in decreasing the symmetry of the molecule. This often results in multiple structural isomers. This feature results in NMR spectra which can easily consist of 30 to 84 lines per structural isomer. With NMR samples of multiple isomers, the spectra often become more complex. In
addition, the number of expected NMR lines is directly proportional to the amount of sample that is necessary. Thus, higher mass empty-cage fullerenes of lower symmetry ($C_{82}$ - $C_{110}$) would require at least an order of magnitude more sample relative to $C_{60}$. Due to the unavailability of columns with sufficient resolving power, it is extremely difficult to obtain purified samples of individual cage symmetries for the $C_{82}$ - $C_{110}$ empty-cage fullerenes. For example, the empty-cage $C_{82}$ fullerene is believed to possess at least three different structural isomers.\textsuperscript{12} Although a $C_{82}$ fraction has been isolated,\textsuperscript{12} the resulting $^{13}$C NMR spectrum actually represents a composite mixture of these individual isomers co-added together in one NMR spectrum. Predicted $C_{82}$ cage symmetries are $C_2$, $C_{2v}$, and $C_{3u}$.\textsuperscript{12} Ideally, one would like to have three sample vials - one for each $C_{82}$ cage symmetry. However, a primary difficulty is finding a stationary phase with adequate selectivity to separate a $C_{82}$ mixture into their three structural isomers. Since it is already difficult to locate a stationary phase that can resolve differences between structures consisting of similar size carbon cages (e.g. $C_{86}$, $C_{88}$), one can readily envision the challenge of separating multiple isomers of the same mass (e.g. $C_{82}$).
2.2 Metallofullerenes ($A_m@C_{2n}$)

2.21 Discovery

As early as 1985, it was suggested that metal atom(s) could be "trapped" or encapsulated inside these hollow empty-cage fullerenes. Shortly thereafter, confirmation of this notion was supported by mass spectral evidence. In 1985, Heath et al. laser-vaporized a lanthanum-impregnated graphite rod and produced an extract containing lanthanum encapsulated fullerenes. As a result, a global scientific interest in the field of metallofullerene science rapidly ensued.

2.22 Nomenclature

The nomenclature presently adopted for the metallofullerenes is denoted by $A_m@C_{2n}$, where "A" represents which metal (e.g. Sc, Y, La, etc) is present. The lower-case "m" indicates the number of metal atoms "trapped" inside the empty-cage structures. The "@" symbol denotes the position of the metal relative to the empty-cage surroundings. Specifically, the "@" symbol indicates that the metal is inside (endohedral) and encapsulated by the fullerene cage. In contrast, the absence of the
"@" symbol would represent a metal located outside (exohedral) of the fullerene cage. Finally, the "2n" term represents the number of carbon atoms in the fullerene cage network. For example, a case where \( n = 30 \) would be \( C_{60} \) (i.e. a cage with sixty carbon atoms). The numerical two in the "2n" term originates from the fact that only empty-cage fullerenes with an even carbon number (e.g. \( C_{90}, C_{92}, C_{94}, \) etc) are produced. Odd-numbered empty-cage species (e.g. \( C_{91}, C_{93}, C_{95}, \) etc) are not formed in the production process.

2.23 Applications

The presence of a metal(s) "trapped" inside of empty-cage fullerenes has generated considerable interest in the scientific community. Numerous properties and characteristics of this new class of compounds have been predicted.\(^{160,175}\) Because several exohedral metallofullerenes\(^{217-242}\) (e.g. \( K_3 C_{60} \) - metal outside the fullerene cage) were proven to be superconducting,\(^{231-242}\) it was believed that endohedral metallofullerenes could be superconducting as well.

Recent applications of empty-cage fullerenes have been in the pharmaceutical area.\(^{24}\) Specifically, derivatized empty-cage fullerenes\(^{2-4}\) have recently exhibited some activity against the AIDS virus. There are potentially other areas of biological
applications. The possibility of encapsulating radioactive species inside of fullerene cages could have a significant impact in cancer research. Metallofullerenes have also been predicted to be useful as possible non-linear optical devices.\textsuperscript{175} It has also been suggested that metallofullerenes could be used as novel catalysts.\textsuperscript{175}

2.24 Chromatographic Overview

Although first produced in nanogram quantities in 1985,\textsuperscript{215} it was not until 1990\textsuperscript{6} that a methodology to produce a metallofullerene extract in macroscopic amounts (mg) was developed. Research efforts to obtain purified metallofullerenes samples quickly ensued on the global level. Although some research groups had attempted non-chromatographic techniques (e.g. sublimation),\textsuperscript{163} the majority of initial purification efforts involved liquid chromatographic methods.\textsuperscript{33-37,134,135,243-251} This was not surprising since some foundational work had only recently been performed with the empty-cage separations\textsuperscript{33-37,134,135} discussed \textit{vide supra}. Note that at this time, chemists had just chromatographically isolated \textit{C}_{60} and \textit{C}_{70}.\textsuperscript{2,3} Thus, there was some recently acquired experience regarding which type of column and mobile phase would produce the most favorable results.

In this regard, it was not surprising that initial efforts to obtain purified
Metallofullerenes paralleled the earlier chromatographic methods which had permitted isolation of C_{60} and C_{70}. However, it was later realized that certain types of columns and mobile phases which achieved favorable results with empty-cage species were not necessarily the best choice for metallofullerene separations. For example, reversed-phase chromatography\textsuperscript{27} readily separated the empty-cage fullerenes with excellent separation factors. However, the amount of sample that could be injected into reversed-phase columns was unsatisfactory. This poor sample throughput was due to the low solubility of fullerene material in reverse-phase type mobile phases (e.g. acetonitrile, water, etc). Even with toluene/acetonitrile mixtures of solvents, the amount of sample loadability was still inadequate for initial, preparative separations. Since the metallofullerenes are typically present at only <1\% abundance in their stock,\textsuperscript{134,144-146,155,162} reverse-phase\textsuperscript{27} methods were not suited for the large sample throughput which was necessary for these initial, preparative clean-up procedures. In addition, there was evidence that certain metallofullerenes would either decompose or never elute from reverse-phase columns.

To achieve improved sample throughput, the metallofullerenes needed to be sufficiently soluble in the mobile phase. At this time, it was suggested that metallofullerene samples should be dissolved in normal-phase type solvents (e.g. aromatic hydrocarbons). However, traditional octadecyl (C_{18}) normal-phase columns
with these solvents permitted little or no resolution. Injected fullerene material eluted as an unresolved bolus demonstrating a poor separation.

2.25 Polystyrene Separations

In June of 1993, it was believed that polystyrene separations might be successful. With polystyrene columns, it would still be possible to use solvents with a high solubility for fullerenes (carbon disulfide, toluene/decalin) with the widely available pore sizes of polystyrene columns. It was hoped that a successful separation would result based on a size-exclusion mechanism. In principle, it was an ideal response to the problem of solvent selection and resolving power of the stationary phase. With the polystyrene system, it would be possible to achieve high sample loadability since the fullerenes were very soluble in this mobile phase. Since the separating mechanism was predicted to be based on size exclusion, there were high expectations for success with polystyrene separations. Various manufacturers even began shifting sales efforts toward polystyrene columns for fullerene separations.

However, the actual experimental data were conflicting. Depending on the type of column and mobile phase, some research groups\textsuperscript{168,169} did achieve a size-exclusion based polystyrene separation (i.e. $C_{84}$, $C_{70}$, then $C_{60}$). Yet others achieved an
absorption-type separation\textsuperscript{32,173,174,246} (i.e. elution of C\textsubscript{60}, C\textsubscript{70}, then C\textsubscript{84}). In these experiments, it was proposed that a weak \( \pi-\pi \) interaction between the metallofullerene and polystyrene substrate was the dominant separation mechanism. Nevertheless, the polystyrene did permit a much higher sample throughput relative to reverse-phase separations. Because the separation factors were often poor with polystyrene columns, several "recovery and re-injection" steps were often necessary to obtain a metallofullerene-containing fraction. In fact, some polystyrene-based separations (manufacturers' brochures) were so poor that four or more columns had to be connected in series to achieve sufficient resolution for fraction collection. To improve the separation and avoid backpressure problems, low flow rates were often utilized. Under these conditions, some separations required over 10 hours for an injected sample to elute. Some common stationary phases utilized in fullerene and metallofullerene separations are presented in Figure 2.1.

2.26 Buckyclutcher Separations

Fortunately, a much more selective column became commercially available. Developed by Pirkle and Welch,\textsuperscript{5} this silica gel-based stationary phase consisted of tripodal, tridentate di-nitrophenyl ligands capable of strong \( \pi-\pi \) complexation
Figure 2.1  Summary of the four most common stationary phases in metallofullerene separations.
interactions with the metallofullerenes. With such a strong retention mechanism, the use of solvents with a high solubility for fullerenes could now be incorporated without sacrificing resolution. This feature permitted even larger sample throughput for preparative clean-up injections of initial raw extract. In addition, the improved selectivity later permitted the first isolation of purified metallofullerene samples.

2.27 TPP Separations

Other types of silica-gel based columns have now become commercially available. Historically, the silica-based tetraphenyl-porphyrin (TPP) based stationary phase had previously been utilized in the separation of aromatic compounds.\textsuperscript{252} Shortly thereafter, this type of column was successfully extended for use in both fullerene separations\textsuperscript{248} and metallofullerene separations.\textsuperscript{178,184} For example, Savina et al.\textsuperscript{184} utilized a binary solvent system (toluene/CS\textsubscript{2}) with this TPP column to obtain a purified La@C\textsubscript{82} sample.\textsuperscript{184}

2.28 Buckyprep Separations

Recently, another highly selective silica gel-based column has been utilized in
metallofullerene separations. Historically, the pyrenyl-ethyl (PYE) based stationary phase had been utilized in the separation of aromatic compounds.\textsuperscript{254,255} For this reason, the PYE column was also extended for use in fullerene and metallofullerene separations.\textsuperscript{182,187,188,190,191} Similar to the Buckyclutcher, the pyrenyl-ethyl based column (PYE or "Buckypep")\textsuperscript{182,187,188,190,191} offers high selectivity, reduced solvent consumption, high sample loadability, and rapid separations. This is accomplished through a retention mechanism thought to consist of a combination of steric, dipolar, and $\pi$-electron interactions. This electron-accepting stationary phase also has the flexibility to be utilized for initial preparative clean-up procedures or in the final purification stages. Buckypep columns have now been utilized in the isolation of Sc$_2$@C$_{82}$, La@C$_{82}$, Gd@C$_{82}$, and Pr@C$_{82}$.\textsuperscript{182,187,188,190,191}

2.29 Isolation

To date, several research groups\textsuperscript{168-172,174-178,182-188,190,191} have now obtained purified metallofullerene samples. The methodology has typically involved two types of chromatographic columns. These "two-stage" systems have involved a combination of any two of the previously mentioned columns. For example, an initial preparative clean-up step to remove empty-cage fullerenes will typically result in a
metallofullerene-containing fraction. However, this sample will often be contaminated with low levels of at least one co-eluting empty-cage fullerene. Through injection with a second type of column (e.g. Buckyclutcher), these contaminants will no longer co-elute with the desired metallofullerene. In this manner, a purified metallofullerene can often be obtained.

The development of purification methodologies for the metallofullerenes has resulted from a global scientific effort. Early pioneers in the chromatographic separation of metallofullerenes were headed by Shinohara, Dorn, and Kikuchi. In summary, Shinohara et al. have isolated Sc$_2$@C$_{74}$, Sc$_2$@C$_{84}$, Sc$_3$@C$_{82}$, Pr@C$_{82}$. Dorn et al. have isolated Sc$_2$@C$_{74}$, Sc$_2$@C$_{84}$ (two isomers), Sc$_3$@C$_{82}$, La$_2$@C$_{72}$, Er@C$_{82}$, Er$_2$@C$_{82}$ (two isomers), and Er$_2$@C$_{92}$. Kikuchi et al. have isolated La@C$_{82}$ and La$_2$@C$_{80}$. Yamamoto et al. have obtained purified La@C$_{82}$. Meyerhoff et al. have also isolated La@C$_{82}$. With all these different purification methodologies now published and available, other scientists should now be able to obtain even larger amounts of purified metallofullerene samples. Now that the pioneering research on metallofullerene separations has been performed, the next few years will likely represent a new era in the field of metallofullerene science. Namely, a plethora of long-awaited characterization experiments will be performed to discover what properties these new,
exciting molecules will exhibit.

2.3 HPLC-EPR

2.31 Historical

Historically, the monitoring of paramagnetic species as they are eluted from a chromatographic column has existed since 1975.\textsuperscript{256} In a landmark paper, the on-line HPLC-EPR approach was demonstrated by Rokushika et al\textsuperscript{256} with the separation of stable organic radicals. Since then, the HPLC-EPR approach has been utilized in the separation of metal complexes,\textsuperscript{257-260} spin adducts,\textsuperscript{261-271} and spin-trapped\textsuperscript{272-295} species.

However, the HPLC-EPR technique had not been applied to the area of metallofullerenes. Therefore, a secondary objective of this research focused on utilizing HPLC-EPR as a technique to assist in the development of a separation methodology which would result in purified metallofullerene samples.

2.32 Advantages

There are several distinct advantages for selecting an on-line EPR approach versus
conventional UV detection alone. The primary advantage of on-line EPR detection of metallofullerene separations is the high specificity for metallofullerenes with an odd number of encapsulated metal atoms. Specifically, recent experiments$^{137,140,142,143,145-147,151,152,156,158,160,164,166,169,173,174,176,177,179,181,182}$ have demonstrated Sc@C$_{82}$, Sc$_2$@C$_{82}$, Y@C$_{82}$, and La@C$_{82}$ to be EPR active. As an example, a typical scandium raw extract represents a complex mixture of at least 30 empty-cage fullerenes as well as ~20 Sc$_m$@C$_{2n}$ metallofullerene species differing in the number of encapsulated metal atoms, the number of structural isomers, and the number of carbons in the surrounding cage network. Experimentally, the empty-cage fullerenes and di-scandium species (Sc$_2$@C$_{2n}$) are essentially diamagnetic and therefore EPR silent. In contrast, the mono-metal Sc@C$_{82}$ and tri-metal Sc$_3$@C$_{82}$ species are paramagnetic and EPR active. Therefore, the striking feature is that only two of the ~50 species in the initial raw extract would exhibit significant EPR activity. This represents a distinct advantage of on-line EPR detection. With this approach, it is possible to monitor a single metallofullerene species in the midst of an overwhelming abundance of all other fullerene and metallofullerene compounds. As chromatographic conditions (e.g., mobile phase, stationary phase, flow, etc.) are optimized, it is possible with on-line EPR detection to monitor the effect of these changes on a given separation. Since this is accomplished on-line, the extra time
involved in fraction collection, sample handling, and off-line characterization procedures can be avoided.

There are other advantages to the HPLC-EPR approach. With on-line EPR detection, accurate retention times for EPR active metallofullerenes can be conveniently determined without resorting to off-line techniques. Since the on-line technique is non-invasive, the recovery of valuable metallofullerene samples and coupling to other HPLC detectors is straightforward. Since the on-line EPR approach monitors the eluting metallofullerene species in a controlled anaerobic environment, the potential decomposition of air-sensitive metallofullerenes is not compromised, (Previous reports have demonstrated that some metallofullerene species are susceptible to decomposition upon exposure to air).

2.33 Disadvantages

There are disadvantages to the on-line HPLC-EPR technique. Since the majority of species are diamagnetic, on-line EPR detection is not possible. Thus, on-line EPR experiments involving di-metal species (A₂@C₂₅₆) and empty-cage (C₂₅₆) fullerenes would not be useful. Another consideration is the sensitivity of the flow EPR experiment. In a typical Aₘ@C₂₅₆ stock solution, the concentration of paramagnetic
species in the EPR flow cell is estimated at only $10^{-5}$ to $10^{-6}$ M.

In addition, the extra "lag time" between the conventional UV detector and EPR flow cell must be accurately measured. This step is crucial in correlating EPR activity with a corresponding peak on the UV-generated chromatogram. For optimal fraction collection, the additional time from the EPR flow cell (detection) and the outlet must also be accurately determined for optimal fraction collection. In addition, band broadening occurs with the extra void volume originating from the on-line EPR flow cell and connecting tubing.

2.4 Goal and Justification

At the time this research began, there was no protocol available for obtaining purified metallofullerene samples. Thus, our primary goal was to develop a chromatographic methodology which would permit purified metallofullerenes. Since these molecules were predicted to have many unique applications (see Introduction), there was a high demand for purified samples so that characterization experiments could be performed. In this regard, this research addresses and fulfills this need for obtaining purified metallofullerene samples.
CHAPTER 3: EXPERIMENTAL

3.1 Metallofullerene Samples

3.11 Production

All metallofullerene samples were produced by IBM and provided to our lab with the intent of developing a suitable chromatographic methodology for their isolation. Scandium, yttrium, lanthanum, and erbium metallofullerene-containing soot was produced in a JS-2000 fullerene generator under a He atmosphere. This electric-arc synthesis was similar to the Krätschmer-Huffman method. Specifically, cored-carbon rods were impregnated with mixtures of graphite and either pure metal, metal oxide, or metal carbide. Previous experiments suggested that the form in which the metal is introduced does not drastically affect the yield and product distribution. It should be noted that the ratio of metal to carbon atoms in these initial rods affects the amount of mono-, di-, and tri-metallofullerene species. For example, a lower ratio of Sc metal to carbon atoms (1% Sc) will result in an improved yield of Sc@C₈₂ relative to Sc₂@C₈₂. In contrast, a higher ratio (3-5% Sc) will generate more Sc₃@C₈₂ and reduced quantities of Sc@C₈₂. Typical production
experiments yield only $\sim 1\%^{134,144-146,155,162}$ metallofullerenes with the empty-cage fullerenes ($\sim 99\%$) dominating the soluble product distribution. The empty-cage $C_{60}$ is the dominant species followed by $C_{70}$ and $C_{84}$.

3.12 Extraction

Once these cored rods are "burned", the resulting metallofullerene-containing soot must be extracted. This extraction step is critical since the choice of solvent and extraction methods dictate the amount of empty-cage and metallofullerene material that will be obtained from the soot. Empty-cage fullerenes are virtually insoluble in hexane, pentane, cyclohexane, acetonitrile, and water.$^{29}$ Solubility increases with solvents such as benzene, toluene, and decalin. Even higher solubilities are achieved with ortho-dichlorobenzene, $\alpha$-chloronaphthalene, and CS$_2$. A detailed solubility study of the empty-cage $C_{60}$ fullerene has recently been published.$^{29}$ Although it is believed that the metallofullerenes possess similar solubilities as the empty-cage species, there has not been a corresponding detailed study of purified metallofullerene solubilities.

In our laboratory, metallofullerene soot was located in thimbles and extracted through Soxhlet and/or static techniques. Although CS$_2$ was the primary choice of
solvent, ortho-dichlorobenzene and toluene have also been employed. Following extraction, the solvents were evaporated - leaving a solid, black powder which was then weighed. Prior to chromatography, this initial raw extract was filtered over a home-built bed of glass wool, sand, and silica gel. This step was useful in preventing any remaining insoluble soot from possibly clogging the chromatographic tubing and columns. With the 80:20 toluene/decalin solvent, approximately 3 mg/mL would dissolve into the solution. Thus, the solubility of metallofullerenes in this stock solution limited the amount of material that could be separated in the initial clean-up procedure.

3.13 Mobile Phase

A 80% toluene (Fischer) / 20% decalin (Aldrich Chemical Co.) mixture was selected as the mobile phase for polystyrene and Buckyclutcher column separations. The decision to incorporate decalin was based on a previous study in which decalin resulted in shorter retention times for higher molecular weight fullerenes (e.g. $C_{100}$ - $C_{130}$). In addition, it was suspected that the metallofullerenes also had increased solubilities in decalin. Meanwhile, carbon disulfide was employed as the mobile phase for experiments with the TPP and PBB columns. Regardless of which mobile
phase was employed, all solvents were degassed by either bubbling N₂ gas and/or sonication. Removing dissolved O₂ was essential for preventing decomposition of any air-sensitive metallofullerenes (e.g. Sc@C₈₂). In addition, on-line EPR spectra can often be obtained with higher resolution and an increased signal-to-noise ratio if the solvent is adequately degassed.

3.14 Chromatographic Equipment

This research focused primarily on four types of columns. The polystyrene separating columns (cross-linked) were connected in series with a Perkin-Elmer, 25 cm x 10 mm PL gel, 10 μm, 1000 Å column followed by a 25 cm x 10 mm PL gel, 5 μm, 5000 Å column. Typical chromatographic conditions for separations using the polystyrene columns were 1 mL/min of 80:20 toluene/decalin (UV detection, 340 nm). In addition, a more selective Trident-Tri-DNP HPLC column ("Buckyclutcher", 25 cm x 10 mm i.d., 5 μm gel, 100 Å, Regis Chemical, Morton Grove, IL, 60053, 1-800-323-8144) permitted sufficient resolution of individual fullerene and metallofullerene species. For this reason, the Buckyclutcher column was often utilized in final purification separations. A third type of column employed was a tetraphenyl-porphyrin derivatized silica-gel column ("TPP-RP" stationary phase, 10
μm gel, 300 Å pore size, Fullesep column, 4.6 x 100 mm, Anspec, Ann Arbor MI, 48107, 1-800-521-1720). A fourth type of chromatographic column ("PBB," Cosmosil packed, 10 mm x 25 cm, 5 μm gel, Phenomenex, Torrance, CA, 90501, 1-310-212-0555) utilized pentabromobenzyl ligands immobilized onto silica gel. The columns packed with a smaller size silica gel (i.e. 5 μm gel) typically offered improved resolution. All chromatographic experiments utilized a Hitachi L-4000 UV detector and D-2500 Chromato-Integrator recorder with flow rates ~1 mL/min. Typically, the 80:20 toluene/decalin solvent system was employed for all separations except for the PBB and TPP columns. These columns employed CS₂ as the mobile phase.

3.2 Instrumentation

3.21 HPLC-EPR Apparatus

For on-line HPLC-EPR experiments, an IBM 200D-SRC EPR spectrometer was placed immediately after the UV detector in the chromatographic flow stream. A simplified diagram of the HPLC-EPR apparatus is illustrated in Figure 3.1. The selection of EPR operating parameters (e.g. attenuation, time constant, sweep time,
Figure 3.1  Block diagram of the on-line HPLC-EPR apparatus. C1 and C2 are the separating chromatographic columns.
gain, and modulation) were optimized for the flow experiment. Since the paramagnetic metallofullerenes were present in such low abundance (<0.05 % in the raw extract), resolution in the EPR spectra was sacrificed at the expense of obtaining an increased signal-to-noise ratio. Specific EPR conditions included a high modulation amplitude (1.0 - 1.5 gauss), high microwave power (~10 mW), and a rapid sweep of the magnetic field at a rate of 4 - 7 gauss per second. Under these conditions, an expected doublet with a small hyperfine coupling constant (0.4-0.9 g) would appear as a "broadened singlet." During the course of this research, Wilson et al. also attempted on-line EPR detection for a Y@C\textsubscript{82} sample. Using a fixed field and frequency (i.e. no EPR spectral resolution), they obtained EPR activity as a function of time.

Microwave irradiation was provided by a Bruker microwave bridge operating at 9.54 GHz. The detection volume of the home-built EPR glass flow cell was measured at 250 µL. For on-line HPLC-EPR experiments, this flow cell was located in the front side of a dual TE\textsubscript{102} microwave cavity. PEEK tubing (0.010-in. i.d., Upchurch Sci.) connected chromatographic eluents to the EPR flow cell. For on-line experiments, 20 second magnetic field sweeps with a spectral window of 90 to 130 gauss were typical. An ASPECT 2000 computer permitted storage of subsequent on-line EPR spectra. Signal averaging of 2 to 4 scans/file provided "stacked plots" of
EPR spectra versus elution time. Each file corresponded to 1 - 3 minute segments of time.

3.21 Automated Apparatus

See Automated Section (Results and Discussion).

3.23 Mass Spectrometry

For off-line characterization of fractions, several different types of mass spectrometers were utilized. The majority of mass spectral data were obtained from a VG 7070E-HF mass spectrometer (VG Analytical, Manchester, UK). This apparatus could perform either positive-ion or negative-ion chemical ionization. Specifically, metallofullerene samples were injected onto a DCI probe filament. The current was ramped from 0 to 1 A at a rate of 0.05 A/sec to evaporate and desorb the sample. Methane was selected as the reagent gas.

Other spectra were obtained from a laser desorption-time of flight (LD-TOF) mass spectrometer located at IBM. In addition, fast atom bombardment (FAB) spectra have also been obtained from a VG Quattro mass spectrometer.
3.24 Two-Stage Chromatographic Systems

Separations involving only one type of column will not generally result in purified metallofullerene samples. This is due to the co-elution of the underlying, high-mass empty-cage fullerenes - which elute as a homologous series across the $A_m@C_{2n}$ fractions. For these reasons, we have developed in this research several two-stage systems which have permitted purified metallofullerene samples.

3.241 Polystyrene/Buckyclutcher System

Metallofullerene raw extracts (i.e. $Sc_m@C_{2n}$, $Y_m@C_{2n}$, and $La_m@C_{2n}$) were injected into the polystyrene columns as a first step to remove the dominant $C_{60}$-$C_{84}$ fullerenes. Due to the poor selectivity for these polystyrene columns, four to five "recovery and re-injection" steps of the $A_m@C_{2n}$ fraction were necessary. This sample obtained from the polystyrene separations (now enriched in $A_m@C_{2n}$) was further separated by the selective Buckyclutcher column into 10 to 15 additional peaks. Collection of these regions resulted in the isolation of individual metallofullerene samples with various levels of purity.
3.242 Buckyclutcher/TPP and Buckyclutcher/PBB Systems

Because five polystyrene re-injections were often necessary to remove the $C_{60} - C_{84}$ fullerenes, this process became tedious and time-consuming. With increased sample loadability and improved selectivity, the Buckyclutcher column quickly replaced the polystyrene columns for the initial preparative injections. Raw metallofullerene extract was separated in an initial Buckyclutcher pass for removal of $C_{60} - C_{84}$. Re-injection into the Buckyclutcher column would permit 10 to 15 single homogeneous peaks. However, to remove the underlying $C_{90} - C_{116}$ impurities, a second column (TPP or PBB column) was employed. These stationary phases would readily separate these higher-mass empty-cage contaminants from the metallofullerenes. In this manner, purified metallofullerene samples were obtained.
CHAPTER 4: RESULTS AND DISCUSSION

4.1 Metallofullerene Separations

4.11 Sc₉₀@C₂₄

A stock solution (~3 mg/mL) containing Sc₉₀@C₂₄ and empty-cage fullerenes was prepared for injection into two polystyrene columns connected in series. The production of this metallofullerene sample was optimized for an improved yield of Sc₃@C₈₂ relative to Sc@C₈₂. With negligible quantities of Sc@C₂₄, only Sc₃@C₂₄ species would result in an EPR signal. The empty-cages and Sc₂@C₂₄ compounds are diamagnetic and therefore EPR-silent.

Figure 4.1 represents a typical chromatogram for an initial, clean-up injection with the polystyrene columns. At 15 milligrams of injected material, this chromatogram suggests the column is overloaded with no useful separation in the UV domain. However, on-line EPR spectra reveals that the "tailing region" of the chromatographic peak contains the paramagnetic Sc₃@C₈₂. The majority of the peak area (~90 %) consists of C₆₀, C₇₀, C₈₄. Although a smaller injection would permit improved resolution, this large amount of injected sample represents a satisfactory compromise between resolution and sample throughput. Note that of the 22 lines expected (I =
Figure 4.1  (a) Initial polystyrene pass of Sc$_m$@C$_{2n}$ raw extract. HPLC-UV trace (340 nm), 5 mL injected (10-15 mg), 1.0 mL/min, and 80:20 toluene/decalin. (b) on-line HPLC-EPR profile, 9.55 GHz, 2.25 min/file, 4 scans/file, and 20 s/sweep.
7/2; 3 equivalent Sc nuclei) for Sc$_3$@C$_{82}$, only ~17 can be observed under these EPR conditions which were optimized for an improved signal-to-noise ratio (oversaturation; see Experimental).

A fraction corresponding to this EPR-active region was collected for subsequent, off-line mass spectral analysis. At this stage, the purity of this metallofullerene fraction was unknown. Although Sc$_3$@C$_{82}$ was in this fraction, it was not known whether additional metallofullerenes and/or empty-cages contaminants were also present. To address these considerations, negative-ion mass spectral data were obtained. The results indicated that C$_{60}$, C$_{70}$, and significant amounts of higher-mass empty-cages (C$_{84}$-C$_{120}$) were still present with the Sc$_3$@C$_{82}$ and at least ten other Sc$_2$@C$_{2n}$ species. Each re-injection of this EPR-active fraction into the polystyrene columns removed increasing quantities of the empty-cages C$_{60}$-C$_{92}$. For this reason, a total of four re-injections of the EPR-active fraction was performed. The chromatograms of the 1$^{st}$, 2$^{nd}$, 3$^{rd}$, and 4$^{th}$ polystyrene passes are presented in Figure 4.2. For the fifth and final polystyrene pass, the chromatogram and corresponding on-line EPR spectra are presented in Figure 4.3. Through retention time data, this chromatogram indicates that C$_{60}$, C$_{70}$, and C$_{84}$ have been removed from the EPR-active fraction. Furthermore, the ability of the HPLC-EPR technique to selectively "monitor" a metallofullerene from a starting stock to a final, collected fraction was
Figure 4.2  HPLC-UV trace for $\text{Sc}_{m} \lrcorner \text{C}_{2n}$ separations in which the EPR active fraction is recovered and re-injected. The first through fourth polystyrene passes are represented by (a) - (d), respectively. Flow rate 1 mL/min, UV 340 nm detection, and the EPR active fraction is denoted as the hatched region (30 - 37 min.).
Figure 4.3  (a) HPLC-UV trace (340 nm) for the fifth polystyrene pass of the Sc$_3$@C$_{82}$ EPR active fraction, 410 µL injection, 1.0 mL/min, and 80:20 toluene/decalin. (b) On-line HPLC-EPR profile, 4 scans/file, 9.55 GHz, and 20 s/sweep.
demonstrated for the first time. Note the surprisingly large signal-to-noise ratio for the Sc$_3$@C$_{82}$ fraction flowing through the small 250 μL EPR flow cell.

Unfortunately, the composition of this final EPR-active fraction was not purified Sc$_3$@C$_{82}$. Instead, a metallofullerene-enriched sample had been obtained. Nevertheless, the composition of this fraction represented a "turning point" in developing the metallofullerene separation process since this sample contained virtually all metallofullerenes and negligible amounts of the higher-mass empty-cage fullerenes. For the first time, a methodology had been developed to remove C$_{60}$-C$_{92}$ from an initial extract and eventually obtain a mixture of metallofullerenes. The negative-ion mass spectrum of this final EPR-active fraction is shown in Figure 4.4. Sc@C$_{82}$ is not observed since it was a low-abundant compound in the starting stock. A series of di-scandum Sc$_2$@C$_{74}$ - Sc$_2$@C$_{102}$ metallofullerenes is readily observed in the mass spectrum. The paramagnetic Sc$_3$@C$_{82}$ (m/z = 1119) species is also relatively abundant in this sample. Only small amounts of extremely high molecular weight empty-cage fullerenes contaminated the fraction. It should be noted that since all Sc$_2$@C$_{2n}$ species had a similar retention time on the polystyrene columns relative to the EPR-active Sc$_3$@C$_{82}$, it was possible to obtain a sample that contained only metallofullerenes. Ironically, the poor selectivity of the polystyrene columns for individual metallofullerenes was beneficial since the metallofullerenes co-eluted with
Figure 4.4  Off-line, negative-ion CI mass spectrum for the fourth polystyrene pass Sc₄@C₈₂ EPR active fraction. The "*" indicates calibration peaks for the standard Ultramark 1621.
each other.

In summary, the polystyrene technique was tedious and cumbersome since at least four to five polystyrene re-injections were necessary to effectively remove large quantities of the lower-mass empty-cage fullerenes. On the other hand, a valuable sample containing only metallofullerenes could eventually be obtained.

At this time, it was realized that the polystyrene column did not have sufficient resolving power to further separate this mixture of metallofullerenes. Fortunately, a column (Buckyclutercher), which claimed effective separations of fullerene compounds, became available. Developed by Welch and Pirkle, this stationary phase consisted of tripodal (2,4-dinitrophenyl) ligands which possessed increased π-π complexation interactions with the metallofullerene family.

For this reason, the EPR-active metallofullerene fraction was separated with this selective column for the final purification stages. A typical chromatogram following a typical 500 μL injection is presented in Figure 4.5a. Since many peaks were observed, ten arbitrary fractions were collected for subsequent off-line EPR and mass spectral characterization. The corresponding on-line EPR activity for this injection is shown in Figure 4.5b. Although the signal-to-noise ratio is poor, a partial EPR spectrum of Sc₃@C₈₂ can be observed. Note that the maximum EPR activity corresponds to peak #6 on the chromatogram. As further confirmation to Sc₃@C₈₂,
Figure 4.5  (a) HPLC-UV trace (340 nm) of the EPR active $\text{Sc}_3@\text{C}_{82}$ fraction after five polystyrene passes, 250 µL detection, Buckyclutcher column, 2.1 mL/min, 80:20 toluene/decalin, EPR active region is peak #6. (b) On-line HPLC-EPR profile, 3 scans/file, 9.55 GHz, and 20 s/sweep.
fraction #6 was evaporated, concentrated, and degassed for subsequent high resolution, off-line EPR characterization. After a number of collection and re-injections of fraction #6, a purified Sc₃@C₈₂ sample of > 90% purity was obtained. The expected 22-line pattern is easily observed as indicated by its EPR spectrum (Figure 4.6). An analytical injection of this sample and corresponding negative-ion mass spectrum are presented in Figure 4.7.

It should be noted that an isolated Sc₃@C₈₂ sample was sent to IBM for further EPR characterization. In that study,¹⁷⁷ EPR spectra were taken at temperatures ranging from 77 °K to 333 °K. According to linewidth analysis, it was possible to monitor the dynamics of the Sc₃ trimer inside the C₈₂ fullerene cage network. The data suggest that the Sc atoms of the Sc₃ trimer reorient rapidly inside the C₈₂ surrounding cage network. At 200 K, a reorientational energy barrier for the Sc ions was determined to be 28 meV with a correlation time of 5 × 10⁻⁹ s.

Peak #6 was not the only fraction that was isolated. Specifically, fractions #0-9 were also collected and re-injected until single, homogeneous peaks for each fraction were obtained. The analytical traces and corresponding mass spectra are presented in Figure 4.8a through Figure 4.8m. A mass spectrum for each of these samples was then obtained to ascertain their purity. Although symmetrical, single peaks were observed in the chromatograms, not all samples were pure and were contaminated
Figure 4.6  Off-line EPR spectra of purified peak #6 (Sc$_3$@C$_{80}$) in decalin. (a) 9.63 GHz, 500 s/sweep, one scan, and 25 °C. (b) 9.63 GHz, 500 s/sweep, one scan, and -70 °C.
Figure 4.7  HPLC-UV chromatogram, 10 μL of purified peak #6 (Sc\textsubscript{3}@C\textsubscript{82}), Buckyclutcher column, 340 nm detection, and 2.1 mL/min of 80:20 toluene/decalin.
Figure 4.8a  (a) Analytical HPLC-UV trace (*peak #0*), Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8b  (a) Analytical HPLC-UV trace (peak #1). Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8c  (a) Analytical HPLC-UV trace *(peak #2)*, Buckycluster column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8d  (a) Analytical HPLC-UV trace (peak #3), Buckycluther column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8e  (a) Analytical HPLC-UV trace (peak #4), Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8f  (a) Analytical HPLC-UV trace (*peak #5*), Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8g  (a) Analytical HPLC-UV trace (peak #6), Buckyclutcher column. 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8h  (a) Analytical HPLC-UV trace (peak #7). Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8i  (a) Analytical HPLC-UV trace (peak #8), Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8j  (a) Analytical HPLC-UV trace (*peak #9, 1 series*). Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.8k  (a) Analytical HPLC-UV trace (*peak #9.2 series*), Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ültramark 1621 as the calibration standard.
Figure 4.81  (a) Analytical HPLC-UV trace (*peak #9.3 series*), Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection.  (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.  (c) off-line EPR spectrum of above, 44 scans, 9.64 GHz, sweep width 140 g, and 200 s/sweep.  Sample was "freeze-thaw" degassed in decalin.
Figure 4.8m  (a) Analytical HPLC-UV trace (*peak #9.4 series*), Buckyclutcher column, 2.1 mL/min of 80:20 toluene/decalin, and 340 nm detection. (b) off-line negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
with other co-eluting metallofullerenes. Nevertheless, several purified samples were obtained. For the first time, we had isolated Sc₂@C₇₄ (#0, ~40% purity), Sc₂@C₈₄ (isomer I, ~95% purity; isomer II, ~85% purity), and Sc₃@C₈₂ (#6, ~90% purity). The composition of Scₘ@C₂ₙ fractions # 0-9 are summarized in Table 4.1.

As noted above, two isomers of the Sc₂@C₈₄ metallofullerene were chromatographically isolated. These two purified samples were characterized by our collaborating IBM research lab utilizing energy dispersive X-ray spectroscopy (EDS) and high resolution tunneling electron microscopy (TEM). Results of these experiments have recently been published in *Nature.* Specifically, it was found that Sc₂@C₈₄ (isomer from fraction 3) molecules are packed in a hexagonal-close-packed (hcp) structure. The ratio of lattice constants c/a = 1.63 is comparable to the value expected for ideal-sphere packing. The spacing between individual Sc₂@C₈₄ molecules was 11.2 Å, a distance comparable to the empty-cage C₈₄ fullerene.

Although a significant amount of final metallofullerene samples were obtained (~1 mg), several questions regarding the "missing" Sc@C₈₂ metallofullerene remained largely unanswered. It was uncertain when or if Sc@C₈₂ would elute from the Buckyclutcher column. Since the Scₘ@C₂ₙ separations (#0-9) mentioned *vide supra* involved negligible amounts of Sc@C₈₂ in the initial, starting stock solution, this species was not found in the final collected Scₘ@C₂ₙ fractions (#0-9). For this
**TABLE 4.1**

Composition of Scₘ@Cₙ Fractions
Buckycluther column

<table>
<thead>
<tr>
<th>Scₘ@Cₙ Fraction</th>
<th>Dominant Aₘ@Cₙ</th>
<th>Next Dominant Aₘ@Cₙ</th>
<th>Least Dominant Aₘ@Cₙ</th>
</tr>
</thead>
<tbody>
<tr>
<td>#0</td>
<td>Sc₂@C₇₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>Sc₂@C₈₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#2</td>
<td>Sc₂@C₈₈</td>
<td>Sc₂@C₈₂</td>
<td>Sc₂@C₉₀</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>#4</td>
<td>Sc₂@C₉₀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#5</td>
<td>Sc₂@C₇₆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#6</td>
<td>Sc₃@C₈₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#7</td>
<td>Sc₂@C₉₂</td>
<td>Sc₂@C₉₄</td>
<td></td>
</tr>
<tr>
<td>#8</td>
<td>Sc₂@C₈₆</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#9.1</td>
<td>Sc₃@C₈₄</td>
<td>Sc₂@C₈₄</td>
<td>Sc₂@C₉₂ - Sc₂@C₁₀₀</td>
</tr>
<tr>
<td>#9.2</td>
<td>Sc₂@C₉₄</td>
<td>Sc₃@C₈₄</td>
<td></td>
</tr>
<tr>
<td>#9.3</td>
<td>Sc₂@C₈₄</td>
<td>Sc₃@C₈₄</td>
<td>Sc₂@C₉₄</td>
</tr>
<tr>
<td>#9.4</td>
<td>(Sc₃C₂)@C₈₄ ? or Sc₃@C₆₆ ?</td>
<td>Sc₂@C₉₆ - Sc₂@C₁₀₄</td>
<td></td>
</tr>
</tbody>
</table>
reason, a new Sc_{m}@C_{2n} extract was prepared with a smaller, initial Sc/C metal loading ratio in the hope that the Sc@C_{82} concentration would increase. An off-line EPR spectrum of this stock solution is given in Figure 4.9. In this spectrum, the octet of the Sc@C_{82} species is distorted by the 22-line pattern expected for Sc_{3}@C_{82}. In addition, these signals are also distorted due to excessive microwave power (saturation) conditions of the EPR spectrometer. Although these operating conditions distort the off-line spectra, they provide the on-line HPLC-EPR experiment with a higher signal-to-noise ratio.

Since the EPR detection limits for a flowing Sc@C_{82} sample were unknown, a large amount of starting extract (5 mL) was injected into the HPLC-EPR apparatus. Although the Buckyclutcher was overloaded, there was a sufficient quantity of Sc@C_{82} in the EPR flow cell for observation. Although the on-line EPR spectra are distorted, the entire, expected octet for Sc@C_{82} can be observed at 19 minutes as presented in Figure 4.10. In contrast, ~16 of the 22 expected lines of Sc_{3}@C_{82} are observed at 23 minutes.

This on-line data was valuable. First, it established that Sc@C_{82} could potentially be isolated by chromatography. Before, it was unknown whether Sc@C_{82} was permanently retained on the column or possessed such a long retention time that its elution profile was too broad for efficient collection. In addition, the on-line data
Figure 4.9 Off-line EPR spectrum of Sc$_{m}@C_{2n}$ raw extract to be injected for Buckyclutcher separations. Spectrum is taken under high modulation, saturation, and rapid sweep operating conditions (see Experimental), 20 s/sweep, 4 scans/file, sweep width 150 g, and 9.55 GHz.
Figure 4.10 On-line HPLC-EPR experiment: (a) HPLC-UV trace following a 20 mg injection of Sc₃@C₈₂ raw extract, Buckyclutcher column, 340 nm detection, and 2.0 mL/min, of 80:20 toluene/decalin. (b) on-line HPLC-EPR profile of above, 3 scans/file, 20 s/sweep, 9.55 GHz, and alternate files are shown.
readily established the elution order of Sc@C_{82} before Sc_{3}@C_{82}, without the need for off-line analysis. The monitoring of potentially unstable species (e.g. Sc@C_{82}) is a distinct advantage of the on-line HPLC-EPR approach. Although off-line EPR characterization (Figure 4.11) and mass spectral data were obtained to corroborate the on-line EPR data, there were potential risks of utilizing off-line techniques alone. Before the on-line EPR data, it was uncertain if Sc@C_{82} eluted from the column but decomposed during the off-line sample handling. Whereas the di-metal Sc_{2}@C_{2n} and tri-metal Sc_{3}@C_{2n} compounds appeared to be stable, the monometal Sc@C_{2n} species was previously suspected to undergo irreversible aerobic oxidation.

Re-injection of this Sc_{1}@C_{2n}/Sc_{3}@C_{2n} EPR-active fraction into the Buckyclutcher indicated resolvable peaks in the chromatogram (Figure 4.12). In an experiment to ascertain which peaks were due to Sc@C_{82} and Sc_{3}@C_{82}, the on-line HPLC-EPR apparatus was not utilized. In this manner, the lag time between UV detection and manual fraction collection was reduced to a few seconds and more accurate peak assignments and retention times could therefore be made. The off-line EPR spectra for these corresponding fractions are presented in Figure 4.13. Based on EPR and mass spectral data, the regions at 18.8 minutes and 20.8 minutes were assigned to Sc@C_{82} and Sc_{3}@C_{82}, respectively.
Figure 4.11  Off-line EPR spectra of collected $\text{Sc}_m@\text{C}_{2n}$ fractions (see Figure 4.10) centered at 17-20 min. and 20-26 min., respectively. (a) 17-20 min. fraction, sweep width 100 g, 100 s/sweep, 4 scans/file, 9.63 GHz, and the solvent is decalin. (b) #20-26 min. $\text{Sc}_m@\text{C}_{2n}$ fraction, sweep width 150 g, 100 s/scan, 9.63 GHz, in decalin.
Figure 4.12  HPLC-UV trace, (second Buckyclutcher pass), 450 μL of the 20-26 min. Scₘ@C₂ₕ EPR active fraction, 2.0 mL/min, 80:20 toluene/decalin, and 340 nm detection.
Figure 4.13  Off-line EPR spectra corresponding to the experiment from Figure 4.12.  (a) Sc@C$_{82}$ fraction, sweep width 100 g, 100 s/scan, 4 scans/file, 9.63 GHz, in decalin.  
(b) Sc$_3$@C$_{82}$ fraction, sweep width 150 g, 100 s/scan, 4 scans/file, 9.63 GHz, in decalin.
4.12 $Y_m@C_{2n}$

Because $Y_m@C_{2n}$ species were desired for $^{89}\text{Y}$ NMR and dynamic nuclear polarization (DNP) experiments, research efforts also focused on separations involving yttrium metallofullerenes. Since a typical starting yttrium stock solution contains only <1% metallofullerenes, a large amount of $Y_m@C_{2n}$ raw extract (~8 mg) was injected into the two polystyrene columns (Figure 4.14). To ensure a sufficient quantity of $Y@C_{82}$ and exceed the detection limits in the flow HPLC-EPR experiment, this high sample loading was employed. Note that the toluene/decalin solvent system permits a relatively high solubility of $Y_m@C_{2n}$ extract. Therefore, a large sample throughput is possible. In contrast, the fullerenes and metallofullerenes are virtually insoluble in typical reverse-phase solvents (e.g. acetonitrile, water, ether, etc).

Following injection, the UV chromatogram (Figure 4.14a) provides little worthwhile information other than indicating an overloaded column. It is not obvious where any of the metallofullerenes elute relative to the abundant empty-cage fullerenes. Although a lack of chromatographic resolution is indicated in the HPLC-UV trace, the on-line EPR dimension (Figure 4.14b) clearly demonstrates that a significant initial separation of $Y@C_{82}$ relative to the empty-cage fullerenes has
Figure 4.14  (a) HPLC-UV trace of $Y_m@C_{2n}$ raw extract, first polystyrene pass, 1.0 mL/min, 80:20 toluene/decalin, 8 mg injected, and 340 nm detection. (b) on-line HPLC-EPR spectra, sweep width 130 g, 4 scans/file, and 20 s/sweep. The broad singlet was observed due to low resolution conditions (see Experimental).
occurred. Specifically, an EPR-active chromatographic region from 28 to 37 minutes has been established for Y@C₈₂ as denoted by the cross-hatched region. The extremely small area under the curve is also consistent with a low (<1%) abundance of the Yₘ@C₂ₙ species in the initial stock solution. For comparison, the dominant peak (unshaded region) consists of the empty-cages C₆₀ - C₉₂ with retention times ranging from 23 - 29 minutes, respectively. Since true size exclusion chromatography (SEC) is not observed, these large differences in retention times (5 - 10 min) between the empty-cage C₆₀ region and Y@C₈₂ have been attributed to weak \(\pi-\pi\) interactions of the Yₘ@C₂ₙ species with the polystyrene substrate.

At this point, the EPR active region (tailing portion of the UV trace) was not resolved from the empty-cage fullerenes. Thus a sequence of "recovery and re-injection" steps for this Y@C₈₂ fraction was performed to improve the separation. Following two initial polystyrene passes, mass spectral data of the EPR active fraction indicated the presence of Y@C₈₂ in addition to the di-yttrium metallofullerenes (Y₂@C₈₀ - Y₂@C₁₀₄) and empty-cage fullerenes C₆₀ - C₁₂₀. The composition of the pre- and post-regions to the EPR active fraction was determined. Mass spectra of these samples indicated the presence of only empty-cage fullerenes of various molecular masses. Thus, it was established the entire yttrium metallofullerene profile eluted in the same fraction as Y@C₈₂. In this manner,
monitoring the paramagnetic Y@C₈₂ species served as a "marker" for the overall metallofullerene region.

In an effort to further remove tailing empty-cage fullerenes from the EPR active fraction, five polystyrene passes were necessary. A summary of the 1st - 5th polystyrene passes is presented in Figure 4.15. The composition of the EPR active fraction after the fifth polystyrene pass is indicated in Figure 4.16. Although most of the lower mass empty-cage C₆₀ - C₉₂ fullerenes have been removed, there are still significant quantities of higher mass C₉₈ - C₁₀₆ empty-cage fullerenes. Nevertheless, this sample is composed primarily of yttrium metallofullerenes. Specifically, the mono-metal Y@C₈₂ and di-metal species (Y₂@C₆₀ - Y₂@C₉₈) are present. The large number of Y₂@C₂ₙ species indicated that the isolation of individual, purified metallofullerenes would be difficult. In contrast to the Scₘ@C₂ₙ separations, there is no corresponding mass spectral evidence for Y₂@C₇₄, Y₂@C₇₆, or the tri-metal Y₃@C₈₂. Although this Yₘ@C₂ₙ "enriched" EPR active fraction could have been further separated with the Buckyclutcher column (analogous to the scandium case), the small amount of sample remaining after five polystyrene passes prevented further purification efforts.

A second objective of the Yₘ@C₂ₙ separation process was to establish the retention time of Y@C₈₂ with the Buckyclutcher column. Since the corresponding Sc@C₈₂
Figure 4.15  HPLC-UV chromatograms for $Y_m@C_{2n}$ polystyrene injections, 2.0 mL/min, 80:20 toluene/decalin, and 340 nm detection. HPLC profiles represent (a) a first polystyrene pass, (b) a third polystyrene pass, and (c) a fifth polystyrene re-injection of the collected EPR active $Y_m@C_{2n}$ fraction.
Figure 4.16  Off-line negative-ion mass spectrum of the final, collected $Y_{n} @ C_{2n}$ EPR active fraction (fifth polystyrene pass). The calibration standard Ultramark 1621 is used.
analog was susceptible to decomposition, it was believed that Y@C\textsubscript{82} would also be
difficult to monitor. Thus, an effort to perform any experiments with minimal sample
handling and time was made. Injection of Y\textsubscript{m}@C\textsubscript{2n} extract directly into the
Buckyclucher would not permit an accurate retention time due to such a large
injection volume and subsequent column overloading. An excessive amount of
injected material and corresponding sample volume would be necessary to ensure
sufficient Y@C\textsubscript{82} in the EPR flow cell. Column overloading and inaccurate retention
times would have resulted. Thus, it was necessary to perform at least two polystyrene
passes to remove a portion of the abundant empty-cage fullerenes. Although five
polystyrene passes would have removed even more empty-cage species, the additional
time and sample handling would likely have been detrimental to preventing
decomposition. For this reason, only two polystyrene clean-up passes of the EPR
active region were performed.

Next, 500 \textmu L of this Y@C\textsubscript{82} - concentrated sample was injected into the
Buckyclucher. The UV trace (Figure 4.17a) reveals at least 15 distinct peaks, and
it is not possible to determine which one corresponds to Y@C\textsubscript{82} with conventional
UV detection alone. However, the on-line EPR profile (Figure 4.17b) clearly
indicates the presence of Y@C\textsubscript{82} at 17.3 minutes. It should be emphasized that on-
line HPLC-EPR identified fraction #5 as containing Y@C\textsubscript{82} without the necessity of
Figure 4.17  (a) HPLC-UV trace (340 nm), Buckyclutcher column, 2.0 mL/min of 80:20 toluene/decalin, and 250 µL injection of the Y_m@C_{2n} EPR active fraction obtained from the final polystyrene pass. (b) on-line HPLC-EPR profile, 9.56 GHz, 3 scans/file, and 20 s/scan. Alternate files are not shown.
off-line fraction collection and characterization. The high selectivity of on-line EPR
detection and its ability to chromatographically monitor labile species was
demonstrated. Although a doublet with a small hyperfine coupling (0.48 g) has
recently been reported for Y@C₈₂ (I=½) under high resolution, static conditions, the
on-line EPR operating conditions (saturation, rapid sweeps, etc) were optimized for
an improved signal-to-noise ratio. As a result, this small hyperfine coupling was not
observed, and a broadened singlet was obtained. Nevertheless, an EPR active fraction
(peak #5; 16.5 - 18.2 min.) was obtained. A recovery and re-injection of peak #5 for
a second Buckyclutter was performed. At this stage, a relatively symmetric UV
trace was observed as indicated by Figure 4.18. However, the off-line negative-ion
mass spectrum revealed that Y₂@C₈₄ and Y₂@C₉₆ co-eluted with Y@C₈₂. In addition,
the higher mass C₁₀₄ empty-cage fullerene was also present. At this point, further
clean-up and isolation of the individual yttrium metallofullerenes was not attempted.
Efforts shifted toward chromatographic separations involving other encapsulated
metals (e.g. La, Er).
Figure 4.18  (a) Analytical HPLC-UV trace for isolated $Y_{m}@C_{82}$ peak #5 (EPR active region), Buckyclutcher column, 2.0 mL/min, and 340 nm detection. (b) off-line negative ion mass spectrum of peak #5
4.13 \( \text{La}_{m}\text{@}C_{2n} \)

Depending on the production methodology, a lanthanum-containing raw extract can possess fewer metallofullerene species to separate. In a typical \( \text{La}_{m}\text{@}C_{2n} \) stock solution, the mono-metal \( \text{La@C}_{82} \) is often the most dominant metallofullerene with certain di-metal \( \text{La}_{2}\text{@}C_{2n} \) species also being abundant. For these reasons, it was initially believed that \( \text{La@C}_{82} \) could be readily isolated.

From ~50 mg of raw \( \text{La}_{m}\text{@}C_{2n} \) extract, a stock solution was made at 3 mg/mL. Since the \( \text{La@C}_{82} \) on-line detection limits were not established, a relatively large sample volume (5 mL) was injected. The resulting chromatogram (Figure 4.19a) once again suggests an overloaded column with poor resolution. Analogous to both the Sc\(_{m}\text{@}C_{2n}\) and Y\(_{m}\text{@}C_{2n}\) case, this \( \text{La}_{m}\text{@}C_{2n} \) chromatogram is similar in that the \( \text{La@C}_{82} \) EPR active region also occurs underneath the tailing region of the peak. Specifically, the EPR active region (Figure 4.19b) occurs between 28 and 35 minutes. Once again, the unshaded region consists of lower mass C\(_{60}\) - C\(_{84}\) empty-cage fullerenes. To effectively remove these empty-cage contaminants, five polystyrene passes were necessary. The 1\(^{st}\), 3\(^{rd}\), and 5\(^{th}\) pass UV traces are summarized in Figure 4.20. The chromatogram and on-line HPLC-EPR spectra (Figure 4.21) for the 5\(^{th}\) polystyrene pass clearly demonstrate that the EPR active region corresponds to the final peak on
Figure 4.19  (a) HPLC-UV trace of 10 mg La$_{82}$@C$_{2n}$ raw extract, first polystyrene pass, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm detection.  (b) on-line HPLC-EPR profile, 4 scans/file, 20 s/scan, sweep width 130 g, and 9.55 GHz.
Figure 4.20  HPLC-UV trace representing the recovery and re-injection of the EPR active La$_{m}$$@$C$_{2p}$ fraction: (a) first polystyrene pass, (b) third polystyrene pass, and (c) fifth polystyrene pass. Chromatographic conditions: 1.0 mL/min, 80:20 toluene/decalin, and 340 nm detection.
Figure 4.21  HPLC-UV trace of the fifth polystyrene pass, 1.0 mL/min, 80:20 toluene/decalin, 340 nm detection, and 200 μL of La$_m$@C$_{82}$ sample. (b) on-line HPLC-EPR profile, 2 scans/file, 20 s/sweep, sweep width 130 g, and 9.55 GHz.
the chromatogram. Note that the expected octet for La@C\(_{82}\) (I = 7/2) was observed as a broadened singlet. This feature is due, in part, to rapid scanning, oversaturation, and a small hyperfine coupling constant (1.2 g). An off-line negative-ion mass spectrum of this EPR active fraction revealed that La@C\(_{82}\) and La\(_2\)@C\(_{72}\) were the dominant peaks. However, low but significant levels of higher mass empty-cage C\(_{96}\) - C\(_{112}\) were also present.

Next, this metallofullerene sample was injected into the selective Buckyclutcher column to observe whether a series of resolvable peaks could be obtained. The chromatogram following a 110 \(\mu\)L injection is presented in Figure 4.22. Note that the four dominant metallofullerene peaks are peaks #0, 3, 4, and 5. To determine which peak was the EPR active La@C\(_{82}\), a subsequent injection was performed with the HPLC-EPR apparatus. According to the on-line EPR spectra, the EPR active region (17.0 to 19.5 min) corresponds to peaks 4 and 5. The composition of peak #0 was still unknown. Two Buckyclutcher passes permitted the isolation of samples corresponding to peaks 0, 4, and 5. Off-line mass spectral data indicated that peak #0 was purified La\(_2\)@C\(_{72}\) (> 98% purity, Figure 4.23) whereas peaks #4 and #5 consisted of La@C\(_{82}\) (~60% purity) co-eluting with several higher mass C\(_{102}\) to C\(_{106}\) empty-cage fullerenes.

Although < 1 mg of La\(_2\)@C\(_{72}\) sample was obtained, this compound could be very
Figure 4.22  Fifth polystyrene pass, La$_m$@C$_{29}$ EPR active fraction on the Buckycletcher column: (a) 110 µL injection, 2.0 mL/min, 80:20 toluene/decalin, and 340 nm detection. (b) on-line HPLC-EPR profile, 2 scans/file, 20 s/sweep, sweep width 130 g, and 9.55 GHz.
Figure 4.23  LD-TOF mass spectrum of isolated $\text{La}_m@\text{C}_{2n}$ fraction #0. (mass spectrum courtesy of IBM, Almaden).
useful from $^{13}$C NMR viewpoint. Specifically, the highly symmetrical $C_{72}$ ($D_{6h}$)$^{135}$
empty-cage could yield as few as four $^{13}$C NMR lines - depending on the position of
the two lanthanum atoms inside the cage. At this writing, a definitive $^{13}$C NMR
spectra elucidating the carbon cage structure of any endohedral metallofullerene has
not been published.

4.14 Er$_n$@C$_{2n}$

At this point, a shift toward chromatographic separations of erbium
metallofullerenes was initiated for primarily two reasons. First, a preliminary mass
spectrum (Figure 4.24) of the erbium raw extract indicated that Er@C$_{82}$ and Er$_2$@C$_{82}$
are the only significant metallofullerenes present in the initial stock. As noted in
Figure 4.24, there is a paucity of di-metal erbium compounds - especially relative to
the scandium and yttrium cases. The high abundance of Er$_2$@C$_{82}$ and Er@C$_{82}$ in the
initial raw extract rendered Er@C$_{82}$ quite amenable to chromatography and off-line
sample handling. In addition, it was anticipated that Er@C$_{82}$ would be sufficiently
stable to oxygen. Note that with the previous mono-metal Sc@C$_{82}$, Y@C$_{82}$, and
La@C$_{82}$ separations, the time-consuming safeguards and constant anxiety of their
possible decomposition made these compounds difficult to handle. Finally, it was
Figure 4.24  LD-TOF mass spectrum of $\text{Er}_{m}@C_{2n}$ raw extract. (mass spectrum courtesy of IBM, Almaden).
predicted that Er_{m}@C_{2n} species could possess optical-limiting or non-linear optical properties.

Thus, a stock solution (3 mg/mL) of Er_{m}@C_{2n} was made and subsequently injected into two Buckyclutcher columns connected in series (Figure 4.25). Off-line mass spectra for these peaks were obtained. According to mass spectral data, three isomers (I, II, and III) of Er_{2}@C_{82} were observed in three distinct peaks. For this experiment, 400 - 500 mg of additional Er_{m}@C_{2n} extract was then separated and fraction collection focused on these three regions. After "recovery and re-injection" using a second Buckyclutcher pass, three samples containing I, II, and III of improved purity were obtained. Off-line mass spectra confirmed Er_{2}@C_{82} as a dominant peak for each fraction. A mixture of these three samples was made and subsequently injected into the Buckyclutcher column. The chromatogram (Figure 4.26) clearly indicates three well-resolved peaks corresponding to Er_{2}@C_{82} isomers I, II, and III.

However, these three samples were not yet pure. There were still low-lying levels of higher mass C_{100} - C_{106} empty-cage fullerenes present. To remove these contaminants, a tetraphenyl-porphyrin (TPP) column was utilized with CS_{2} as the mobile phase. The high solubility of the metallofullerenes in carbon disulfide permitted improved sample throughput. As an example, 500 µL of the isomer III Er_{2}@C_{82} sample was injected into the TPP. The chromatogram (Figure 4.27) revealed
Figure 4.25  HPLC-UV trace of $\text{Er}_n@C_{2n}$ raw extract, Buckyclutcher column, 200 µL injection, 1.2 mL/min, 80:20 toluene/decalin, and 354 nm UV detection.
Figure 4.26  HPLC-UV trace of Er₄@C₈₂ isomers I, II, and III. Chromatographic conditions: Two Buckyclutcher columns in series, 1.55 mL/min, 80:20 toluene/decalin, and 340 nm UV detection.
Figure 4.27  HPLC-UV chromatogram for an Er$_2$@C$_{2n}$ (isomer III fraction), sample obtained from initial Buckyclutcher separations. Chromatographic conditions: TPP column, 1.0 mL/min CS$_2$, and 340 nm UV detection.
two peaks which were collected for mass spectral characterization. According to the data, the first peak corresponds to $\text{Er}_2@C_{82}$ and small amounts of $\text{Er}_2@C_{84}$ and $\text{Er}_2@C_{90}$. In contrast, the second peak contains only higher mass $C_{100} - C_{106}$ fullerenes. Thus, a methodology to separate empty-cages from $\text{Er}_m@C_{2n}$ species was developed with this Buckyclutcher/TPP "two-stage" procedure. In contrast to the Sc, Y and La case, it was no longer necessary to perform five polystyrene passes to remove the empty-cage fullerenes. In summary, sample loadability and decreased experimental time were the dominant features of this newly discovered Buckyclutcher/TPP "two-stage" methodology.

At this stage, $\text{Er}_2@C_{82}$ had not yet been isolated in high purity. Following collection of the first peak ($\text{Er}_2@C_{2n}$), it was soon realized that the TPP column did not adequately resolve the $\text{Er}_2@C_{84}$ and $\text{Er}_2@C_{90}$ contaminants. This sample (1st peak) was then injected into the Buckyclutcher column to obtain purified $\text{Er}_2@C_{82}$ isomer III (> 98% purity). The analytical trace and mass spectrum for this highly purified sample are presented in Figure 4.28. The other samples (I and II) were also separated with the same methodology as just described for isomer III. Isomer I was a 50/50 mixture of $\text{Er}_2@C_{82}$ and $\text{Er}_2@C_{84}$. In contrast, isomer II was pure $\text{Er}_2@C_{82}$ (> 90% purity).

As previously discussed, one objective was to isolate the mono-metal $\text{Er}@C_{82}$
Figure 4.28  (a) HPLC-UV analytical trace for isolated Er$_2$@C$_{82}$ (isomer III), Buckycluster columns, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm detection. (b) LD-TOF mass spectrum of above (courtesy of IBM, Almaden).
species. Chromatographically, Er@C₈₂ was located beyond Er₂@C₈₂ (III) through off-line mass spectra of collected Buckyclutcher fractions. The fraction containing Er@C₈₂ was then injected into the TPP column for further separation, and a new Er@C₈₂ sample (~60% purity, Figure 4.29) from the TPP was obtained.

Attempts were now made to isolate other erbium metallofullerenes (e.g. Er₂@C₈₈, Er₂@C₉₂, etc) which were present as low abundant species in the initial raw extract. Chromatographically, Er₂@C₉₂ was located beyond Er@C₈₂ during the initial Buckyclutcher clean-up procedure. This Er₂@C₉₂ fraction obtained from the Buckyclutcher was then injected into the TPP column for removal of other Er₂@C₂₅ and higher-mass empty-cage fullerenes. A re-injection into the Buckyclutcher column yielded an isolated Er₂@C₉₂ sample (~80% purity). The analytical trace and mass spectrum are presented in Figure 4.30.

4.2 Improved Separation Methodology

4.21 PBB column

In August 1995, another chromatographic column became commercially available. This pentabromobenzyl-based stationary phase was originally obtained for its high
Figure 4.29  Isolated Er@C_{82} sample obtained after Buckyclutcher and TPP column separations. (a) HPLC-UV analytical trace, Buckyclutcher columns, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm UV detection. (b) negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
Figure 4.30 Isolated $\text{Er}_2@\text{C}_{92}$ sample obtained after Buckyclutcher and TPP column separations. (a) HPLC-UV analytical trace, Buckyclutcher columns, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm UV detection. (b) negative-ion chemical ionization mass spectrum of above using Ultramark 1621 as the calibration standard.
sample loadability. With carbon disulfide as a mobile phase, this column could handle ~50 mg of injected material. For these separations, 1000 mg of $\text{Sc}_m@C_{2n}$ initial raw extract was prepared with an increased Sc loading (3-4 %) to produce a higher abundance of $\text{Sc}_3@C_{82}/\text{Sc}@C_{82}$. This initial raw extract was separated using the PBB column in conjunction with the automated system as described in Section 4.3. After the initial PBB automated separation, a metallofullerene fraction ($\text{Sc}_m@C_{74}-\text{Sc}_m@C_{90}$) was collected. Note that this fraction overlapped chromatographically with the empty-cage region of $C_{76} - C_{86}$.

This metallofullerene-enriched fraction was further separated with the Buckyclutcher column using 80:20 toluene/decalin solvent system. The resulting chromatogram (similar to Figure 4.5) also revealed $\text{Sc}_m@C_{2n}$ peaks #0 - 6. Each of these peaks was re-injected into the Buckyclutcher column until single peaks were obtained. Each of these seven peaks (#0-6) was then further separated with the PBB column for final purification. (Note that although single peaks were obtained with the Buckyclutcher column, the peak would typically further separate into 5 - 8 additional peaks with the PBB column.) From these separations, additional purified $\text{Sc}_m@C_{2n}$ samples were obtained. A listing of these isolated metallofullerene samples are summarized in Table 4.2 - Table 4.5.

It should be noted that the mass spectrum for PBB $\text{Sc}_m@C_{2n}$ fraction 4.B (Table
### TABLE 4.2

Composition of $\text{Sc}_n@C_{2n}$ fractions (#2 Buckyclutcher series) from PBB chromatographic column

<table>
<thead>
<tr>
<th>PEAK NUMBER</th>
<th>COMPOSITION</th>
<th>RETENTION TIME (min.)</th>
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</thead>
<tbody>
<tr>
<td>2.A</td>
<td>C82</td>
<td>10.80</td>
</tr>
<tr>
<td>2.B</td>
<td>(ScC@C82)?</td>
<td>11.98</td>
</tr>
<tr>
<td>2.C</td>
<td>Sc2@C82</td>
<td>12.24</td>
</tr>
<tr>
<td>2.D</td>
<td>Sc2@C86</td>
<td>13.40</td>
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TABLE 4.3

Composition of PBB Scₙ@C₂ₙ fractions (#3 Buckyclutcher series) from PBB chromatographic column

<table>
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<th>PEAK NUMBER</th>
<th>COMPOSITION</th>
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<tbody>
<tr>
<td>3.A</td>
<td>Sc₂@C78</td>
<td>11.40</td>
</tr>
<tr>
<td>3.B</td>
<td>(Sc₂C)@C82 ?</td>
<td>11.77</td>
</tr>
<tr>
<td>3.C</td>
<td>Sc₂@C82</td>
<td>12.31</td>
</tr>
<tr>
<td>3.D</td>
<td>Sc₂@C84</td>
<td>12.62</td>
</tr>
<tr>
<td>3.E</td>
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<td>13.00</td>
</tr>
<tr>
<td>3.F</td>
<td>Sc₂@C88</td>
<td>13.60</td>
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TABLE 4.4

Composition of PBB Sc\textsubscript{m}@C\textsubscript{2n} fractions (#4 Buckyclutcher series) from PBB chromatographic column

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<tr>
<td>4.A</td>
<td>Sc\textsubscript{2}@C80</td>
<td>12.22</td>
</tr>
<tr>
<td>4.B</td>
<td>Sc\textsubscript{4}@C82</td>
<td>12.42</td>
</tr>
<tr>
<td>4.C</td>
<td>Sc\textsubscript{2}@C86</td>
<td>13.08</td>
</tr>
<tr>
<td>4.D</td>
<td>Sc\textsubscript{4}@C80</td>
<td>13.57</td>
</tr>
<tr>
<td>4.E</td>
<td>Sc\textsubscript{2}@C90</td>
<td>14.85</td>
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TABLE 4.5

Composition of PBB Scₘ@[C₂ₙ] fractions (#5 Buckyclutcher series) from PBB chromatographic column

<table>
<thead>
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<th>SAMPLE NUMBER</th>
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</thead>
<tbody>
<tr>
<td>5.A</td>
<td>Sc₂@[C76]</td>
<td>11.47</td>
</tr>
<tr>
<td>5.B</td>
<td>Sc₂@[C80]</td>
<td>12.28</td>
</tr>
<tr>
<td>5.C</td>
<td>Sc₄@[C82] / Sc₂@[C82]</td>
<td>12.50</td>
</tr>
<tr>
<td>5.D</td>
<td>Sc₃@[C84]</td>
<td>13.02</td>
</tr>
<tr>
<td>5.E</td>
<td>Sc₂@[C86]</td>
<td>13.41</td>
</tr>
<tr>
<td>5.F</td>
<td>Sc₂@[C88]</td>
<td>14.17</td>
</tr>
<tr>
<td>5.G</td>
<td>Sc₂@[C90]</td>
<td>14.78</td>
</tr>
</tbody>
</table>
4.4) indicated the presence of $\text{Sc}_4@C_{82}$. This was exciting since there had been no precedence for encapsulating four metal atoms. Although $\text{Sc}_4@C_{82}$ was the dominant species, this fraction was still contaminated with minor amounts of $\text{Sc}_2@C_{80} - \text{Sc}_2@C_{84}$. Since the PBB HPLC-UV trace (Figure 4.31a) revealed a symmetrical peak, further re-injection into this PBB column would not have effectively resulted in a purified $\text{Sc}_4@C_{82}$ sample.

An injection into the Buckyclutcher column demonstrated that further separation was possible. Collection of the peak centered at 15.26 minutes (Figure 4.31b) resulted in a purified $\text{Sc}_4@C_{82}$ sample with > 90% purity (Figure 4.31c). Although this mass spectrum was not obtained under satisfactory conditions for an accurate isotope distribution analysis, a mass spectrum (Figure 4.32) for a separate $\text{Sc}_4@C_{82}$ sample was obtained under optimal conditions. A subsequent isotope distribution analysis suggests that the intensities for the experimental and theoretical $M$, $M+1$, $M+2$, $M+3$, and $M+4$ peaks are comparable. Note that the nearest empty-cage peak ($C_{94}$) would possess a "$M+1$" peak of higher intensity than its "$M$" peak. Other possible $\text{Sc}_n@C_{2n}$ species were inconsistent with this molecular weight.

In addition, a purified $\text{Sc}_2@C_{74}$ sample was also obtained from injection of $\text{Sc}_n@C_{2n}$ Buckyclutcher peak "0" (see Table 4.1 or Figure 4.5) into the PBB column. The HPLC-UV chromatogram for the final PBB injection is presented in Figure
Figure 4.31  (a) HPLC-UV analytical trace of PBB fraction 4 B, CS₂ mobile phase, 380 nm UV detection, PBB column, and 2.0 mL/min.  (b) HPLC-UV analytical trace of the final purified Sc₄@C₈₂ sample, Buckyclucher column, 2.0 mL/min, 380 nm UV detection, and 80:20 toluene/decalin mobile phase.  (c) negative-ion chemical ionization mass spectrum of the above final purified Sc₄@C₈₂ sample using Ultramark 1621 as the calibration standard.
Figure 4.32  Negative-ion chemical ionization mass spectrum for the Sc$_4$@C$_{82}$ sample using Ultramark 1621 as the calibration standard. Obtained under optimal high resolution mass spectral condition, this mass spectrum was used for analyzing predicted isotope distribution patterns for Sc$_4$@C$_{82}$. 
4.33a. The purity of this Sc$_2$@C$_{74}$ sample is estimated at > 95%. A $^{13}$C NMR experiment for this metallofullerene was performed. Although several real $^{13}$C NMR peaks were suspected to be emerging above the noise, the results were nevertheless inconclusive.

Next, an attempt was made to obtain a $^{45}$Sc NMR spectrum for Sc$_2$@C$_{74}$ to determine if the two Sc atoms were equivalent on the NMR time-scale. This experiment was successful, and the $^{45}$Sc NMR spectrum is presented in Figure 4.33b. Obtained under ambient conditions, this NMR spectrum suggests that the metal atoms are equivalent within the cage structure. Note that the $^{45}$Sc NMR signal is broadened due to quadrupolar interactions from the scandium nuclei.

4.3 Automated Metallofullerene Separations$^{173}$

Because the amount of experimental time became a significant consideration, a quicker, if not better, methodology needed to be developed. Depending on the amount of initial raw extract, a typical series of five polystyrene passes required days to weeks. Once that metallofullerene-enriched fraction was obtained, another block of time was required. Based on the number of peaks to be collected and number of re-injections, one to three weeks was often necessary. In addition, off-line collection
Figure 4.33  (a) HPLC-UV analytical trace for purified Sc$_2$@C$_{74}$, PBB column 2.0 mL/min, carbon disulfide mobile phase, and 380 nm UV detection. This sample was previously separated using the Buckyclutcher column and injected into the PBB column for final purification. (b) $^{45}$Sc NMR spectrum for the above purified Sc$_2$@C$_{74}$ sample obtained under ambient conditions. Under these conditions, ScCl$_3$ would have a chemical shift of 114 ppm.
of eluents was often done manually. This increased the risk of sample contamination and required an inordinate amount of limited manpower. For these reasons, the following automated methodology was developed for unattended operation. It should be noted that Paul Burbank (see Acknowledgement Section) played the integral role in developing this automated system. Although details of this work have been recently published, a diagram of this automated apparatus is presented in Figure 4.34.

4.31 $\text{Er}_m@C_{2n}$

This automated approach can effective for chromatographic separations of metallofullerenes. As an example, it will be demonstrated in this section that $\text{Er}_2@C_{82}$ (isomer III) can be isolated solely from the automated approach.

From ~1000 mg of $\text{Er}_m@C_{2n}$ extract, a stock solution (3 mg/ml.) was prepared and separated using the Buckyclutcher columns as the separation columns. The automated system was initiated and the resulting chromatograms are presented in Figure 4.35a. Based on preliminary results (see $\text{Er}_m@C_{2n}$ separations; Section 4.14), the $\text{Er}_2@C_{82}$ (isomer III) species elutes at ~54 minutes in the automated experiments due to a lower flow rate in comparison with previous experiments. A narrow fraction centered at this region resulted in a sample containing $\text{Er}_2@C_{82}$ and the empty-cages.
Figure 4.34  Automated HPLC apparatus: Load and separation columns are C1, C2 and C3, C4, respectively. The manual valve is an optional feature (details in ref. 173).
Figure 4.35  (a) Automated sequence of Er@C$_{2n}$ injections. Chromatographic conditions: first Buckyclutcher pass, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm detection.
C_{100} - C_{106} fullerenes. In order to remove the C_{60} - C_{92} empty-cage fullerenes which had "tailed" into the metallofullerene fraction, the Er_2@C_{82} concentrated fraction (isomer III) was subsequently re-injected for a second Buckyclutcher automated sequence (Figure 4.35b).

To remove the higher-mass empty-cage contaminants, a series of automated-TPP injections were performed. The results are illustrated in Figure 4.36. A narrow fraction centered around the peak maxima (~5 min) resulted in a highly purified sample of Er_2@C_{82} (isomer III, > 90% purity). In summary, this automated approach went from an initial Er_m@C_{2n} extract to a final, isolated metallofullerene. This total-automated system represented a significant advancement in the savings of manpower and time.

4.4 Empty-cage C_{2n} Separations

In the course of the chromatography of metallofullerene samples, the empty-cage species were concomitantly separated for other characterization studies. For example, isolation of empty-cage species are important models to determine the cage symmetry based on ^{13}C NMR data. In addition, empty-cage fullerenes serve as chromatographic "standards" for newly purchased columns and for characterization experiments of
Figure 4.35  (b) Re-injection of the Er$_2@C_{82}$ fraction into the automated apparatus. Chromatographic conditions: second Buckyclutcher pass, 1.0 mL/min, 80:20 toluene/decalin, and 340 UV nm detection. The dominant peak corresponds to Er$_2@C_{82}$ (isomer III).
Figure 4.36  Automated sequence of the $\text{Er}_2\text{@C}_{82}$ fraction (isomer III) obtained after two Buckyclutter passes yielded a single, symmetric peak. Chromatographic conditions: TPP column, 1.0 mL/min, carbon disulfide, and 340 nm UV detection.
purified metallofullerenes. For example, the UV-Vis spectrum of Sc$_2$@C$_{82}$ can be compared to the corresponding empty-cage C$_{82}$ fullerene. Thus, subtle differences between a metallofullerene and its corresponding empty-cage can be compared to determine whether a given property arises from the empty-cage carbon network or as a result of metal encapsulation. In our laboratory, isolated empty-cage samples include C$_{50}$ (> 98 % purity), C$_{70}$ (> 98% purity), C$_{78}$ (~80% purity), C$_{82}$ (> 90% purity), C$_{84}$ (> 90% purity), C$_{86}$ (~85% purity), C$_{88}$ (~70% purity), C$_{90}$ (> 90% purity), C$_{92}$ (~80% purity), and C$_{96}$ (> 90% purity).

Although the polystyrene column lacked the desired selectivity for separating individual metallofullerenes, it was adequate resolution for isolation of C$_{60}$, C$_{70}$, and C$_{84}$ samples. As an example, Figure 4.37 illustrates a typical chromatogram following a polystyrene injection. Note that the column is not overloaded as was often the case in the initial polysyrene (1st pass) clean-up step previously used for obtaining the "EPR active" fraction (see section on metallofullerene separations).

However, the majority of purified empty-cage fullerenes were obtained with the more selective Buckyclutcher column. The analytical chromatograms and corresponding mass spectra for several isolated empty-cage fullerene fractions obtained from Buckyclutcher separations are presented in Figures 4.38a through 4.38f.
Figure 4.37  HPLC-UV chromatograms for a typical polystyrene separation. Chromatographic conditions: second polystyrene pass of the $Y_m@C_{2n}$ fraction, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm UV detection.
Figure 4.38a Analytical HPLC-UV trace of an isolated C₈₂ fraction, Buckyclutcher column, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm UV detection.
Figure 4.38b Analytical HPLC-UV trace of an isolated C₈₄ fraction, Buckyclutcher column, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm UV detection.
Figure 4.38c Analytical HPLC-UV trace of an isolated C_{86} fraction, Buckyclutcher column, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm UV detection.
Figure 4.38d Analytical HPLC-UV trace of an isolated C₈₅ fraction, Buckyclutcher column, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm UV detection.
Figure 4.38e  Analytical HPLC-UV trace of an isolated C\textsubscript{90} fraction, Buckyclutcher column, 1.0 mL/min, 80:20 toluene/decalin, and 340 nm UV detection.
Figure 4.38f Analytical HPLC-UV trace of an isolated C_{92} fraction, Buckyclutcher column, 1.0 mL/min, 80:20 toluene/decalin, and 540 nm UV detection.
4.5 Trends in $A_m@C_{2n}$ Separations

In this research, the polystyrene, Buckyclutcher, TPP, and PBB columns have been the most useful for separation of endohedral metallofullerenes. Following years of metallofullerene separations on different types of chromatographic columns, valuable experimental data has been obtained. This data can provide a more fundamental understanding of separation processes of the endohedral metallofullerenes. Specifically, the chromatographic retention behavior [e.g. retention time ($t_r$) and capacity factor ($k'$)] of empty-cage fullerenes ($C_{60}$-$C_{120}$) as well as endohedral metallofullerenes ($Sc_m@C_{2n}$, $Y_m@C_{2n}$, $La_m@C_{2n}$, and $Er_m@C_{2n}$) has been characterized and evaluated for several columns. With the 80:20 toluene/decalin mobile phase, individual capacity factors were determined for each metallofullerene.

To unambiguously assign retention times for the paramagnetic metallofullerene species, HPLC-EPR detection was utilized as an on-line technique. In addition, off-line characterization methods included the following mass spectrometric techniques: time-of-flight (LD-TOF) and negative-ion desorption chemical ionization (NI-DCI). Off-line fractions were also characterized by EPR analysis of static samples. Chromatographic parameters of all metallofullerene separations have been obtained and are summarized in Figure 4.39 - a graphical representation of the log $k'$ (capacity
Figure 4.39  Plot of chromatographic capacity factor (log k') versus cage size (number of carbon atoms). ● polystyrene column ◇ Buckyclutch column, and 80:20 toluene/decalin mobile phase. (a) is an expanded inset from (b).
factor) versus the number of carbon atoms in the outer fullerene cage network. Since plotting all metallofullerene data points would result in a cluttered graph, only metallofullerenes with a C_{82} outer-cage are presented. This graph addresses one of the primary concerns of metallofullerene separations. Namely, it provides information regarding where a particular metallofullerene would elute relative to the empty-cage fullerenes. Thus, a practical feature of Figure 4.39 is its ability to predict which empty-cage fullerene(s) will be the dominant contaminant for a specific metallofullerene. As an example, a Sc_{3}@C_{82} fraction obtained from a polystyrene column will possess C_{104} as the primary empty-cage contaminant. On the Buckyclutcher column, however, the identical Sc_{3}@C_{82} species would co-elute with the empty-cage C_{112} fullerene of slightly higher mass. In this regard, it would then be possible to isolate Sc_{3}@C_{82} using a combination of these two columns. Specifically, a Sc_{3}@C_{82} fraction from a polystyrene separation could be injected into the Buckyclutcher column to obtain a purified Sc_{3}@C_{82} sample. This "two-stage" system would be effective since the C_{104} and C_{112} species would be removed with a combination of these two different types of columns.

Figure 4.39 also reveals valuable information regarding metallofullerene separations. It should be noted that regardless of which type of column was used (polystyrene or Buckyclutcher), all of the empty-cage fullerenes were eluted as a
homologous series as indicated by the $C_{2n}$ line connecting their data points. Based on this feature, a powerful statement is made regarding the chromatographic separations of metallofullerenes. Namely, there is an extremely high probability that any selected metallofullerene will co-elute with some empty-cage fullerene. For this reason, at least two different types of columns must often be utilized in the isolation of individual metallofullerenes. This explains why the "two-stage" systems (see Isolation; Section 2.29) have presently become the dominant methodology in metallofullerene isolation schemes. However, there is an exception. It is possible that, by chance, a metallofullerene could elute between two empty-cages in the $C_{2n}$ homologous series (e.g. $C_{86} < \text{La}@C_{82} < C_{88}$). This situation could occur for columns possessing adequate resolution of the higher-mass empty-cage $C_{86}$-C$_{112}$ species. Metallofullerenes often elute in this region regardless of which type of column is employed. However, it should be noted that these "one-step" purifications involving only one type of column have been rare.

In addition, the data in Figure 4.39 reveals other overall trends in metallofullerene separations. As an example, $\text{Sc}_3@C_{82}$ possesses a large log $k'$ value and is a strongly retained metallofullerene. With slightly lower log $k'$ values, the mono-metal, endohedral metallofullerene series ($Y@C_{82}$, La@$C_{82}$, Sc@$C_{82}$, and Er@$C_{82}$) are clustered together. These mono-metal species are less strongly retained than the tri-
metal Sc$_3$@C$_{82}$ metallofullerene. Individually, Y@C$_{82}$, La@C$_{82}$, Sc@C$_{82}$, and Er@C$_{82}$ possess minor differences in retention times. In contrast, the di-metal series (Sc$_2$@C$_{82}$, Y$_2$@C$_{82}$, La$_2$@C$_{82}$, and Er$_2$@C$_{82}$) are noticeably the most weakly retained metallofullerenes. These di-metal species tend to elute in families or groups - possibly determined by which C$_{82}$ empty-cage isomer is surrounding the metal atoms. Disregarding obvious differences in retention times of various individual isomers (Er$_2$@C$_{82}$, I, II, and III), it appears that the identity of the metal atoms (e.g. Sc$_2$, Y$_2$, etc) does not significantly influence their retention times since these species are clustered so closely together.

In addition, the plot illustrates the poor selectivity of polystyrene separations as indicated by a C$_{2n}$ slope of only 0.0088. This lack of significant resolution between individual metallofullerene compounds is due to a weaker $\pi$-$\pi$ complexing stationary phase (relative to the Buckyclutcher). This poor selectivity addresses why the paramagnetic species (Sc@C$_{60}$, Sc$_3$@C$_{82}$, Y@C$_{82}$, and La@C$_{82}$) effectively served as ideal "markers" for the overall metallofullerene fraction as previously discussed. This feature also explains why five polystyrene "recovery and re-injection" steps were necessary to sufficiently distinguish and separate the overall metallofullerene EPR active fraction from the lower-mass empty-cage region (e.g. C$_{60}$-C$_{84}$).

At this stage, an attempt was made to correlate the paramagnetic metallofullerenes
(Sc@C₈₂, Y@C₈₂, La@C₈₂, and Sc₃@C₈₂) with their chromatographic retention behaviour. We noted that the elution order was always Y@C₈₂, La@C₈₂, Sc@C₈₂, and Sc₃@C₈₂. This has been consistent regardless of which type of column (polystyrene or Buckyclutcher) was utilized. Although this feature is not presently understood, a relationship between the log k' versus the hyperfine coupling constant (a) has been created as illustrated in Figure 4.40. From this plot, the trend is increased retention times for the metallofullerenes with larger hyperfine coupling constants. Specifically, Y@C₈₂ has the smallest hyperfine value and is the earliest to elute.

A possible explanation for this elution trend might be the amount of electron spin density that the metal atom(s) transfers to the surrounding carbon cage network. For example, the large hyperfine coupling constant of Sc₃@C₈₂ suggests a significant amount of unpaired electron spin density remains on the Sc₃ trimer and less electron density transferred to the cage (relative to Y@C₈₂). Thus, a separation based on the amount of "charge-transfer" could be a significant factor for these mono-metal species. Note that the hyperfine coupling constant for Sc₃@C₈₂ (6.8 g) has a similar value (8.5 g) relative to a Sc₃ trimer trapped in an argon matrix (4 K).¹⁸⁸b These similar hyperfine coupling constants suggest further evidence that most of the unpaired electron spin density of Sc₃@C₈₂ remains on the Sc atoms.
Figure 4.40  Plots of chromatographic capacity factor ($\log k'$) versus the hyperfine coupling constant, $a$, for (a) Buckycluther and (b) polystyrene separations, respectively. The mobile phase is 80:20 toluene/decalin.
At first glance, this hyperfine data might appear to be inconsistent with the retention mechanism expected for the di-nitrophenyl stationary phase of the Buckyclutcher column. Namely, the nitrophenyl groups create a π-accepting stationary phase. However, Sc$_3$@C$_{82}$ (less electron density transferred to the cage) would be then expected to interact to a lesser degree with this stationary phase and therefore elute earlier. Yet the elution trend for these mono-metal A$_m$@C$_{2n}$ is reversed.

Another possible explanation for this elution trend might be the motional dynamics of the encapsulated metal atoms. Preliminary data$^{296}$ suggest that for the mono-metal endohedral species, the metal atom is off-center and bonded to the carbon cage atoms.$^{296}$ With the absence of other metal atoms to share the unpaired electron spin density, the charge and metal atom have no choice but to interact strongly with the carbon cage atoms. This would result in restricted motional dynamics for the metal atom. For the di-metal species, the electrons can now be shared between the two metal atoms. As a result, this metal dimer perhaps does not bond with the carbon cage and can then tumble freely within the cage. With the Sc$_3$ trimer of Sc$_3$@C$_{82}$, any unpaired electrons could also be shared between the metal atoms instead of interacting strongly with the carbon cage atoms as discussed above. This Sc$_3$ trimer is then free to move within the cage. This is consistent with our recent Sc$_3$@C$_{82}$
motional dynamics study\textsuperscript{177} indicating an energy rotational barrier of 28 meV and correlation time of $5 \times 10^{-9}$ s. These features could relate to the elution trend in the following way. The rapidly moving Sc\textsubscript{3} trimer of Sc\textsubscript{3}@C\textsubscript{82} would strike the cage wall and briefly transfer a "bolus" of negative charge at a given instant. It could be this "concentration" of negative charge on the outer-cage wall that then interacts with the $\pi$-accepting dinitrophenyl ligands. This interaction could explain the longer retention of Sc\textsubscript{3}@C\textsubscript{82}.

It is possible that the nitro groups of the stationary influence the retention mechanism. As discussed previously for Sc\textsubscript{3}@C\textsubscript{82}, most of the charge remains on the metal with less charge transfer to the cage. Relative to the mono-metal A\textsubscript{m}@C\textsubscript{2n} species where there is the most charge-transfer to the cage, the outer-cage would therefore possess more negative charge to be repulsed by the negative charge on the NO\textsubscript{2} groups. Hence, these mono-metal species would be first to elute relative to Sc\textsubscript{3}@C\textsubscript{82}. The Sc\textsubscript{3}@C\textsubscript{82} with less charge-transfer to the cage would have a more positive charge on the cage and would therefore interact more strongly with the negative charge on the NO\textsubscript{2} group. Hence, the Sc\textsubscript{3}@C\textsubscript{82} would be last to elute.

It should be emphasized that the above discussion on retention mechanisms of the A\textsubscript{m}@C\textsubscript{2n} species with the various stationary phases are only presented as several possibilities. At present, a detailed study on the retention mechanisms for the
metallofullerenes on various stationary phases has not been published. Other possible retention mechanisms could involve the shape, size, and symmetry of the cage. In addition, recent electrochemical studies\textsuperscript{183,185} have demonstrated that metallofullerenes can either accept or donate electrons to their cage structure. This feature could also contribute to the retention order of the metallofullerenes.

Despite the lack of fundamental understanding that these trends provide, other valuable and interesting information can be obtained. As a first example, an EPR spectrum of the paramagnetic Er@C\textsubscript{82} species (I = 7/2) would, in principle, yield an octet. Experimentally, however, a relatively sharp resonance signal\textsuperscript{189} for Er@C\textsubscript{82} with a g-factor (2.005) similar to Sc@C\textsubscript{82}, Y@C\textsubscript{82}, and La@C\textsubscript{82} is observed.\textsuperscript{189} However, it is reported that the EPR signal for Er@C\textsubscript{82} could not be resolved into the expected octet due to spin-spin interactions of the unpaired electron (S=1/2) with the Er\textsuperscript{3+} 4f-electrons (S=3/2).\textsuperscript{189} Thus it is difficult to assign an experimental hyperfine coupling value to Er@C\textsubscript{82} for graphical purposes. However, the Er@C\textsubscript{82} species can still be plotted in Figure 4.40 since the log k' value has been obtained. After plotting, the striking feature is that, graphically speaking, the hyperfine coupling constant for Er@C\textsubscript{82} coincidentally corresponds to \(~0\) gauss. This feature may be related to the difficulty (spin-spin coupling) in observing hyperfine structure for the sharp absorption (g = 2.005) as discussed above. It should also be noted that a second EPR
signal was located for Er@C$_{82}$. Located at a g-factor of 8.6 g (80 GHz), this observed resonance is very broad and originates from transitions of Er$^{3+}$ 4f electrons (S=3/2). Similar g-values are found for Er$^{3+}$ ions in salt crystals.

An additional application of Figure 4.40 can be made in the yttrium case. As mentioned previously, the Sc$_3$@C$_{82}$ metallofullerene has been the only tri-atomic, metal-encapsulated fullerene. The issue of whether other tri-atomic metal atoms (e.g. Y$_3$, La$_3$, etc.) exist has often been debated. In a fraction obtained from a Buckyclutcher separation of an yttrium extract, a weak mass spectral signal corresponding to Y$_3$@C$_{82}$ was found (Figure 4.41a). The m/z value of 1253 from this spectrum corresponds to a calculated mass of Y$_3$@C$_{82}$ (1253). Other species containing mass values in this region include C$_{104}$ (1249), Y@C$_{96}$ (1242), and Y$_2$@C$_{90}$ (1259). Other species in this fraction included C$_{110}$ and Y$_2$@C$_{94}$. Chromatographically, a log k' value was determined and subsequently plotted as indicated by Figure 4.41b. Assuming Y$_3$@C$_{82}$ was found and assuming this plot is accurate, a predicted hyperfine coupling constant of 5.03 - 6.18 g would be obtained.
Figure 4.41  (a) mass spectrum of a $Y_3@C_{82}$ - containing Buckyclutcher fraction. (b) Predicted hyperfine value for $Y_3@C_{82}$ using a plot of log $k'$ versus hyperfine coupling constant.
CHAPTER 5: FUTURE DEVELOPMENTS

5.1 Summary

At this writing, our laboratory has developed premier separation methodologies for the isolation of endohedral metallofullerenes. Purified metallofullerene samples have been obtained utilizing different types of columns. In summary, the polystyrene/Buckyclutcher two-stage system has resulted in purified Sc$_2$@C$_{76}$, Sc$_2$@C$_{84}$ (two isomers), Sc$_3$@C$_{82}$, and La$_2$@C$_{72}$. Meanwhile, the Buckyclutcher/TPP system has resulted in purified Er$_2$@C$_{82}$ (two isomers) and Er@C$_{82}$. However, the optimal methodology involves a Buckyclutcher/PBB two-stage system. The high loadability of the Buckyclutcher column in conjunction with the high resolution of the PBB column has resulted in a valuable collection of purified metallofullerene samples. Specifically, isolated samples using this system include Sc$_2$@C$_{74}$, Sc$_2$@C$_{76}$, Sc$_2$@C$_{78}$, Sc$_2$@C$_{80}$, Sc$_2$@C$_{82}$, Sc$_2$@C$_{84}$ (two isomers), Sc$_2$@C$_{86}$, Sc$_2$@C$_{88}$, Sc$_2$@C$_{90}$, Sc$_3$@C$_{82}$, Sc$_3$@C$_{84}$, and Sc$_4$@C$_{82}$. These milligram quantities of purified samples can now be used in long-awaited characterization experiments (e.g. $^{45}$Sc NMR, $^{13}$C NMR, TEM, non-linear optical measurements, etc.).
The isolation of several unique metallofullerenes should be addressed. Namely, Sc$_2$@C$_{74}$ and La$_2$@C$_{72}$ possess only one possible cage structure in accordance with the isolated pentagon rule (Section 2.12). Holding the cage size constant, the influence of the encaged metal atoms can then be studied directly. These cage structures are also expected to be highly symmetrical. The C$_{74}$ cage has D$_{3h}$ symmetry, whereas the C$_{72}$ cage structure has D$_{6h}$ symmetry. From a NMR viewpoint, the La$_2$@C$_{72}$ species would be an ideal sample and yield a $^{13}$C NMR spectrum of only four lines - assuming the La atoms do not lower the symmetry of the molecule.

At present, the Sc$_2$@C$_{74}$ and Sc$_2$@C$_{76}$ purified samples are currently being used in a $^{45}$Sc NMR study. Preliminary data has suggested that $^{45}$Sc NMR spectra are readily obtained only for those metallofullerenes with smaller C$_{74}$-C$_{76}$ cage sizes. In contrast, $^{45}$Sc NMR spectra for isolated metallofullerene samples with larger C$_{82}$-C$_{84}$ cage structures (Sc$_2$@C$_{84}$, Sc$_3$@C$_{82}$) were unsuccessful (room temperature) despite having a factor of 3-6 times more sample.

In addition, the discovery and isolation of purified Sc$_4$@C$_{82}$ represented the first time a sample with four encaged metal atoms had been obtained. However, future characterization experiments will be limited since only ~ 100 $\mu$g was isolated.
5.2 Concluding Remarks.

From 1990-1995, the field of metallofullerene science focused on developing a methodology to obtain purified samples. The recent advances in chromatographic separations have finally resulted in milligram quantities of isolated metallofullerenes. At present, columns are now commercially available and specifically designed for fullerene and metallofullerene separations. Although isolated samples are only present in milligram quantities, chemists can now finally probe into the unique characterization of metallofullerene species.

In recent years, there has been much discussion regarding the type of properties which metallofullerenes are believed to possess. Based on the novel arrangement of metal and carbon atoms, metallofullerenes are anticipated to have unique and exciting properties. Although purification methodologies have now been developed, more fundamental research into the production aspect needs to be performed. Despite having milligram-level samples, scientists are still often sample limited. Despite the fact that several research groups have now obtained purified metallofullerene species, the exact structure of any metallofullerene has yet to be elucidated. This is due, in part, to the difficulty of isolating macroscopic amounts of highly purified samples.

One could argue that the major problem area does not rest with the
chromatographic aspect but rather in the production step. Since the metallofullerene moiety typically represents only 1% of initial raw extract, more research should be performed in the production stage. For example, if a methodology could be developed to generate metallofullerenes in higher yield (20 - 70%), then separation scientists could likely obtain larger amounts of purified samples more efficiently. Unfortunately, until an improved production methodology is developed, the burden of obtaining purified metallofullerene samples will remain in the field of chromatographic science.

To date, scientists are on the edge of finally elucidating the exact structure for endohedral metallofullerene samples. NMR experiments have not provided conclusive information despite the fact that seemingly sufficient quantities (>10 mg) of purified endohedral samples have been obtained. This is likely due to any of the following: (1) presence of other co-eluting structural isomers (2) decreased symmetry of the carbon cage and/or (3) paramagnetism of the metal (e.g. Sc@C82).

Nevertheless, purified metallofullerene samples are now available, and a shift toward the "characterization aspect" should now be forthcoming. For example, Shinohara et al\textsuperscript{29c} have recently utilized scanning tunneling microscopy (STM) to observe an oriented "head-to-tail" cluster formation on a Cu(111) 1x1 surface for an isolated Y@C82 sample. Their data indicates the presence of strong dipole-dipole and
charge-transfer interactions between the Y@C_{82} species. These Y@C_{82} molecules are similar to the "superatom" features that were theoretically proposed in a semiconductor heterostructure.\textsuperscript{296} These Y@C_{82} metallofullerenes form quasi-molecules of the type (\ldots Y@C_{82} \ldots Y@C_{82} \ldots Y@C_{82} \ldots ).

In addition, this Y@C_{82} metallofullerene was subsequently characterized in a very recent X-ray diffraction study.\textsuperscript{297} The analysis of Y@C_{82} microcrystals indicate that the molecules achieve a molecular alignment along the [001] direction in a "head-to-tail" manner. This orientation of Y@C_{82} molecules in a specific direction could lead to novel solid-state properties.\textsuperscript{297}

5.3 Fullerene Related Materials.\textsuperscript{298-333}

These initial emerging characterization experiments are encouraging for this endohedral type of fullerene compound. Nevertheless, it should be noted that the metallofullerenes represent only one member of the "fullerene family." Other intriguing species can also be created in arc-generated soot - depending on experimental considerations (e.g. choice of metal). For example, nanotubes\textsuperscript{298-328} are cylindrical, tubular (\mu m length x nm diameter) shaped assemblies of carbon formed in arc-generated soot. These are formed when a metal such as cobalt\textsuperscript{299} is introduced
to a cored graphite rod. Numerous predicted applications\textsuperscript{303,304,324,325} include their use as composites,\textsuperscript{303} catalysts,\textsuperscript{303} and molecular wires.\textsuperscript{304} Cylindrical layers of carbon nanotubes are predicted to be either metallic or semi-conducting.\textsuperscript{306,327,328}

Shortly after the emergence of these hollow carbon nanotubes, scientists have successfully modified these nanotubes to encapsulate a metal (e.g. La, LaC, LaB\textsubscript{6}, YC, Pb, Mn, Gd, TiC, Nb, Fe\textsubscript{3}C, and Si).\textsuperscript{329-333} Preliminary studies for several of these metallated nanoparticles do exhibit superconductivity and magnetism.\textsuperscript{325}

For these reasons, there is a substantial interest in the endohedral metallofullerenes such as those purified in this research. With isolated metallofullerenes finally becoming available, scientists will soon be able to compare experimental data with their numerous, predicted properties. As previously discussed, anticipated applications have included the following: 1) tunable, non-linear-optical devices,\textsuperscript{175} 2) biological studies\textsuperscript{24} 3) superconductors,\textsuperscript{160} and 4) novel catalytic materials.\textsuperscript{175} With further research in the production, chromatography, and crystallization of metallofullerenes, one eagerly awaits characterization of their structural, electronic, and optical properties for this exciting new class of compounds.
REFERENCES


APPENDIX I: EMPTY-CAGE, C_{2n}
MASS SPECTROMETRY TABLE

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166
### APPENDIX II: \( \text{Sc}_{m}\text{@C}_{2n} \)

**MASS SPECTROMETRY TABLE**

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### APPENDIX V: $\text{Er}_m@C_{2n}$

#### MASS SPECTROMETRY TABLE

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VITA

Born in Clearlake Highlands, California, I was raised in a family of nine people. My father is a dedicated teacher whose career has been enlightening students at the grade school, high school, and university level. My mother is a dedicated housewife who raised five boys and two girls. Academically, my education has consisted of the following: Sharyland High School (Mission, TX, 1982-1986), Angelo State University (San Angelo, TX, 1986-1990, B.S. Chemistry, Magna Cumme Laude Honors), Virginia Polytechnic Institute & State University (Blacksburg, VA, 1990-1992, M.S. Chemistry), and Virginia Polytechnic Institute & State University (Blacksburg, VA, 1993-1995, Ph.D. Chemistry). On a personal level, I have two beautiful children, Jordan (5 years old) and 'Cole (3 years old).

Steven Stevenson