

Electrocatalysis of Hemoglobin at C₇₀/DDAB Films in an Aqueous Solution

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The electrochemistry of films made from cationic surfactant DDAB containing C₇₀ on a glassy carbon electrode was investigated in an aqueous solution, two couples of reduction/reoxidation peaks were found in the potential range from 0 to −1.0 V. Electrochemically stable fullerene modified electrodes were produced. The cast films of C₇₀/DDAB were also characterized by SEM, XPS, and UV–vis. Furthermore, the electrocatalysis of hemoglobin at C₇₀/DDAB films was studied.

1. Introduction

Electrochemical studies of fullerene films have been very rich in nonaqueous solution;^{1–8} however, due to the insoluble nature of fullerenes in water, the electrochemistry of fullerene films has been very limited in an aqueous solution,^{9–12} and that both the reduction and the oxidation were completely irreversible. Recent investigations have revealed that the fullerenes and fullerene derivatives exhibit an interesting range of biological activities, especially promising in the fields of photodynamic therapy, HIV treatments, neuroprotection, and apoptosis.^{13–17} This has evoked research interests in fullerenes in an aqueous solution. To solve the problem of solubility for fullerenes in water, the dissolution of fullerenes in water may be accomplished via derivatization,^{18–20} cyclodextrin complex formation,^{21–28} or surfactant solutions containing micelles or liposomes,^{29–31} etc. In recent years, our research group has investigated electrochemistry of supramolecular complex films of fullerenes with cyclodextrins and calixarenes in solution containing water,^{32–38} and a reversible electroreduction wave was obtained. At present, our interest is focused on the electrochemistry of films formed of fullerenes and surfactants in an aqueous solution in order to produce electrochemically stable fullerene modified electrodes for further application of biosensors. Although Nakashima and co-workers have already published several papers on the structure and electrochemistry of C₆₀ embedded in cationic surfactant lipids, such as tetraoctylphosphonium bromide, tetraoctylammonium bromide, and ditetradecyldimethylammonium poly (styrene sulfonate), etc., on electrodes in an aqueous solution,^{39–42} few reported the electrocatalysis of biomacromolecules at the modified electrodes.

Cationic surfactant didodecyldimethylammonium bromide (DDAB) can be formed multiple bilayer films resembling those of lipid membranes in living cells by casting its solution onto electrodes, which has potential application include membranes with controllable permeability and kinetic control of electrochemical reactions. It is reported that the electron-transfer rate of MbFe^{III}/Fe^{II} (Mb represents myoglobin) at PG electrodes is about 1000 times faster in DDAB films than that in solutions at InSnO₂ electrodes.⁴³

In this paper, electrochemistry of films made from cationic surfactant DDAB containing C₇₀ on a glassy carbon electrode

was investigated in an aqueous solution, two couples of reduction/reoxidation peaks were found in the potential range from 0 to −1.0 V. To explore the possible biological and analytical application, electrocatalysis of hemoglobin at C₇₀/DDAB films was also studied.

2. Experimental Section

2.1. Materials. Didodecyldimethylammonium bromide (DDAB) was 99+% from Eastman Kodak. C₇₀ was prepared in our laboratory. Its purity was 99%, as determined by mass spectrometry. Bovine hemoglobin (Hb) was from Sigma. Other reagents used were of analytical grade. Water was triply distilled from an all-quartz still. Experiments were carried out at room temperature, 25 ± 2 °C.

2.2. Electrochemical Measurements. The electrochemical cell was a single-compartment glass cell with a Pt counter electrode and a KCl saturated calomel reference electrode located near the working electrode. Prior to electrochemical experiments, the solutions were routinely deaerated by purging with high purity nitrogen. Cyclic voltammetry (CV) and Osteryoung square wave voltammetry (SWV) experiments were performed with a PAR (Princeton Applied Research) Model 273 Potentiostat/Galvanostat.

2.3. Electrodes and Preparation. A glassy carbon (GC) disk ($d = 4$ mm) was used as the working electrode. Before use in electrochemical experiments and also before modification, the surface of the GC electrode was polished with a fine emery paper and chamois leather soaked with a Al₂O₃ slurry, then rinsed thoroughly with water in an ultrasonic bath. The films were prepared by dropping a few microliters of 5×10^{-4} mol L^{−1} C₇₀ in 0.01 mol L^{−1} DDAB toluene solution onto a GC disk and air-dried. The experiments showed that there was an optimal CV response for a certain amount of toluene solution of mixed C₇₀ and DDAB. Below the optimal value the currents became insignificant, and above the value the resistance of films increased and the peak shape became worse. Thus 15 μ L toluene solution of mixed 5×10^{-4} mol L^{−1} C₇₀ and 0.01 mol L^{−1} DDAB dripped on the surface of the GC electrode was chosen.

Scanning electron microscopy (SEM) was done with a KYKY2000 SEM instrument in the secondary electron emission mode. X-ray photoelectron spectroscopy (XPS) was done with a PHI-5300 ESCA electron spectrometer in Tsinghua University. The UV–vis spectra were recorded by means of a Shimadzu Model UV-3100 spectrophotometer.

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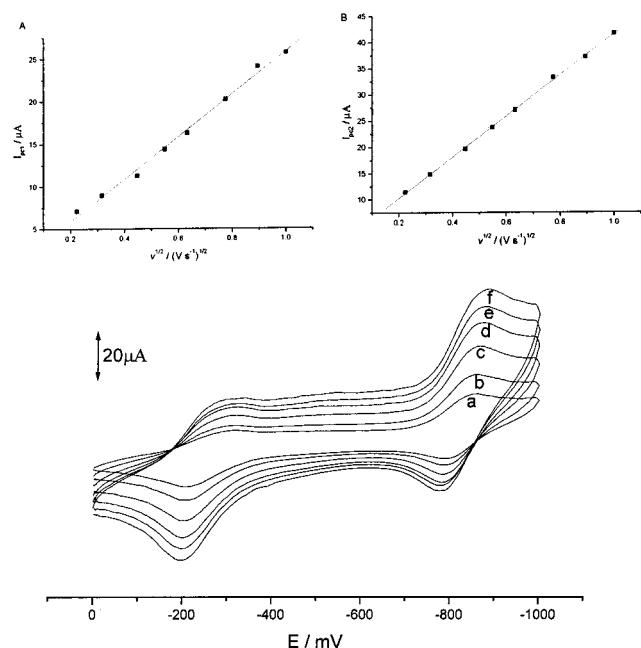


Figure 1. Cyclic voltammograms for the C_{70} /DDAB films at different scan rate in 0.40 mol L^{-1} KCl, scan rate, (a) 0.1 V s^{-1} , (b) 0.2 V s^{-1} , (c) 0.4 V s^{-1} , (d) 0.6 V s^{-1} , (e) 0.8 V s^{-1} , (f) 1 V s^{-1} .

3. Results and Discussion

The cast films of C_{70} /DDAB on the GC electrode were tested in various supporting electrolytes including KCl, NaClO_4 , $\text{NH}_3\text{--NH}_4\text{Cl}$ (pH9.5), $\text{KH}_2\text{PO}_4\text{--Na}_2\text{HPO}_4$ (pH6.9), HCl--KCl (pH2.0), and Bu_4NBr by cyclic voltammetry. KCl was found to be the best choice, because excellent stability and the best electrochemical response for the C_{70} /DDAB films are obtained.

The cyclic voltammograms for the C_{70} /DDAB films at different scan rate between 0 and -1.0 V in 0.4 mol L^{-1} KCl solution are shown in Figure 1. The curves exhibit two pairs of redox peaks with $E_{\text{pc}1} = -0.322 \text{ V}$, $E_{\text{pa}1} = -0.214 \text{ V}$, $E_{\text{pc}2} = -0.858 \text{ V}$, and $E_{\text{pa}2} = -0.782 \text{ V}$ (curve a); furthermore, the cathodic currents for two redox couples had a linear relationship with the square root of the scan rate in the range of $0.05\text{--}1.0 \text{ V s}^{-1}$, respectively (Figure 1A,B), indicating that the voltammograms are diffusion-controlled quasi-reversible waves and two redox couples correspond to C_{70}/C_{70}^- and C_{70}^-/C_{70}^{2-} , respectively. Some difference between the initial scans and subsequent scans was also observed. The current response increased a little and the peak shape was well-defined with increasing CV cycles, and then became constant after about 10 CV cycles. These indicate that there might be a structural rearrangement accompanying the release of the solvent that was entrapped during evaporation process and ion pair formation between the cation and C_{70} anion upon reduction in the films and the exchange of a small amount of anions into the films. SEM analyses also attest it. C_{70} , DDAB, and C_{70} /DDAB films on GC electrodes were observed by SEM, respectively (Figure 2). The DDAB films (Figure 2B) had a porous appearance; nevertheless, before electrochemical scan, the C_{70} /DDAB films (Figure 2C) showed no porous structure because the C_{70} crystals dispersed in the holes of DDAB. After the electrochemical scan, that is, C_{70} /DDAB films soaked in 0.40 mol L^{-1} KCl solution for three linear sweeps and washed with water, the SEM (Figure 2D) appeared different in comparison with that before the electrochemical scan, and showed a uniform appearance in size and shape of grains, which confirmed a structural rearrangement.

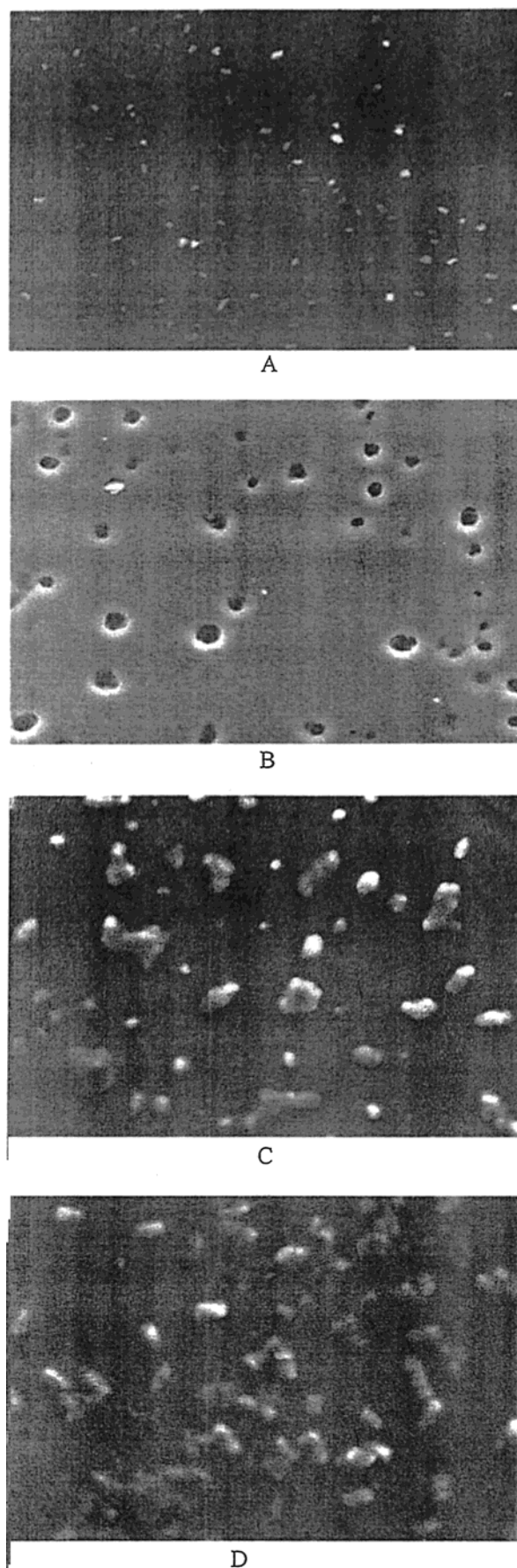


Figure 2. SEM of (A) C_{70} films, $300\times$; (B) DDAB films, $300\times$; (C) C_{70} /DDAB films before electrochemical scan, $300\times$; (D) C_{70} /DDAB films after a three-time linear sweep, $300\times$.

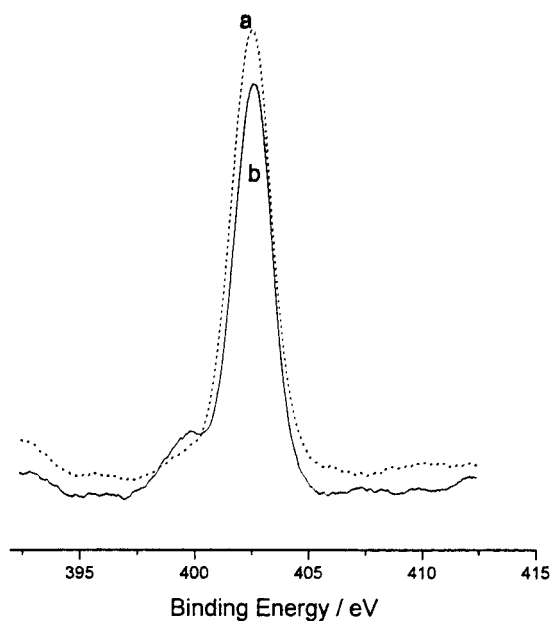


Figure 3. N_{1s} spectra of the C_{70} /DDAB films (a) after five cycles of voltammetry and (b) after a five-time linear sweep.

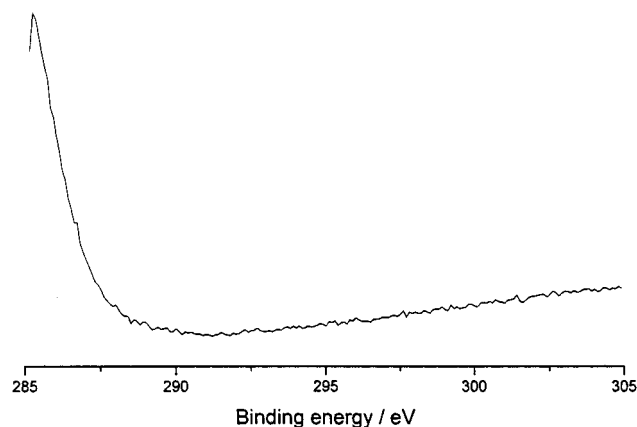


Figure 4. K_{2p} spectrum of the C_{70} /DDAB films after a five-time linear sweep.

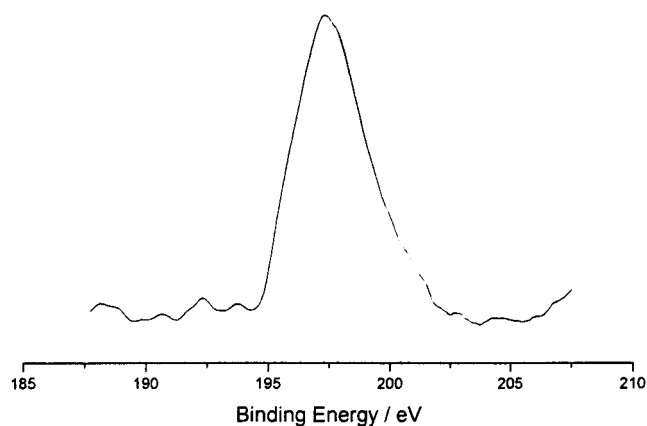


Figure 5. Cl_{2p} spectrum of the C_{70} /DDAB films after a five-time linear sweep.

To verify whether cations incorporated into the C_{70} /DDAB films during the reduction of C_{70} , XPS spectra were acquired. After each cyclic scanning in 0.40 mol L^{-1} KCl solution between 0 and -1.0 V , the C_{70} /DDAB film electrode was taken out and washed with water, then dried in air. XPS spectrum in Figure 3 shows the presence of elemental N in the films (binding

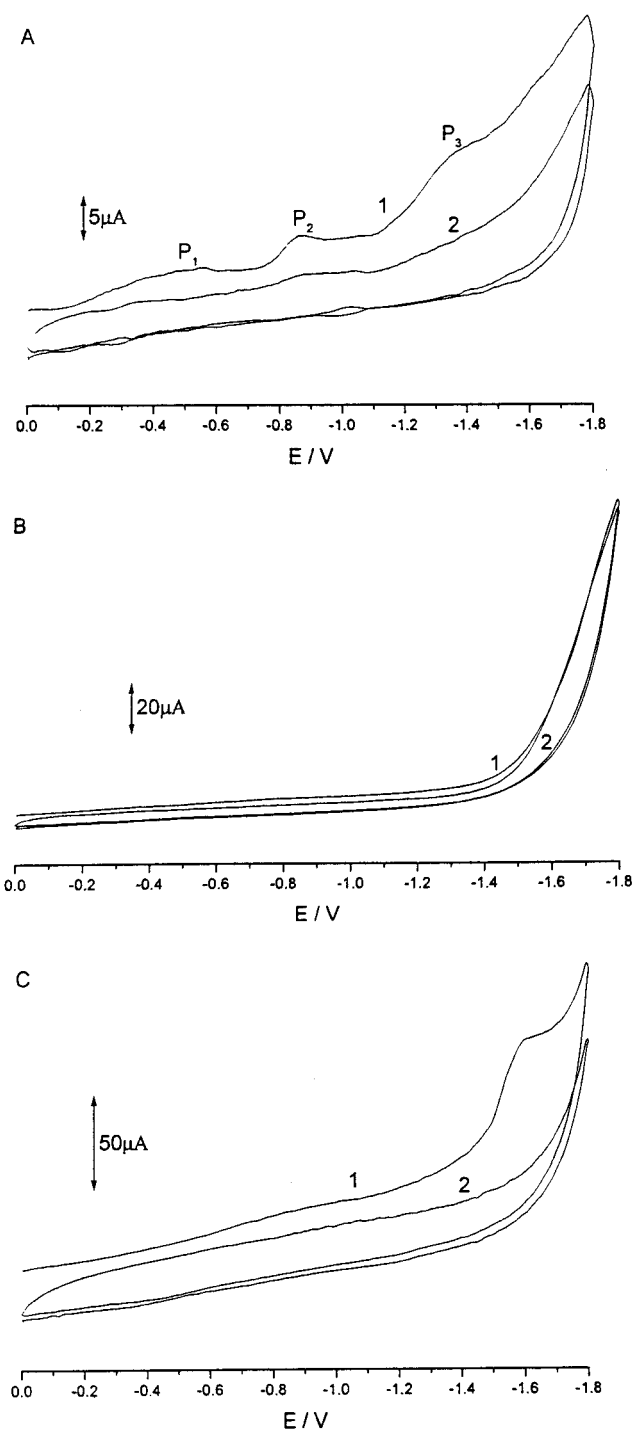


Figure 6. Continuous cyclic voltammograms of (A) C_{70} /DDAB, (B) DDAB, and (C) C_{70} films in 0.40 mol L^{-1} KCl. Scan rate: 0.1 V s^{-1} .

energy is 402.4 eV , as expected N presence of DDAB). After each linear sweep, the XPS spectrum (Figure 4) shows that there is not any elemental K in the C_{70} /DDAB films (K_{2p} peak with binding energy between 291 and 295 eV), and the N_{1s} peak with the binding energy of 402.4 eV (curve a in Figure 3) splits into two peaks at 399.8 and 402.4 eV upon reduction (curve b in Figure 3), which can be assigned to the elemental N in ion pair $C_{70}^{-}DDA^{+}$ or $C_{70}^{2-}(DDA)^{2+}$ (DDA^{+} represents didodecyldimethylammonium ion) and DDAB, respectively. These indicate that didodecyldimethylammonium ion in DDAB acts as the counteranion. Furthermore, the XPS spectrum shows that elemental Cl exists in the C_{70} /DDAB films (Cl_{2p} peak with

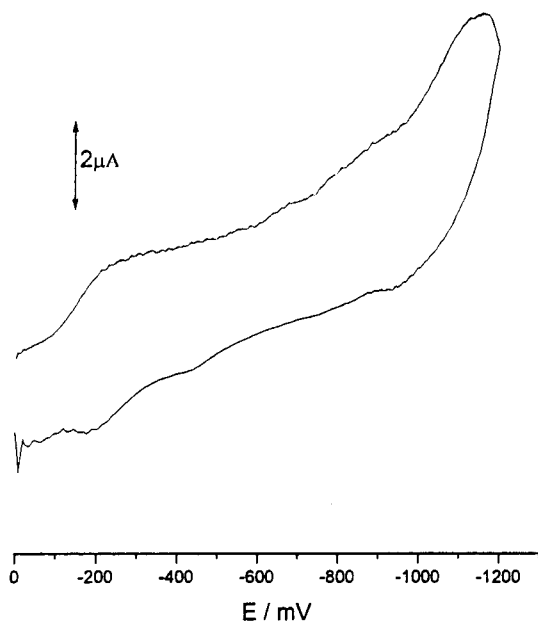


Figure 7. Cyclic voltammogram of DDAB films in 0.40 mol L⁻¹ KCl containing 1.0×10^{-4} mol L⁻¹ hemoglobin. Scan rate: 0.1 V s⁻¹.

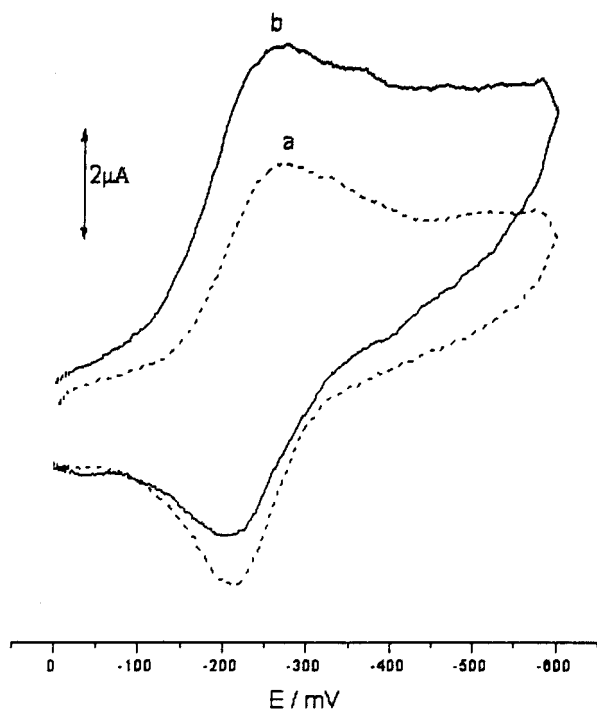


Figure 8. Cyclic voltammograms of C₇₀/DDAB films in the absence and presence of hemoglobin in 0.40 mol L⁻¹ KCl. Scan rate: 0.1 V s⁻¹. (a) C₇₀/DDAB CME without hemoglobin; (b) C₇₀/DDAB CME in 8.0×10^{-5} mol L⁻¹ hemoglobin.

binding energy is 197.5 eV, the concentration is 33%), as shown in Figure 5, attesting the exchange of anions into films.

The stability of the C₇₀/DDAB films was tested, more than 50 complete CV cycles were performed, no significant decrease in cathodic and anodic peak currents was observed. A series of six C₇₀/DDAB films prepared in the same manner and immersed in 0.40 mol L⁻¹ KCl solution, with a scan rate of 100 mV s⁻¹, gave an average cathodic peak current i_{pc1} of 9.18 μ A with an RSD of 7.7% and i_{pc2} of 13.6 μ A with an RSD of 6.9%, indicating films of surfactant DDAB in the liquid-phase incorporating C₇₀ crystals yield stable electroactive films on the GC electrodes.

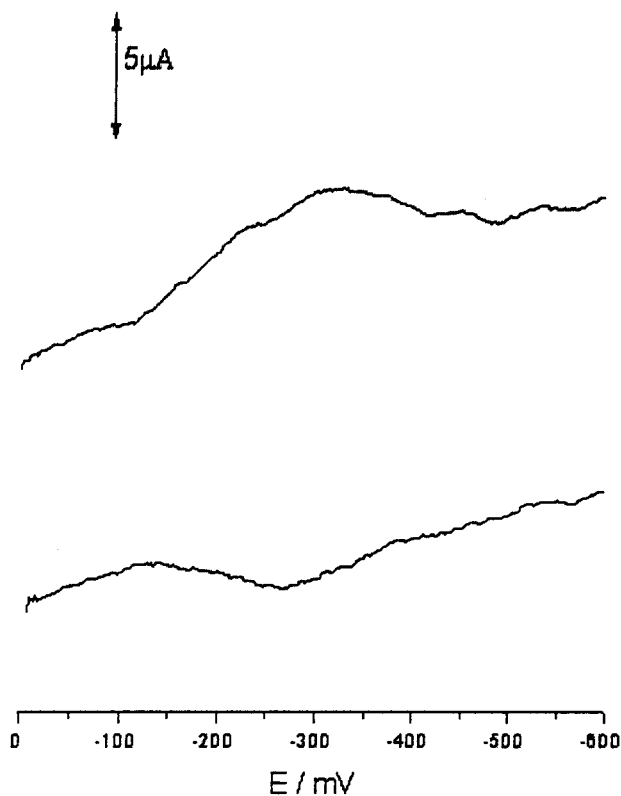


Figure 9. SWV curves of DDAB films in 0.4 mol L⁻¹ KCl containing 1.0×10^{-4} mol L⁻¹ hemoglobin. Frequency: 150 Hz. Pulse height: 25 mV.

When the potential was scanned between 0 and -1.80 V, the C₇₀/DDAB films showed three electroreduction peaks in 0.40 mol L⁻¹ KCl solution, the third electroreduction peak positioned at $E_{pc3} = -1.38$ V (curve a in Figure 6A). But on the second scan, almost all peaks disappeared. Maybe the third electron transfer reaction was not chemically reversible. Nevertheless, the C₇₀ films showed an irreversible electroreduction peak, $E_{pc} = -1.60$ V (curve a in Figure 6C), and the DDAB films showed no peak under the same condition (Figure 6B). These indicated that electrochemical response of C₇₀ embedded in cast films of DDAB on GC electrode was different from that of the pristine C₇₀ films in aqueous solution. Existence of surfactant DDAB on the electrode must play a role in changing the micro-environment on the surface of the electrodes and enhancing the electron-transfer rate for C₇₀, though the exact influence remains unclear.

It was reported that C₆₀ incorporated in the lipid bilayer could be as an excellent electron-transfer mediator.⁴⁴ To explore practical application of stable electroactive C₇₀/DDAB films on the GC electrodes, voltammetric behavior of hemoglobin at the C₇₀/DDAB films was also investigated.

The experiments show that hemoglobin has two pairs of redox peaks on the DDAB films between 0 and -1.2 V in 0.4 mol L⁻¹ KCl solution (Figure 7). On the basis of Rusling's work,⁴³ myoglobin can be imbedded in the surfactant DDAB bilayers, and Mb-DDAB films show two pairs of cathodic-anodic peaks representing the MbFe^{III}/Fe^{II} and MbFe^{II}/Fe^I couples. So two pairs of redox peaks in Figure 5 represents the HbFe^{III}/Fe^{II} and HbFe^{II}/Fe^I couples. In contrast, CVs obtained with C₇₀/DDAB films between 0 and -0.6 V in 0.4 mol L⁻¹ KCl with and without added hemoglobin are shown in Figure 8. Although redox peak currents (curve a) decrease a little in comparison with that in Figure 1(curve a), the reversibility of redox peaks is improved ($E_{pc1} = -0.272$ V, $E_{pa1} = -0.216$ V). Furthermore,

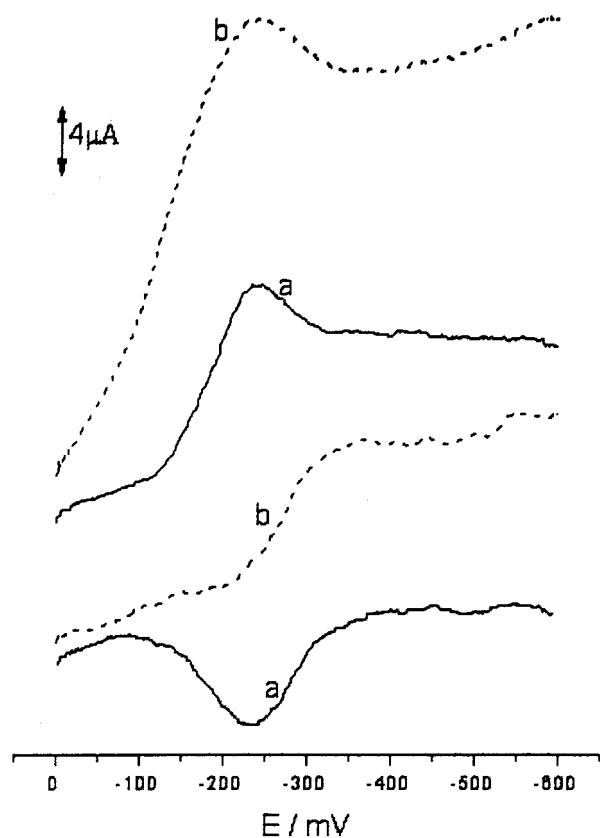
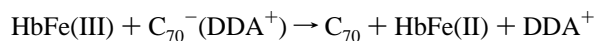
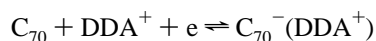


Figure 10. SWV curves of C_{70} /DDAB films in the absence and presence of hemoglobin, in 0.40 mol L^{-1} KCl. Frequency: 150 Hz. Pulse height: 25 mV. (a) C_{70} /DDAB films without hemoglobin; (b) C_{70} /DDAB films in $2.0 \times 10^{-4} \text{ mol L}^{-1}$ hemoglobin.

the voltammograms showed an increase in the height of the reduction current and a decrease in the oxidation current for C_{70} /DDAB films after added hemoglobin, with no change in the potentials of the two electrode processes, indicating C_{70} -DDA⁺ reacted with hemoglobin. The catalytic reduction peak current for C_{70} was linearly proportional to the concentrations of hemoglobin in the range from $2.0 \times 10^{-5} \text{ mol L}^{-1}$ to $2.0 \times 10^{-4} \text{ mol L}^{-1}$, the linear regression equation is expressed as $I/\mu\text{A} = 3.22 + 0.437c/(10^5 \text{ mol L}^{-1})$ ($R = 0.9991$, $n = 7$). The same results were observed by SWV in Figures 9 and 10: the obviously increasing reduction peak current could be seen. Catalytic efficiency expressed as the ratio of catalytic current (I_c) to the reduction current (I_p) for C_{70} /DDAB films in solution not containing hemoglobin decreased with increasing scan rate. These results are characteristic of catalytic reduction of hemoglobin by C_{70} in the films, indicating that the C_{70} /DDAB films are capable of mediating the electron-transfer rate of hemoglobin and the embedded C_{70} is an electron-transfer mediator.

A possible mechanism for the electrocatalytic reaction might be expressed as follows:



To attest that hemoglobin molecules have penetrated into the C_{70} /DDAB films, the C_{70} /DDAB films were characterized by UV-vis and SEM. First, the C_{70} /DDAB films were prepared by transferring $40 \mu\text{L}$ toluene solution of mixed $5 \times 10^{-4} \text{ mol L}^{-1}$ C_{70} and 0.01 mol L^{-1} DDAB on a quartz microscope slide ($1 \times 2 \text{ cm}^2$) and allowing the solvent to evaporate. The quartz

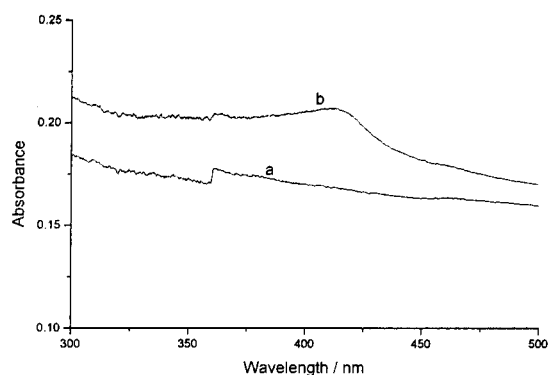


Figure 11. UV-vis spectra for C_{70} /DDAB films on quartz microscope slides. (a) No hemoglobin; (b) incorporating hemoglobin.



Figure 12. SEM of C_{70} /DDAB films after 15 cycles of voltammetry in 0.40 mol L^{-1} KCl and a one-time linear sweep in $8 \times 10^{-5} \text{ mol L}^{-1}$ hemoglobin, $300\times$.

microscope slide was soaked in 0.40 mol L^{-1} KCl solution containing $6.0 \times 10^{-5} \text{ mol L}^{-1}$ hemoglobin for 15 min, and then rinsed with water. The spectra in Figure 11 show the Soret band of the porphyrin, indicating that hemoglobin exists in the C_{70} /DDAB films. Second, after 15 cycles of voltammetry in 0.40 mol L^{-1} KCl solution and one time linear sweep in $8 \times 10^{-5} \text{ mol L}^{-1}$ hemoglobin between 0 and -1.0 V , the C_{70} /DDAB film electrode was taken out and washed with water, then dried in air. The SEM (Figure 12) shows some snowflake crystals in comparison with that of C_{70} /DDAB film after electrochemical scan (Figure 2D), suggesting that hemoglobin exists in the C_{70} /DDAB films.

4. Conclusions

The stable electroactive fullerene films in an aqueous solution can be made via coating the films of surfactant DDAB in the liquid-phase incorporating C_{70} on the GC electrodes. Electron-transfer rates are enhanced for the redox couples of C_{70} due to existence of surfactant DDAB on the electrodes. The C_{70} /DDAB films on the electrodes catalyzed reduction of hemoglobin.

Research is continuing in our laboratories on further applications of fullerene-containing surfactant films for developing biosensors.

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