

## Horizontal touch voltammetric analysis - determination of insoluble electroactive species in films at the air/water interface

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## Horizontal Touch Voltammetric Analysis—Determination of Insoluble Electroactive Species in Films at the Air/Water Interface

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**The electroanalysis of small quantities (picomoles) of water-insoluble compounds at the air/water interface of a Langmuir minitrough by transfer to an electrode and on trough voltammetry is described. Experiments were carried out with an amphiphilic surfactant, Ru(bpy)<sub>2</sub>(bpy-C<sub>19</sub>)<sup>2+</sup> (where bpy = 2,2'-bipyridine and C<sub>19</sub> = CH<sub>2</sub>NHOC(CH<sub>2</sub>)<sub>18</sub>CH<sub>3</sub>), as well as tris(4,4'-diphenyl-2,2'-bipyridyl)ruthenium(II), the tris-(4,7-diphenyl-1,10-phenanthroline) complexes of Ru(II) and Fe(II), and several aromatic phenols and amines. The effects of several parameters, such as electrode surface hydrophobicity and transfer conditions, are considered and on-trough electrogenerated chemiluminescence (ECL) is shown to be useful in imaging the distribution of analyte on the electrode surface.**

### INTRODUCTION

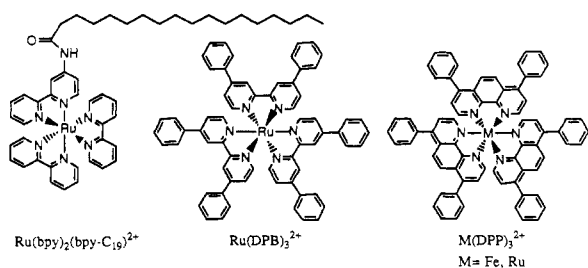
We propose here a new approach to the electrochemical analysis of very small quantities of substances that form monolayer films at the air/water interface by on-trough voltammetry. High-sensitivity electroanalytical techniques frequently depend upon the preconcentration of a sample on or near an electrode surface. Anodic stripping analysis (1, 2) is a familiar example of such an approach where a preelectrolysis is used to deposit a metal in a small mercury drop, followed by its voltammetric determination in an anodic scan. Preconcentration before voltammetry by adsorption on an electrode surface (3) or into a polymer film (4, 5) from bulk solution has also been used. The approach described here, called horizontal touch (HT) voltammetric analysis, is based

on localizing an insoluble electroactive substance on a small area of an air/water interface and transferring it to an electrode surface brought into contact with that area, followed by a voltammetric scan. This work grows out of previous electrochemical studies of organized assembly films on a Langmuir trough by the HT method (6).

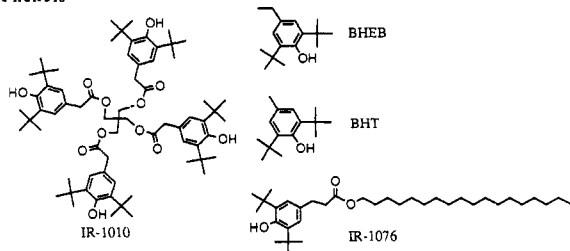
In a Langmuir trough, amphiphilic molecules that form organized assemblies, e.g., long hydrocarbon chains with polar head groups, are confined to the air/water interface in a single monomolecular layer, where their compressibility, viscosity, and transfer characteristics can be studied (7). Traditionally, these monolayers have been studied at high packing densities under surface pressures between 1 and 80 mN/m. Much of the emphasis in the properties of these monolayers at high surface pressures stems from their uses in building multilayer films on solid supports by the Langmuir-Blodgett technique (8). In the most common "vertical dipping" method, the substrate (e.g., electrode) surface is oriented normal to the monolayer and is passed through the air/water interface, resulting in the transfer of the compressed monolayer. A significant surface pressure is required to align the amphiphiles into an oriented, near close-packed, structure and to force the monolayer film on the aqueous meniscus of the solid support. In an alternative monolayer deposition scheme, a hydrophobic support is brought into contact with the monolayer oriented parallel to the air/water interface (9). Because this horizontal touching method does not require the monolayer to be compressed to any degree, it allows the transfer of water-insoluble species onto solid supports under a wider range of surface packing and pressures than the traditional vertical dipping method. This added flexibility is particularly important, if one wants to transfer molecules that do not form highly organized monolayers and collapse under even low surface pressures.

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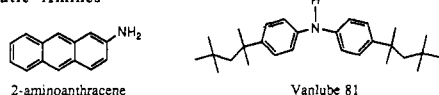
## Transition Metal Complexes



## Phenols



## Aromatic Amines



**Figure 1.** Chemical structures and trivial names for the redox active compounds studied in this work.

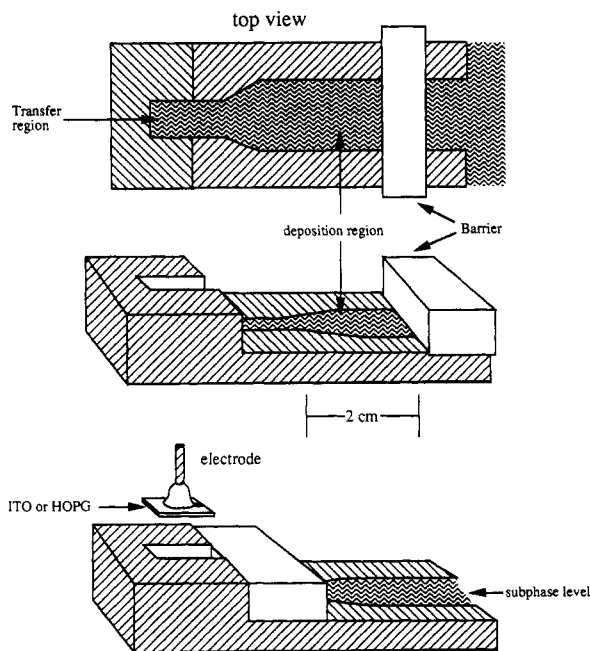
The ability to transfer monolayer films onto surfaces can be exploited in the development of new trace analysis techniques. A single square centimeter area covered with a close-packed monolayer of a surfactant corresponds to less than 1 nmol of the surfactant. Because so little surface-active material is required to form a close-packed monolayer film, the Langmuir balance can be viewed as a trace sample preparation technique. Even using conventional Langmuir-Blodgett instruments with air/subphase areas of approximately 1000 cm<sup>2</sup>, sample quantities required for the study and transfer of monolayer films are still quite small. The quantity of amphiphile required can be dramatically reduced by simply reducing the dimensions of the air/water interface. Iwahashi et al. have reported the transfer of cadmium-109 eicosanoate by the horizontal lifting method to produce radioactive sources by using a specially designed trough with surface areas of 3–10 cm<sup>2</sup> (10).

Once transferred onto the solid surface, the monolayer must be characterized and quantified by some means. Electrochemical methods, owing to their high sensitivity, are particularly suited to this task. Fujihira and Araki have described the voltammetric response of quinone derivatives transferred via the HT method onto glassy carbon electrodes (11). In previous studies, we used the HT method to transfer a surfactant ruthenium(II) bipyridyl complex over a range of surface coverages (6). In that work, which involved electrochemistry directly on a large area Langmuir trough, the possibility of an analytical method based on the Coulometric determination of trace quantities of electroactive, water-insoluble species transferred onto an electrode via the HT method was proposed (6). Here, the requirements for implementing this technique are explored, and its use with several different types of species is demonstrated.

## EXPERIMENTAL SECTION

**Material.** Chemical structures for some of the compounds studied are shown in Figure 1. The synthesis of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})(\text{ClO}_4)_2$  has been described previously (12).  $\text{Tris}(4,7\text{-diphenyl-1,10-phenanthroline})\text{ruthenium(II)}(\text{ClO}_4)_2$  and  $\text{tris}$

## Mini-trough

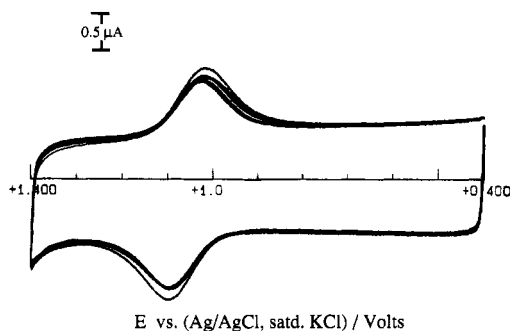


**Figure 2.** Schematic drawing of the minitrough. The minitrough was constructed completely of Teflon and was positioned in a larger Teflon trough filled with an electrolyte subphase.

(4,4'-diphenyl-2,2'-bipyridyl)ruthenium(II)( $\text{ClO}_4$ )<sub>2</sub> were prepared by Paul McCord, who synthesized them by the procedure of Anderson and Seddon (13).  $\text{Tris}(4,7\text{-diphenyl-1,10-phenanthroline})\text{iron(II)}(\text{ClO}_4)_2$  was synthesized by using a literature procedure (14). 2-Aminoanthracene was purchased from Aldrich Chemical Co. (Milwaukee, WI) and was recrystallized from ethanol/ $\text{H}_2\text{O}$ . 4-(1,1,3,3-Tetramethylbutyl)-*N*-(4-(1,1,3,3-tetramethylbutyl)phenyl)benzenamine (Vanlube 81) (R.T. Vanderbilt Company, Norwalk, CT) was a generous gift of Dr. Hugh Horowitz (Exxon). The phenols, IR-1076, IR-1010, BHT, and BHEB, were supplied by Wayne Riley (Exxon). All other chemicals were reagent grade and used as received. Aqueous solutions were prepared with water purified via an ion-exchange purification train (Milli-Q system, Millipore, Inc.).

Indium-tin oxide (ITO) electrodes (area, 0.2 cm<sup>2</sup>) were fabricated from ITO-coated glass plates (Delta Technologies, Stillwater, MN). An approximately 0.05-cm-wide band of Ti (ca. 700-Å thick) was sputtered onto the ITO-coated side from approximately the center of the electrode, around the side of the glass to the uncoated side of the glass (Figure 2). This Ti band afforded a back-side electrical contact to the ITO surface. The Ti-banded ITO-coated glass piece was subsequently mounted on the end of a 1-mm-diameter Cu wire contact and support with silver epoxy cement (Type H20S, Epoxy Technology, Billerica, MA) and the Cu wire insulated with vacuum epoxy (Torr-Seal, Varian, Santa Clara, CA). Highly oriented pyrolytic graphite (HOPG) electrodes were made from samples generously supplied by Dr. Arthur Moore (Union Carbide). Squares (area 0.2 cm<sup>2</sup>) of HOPG, ca. 0.5 mm thick, were mounted on the end of a 1-mm-diameter Cu wire with silver epoxy cement. The Cu wire and the edge planes of the HOPG were insulated with vacuum epoxy cement. After fabrication, the surface of the electrode was carefully cleaved by using Scotch tape to expose a fresh basal plane graphite surface. Both ITO and HOPG electrodes were rinsed with ethanol and deionized water and dried in a stream of argon prior to all monolayer transfer experiments.

**Apparatus.** A specialized small Langmuir trough ("minitrough", Figure 2), constructed from Teflon, was used for all of the experiments. Before each experiment, the barrier was swept across the subphase surface and the surface solution aspirated from the subphase region to remove any contaminants that might have accumulated at the subphase/air interface. With the barrier at its fully expanded position, a  $\text{HCl}_3$  solution of the compound



**Figure 3.** Series of cyclic voltammograms obtained immediately after the horizontal touching (HT) transfer of 5.6 pmol of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  using a single ITO electrode on the minitrough. (See text.) The voltammograms corresponding to five replicate transfers are shown, with the electrode washed in EtOH prior to each transfer. The subphase was 0.1 M  $\text{NaClO}_4$ , and the scan rate was 0.5 V/s.

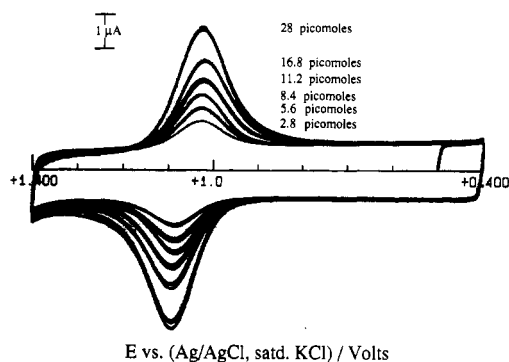
of interest was deposited on the minitrough. The volume of each addition of the  $\text{HCCl}_3$  solution was limited to 2  $\mu\text{L}$  in order to prevent the size of the expanding  $\text{HCCl}_3$  droplet from exceeding the 4- $\text{cm}^2$  area of the deposition region of the minitrough. Deposition of larger volumes resulted in loss of the species from the air/subphase interface, because the  $\text{HCCl}_3$  solution flowed down the sides of the minitrough. The volatile solvent was allowed to evaporate, and the barrier was then moved to compress the deposited material into the small transfer region (approximately 0.2  $\text{cm}^2$  in area). When the barrier was moved to the position of maximum surface compression, it closed the transfer region, forming a guide for the ITO or HOPG electrode. The size of these electrodes was controlled so that they fit in this transfer area with only a small clearance. The electrode was manually lowered within this guide, at a controlled rate, until its surface just touched the surface of the electrolyte. The quantity of the substance of interest transferred onto the ITO or HOPG working electrode surfaces was then measured voltammetrically on the trough. Counter (Pt) and reference (Ag/AgCl/saturated KCl) electrodes (not shown in Figure 2) were immersed in the subphase solution of the minitrough, and the movement of the working electrode was controlled by the Lauda film balance lifter (6).

Surface pressure/area isotherms were obtained by using a Lauda preparative film balance, Model P (Brinkman Instruments Co., Westbury, NY). Samples were deposited at the air/subphase interface from  $\text{HCCl}_3$  solutions using a gas-tight microliter syringe. Contact angle measurements were made with a contact angle goniometer (Model 100, Ramé-Hart, Mountain Lakes, NJ). Cyclic voltammetric experiments were performed with a BAS 100A electrochemical analyzer (Bioanalytical System, Inc., West Lafayette, IN). All potentials were measured and are reported versus a Ag/AgCl/saturated KCl reference electrode. Photographs of the electrogenerated chemiluminescence (ECL) from  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  deposits on electrodes were obtained with a CCD camera (Model CC2220, Photometrics, Inc., Tucson, AZ) cooled to  $-90^\circ\text{C}$ . During the exposure, the electrodes were held at 1.2 V in a solution of 0.1 M  $\text{Na}_2\text{C}_2\text{O}_4$ -0.1 M  $\text{NaClO}_4$ .

## RESULTS AND DISCUSSION

**Voltammetry of Surfactant,  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$ .** The initial experiments were carried out with an amphiphilic molecule composed of a  $\text{Ru}(\text{bpy})_3^{2+}$  polar head group with a hydrophobic 19-carbon hydrocarbon chain (Figure 1). This species forms an organized monolayer at the air/water interface with the polar head group facing the aqueous subphase (6, 12).

The analysis of trace quantities of electroactive water-insoluble species by the HT voltammetric method requires one to spread the analyte on the air/electrolyte interface, compress it into a small region, and reproducibly transfer the analyte onto the electrode surface. Figure 3 shows five cyclic voltammograms for five separate transfers of 5.6 pmol of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  onto an ITO electrode using the minitrough. When 5.6 pmol of this surfactant is compressed into the

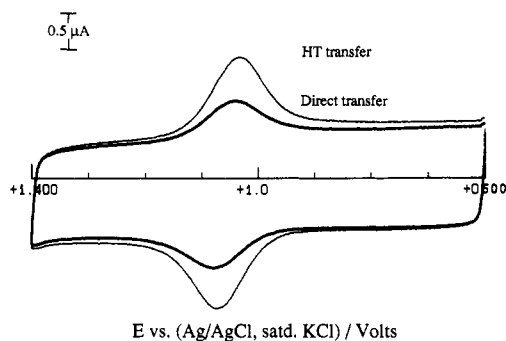


**Figure 4.** Dependence of the voltammetric signal on the quantity of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  deposited on the minitrough. Experimental conditions as in Figure 3.

transfer region of the minitrough, the 0.22- $\text{cm}^2$  transfer region area corresponds to 650  $\text{\AA}^2$ /molecule. This is over 5 times the limiting molecular area of this amphiphile as determined from pressure-area curves on the film balance. The horizontal transfer shown in Figure 3 was therefore performed with the monolayer film in a highly expanded state under no measurable surface pressure. The integral of the charge under either the anodic or cathodic waves centered at ca. 1.05 V (corrected for double-layer charging) provides a measure of the quantity of the Ru complex transferred onto the electrode surface. For these five experiments, the area under the voltammetric wave corresponded to  $0.22 \pm 0.2 \mu\text{C}$ , representing 41% of the sample. The variability in this series is due in part to the irreproducibility of transferring 2  $\mu\text{L}$  of a 2.8  $\mu\text{M}$  solution of the ruthenium complex onto the deposition region of the minitrough.

The effect of varying the quantity of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  spread on the minitrough on the voltammetric signal measured at an ITO electrode is shown in Figure 4. The smallest quantities studied were 2 pmol. Waves at this level could easily be distinguished from background and could be integrated with a reproducibility of about  $\pm 20\%$ . As the quantity of the electroactive species deposited on the minitrough increased, the voltammetric oxidation and reduction waves displayed a corresponding increase. We avoided adding quantities of material to the minitrough above a certain maximum value determined by the size of the transfer region and the molecular area of the analyte at its collapse pressure. Clearly, if the deposited monolayer exceeds its collapse pressure during the compression of the monolayer into the transfer region, some of the amphiphile will collapse into multilayer aggregates that may not be completely electroactive when deposited on the electrode surface. Such a collapse of the monolayer could thereby result in a low voltammetric assay.

An alternative approach to electrochemical determination of an insoluble species involves its direct deposition on an electrode surface from a suitable solvent (e.g.,  $\text{HCCl}_3$ ), followed by solvent evaporation and immersion of the electrode in the aqueous supporting electrolyte. The transfer of a water-insoluble analyte by the HT deposition method has several advantages over the direct deposition of the sample on the electrode surface. Apart from the difficulty in keeping the sample solution only on the electrode surface while the solvent evaporates, the direct deposition results, in general, in lower electroactivity of the deposited sample. Figure 5 shows two voltammograms obtained by using the same ITO electrode coated with the same quantity of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  by the direct deposition and HT methods. As shown, the HT method gives a significantly larger voltammetric wave. In all cases tried, the direct-transfer method gave smaller voltammetric responses. Indeed with some water-insoluble species, such

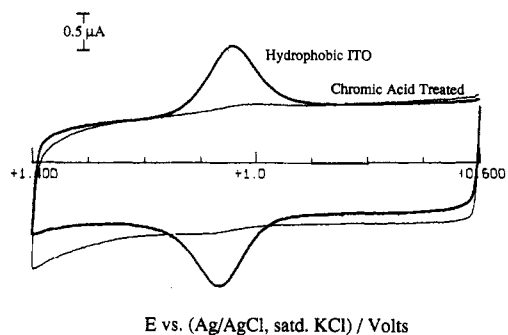


**Figure 5.** Comparison of the voltammetric signal between the HT and direct transfers of the same quantity of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  onto the same ITO electrode. Experimental conditions as in Figure 3.

as  $\text{Fe}(\text{DPP})^{3+}$ , whose HT voltammetry is discussed later, direct transfer to an electrode gave no voltammetric response. As the solvent evaporates on the electrode surface in the direct deposition, the analyte becomes concentrated in the remaining solvent and can deposit as large electroinactive crystals or aggregates on the electrode surface. In the HT method, the sample is spread initially over a large area so that the concentration of the amphiphile is only a small fraction of its close-packed monolayer coverage. This minimizes the possibility of aggregation.

Another potential advantage of the HT deposition method in analysis is that, once at the air/water interface, all sample components with a significant water solubility will dissolve into the subphase and will not be transferred to the electrode. Thus, the HT voltammetric method can be viewed as including an extremely efficient subphase extraction, which could be useful in an analysis scheme. The remaining water-insoluble species can then be compressed to an arbitrary extent on the trough, which is, in effect, a mechanical pre-concentration; this is not possible in the direct deposition scheme.

The average integral of the voltammetric waves shown in Figure 3 represents an amount of charge equivalent to about one-half of the species added to the trough, i.e., a 0.5 transfer ratio. This discrepancy in the amount of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  which was deposited on the minitrough with that observed electrochemically on the electrode surface stems primarily from a loss of material on the air/water interface during the horizontal transfer. Several characteristics of the electrode and parameters of the HT transfer were found to influence this transfer ratio. Hydrophobicity of the electrode surface was important for good transfer. The ITO-coated glass electrodes upon exposure to the laboratory environment became somewhat hydrophobic with water contact angles within the range 50–85°. This probably can be attributed to the adsorption of organics from the atmosphere on the ITO surface. To demonstrate the effect of a hydrophilic surface, some electrodes were cleaned by a brief exposure to a saturated solution of sodium dichromate in concentrated  $\text{H}_2\text{SO}_4$  (SCA) to give surfaces perfectly wetted by water. Figure 6 shows two voltammograms obtained by depositing 4.2 pmol of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  onto the minitrough and performing an HT transfer, as described above, for the same ITO electrode before and after the SCA treatment. Before the cleaning procedure, the voltammetric wave indicates a transfer ratio of ca. 0.5, which is consistent with the series of measurements shown in Figure 3. When the electrode was made hydrophilic, almost no electroactivity was seen, so that the transfer ratio was nearly zero. For electrodes not cleaned in SCA, the transfer ratios were observed to vary from 0.4 to 0.7. This variability in the transfer ratios of different ITO electrodes likely resulted from differences in their hydrophobicity, and particularly variability, across the electrode surfaces. For individual ITO



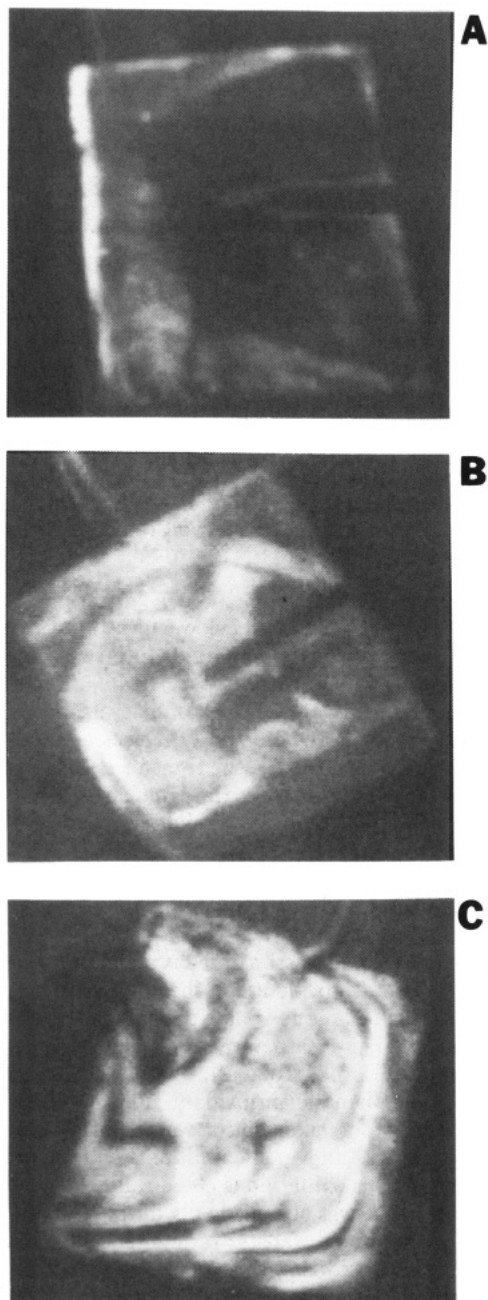
**Figure 6.** Dependence of the quantity of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  transferred on the hydrophobicity of the ITO electrode. These cyclic voltammograms were obtained after identical HT transfers of 4.2 pmol of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  using a hydrophilic ITO electrode (freshly cleaned in sulfochromic acid) and a hydrophobic ITO electrode. (See text.)

electrodes, there was typically a 15° scatter in contact angle measurements made at different locations on the electrode surface. Consistent with the importance of electrode surface hydrophobicity, the transfer ratios found with basal plane HOPG electrodes (0.8–1.05) were uniformly higher than those for ITO electrodes. The water contact angles for the freshly cleaved basal plane of the HOPG were  $71 \pm 1^\circ$  and were uniform across the electrode surface.

#### Electrogenerated Chemiluminescence (ECL) of Films.

As shown in earlier studies (6, 15), monolayer films of  $\text{Ru}(\text{bpy})_3^{2+}$ -based amphiphiles on ITO electrodes will show light emission when electrochemically oxidized in oxalate solutions. This ECL emission is useful in assessing the uniformity of the monolayer transfers. The ECL is emitted from electronically excited Ru(II) centers formed by the reaction of electrogenerated Ru(III) with  $\text{CO}_2^{\cdot-}$  (or Ru(I) produced by reaction of  $\text{CO}_2^{\cdot-}$  with Ru(II) produced from oxidation of oxalate ion (12, 16). Figure 7 shows three photographs of the ECL emission from electrodes coated with 4.2 pmol of the Ru complex on the minitrough. The electrode was imaged by means of a small mirror held beneath the electrode in the subphase. Figure 7A shows a photograph of the ECL emission from a SCA treated ITO electrode. The electrode surface shows little ECL emission, consistent with the small level of the voltammetric signal observed in this case. Most of the  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  complex that was transferred in this experiment was at the edge of the electrode. Because the transfers are made within the enclosed transfer region of the minitrough, the available area of the air/subphase interface decreases dramatically upon the immersion of the electrode. As the hydrophilic electrode is wetted by the subphase, the  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  monolayer is compressed rapidly into the remaining area with a concomitant increase in its surface pressure. The increased surface pressure leads to the deposition of the monolayer on the electrode edges by pushing the monolayer toward the air/subphase/electrode coexistence line.

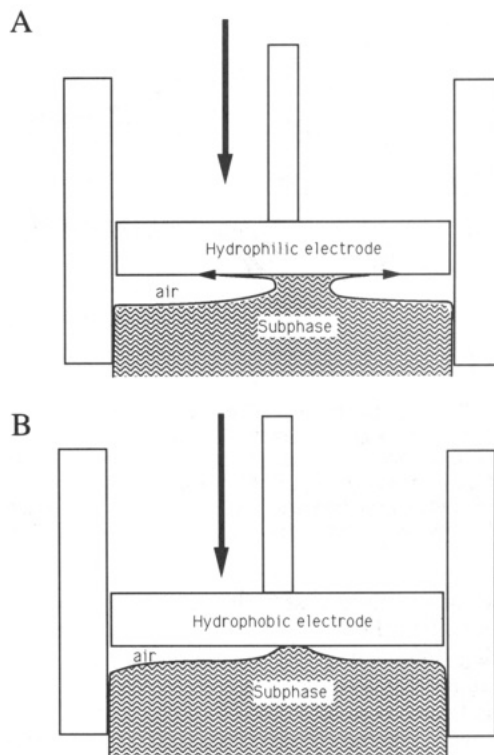
Parts B and C of Figure 7 show photographs of the ECL emission from an ITO electrode (not cleaned in SCA) and an HOPG electrode, respectively. For both of these hydrophobic electrodes, the ECL emission is substantially increased over that seen in Figure 7A, in keeping with the increased voltammetric signal seen from these electrodes. In the photograph of the ITO electrode shown in Figure 7B, several dark patches can be seen where little of the Ru complex was deposited. These dark patches occurred with some reproducibility at a given ITO electrode and probably represent less hydrophobic regions of the ITO surface. The nonuniformity in the ECL emission observed in Figure 7C at the HOPG electrode had no noticeable relationship to features on the HOPG surface and may reflect an initial nonuniform distribution of the ruthenium amphiphile in the transfer region (6). Similar



**Figure 7.** Electrogenerated chemiluminescence (ECL) photographs of 4.2 pmol of  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  transferred to ITO and HOPG electrodes. A and B show photographs of a sulfochromic acid cleaned ITO electrode and an uncleaned ITO electrode, respectively. The dark band seen in these photographs is the electroinactive Ti band used to make electrical connection to the conductive ITO coating. (See text.) C is a photograph of a HOPG electrode. During the 60-s photographic exposures, the electrodes were held at 1.2 V vs Ag/AgCl/saturated KCl. The subphase was 0.1 M  $\text{NaClO}_4$ -0.1 M  $\text{Na}_2\text{C}_2\text{O}_4$ .

nonuniformities in ECL emission were found with self-assembled films of  $\text{Ru}(\text{bpy})_3^{2+}$ -based amphiphiles whose long hydrocarbon chain was terminated by a sulfhydryl group on ITO electrodes (15). A more detailed account of the use of ECL emission for the imaging of Langmuir monolayers will be the subject of a separate report.

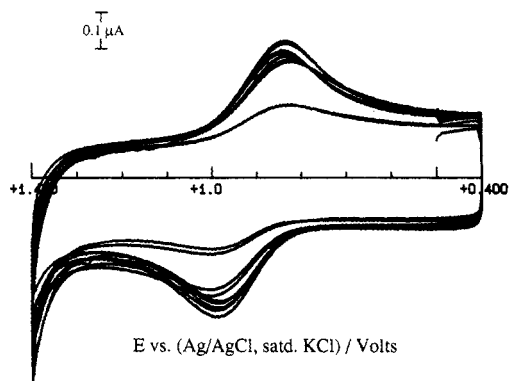
In addition to the hydrophobicity of the electrode, the alignment of the electrode to the air/subphase interface and the dipping rate (speed at which the electrode approaches the monolayer) played important roles in determining the transfer ratios. When the ITO electrode is brought into contact with the monolayer at an angle, consistently smaller transfer ratios were measured. For experiments in which the electrode was



**Figure 8.** Schematic representation of the HT transfer. A and B show a hydrophilic and a hydrophobic electrode, respectively, at the point of first contact of the electrode surface with the subphase in the transfer region of the minitrough.

aligned parallel with the subphase surface, there was a larger amount of scatter in the transfer ratios at slow dipping rates, i.e., when the dipping rate was decreased from 10 to 0.05 mm/s.

These observations on the effect of surface hydrophobicity and transfer conditions on the transfer ratio can be rationalized by considering how the amphiphiles at the air/water interface are transferred by the horizontal touching method. Figure 8 shows a schematic representation of the monolayer transfer scheme after the point of first contact of the electrode surface with the subphase. As the monolayer-covered surface makes contact with the electrode surface at an initial site, the contact angle of the subphase on the electrode will initially be at  $180^\circ$  and approach a value consistent with the local hydrophobicity of the electrode surface at that point. If this equilibration of the contact angle (caused by the wetting of the electrode surface by the subphase) causes the contact angle to decrease below  $90^\circ$ , the transfer of the amphiphiles on the subphase will be hindered. In this case (shown in Figure 8A), the air/subphase/electrode line will advance away from the amphiphiles, creating new subphase/air area. Because the monolayer is under low or zero surface pressure in these transfers, the monolayer will not expand to fill this newly created air/subphase interface so that the area near the air/subphase/electrode line will be depleted in the amphiphile. At the same time, the total subphase/air area decreases due to the escape of air around the sides of the electrode. This results in a rapid compression of the amphiphile, leading eventually to the collapse of the monolayer into multilayer aggregates concentrated around the edges of the electrode. If the dynamic contact angle remains greater than  $90^\circ$  as the electrode is pushed through the air/subphase interface (as shown in Figure 8B), the area of the air/subphase interface will decrease at the air/subphase/electrode line, trapping the amphiphiles at the electrode surface. The planarity of the electrode and the dipping speed become important variables in the transfer scheme because they determine how quickly

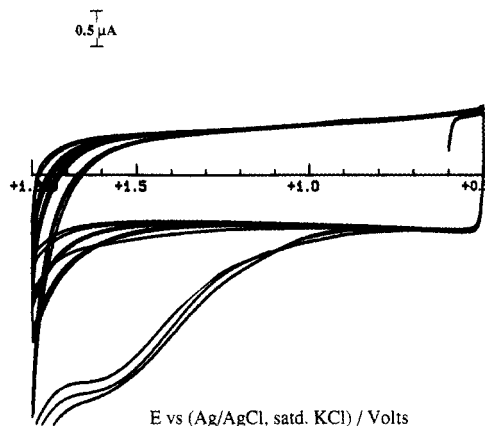


**Figure 9.** Series of cyclic voltammograms obtained immediately after the horizontal touching transfer of 12.7 pmol of  $\text{Fe}(\text{DPP})_3^{2+}$  using a single ITO electrode. The scan rate for these experiments was 0.1 V/s, and the subphase was 1.5 M  $\text{NaClO}_4$ . The four closely spaced voltammograms showed an area of  $1.0 \mu\text{C}$  with a standard deviation of  $0.1 \mu\text{C}$ . The anomalous small voltammogram is about 50% of this value.

the electrode surface passes through the air/subphase interface, defining the time allowed for the equilibration of the advancing wetting angle of the subphase on the electrode.

The above discussion on the mechanics of the horizontal touching transfers points to the complexity involved in this trace analysis scheme. Many of the difficulties in developing the HT transfer analysis stem from apparatus design and materials limitations. In performing the HT transfers, we have striven to follow the same experimental protocol as closely as possible. Even so, in a series of replicate transfers, we occasionally observed an individual transfer with an anomalously low transfer ratio (often close to zero). Correlating these low transfers with a particular change in one of the monolayer spreading parameters, the electrode alignment, or the dipping speed was not possible. This sensitivity of the transfer ratio to small changes in experimental parameters is perhaps not so surprising given the dynamic nature of the HT transfer discussed above. Choosing electrodes that have the required degree of hydrophobicity, planarity, electron transfer kinetics, and background characteristics is perhaps the most difficult challenge. We have found ITO and HOPG particularly well suited for these studies because of their hydrophobicity, wide potential windows, and low background currents.

**Insoluble Nonamphiphilic Analytes.** While the  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  complex is a very useful model analyte for the study and optimization of the transfer conditions, it is an amphiphile that forms organized layers. We wanted to address the application of this HT transfer technique as a general analysis tool for other insoluble electroactive species that do not self-assemble. To this end we have investigated several transition-metal complexes and organic compounds. We found that the major limitation for determination of small quantities is the need for high water insolubility. Because the samples are spread on the surface of the trough at surface concentrations below 2% of their close-packed values, dissolution into the aqueous subphase can be a serious problem. Thus, redox active compounds, such as decamethylferrocene, 2,3,4,5-tetramethylbenzoquinone, and the tris(2,2'-bipyridyl) complexes of Fe and Ru dissolved too rapidly into the aqueous subphase to be detected. Some improvement in the stability of these molecules on the air/water interface was noted when the concentration of supporting electrolyte ( $\text{NaClO}_4$ ) was increased from 0.1 to 2.0 M, but this increased salt concentration was not sufficient to allow any quantitation of these compounds. When the more hydrophobic  $\text{Ru}(\text{DPP})_3^{2+}$ ,  $\text{Fe}(\text{DPP})_3^{2+}$ , and  $\text{Ru}(\text{DPB})_3^{2+}$  complexes were tested, acceptable voltammograms could be obtained. Some dissolution of these diphenyl-dipyridyl and phenanthroline complexes into the

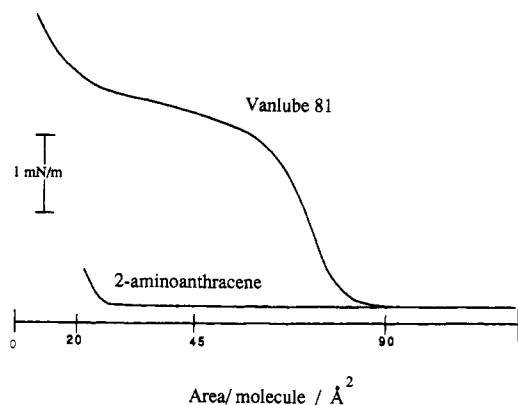


**Figure 10.** Series of cyclic voltammograms obtained after the HT transfer of 15 pmol of IR-1076 onto an ITO electrode. Three complete voltammetric cycles are shown for three replicated HT transfers. Experimental conditions as in Figure 3. Variation of integrals and peak currents are about  $\pm 10\%$ .

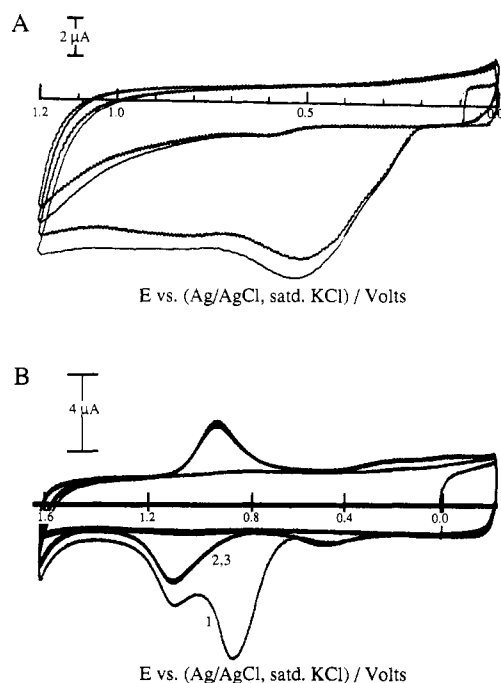
subphase was observed when the concentration of the deposition solution was decreased below  $5 \mu\text{M}$ . This is consistent with aggregation of these compounds into two-dimensional crystallites, which slows the dissolution rate of the complex. Figure 9 shows a series of cyclic voltammograms for 12.7 pmol of  $\text{Fe}(\text{DPP})_3^{2+}$  transferred onto an ITO electrode. Except for one anomalously low transfer, the transfer ratios were within the 0.4–0.6 range measured for the same electrode using the  $\text{Ru}(\text{bpy})_2(\text{bpy}-\text{C}_{19})^{2+}$  complex. Similar results were obtained using the Ru complexes. These symmetrical complexes do not have the well-defined hydrophilic head group and hydrophobic tail group of a typical amphiphile. Yet, they could be determined readily by this technique.

We also investigated a series of redox active, water-insoluble organic compounds to identify other compounds that are amenable to this technique. Some phenols can be studied by this technique. Figure 10 shows a set of three voltammograms obtained at an ITO electrode after transferring 15 pmol of the amphiphilic phenolic antioxidant IR-1076. The voltammograms display a broad oxidation wave starting at about 0.9 V and continuing to the anodic limit of the ITO electrode at 1.8 V. Because this oxidation is chemically irreversible, the voltammetric wave was observed only during the first anodic sweep. If we assume a transfer ratio of 0.5, the quantity of charge passed in oxidizing this phenol is consistent with a two-electron oxidation, as expected for this compound (17). The reproducibility of the voltammograms from one HT transfer can be gauged by the similarity of the three voltammetric determinations shown in Figure 10. Other phenolic antioxidants, IR-1010, BHT, and BHEB, gave very similar voltammetric responses. However, BHT and BHEB dissolved too rapidly into the subphase to obtain good reproducibility in their determinations.

Aromatic amines are another class of redox active compounds that can be measured and studied by this HT voltammetric analysis technique. While the analyte must not dissolve into the subphase within the time required to spread, compress, and voltammetrically analyze a sample aliquot, the analyte need not withstand any surface pressure. This point is demonstrated in Figures 11 and 12. Figure 11 is a pressure-area diagram for two aromatic amines that were investigated as possible analytes by the HT approach. Vanlube 81 was observed to collapse at a surface pressure of ca. 2 mN/m and 2-aminoanthracene collapsed at pressures well below 1 mN/m. These pressures are much lower than those typically used to transfer monolayer films from Langmuir troughs by the vertical dipping method (7). Figure 12 shows the voltammograms for these two aromatic amines transferred



**Figure 11.** Pressure–area isotherms of Vanlube 81 and 2-aminoanthracene. Subphase, 0.1 M NaClO<sub>4</sub>.



**Figure 12.** Cyclic voltammograms of the aromatic amines. A shows two voltammograms, each obtained after the horizontal transfer of a monolayer of 2-aminoanthracene onto an ITO electrode. B shows three complete voltammetric cycles obtained after a single horizontal transfer of a monolayer of Vanlube 81 onto an ITO electrode. Experimental conditions as in Figure 3.

by the HT method on the commercial LB trough. Figure 12A shows cyclic voltammograms for two independent horizontal touching transfers of 2-aminoanthracene. Figure 12B contains a single voltammetric experiment for a transferred monolayer of Vanlube 81. The oxidations of these amines are chemically irreversible and, in the case of the voltammogram of Vanlube 81, mechanistically complex. These monolayers were transferred at the onset of pressure and therefore were nearly close-packed before their transfer to the electrode surface. In the voltammogram of Vanlube 81, three distinct voltammetric waves are seen, a small prewave at 0.4 V, a large irreversible oxidation with a peak at 0.85 V, and a reversible product wave centered at 0.95 V. Note that Vanlube 81 shows a single one-electron Nernstian anodic wave in acetonitrile (18), so the complexity in the aqueous environment is probably connected with deprotonation of the initially formed radical cation. Other substituted diphenylamines have been shown to couple upon oxidation to give dimeric products, e.g., dihydrophenazine derivatives (19). Dihydrophenazines can be further oxidized in two-electron processes (20). The charge passed in the first positive potential scan is consistent with

an overall 3 electrons per aromatic amine oxidation.

The complexity of the oxidation of Vanlube 81 suggests another use of this HT transfer voltammetry: the study of the redox reactivity of water-insoluble species in aqueous electrolytes. Such studies are difficult for many redox active organic species because of their poor solubility in water. The HT transfer method allows one to trap a reproducible quantity of a water-insoluble species at the electrode surface where its voltammetric properties can be studied quantitatively. Because the number of moles of the redox center are determined by the transfer conditions, one can obtain estimates of the number of electrons and the potentials for oxidation and reduction waves.

## CONCLUSIONS

The HT voltammetric analysis method is demonstrated for the trace determination of water-insoluble electroactive species. This method can be viewed as an extremely efficient subphase extraction followed by a Coulometric determination of the sample. The monolayer need not be under any surface pressure for the HT deposition method, and possible analytes for this technique need only be sufficiently insoluble in the subphase. The ability of the analyte to withstand significant surface pressures without collapsing is not required. This is important because it means that a wider range of redox active species (other than self-assembling amphiphiles) should be measurable by HT voltammetric analysis. The detection limit for this technique is governed by the size and voltammetric background of the electrode. With relatively large electrodes (0.2 cm<sup>2</sup>), the detection limit for the model redox amphiphile, Ru(bpy)<sub>2</sub>(bpy-C<sub>19</sub>)<sup>2+</sup>, was below 1 pmol (i.e., about one-fifth the amount shown in Figure 3). Decreasing the size of the electrode by a factor of 10–100 should be accomplished with minor modifications of the present apparatus. Such electrodes should show a decrease in charging current with a corresponding decrease in the detection limit to the attomole range. Indeed, if microelectrodes such as those used in bulk voltammetric methods (21), with diameters ca. 10 μm and areas ca. 10<sup>-6</sup> cm<sup>2</sup> and small double layer capacitances, could be employed in this method, which involves a considerable technical challenge, quantities in the 4 × 10<sup>-18</sup> mol (2.4 million molecules) range might be possible.

Although this is a trace analysis, we did not find it necessary to take extreme precautions (e.g., use of a laminar flow hood) to prevent contamination of the subphase surface. The extent of contamination depends on the area of the subphase/air interface and the time elapsed since that area was cleaned by sweeping the surface with the movable barrier and aspirating any contaminants. The minitrough area is only 4 cm<sup>2</sup> and the time elapsed between sweeping, sample introduction, and HT analysis was usually less than 30 s; contamination by electroactive materials or species that might block the electrode surface under these conditions was not significant.

Because the efficiency of the HT transfer method is strongly dependent on the mechanical and geometric parameters of the transfer, it is essential to reproduce, as closely as possible, the experimental protocol from one determination to the next, if one is to have acceptable reproducibility in replicate determinations. Complete automation of the transfer measurement would likely improve the reproducibility of individual transfers. In addition, automation of the sample handling would open the possibility of a new and potentially powerful trace analysis scheme. In particular, one could exploit the initial subphase extraction to add selectivity to the analysis. Note that choice of the subphase used in the minitrough is controlled by the experimenter. By varying the time between sample deposition and the HT transfer, or by flowing differing subphase solutions (e.g., with differing composition of complexing ions, organic solvents or detergents), one could



obtain a series of determinations as a function of the extraction parameter (time or subphase composition). This determination would thereby generate a two-dimensional signal, perhaps allowing the determination and identification of several species within a sample on the basis of the differing solubilities of components in the subphase. The HT voltammetric method might also be useful following extraction of samples (e.g., polymers) with low polarity solvents, for example, in the determination of antioxidants.

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## Electrocatalysis and Amperometric Detection Using an Electrode Made of Copper Oxide and Carbon Paste

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**Amperometric flow measurements were made at +0.55 V vs Ag/AgCl in 0.1 M NaOH background electrolyte with Cu<sub>2</sub>O/C-paste electrodes. Stable and sensitive electrocatalytic responses for oxidations of carbohydrates, amino acids, aliphatic diols, simple alcohols, amines, and alkyl polyethoxy alcohol detergents were observed. Linear responses at low concentrations yielded detection limits in the picomole range. Typical relative standard deviations were 1.2%. Linear plots of 1/I<sub>p</sub> vs 1/C<sub>analyte</sub> indicated Langmuir-type adsorption of analytes on the electrode surface. It was concluded that the active sites could be represented as CuO\*OH. CuO, CuCl, and Cu<sub>2</sub>O were examined as starting materials, and the last was found to be the best. A proposed mechanism for oxidations of these analytes required both hydroxyl radical active sites (CuO\*OH) and neighboring adsorbed analyte sites. The rate-determining step involved the formation of a bridged cyclic intermediate and the abstraction of a hydrogen from the  $\alpha$ -carbon by the adsorbed hydroxyl radical attack.**

#### INTRODUCTION

The detection of aliphatic compounds in flow injection analysis (FIA) and high-performance liquid chromatography (HPLC) has been of great interest to analytical chemists for the past decade. Aliphatic compounds do not have strong chromophores in the UV-visible range. Low-wavelength UV and refractive index techniques are often limited in specificity

and sensitivity. The development of electrode materials for direct amperometric detection of aliphatic compounds is a growing area of electroanalytical chemistry. The commonly used carbon amperometric electrodes, however, exhibit no response for aliphatic compounds. Metallic electrodes have thus been utilized in place of carbon electrodes. At present, gold (1-6), platinum (7-9), nickel (10-16), and copper (17-19) have been reported useful for detection of aliphatic compounds. The inherent instability and nonreactivity of noble metallic electrodes, such as gold and platinum, have been overcome by pulse-amperometric detection (PAD), which combines cleaning and activation steps with detection (20, 21). For active metallic electrodes, such as nickel and copper, the electrode processes in alkaline solutions involve the electrochemical formation of metal oxide or hydroxide films of higher oxidative states, e.g., NiO(OH) and CuO(OH), which have been proposed to behave as the redox mediators associated with analyte oxidations. In addition, chemically modified electrodes (CMEs) (22), containing surface-bound inorganic redox species have been described, which demonstrate unusual catalytic stability and reactivity for aliphatic compounds. For example, copper deposits on glassy-carbon (23-25) and RuO<sub>2</sub>-mixed carbon paste (26) greatly enhanced the amperometric detection of carbohydrates and other related compounds at the picomole level under constant applied potentials.

Continuing investigations in this laboratory involving transition-metal oxide electrode responses suggested that copper oxide particles in a carbon-paste matrix warranted