Enhancing the properties of carbon and gold substrates by surface modification

by

Jennifer Anne Harnisch

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Analytical Chemistry
Major Professor: Marc D. Porter

Iowa State University
Ames, Iowa
2001

Graduate College
Iowa State University

This is to certify that the Doctoral dissertation of

Jennifer Anne Harnisch

has met the dissertation requirements of Iowa State University

Marc D Port

Major Professor

Patricia A. Thiel

For the Major Program

For the Graduate College

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ABBREVIATIONS

T	temperature
°C	degrees Celsius
°F	degrees Farenheit
K	degrees Kelvin
PG	pyrolytic graphite
HOPG	highly ordered pyrolytic graphite
GC	glassy carbon
RVC	reticulated vitreous carbon
PAN	polyacrylonitrile
ACF	activated carbon fiber
CPE	carbon paste electrode
ECP	electrochemical pretreatment
SCE	saturated calomel electrode
VHT	vacuum heat treatment
CME	chemically modified electrode
rf	radiofrequency
O_2	oxygen
H_2O	water
CO_2	carbon dioxide
CO	carbon monoxide
Cl_2	chlorine
CH_2Cl_2	methylene chloride

N_2	nitrogen
NH_3	ammonia
HPLC	high performance liquid chromatography
PGC	porous graphitic carbon
EMLC	electrochemically modulated liquid chromatography
NBDT	4-nitrobenzenediazonium tetrafluoroborate
HBDT	4-hexylbenzenediazonium tetrafluoroborate
XPS	X-ray photoelectron spectroscopy
LC	liquid chromatography
E_{appl}	applied potential
PAHs	polyaromatic hydrocarbon
TFA	trifluoroacetic acid
TBAT	tetrbutylammonium tetrafluoroborate
AN	anisole
FL	fluoranthene
NP	3-nitrophenol
RS	resorcinol
HB	hexobarbital
OZ	oxazepam
NZ	nitrazepam
λ_{det}	detection wavelength
ODS	octadecylsilica
DBDT	4-decylbenzenediazonium tetrafluoroborate

MBDT	4-mercaptobenzenediazonium tetrafluoroborate
BTP	4-bromothiophenol
AFM	atomic force microscopy
MBACF	(α)-(+)-(methylbenzylaminocarbonyl)ferrocene
NMR	nuclear magnetic resonance
MS	mass spectrometry
RA	redox active group
Cp	cyclopentadienyl
β-CD	β-cyclodextrin
BHT	benzenehexathiol
FFM	friction force microscopy
IRRAS	infrared reflection/adsorption spectroscopy
SAMs	self-assembled monolayers
TBAH	tetrabutylammonium hexafluorophosphate
PDMS	polydimethylsiloxane
μ-CP	microcontact printing

ACKNOWLEDGMENTS

As with any great endeavor, there are many people who deserve special thanks for making it possible. My deepest thanks to my major advisor, Marc Porter for his guidance, support, and willingness to listen to crazy research ideas that I term “cute”. Secondly, I would like to thank Shelley Coldiron for prompting me to consider staying at Iowa State University to work for Marc rather than continue my education elsewhere. It was one of the best decisions I have made thus far.

Research in an academic setting is definitely a “meeting of the minds” and to the past and present members of the Porter research group, I say thank you for the great discussions, research ideas and camaraderie. I’d especially like to thank my mocha run buddy, Andy and Dan, for being the scapegoat for my delinquencies. Special thanks to Brent for his friendship, and being my “matchmaker”. And to Becky, our “real group leader”, thank you for always knowing the right answers, where everything is, and offering free counseling whenever I needed it.

There are also several friends who deserve credit for keeping me sane throughout my excursions through graduate school. At the top of this list are my two “sisters” Laurie Cardoza (Buttercup) and Sahana Mollah (Blossom). Thank you for believing in me when I doubted myself. Thanks to Rich Gurney for the past 10 years of friendship. Special thanks to Dr. David Rausch for his guidance and teaching me how to be a synthetic chemist.

There are two, near and dear to my heart, I’d like to especially thank. First, I would like to thank Michael Granger for his undaunting support, concern and constant teasing. Finally, I express my deepest gratitude to my mother for the sacrifices she made to get me to this point. Thank you for giving me the opportunities you never had.

Ames Laboratory is operated for the U.S. Department of Energy by Iowa State University under contract number W-7405-ENG-82. The United States Government has assigned the DOE Report Number IS-T 2191 to this thesis.

IS-T 2191

ABSTRACT

The properties of both carbon and gold substrates are easily effected by the judicious choice of a surface modification protocol. Several such processes for altering surface composition have been published in literature. The research presented in this thesis primarily focuses on the development of on-column methods to modify carbon stationary phases used in electrochemically modulated liquid chromatography (EMLC). To this end, both porous graphitic carbon (PGC) and glassy carbon (GC) particles have been modified on-column by the electroreduction of arenediazonium salts and the oxidation of arylacetate anions (the Kolbe reaction). Once modified, the carbon stationary phases show enhanced chromatographic performance both in conventional liquid chromatographic columns and EMLC columns.

Additionally, one may also exploit the creation of aryl films to by electroreduction of arenediazonium salts in the creation of nanostructured materials. The formation of mercaptobenzene film on the surface of a GC electrode provides a linking platform for the chemisorption of gold nanoparticles. After deposition of nanoparticles, the surface chemistry of the gold can be further altered by self-assembled monolayer (SAM) formation via the chemisorption of a second thiol species.

Finally, the properties of gold films can be altered such that they display carbon-like behavior through the formation of benzenehexathiol (BHT) SAMs. BHT chemisorbs to the gold surface in a previously unprecedented planar fashion.

Carbon and gold substrates can be chemically altered by several methodologies resulting in new surface properties. The development of modification protocols and their application in the analytical arena is considered herein.

GENERAL INTRODUCTION

Dissertation Organization

The work presented in this dissertation describes new approaches in the modification of carbon surfaces for chromatographic applications. Modification of carbon substrates is realized through three methodologies: electroreduction of arenediazonium salts, the Kolbe reaction and use of a mobile phase electroactive chiral selector. Additionally, altering gold surfaces to have carbon properties through the use of an aromatic hexathiol as a modification agent is explored.

In the introduction section, a review of the types, uses, electrochemistry, and modification methodologies of carbonaceous materials are discussed. A comprehensive list of references is given at the end of this section. Six data chapters, each of which is presented as a separate manuscript with a different research focus, follow the general introduction.

The first three chapters describe research efforts involving the modification of glassy carbon (GC) and porous graphitic carbon (PGC) stationary phases used in electrochemically modulated liquid chromatography (EMLC) via covalent modification using either the electroreduction of an arenediazonium salt or the Kolbe reaction. In each chapter, a different modifier is utitlized and thus, the properties of the carbon stationary phase can be easily altered to separate mixtures of both aromatic and proteinaceous analytes.

Chapter 4 presents preliminary work that extends the modification of a glassy carbon electrode with an arenethiol film to which gold nanoparticles may be adsorbed after modification thus providing a new methodology to anchor gold to carbon substrates.

The data discussed in Chapter 5 outlines the development of a ferrocene-based chiral selector used in the mobile phase during EMLC separations of pharmaceutical agents, and Chapter 6 relates the use of hexathiolbenzene (HTB) as a modification agent for gold substrates that imparts carbon-like properties to the surface. The dissertation is concluded with a brief summary of the research presented herein and speculation concerning future applications of the newly developed methodologies.

Literature Review

Carbon is perhaps one of the most versatile substrates employed by proselytes of the chemical sciences. Known for their diversity of forms and properties, carbonaceous materials have had a tremendous impact on many facets of analytical chemistry including electrochemistry, surface science, and chromatography. In this review, each of the many forms and uses of naturally occurring and synthetic carbons are described. The majority of the information for these two topics was found in three well-written reviews.[1-3] The modification of carbon-based materials is also discussed.

Naturally Occurring Allotropes of Carbon

As one of the most abundant elements, carbon exists in two forms - diamond and graphite.[1-3] Although compositionally similar, both allotropes have distinct structural and materials properties as illustrated in table 1. The dramatic differences in bond length, modulus, and thermal conductivity are explained by considering the structures of these two materials.

Table 1. Structural properties of diamond and graphite.[3]

Property	Cubic Diamond	Hexagonal Graphite
Bond length (Å)	1.54	1.41, 3.35
Density (g/cm^3)	3.52	2.26
Bulk modulus (GPa)	442	286
Young's modulus (GPa)	1054	1020, 36.3
Melting point (K)	4500	4450
Thermal conductivity (W/mK)	15000	2800, 5

Diamond

The most common diamond structure has a cubic lattice (zinc blende) and consists of sp^3 hybridized carbon atoms connected tetragonally by σ-bonds with a C-C bond length of 1.54 Å as represented in figure 1. It is this tetragonal bonding that imparts the characteristic hardness to the crystal. A less common form of diamond (Lonsdaleite) is a hexagonal structure that adopts a wurtzite crystal structure with C-C bond lengths of 1.52 Å. Due to their rarity and subsequent cost, naturally occurring diamonds are used primarily in jewelry due to their attractive appearance and hardness.

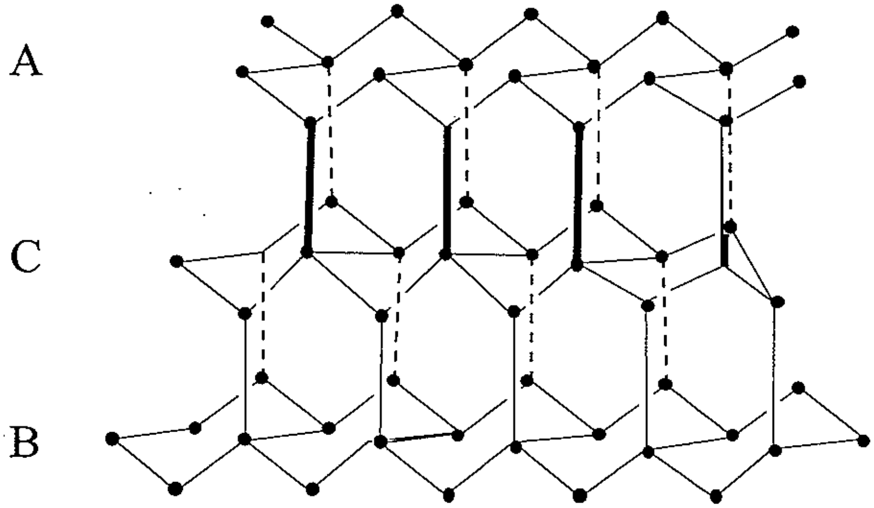

Figure 1. Schematic representation of the cubic diamond lattice.

Graphite

A second elemental form of carbon is graphite. It is constructed of sheet-like layers of carbon atoms with sp^2 σ-bonds and a delocalized π-array. The C-C bond distance in graphite is 1.41 Å, which is slightly less than that of diamond. This difference is best explained by the bonding within the graphitic sheets, which is intermediate between C_{sp3}-C_{sp3} and C_{sp2}-C_{sp2}. Like diamond, two forms of graphite are known. The first is hexagonal graphite, which has an ABAB layering of graphene planes and an interlayer spacing of 3.35 Å as depicted in figure 2. This large interlayer distance suggests that π-bonding between layers is negligible. A less-known form of graphite has an ABCABC layering of graphitic planes thus adopting a rhombohedral structure. Rhombohedral graphite is a minor component of well-crystallized

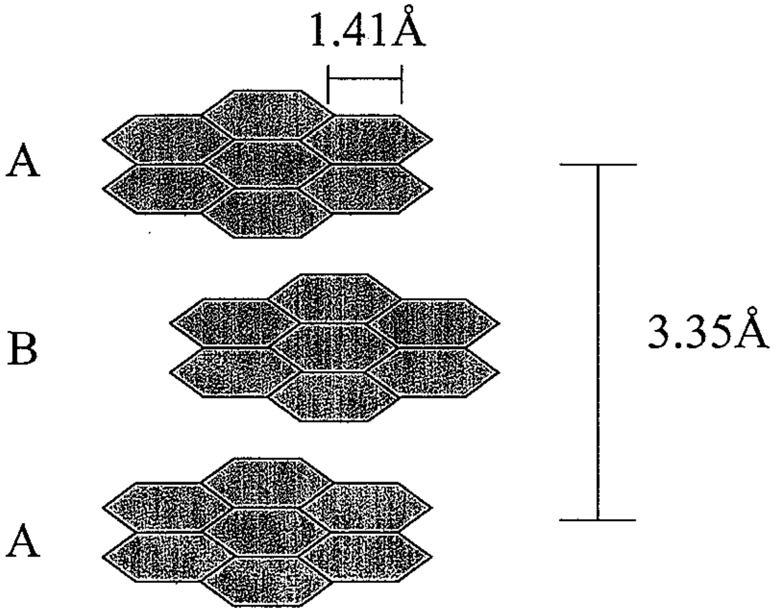

Figure 2. Schematic representation of the hexagonal graphite structure.

graphites and its concentration may be increased by a deformation process such as grinding. Due to the high ash content in natural deposits of graphite, it is typically not used in analytical applications.

Processed and Synthetic Carbons

In comparison to naturally occurring forms of carbon, several categories of processed carbon exist. These include, but are not limited to, carbon blacks, active carbon, synthetic graphite, glassy carbon, carbon fibers, carbon nanotubes, fullerenes and synthetic diamond. Each of the aforementioned materials has unique properties and is created by varying synthetic methodologies. Many of these synthetic processes involve a heating process termed graphitization. In this procedure, the carbonaceous material is heated to high temperatures (~1500 °K). This procedure results in the ordering of the aromatic rings into graphene planes typical in graphitic structures as depicted in figure 3.

Carbon Blacks

Used primarily in the tire rubber industry, carbon blacks are prepared by burning gaseous or liquid hydrocarbons in a limited supply of air at 1000 °C. Several procedural methodologies have been adopted and these are detailed in table 2. The surface area for the channel black particles varies depending on the synthetic process with channel blacks being the smallest (50 Å) and thermal blacks the largest (> 3000 Å). In general, the conductance of carbon blacks is improved by the removal of surface oxides rather than graphitization, indicating that the particle-particle contact resistance is greater and the major resistance component in carbon blacks.

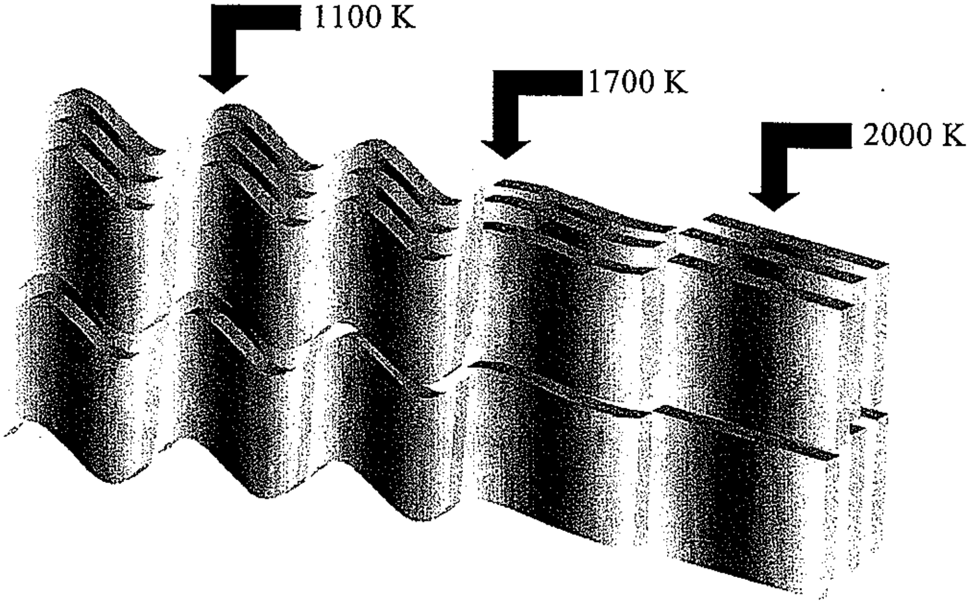

Figure 3. Model of graphitization of carbon materials.

Table 2. Methods for the preparation of carbon black materials.[1]

Method Title	Procedure
Channel	Incomplete oxidation of natural gas
Oil furnace	Aromatic residue oils from petroleum products are heated to 300 °C and atomized into a hot zone at 1400 °C
Thermal	Thermal decomposition of hydrocarbon gases in the absence of a flame
Acetylene black	A class of thermal black; Acetylene decomposes exothermically to yield carbon black and hydrogen at T > 1800 °C

Carbon blacks are also used in electrochemical applications as components of carbon paste electrodes (CPE),[4] which are biphase systems comprised of powders held together by viscous or capillary forces of a liquid phase like Nujol.

Active Carbon

Active carbons are characterized by high surface area and porosity. These materials find common use as adsorbents and catalyst supports due to the high capacity of their numerous and easily accessible micropores (diameter ~ 15Å). Active carbons are generated through two principle methods: thermal and chemical activation. In the thermal activation protocol, a previously charred material is heated in the presence of an oxidizing gas such as steam. Chemical activation involves the heating of a mixture of raw material (e.g. sawdust, coconut shells, charcoal, petroleum coke) and dehydrating agent (e.g. H_3PO_4, $ZnCl_2$, H_2SO_4) to temperatures ranging from 200 to 650 °C. After carbonization, the drying agent is leached from the product

Synthetic Graphite

Artificial graphitic materials are produced via a multitude of methods. In general, petroleum coke is heated in the first stage to 1000 °C followed by treatment at 2500-3000 °C. This procedure orders the graphene planes as previously discussed (see figure 3). The higher the treatment temperature, the more ordered the graphite planes become. Processed graphite has many industrial uses including batteries, lubricants and rubbers.

Pyrolytic graphite[5] (PG) is synthesized by the thermal decomposition of carbonaceous gases (e.g., methane) onto a substrate at $T > 1200$ °C. Although the main structural component of PG is carbon cones, the structure varies greatly with the deposition substrate, hydrocarbon gas composition and concentration, contact time between the gas and substrate, and the geometry of the pyrolysis chamber. High electrical conductivity is found parallel to the deposition surface while conductivity is low in the perpendicular direction.

The PG surface is typically prepared by polishing with 600 mesh silicon carbide paper. This technique cuts across the conical deposition planes, exposing vertical edges. However, it is not possible to obtain a reproducible surface area, stress or grain pattern via this method. Polishing PG also exposes a large number of edge planes (i.e. PG will have more electrochemical activity) of the truncated cones of the graphite layers.

A derivative of PG, stress recrystallized pyrolytic graphite, commonly referred to as highly ordered pyrolytic graphite (HOPG)[6], is made by heating PG to 3400 °C while compressing the material to enhance crystallite formation and growth. HOPG is a common electrode material employed in analytical chemistry and has a layered structure as shown in figure 4. Its application as an electrode in analytical chemistry will be discussed in a future section.

Glassy Carbon

Perhaps one of the most familiar carbon-based electrode materials used in analytical chemistry is glassy carbon also termed "vitreous carbon". Glassy carbon materials are produced by the thermal degradation of organic polymers and can be in two forms, both of which find application as electrodes, frits, joints and bearings. The first is a solid structure

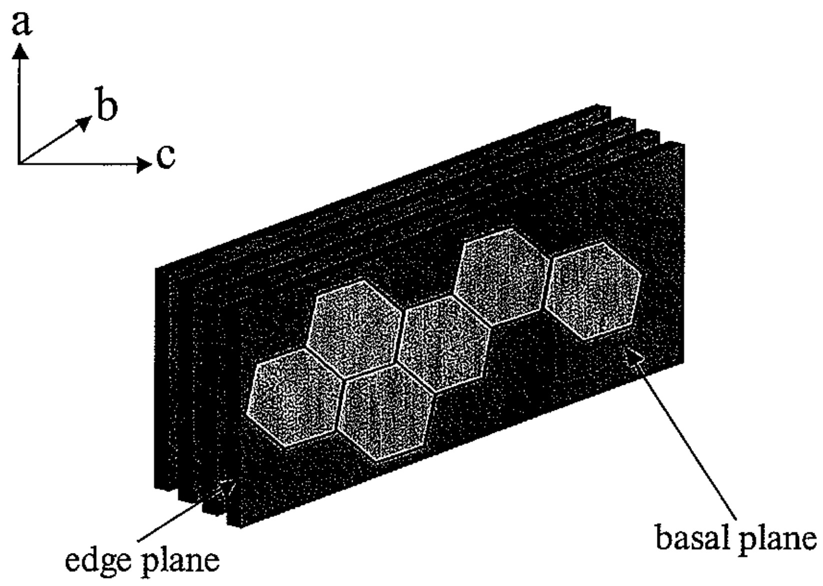

Figure 4. Microstructure of highly ordered pyrolytic graphite (HOPG).

that is simply referred to as glassy carbon (GC)[7-12]. GC has a significant volume of closed voids resulting in a low density material. As illustrated in figure 5, the structure is porous with randomly oriented microfibrils. The lamellar width (L_a) is on the order of 50 Å and the height (L_c) about 15 Å. Although difficult to machine due to its hardness, GC has several attractive properties as a material including impermeability to gases, resistance to chemical attack, electrical conductivity, and high purity.

Reticulated vitreous carbon (RVC)[13] is the second type of glassy carbon, and is an open pore material with a honeycomb structure. In contrast to GC, RVC has a high void volume and electrical conductivity and it therefore finds application as a flow-through electrode.

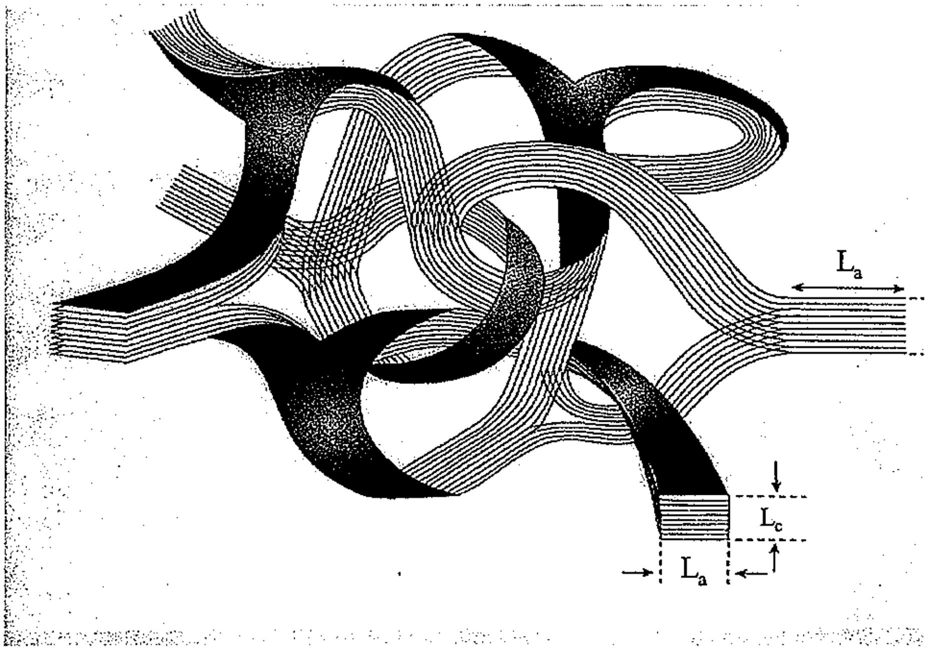

Figure 5. Proposed structure of glassy carbon.

Carbon Fibers

An important industrial carbonaceous material is carbon fiber, which is synthesized from either polyacrylonitrile (PAN) or mesophase pitch (generated by thermal polymerization of petroleum pitch) precursor fibers using two types of carbonizing heat treatments. Type I treated fibers are heated at temperatures greater than 2000 °C and have comparatively low strength to type II fibers, which are heated to $T < 1500$ °C. PAN fibers are more resistant to compressive failure than their pitch-based counterparts, are fibrillar in nature and subsequently do not possess an extended graphitic structure.

Carbon fiber materials have many advantages over powdered forms. First, the pores and active surface sites of fibers are highly accessible to adsorptive or reactive fluids. Since the fibrous materials can be woven into cloths and felts, they are devoid of the difficulties associated with packed beds such as channeling and settling. In the electrochemical arena, woven fibers (cloths and felts) are used as flow-through electrodes because of their conductivity, high surface area:volume ratio and low cost. Finally, carbon fibers have strength superior to graphite, hence these materials are used in composites found in aircrafts where long-term durability is critical.

In addition to conventional carbon fibers, one may also prepare activated carbon fibers (ACF). This process employs low temperature treatments in lieu of graphitization where small pores are developed via selective oxidation of carbon. Since adsorption at ACF is faster than at powdered carbon materials, they are used as catalysts, wound dressings and skin substitutes.

Another related type of carbon fiber is found in vapor grown carbon fiber (VGCF) composites. These materials are synthesized from the pyroloysis of hydrocarbons or CO in the presence of a catalyst. VGCF may be highly graphitized since the heat treatment temperature (HTT) is approximately 2800 °C and its properties may therefore approach that of single crystal graphite.

Carbon Nanotubes

First observed in 1991 by transmission electron microscopy (TEM)[14-16], carbon nanotubes have aroused much interest due to their unique properties. Nanotubes are prepared using a carbon arc or laser vaporization method in the presence of a metal catalyst (e.g. Ni-Y or Co-Ni) that prevents closure of the tube end. Carbon nanotubes consist of a cylidrical

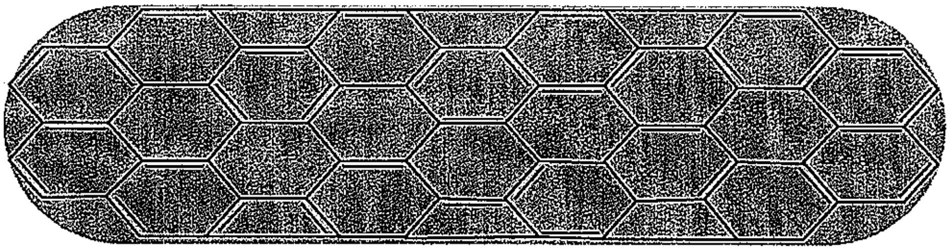

Figure 6. Simplified illustration of a single-walled nanotube.

graphene sheet closed on either end by a fullerene-like structure as depicted in figure 6. In general, the structure of a nanotube depends upon the orientation of the hexagons in the cylinder with respect to the tubule axis and is characterized in terms of its diameter, chiral angle and one-dimensional unit cell. Numerous other forms of nanotubes have been prepared having interesting nanostructures. Much research interest has been focused towards uses of these elegant carbon materials because of their metallic or semiconducting electronic properties.[17-20]

Fullerenes

First discovered in 1985 in the mass spectrum of laser-ablated graphite, fullerenes have attracted much interest due to their unique structure and properties.[21] The basic fullerene architype is that of C_{60}, also referred to as Buckyball since it resembles the geodesic domes of R Buckminster Fuller the noted architect and engineer (figure 7).

C_{60} fullerene consists of 12 pentagons and 20 hexagons arranged in a spheroid structure. Each carbon atom is bonded to three others by two long C-C bonds (~1.45 Å) and one short bond (~1.4 Å) whose bond orders are considered to be intermediate between C_{sp3}-

C_{sp3} and C_{sp2}-C_{sp2} bonds. Since the 1980s, other derivatives of the basic C_{60} structure have been synthesized and characterized including C_{70}, C_{78}, C_{82} and C_{84}.[22] The research focused on application of these unique molecules is too diverse to report here. In general, fullerenes find use as optical limiters and fullerene doped polymers have been applied as photo-voltaic devices and photo-refractive materials.

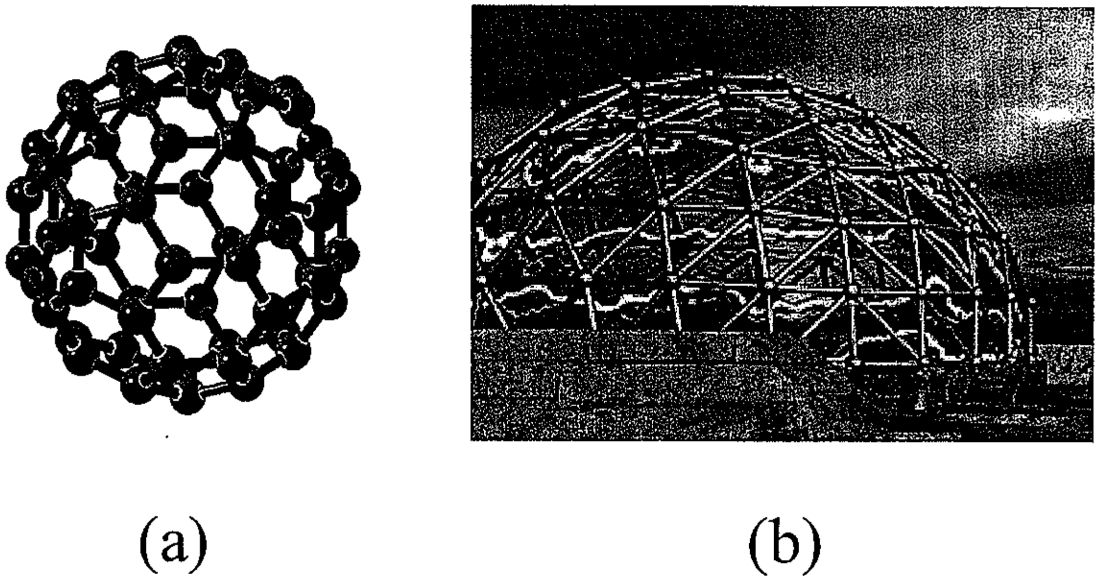

Figure 7. (a) Representative structure of a C_{60} Fullerene. (b) Geodesic dome design of R. Buckminster Fuller.[23]

Synthetic Diamond Materials

Synthetic diamonds are prepared by dissolution of graphite in metals and crystallizing the diamond at high pressure and temperature. More useful in analytical applications are the boron doped diamond (BDD) films, prepared by chemical vapor deposition (CVD), which have found application as electrodes due to the film properties which include: 1) no degradation over time, 2) a large potential window, 3) a low and featureless background, 4) low background noise, and 5) little to no adsorption of organic materials.[24]

Electrochemistry at Carbon Electrodes

One of the most extensive applications of carbonaceous materials in analytical chemistry has been in the field of electrochemistry. Carbon-based electrodes have become a critical part in the development of solid electrodes for several reasons.[25] First, many useful cost-effective forms of carbon are available for use as electrodes (table 3). Secondly, the kinetics of carbon oxidation is slow (depending on the electrolyte); this results in a wide potential window. Finally, one can easily take advantage of the surface groups inherently present on carbon to influence electron transfer properties of the material.

Whilst choosing an electrode for a particular electroanalytical task, several criteria must be considered including background current, potential limits, electrode kinetics, and the materials properties of carbon. However, there really is no ideal electrode for every situation, and the method by which a carbon electrode is handled has a significant impact on its electrochemical behavior. The criteria for electrode selection and effect of pretreatment on the electron transfer properties of the electrode will be discussed in the following sections.

Table 3. Common electrode materials and some representative applications.

Family		Electrochemical Application
Pyrolytic graphite	pyrolytic graphite (PG) highly ordered pyrolytic graphite (HOPG)	general electrode
Polycrystalline graphite	carbon paste electrodes (CPE) spectroscopic graphite electrodes graphite composite electrodes (wax impregnated graphite electrode-WIGE)	general electrode: used when a highly reproducible surface is needed
Glassy carbon	glassy carbon (GC) reticulated vitreous carbon (RVC)	general electrode RVC used as a flow-through electrode
Carbon fibers	perfectly oriented fibers disordered fibers	microelectrodes: flow through electrodes when woven into fabrics

Background Current, Potential Limits, and Electron Transfer Kinetics

The background current at a metallic electrode generally involves the charging of the double-layer at the electrode solution interface. However, with carbon electrodes, the situation is slightly more complex. Carbon electrodes have a "non-classical" component to the conventional background over and above double-layer charging which gets included in what is termed the apparent capacitance. Depending strongly on the pretreatment protocol used, this non-classical component has been attributed to surface redox processes, ion adsorption, and porosity. The magnitude of this component varies with the type of carbon employed.

The rate of electron transfer for an electrochemical process is discussed in terms of its heterogeneous electron transfer rate constant, k^o. A manifestation of surface structure, measurements of rate constants provide useful information about the electrode, and like many other parameters, the rate of electron transfer at carbon electrodes is strongly dependent upon the electrode type and history. For example, the voltammetry of the ferri/ferrocyanide couple in aqueous electrolyte (1 M KCl) at GC and HOPG demonstrates that the electron transfer is faster at the GC electrode surface.[25] This difference is best explained by the aforementioned differences in microstructures between the two substrates as enumerated in the following section.

Materials Properties of Carbon

The properties of carbonaceous materials vary greatly depending on the microstructure, surface roughness, cleanliness, oxidation and heterogeneity. As a simple illustration, one may consider the structure of HOPG (figure 4), which best resembles single crystal graphite. The electrochemical properties of HOPG are drastically different depending on whether one is using its basal or edge plane. Table 4 presents typical capacitances, adsorption values for anthraquinone and heterogeneous rate constants (k°) for the ferri-/ferrocyanide couple for both the basal and edge planes. From these data, one notices that the

Table 4. Comparison of electrochemical properties for basal and edge planes of HOPG.[25]

	Basal plane	Edge plane
Capacitance ($\mu F/cm^2$)	< 1.0	~ 50-70
Adsorption of anthraquinone disulfonate ($pmol/cm^2$)	< 1	> 150
k° for $Fe(CN)_6^{3-/4-}$ (cm/s)	$< 10^{-6}$	0.06-0.10

edge plane is more reactive towards adsorption of solution species and has faster kinetics, but it is more capacitive than the basal plane.

An additional feature of carbon materials to consider is the nature of the surface groups. Several oxygen-containing groups are typically present, in varying concentrations, as shown in figure 8, which shows a schematic representation of the functional moities that can be present on a carbon surface. The extent of oxidized surface species profoundly affects the background current and the interaction of the electrode with the solution species and depends on the treatment of the electrode. Oxidation of the carbon surface is difficult to avoid since most pretreatments (discussed in a later section) are carried out in the laboratory ambient.

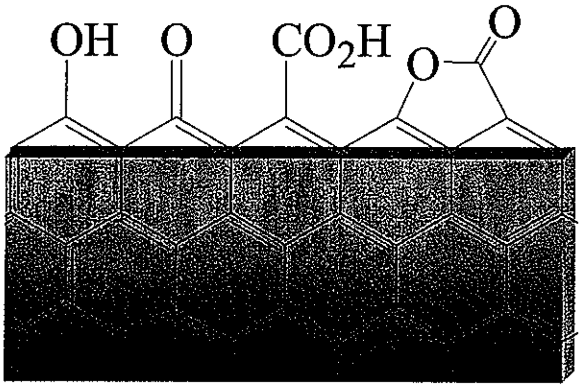

Figure 8. Representation of surface groups on a carbon substrate.

Electrode Pretreatment

As mentioned in previous sections, the identity and history of the carbon electrode is a vital component to understanding its electrochemical behavior. In many cases, the enhancement of electron transfer properties for a particular electroactive species at an electrode is desired. One way of improving the electrochemical behavior of a carbon electrode is by employing a pretreatment protocol to the electrode surface. In many cases, pretreatment results in the increase in the amount of oxygen-containing groups on the surface. As an added benefit, surface groups provide a convenient way to covalently modify the electrode surface. Several methodologies exist for pretreating the electrode prior to use, including mechanical abrasion[10,26-28], chemical oxidation[1], electrochemical pretreatment (ECP)[29-44], plasma treatments[45-48], in-situ laser activation[43,49-53], and vacuum heat treatment (VHT).[43,54] The notion of chemically modified electrodes (CMEs) will be expounded upon in a future section.

Mechanical Abrasion

The physical polishing of carbon electrode surfaces varies[1] amongst research groups and ranges from simple procedures to more involved methods. In most cases, abrasion of the electrode surface is achieved by sequentially using a series of three α-alumina powders starting at a 1.0 μm size, followed by 0.3 μm and 0.05 μm, rinsing with copious amount of deionized water after each polishing step. More rigorous protocols for treating rough surfaces call for using either diamond paste (6 μm, 1μm, 0.25 μm) or SiC sand paper (240, 400, 600 grit) prior to the Alumina series. After mechanical abrasion, the electrode is frequently ultrasonicated in water or an organic solvent such as acetonitrile or isopropyl alcohol to remove adsorbed species. Extreme measures call for the extraction of the electrode in a soxhlet apparatus. Table 5 summarizes the results on reaction rates for selected polishing protocols for differing carbon electrodes.[1]

Chemical Oxidation

Several common chemical oxidants are used to increase the concentration of oxygenated moieties on the carbon surface. The majority of these agents are inorganic acids including nitric and sulfuric acid, and are frequently combined with chromium, cerium, silver or hydrogen peroxide. However, treatment of an electrode surface with a corrosive acid generally results in the formation of pits or the destruction of the surface.

Electrochemical Pretreatment (ECP)

One of the most facile pretreatment procedures for activating a carbon electrode is accomplished by electrochemical oxidation of the electrode surface in an aqueous electrolyte. Table 6 summarizes the results of several more common electrochemical pretreatment methods on different electrode materials.[1]

Some generalization about the effect of ECP on the carbon surface may be readily drawn. First, the surface O/C ratio increases significantly as evidenced by X-ray photoelectron spectroscopy (XPS).[31] Secondly, slight damage is inflicted upon the sp^2 carbon lattice and layers may be removed. Finally, electrodes that are pretreated by electrochemical methods have higher background currents compared to polished surfaces.

Table 5. Activation of carbon electrodes by mechanical abrasion.[1]

Electrode	Polishing Protocol	Result
Glassy Carbon	1.) Polish with 600-grit SiC 2.) 30-,6-,1 μm diamond paste 3.) water rinse 4.) 0.05 μm Alumina	$Fe(CN)_6^{3-/4-}$ reaction rate increases
Glassy Carbon WIGE	1.) Polish with 0.3 μm Alumina 2.) H_2SO_4 rinse	Kinetics for $Fe^{3+/2+}$ greater after dipping in H_2SO_4
Glassy Carbon	1.) Polish with 200-, 400-, 600-grit SiC 2.) Ultrasonicate in H_2O (10 minutes) 3.) 1-, 0.3-, 0.05-μm Alumina 4.) Ultrasonicate in H_2O (10 minutes)	$Fe(CN)_6^{3-/4-}$ reaction rate increases

Table 6. Effect of ECP on some carbon electrodes.[1]

Electrode	Method	Result
HOPG (Basal plane)	Oxidation at 1.5V (vs. SCE) for 15 minutes	Reduction rate of $Fe(CN)_6^{3-}$ increases
HOPG (Basal plane)	Oxidation at 1.4V for 20 minutes	Oxidation rate of ascorbic acid increases
Glassy Carbon	Oxidation at 1.8V for 5 minutes and –0.2V for 15 s after polishing	Oxidation rate of $Fe(CN)_6^{4-}$ increases

Plasma Treatment

Another approach to increasing the amount of surface oxygen on the carbon surface is realized via the use of radiofrequency (rf) or microwave oxygen plasmas. Typically, glassy carbon or HOPG electrodes are subjected to a 0.5 h exposure at a pressure of 150 mTorr O_2 that results primarily in the formation of carboxyl and hydroquinone oxygen containing groups.[45] Furthermore, modification of both the basal and edge plane occurs.[46] In addition to oxygen as a plasma gas, research into other reagent gases has been conducted. Plasma gases such as water, carbon dioxide, chlorine, nitrogen and ethylenediamine have been used to alter the surface composition of glassy carbon substrates. Table 7 summarizes the atomic ratios from XPS analysis of glassy carbon electrodes that have been exposed to several different reagent plasma gases.[55]

Investigation of HOPG surfaces by atomic force microscopy (AFM) after microwave oxygen plasma treatment shows that even at low microwave powers pit formation is common.[48] Additionally, scanning electron microscopic (SEM) examination of glassy carbon substrates indicates that rf-plasmas cause some erosion.[47]

Despite the slight damage inflicted upon the carbon surface, plasma treatment still holds many advantages. First and foremost, it is a rapid, reproducible, and contaminant-free method for enhancing the electrochemical response of the electrode. Additionally, the plasma treated surfaces may be further treated with a secondary reagent, such as trifluoroacetic acid, to alter the surface composition.

Table 7. Results of several plasma treatments on a glassy carbon electrode.[55]

Plasma Gas	O/C	N/C	Cl/C
As Polished	0.144	---	---
O_2	0.248	---	---
H_2O	0.226	---	---
CO_2 (dry ice)	0.265	---	---
CO	0.304	---	---
Cl_2	0.139	---	0.185
CH_2Cl_2	0.102	---	0.649
N_2	0.090	0.140	---
NH_3	0.056	0.120	---
Ethylenediamine	0.055	0.175	---
Hydrazine	0.046	0.154	---

In-situ Laser Activation

Activation of carbon substrates is also accomplished by exposing the electrode surface to short duration, high-intensity pulses in air or in solution. This method of treatment increases the rate of heterogeneous electron transfer by one to three orders of magnitude,[49] decreases the O/C ratio,[50] and is most likely due to the exposure of active sites via the removal of chemi- and physisorbed impurities.[51,52]

As evidenced by SEM, scanning auger microscopy (SAM), Raman spectroscopy, and profiliometry, there are no detectable differences between a laser treated electrode and one that has been prepared by mechanical polishing.[49] However, it is possible ablate the electrode surface, although this is not necessary for improving the electron transfer rate.[50]

Vacuum Heat Treatment (VHT)

Vacuum heat treatment (VHT) entails heating a glassy carbon substrate to ~725 °C at pressures less than 2×10^{-6} Torr to avoid reactions associated with exposure to air after heating. Although the effective surface area of the electrode is not increased, this protocol serves to remove oxygen surface groups, and hence one observes a dramatic reduction in background charging current.[56]

Use of Carbonaceous Material in Chromatographic Applications

High Performance Liquid Chromatography (HPLC)

Carbon based materials have received much recent attention in the arena of separation science. As a prime example, porous graphitic carbon (PGC) (marketed by ThermoQuest Hypersil® as Hypercarb™) has been developed as a stationary phase for high performance liquid chromatography (HPLC) because of its attractive and unique properties.[57]

Conventional reversed stationary phases (e.g. octadecylsilica, ODS) generally involve the use of a silica support whose siloxane bonds are subject to hydrolysis at both low and high pH. Additionally, these silica phases are ineffective in separating structurally similar compounds such as geometric isomers. Due to its stable carbon framework, the performance of PGC stationary phases is not affected by pH, therefore it is functional at both pH extremes. Furthermore, PGC allows for the separation of both polar and nonpolar compounds since the retention mechanism is based on π–π interactions and subsequent charge-induced interaction with the extensive aromatic network of carbon atoms.

In addition to using PGC as a new reversed stationary phase, some researchers have modified the carbon surface via dynamic adsorption or coating the PGC particles with a modifier. For example, chiral selection may be achieved by adsorption of Lasalocid[58] or poly-L-leucine[59] on PGC. Coating the PGC particles with Cellulose Tris(3,5-dimethylphenyl carbamate)[60] also results in a chiral carbon stationary phase.

Electrochemically Modulated Liquid Chromatography (EMLC)

The use of carbon-based stationary phases in chromatographic separations has been furthered by the concept of electrochemically modulated chromatography (EMLC), which interfaces electrochemistry with HPLC. The notion of application of potential to chromatographic supports is not unprecedented in literature. Several researchers have attempted to effect separations of various analytes by electrochemical manipulation of retention on graphitic supports or electroactive ion exchange polymers.[61-63]

In our research group, EMLC is brought to fruition by designing a chromatographic column that incorporates aspects of an electrochemical system. Briefly, a porous stainless steel tube (the counter electrode) is fitted with a Nafion® ion-exchange membrane and packed with a conductive stationary phase (PGC or GC spheres) that serves as the working electrode. A silver/silver chloride reference electrode is then placed externally in an electrolyte reservoir as illustrated in figure 9.

Demonstration of EMLC as a viable separations method has been realized through the separation of many analytes including substituted aromatics, corticosteroids, and charged species.[64-74] In each case, the separation can be manipulated by changes in applied potential.

Modification of Carbon Substrates

Carbonaceous materials may be chemically modified to enhance their performance in a target application. There are three main methods to modify carbon substrates, including covalent modification, adsorption[58-60,75-95], and immobilization of

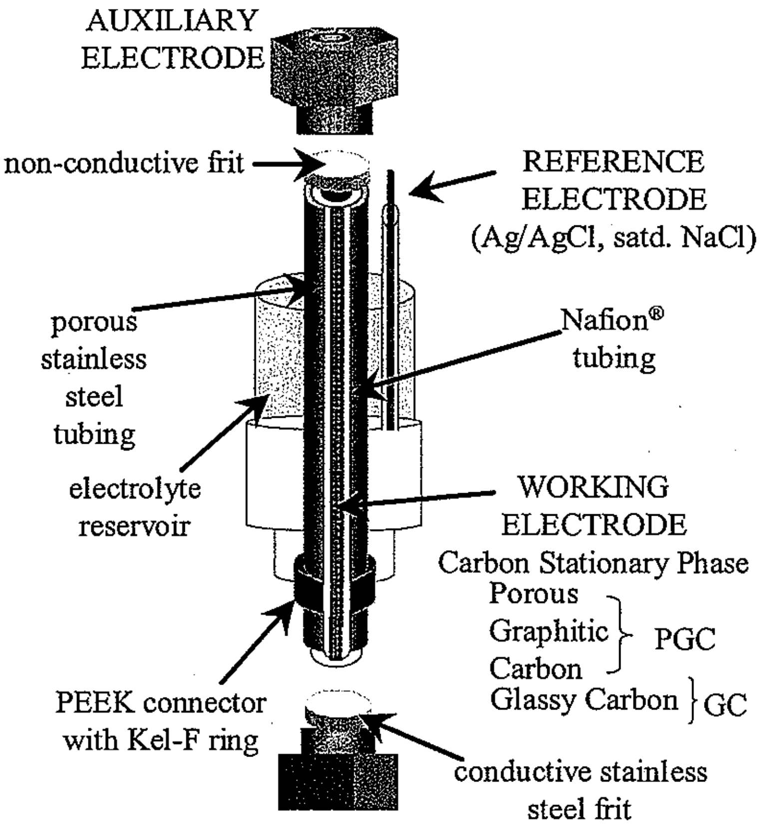

Figure 9. Column design for electrochemically modulated liquid chromatography.

the modifier in a polymer film.[1] Of these, covalent modification will be emphasized in this section since the concept will be used throughout this thesis.

Surface Group Reactions

As previously illustrated in figure 8, several oxygen-containing moieties are found on carbon surfaces, whether inherently present or the result of pretreatment procedures. Many researchers have taken advantage of these surface groups to chemically modify carbon electrodes.[96-101] Several different reagents and methodologies have been used for this purpose, and table 8 lists a few of the more common reaction schemes.[1] In many cases, oxidation, electrochemically or by rf-O_2 plasma, is necessary prior to modification to increase the number of O-containing groups for attachment.

Although the nature of the electrode surface can be altered in this fashion, many of the reactions, particularly silanization, are sensitive to moisture in the reaction environment. Additionally, it is difficult to have reproducible surface coverages since the concentration of oxygen-containing groups varies from electrode to electrode.

Direct Reaction with the Aromatic Network

A more robust approach to modifying a carbon electrode surface is realized through the generation of radical species that rapidly insert into the carbon-carbon framework, forming a covalent bond between the modifier and the surface. In sharp contrast to surface group reactions, functionalization with radicals is not dependent upon the surface oxygen concentration. Therefore, the resulting coverage of the modifier is much higher and the reaction does not require the careful experimental conditions that are necessary with other chemical reagents.

There are several methods to generating radicals that have precedent in literature including the electroreduction of arenediazonium salts[102-107], oxidation of arylacetates[108-111], anodization of amines[65,112,113] and alcohols[114-116], and the photogeneration of nitrenes.[117] With the exception of nitrene formation, each of these methodologies involves the generation of the radical species at an applied potential, with subsequent insertion of the radical species into the carbon framework of glassy carbon or HOPG.

Table 8. Reaction schemes for modifiying carbon electrodes via surface groups.[1]

Reaction Type	Reaction Scheme
Silanization	$>C \rightarrow >C\text{-}OH$ $>C\text{-}OH + XSiX_2R \rightarrow \mathbf{>C\text{-}O\text{-}SiX_2R} + HX$
Amidization	$>C \rightarrow >C\text{-}OOH$ $>C\text{-}OOH + SOCl_2 \rightarrow >C(O)Cl + SO_2 + HCl$ $>C(O)Cl + H_2NR \rightarrow \mathbf{>C(O)NHR} + HCl$
Esterification	$>C \rightarrow >C\text{-}OH$ $>C\text{-}OH + RC(O)Cl \rightarrow \mathbf{>C\text{-}O\text{-}C(O)R} + HCl$
Ether Bond Formation	$>C \rightarrow >C\text{-}OH$ $>C\text{-}OH + R\text{-}F \rightarrow \mathbf{>C\text{-}O\text{-}R} + HF$

Electroreduction of Arenediazonium Salts

The modification of glassy carbon and HOPG electrodes by an electrogenerated arene radical from an arenediazonium salt in anhydrous media (0.1 M tetrabutylammonium tetrafluoroborate in acetonitrile) is illustrated in figure 10. In this mechanism, an arene radical, formed from the corresponding arenediazonium salt at reducing potentials, rapidly inserts into a C-C bond of the electrode surface forming a covalent bond. Recent investigations of the modified surfaces by scanning probe microscopy (SPM) suggest that some of the radicals attach at defect sites on the electrode surface followed by subsequent attachment to the arene rings of the species at the defect sites to form a film.[111] Regardless of the exact mechanism, the resulting film is very robust and can only be removed via mechanical abrasion. Additionally, the surface concentration of the modifying species is significantly higher than that achieved by using surface group reactions.

Oxidation of Arylacetates

Perhaps the oldest electro-organic reaction involves the oxidation of carboxylates termed the Kolbe reaction. When conducted in the presence of a glassy carbon or HOPG electrode, the benzyl radical generated may insert into the C-C framework (figure 11) in a silimar fashion as that described for the electroreduction of arenediazonium salts.

Once again, the covalent bond formed is very robust. However, films formed by this method may be "erased" simply by stepping the applied potential anodically in fresh electrolyte. Additionally, if the applied potential is move more positive in the presence of the carboxylate modifier, a second layer of modifying species is attached to the remaining sites on the electrode surface.

The surface concentration of arene groups is on the order of that for the arenediazonium approach, ~ 10^{-10} mol/cm^2 as determined from employing Dreiding models to analyze scanning tunneling microscopy (STM) images.[108]

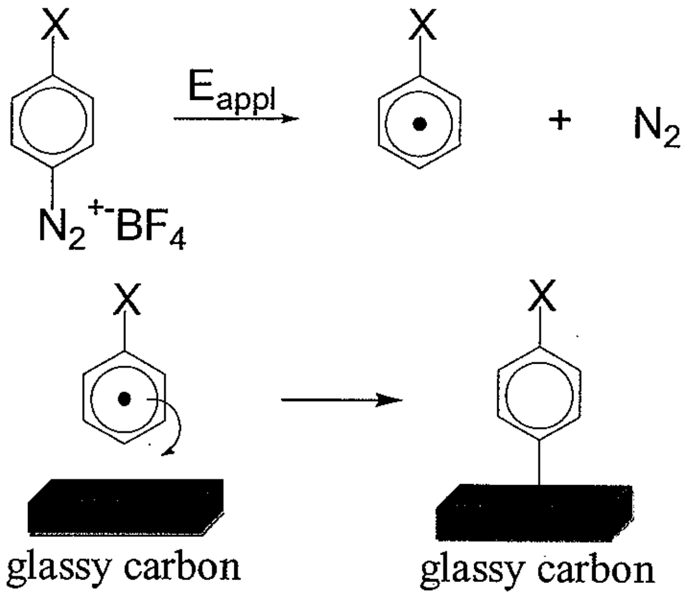

Figure 10. Mechanism for modification by electroreduction of an arenediazonium salt.

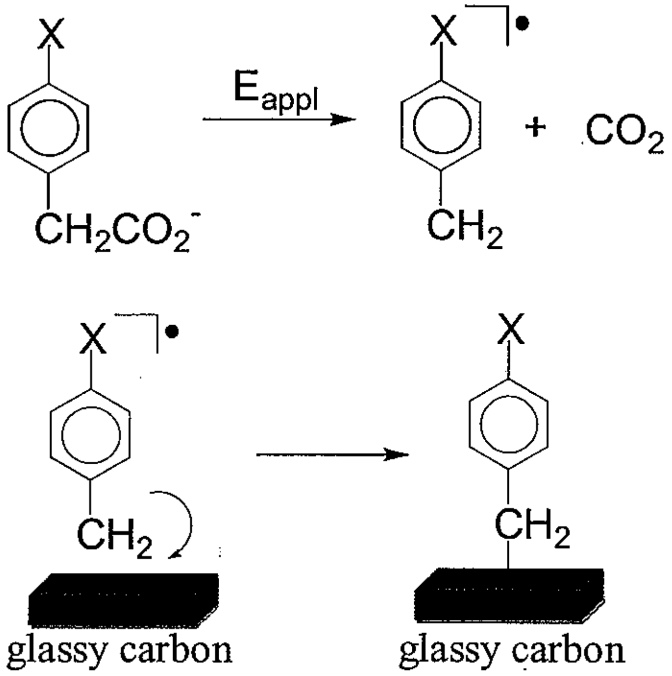

Figure 11. Mechanism for the Kolbe reaction.

Oxidation of Amines and Alcohols

Another electrochemical approach that grafts a functional group to a carbon electrode is found in the oxidation of amines and alcohols. In both cases, an anodic potential generates a radical cation that inserts into the aromatic structure of either glassy carbon or HOPG. Films of alkylamines[65], alkanols[114-116], and polyethylene glycols[118] have been prepared to suppress protein adsorption and aniline-based species have been employed for future use in polyoxometalate monolayer formation.

Nitrene Insertion

A final example of a covalently modified carbon surface is via the insertion of a photogenerated nitrene. Since the nitrene is photochemically generated from an azide precursor, this chemistry is ammenable to photopatterning.[117] To this end, the surface of glassy carbon has been patterned with photobiotin using maskless photolithography to generate micrometer-sized domains for subsequent attachment of avidin.

Conclusion

Carbon is a versatile substance, available in many forms, which lends itself to many facets of chemistry. In modern times, carbon powders are found as adsorbents in water purification systems, carbon fibers are used in dressings for wounds, and carbon microelectrodes are being used to study the electrochemistry of the brain. With its extensive applicability, carbonaceous materials have assured their position in the future of analytical science.

In this dissertation, carbon powders, specifically porous graphitic carbon and glassy carbon, are employed as electrochemically tunable stationary phases in electrochemically modulated liquid chromatography (EMLC). These materials are not only used in their native state, but are also effectively modified by electroreducing an arenediazonium salt at the surface. The modification protocol is straightforward, and imparts intriguing properties to the stationary phase.

Building on the utility of arenediazonium electroreduction, a mercaptoarenediazonium salt can be used to prepare a covalently bound thiol-terminated film on a glassy carbon electrode. The thiol terminus can be further exploited as an attachment point for gold nanoparticles. This methodology can be extended to the development of novel stationary phases in nanoscale chromatographic applications.

A second extension of EMLC entails the consideration of ferrocene-based mobile phase chiral selectors that may have enhanced selectivity at applied potentials. These chiral selectors must be stable at applied potentials from +500 to –500 mV (vs. Ag/AgCl, satd. NaCl) and have the appropriate chiral appendages to provide interaction points for enantiomeric analytes.

Finally, carbon-like properties can be imparted to gold substrates by modification with benzenehexathiol (BHT). Due to the planar arrangement of arene rings, the BHT monolayer has electrochemical behavior similar to that of a polished glassy carbon electrode, and provides a convenient method to alter the surface properties of gold.

The use of carbon and gold substrates and the modification agents for each will be discussed further in subsequent chapters.

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CHAPTER 1. CHEMICAL MODIFICATION OF CARBONACEOUS STATIONARY PHASES BY THE REDUCTION OF DIAZONIUM SALTS

A manuscript submitted to *Analytical Chemistry*

Jennifer A. Harnisch, Daniel B. Gazda, James W. Anderegg, and Marc D. Porter
Microanalytical Instrumentation Center, Ames Laboratory-USDOE, and Department of
Chemistry, Iowa State University, Ames, IA 50011 USA

Abstract

This paper describes a new strategy for the creation of chemically modified carbonaceous stationary phases. The strategy exploits the electroreduction of arenediazonium salts as a means for functionalizing the surface of glassy carbon (GC) and porous graphitic carbon (PGC) stationary phases. The one-electron reduction of these salts forms an arene radical which then couples via a carbon-carbon linkage to the carbon framework at the surface of the stationary phase. Two arenediazonium-based modifiers were used in evaluating the potential utility of this strategy: 4-nitrobenzenediazonium tetrafluoroborate for the GC and PGC phases and 4-hexylbenzenediazonium tetrafluoroborate for only the PGC phases. Modifications were carried out by packing the phases into a column used for electrochemically-modulated liquid chromatography. The effectiveness of the modifications was assessed by X-ray photoelectron spectroscopy and by comparing the liquid separation of a series of mixtures before and after coating deposition. For the nitrobenzyl-modified GC phase, the test mixture contained both anisole and fluoranthene. The performance of the nitrobenzyl- and hexylbenzyl-modified PGC stationary phases was characterized by the separations of substituted phenols (i.e.,

nitrophenol and resorcinol) and a few important pharmaceutical agents (i.e., hexobarbital, oxazepam, and nitrazepam). The potential utility of this modification procedure to form stationary phases that are stable upon extended exposure to aggressive mobile phases is discussed and briefly examined

Introduction

Porous graphitic carbon (PGC) is an extremely stable (pH 0-14 [1, 2]), hydrophobic stationary phase. Its microscopic surface structure, coupled with a strong π-electron character, facilitates the separation of a wide variety of analytes [1]. The hydrophobic character of PGC has also been exploited as a basis for creating chiral stationary phases by the dynamic adsorption of different types of enantiomers [3-6].

In our laboratory [7-15], we have been taking advantage of another property of PGC – its intrinsic conductivity – in exploring the range and scope of electrochemically modulated liquid chromatography (EMLC) as an intriguing variant to LC [7-10]. EMLC works by altering analyte retention through the changes in the donor-acceptor properties that result from manipulating the potential applied to a conductive stationary phase like PGC. Thus, EMLC provides a means to enhance the efficiency of a separation without the use of the gradient elution strategies often required by LC to address the analysis of complex samples. We have shown, for example, that EMLC can be readily applied to the separation of mixtures ranging from structurally similar corticosteroids [11] to racemates of important pharmaceutical agents [12, 13, 15]. The advantages of the on-line coupling with electrospray mass spectrometry (ES-MS) have also recently been demonstrated in a collaboration with Van Berkel's group. The goal was to exploit the ability of EMLC to separate complex samples without the associated changes in ionization efficiencies which arise from a changing mobile phase composition and degrade ES-MS performance [16]. The range and scope of EMLC as a new separation tool, including work in other laboratories, has recently been reviewed [10].

This paper reports on our exploration of another facet of combining PGC and EMLC – the ability to functionalize the surface of PGC by using electrochemically-based processes. The goal was to synthesize PGC phases that would function more like the widely-used bonded phases based on silica supports and silane-coupling chemistry. In examining the many strategies for modifying carbonaceous materials [17-20] (e.g. plasma treatment [21-23], hydrogenation [24], electropolymerization [25-28], and dynamic adsorption [3-6]), we opted to utilize the electroreduction of arenediazonium salts. As developed and expanded upon by the Savéant [29-31], Pinson [32], McCreery [33], and McDermott [34] laboratories, the one-electron reduction of arenediazonium salts forms an arene radical that inserts itself into the carbon framework of the electrode surface through a carbon-carbon linkage. By carefully controlling the reaction conditions [35], a monomolecular coating can be formed that can only be removed by mechanical abrasion. Based on these findings, we projected that the versatility of this type of derivatization strategy could be used to create new families of carbon-based stationary phases that would parallel the performance of the many types of silica-based bonded phases. We further hypothesized that the linkage chemistry for these new arene-modified phases would show exceptional stability upon exposure to aggressive mobile phases (i.e., phases inducing the hydrolysis of the Si-O linkages), potentially addressing one of the long standing weaknesses of silica bonded phases [36-39].

Herein, we describe our first results using the reduction of arenediazonium salts to modify both glassy carbon (GC) and PGC particles. Two modifiers are used:

4-nitrobenzenediazonium tetrafluoroborate (NBDT) and 4-hexylbenzenediazonium tetrafluoroborate (HBDT). Following electrodeposition (Scheme 1), these new phases were characterized by X-ray photoelectron spectroscopy (XPS), and then applied to the LC separation of a series of mixtures to assess the effectiveness of the coating process. Experiments to test the stability of the modified PGC stationary phases upon extended exposure to aggressive mobile phases were also conducted. The potential utility of this modification strategy for the creation of a wide range of functionalized stationary phases using carbonaceous materials is discussed.

Experimental Section

Reagents

Uncoated GC (Sigridur G, diameter ~6 μm after sieving; Brunauer-Emmet-Teller (BET) surface area 1.76 ± 0.02 m^2g^{-1}) or PGC spheres (Hypersil®, diameter ~7 μm as received; BET surface area 91.82 ± 0.51 m^2g^{-1}) were used as stationary phases. Lithium perchlorate, diiodomethane, sodium tetrafluoroborate, 4-hexylaniline, trifluoroacetic acid (TFA), tetrabutylammonium tetrafluoroborate (TBAT), anisole (AN), fluoranthene (FL), 3-nitrophenol (NP), resorcinol (RS), and 4-nitrobenzenediazonium tetrafluoroborate (NBDT) were purchased from Aldrich; hexobarbital (HB), oxazepam (OZ) and nitrazepam (NZ) from Sigma; and acetonitrile (HPLC grade), acetone, methanol (HPLC grade), toluene, sodium hydroxide, sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium nitrite, hydrochloric acid, 4 Å molecular sieves, and magnesium sulfate from Fisher. All column modification procedures used acetonitrile that was dried over anhydrous magnesium sulfate, and distilled and stored over 4 Å molecular sieves. Tetrabutylammonium tetrafluoroborate was dried at 110 °C in an oven prior to solution preparation. All other chemicals were used as received. HBDT was prepared according to a modified literature procedure [32]. All aqueous solutions were made with deionized water from a Millipore Milli-Q purification system.

Instrumentation

A Waters model 600E pump controller, model 610 pump, and valve station were used as the chromatographic system and a Waters 996 photodiode array detector as the detector. The detection wavelengths (λ_{det}) were either 254 nm or 220 nm. Solutions were introduced onto the chromatographic column via a 5-µL injection loop (Rheodyne model 7413). Chromatographic columns were packed using a Shandon slurry column packing system using a previously described procedure [7]. The dead volume of the columns were ~0.2 mL. The design and fabrication of columns used in EMLC have also been extensively detailed [9].

Applied voltages (E_{appl}) were controlled by a Princeton Applied Research model 173 potentiostat/galvanostat. Cyclic voltammograms were recorded on a Houston Instruments 2000 X/Y.

XPS data were obtained using a Physical Electronics Industries 5500 surface analysis system equipped with a hemispherical analyzer, torroidal monochromator, and multichannel detector. Monochromatic aluminum Kα radiation (1486.6 eV) at 300 W was used for excitation. Binding energies were referenced to the C(1s) emission band at 284 eV. The pressure in the XPS chamber was less than 1×10^{-9} Torr during analysis. The surface areas of the GC and PGC particles were determined using an ASAP 2010 BET surface analyzer.

Electrochemical evaluation of NBDT and HBDT

The electroreduction of NBDT and HBDT was studied at a glassy carbon plate electrode (GC-20, Tokai) prior to on-column modification. All GC electrodes were polished sequentially with 1.0 μm, 0.3 μm and 0.05 μm alumina powder (Buehler) [33]. After polishing, the electrodes were sonicated for 10 min in deionized water followed by 10 min in acetonitrile, and dried under a directed stream of high purity nitrogen. Acetonitrile solutions of 10 mM NBDT or HBDT, containing 0.1 M TBAT, were used as the modifier and electrolyte, respectively, in a standard three-electrode electrochemical cell. Solutions were purged with high purity nitrogen for 3 min before the potential was scanned, and an inert atmosphere of nitrogen was maintained above the solution in the electrochemical cell at all times. Applied potentials were referenced against a silver/silver chloride electrode (sat'd. NaCl) with a platinum coil as the auxiliary electrode.

On-column functionalization by NBDT and HBDT

A freshly packed EMLC column was rinsed with anhydrous acetonitrile for 1 h. This step was followed by elution of 0.1 M TBAT in anhydrous acetonitrile at 0.40 mL/min for 1 h at E_{appl} of –255 mV. The external electrolyte reservoir was filled with 0.1 M TBAT in acetonitrile. A solution of 50 mM NBDT and 0.1 M TBAT in acetonitrile was then passed through the column (0.40 mL/min) at E_{appl} = -255 mV for 5 h. After a 5 h modification period, the column was rinsed for 1 h with a flowing solution of neat acetonitrile.

Carbon columns were modified with HBDT following the same procedure used for modification with NBDT. With HBDT, however, the modification was conducted at E_{appl} = -800 mV for 5 h by using a 40 mM solution of HBDT and 0.1 M TBAT in acetonitrile. These potentials were chosen based on the voltammetry of NBDT and HBDT at a GC plate electrode.

We add that arenediazonium salts will react slowly over time to form the corresponding azo dye [40]. Using anhydrous acetonitrile and dry electrolyte in each step of the modification, however, maintains the integrity of the modifier. Degradation of the modifier is easily detected since solutions of the diazonium salt are yellow and gradually turn a vibrant red upon conversion to the azo dye.

Chromatographic separations

Two mobile phase compositions were used: Mobile phase A consisted of 50:50 acetonitrile:H_2O (0.1 M lithium perchlorate), and Mobile phase B consisted of 100% methanol. Three different analyte mixtures were used. Analyte Mixture 1 was 300 ppm anisole and 100 ppm fluoranthene in acetonitrile, Mixture 2 was 170 ppm 3-nitrophenol and 400 ppm resorcinol in methanol, and Mixture 3 was 100 ppm each in hexobarbital, oxazepam, and nitrazepam in methanol. The temperature of the chromatographic system was not regulated.

Evaluation of stability in aggressive mobile phases

The stability of 4-nitrobenzene (NB) and 4-hexylbenzene (HB) modified PGC stationary phases was tested using two different mobile phase compositions: Mobile phase C: 50:50 acetonitrile:H_2O (0.1% TFA, pH = 1.5), and Mobile phase D: 50:50 acetonitrile:H_2O (20 mM Na_2HPO_4, pH = 11.0). The tests, which mimic those used in evaluations by others [41], were conducted at room temperature by first passing Mobile phase C through the column for ~2000 min, followed by Mobile phase D for ~2000 min. The flow rate in both cases was 0.5 mL/min. A 300 ppm solution of toluene in methanol was injected periodically over the exposure time to assess the effect of exposure on k' (defined as $t-t_0/t_0$, where t = retention time and t_0 = retention time of injection peak).

Results and Discussion

Electrochemistry of NBDT and HBDT at a GC electrode

As a starting point, the electrochemistry of both NBDT and HBDT was investigated using a GC electrode to identify the appropriate conditions for column modification. Successive current-potential (*i-E*) curves for a 10 mM solution of NBDT using 0.1 M TBAT in acetonitrile as the electrolyte are presented in Figure 1. A large, irreversible cathodic wave is observed in the first scan that dramatically decreases to near background levels with successive scans. The cathodic wave is attributed to the electrode reaction in Scheme 1, and the marked decrease in current in each of the subsequent scans is diagnostic of the strong passivation of the electrode by the formation of the organic monolayer coating [29-32]. The same general behavior is observed upon the repetitive voltammetric cycling for a GC electrode immersed in electrolyte containing 10 mM HBDT. However, because of the difference in donor-acceptor strengths of the hexyl- appendage of HBDT with respect to the nitro- appendage of NBDT, the one-electron reduction of the diazonium group for HBDT is shifted negatively by ~550 mV. Based on these data, we opted to use an applied potential of –255 mV for modification of our columns with NBDT and of –800 mV for modification by HBDT.

XPS characterization of NB-modified GC electrode

XPS was used to characterize the composition of the NB coating as well as to estimate its surface concentration [29-33] (a characterization of the HB-modified electrode was not performed since the composition of the modifier would be indistinguishable from the substrate). Prior to XPS analysis, the modified GC electrode was extensively sonicated in acetonitrile to remove any unreacted modifier and then dried under a directed stream of high purity nitrogen. Figure 2 presents the XPS spectra for an unmodified (a) and NB-modified (b) GC electrode; the corresponding atomic percentages are reported in Table 1. A freshly prepared GC electrode shows only two bands, one at 284 eV for C(1s) electrons (89.8 atomic %) and one at 532 eV for O(1s) electrons (10.2 atomic %). However, the spectrum after modification with NBDT displays a band for the N(1s) electrons (12.0 atomic %) of the NO_2 moiety at 406 eV. A much smaller band at 400 eV is also evident, which likely arises from the reduction of the nitro group to an amine group during the electroreduction of the diazonium salt [35]. These results are consistent with the expected composition of the coating.

The XPS data were also used to estimate the surface concentration of the coating from the signal strength in the N(1s) region. Thus, the surface concentration of nitrogen-containing aromatic groups on the GC electrode was calculated by multiplying the ratio of the total area under the two N(1s) bands with respect to the C(1s) band by a factor of 7.3×10^{-9} mol/cm^2. This factor is the surface density of the carbon atoms for basal plane graphite, and was used as a rough approximation of the much more complex surface of GC. This approach yields a surface concentration for the NB-modified GC electrode of 1.2×10^{-9} mol/cm^2. This value is similar to that previously reported for carbon electrodes modified with a NB-monolayer [33], suggesting that this derivatization strategy can be used to prepare carbonaceous phases coated with a single molecular layer.

On-column modification of carbon stationary phases

The electrochemical functionalization of carbon in spherical form is significantly more difficult than that of carbon in planar form when considering the difficulties associated with uniformly coating small particles (5-10 μm diameter) in a packed column. Our first experiments used NBDT as a modifier.

Upon completion of modification processes described in the Experimental Section, the stationary phases were first analyzed by XPS. Figure 3(a) presents a typical XPS spectrum for the unmodified GC spheres. Like the GC electrode, the spectrum shows a band at 284 eV for the C(1s) electrons and a band at 532 eV for the O(1s) electrons from oxygen-containing groups. After modification of both GC and PGC spheres, a band (406 eV) indicative of the presence of a NO_2 group is observed (see Figures 3(b) and 3(c)). A band for an amine-nitrogen is also evident, but at a much lower intensity in the case of the NB-modified GC particles; the basis for the disparity in intensities is uncertain at this time [33, 42].

Because of the sphericity and/or porous structure of the two types of particles, estimates of the surface concentrations of the modifier were viewed as unreliable. We, therefore, report only the atomic concentrations of C, O and N from the XPS data. These data are summarized in Table I, which includes values for unmodified and modified GC and PGC spheres for comparative purposes. For both NB-modified GC and PGC, the atomic concentration of nitrogen is at least an order of magnitude greater than that for the analogous unmodified spheres, confirming the presence of the organic coating on the surfaces of the two types of particles.

Chromatography of aromatic compounds on a NB-modified GC column

Nitro-based silica stationary phases are commonly used for the separation of mixtures of polyaromatic hydrocarbons (PAHs) and aromatic pesticides via moderate electron donor-acceptor interactions [43]. Coupled with inherent difficulty in eluting PAHs from carbon-based stationary phases, we therefore chose anisole (AN) and fluoranthene (FL) (i.e., Mixture 1) as analytes for an initial assessment of the effect of nitrophenyl modification on the retention of GC stationary phases [44, 45].

The chromatograms in Figure 4 present the separation of Mixture 1 using an unmodified GC column (a) and a NB-modified GC column (b). In the case of the unmodified GC spheres, AN elutes within ~0.5 minutes after injection. FL, in contrast, is strongly retained on the column, requiring a very high concentration of organic modifier (98%) for elution. This high of an acetonitrile concentration, however, causes the co-elution of both analytes.

After treatment of the GC column with NBDT, a dramatic difference in analyte retention is observed. Two elution bands are now present with AN eluting before FL. We note, however, that the elution band for FL is slightly tailed. We suspect that this tailing may arise from pinholes in the NB-coating, which results in π-π interactions with the underlying GC surface. Importantly, this result confirms that: 1) the NB-coating effectively masks the strong interactions that caused the irreversible adsorption of FL on uncoated GC; and 2) the derivatization procedure provides a facile means to create a modified carbon-based stationary phase. The next section examines the effectiveness of using this modification strategy to functionalize PGC, which is a more complex [2], but much more extensively used, stationary phase [1].

Comparison of NB and HB modified PGC to Hypercarb®

PGC was modified with NBDT and HBDT using the same procedure as for the GC spheres. After derivatization, both the NB and HB-modified PGC stationary phases were packed into a stainless steel HPLC column, and the resulting separations were compared to those using a Hypersil® Hypercarb® PGC column of the same dimensions. The chromatographic separations of Mixture 2, which contains 3-nitrophenol (NP) and resorcinol (RS), and Mixture 3, which is composed hexobarbital (HB), oxazepam (OZ) and nitrazepam (NZ), are shown in Figures 5 and 6, respectively, for the NB-modified PGC (a), the HB-modified PGC (b) and Hypercarb® (c).

As is evident, modification of PGC spheres with both NBDT and HBDT results in a marked change in retention. In all three cases, the elution of the two components of Mixture 2 follows the same order, with NP eluting before RS. However, the time required to fully elute the mixture at the modified phases is much less than at the unmodified phase. The results for the three different separations of Mixture 3 exhibit the same trend (i.e., HB elutes before OZ, followed by NZ).

In comparing the performance of the two types of modified PGC, the retention times for all five of the test compounds in Mixtures 2 and 3 are longer at the HB-modified stationary phase. This difference parallels those established in the literature of silica bonded phases [46]. In other words, the affinity of the HB-modifier for NP, RS, HB, OZ and NZ is greater than that of the NO_2 modifiers.

Stability of NBDT and HBDT stationary phases to aggressive mobile phases

One of the benefits of using a PGC-based stationary phase, in addition to its compatibility with organic solvents and absence of swelling, is its stability upon exposure to extremes in pH [1]. This situation is in marked contrast to conventional reversed phase silica stationary phases, which are prone to degradation in both strongly acidic and strongly basic solutions [36-39]. It is therefore of great importance to evaluate the stability of our functionalized PGC stationary phases upon extended exposure to mobile phases of high and low pH.

This assessment was conducted using a HB-modified PGC stationary phase and toluene as the test analyte [47]. As described in the Experimental section, the functionalized stationary phase was first exposed to Mobile phase C (50:50 acetonitrile:H_2O (0.1% TFA (pH 1.5)) at a flow rate of 0.5 mL/min for ~2000 min, and then to Mobile phase D (50:50 acetonitrile:H_2O (20 mM Na_2HPO_4 (pH 11.0)) for ~2000 min. Toluene was injected periodically into the running mobile phase in order to determine the effect of these treatments on retention.

The results are presented in Figure 7 by a plot of the observed k' for toluene as a function of exposure time. Throughout the duration of the experiment, the performance of the column is only marginally diminished. That is, the retention character of the HB-modified PGC stationary phase is decreased by only ~11% by the exposure of ~4000 column volumes of Mobile phase C, followed by ~4000 column volumes of Mobile phase D. Albeit only preliminary, these results indicate that HB-modified PGC is less susceptible to degradation under these types of mobile phases than many of the commercially available bonded phases, [41] demonstrating the potential utility of using the electroreduction of arenediazonium salts as a means for preparing a new family of stable, chemically functionalized stationary phases.

Conclusion

This paper has demonstrated the capability to chemically manipulate the surface of carbon-based materials used as stationary phases for LC separations. The derivatization can be easily carried out by the on-column electroreduction of arenediazonium salts, yielding, as a consequence of the linkage chemistry, a modified coating that is chemically stable under conditions generally detrimental to the performance of silica-based bonded phases. In view of the ease in which these compounds can be synthetically manipulated, we believe that this methodology can be applied at the same level of flexibility to other arenediazonium salts, (e.g., an octadecyl-like phase based on the electroreduction of 4-octadecylbenzenediazonium tetrafluoroborate). This strategy therefore opens a wide range of possibilities for creating a new family of stable, chemically modified stationary phases. Efforts to both explore the potential of this type of stationary phase and to further assess its stability under a wide range of mobile phases are underway.

Acknowledgment

Helpful discussions with M.T. McDermott and R.L. McCreery are gratefully acknowledged. J.A.H. wishes to acknowledge the Phillips Petroleum Company for a research fellowship. This work was supported by the Office of Basic Energy Research, Chemical Sciences Division of the U.S. Department of Energy and by the Microanalytical Instrumentation Center of Iowa State University. The Ames Laboratory is operated for the U.S. Department of Energy by ISU under Contract W-7405-Eng-82.

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(47) Although the NB-modified PGC is relatively stable in acidic media, exposure to basic conditions results in the partial hydrolysis of the NO_2 moiety (40). Preliminary tests show a working pH range of 1.5 to 7.0.

Figure Captions

Scheme 1: Procedure for the on-column modification of a carbon stationary phase particle via the one-electron reduction of an arenediazonium salt.

Figure 1: Voltammetry of 10 mM NBDT at a freshly polished glassy carbon electrode (scans 1-4). Electrolyte: 0.1 M TBAT in acetonitrile. Scan rate = 200 mV/s.

Figure 2: XPS of an unmodified GC electrode (a) and of a NB-modified GC Electrode (b). Inset for Fig. 2b is the N(1s) region which shows the diagnostic emission for the nitrogen of an NO_2 group at 406 eV and an NH_2 group just below 400 eV.

Figure 3: XPS of unmodified, 6-μm glassy carbon particles (a), of NB-modified GC particles (b), and of NB-modified PGC particles (c). Insets for Figs. 3b,c are the N(1s) region which shows diagnostic emission for the nitrogen of an NO_2 group at 406 eV and an NH_2 group just below 400 eV.

Figure 4: Separation of analyte mixture 1 on an unmodified GC stationary phase (a), and of analyte mixture 1 on a NB-modified GC stationary phase (b). Mobile phase A was used at a flow rate of 0.50 mL/min. $\lambda_{det} = 254$ nm.

Figure 5: Separation of analyte mixture 2 on a NB-modified PGC stationary phase (a), a HB-modified PGC stationary phase (b), and a Hypersil® Hypercarb® column (c). Mobile phase B was used at a flow rate of 1.00 mL/min in all separations. λ_{det} = 254 nm.

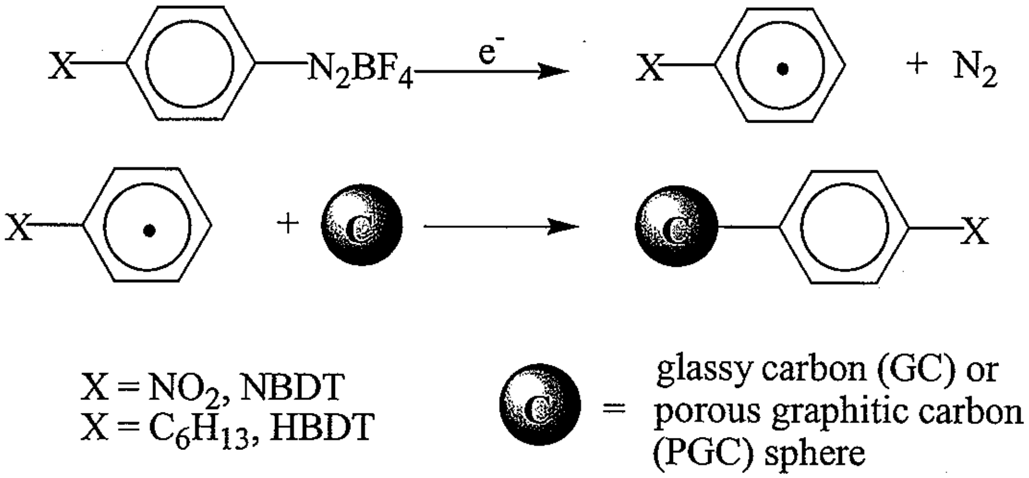
Figure 6: Separation of analyte mixture 3 on a NB-modified PGC stationary phase (a), a HB-modified PGC stationary phase (b), and on a Hypersil® Hypercarb® column (c). Mobile phase B was used at a flow rate of 1.00 mL/min in all separations. λ_{det} = 220 nm.

Figure 7: Long term stability of HB-modified PGC during sequential exposure to Mobile phase C (~2000 min) and Mobile phase D (~ 2000 min) at a flow rate of 0.50 mL/min. λ_{det} = 254 nm. An injection of 300 ppm toluene in methanol was made at each exposure time and k' ((-●-) Mobile phase C; (-■-) Mobile phase D) determined from the corresponding chromatograms.

Table 1. XPS atomic concentrations of C, O and N for unmodified and NB-modified GC electrodes and GC and PGC stationary phases.

Packing Material	C(1s) Concentration (atomic %)	O(1s) Concentration (atomic %)	N(1s) Concentration (atomic %)
Unmodified GC Electrode	89.8	10.2	ND[a]
NB-modified GC Electrode	71.4	16.6	12.0
Unmodified GC Spheres	88.8	10.6	0.6
NB-modified GC Spheres	75.4	16.6	8.0
Unmodified PGC Spheres	100.0	0.0	ND
NB-modified PGC Spheres	87.6	7.2	5.2

[a] ND- not detected


X
N$_2$BF$_4$
e$^-$
X
+ N$_2$
X
+
C
C
X
X = NO$_2$, NBDT
X = C$_6$H$_{13}$, HBDT
C
=
glassy carbon (GC) or
porous graphitic carbon
(PGC) sphere

Scheme 1

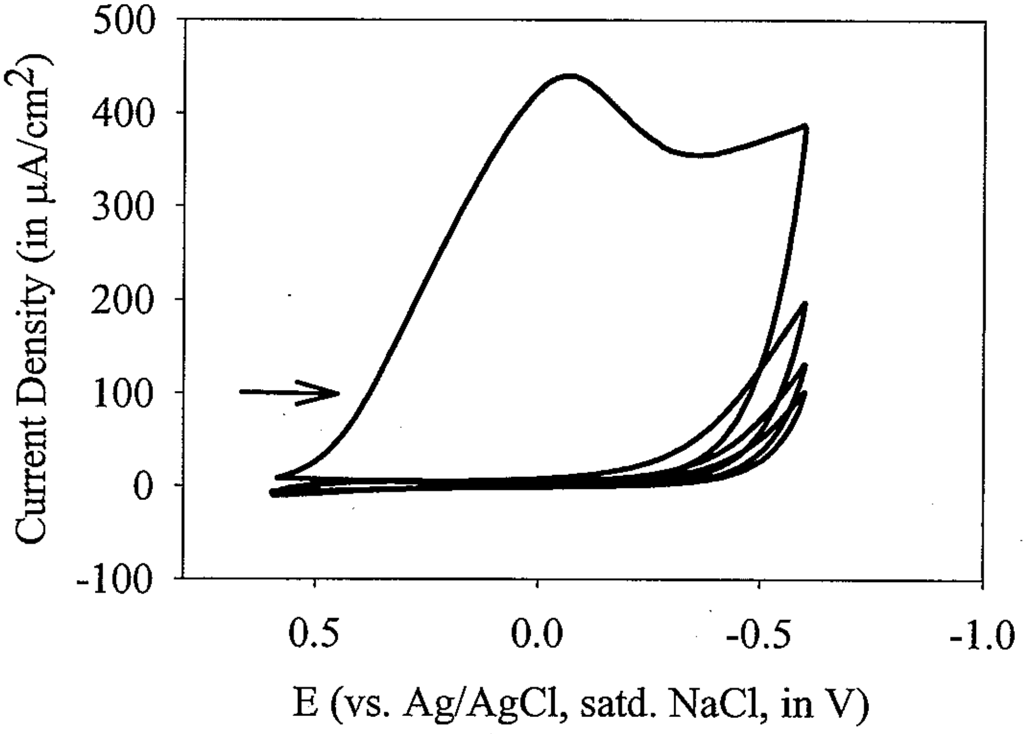

500
400
300
200
100
0
-100
Current Density (in μA/cm²)
0.5
0.0
-0.5
-1.0
E (vs. Ag/AgCl, satd. NaCl, in V)

Figure 1

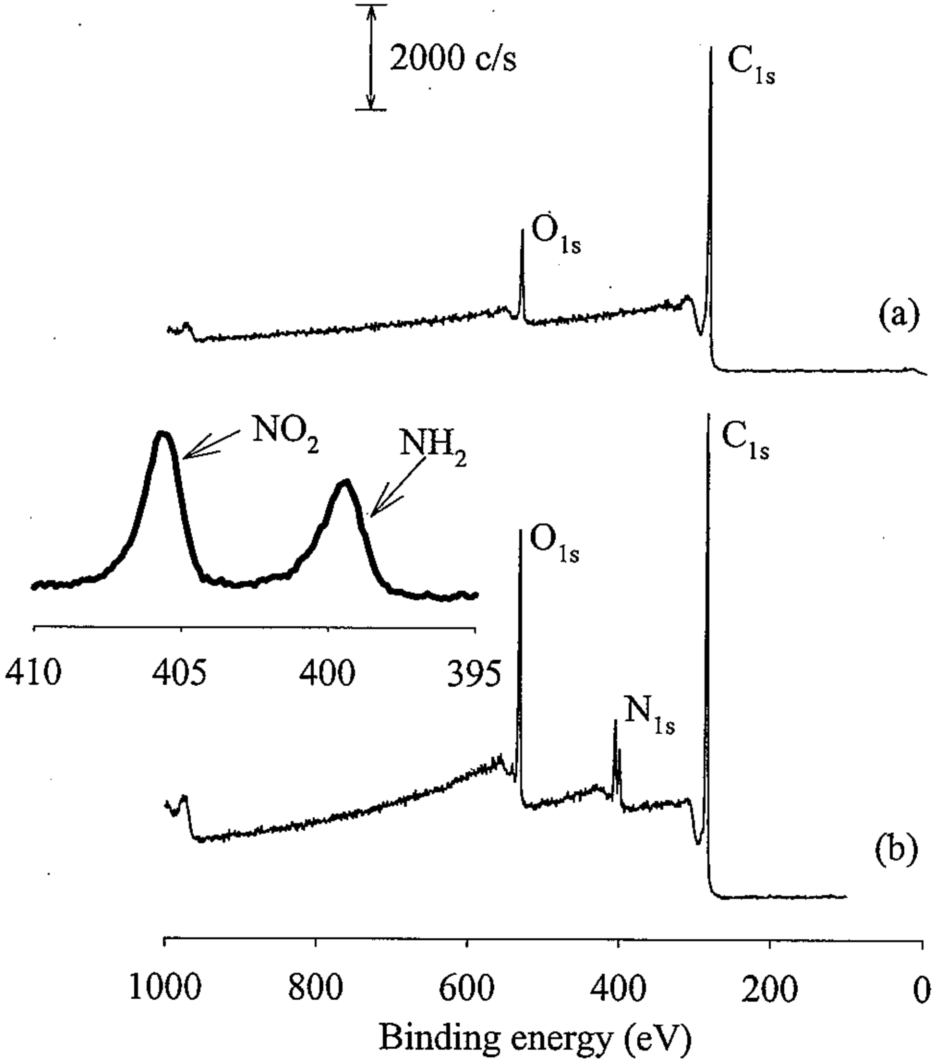

2000 c/s
C_{1s}
O_{1s}
(a)
NO_2
NH_2
C_{1s}
O_{1s}
410
405
400
395
N_{1s}
(b)
1000
800
600
400
200
0
Binding energy (eV)

Figure 2

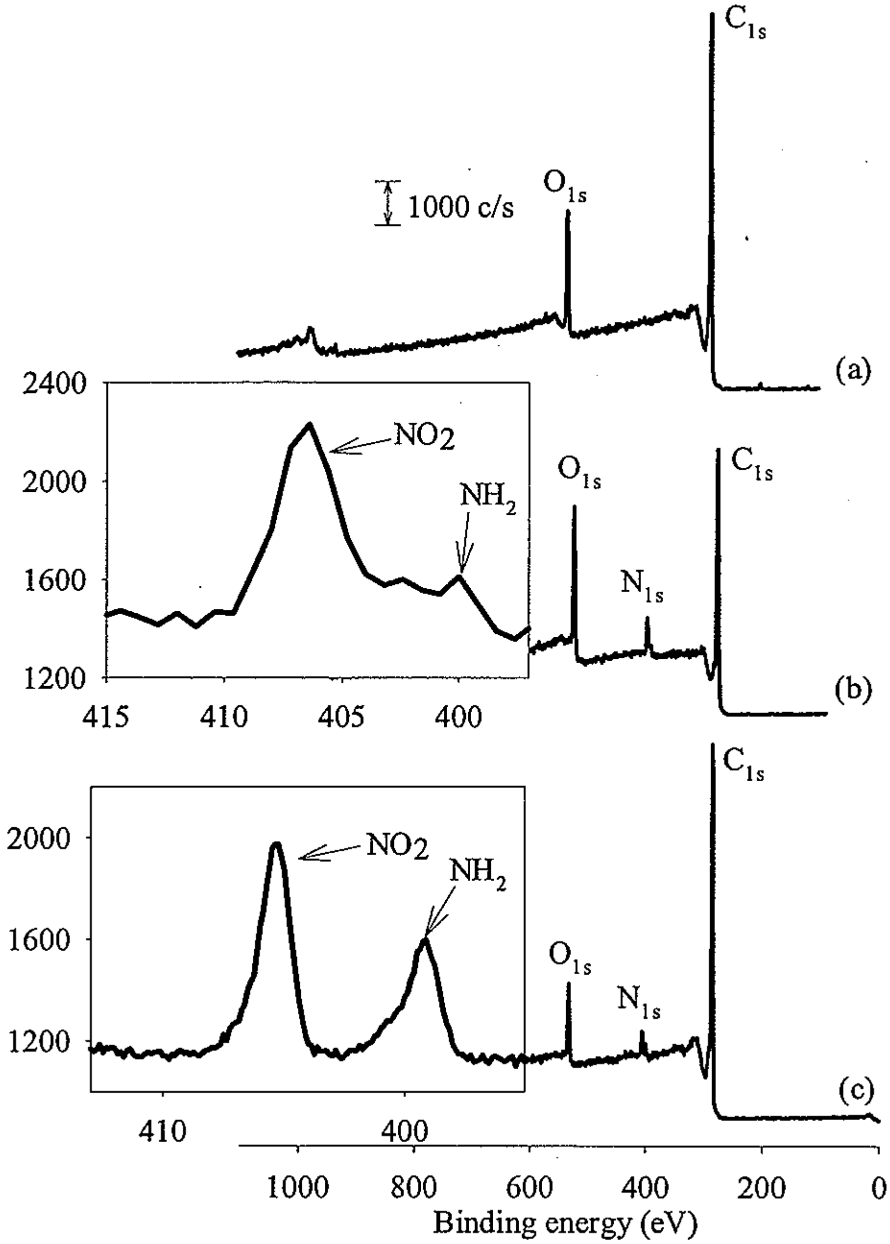

C1s
O1s
1000 c/s
(a)
2400
2000
1600
1200
415
410
405
400
NO2
NH2
O1s
N1s
C1s
(b)
C1s
2000
1600
1200
NO2
NH2
O1s
N1s
(c)
410
400
1000
800
600
400
200
0
Binding energy (eV)

Figure 3

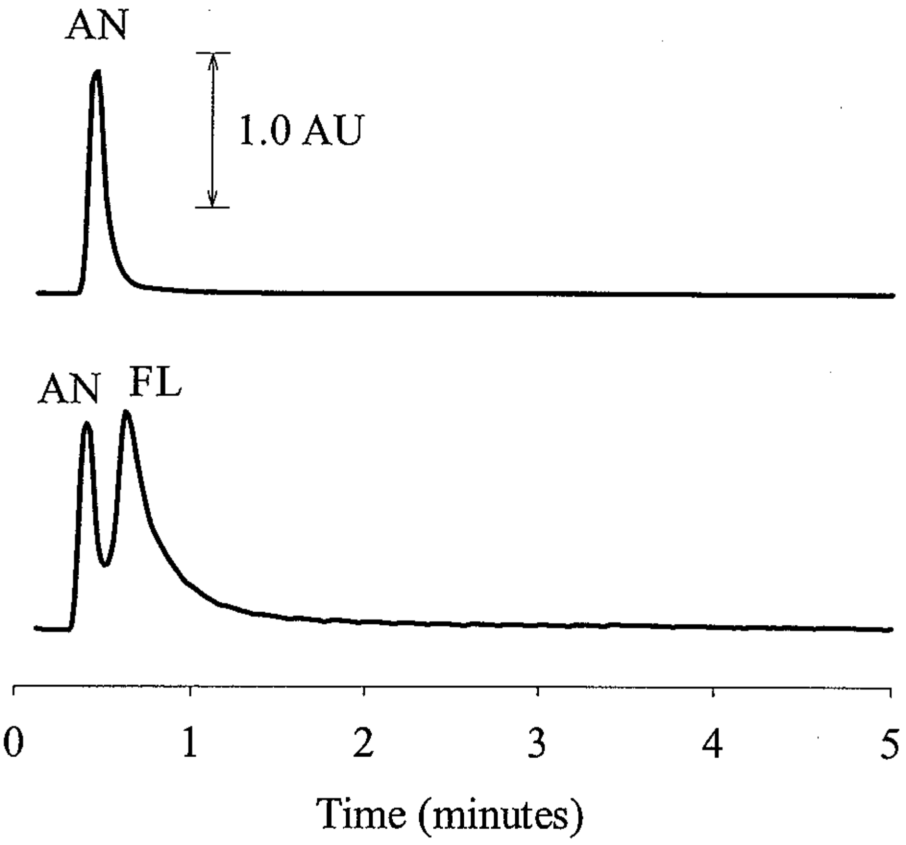


Figure 4

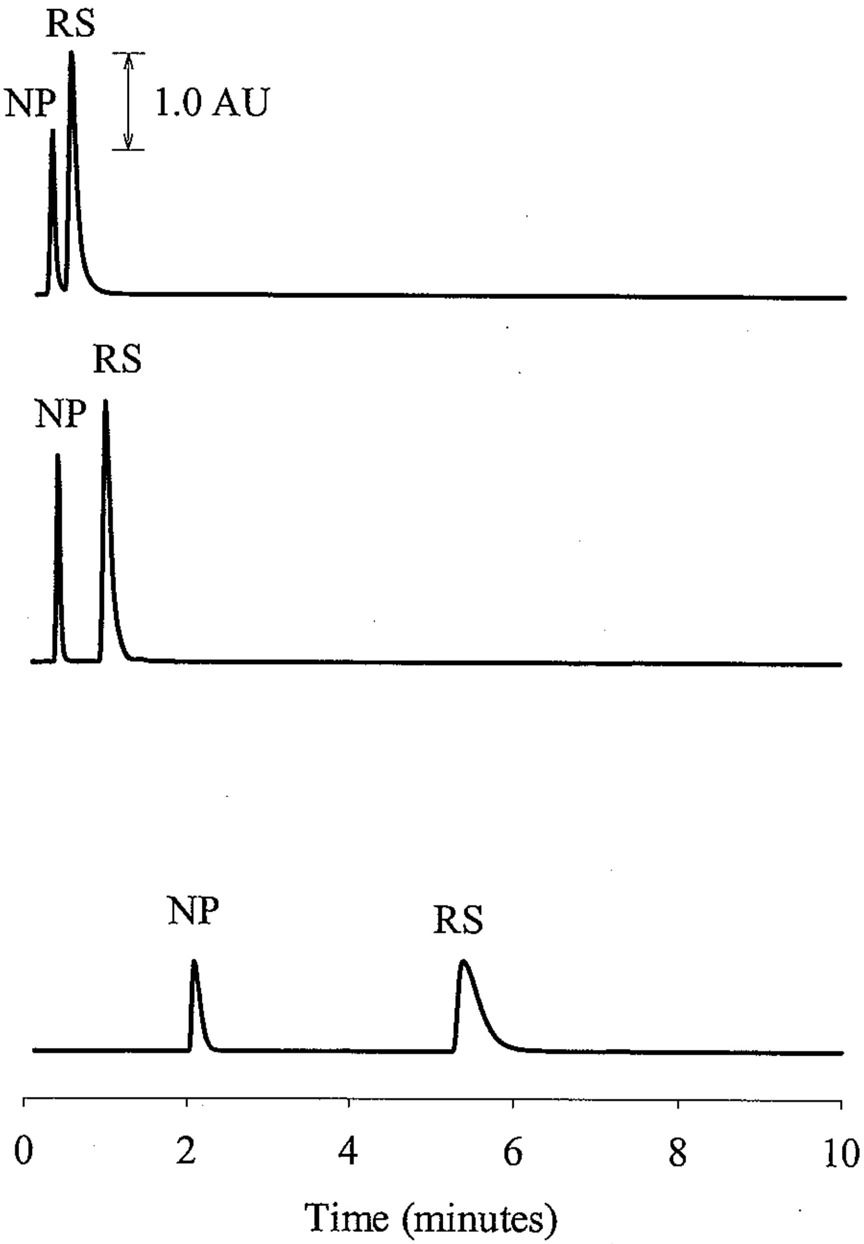


Figure 5

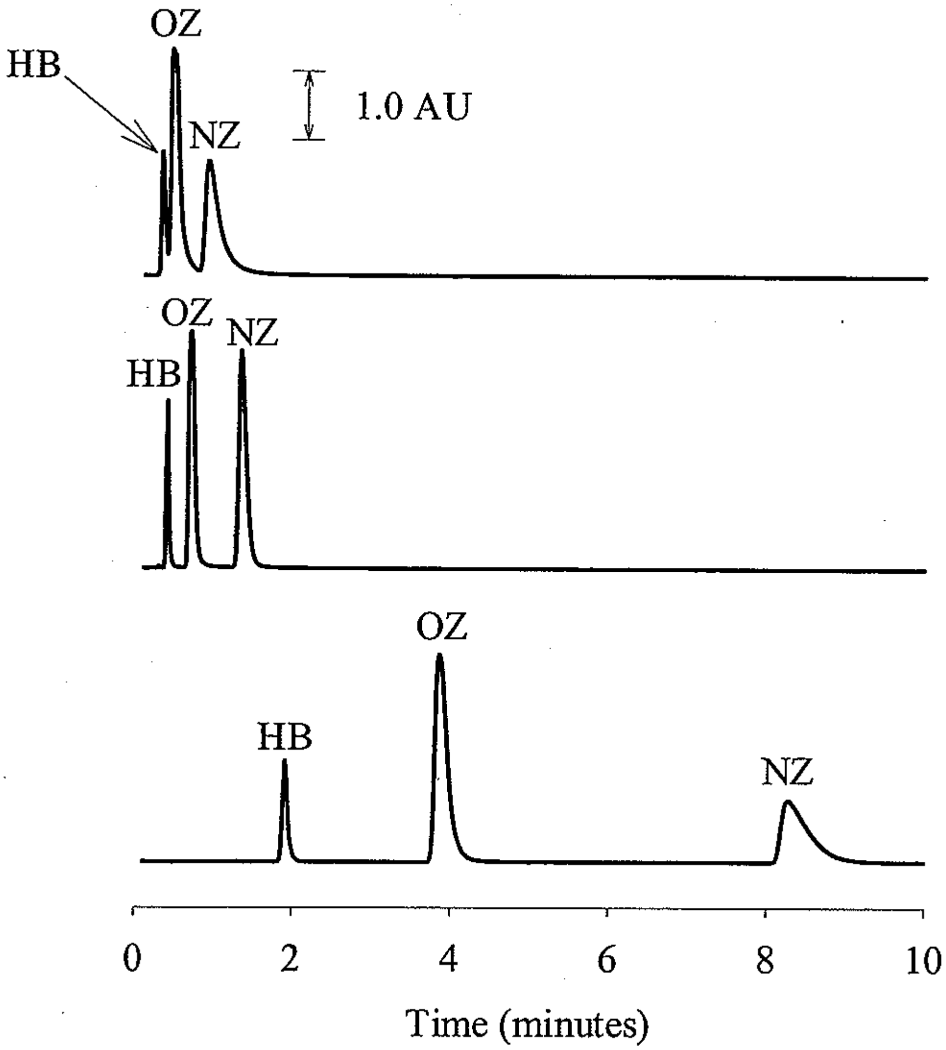


Figure 6

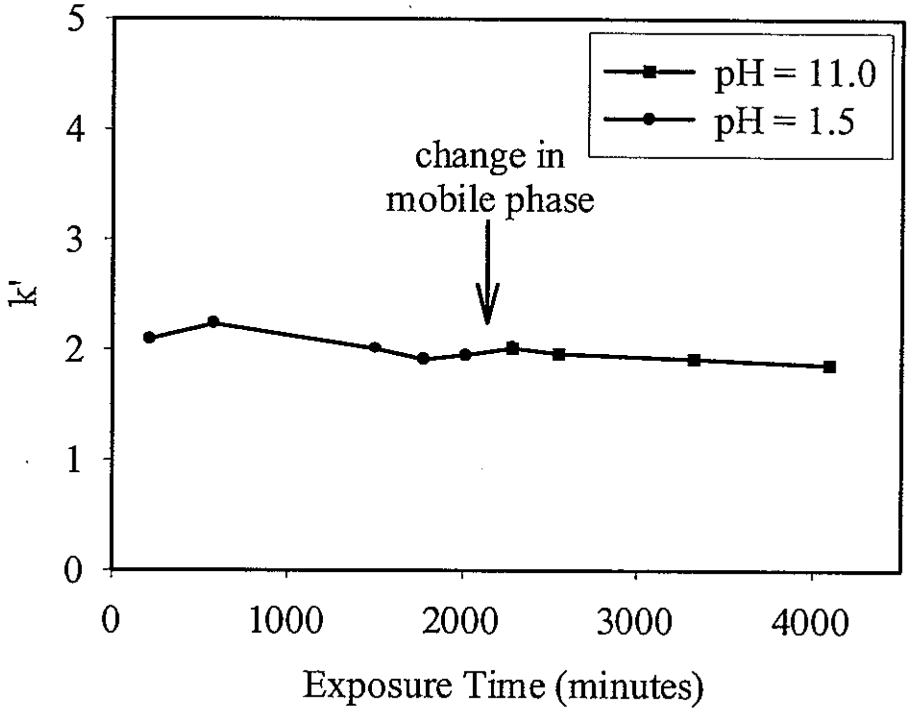

5
4
3
2
1
0
k'
0
1000
2000
3000
4000
Exposure Time (minutes)
pH = 11.0
pH = 1.5
change in mobile phase

Figure 7

CHAPTER 2. MANIPULATION OF THE PROPERTIES OF POROUS GRAPHITIC CARBON STATIONARY PHASES: USE OF ELECTROCHEMICALLY MODULATED LIQUID CHROMATOGRAPHY TO CHEMICALLY MODIFY CARBONACEOUS MATERIALS

A manuscript in preparation for submission to *Journal of Chromatography A*

Jennifer A. Harnisch and Marc D. Porter

Microanalytical Instrumentation Center, Ames Laboratory-USDOE, and Department of Chemistry, Iowa State University, Ames, IA 50011 USA

Abstract

This paper describes the preparation and preliminary testing of a new family of stationary phase materials. These phases are prepared by the chemical modification of porous graphitic carbon (PGC) particles, a material becoming increasingly used in high performance liquid chromatographic separations. The modification process results from the electroreduction of arenediazonium salts ($X\text{-}Ph\text{-}N_2^+$, X = NO_2, C_6H_{13}, $C_{10}H_{21}$, $C_{18}H_{37}O$, CO_2H), which is carried out after packing the particles in a column used in electrochemically modulated liquid chromatography. Using this methodology, several chemically modified stationary phases were created including 4-nitrobenzene (NB), 4-hexylbenzene (HB), 4-decylbenzene (DB), 4-carboxybenzene (CB) and 4-octadecyloxybenzene (OB) modified PGC. The modification protocol and subsequent use of the modified PGC stationary phases in the separation of an analyte mixture containing hexobarbital, oxazepam and nitrazepam are reported herein. The OB-modified PGC stationary phase is also resistant to the adsorption of proteinaceous materials, specifically, cytochrome c and lysozyme, which

coupled with the stability of the modified stationary phases to aggressive mobile phases, points to the potential use of this strategy in creating a new class of reversed phase materials.

Introduction

Carbon-based stationary phases (e.g., porous graphitic carbon (PGC)) have become invaluable in addressing some of the difficulties encountered in the separation of very polar analytes and geometric isomers when using conventional reversed phase materials such as octadecylsilica (ODS)[1]. Polar compounds, for example, are not strongly retained on ODS phases, and mixtures of structurally similar compounds are generally not well separated. An additional advantage of PGC phases is its inherent stability in aggressive mobile phases (e.g., those at high and low pH). Silica-based stationary phases only have a useful pH range from 4 to 8, since the Si-O tether between the modifier and silica surface is prone to hydrolysis at lower and higher pH.

One of the limitations of PGC as a stationary phase is the irreversible adsorption of aromatic and proteinaceous materials caused by strong π–π interactions between the graphitic planes of the carbon phase and the highly conjugated analytes. For example, in our own lab, we have found that even with high concentrations of organic modifier in the mobile phase, pyrene will not elute from the column. The recent literature has shown that elution can only be accomplished by operating at elevated temperatures[2]. It would therefore be advantageous to subtly decrease the strength of the π–π interactions of PGC while still maintaining its effectiveness to separate geometric isomers and polar compounds. One goal of this body of work is to test whether this may be accomplished by directly modifying the

PGC surface with an appropriate functional group much in the same way bonded phases based on silica are created.

The modification of PGC stationary phases is not unprecedented. Several groups have anchored chiral selectors to PGC via adsorption[3-5], and coated PGC with a chiral cellulose derivative[6]. An intriguing and well-studied prospect for the modification of carbon substrates is found in the electroreduction of arenediazonium salts at carbon electrodes[7-15]. In this approach, an arenediazonium salt ($X\text{-}Ph\text{-}N_2^{+-}BF_4$) is reduced electrochemically to generate an areneradical species that will react with the graphene planes of the carbon electrode to form a covalently bound monolayer film[11].

The electroreduction of arenediazonium salts may be easily extended to the modification of carbon particles by using electrochemically modulated liquid chromatography[16-26] (EMLC) as a means to apply the necessary modifying potential to the PGC substrate. We have already reported on a preliminary examination of the covalent modification of glassy carbon (GC) and PGC stationary phases by the on-column electroreduction of 4-nitrobenzenediazonium tetrafluoroborate (4-NBDT)[19]. This paper furthers the scope of this investigation by preparing 4-nitrobenzene (NB), 4-hexylbenzene (HB), 4-decylbenzene (DB), 4-carboxybenzene (CB) and 4-octadecyloxybenzene (OB) modified PGC stationary phases and testing their use in the separation of a mixture of pharmaceutical agents. The stability of this type of phase upon exposure to mobile phases that generally degrade silica bonded phases is also examined.

Experimental Section

Reagents

Uncoated PGC spheres (Hypersil®, diameter ~7 μm; BET surface area 91.82 ± 0.51 m^2g^{-1}) was used as a stationary phase. Lithium perchlorate, diiodomethane, sodium tetrafluoroborate, 4-hexylaniline, 4-aminobenzoic acid, 4-nitrobenzediazonium tetrafluoroborate (NBDT), trifluoroacetic acid (TFA), tetrabutylammonium tetrafluoroborate (TBAT) were purchased from Aldrich; hexobarbital (HB), oxazepam (OZ), nitrazepam (NZ), Cytochrome *c*, and Lysozyme from Sigma; 4-decylaniline from Alfa Aesar; and acetonitrile (HPLC grade), acetone, methanol (HPLC grade), sodium hydroxide, sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium nitrite, hydrochloric acid, 4 Å molecular sieves, and magnesium sulfate from Fisher. All column modification procedures used acetonitrile that was dried over anhydrous magnesium sulfate, and distilled and stored over 4 Å molecular sieves. Tetrabutylammonium tetrafluoroborate was dried at 110 °C in an oven prior to solution preparation. All other chemicals were used as received. 4-hexylbenzenediazonium tetrafluoroborate (HBDT), 4-decylbenzenediazonium tetrafluoroborate (DBDT), 4-carboxybenzenediazonium tetrafluoroborate (CBDT) and 4-octadecyloxybenzenediazonium tetrafluoroborate (ODBT) were prepared according to modified literature procedures[11,27]. All aqueous solutions were made using deionized water from a Millipore Milli-Q purification system.

Instrumentation

A Waters model 600E pump controller, model 610 pump, and valve station were used as the chromatographic system and a Waters 996 photodiode array detector as the detector. The detection wavelengths (λ_{det}) were either 254 nm or 220 nm. Solutions were introduced onto the chromatographic column via a 5-µL injection loop (Rheodyne model 7413). All electrochemical experiments employed a Princeton Applied Research model 173 potentiostat/galvanostat. Chromatographic columns were packed using a Shandon slurry column packing system. The dead volume of the columns were ~0.2 mL. The design and fabrication of columns used in EMLC have also been extensively detailed[23].

Electrochemical investigation of diazonium salts

The electroreduction of NBDT, HBDT, DBDT, CBDT and OBDT was studied at a glassy carbon electrode (GC-20, Tokai) prior to on-column modification. All GC electrodes were polished sequentially with 1.0-µm, 0.3-µm and 0.05-µm alumina powder (Buehler)[15]. After polishing the electrodes were sonicated for 10 min in deionized water followed by 10 min in acetonitrile and dried under a directed stream of high purity nitrogen. Acetonitrile solutions of the arenediazonium salt (10 mM) containing 0.1 M TBAT were used as the modifier and electrolyte, respectively, in a standard three-electrode electrochemical cell. Solutions were purged with high purity nitrogen for 3 min before the potential was scanned and an inert atmosphere of nitrogen blanketed the solution in the electrochemical cell at all times. Applied potentials were referenced against a silver/silver chloride electrode (satd. NaCl) with a platinum coil as the auxiliary electrode.

Chemical modification of PGC stationary phases

An EMLC column freshly packed with PGC particles was rinsed with anhydrous acetonitrile for 1 h. This step was followed by elution of 0.1 M TBAT in anhydrous acetonitrile at 0.40 mL/min for 1 h at the modification potential for the corresponding diazonium salt reported in Table 1. The external electrolyte reservoir was filled with 0.1 M TBAT in acetonitrile. A solution of 50 mM diazonium salt and 0.1 M TBAT in acetonitrile was then passed through the column (0.40 mL/min) at the modification potential for 5 h. After the 5 h modification period, the column is rinsed for 1 h with a flowing solution of neat acetonitrile.

Chromatographic separations

Once modified, the PGC stationary phase was repacked into a stainless steel HPLC column of the same dimensions as a commercially available Hypersil Hypercarb™ column for comparison purposes. The OB-modified PGC was further compared to an octadecylsilica (ODS) column (Waters). Mobile phase A was comprised of 100% methanol at a flow rate of 1.00 mL/min was used as the eluent for all modified stationary phases and the test analyte mixture was 100 ppm each HB, OZ, and NZ in methanol. The detection wavelength was 220 nm.

Evaluation of stability in aggressive mobile phases

The stability of the DB modified PGC stationary phase was tested at extreme values in pH. Two mobile phase compositions were used: Mobile phase B: 50:50 acetonitrile:H_2O (0.1% TFA, pH = 1.5), and Mobile phase C: 50:50 acetonitrile:H_2O (20 mM Na_2HPO_4, pH = 11.0). The DB-modified PGC was sequentially exposed to mobile phases B and C each over an extended period of ~3000 min. A 300 ppm solution of toluene in methanol was injected periodically over the exposure time to assess the effect of pH on k' (defined as $t\text{-}t_0/t_0$, where t = retention time and t_0 = retention time of injection peak) and retention time. The detection wavelength was 254 nm.

Results and Discussion

Modification of PGC stationary phases

The covalent modification of PGC stationary phases has been realized by using EMLC to functionalize the carbon surface. To establish the conditions for the on-column modification protocol, the voltammetry of each modifier was studied at a glassy carbon electrode in plate form. Figure 1 shows a representative cyclic voltammogram for a 10 mM acetonitrile solution of 4-CBDT containing 0.1 M TBAT. In the first scan, an irreversible reduction wave is observed near -580 mV (vs. Ag/AgCl/ sat'd. NaCl) that dramatically decreases to near background levels with successive scans. The cathodic wave is attributed to the electroreduction of the arenediazonium salt to form the aryl radical, and the marked decrease in current in each of the subsequent scans is diagnostic of the strong passivation of the electrode by the formation of the organic monolayer coating[11-13].

The same type of response was observed with the other four modifiers noting that the potentials for the peak current in the first scan differed depending upon the substituent appended to the aromatic ring. The voltammetry of each diazonium salt was used to select the applied potential (Table 1) for the on-column modification protocol. On average, these potentials were chosen to be ~100 mV positive of the reduction potential of the diazonium salt in order optimize the concentration of electrogenerated radical species (i.e., minimize undesired reactions of the radicals with solvent)[15,19].

Once the appropriate modification potential is selected, an acetonitrile solution containing the modifier and supporting electrolyte is passed continuously through an EMLC column (that is packed with 7 μm PGC particles) at the modification potential. This process results in the on-column electroreduction of the diazonium salt and subsequent generation of the corresponding arene radical as illustrated in Scheme 1. This radical reacts with the aromatic network of the PGC spheres at defect sites[9] thus forming a covalent bond between the surface and the modifier. Further reaction of the arene radicals with the modified surface creates an aryl film[9].

Comparison of modified stationary phases to Hypercarb™

After modification, the NB-, HB-, DB-, CB-, and OB-modified PGC stationary phases were slurry packed into a stainless steel HPLC column, and then used to separate a set of mixtures directly compared to the same separations on a commercially available Hypercarb™ column.

The chromatographic behavior of the modified PGC was evaluated using an analyte test solution containing the polar pharmaceutical agents hexobarbital, oxazepam and nitrazepam (see Scheme 2 for structures). As a starting point, the separation of the analyte mixture on an unmodified PGC stationary phase is considered. Figure 1(a) presents the chromatogram for this analyte mixture on a Hypercarb™ column. Peaks for this three-component mixture are well resolved, with the least polar analyte, HB eluting first (~2.8 min), and most polar analyte, NZ, last (~8.5 min).

In comparison to Hypercarb™, a dramatic change in the separation of the mixture is observed for all five of the modified stationary phases. Specifically, one notes that although the elution order remains the same, they elute faster from the modified stationary phases. This decrease in retention time is attributed to the perturbation of the interaction of the analytes with the PGC surface by the modifying film.

Amongst the modified stationary phases, subtle differences in retention times are noted and can be rationalized by considering difference in the functional groups on the modifying agents. Figure 1(b-d) illustrates the impact of the length of the alkyl chain of the modifiers on the separation. As the length of the alkyl chain increases from hexyl to decyl to octadecyl, the retention time for all three of the components of the mixture decreases. In other words, a shorter chain length allows the analyte to approach the PGC surface more closely, thus increasing its interaction with the underlying PGC surface. The longer alkyl chain limits the accessibility of the PGC surface to the analytes, thus decreasing their retention time.

The effect of incorporating functional groups other than alkyl chains, is illustrated by the NB- and CB-modified stationary phases (Figures 1 (e-f)). Interestingly, the retention times of HB and OZ on the NB-modified stationary phase are similar to those on the OB-modified PGC, but the more polar NZ is more strongly retained. This difference illustrates the effect of incorporating a polar moiety (i.e. NO_2 in the case of NB-modified PGC) in the modifier. Conversely, the retention times of all of the analytes on the CB-modified stationary phases is increased, attributed to H-bonding between the carboxy- group of the aryl film and the carbonyl groups of the analytes.

Comparison of OB-modified PGC to ODS

Although the formation of aryl films on the PGC stationary phase does effectively alter its retention characteristics, we were interested in determining whether this phase would retain its ability to separate polar compounds. Theoretically, if the π-character of the PGC is effectively diminished by the presence of the arene film, then the separation of a polar analyte mixture on the OB-modified stationary phase should roughly resemble that found for an ODS column. To this end, the chromatography of the HB, OZ and NZ mixture was compared on both OB-modified PGC and ODS stationary phases. The results are presented in Figure 3. In both cases, the elution order of the mixture is the same. However, the resolution of the mixture is notably better on the OB-modified PGC, even though the retention times are much shorter. If polar interactions with the PGC were not possible, then a poorer resolution of the HB from the OZ would be expected on this stationary phase, i.e., it would mimic the chromatography on the ODS column. These phases therefore have a retention character that is a mixture of PGC and reversed phase silica phases.

Adsorption of proteinaceous material and PAHs

One of the limitations of using PGC stationary phases is the irreversible adsorption of both proteinaceous and extensively conjugated (e.g., polynuclear aromatic hydrocarbons (PAHs)) analytes onto to the stationary phase. In many cases, even high concentrations of an organic modifier (~80%) are incapable of eluting these compounds. Modified PGC stationary phases should possess weaker π-π bonding interactions due to the interruption of the PGC surface aromaticity, and therefore should eliminate the strong adsorption of proteins and PAHs to the surface. Of the new phases, the OB-PGC phase is a good candidate since ODS stationary phases are routinely used to separate protein and PAH mixtures[28]. Figure 4 presents the results for independent injections of two high molecular weight proteins, cytochrome *c* (a), and lysozyme (b), on an OB-modified PGC stationary phase. Remarkably, the retention time for both proteins is less than one minute on the OB-modified PGC. However, under the same mobile phase conditions with an unmodified PGC stationary phase, cytochrome c and lysozyme do not elute.

Stability of modified PGC stationary phases in aggressive mobile phases

As noted, PGC has exceptional stability as a stationary phase, even when used with aggressive mobile phases. Conventional bonded phases are, however, susceptible to the hydrolytic degradation of the siloxane linkages connecting the modifier to the silica support. This limitation is particularly problematic in the separation of many types of biological materials, where the use of strongly acidic and strongly alkaline mobile phases is often required for effective sample resolution[29]. It is therefore of great importance to test the stability of our aryl modified phases under conditions that degrade conventional phases. As a representative case for our alkyl based arene phases, the DB-modified PGC was subjected sequentially to two mobile phases. Mobile phase B (50:50 acetonitrile:H_2O (0.1% TFA (pH 1.5)) was eluted for ~3000 min followed immediately by mobile phase C (50:50 acetonitrile:H_2O (20 mM Na_2HPO_4 (pH 11.0)) for a second ~3000 min time period. During exposure to each mobile phase, periodic injections of a 300 ppm solution of toluene were injected and the retention time (R_t) and capacity factor (***k***') were used to evaluate column performance.

Figure 5 presents the plot of k' for toluene versus exposure time in both acidic and alkaline mobile phases. Little variability in k' is observed, indicating that the DB-modified stationary phase is extremely resilient to hydrolysis at extremes in pH. The pH stability of the NB-, and CB-modified PGC stationary phases has already been discussed[19].

Conclusion

Preliminary results regarding the on-column modification of PGC stationary phases via the electrochemical reduction of an arenediazonium salt have been recently reported by our group[19]. This paper serves to extend this investigation by creating several functionalized PGC stationary phases. To this end, modified stationary phases not only exhibit a dramatic decrease in the retention times of a mixture of polar pharmaceutical agents when compared to commercially available PGC phases, but also show better resolution and stability to aggressive mobile phases than conventional silica stationary phases. Additionally, the OB-modified PGC was demonstrated to be resilient to the adsorption of proteins such as cyctochrome *c* and lysozyme. This coupled with great stability to extremes in pH indicates that biological separations are feasible with our modified PGC stationary phases.

Efforts towards the application of the NB-, CB-, HB-, DB-, and OB-modified PGC in more difficult separations are underway.

Acknowledgment

J.A.H. wishes to acknowledge the Phillips Petroleum Company for a research fellowship. This work was supported by the Office of Basic Energy Research, Chemical Sciences Division of the U.S. Department of Energy and by the Microanalytical Instrumentation Center of Iowa State University. The Ames Laboratory is operated for the U.S. Department of Energy by ISU under Contract W-7405-Eng-82.

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Figure Captions

Scheme 1. Aryl film formation on PGC spheres by the on-column electroreduction of substituted arenediazonium salts. The arene radicals react with defect sites on the PGC surface first, followed by subsequent film growth across the modified surface.

Scheme 2. Test analytes used to evaluate column performance.

Figure 1. Voltammetry at a polished glassy carbon electrode for a 10 mM acetonitrile solution of 4-CBDT with 0.1 M TBAT as the supporting electrolyte. Scan rate = 200 mV/s.

Figure 2. Separations of analyte test mixture (100 ppm each hexobarbital (HB), oxazepam (OZ), and nitrazepam (NZ)) on (a) Hypercarb™, (b) HB-modified, (c) DB-modified, (d) OB-modified, (e) NB-modified, and (f) CB-modified PGC. Mobile phase A was used at a flow rate of 1.00 mL/min.

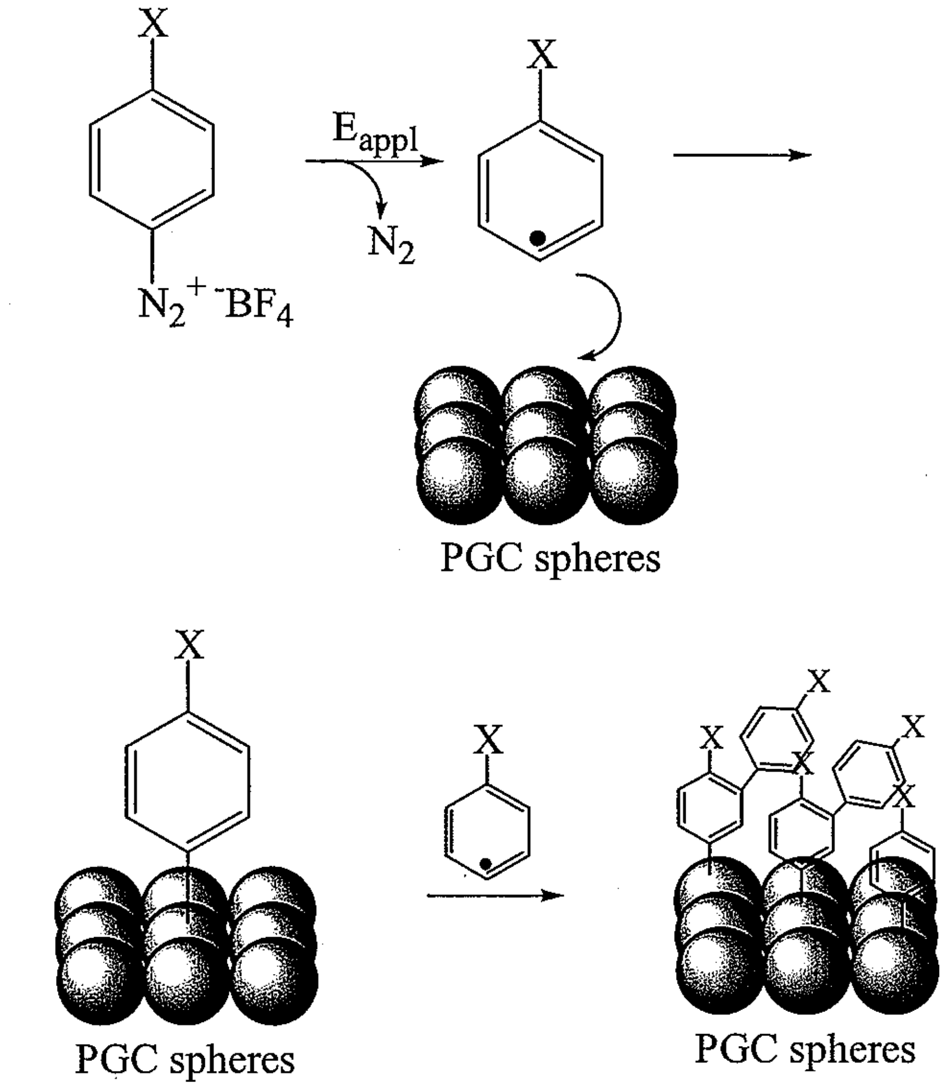
Figure 3. Separations of analyte test mixture (100 ppm each hexobarbital (HB), oxazepam (OZ), and nitrazepam (NZ)) on (a) OB-modified PGC and (b) ODS stationary phases. Mobile phase A was used at a flow rate of 1.00 mL/min.

Figure 4. Independent injections of (a) cytochrome *c* and (b) lysozyme on an OB-modified PGC column. Mobile phase A was used at a flow rate of 1.00 mL/min.

Figure 5. Long term stability of DB-modified PGC during sequential exposure to Mobile phase B (~3000 min) and Mobile phase C (~ 3000 min) at a flow rate of 0.50 mL/min. λ_{det} = 254 nm. An injection of 300 ppm toluene in methanol was made at each exposure time and k' ((-●-) Mobile phase B; (-■-) Mobile phase C) determined from the corresponding chromatograms.

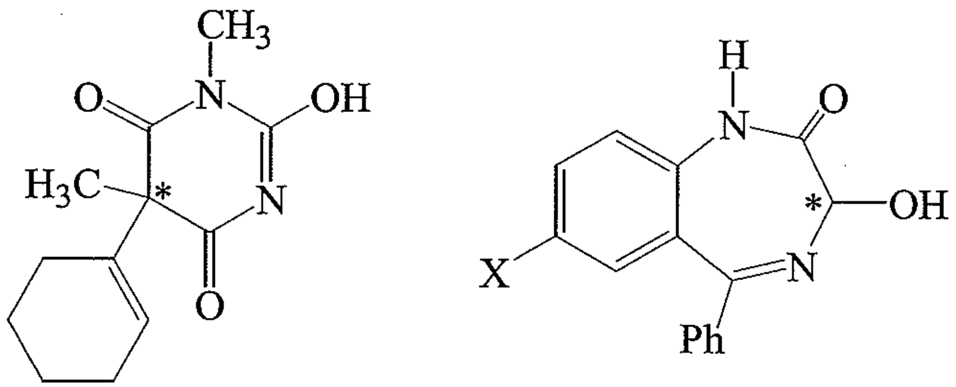
Table 1. Arenediazonium salt modifier and their corresponding modification potentials

Modifier	E_{appl} (in mV vs. Ag/AgCl/sat'd NaCl)
NBDT	-255
HBDT	-800
DBDT	-800
CBDT	-500
OBDT	-500



$X = NO_2, C_6, C_{10}, C_{18}O,$

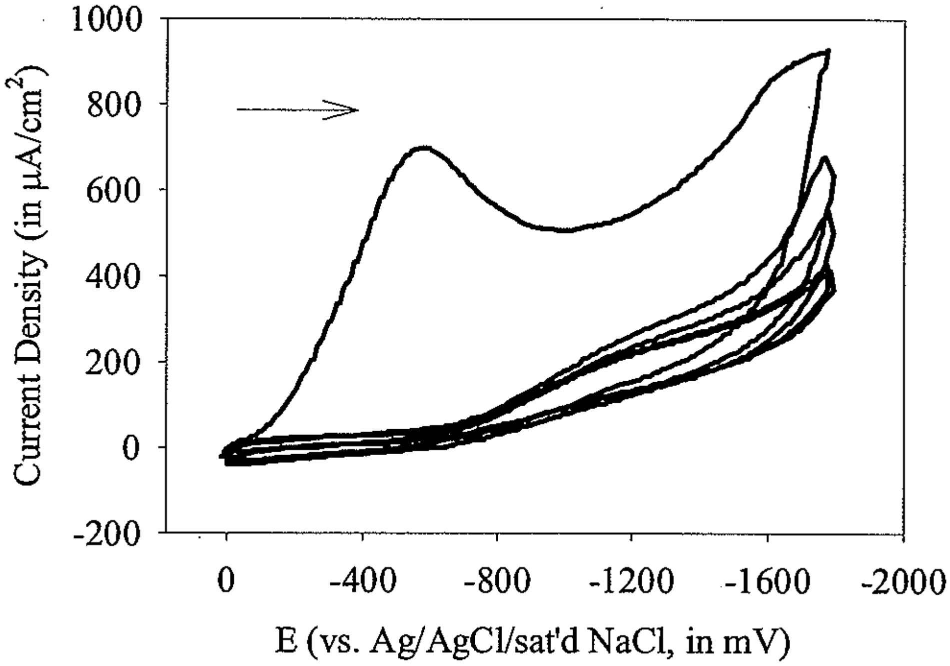
Scheme 1



Hexobarbital (HB)

X = Cl, Oxazepam (OZ)
X = NO_2, Nitrazepam (NZ)

Scheme 2



1000
800
600
400
200
0
-200
Current Density (in μA/cm^2)
0
-400
-800
-1200
-1600
-2000
E (vs. Ag/AgCl/sat'd NaCl, in mV)

Figure 1

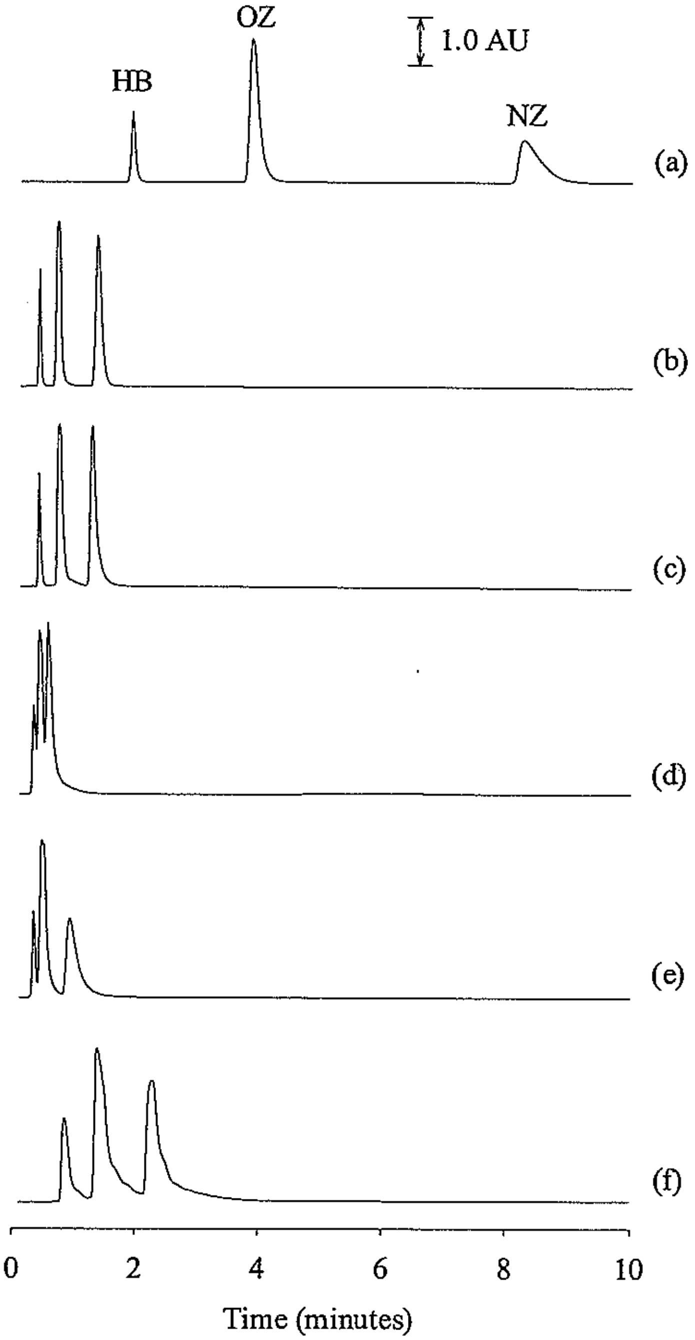


Figure 2

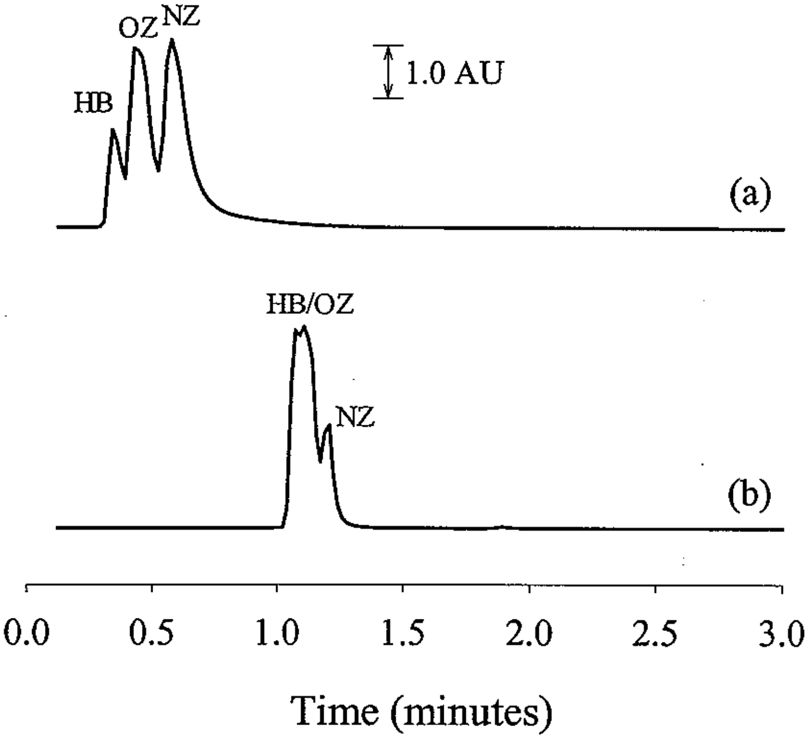


Figure 3

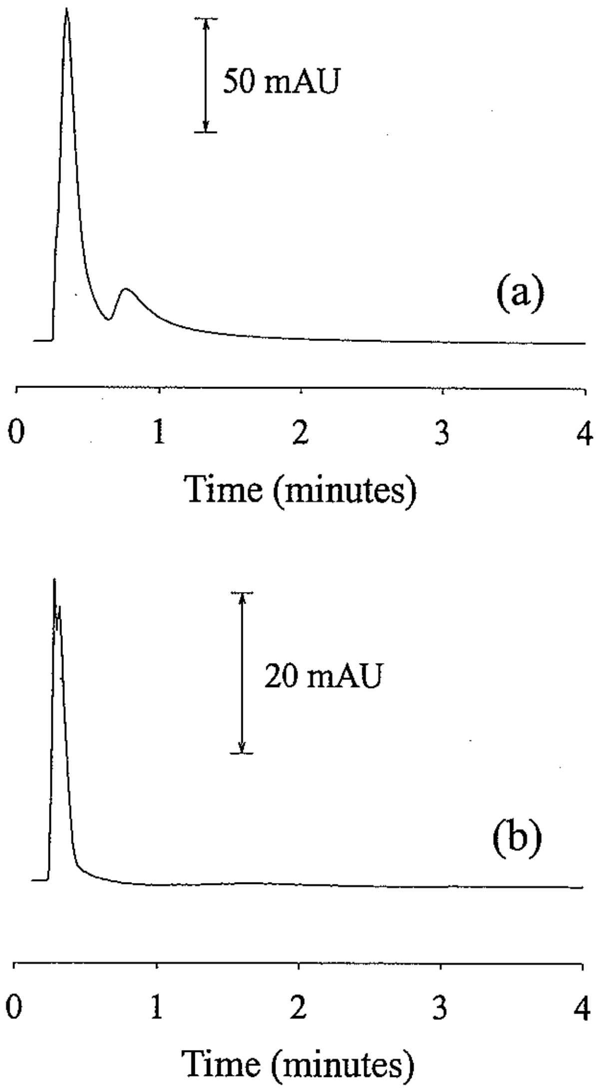


Figure 4

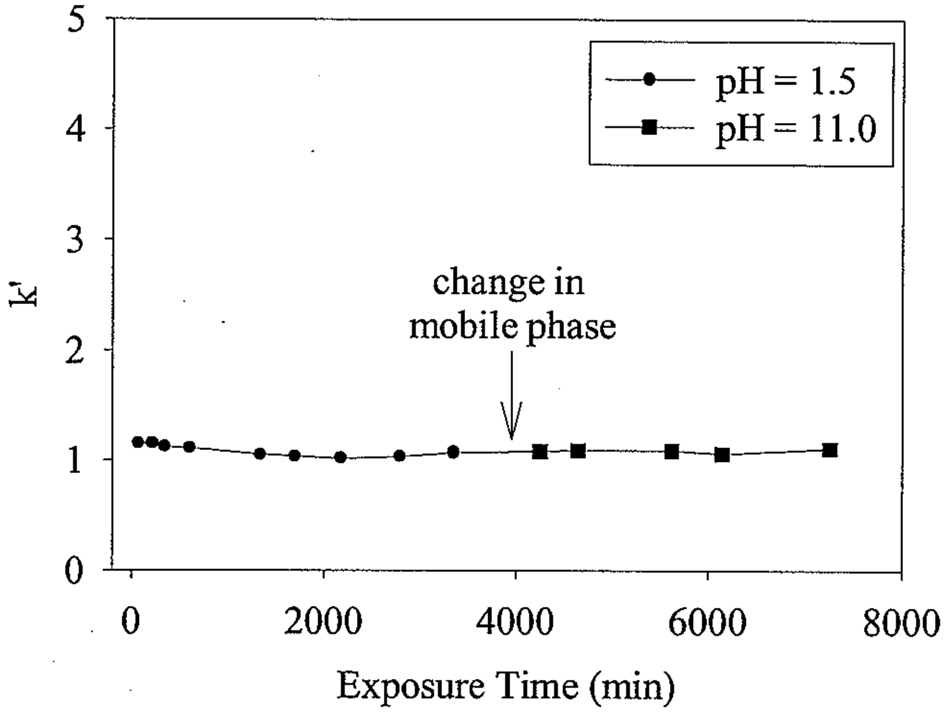

5
4
3
2
1
0
k'
0
2000
4000
6000
8000
Exposure Time (min)
pH = 1.5
pH = 11.0
change in
mobile phase

Figure 5

CHAPTER 3. THE MODIFICATION OF GLASSY CARBON STATIONARY PHASES USING THE KOLBE REACTION

Jennifer A. Harnisch and Marc D. Porter

A manuscript in preparation for submission to *Journal of Chromatography A*

Microanalytical Instrumentation Center, Ames Laboratory-USDOE, and Department of Chemistry, Iowa State University, Ames, IA 50011 USA

Abstract

A strategy for the modification of carbon stationary phases used in electrochemically modulated liquid chromatography (EMLC) has been developed that uses the electrochemical oxidation of arylacetate anions (the Kolbe reaction) to alter the surface composition. To this end, glassy carbon (GC) spheres have been modified on–column by the one-electron oxidation of phenylacetic acid (PAA) and 4-nitrophenylacetic acid (NPA) as modifying species to create benzyl- (BN) and nitrophenyl- (NP) modified stationary phases. The BN-modified columns are subsequently used to separate a mixture of resorcinol (RS) and 3-nitrophenol (NP) at applied potential. To confirm the success of the modification protocol, the NP-modified GC spheres were analyzed by X-ray photoelectron spectroscopy (XPS). The voltammetry of PAA and NPA at a planar GC electrode is also presented.

Introduction

The modification of carbonaceous stationary phases used in electrochemically modulated liquid chromatography[1-11] (EMLC) has been recently realized by an on-column modification protocol that employs the electroreduction of arenediazonium salts.[12-14] This method resulted in the development of a facile approach to alter the surface composition of carbon stationary phases.

Recently, Savéant and coworkers applied the anodic oxidation of arylacetic acids to functionalize glassy carbon electrodes.[15] As outlined in Scheme 1, an arylacetate anion is oxidized to generate a benzyl radical that rapidly inserts into the carbon-carbon framework of the aromatic structure. Furthermore, the monolayer formed by the Kolbe reaction may be removed by placing the modified electrode in a fresh solution of electrolyte and moving to more anodic potentials than those used in the deposition step. One may envision a second modification strategy, based on the Kolbe reaction, to alter the surface composition of the carbon stationary phases used in EMLC.

Furthermore, the Kolbe reaction has potential application in the development of a reversibly modifiable EMLC column. Ideally, the chemical composition of the stationary phase could be altered by a modifying species, the desired chromatographic analysis performed, and then the modifier could be removed and replaced with a second modifier that has different chromatographic properties.

An on-column application of the Kolbe reaction has been developed to modify GC stationary phase materials with benzyl- and 4-nitrobenzyl- groups. The voltammetric characterization of the modifiers at a planar GC electrode was performed prior to on-column modification in order to determine the appropriate modification potential.

After modification, the chromatographic properties of the BN-modified GC were probed using a mixture of resorcinol (RS) and 3-nitrophenol (NP) as analytes. The results of the modification of GC stationary phases via the Kolbe reaction are presented herein.

Experimental Section

Reagents

Uncoated GC spheres (Alfa Aesar, diameter ~6 μm) were used as a stationary phase. Lithium perchlorate, 4-nitrophenylacetic acid (NPA), 4-phenylacetic acid (PAA), resorcinol (RS), 3-nitrophenol (NP), tetrabutylammonium hydroxide (TBOH) (1.0 M in methanol), and tetrabutylammonium tetrafluoroborate (TBAT) were purchased from Aldrich; and acetonitrile (HPLC grade) from Fisher. All column modification procedures used acetonitrile that was dried over anhydrous magnesium sulfate, and distilled and stored over 4 Å molecular sieves. Tetrabutylammonium tetrafluoroborate was dried at 110 °C in an oven prior to solution preparation. All other chemicals were used as received. All aqueous solutions were prepared with Milli-Q deionized water.

Instrumentation

A Waters model 600E pump controller, model 610 pump, and valve station were used as the chromatographic system and a Waters 996 photodiode array detector as the detector. The detection wavelength (λ_{det}) was 254.0 nm. Solutions were introduced onto the chromatographic column via a 5 μL injection loop (Rheodyne model 7413). Applied voltages (E_{appl}) were controlled by a Princeton Applied Research model 173 potentiostat/galvanostat. Chromatographic columns were packed using a Shandon slurry column packing system.

XPS data were obtained using a Physical Electronics Industries 5500 surface analysis system equipped with a hemispherical analyzer, torroidal monochromator, and multichannel detector. Monochromatic aluminum Kα radiation (1486.6 eV) at 300 W was used for excitation. Binding energies were referenced to the C(1s) emission band at 284 eV. The pressure in the XPS chamber was less than 1 x 10^{-9} Torr during analysis.

Electrochemical investigation of arylacetic acids

The voltammetry of NPA and PAA was studied at a glassy carbon electrode (GC-20, Tokai) prior to on-column modification. All GC electrodes were polished sequentially with 1.0 μm, 0.3 μm and 0.05 μm alumina powder (Buehler)[16]. After polishing the electrodes were sonicated for 10 min in deionized water followed by 10 min in acetonitrile and dried under a directed stream of high purity nitrogen. Acetonitrile solutions of 4 mM of the areneacetic acid containing 0.1M TBAT (1 eq. TBOH to deprotonate the carboxylic acid group) were used as the modifier and electrolyte, respectively, in a standard three-electrode electrochemical cell. Solutions were purged with high purity nitrogen for 3 min before the potential was scanned and an inert atmosphere of nitrogen was maintained above the solution in the electrochemical cell at all times. Applied potentials were referenced against a silver/silver chloride electrode (satd. NaCl) with a platinum coil as the auxiliary electrode.

Chemical modification of GC stationary phases using EMLC

An EMLC column freshly packed with GC particles was rinsed with anhydrous acetonitrile for 1 h. This step was followed by elution of 0.1 M TBAT in anhydrous acetonitrile at 0.50 mL/min for 1 h at $E_{appl} = +1.0$ V. The external electrolyte reservoir was filled with 0.1 M TBAT in acetonitrile. A solution of 20 mM areneacetic acid and 0.1 M TBAT (1 eq. TBOH) in acetonitrile was then passed through the column (0.50 mL/min) at the modification potential for 3 h. After the 3 h modification period, the column is rinsed for 1 h with a flowing solution of neat acetonitrile.

Chromatographic separations

Mobile phase A consisted of 50% aqueous 0.1 M lithium perchlorate and 50% 0.1 M lithium perchlorate in acetonitrile at a flow rate = 0.50 mL/min was used as the eluent for both modified stationary phases and the test analyte mixture was 170 ppm NP and 400 ppm RS in acetonitrile. The detection wavelength was 254 nm.

Results and Discussion

Voltammetry of modifying species

Prior to developing an on-column modification protocol, it is useful to characterize the modifying arylacetates by cyclic voltammetry at a glassy carbon electrode. This simple electrochemical is the basis for determining appropriate modification potentials used in the on-column protocol, and provides a simple platform to assess the success of the electromodification. The voltammetry for the oxidation of an acetonitrile solution containing 4 mM NPA in 0.1 M TBAT as the supporting electrolyte is presented in Figure 1 (It should be noted that 1 equivalent of TBOH is added to ensure that the arylacetate anion is present). Scan 1 shows the irreversible anodic oxidation of NPA, which decreases in current with each consecutive scan indicative of the deposition of a nitrobenzyl monolayer on the glassy carbon substrate. Similarly, the formation of a benzyl monolayer is achieved by the electro-oxidation of PAA as shown in Figure 2. Once again, an irreversible anodic wave is observed in the first scan that gradually decreases in current with subsequent scans.

On-column modification of GC spheres

After studying the electrochemical oxidation of NPA and PAA to ascertain an appropriate modification potential, i.e. the potential at which the benzyl radical is present in high concentrations, an on-column modification protocol can be developed. Briefly, an acetonitrile solution of the background electrolyte is passed through a freshly packed GC column at the modification potential (+1.0 V in the case of NPA and PAA) for 1 h. Subsequently, a 20 mM solution of the modifier in the same electrolyte (1 eq. TBOH) is eluted through the column over a 3 h period at $E_{appl} = +1.0V$ and then rinsed well with fresh acetonitrile to remove adsorbed modifier.

Characterization of NB-modified GC by XPS

After the modification protocol, the NB-modified GC stationary phase was analyzed by XPS to determine if the on-column procedure was successful. Figure 3 presents the XPS spectra for unmodified (a) and NB-modified (b) GC spheres. As illustrated in Figure 3a, the typical XPS spectrum for the unmodified GC spheres displays a band for C1s and O1s electrons at 287 and 532 eV, respectively. However, upon modification with NB groups, a new band at 406 eV is observed for the N1s electrons originating from the nitro group. An additional band at 400 eV is also present, and is attributed to the presence of an amine caused by the electrochemical reduction of the nitro group[15].

Chromatographic behavior of BN-modified GC spheres

After the aforementioned protocol using PAA as a modifier, the chromatographic behavior of a BN-modified EMLC column at applied potential was studied and compared to an unmodified EMLC column. To probe the effects of modification, a simple mixture of polar aromatic analytes (170 ppm NP and 400 ppm RS in acetonitrile) was used.

The chromatography of NP and RS at applied potentials ranging from +500 mV to -500 mV on an unmodified GC column is shown in Figure 4. Regardless of the applied potential, NP and RS elute as a concise band in less than 1 minute due to poor interaction of the analytes with the stationary phase. However, the chromatographic behavior of the same analyte mixture on a BN-modified GC column is remarkably different. Figure 5 presents the separation of NP and RS on an EMLC column that has been subjected to the on-column Kolbe reaction using PAA as a modifier. Although little resolution is observed at negative applies potentials, one observes near baseline resolution of a mixture of NP and RS at positive applied potentials. This points toward the possibility that the benzyl monolayer adds a new point of interaction to the GC surface which is attractive at positive potentials and repulsive at negative potentials.

Conclusion

The on-column modification of carbon of stationary phases used in EMLC has been effected in previous research via the electroreduction of arenediazonium salts. Recently, a new protocol has been developed that uses the generation of benzyl radicals as the modifying species by the oxidation of arylacetate anions. To this end, the surface composition of a GC EMLC column has been successfully altered using NPA and PAA to create NB and BN-modified surfaces.

Additionally, BN-modified stationary phases show an improvement in the separation of a mixture of NP and RS at positive applied potentials in comparison to an unmodified GC column illustrating the effects of modification on column performance.

In the future, investigations into the reversibility of the Kolbe reaction[15] will be performed. The ability to remove the deposited monolayer points to the intriguing possibility of reversibly modifiable EMLC stationary phases.

Acknowledgment

This work was supported by the Office of Basic Energy Research, Chemical Sciences Division of the U.S. Department of Energy and by the Microanalytical Instrumentation Center of Iowa State University. The Ames Laboratory is operated for the U.S. Department of Energy by ISU under Contract W-7405-Eng-82.

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Figure Captions

Scheme 1. Modification of a glassy carbon surface via the Kolbe reaction.

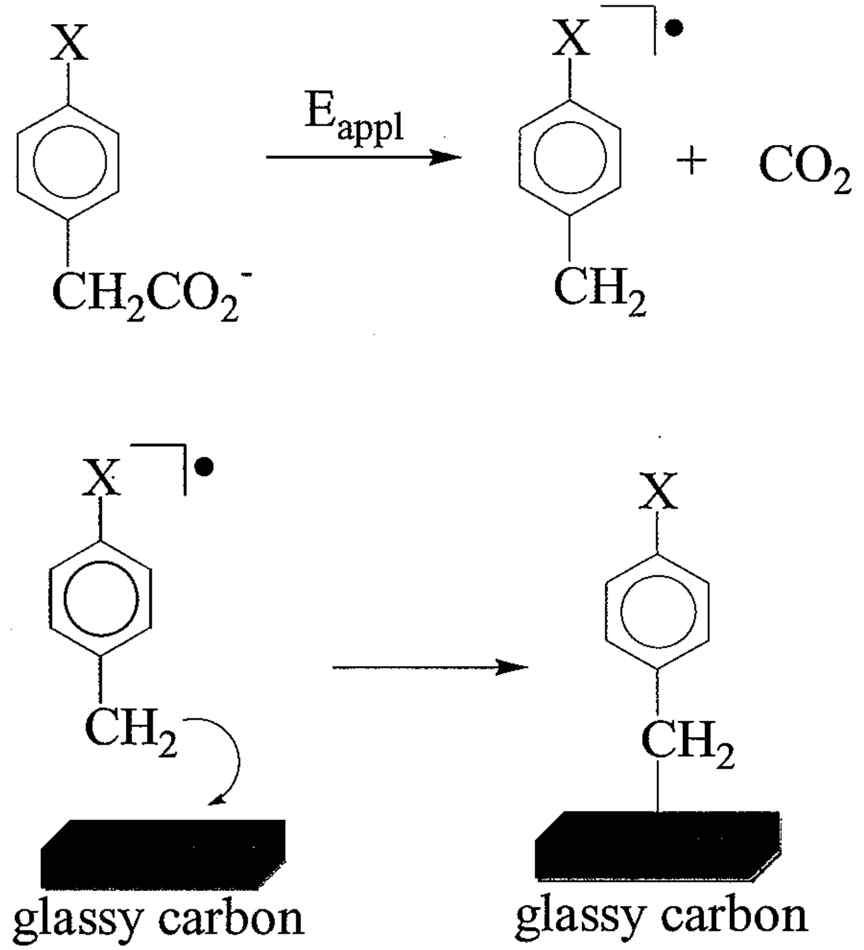
Figure 1. Voltammetry of 4 mM NPA at a polished glassy carbon electrode (Scans 1-4). Electrolyte: 0.1 M TBAT in acetonitrile (1 eq. TBOH). Scan rate = 200 mV/s.

Figure 2. Voltammetry of 4 mM PAA at a polished glassy carbon electrode (Scans 1-4). Electrolyte: 0.1 M TBAT in acetonitrile (1 eq. TBOH). Scan rate = 200 mV/s.

Figure 3. XPS spectrum of (a) unmodified glassy carbon spheres and (b) NB-modified glassy carbon spheres. Inset is the N(1s) region that shows diagnostic emission for the nitrogen of an NO_2 group at 406 eV.

Figure 4. EMLC separation of resorcinol (RS) and 3-nitrophenol (NP) on an unmodified GC stationary phase at E_{appl} = (a) +500 mV, (b) +250 mV, (c) open circuit, (d) –250 mV, and (e) –500 mV. Mobile phase A was used at a flow rate of 0.50 mL/min. λ_{det} = 254 nm.

Figure 5. EMLC separation of resorcinol (RS) and 3-nitrophenol (NP) on a BN-modified GC stationary phase at E_{appl} = (a) +500 mV, (b) +250 mV, (c) open circuit, (d) –250 mV, and (e) –500 mV. Mobile phase A was used at a flow rate of 0.50 mL/min. λ_{det} = 254 nm.



X = NO_2, NPA
X = H, PAA

Scheme 1

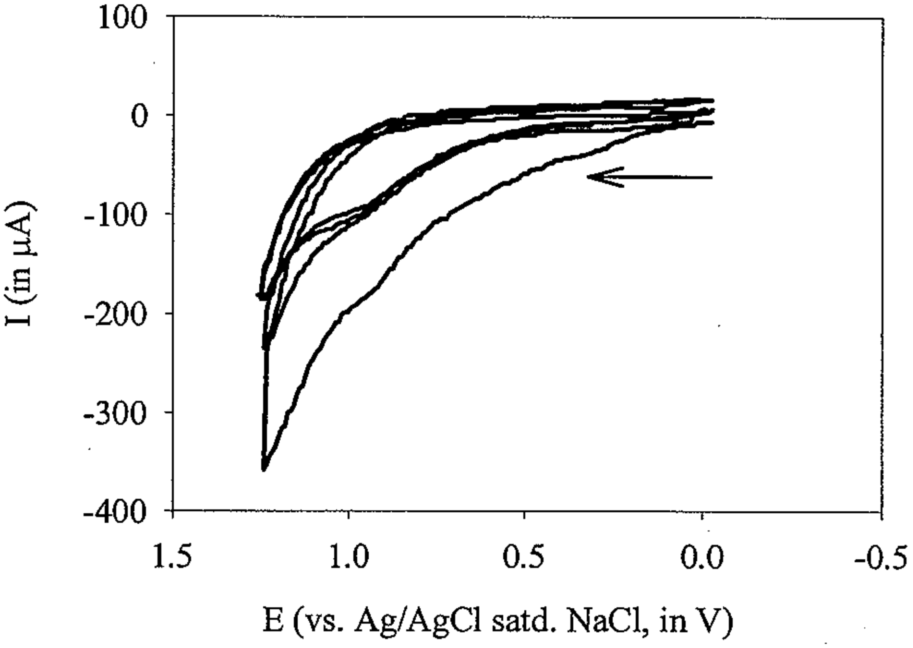

100
0
-100
-200
-300
-400
I (in μA)
1.5
1.0
0.5
0.0
-0.5
E (vs. Ag/AgCl satd. NaCl, in V)

Figure 1

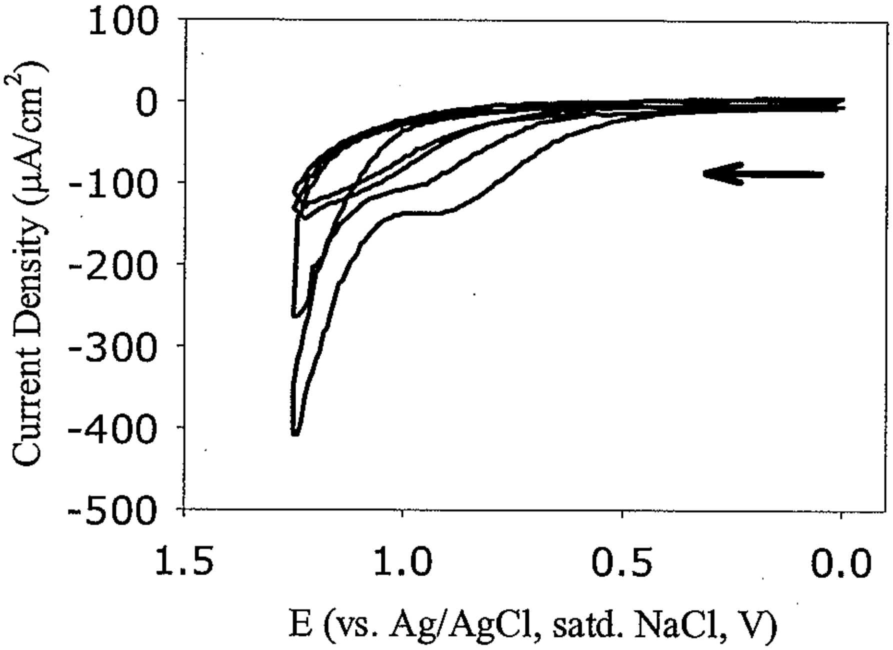

100
0
-100
-200
-300
-400
-500
Current Density (μA/cm2)
1.5
1.0
0.5
0.0
E (vs. Ag/AgCl, satd. NaCl, V)

Figure 2

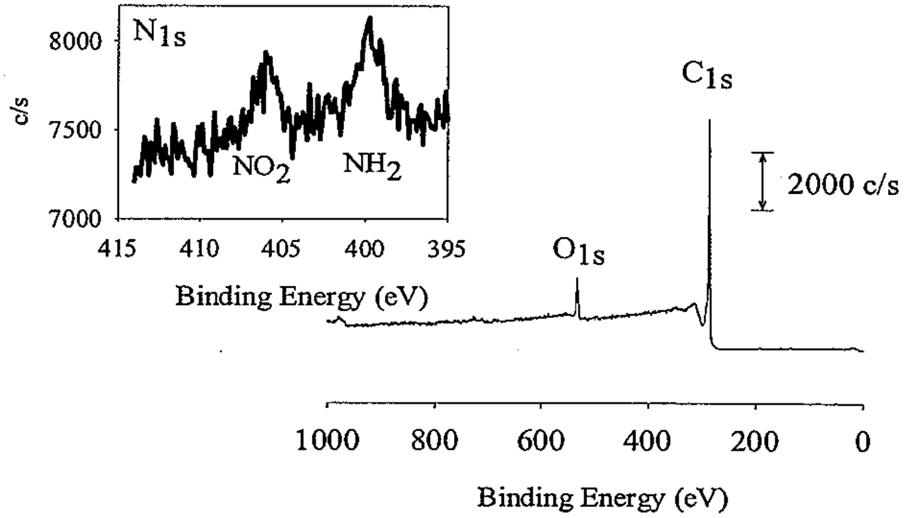

N1s
8000
7500
7000
c/s
NO2
NH2
415
410
405
400
395
Binding Energy (eV)
C1s
2000 c/s
O1s
1000
800
600
400
200
0
Binding Energy (eV)

Figure 3

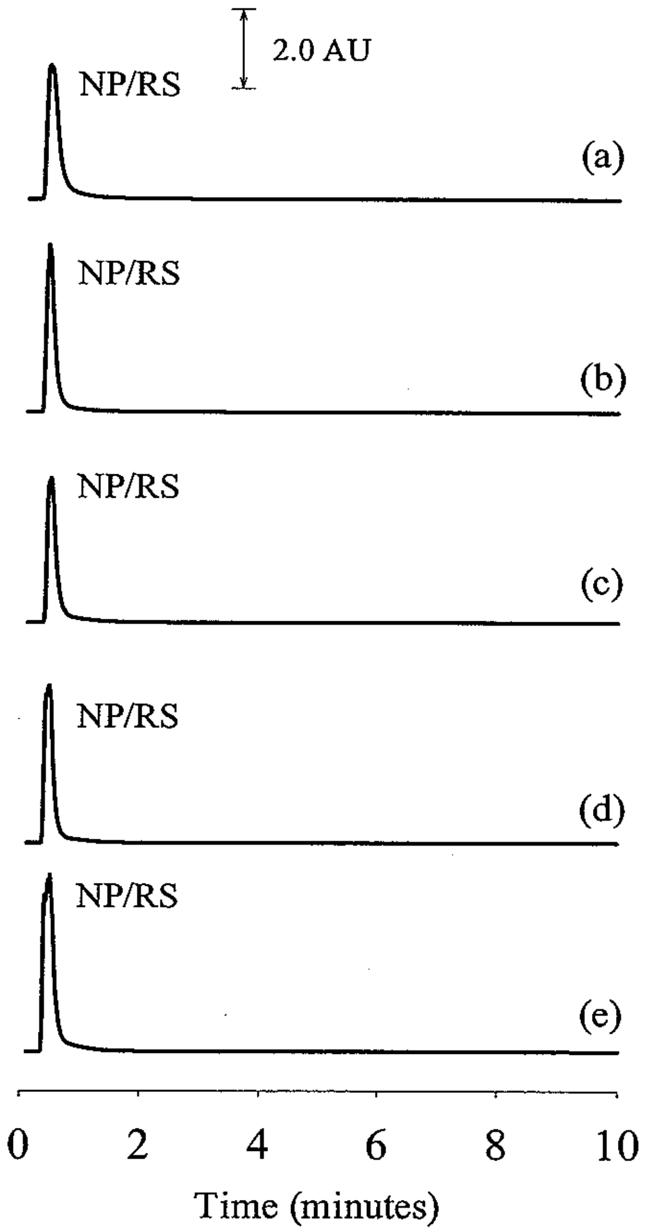


Figure 4

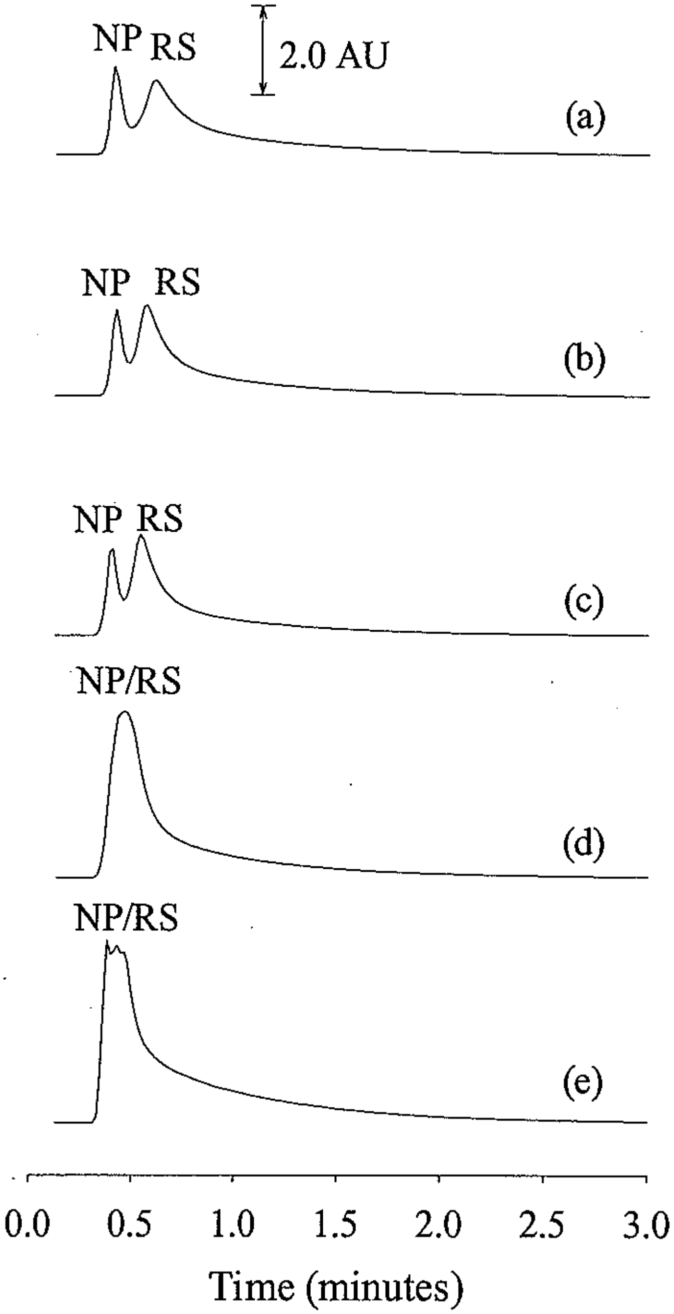

NP
RS
2.0 AU
(a)
NP
RS
(b)
NP
RS
(c)
NP/RS
(d)
NP/RS
(e)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Time (minutes)

Figure 5

CHAPTER 4. ATTACHMENT OF GOLD NANOPARTICLES TO GLASSY CARBON ELECTRODES VIA A MERCAPTOBENZENE FILM

A communication submitted to the *Journal of the American Chemical Society*

Jennifer A. Harnisch, Andrew D. Pris, and Marc D. Porter
Microanalytical Instrumentation Center, Ames Laboratory-USDOE, and Department of
Chemistry, Iowa State University, Ames, IA 50011 USA

Introduction

Metal nanoparticles have a wide range of properties of potential importance to electronics, magnetism, catalysis, and analysis [1-13]. In many instances, the assembly of such particles into nanostructured systems has exploited the simplicity and versatility of monolayers prepared from bifunctional molecules (e.g. dithiols[4,7] and diisocyanides[14,15]) which act as coupling agents for linking to substrates like gold or platinum. This communication extends the range of usable substrates to carbon-based materials by describing the ability to bind gold nanoparticles to glassy carbon electrodes (GCEs) through a sulfhydryl-terminated monolayer that is formed by the electrodeposition of 4-mercaptobenzenediazonium tetrafluoroborate (4-MBDT).

Experimental Section

Scheme 1 summarizes each step in the overall preparative process [16]. The first step depicts the electrochemical formation of the 4-mercaptobenzene (MB) monolayer [19]. The second step illustrates linkage of the nanoparticles to the GCE via the sulfhydryl moiety of the monolayer. The MB monolayer is formed on freshly polished GCEs by cycling the applied potential between +600 and –600 mV in a 10 mM solution of 4-MBDT until the electrode was passivated and the observed current decayed to background levels (3-4 cycles). This process reflects the irreversible one-electron reduction of the diazonium group[19-21] (half-wave potential: -480 mV vs. Ag/AgCl/satd. NaCl). After sonication in neat acetonitrile for 10 min and drying under a stream of high purity nitrogen, an aqueous solution (20 μL) of uncoated, 30-nm gold particles was pipetted onto the MB-modified GCE and allowed to stand for 24 h in an environment saturated with water vapor. Finally, the electrodes were again rinsed and dried in the same way as the freshly polished GCEs.

Results and Discussion

Two sets of characterizations were used to examine the effectiveness of coupling the particles to the MB-coated GCE. The results from the first set of characterizations are shown by the atomic force microscopy[22] (AFM) images (2.0 x 2.0 μm) in Fig. 1. Fig. 1 presents the topographic images of MB-modified GCE before (a) and after (b) exposure to the gold particle solution. The AFM image of MB-modified GCE is similar to those reported in literature for both uncoated and monolayer-coated GCEs.[27,28] It is characterized by features less than 30-nm in height and a large number of striations originating from the polishing process. After exposure to the 30-nm gold particles, the image shows the sample has developed a “pebbled” appearance, indicative of the presence of a fairly dense layer of the nanoparticles. The particles have the expected height of ~30-nm as revealed by cross-sectional contour plots of the surface topography (not shown). Imaging also showed that the particles remained attached to the GCE surface even after extensive sonication, whereas particles deposited onto unmodified GCE were easily removed by sonication. Adherence after sonication supports the coupling of the particles to GCE with the sulfhydryl moiety of the MB monolayer.

The second set of characterizations used X-ray photoelectron spectroscopy (XPS) [22]. These results are shown in Fig. 2, and include survey spectra for freshly polished and MB-modified GCEs, and for MB-modified GCEs after exposure to the nanoparticles. As illustrated in Fig. 2a, freshly polished GCE shows the expected bands in the C(1s) and O(1s) regions at 287 and 532 eV, respectively. After the electrolysis of 4-MBDT (Fig. 2b), bands for the S(2s) singlet (located at 228 eV), and S(2p) couplet (centered at 164 eV). The two sulfur bands are diagnostic of an unreacted sulfhydryl species and the presence of these features is consistent with the expected composition of the MB-monolayer.

In addition to the characteristic bands for the MB film, the XPS spectrum for the particle-modified sample (Fig. 2c) contains Au(4f) bands at 88 and 85 eV, which further support the successful deposition of the gold nanoparticles. The spectrum also shows that the centroid of the S(2p) band is shifted ~1 eV lower in binding energy after particle deposition to 163 eV. We attribute this shift to the immobilization of the gold particles through the formation of gold thiolate linkages [29].

We also tested the ability to modify the nanostructured assembly as a possible avenue for manipulating the surface properties of the immobilized particles as well as for generating three-dimensional motifs. After attachment of gold particles to GCE, some samples were immersed in a dilute (1 mM) ethanolic solution of 4-bromothiophenol (BTP) for 24 h. Analysis of these samples by XPS (Fig. 2(d)) shows the presence of the BTP adsorbate based on the bands centered at 70 eV for the emission of Br(3d) electrons (Fig 3). In other preliminary experiments, we have successfully modified the immobilized nanoparticles with a ferrocene terminated thiol (i.e. HS-$(CH_2)_{11}O_2CFc$), demonstrating the potential flexibility of our synthetic strategy.

Conclusion

In conclusion, we have developed an approach to robustly anchor gold nanoparticles to carbon surfaces via an electrodeposited mercaptobenzene monolayer, extending the range of the platforms that can be used to prepare nanostructure assemblies. Studies are underway to examine the scope of this approach. We are, for example, beginning to explore the utility of this process to create three-dimensional motifs by the subsequent modification of the gold colloid layer, the coupling properties of which may be manipulated by the nature of the functional groups on an adsorbing thiol (e.g. labile protecting groups) [30]. We are also pursuing the construction of patterned arrays of nanoparticles via "thiolate" photolithography [31].

Acknowledgment

The authors acknowledge James Anderegg for his expert assistance in acquiring the XPS spectra. This work was supported by the Office of Basic Energy Research, Chemical Sciences Division of the U.S. Department of Energy and by the Microanalytical Instrumentation Center of Iowa State University. The Ames Laboratory is operated for the U.S. Department of Energy by ISU under Contract W-7405-Eng-82.

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(16) Gold nanoparticles (30-nm) were from BBInterational. GCEs (Tokai, GC-20) were polished sequentially with 1.0, 0.3, and 0.05 μm Alumina powder (Buehler), sonicated for 10 minutes each in water and acetonitrile, and then dried under a directed stream of high-purity nitrogen prior to use.[21] 4-MBDT was synthesized according to a previously reported procedure.[22]

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(22) AFM images were obtained with a Nanoscope IIIa AFM (Digital Instruments), which was equipped with a 150-mm scanner and oxide-sharpened Si_3N_4 cantilevers (Nanoprobes, Digital Instruments) [23]. Images obtained at much higher magnification also showed slightly elongated particle shapes, as expected from the effect of tip convolution [24-26]. XPS data were obtained using a Physical Electronics Industries 5500 surface analysis system equipped with a hemispherical analyzer, torroidal

monochromator, and multichannel detector. Monochromatic aluminum Kα radiation (1486.6 eV) at 300 W was used for excitation. Binding energies were referenced to the C(1s) emission band at 284 eV. See reference 23 for further details.

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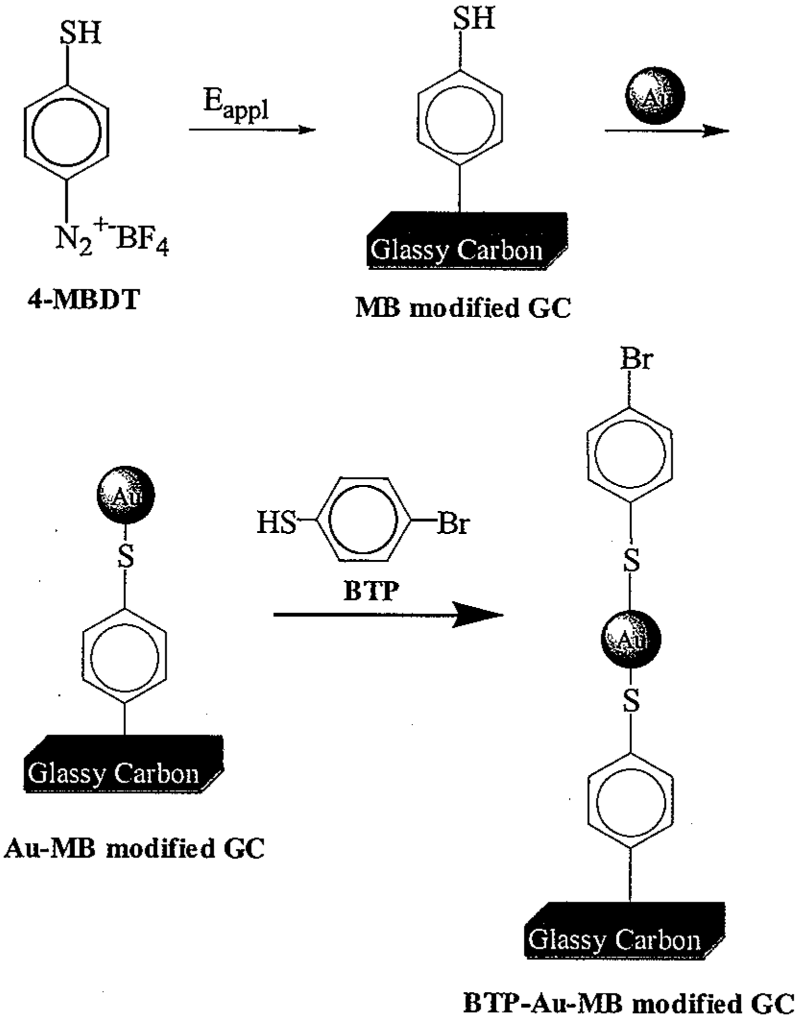
Figure Captions

Scheme 1. Construction of Au-modified glassy carbon electrode with 4-MB linking layer

Figure 1. (a) AFM Tapping Mode image in air of MB-modified glassy carbon.
(b) AFM liquid contact mode image of MB-modified electrode after deposition of gold nanoparticles.

Figure 2. XPS spectra for (a) unmodified glassy carbon, (b) MB-modified glassy carbon, (c) Au-MB-modified glassy carbon, and (d) BTP-Au-MB-modified glassy carbon. The insets are the expanded region from 60-250 eV for MB-, Au-MB-, and BTP-Au-MB-modified glassy carbon.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E_{appl}
SH
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MB modified GC
Au
Au
S
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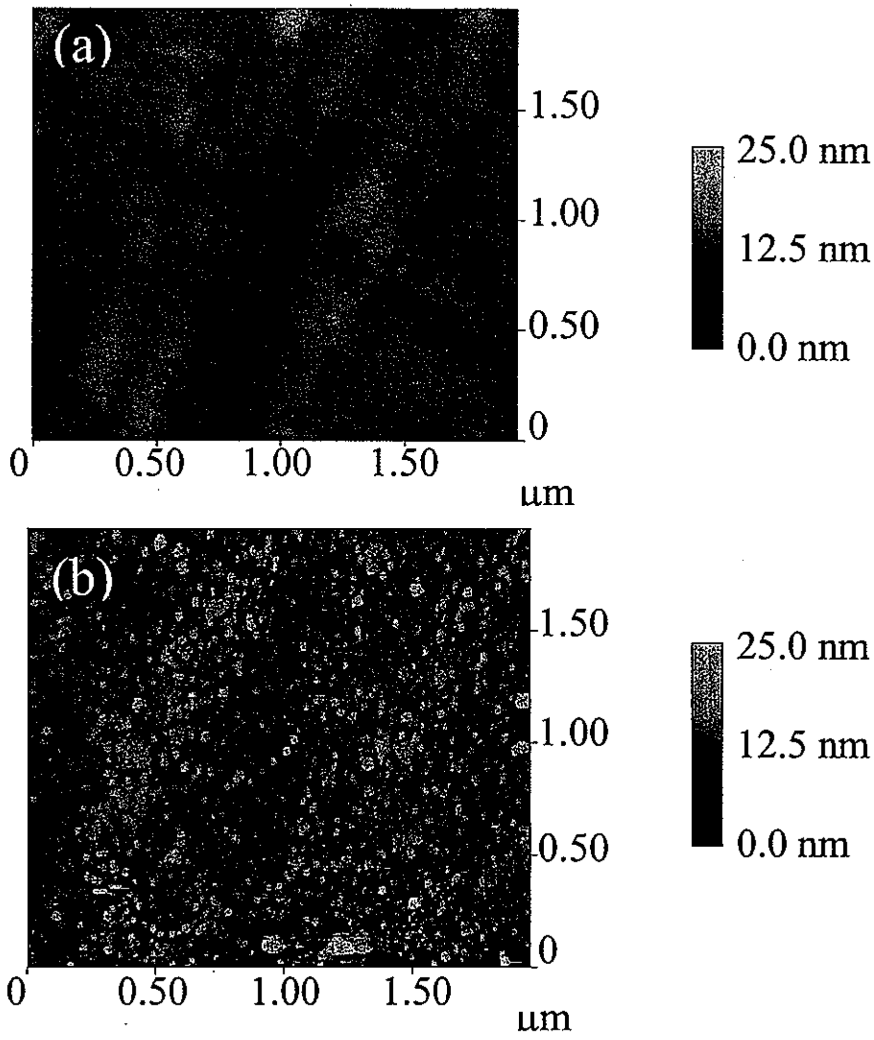
HS
Br
BTP
Br
S
Au
S
Glassy Carbon
BTP-Au-MB modified GC

Scheme 1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1.50
1.00
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(b)
1.50
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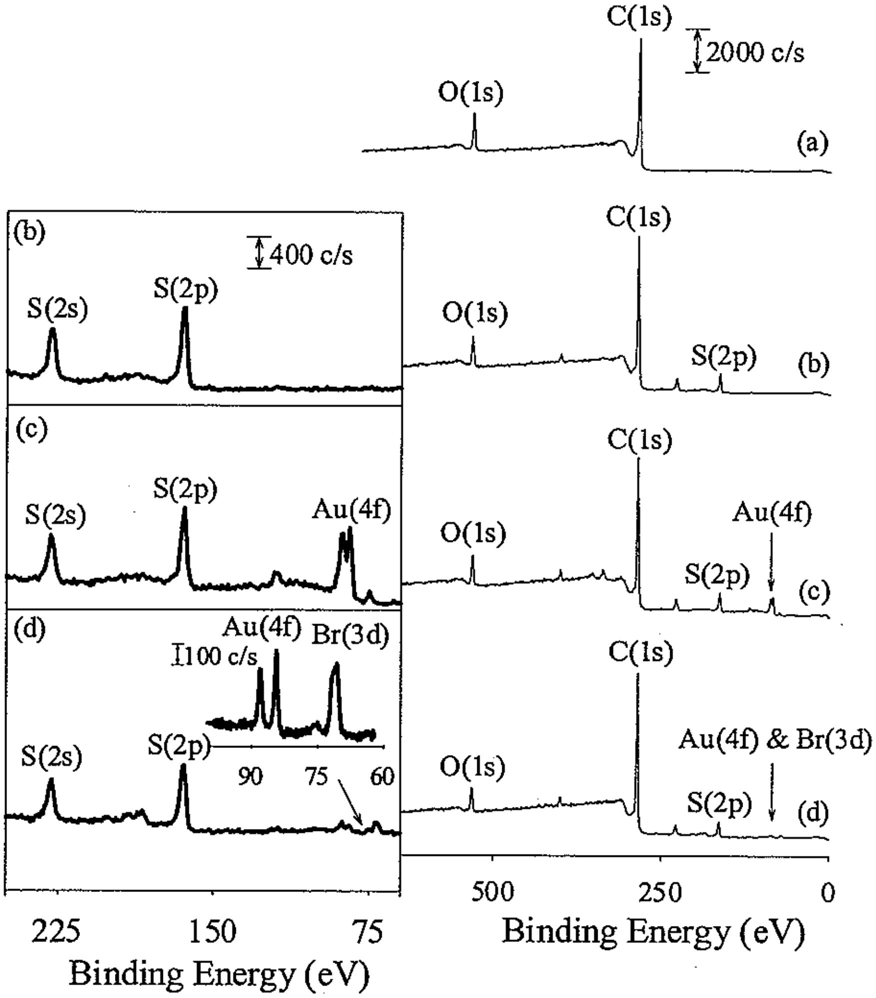
Figure 1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2000 c/s
O(1s)
(a)
(b)
400 c/s
S(2s)
S(2p)
C(1s)
O(1s)
S(2p)
(b)
(c)
S(2s)
S(2p)
Au(4f)
C(1s)
Au(4f)
O(1s)
S(2p)
(c)
(d)
Au(4f)
Br(3d)
100 c/s
90
75
60
S(2s)
S(2p)
C(1s)
Au(4f) & Br(3d)
O(1s)
S(2p)
(d)
500
250
0
Binding Energy (eV)
225
150
75
Binding Energy (eV)

Figure 2

CHAPTER 5. ELECTROCHEMICALLY TUNABLE FERROCENE-BASED CHIRAL SELECTORS FOR ELECTROCHEMICALLY MODULATED LIQUID CHROMATOGRAPHY

A manuscript in preparation for submission to *Chromatrographia*

Jennifer A. Harnisch and Marc D. Porter

Microanalytical Instrumentation Center, Ames Laboratory-USDOE, and Department of Chemistry, Iowa State University, Ames, IA 50011 USA

Abstract

Electrochemically modulated liquid chromatography (EMLC) has been previously demonstrated as a viable chiral separation methodology that uses applied potential to reversibly adsorb a mobile phase chiral selector (β-cyclodextrin) to the porous graphitic carbon (PGC) stationary phase. Recently, the EMLC concept has been extended to the development of ferrocene-based mobile phase chiral selectors that adsorb to the carbon surface with increasing negative applied potentials. Herein we report the synthesis and use of (α)-(+)-(methylbenzylaminocarbonyl)ferrocene (MBACF) as a potential candidate for chiral selection in EMLC applications. MBACF was characterized by NMR, MS, and its chirality was assessed by HPLC on a β-Cyclodextrin column. Additionally, the electrochemical stability of MBCAF was evaluated by cyclic voltammetry and at various applied potentials on an EMLC column. Preliminary results from the separation of the enantiomers of (+/-) Homatropine using MBACF at applied potential are also discussed.

Introduction

One of the most important applications for liquid chromatography is the separation of pharmaceutically important enantiomeric compounds[1-4]. In many cases, only one enantiomer may have therapeutic benefits, whereas the second enantiomer may occasionally have harmful side effects[5,6].

Two approaches are commonly used to effect chiral resolution: addition of a chiral additive to the mobile phase, or the use of a chiral stationary phase. Several types of chiral stationary phases are available depending on the composition of the target analyte, which naturally implies a large cost associated with use of multiple columns and associated methods development for different racemic mixtures. Ideally, electrochemically modulated liquid chromatography (EMLC) has been developed as a chromatographic method that is capable of separating a wide variety of analytes on a porous graphitic carbon (PGC) column by using applied potential to manipulate analyte retention [7-16]. To this end, furthering applicability of EMLC to chiral separations is highly desirable.

Chiral resolution of barbiturates using EMLC has already been achieved by our research group through the use of β-cyclodextrin (β-CD) as a chiral additive to the mobile phase[10]. The observed separations of hexobarbital and mephenytoin were achieved via reversible electrosorption of the β-CD selector to the PGC stationary phase.

A viable extension of chiral separation and the EMLC concept is found in designing chiral selectors with functional groups that will reversibly adsorb to the stationary phase at applied potentials as depicted in Scheme 1. This inherently implies that the "adsorbing moiety" must be electroactive. Ideally, manipulation of the electron donating/accepting properties of the redox active group (RA) with applied potential would increase or decrease the adsorption of the selector to the PGC phase.

Several factors were considered in the design of an electroactive chiral selector for EMLC. First, the compound must be electrochemically stable in the potential range used in EMLC applications (+500 mV to –1000 mV, vs. Ag/AgCl, satd. NaCl). Although many electrochemically active groups have been reported in the literature, ferrocene was selected as a promising candidate since its voltammetry and stability are well-documented[17-20]. Secondly, the creation of the chiral architectures should be facile, and many chiral ferrocenes are synthetically feasible in a few steps[21]. Once prepared, the chiral functionality should not racemize. It is well known that ferrocenes with a chiral group α to the cyclopentadienyl (Cp) ligand will racemize in solution at room temperature[21], therefore the selector must have the chiral moiety at least one carbon away from the Cp ligand, or have a group that sterically hinders rotation about the attachment point. Finally, as mentioned earlier, there should be an enhancement in the separation that scales with applied potential due to an increase in the adsorption of the chiral selector to the stationary phase.

Based on the above criteria, (α)-(+)-(methylbenzylaminocarbonyl)ferrocene (MBACF) (scheme 2) was chosen as a chiral selector for the preliminary evaluation of the effect of applied potential on a separation. Herein we report the synthesis and characterization of MBACF, and its application as a mobile phase chiral selector in the separation of (+/-) homatropine, a pharmaceutically important anticholinergic.

Experimental Section

Reagents and Materials

Porous graphitic carbon ((PGC), diameter ~7 μm) was purchased from Hypersil; lithium perchlorate ($LiClO_4$), ferrocenecarboxylic acid, pyridine, tetrabutylammonium hexafluorophosphate and α-methylbenzylamine from Aldrich; (+/-) homatropine from Sigma; cyanuric fluoride was from Strem; deuterated chloroform from Cambridge Isotopes and acetonitrile (HPLC grade), methylene chloride, tetrahydrofuran, magnesium sulfate and methanol (HPLC grade) from Fisher.

Synthesis of Ferrocene-based Chiral Selector

The mobile phase chiral selector used throughout these experiments, (α)-(+)-(methylbenzylaminocarbonyl)ferrocene (MBACF), was prepared according to a modified procedure[22].

A. Preparation of Flurocarbonylferrocene (1)

A 100 mL round bottom flask, equipped with a magnetic stir-bar, was charged with a suspension of 1.70 g (7.4 mmol) ferrocenecarboxylic acid, 1.20 mL (14.8 mmol) of pyridine, and 37 mL of freshly distilled methylene chloride. The mixture was stirred at 0 °C in an ice bath and 2.0 g (14.8 mmol) of cyanuric fluoride was added dropwise. After stirring at this temperature for 90 min, ~25 g of crushed ice were added and a white gelatinous solid formed. The solution was then passed through fluted filter paper and the filtrate was washed sequentially 3 x 25 mL with methylene chloride. The methylene chloride extract was washed with cold water 2 x 25 mL, and the organic layer was then dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to give (1) as a red-brown oil. The crude oil was purified by column chromatography on silica gel with 6:1

hexane:ethyl acetate as the eluent. The pure product is a red-orange solid. ^{1}H-NMR ($CDCl_3$): 4.64 δ (m, 2H); 4.41 δ (m, 2H); 4.11 δ (s, 5H). EIMS: m/z = 232 (M^+).

B. Preparation of (α)-(+)-(methylbenzylaminocarbonyl)ferrocene (MBACF)

A 100 mL round bottom flask, equipped with a magnetic stir-bar, was charged with 0.5 g (2.15 mmol) of (**1**), 21 mL of freshly distilled tetrahydrofuran, and 0.27 mL (2.15 mmol) of (α)-(+)-methylbenzylamine under a blanket of nitrogen. The reaction was stirred for 1 h at room temperature. After this period, the solvent was removed on a rotary evaporator and 20 mL of methylene chloride were added. The organic layer was extracted 2 x 10 mL with water, dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure to give MBACF as orange-red crystals. ^{1}H-NMR ($CDCl_3$): 7.37 δ (m, 5H); 5.90 δ (d, 1H); 4.73 δ (m, 3H); 4.33 δ (m, 2H); 4.14 δ (s, 5H); 1.59 δ (d, 3H). EIMS: m/z = 333 (M^+); m/z = 121 ($HN\text{-}C(H)PhCH_3$).

Instrumentation

A Waters model 600E pump controller, model 610 pump, and valve station were used as the chromatographic system and a Waters 996 photodiode array detector as the detector. The detection wavelength (λ_{det}) was 254.0 nm. Solutions were introduced onto the chromatographic column via a 5 µL injection loop (Rheodyne model 7413). Applied voltages (E_{appl}) were controlled by a Princeton Applied Research model 173 potentiostat/galvanostat and all potentials were referenced to a Ag/AgCl, satd. NaCl reference electrode. Chromatographic columns were packed using a Shandon slurry column packing system. The dead volume of the columns were ~0.2 mL. The design and fabrication of columns used in EMLC have also been extensively detailed[13].

Cyclic voltammograms of 0.3 mM MBACF (in 60:40 acetonitrile: 0.1 M aqueous $LiClO_4$) were recorded on a Houston Instruments 2000 X/Y using a polished glassy carbon plate (GC-20, Tokai) as the working electrode, a platinum mesh counter electrode, and a Ag/AgCl, satd. NaCl reference electrode (Scan rate = 50 mV/s).

XPS data were obtained using a Physical Electronics Industries 5500 surface analysis system equipped with a hemispherical analyzer, torroidal monochromator, and multichannel detector. Monochromatic aluminum Kα radiation (1486.6 eV) at 300 W was used for excitation. Binding energies were referenced to the C(1s) emission band at 284 eV. The pressure in the XPS chamber was less that 1 x 10^{-9} Torr during analysis.

^{1}H-NMR spectra were obtained on a Varian VXR 300 MHz spectrometer using the residual solvent peak as an internal reference (δ 7.26 ($CDCl_3$)). Electron ionization mass spectra (EIMS, 70 eV) were run on a Finnigan 4000 spectrometer.

Chromatographic separations

A Cylcobond 2000, β-cylcodextrin (β-CD) column (Alltech) was used to evaluate the purity of chiral reagents. Three mobile phases were used: Mobile phase A consisted of 65:35 methanol:water for separations using the β-CD column, Mobile phase B consisted of 60:40 acetonitrile:0.1 M aqueous $LiClO_4$ and Mobile phase C consisted of 60:40 acetonitrile (0.3 mM MBACF):0.1 M aqueous $LiClO_4$. The EMLC chromatographic columns were equilibrated at each applied potential for 1 h prior to analyte injection. Separation factors were calculated using the formula $\alpha = t_2/t_1$, where t_1 = retention time of early eluting enantiomer and t_2 = retention time of later eluting enantiomer.

Results and Discussion

Affirmation of the Chirality of MBACF

As with the preparation of any chiral substance, it is important to verify that the integrity of its handedness has been maintained throughout the synthetic protocol. Many racemic mixtures of ferrocenes have been successfully separated by inclusion with cyclodextrins, either in the mobile phase[23], or on a bonded phase[24]. To assess the chirality of MBACF, we used a commercially available β-CD column, and compared the separation to that reported for a structurally similar racemic ferrocene (+/- N-benzyl-N-(1-ferrocenylethyl)amine)[24] under the same separation conditions. Figure 1 presents a typical chromatographic result for a 100 ppm solution of MBACF in acetonitrile. A single band indicates that the chirality of MBACF is in tact and that no other detectable or separable synthetic ferrocenyl precursors (such as unreacted fluorocarbonylferrocene) are present.

Electrochemical Stability of MBACF

While prone to rapid electrochemical decomposition in aqueous solvents[19], most ferrocenes are stable towards electrolysis in non-aqueous solvents. An evaluation of MBACF by cyclic voltammetry was performed to assure that there is only one iron center present and to assess its stability to electrolysis. Figure 2 displays the voltammetry for a 0.3 mM solution of MBACF using mobile phase B as the electrolyte and a freshly polished glassy carbon electrode. The scan rate was 50 mV/s. A single set of quasi-reversible waves is observed with a peak current separation of ~90 mV and an estimated formal reduction potential of ~ 630 mV. The long term stability of the compound was tested by continuously scanning the applied potential between the same anodic and cathodic limits for 15 min.

There was no detectable degradation of MBACF evident in the voltammetric response, indicating that MBACF is stable in the potential ranges typically used for EMLC separations at PGC, and for a period of time surpassing its residence time as a chiral selector added to the mobile phase.

In order to further evaluate MBACF, its chromatographic behavior as a function of applied potential was studied using an EMLC column. A set of chromatograms at various applied potentials for a 100 ppm solution of MBACF in mobile phase B is presented in Figure 3. A consistent retention time (~1.5 min) is observed for negative E_{appl} and at open circuit. However, application of increasing positive potentials results in the increased retention of MBACF on the EMLC column, which is attributed to the attractive interaction between the π-electrons of the aromatic Cp rings with the positively charged PGC surface. No decomposition of the chiral selector on-column is observed.

Use of MBACF as a Chiral Selector in the Mobile Phase

After the careful synthesis of MBACF, and ensuring that a single enantiomer and redox center was present, the chiral selector was added to the mobile phase. As a test racemic mixture, (+/-) homatropine (Scheme 3) was selected since it is of biological importance as an anticholinergic.

The chromatography of (+/-) homatropine on a PGC column is shown as a function of E_{appl} before (Figure 4) and after (Figure 5) the addition of MBACF to the mobile phase. In the absence of MBACF as a chiral selector, both enantiomers elute as a single band in less than two minutes at all E_{appl}. Although the acidity of the mobile phase was not monitored, it is likely that homatropine is protonated[25] ($pK_a \sim 9.41$), thus resulting in the observed decrease in retention time as the applied potential becomes more positive.

Addition of MBACF to the mobile phase results in the resolution of (+) and (-) homatropine at all applied potentials as demonstrated in Figure 5. In all cases, the (+) enantiomer elutes first, as confirmed by independent injection of (+)-homatropine. As the applied potential moves from positive to negative values, the resolution of the enantiomeric mixture improves. Furthermore, the separation factor, α, scales linearly with applied potential as shown in Figure 6. It is expected that application of more negative potentials will enhance the resolution even further, however one is limited by the reduction of solvent at potentials greater than –1000 mV. From the chromatography in Figure 5, one also notes that mass balance is not conserved from the separations at positive and negative potentials. This is most likely attributed to some electrochemical decomposition of the analyte at negative potentials.

Retention of MBACF on PGC

The many possible interaction mechanisms between the chiral selector (MBACF), enantiomerically pure analyte (H), and PGC stationary phase are represented in Scheme 4. As shown in this representation, H:MBACF is the interaction complex formed between homatropine and MBACF, and (m) and (s) are used to designate the complex in the mobile phase or stationary phase, respectively. As more negative potentials are applied to the PGC, more MBACF adsorbs to the carbon surface resulting in an increase in the separation factor, α, hence H:MBACF(s) is the predominant complex at these potentials. Conversely, at positive potentials, less MBACF is adsorbed to the PGC, so H:MBCF(m) is the more likely complex.

Adsorption of MBACF to the PGC Stationary Phase

A final assessment of the performance of MBACF as a chiral selector for EMLC is its adsorption to the PGC stationary phase. It is well known that ferrocenyl compounds irreversibly adsorb to carbon electrodes[26], and it has been postulated that the ferrocenium ion has a similar spacing between the Cp rings to allow it to intercalate in between the graphene layers of highly ordered pyrolytic graphitic (HOPG). Hence, after extended use with MBACF, the PGC column packing was submitted for analysis by x-ray photoelectron spectroscopy (XPS). Figure 7 shows the XPS spectrum from 740-700 eV for PGC that has been used for 6 months with MBACF. Two bands attributable to the Fe(2p) electrons of ferrocene are present, at an estimated atomic concentration is 0.8%. Thus, the irreversible adsorption of the compound is insignificant in terms of contributing to the retention process.

Conclusion

A new dimension to EMLC is the separation of enantiomeric compounds, which serves to extend its applicability as a universal chromatographic technique. Although chiral selectors have already been successfully added to the mobile phase in EMLC applications, the creation a selector with electrochemically tunable adsorption is an interesting prospect. To this end, we have design a compound, MBACF, with a ferrocenyl group appended to a chiral arm. MBACF is readily synthesized in two steps from ferrocenecarboxylic acid, and retains its chirality in all applications. Additionally, it is not prone to electrochemical degradation under the conditions used in EMLC separations. This coupled with its effectiveness in the resolution of the enantiomers of homatropine make MBACF an attractive mobile phase chiral selector for EMLC.

In the future, other chiral selectors incorporating a ferrocene redox center will be prepared. Furthermore, the interaction mechanism of MBACF with several target analytes will be explored.

Acknowledgment

J.A.H. wishes to acknowledge the Phillips Petroleum Company for a research fellowship. This work was supported by the Office of Basic Energy Research, Chemical Sciences Division of the U.S. Department of Energy and by the Microanalytical Instrumentation Center of Iowa State University. The Ames Laboratory is operated for the U.S. Department of Energy by ISU under Contract W-7405-Eng-82.

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Figure Captions

Scheme 1. Pictorial representation of the effect of applied potential on the adsorption of a chiral selector containing a redox active group (RA) to the PGC stationary phase. Ideally, the adsorption of the selector may be manipulated by electrochemically changing the electron donating/accepting properties of the RA.

Scheme 2. Structure of (α)-(+)-(methylbenzylaminocarbonyl)ferrocene (MBACF).

Scheme 3. Structure of (+/-) homatropine.

Scheme 4. Schematic representation of the possible interaction mechanisms for the separation of (+/-) homatropine with MBACF as a chiral selector.

Figure 1. Chromatography of 100 ppm MBACF in acetonitrile on a β-CD bonded HPLC column. Mobile phase A was used as the eluent at a flow rate = 0.50 mL/min and $\lambda_{det} = 254$ nm.

Figure 2. Representative cyclic voltammogram of 0.3 mM MBACF in mobile phase B as the supporting electrolyte at a freshly polished glassy carbon electrode. Scan rate = 50 mV/s and potential is applied vs. a Ag/AgCl, satd. NaCl reference electrode.

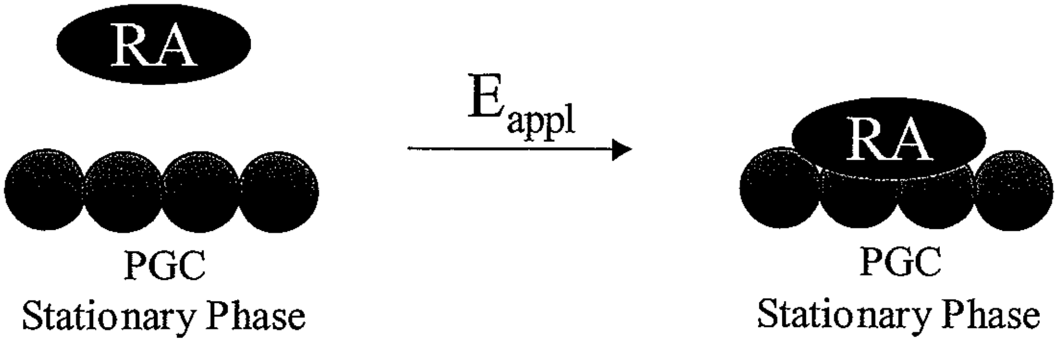
Figure 3. Chromatography of 100 ppm MBACF in mobile phase B at various applied potential on an EMLC column. Mobile phase B was used as the eluent at a flow rate = 0.40 mL/min and λ_{det} = 254 nm. $E_{open\ circuit}$ = +260 mV.

Figure 4. Chromatography of 1000 ppm racemic Homatropine in mobile phase B at various applied potential on an EMLC column. Mobile phase B was used as the eluent at a flow rate = 0.40 mL/min and λ_{det} = 254 nm. $E_{open\ circuit}$ = +260 mV.

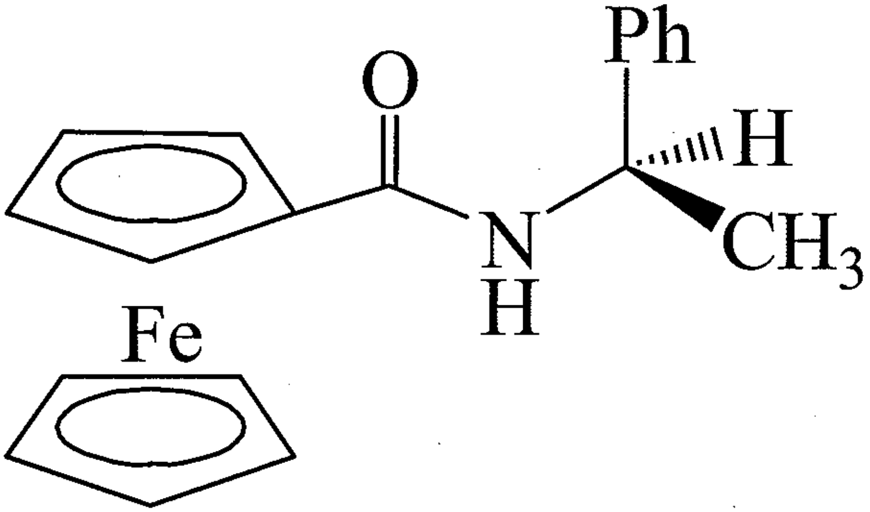
Figure 5. Chromatography of 1000 ppm racemic Homatropine in mobile phase C at various applied potential on an EMLC column. Mobile phase C was used as the eluent at a flow rate = 0.40 mL/min and λ_{det} = 254 nm. $E_{open\ circuit}$ = +260 mV.

Figure 6. Plot of separation factor, $\alpha = t_2/t_1$, vs. applied potential. The line was plotted using linear regression.

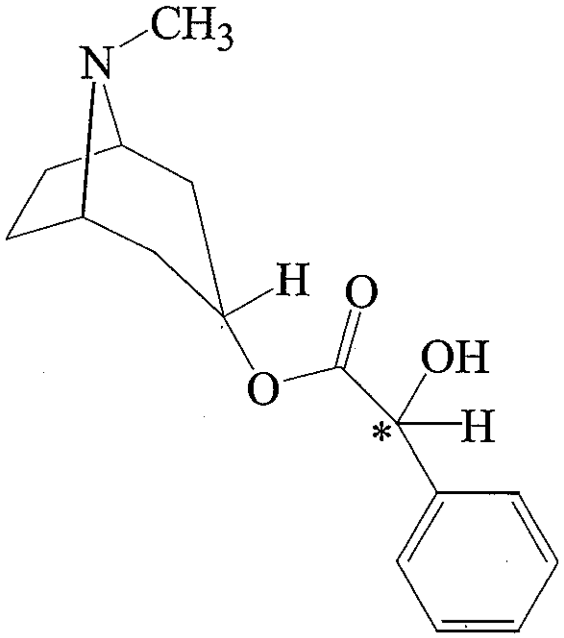
Figure 7. X-ray photelectron spectrum from 740-700 eV for a PGC column packing that has been used with MBACF for a period of 6 months.



Scheme 1



Scheme 2



Scheme 3

$$\begin{tikzcd}
\text{H(m) + MBACF(m)} \arrow[r, shift left] \arrow[d, shift left] & \text{H:MBACF(m)} \arrow[l, shift left] \arrow[r, shift left] \arrow[d, shift left] & \text{H(m) + MBACF(m)} \arrow[l, shift left] \arrow[d, shift left] \\
\text{H(s) + MBACF(s)} \arrow[r, shift left] \arrow[u, shift left] & \text{H:MBACF(s)} \arrow[l, shift left] \arrow[r, shift left] \arrow[u, shift left] & \text{H(s) + MBACF(s)} \arrow[l, shift left] \arrow[u, shift left]
\end{tikzcd}$$

Scheme 4

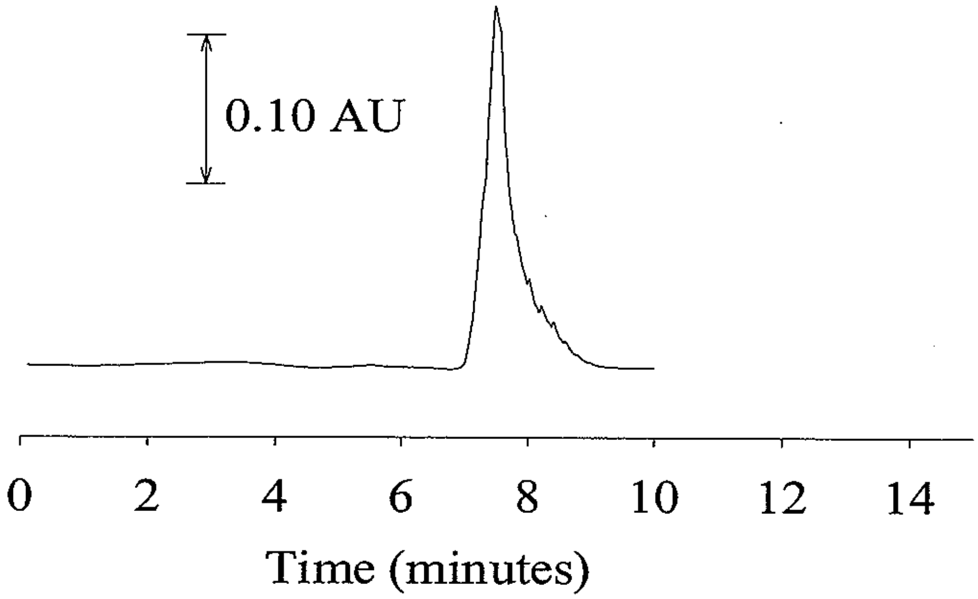


Figure 1

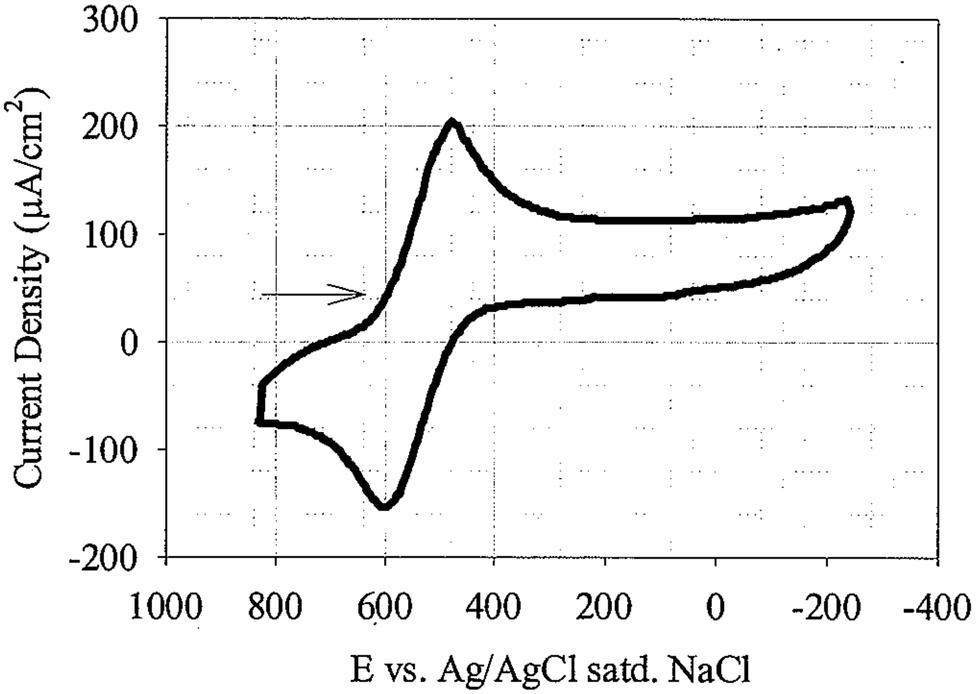

300
200
100
0
-100
-200
Current Density (µA/cm²)
1000
800
600
400
200
0
-200
-400
E vs. Ag/AgCl satd. NaCl

Figure 2

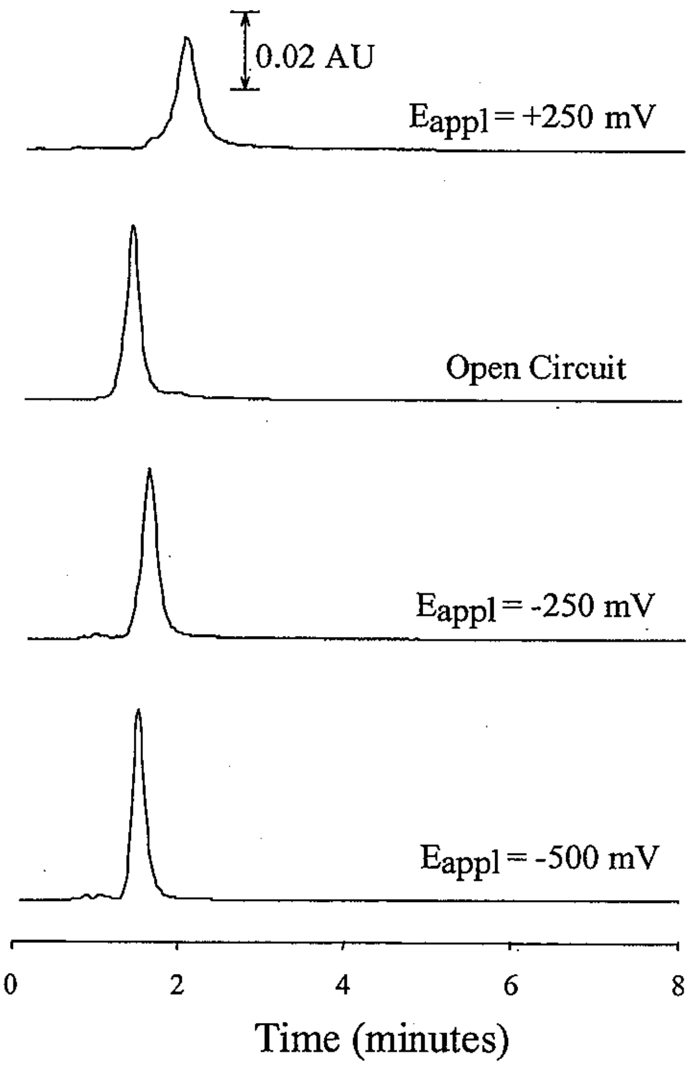

0.02 AU
Eappl = +250 mV
Open Circuit
Eappl = -250 mV
Eappl = -500 mV
0
2
4
6
8
Time (minutes)

Figure 3

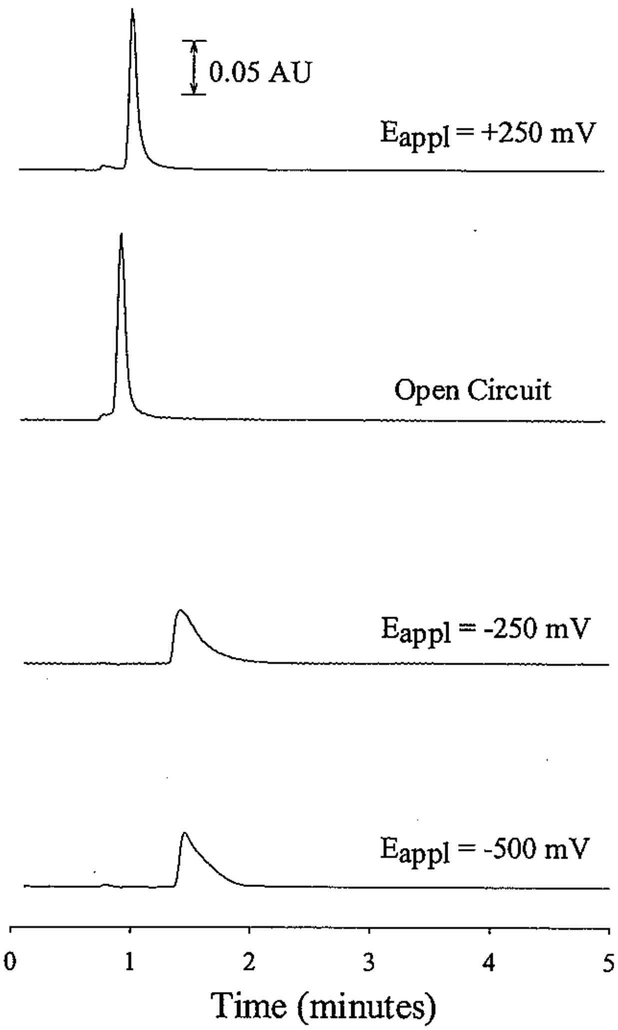

0.05 AU
E_{appl} = +250 mV
Open Circuit
E_{appl} = -250 mV
E_{appl} = -500 mV
0
1
2
3
4
5
Time (minutes)

Figure 4

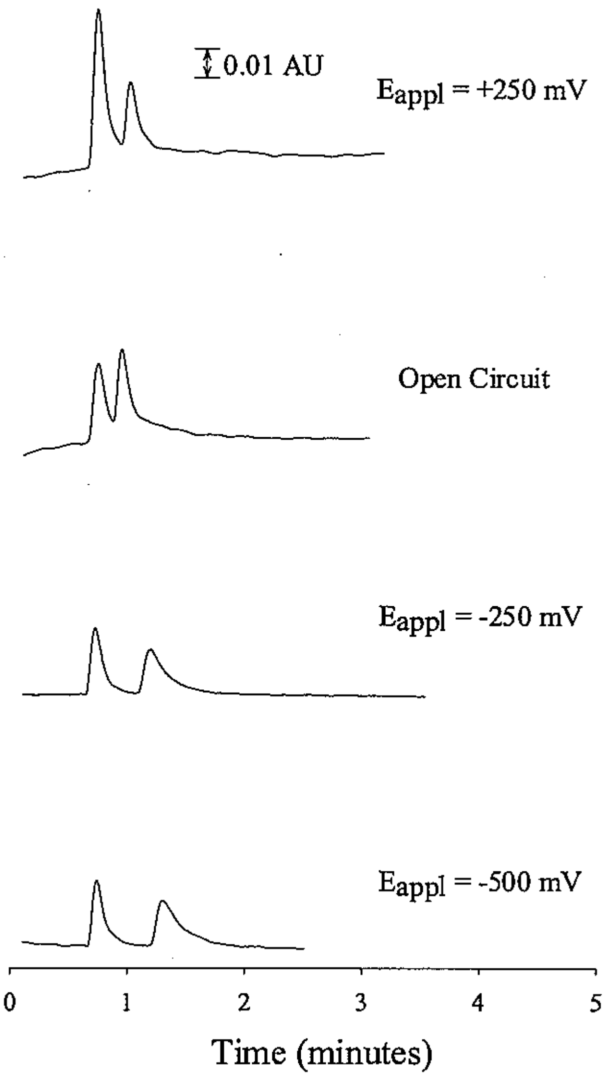

0.01 AU
Eappl = +250 mV
Open Circuit
Eappl = -250 mV
Eappl = -500 mV
0
1
2
3
4
5
Time (minutes)

Figure 5

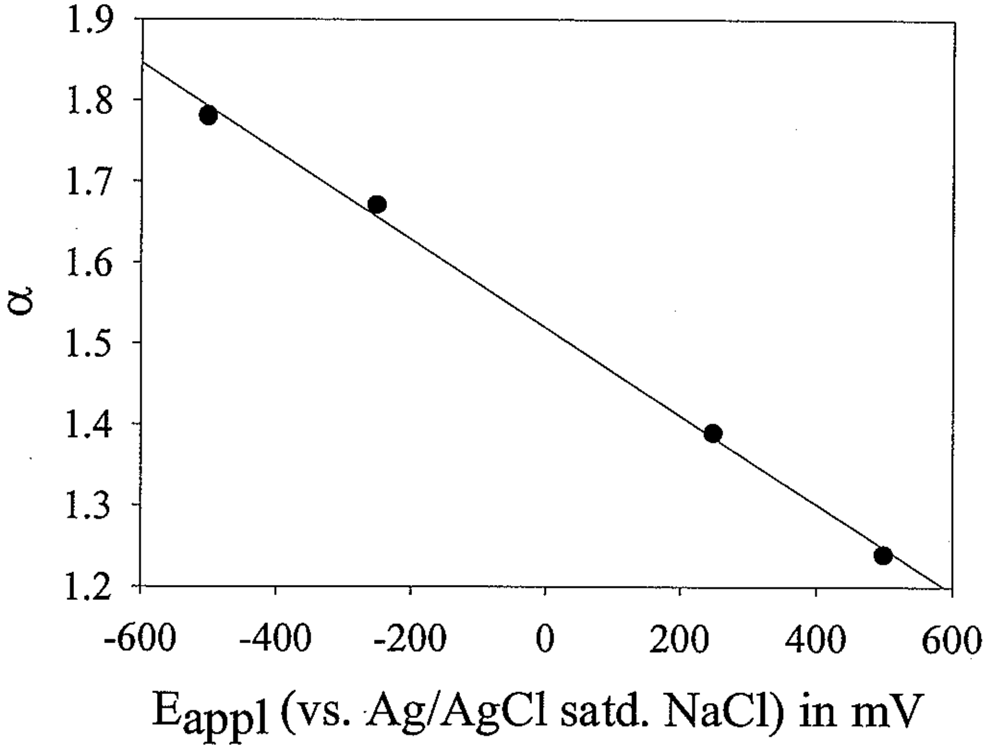

1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
α
-600
-400
-200
0
200
400
600
Eappl (vs. Ag/AgCl satd. NaCl) in mV

Figure 6

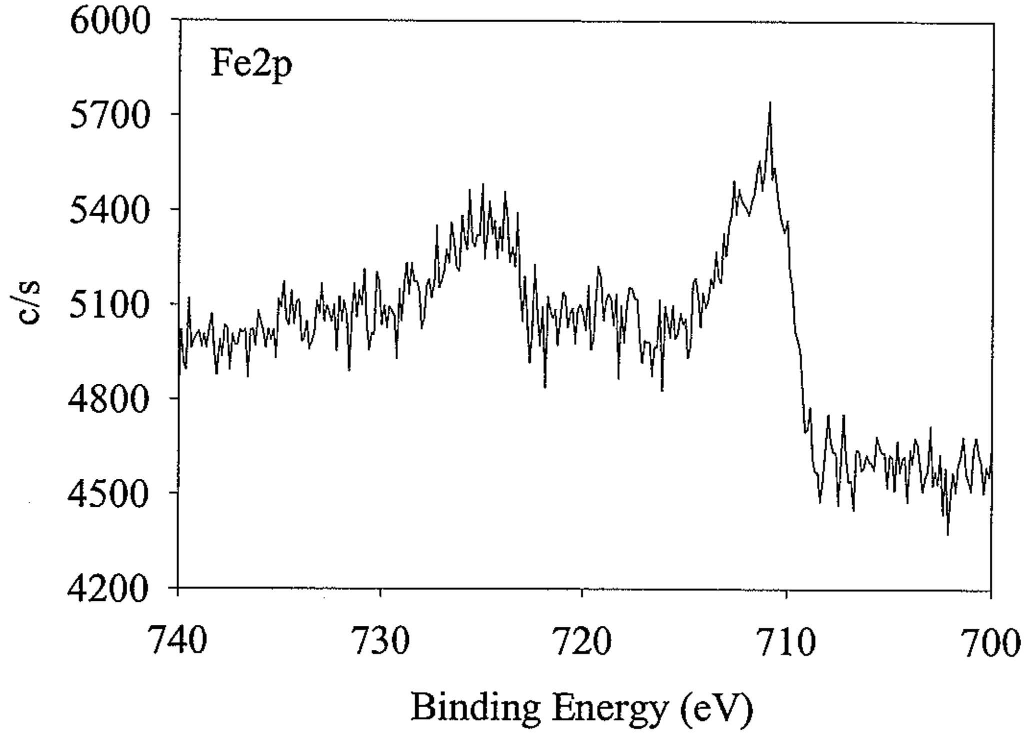

Fe2p
6000
5700
5400
5100
4800
4500
4200
c/s
740
730
720
710
700
Binding Energy (eV)

Figure 7

CHAPTER 6. CHARACTERIZATION OF BENZENEHEXATHIOL MONOLAYERS AT GOLD: THE DEVELOPMENT OF MODIFIED GOLD ELECTRODES WITH CARBON-LIKE BEHAVIOR

A manuscript in preparation for submission to *Langmuir*

Jennifer A. Harnisch, Andrew D. Pris and Marc D. Porter
Microanalytical Instrumentation Center, Ames Laboratory-USDOE, and Department of Chemistry, Iowa State University, Ames, IA 50011 USA

Abstract

Numerous thiol-based compounds have been used to modify gold substrates in recent years, yet few examples of functionalized thiols whose pendant group adsorbs in a parallel orientation to the gold surface are known. Herein, we describe the characterization and properties of benzenehexathiol (BHT, $C_6H_6S_6$) monolayer films formed by chemisorption at resistively evaporated gold films. Infrared reflection absorption spectroscopy (IRRAS), x-ray photoelectron spectroscopy (XPS), friction force microscopy (FFM), and cyclic voltammetry were used to characterize the monolayer. The absence of characteristic adsorption bands in the IRRAS spectra of a BHT monolayer strongly suggests that the molecules lie planar to the gold surface. This is substantiated by AFM contact mode images of micro-contact printed BHT monolayers. X-ray photoelectron spectroscopy (XPS) confirms the presence of BHT on the gold surface and points toward the possibility of more than one type of sulfur species. Surface coverage was estimated to be $\Gamma = 7.34 \times 10^{-9}$ mol/cm^2 by reductive desorption experiments, and is compared to a predicted coverage of $\Gamma_{est} = 5.95 \times 10^{-10}$. Finally, the electron-transfer properties of a BHT-modified gold electrode were evaluated using ferrocene and catechol as test probes.

Introduction

The modification of surfaces is a rapidly expanding field in analytical chemistry. Of particular interest is the spontaneous adsorption of sulfur-containing molecules onto gold substrates forming self-assembled monolayers (SAMs). Sulfur-derived monomolecular films are stable and have many uses due to the ability to tailor the chemical properties of the adsorbing thiol[1,2]. Examples include fundamentals studies of electron transfer across an interface[3-13], modification of electrodes to create more ideal voltammetric responses[14-16], microelectronics, and biomaterials.

In general, spontaneous adsorption of thiols results in the formation of a densely packed monolayer covalently attached to the metal surface. Infrared reflection spectroscopy (IRS) and ellipsometric thickness measurements indicate that alkanethiol chains adopt a surface orientation such that the majority of the carbon-carbon bonds are in an all *trans*-configuration with additional van der Waals interactions causing the chains to be tilted ~30° from the surface normal[17-19]. Similarly, arenethiols form monolayers with their aromatic groups relatively perpendicular to the surface [20].

It is uncommon to observe thiol monolayers where the organic endgroup lies parallel to the gold surface. Bushby and coworkers provide some evidence for thiol-tethered triphenylene compounds that form monolayers in which the triphenylene unit is planar to the gold surface[21]. Additionally, porphyrins bearing alkanethiol chains have been prepared that make the porphyrin moiety capable of planar orientations to the gold surface[22].

Recently, we prepared monolayers of benzenehexathiol (BHT), an aromatic compound substituted with six thiol functionalities, which lies parallel to the gold surface as illustrated in Scheme 1a. To our knowledge, the adsorption of other aromatic polythiols in this fashion, such as 1,4-benzenedithiol, has not been observed[23]. The results of IRRAS, FFM, XPS, and electrochemical characterization of monolayers formed from BHT on gold surfaces are reported herein.

Experimental Section

Reagents

Acetonitrile, tetrabutylammonium hexafluorophosphate (TBAH), methanol and acetic acid were purchased from Fisher Scientific; polydimethylsiloxane (PDMS) 182 elastomer kit from Dow Corning, and ferrocene, catechol, and potassium hydroxide (KOH) were from Aldrich Chemical Company. Catechol was sublimed prior to use. All other compounds were used as received. Benzenehexathiol was synthesized and characterized according to published literature procedures[24,25]. All aqueous solutions were prepared using Millipore deionized water.

Gold Substrates

Gold was deposited on two substrates: glass and mica. All substrates were prepared by deposition of 300 nm of gold (0.2 μm s^{-1}) in a cryopumped E306A Edwards Coating System. The pressure during evaporation was typically below 7 x 10^{-6} Torr.

Glass substrates were pretreated with a 15 nm (0.1 μm s^{-1}) adhesive layer of chromium prior to gold deposition. The glass slides were cleaned in an aqueous solution of Micron surfactant overnight and rinsed well with Millipore deionized water. Finally, the slides were washed with methanol and dried under a directed stream of high purity nitrogen before loading into the evaporator.

The mica substrates were cleaved immediately before loading into the evaporator. After gold deposition, the samples were annealed in the ambient at 300 °C for 4-6 h to ensure that large Au(111) terraces are formed[26,27].

Glassy Carbon Substrates

Glassy carbon electrodes (Tokai, GC-20) were polished sequentially with 1.0 μm, 0.3 μm, and 0.05 μm alumina (Beuhler) followed by sonication in water and acetonitrile for 10 min.

Monolayer Preparation

Monolayers were formed on the two types of gold (Au/Cr/glass or Au/mica) by immersion into 1 mM BHT solutions of acetonitrile according to conventional protocols[1]. Immersion times were typically 5 min. On emersion, the samples were rinsed with acetonitrile, and dried in a stream of high purity nitrogen. It is noted that monolayers of BHT on gold are hydrophobic (as is the case with GC) and are not wetted extensively by water.

Patterned monolayer surfaces were prepared using micro-contact printing (μ-CP) with PDMS stamps as previously described[28]. Briefly, a PDMS μ–CP stamp was immersed in a 3 mM solution of BHT in acetonitrile for 15 min. Upon emersion, the stamp was dried in a stream of high purity nitrogen for 10 s and placed directly onto the gold substrate. The stamp was allowed to sit for 30 s and was carefully removed. The μ-CP process results in domains of BHT and bare Au.

Infrared Spectroscopy

Infrared spectra were acquired with a Nicolet 750 FT-IR interferometer. Monolayer spectra were obtained using p-polarized light incident at 80° with respect to the surface normal and are reported as $-\log(R/R_o)$, where R is the reflectance of the sample and R_o is the reflectance of a C_{18}-perdeuterated thiol monolayer/Au reference sample. A liquid N_2 cooled HgCdTe detector was used. The spectrometer and sample chamber were purged with boil-off from liquid N_2. Further details of the experimental procedures are given elsewhere[29].

Electrochemical Measurements

Electrochemical experiments were performed to characterize the properties of the modified electrode in solutions of 0.1 M tetrabutylammonium hexafluorophosphate (TBAH) in methanol (1.3 mM ferrocene) or 0.1 M aqueous acetic acid (1.0 mM catechol) using a CV-27 potentiostat (Bioanalytical Systems), a Houston Instruments 2000 XY recorder, and a conventional three-electrode cell. The exposed area of the working electrode, as defined by the diameter of a Viton O-ring, was 0.50 cm^2. All voltages are reported with respect to a Ag/AgCl, saturated NaCl electrode. Electrolyte solutions were deoxygenated with high purity argon for approximately ten minutes prior to use.

Reductive desorption experiments which were used to estimate the surface concentration of the adlayer[30] were performed in deoxygenated 0.5 M KOH solutions using the same conditions described above.

X-ray Photoelectron Spectroscopy (XPS)

XPS data were obtained using a Physical Electronics Industries 5500 surface analysis system equipped with a hemispherical analyzer, torroidal monochromator, and multichannel detector. Monochromatic aluminum Kα radiation (1486.6 eV) at 300 W was used for excitation. Binding energies were referenced to the C(1s) emission band at 284 eV. The pressure in the XPS chamber was less than 1 x 10^{-9} Torr during analysis.

Friction Force Microscopy (FFM)

FFM images of patterned BHT monolayers on gold /mica were collected on a Digital Instruments Multimode with a Nanoscope III controller. Images were captured in Contact Mode using 200 μm oxide-sharpened Si_3N_4 cantilevers (Nanoprobes). A more detailed procedure is reported elsewhere[31].

Results and Discussion

Infrared Reflection/Absorption Spectroscopy (IRRAS) of BHT Monolayers

IRRAS provides a facile means to examine the composition and spatial organization of organic monolayers on gold surfaces. Numerous examples can be cited in which IRRAS has been employed to detail the characteristics of both the end group and organic spacer of alkanethiols on gold substrates[17,32]. Orientation insights rely on the so-called surface selection rule, which indicates that the transition dipole of a vibrational mode is preferentially excited when aligned closer to the surface normal[33].

In the simplest case, BHT would adopt one of the two possible orientations illustrated in Scheme 1: with the aromtic ring parallel (a), or perpendicular (b) to the gold surface. If BHT adsorbs with its ring perpendicular to the electrode, then features for the S-H bands from 2600-2550 cm^{-1} typical of an aromatic thiol[34]should be observed; specifically, the in-plane and stretching modes of the carbons in the ring should also be present.

Figure 1 presents the split spectral region from 3200-2800 cm^{-1} and from 1700-1000 cm^{-1} for BHT adsorbed on Au/Cr/glass. The spectrum is essentially featureless pointing toward the possibility that the benzene ring is oriented parallel to the gold surface. Parallel orientation of aromatic ring systems has been previously observed for electrosorbed benzene derivatives[35-37]. The weak bands at 1641 and 1527 cm^{-1} in Figure 1 are assigned to the ring-breathing modes of an aromatic ring. Exploration of the spectral region diagnostic of C-S modes (800-600 cm^{-1}) is not possible due to the lower wavelength cut-off of our detector.

The strongest absorbance at 1275 cm^{-1} is indicative of a thiocarbonyl (C=S) group[38] and potentially establishes that some of the S-H bonds have been cleaved. This is not an entirely unfeasible possibility since one may consider distribution of the negative charge throughout the aromatic system via a resonance mechanism that incorporates carbon-sulfur double bonds as depicted in Scheme 2. Thioquinonoid compounds (S=Ph=S) are synthetically challenging to isolate due to their instability[39-41]. In general, they are prepared as their dimeric diaminodithioquinone counterparts since the amine moiety lends stability to the system[39]. However it appears that the bonding between the thiolate and gold supports the thioquinone structure.

X-Ray Photoelectron Spectroscopy (XPS) of BHT Monolayers

The gold-sulfur bond of an organosulfur monolayer can be easily probed using X-ray photoelectron spectroscopy. Previous investigations have argued that thiols adsorb as their corresponding thiolates by the cleavage of the sulfur-hydrogen bond. For a typical alkanethiol monolayer, the bands appear at 163.2 eV and 162.0 eV for the sulfur $2p_{1/2}$ and

$2p_{3/2}$ emissions, respectively[42-45]. Furthermore, the XPS spectrum of an arenethiol such as thiophenol is similar, with bands at 163.0 and 164.0 eV for the S(2p) electrons[46].

The XPS spectrum for BHT on Au/Cr/glass from 158-178 eV is displayed in Figure 2. The broad feature centered at 163.5 eV suggests that more than one state of sulfur is present. This observation is not uncommon for thiols with a complex aromatic system such as BHT. Fujihara and coworkers observed a broad XPS peak at ~162 eV for a bisthiophene derivative[46]. Deconvolution of this feature pointed toward the possibility of two sulfur species, one tightly bound to the surface, the other unbound. However, these results were not conclusive.

Friction Force Microscopy (FFM) of BHT Monolayers

Recently, sample preparation using micro-contact printing (μ-CP) has been utilized in conjunction with Friction Force Microscopy (FFM) to show differences the spatial orientation monolayers[31]. Using this methodology, subtle differences in the terminal group orientations can be probed. In theory, one should not be able to obtain a friction image with a developed μ-CP pattern if the aromatic ring is oriented parallel to the gold surface, however, a perpendicular orientation should have higher friction than the bare gold surface, and in this case, a pattern would be observed.

Figure 3 presents the friction image (40 x 40 μm) of a Au/mica surface after printing with a BHT monolayer. A distinct pattern is evident in the AFM image in Figure 3, which is attributed to height-induced friction. Although there appears to be some height, the results are not enough to draw a conclusion as to the exact orientation of the BHT molecules. Further exploration of the BHT monolayers by FFM is currently in progress.

Electrochemical Measurements of Surface Coverage

Reductive desorption is a common method to determine the surface concentration of thiols on gold surfaces. In a series of studies[32,47-49], we have shown that alkanethiolate monolayers can be desorbed into alkaline solutions from Au(111) on mica by a one-electron reduction as shown in equation 1. Using a linear voltage sweep, for example, to drive the reaction produces a single well defined cathodic wave that reflects the desorption process. The surface concentration (Γ) of the monolayer is subsequently calculated by integrating the area under the desorption wave after accounting for surface roughness and double layer charging current.

$$\mathrm{AuSR} + 1\,e^- \longrightarrow \mathrm{RS}^- + \mathrm{Au(0)} \qquad (1)$$

A representative voltammogram for the reductive desorption of adsorbed BHT is shown in Figure 4. The voltage scan was initiated at –200 mV at 50 mV/s, and was reversed at –1200 mV before solvent reduction. The response clearly exhibits two reduction waves (-750 mV and -940 mV), which are superimposed on a broad background. The surface concentration was calculated to be 7.3 x 10^{-9} mol/cm^2. This value is higher than the estimated coverage, $\Gamma_{est} = 5.95 \times 10^{-10}$ mol/cm^2, for the closest packed adlayer of BHT on Au(111) adsorbed with its ring parallel to the electrode surface.

The presence of two reduction waves and a high coverage indicates that more than one type of Au-S linkage is present and lends credence to the possibility of a C=S. It is rationalized that a species containing C=S would be more weakly bound than a typical thiolate. Hence, it is expected that part of the adsorbed BHT with C=S bonds would desorb at less negative potentials (i.e., at –750 mV) followed by the desorption of the aromatic thiolate at –940 mV. It is also possible that the high coverage results from the formation of multilayers due to π–π interactions between adsorbing aromatic rings.

Electrochemical Evaluation of BHT-Modified Electrodes

Many probe molecules have been used to investigate the integrity of monolayer coatings by determining the heterogeneous electron transfer rates[50-52]. Ferrocene and catechol were chosen to evaluate the electron-transfer properties of the BHT-modified electrodes since their electron transfer mechanisms are outer and inner-sphere, respectively, and the electrochemical behavior of these systems at gold electrodes is well documented[53].

Figure 5 presents cyclic voltammograms for 1.3 mM ferrocene in methanol at (a) uncoated gold and (b) BHT-modified gold electrodes. Both voltammograms display a reversible redox wave with a formal reduction potential at +410 mV and a comparable peak separation of 120 mV. Therefore, the BHT monolayer does not inhibit electron transfer at the electrode surface for an outer-sphere mechanism.

In sharp contrast is the voltammetry of catechol at uncoated gold, BHT-modified gold, and glassy carbon (GC) electrodes. Figure 6 shows the cyclic voltammetry for 1 mM catechol (in 0.1M aqueous acetic acid) at (a) uncoated gold electrode, (b) BHT-modified gold electrode, and (c) GC. Typical quasi-reversible electrochemical behavior for catechol is observed at the uncoated gold surface (Figure 6a), with a formal reduction potential of +423 mV and a peak separation of 175 mV.

In contrast to uncoated gold, the voltammetry of catechol at a BHT-modified electrode (Figure 6b) is markedly attenuated and is characterized by an irreversible cathodic wave. Similarly, the voltammetry of catechol at an as-polished glassy carbon electrode

(Figure 6c) is also irreversible. These observations lead to the conclusion that the BHT-modified gold electrode essentially electrochemically "mimics" a carbonaceous surface.

Conclusion

BHT is an intriguing species when adsorbed to a gold surface. The presence of six thiol groups on one molecule affords BHT the possibility of many surface orientations and modes of binding to the gold surface. However, a featureless infrared reflectance spectrum and clearly implies that the benzene ring is lying parallel to the gold surface. This conclusion is substantiated by the FFM friction image, which shows only edge-induced friction. To our knowledge, an entirely parallel aromatic thiol system is unprecedented in literature and points toward the intriguing possibility for carbon films of monolayer thicknesses.

Results from XPS analysis and reductive desorption experiments indicate the presence of more than one sulfur state on the gold surface. Although we are unable to confirm the exact nature of the sulfur species, an IRRAS adsorption band at 1275 cm^{-1} points toward a C=S species which is arguably possible considering conjugation through resonance.

The electrochemical behavior of BHT-modified gold surfaces mirrors that of a carbon electrode pointing toward the possible use of BHT as a modification agent for gold surfaces when the properties of carbon electrodes (e.g. wide potential windows, materials properties) are desired. Several applications of BHT monolayer films can be envisioned including coatings for gold microchannels, detectors, and platforms for quasicrystals.

Acknowledgment

J.A.H. wishes to acknowledge the Phillips Petroleum Company for a research fellowship. This work was supported by the Office of Basic Energy Research, Chemical Sciences Division of the U. S. Department of Energy and by the Microanalytical Instrumentation Center of Iowa State University. The Ames Laboratory is operated for the U. S. Department of Energy by ISU under Contract W-7405-Eng-82.

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Figure Captions

Scheme 1. Representation of orientation of BHT molecules with the aromatic ring adsorbed (a) parallel and (b) perpendicular to the gold substrate.

Scheme 2. Postulated resonance mechanism for formation of carbon-sulfur bonds in BHT adsorbed on gold.

Figure 1. IRRAS spectrum of a BHT monolayer on a Au/Cr/glass substrate. Regions displayed are from 3200-2800 cm^{-1} and from 1700-1000cm^{-1}.

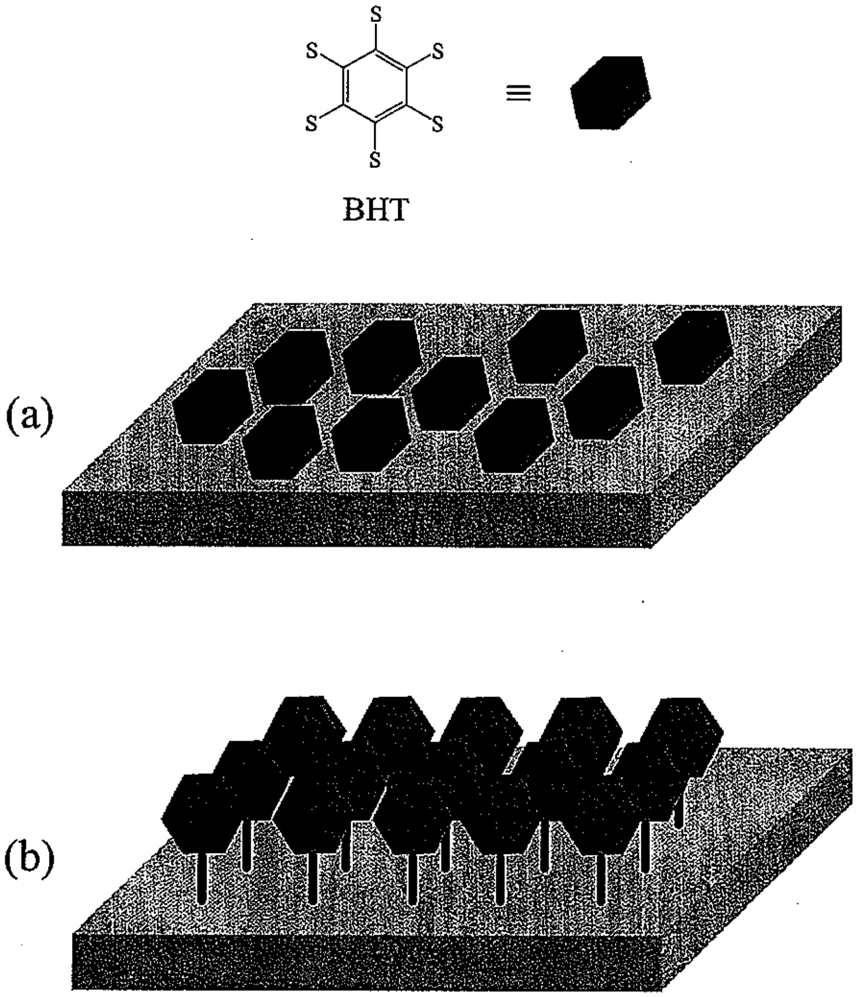
Figure 2. X-ray photoelectron spectrum of the S(2p) region of a BHT monolayer on a Au/Cr/glass substrate.

Figure 3. Friction image (40μm x 40 μm) (z-scale = 0.2 V) of a BHT monolayers patterned on smooth gold by micro-contact printing. The areas of darker contrast are due to edge-induced friction, and not indicative of perpendicularly oriented BHT molecules. Brighter image areas are indicative of higher friction.

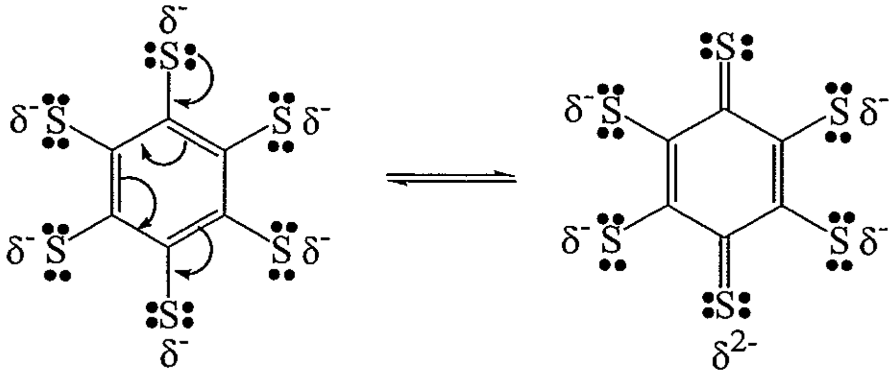
Figure 4. Cyclic voltammetry for reductive desorption of BHT at Au/mica. Scan was initiated at –200 mV at 50 mV/s with 0.5 M aqueous KOH as supporting electrolyte.

Figure 5. Cyclic voltammetry of 1.3 mM ferrocene in methanol (0.1 M tetrabutylammonium hexafluorophosphate (TBAH)) at (a) uncoated gold and (b) BHT-modified gold electrodes. Scans were initiated at +750 mV at 50 mV/s.

Figure 6. Cyclic voltammetry of 1.0 mM catechol at (a) uncoated gold, (b) BHT-modified gold and (c) glassy carbon electrodes. electrode Scans were initiated at +750 mV at 50 mV/s in 0.1 M acetic acid as the supporting electrolyte.



Scheme 1



Scheme 2

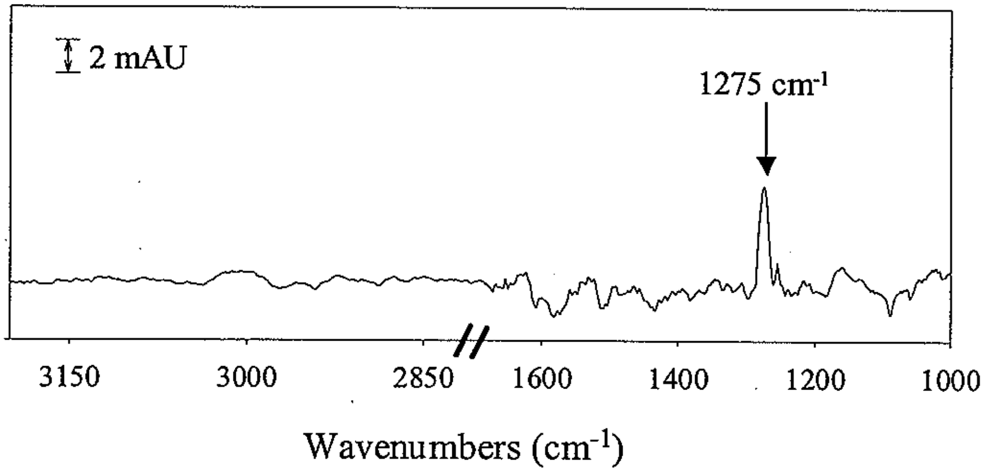

2 mAU
1275 cm⁻¹
3150
3000
2850
1600
1400
1200
1000
Wavenumbers (cm⁻¹)

Figure 1

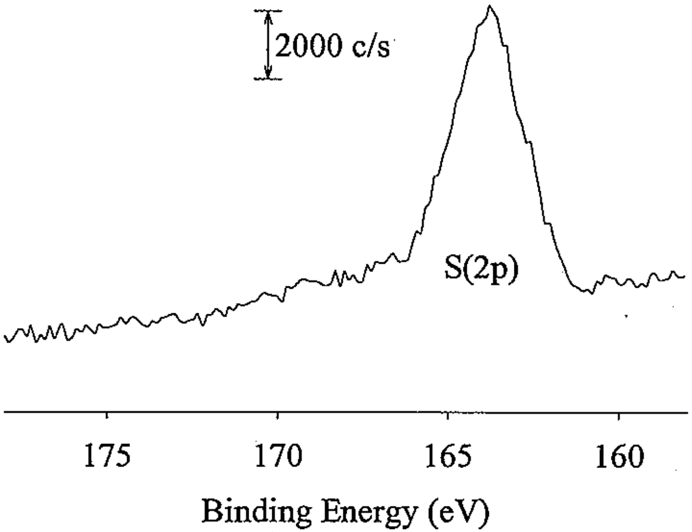


Figure 2

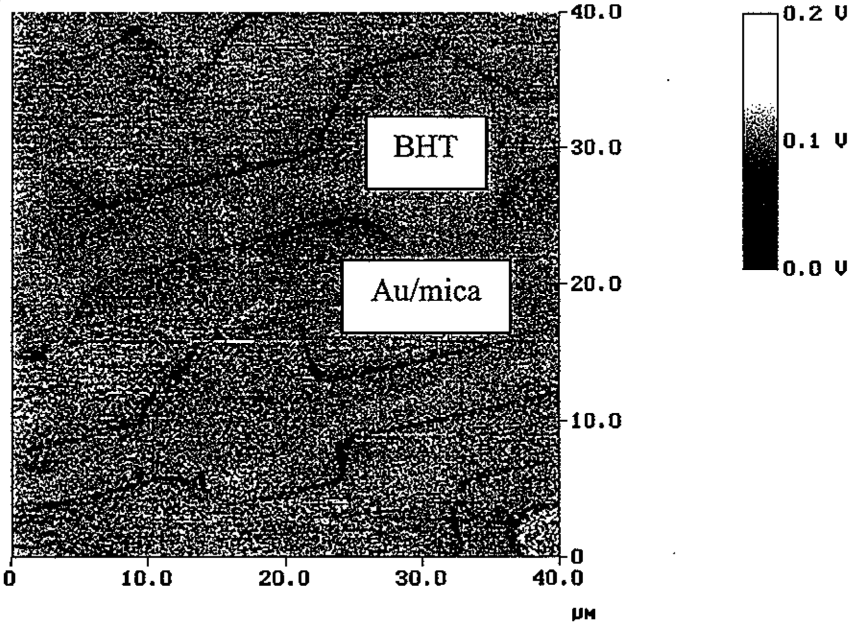


Figure 3

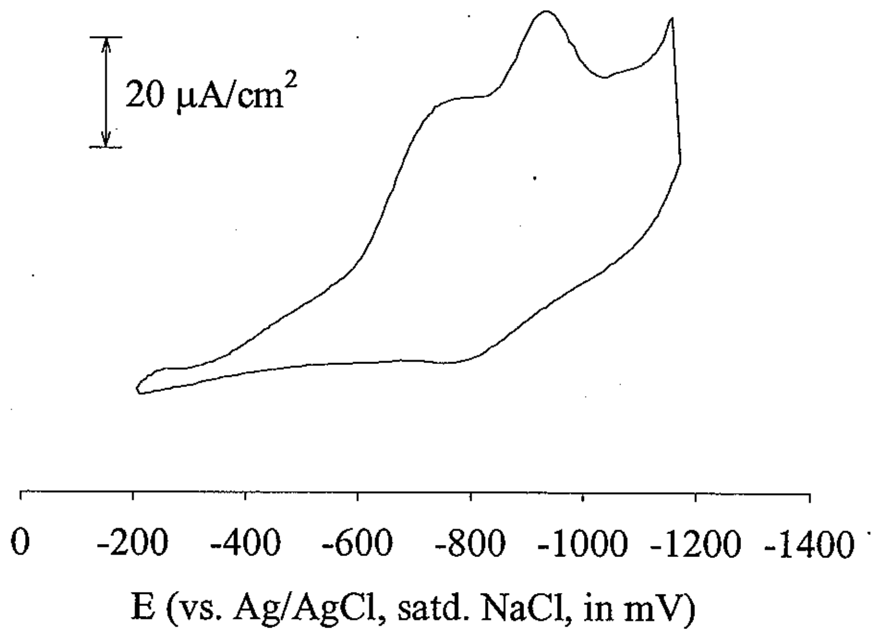

20 μA/cm²
0
-200
-400
-600
-800
-1000
-1200
-1400
E (vs. Ag/AgCl, satd. NaCl, in mV)

Figure 4

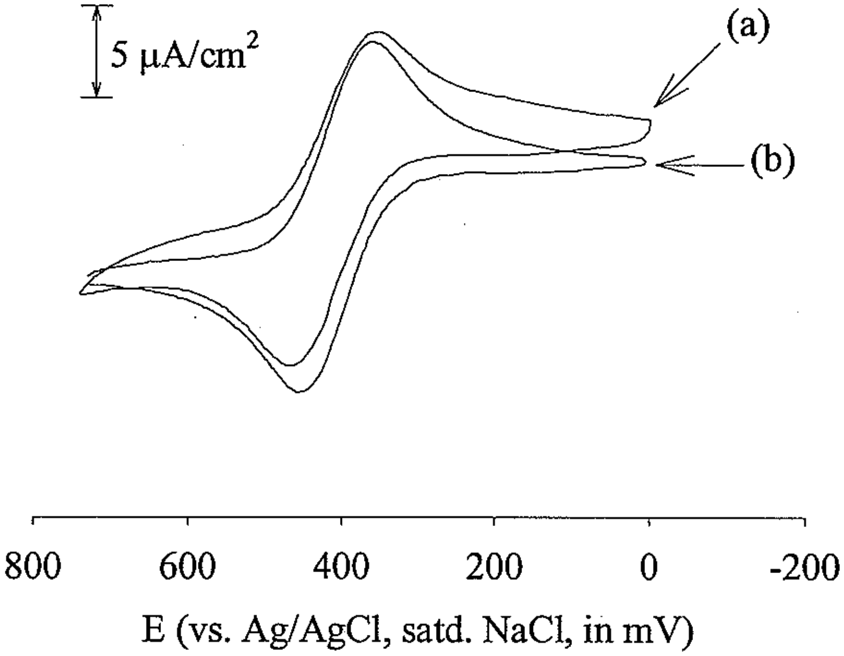


Figure 5

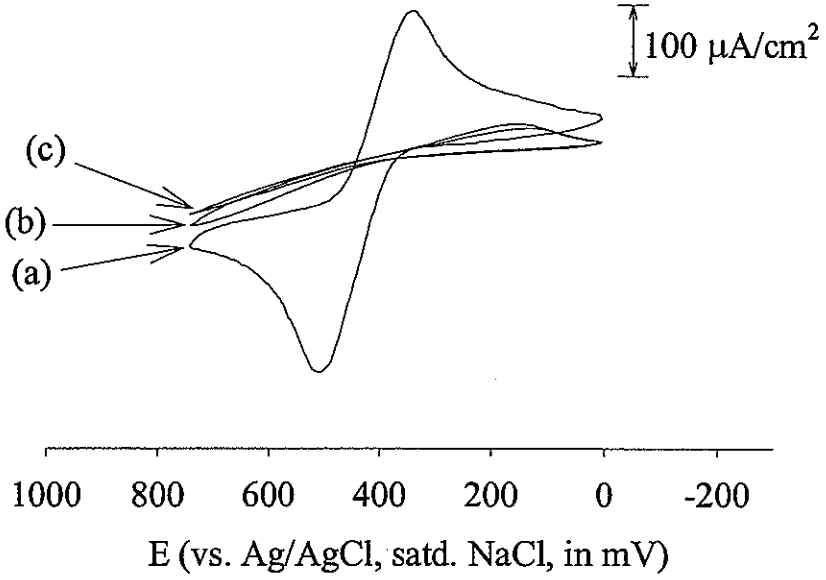

100 μA/cm^2
(c)
(b)
(a)
1000
800
600
400
200
0
-200
E (vs. Ag/AgCl, satd. NaCl, in mV)

Figure 6

GENERAL CONCLUSIONS

Research Overview

The main objective of this doctoral research was to develop methodologies that derivatize both carbon and gold substrates in order to lend new materials properties to the surface. Several approaches have been taken to achieve this goal. Carbon substrates (porous graphitic carbon (PGC) and glassy carbon (GC)) in both spherical and planar form have been successfully functionalized using the electroreduction of arenediazonium salts and the Kolbe reaction. These two modification strategies were applied to planar GC electrodes as well as on-column using electrochemically modulated liquid chromatography (EMLC). In contrast, gold substrates are easily modified by the formation of self-assembled monolayers or SAMs. A new monolayer, benzenehexathiol (BHT) chemisorbs parallel to the gold surface in an unprecedented fashion, imparting materials properties similar to those of carbon substrates. All of the modification methods mentioned in this thesis further efforts to control the properties of carbon and gold materials by chemically altering the surface composition.

On-column Modification of Stationary Phases Used in EMLC

Although EMLC has already been demonstrated as a viable chromatographic method that is capable of manipulating retention time by applied potential, the separation of proteinaceous materials and polyaromatic hydrocarbons (PAHs) is still challenging. One reason for this is the irreversible adsorption of highly conjugated materials to the carbon stationary phases resulting from strong π–π interactions. It was postulated that chemically altering the surface composition of the carbon phases to minimize these undesirable interactions would extend the utility of EMLC as a universal chromatographic technique.

This project focuses on the development and implementation of an on-column chemical modification protocol that integrates with conventional EMLC operating conditions.

This first successful strategy was realized with the electroreduction or arenediazonium salts as modifiers in the mobile phase. In this approach, a reactive areneradical is generated at cathodic potentials at the surface of the carbon stationary phase (e.g. PGC or GC). The areneradical rapidly inserts into the carbon-carbon aromatic framework of the packing material, forming a robust covalent bond between the arene ring of the modifier and the carbon surface. Modification by the reduction of arenediazonium salts is attractive for two reasons. First, the electrochemistry of several modifiers at GC electrodes, and the subsequent characterization of the modified substrates is well documented in literature. Secondly, arenediazonium salts are readily synthesized from any substituted aniline precursor, thus expanding the group of possible modifiers. As chapters 1 and 2 illustrate, modification results in an enhancement of the chromatographic properties of PGC and GC stationary phases both at applied potentials (E_{appl}) and in a conventional high-performance liquid chromatography (HPLC) column. Additionally, the stationary phases modified using arenediazonium reductions show good stability at extremes in pH, which is an important consideration when performing biological separations.

Chapter 3 discusses a second on-column modification protocol involving the oxidation of arylacetate anions (the Kolbe reaction). In this case, an oxidative potential is applied to the EMLC column with the modifying arylacetate in the mobile phase. At these applied potentials, the arylacetate anion forms a benzyl radical with the subsequent loss of CO_2. Much like the aryl radical in the aforementioned arenediazonium salt reduction, the benzyl radical will also insert into the carbon-carbon bonds of the carbon stationary phase to form a covalent bond. GC column packings that have been modified with substituted benzyl groups show an improvement in the separation of polar analytes at positive E_{appl}.

Both of the on-column modification strategies display encouraging improvements in the chromatographic separations of polar analytes, highly conjugated species and proteins, thus adding a new dimension to EMLC.

Creating Gold Nanostructures on Glassy Carbon Surfaces

The covalent linking of gold to a carbon surface is a challenging prospect, and is therefore unprecedented in literature. Chapter 4 discusses an exciting extension of the electroreduction of arenediazonium salts involves the casting of a mercaptoarene film onto a GC electrode, via the reduction of 4-mercaptobenzenedizaonium tetrafluoroborate, to provide a point of attachment for gold nanoparticles. Furthermore, one may further modify the gold nanoparticle surface after deposition through the formation of SAMs. Several applications of this new architecture are possible including the creation of individually addressed nano-arrays.

Electroactive Chiral Selection with EMLC

Chapter 5 delves into the exploration of chiral selectors whose adsorption to the PGC stationary phase increased or decreased by manipulating the electron donor/acceptor properties of the selector electrochemically. These new chiral selectors must be electrochemically stable, easy to synthesize, and show an overall enhancement in enantiomeric resolution. Ferrocenyl-based selectors with a chiral functionality offered an attractive prospect that fit the aforementioned criteria. Although several chiral ferrocene derivatives were prepared throughout the course of this doctoral work, (α)-(+)-(methylbenzylaminocarbonyl)ferrocene (MBACF) showed great promise in the resolution of a racemic mixture of homatropine. Furthermore, the separation factor (α) increased with more positive values of E_{appl} and is limited only by the potential window of the EMLC stationary phase (PGC).

Imparting Carbon-like Properties to Gold Films

The modification of gold surfaces by SAM technology in order to customize surface characteristics is a mature field. However, evidence for a monolayer that chemisorbs parallel to a gold surface has not been reported. Chapter 6 discusses benzenhexathiol (BHT) as a thiol species that adsorbs planar to a gold electrode. SAMs of BHT show electrochemical behavior similar to that of a freshly polished glassy carbon electrode. This leads to the intriguing possibility of modified gold surfaces that possess carbon-like properties.