Anodization in Oligo(Ethylene Glycol) as an Initial Derivatization Tool for Preparing Glassy Carbon Electrodes Covalently Modified with Amino Compounds: Effective Access to a 2,2,6,6-Tetramethylpiperidinyl-1-oxyl (TEMPO)-Modified Glassy Carbon Electrode

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Anodization in $HO(CH_2CH_2O)_nH$ (**1a**, $n=2$; **1b**, $n=3$; **1c**, $n=4$) as an initial derivatization tool for preparing glassy carbon (GC) electrodes covalently modified with amino compounds was explored. As an amino compound to be immobilized, 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxyl (4-amino-TEMPO) was selected. When GC electrodes anodized at 2.0 V *vs.* Ag wire coated with AgCl in **1** containing $RCH_2CH_2SO_3Na$ (**2a**, R=H; **2b**, R=OH) were treated with a *N,N*-dimethylformamide (DMF) or CH_2Cl_2 solution of 4-amino-TEMPO and 1,3-dicyclohexylcarbodiimide (DCC), TEMPO-modified GC electrodes were afforded. Coverage (Γ_{TEMPO}) of the electrode surfaces by TEMPO was estimated by cyclic voltammetry in CH_3CN containing $NaClO_4$. A TEMPO-modified GC electrode with the best Γ_{TEMPO} (1.36×10^{-10} mol/cm^2) was obtained by anodization in **1b** containing **2a** at the expense of 3.0 C followed by amidization in DMF for 7 d. On cyclic voltammetry, the TEMPO-modified GC electrode showed good and stable electrocatalytic ability for oxidation of allyl alcohol in the presence of 2,6-lutidine.

Key words   chemically modified electrode; anodization; glassy carbon; immobilization; surface modification; 2,2,6,6-tetramethylpieridinyl-1-oxyl

Chemically modified electrodes (CMEs)[1,2] have attracted a great deal of attention in modern electrochemistry, and have resulted in significant improvements in analytical methodology.[3—10] Four approaches for producing CMEs are available: chemisorption, covalent bonding, polymer film coating and composite (mixing a modifier with a conductive matrix such as a carbon paste).[1—10] The last three methods are well recognized as reliable means of obtaining CMEs. For carbon, a common and inexpensive electrode material, only the last two methods seem effective, although among the four methods, covalent bonding should provide modified carbon electrodes with the best physical stability and hence able to operate in the widest range of aqueous and organic media. General access to covalently modified carbon electrodes consists of introduction of carboxylate on the surfaces and coupling of amino compounds with the functional group: the former process is generally achieved by heating in air, treatment in a radio-frequency O_2 plasma, anodization in an aqueous solution, or chemical oxidation.[4,11] These treatments for derivatization with carboxylate are known to introduce various oxygen functionalities such as hydroxy and carbonyl groups on carbon surfaces as well.[11] These functional groups enter electrochemical reactions. In other words, carbon electrodes derivatized by these oxidative treatments exhibit markedly high back-ground currents in electrochemical measurements,[11] which prevents analytical application of the modified electrodes. Thus, preparation of covalently modified carbon electrodes for analytical purposes requires a novel method for surface derivatization with carboxylate.

Recently, we and other groups have independently developed novel methods for introducing carboxylate onto glassy carbon (GC) surfaces without the problem of high background currents: 1) anodization at 2.0 V *vs.* Ag wire coated with AgCl in $HO(CH_2CH_2O)_3H$ (**1b**) containing RCH_2-CH_2SO_3Na (**2a**, R=H; **2b**, R=OH) as a supporting electrolyte[12]; and 2) cathodic reduction of aromatic diazonium salts with carboxylate.[13,14] The latter method was shown to be effective as the initial derivatization tool for preparing glucose oxidase-modified GC electrodes.[15] We examined whether our own method can be practically applied for preparation of GC electrodes modified with amino compounds. For this purpose, 4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxyl (4-amino-TEMPO) was chosen as an amino compound since the efficiency for immobilizing TEMPO or its analogue on electrode surfaces has been conveniently evaluated by cyclic voltammetry for the electrodes modified by a self-assembly technique[16] and polymer film coating methods.[17—19] Here, we report that 4-amino-TEMPO can be effectively fixed *via* amide bonds on the surface of a GC electrode anodized in **1b** rather than $HO(CH_2CH_2O)_2H$ (**1a**) and $HO(CH_2CH_2O)_4H$ (**1c**) as an initial derivatization tool (Chart 1).

Experimental

Dehydrated CH_3CN and *N,N*-dimethylformamide (DMF) were used. CH_2Cl_2 was distilled from P_2O_5. All other chemicals were of reagent grade. A GC plate (GC20, $15\times30\times3$, Shimadzu) was polished with a Maruto Model ML-150P polishing system with polishing paper (#1500) and alu-
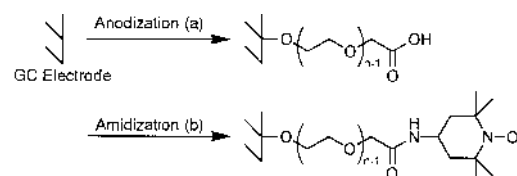
(a) at 2.0 V in $HO(CH_2CH_2O)_nH$ (**1a**, $n=2$; **1b**, $n=3$; **1c**, $n=4$) containing 0.1 M $RCH_2CH_2SO_3Na$ (**2a**, R=H; **2b**, R=OH);
(b) 4-amino-TEMPO, DCC in CH_2Cl_2 or DMF

Chart 1

mina powder (0.05 μm) on a polishing cloth, sonicated in deionized distilled water, MeOH and CHCl$_3$, and dried with a stream of nitrogen. The GC plate was electrochemically modified using a Hokuto Denko model HA301 potentiostat/galvanostat connected to a Hokuto Denko Model HF201 coulomb amperehour meter. Cyclic voltammograms were obtained with a Huso Model 315A potentiostat equipped with a Riken Denshi Model F-5C X-Y recorder. A three-electrode configuration was employed: a modified GC plate as the working electrode, saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the counter electrode.

Electrode Modification   The GC plate was subjected to controlled-potential electrolysis in **1** (30 ml) containing RCH$_2$CH$_2$SO$_3$Na (0.1 M) (**2a**, R=H; **2b**, R=OH) at 2.0 V *vs.* Ag wire coated with AgCl at room temperature until a specified amount of electricity had been consumed. After this treatment, the GC plate was washed with MeOH, H$_2$O, MeOH and the solvent used for the following chemical modification. The washed GC plate was immersed in a stirred CH$_2$Cl$_2$ or DMF solution (15 ml) containing 4-amino-TEMPO and 1,3-dicyclohexyl carbodiimide (DCC) (0.75 mmol each) at room temperature. After a specified period, the GC plate was washed by immersing in CH$_2$Cl$_2$ or DMF for 10 min, 1 N HCl for 30 min and H$_2$O for 10 min, and washed with CH$_3$CN and dried with a stream of nitrogen.

Voltammetric Measurements   An open-bottomed Diflon cell was employed, and the modified GC plate served as the cell bottom. The electrode area (0.2 cm^2) was defined by a sealing rubber o-ring positioned between the electrode and the Diflon cell. The immobilized amount (Γ_{TEMPO}) of TEMPO molecules was estimated as follows: cyclic voltammetry in CH$_3$CN containing only NaClO$_4$ (0.1 M) was carried out successively 50 times between 0 and 0.9 V (*vs.* SCE) at a sweep rate of 0.1 V s^{-1}; this measurement was repeated at three different points on the same modified electrode; the average charge of anodic peaks observed on the 50th voltammograms was obtained. The reported Γ_{TEMPO} values are the average values of those estimated for three GC plates with totally different histories but modified under essentially the same conditions. The catalytic properties of the modified GC electrode were evaluated by similar measurements in CH$_3$CN (0.1 M NaClO$_4$) containing allyl alcohol and 2,6-lutidine (5.0 mM each) at a sweep rate of 25 mV s^{-1}.

Results and Discussion

TEMPO-modified GC electrodes were prepared under various conditions according to the reaction scheme consisting of anodization and amidization (Chart 1). On cyclic voltammetry in CH$_3$CN containing only 0.1 M NaClO$_4$, each of the modified GC electrodes showed a reversible redox wave centered at *ca.* 640 mV *vs.* SCE, which was ascribed to the redox couple of the TEMPO moiety. The peak separation of the redox wave was *ca.* 20 mV, where the anodic and cathodic peaks were nearly mirror images of one another (*cf.* Fig. 1a). The electrochemical response at each of the modified electrodes became almost constant after the voltammetric measurement was repeated 10 times: the difference between the anodic peak currents on first and 10th voltammograms was less than 5%, and only a negligible decrease was observed during further 40 measurements. After each of the modified electrodes was subjected to successive voltammetric measurements more than 10 times, the anodic peak currents exhibited a linear relationship against a potential sweep rate over the range between 20 and 250 mV s^{-1}. These voltammetric results were consistent with the general descriptions of how the success of an immobilization procedure manifests itself in certain electrochemical measurements.[4]

Γ_{TEMPO} for each modified GC plate was estimated using the charge of the anodic peak on the 50th voltammogram obtained in CH$_3$CN containing only NaClO$_4$. The results are summarized in Table 1. The effects of the conditions of amidization on Γ_{TEMPO} values were examined (runs 1—4), where anodization was performed by passing 2C of electricity at 2.0 V in **1b** containing **2a**. Longer periods of treatment of the anodized GC electrode with 4-amino-TEMPO in DMF were associated with larger values of Γ_{TEMPO} (runs 1—3). As
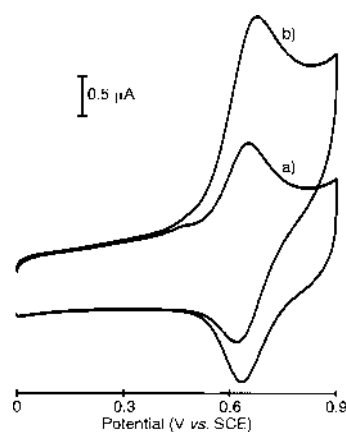

Fig. 1.   Voltammograms of a TEMPO-Modified GC Electrode in CH$_3$CN Containing NaClO$_4$ (0.1 M) in the Absence (a) and Presence (b) of Allyl Alcohol and 2,6-Lutidine (5 mM Each)

The modified electrode was prepared by anodization (2.0 V, 3C) in **1b** containing **2a** followed by amidization with 4-amino-TEMPO in DMF for 7 d. Potential sweep rate, 25 mV s^{-1}.

Table 1.   The Amount (Γ_{TEMPO}) of TEMPO Immobilized on GC Electrodes Subjected to Anodization Followed by Amidization under Various Conditions

Run	Conditions for		Γ_{TEMPO} ($\times 10^{-10}$ mol/cm^2) (RSD: $n=3$)
	Anodization	Amidization	
1	**1b/2a**/2C[a]	DMF/1 d	0.56 (20.3%)
2	**1b/2a**/2C[a]	DMF/3 d	0.71 (4.6%)
3	**1b/2a**/2C[a]	DMF/7 d	0.99 (5.1%)
4	**1b/2a**/2C[a]	CH$_2$Cl$_2$/7 d	0.50 (19.4%)
5	**1b/2b**/2C[a]	DMF/7 d	0.65 (9.1%)
6	**1b/2a**/3C[a]	DMF/7 d	1.36 (5.7%)
7	**1b/2a**/4C[a]	DMF/7 d	1.00 (9.8%)
8	**1a/2a**/3C[a]	DMF/7 d	0.44 (21.6%)
9	**1c/2a**/3C[a]	DMF/7 d	1.21 (8.2%)

a) The amount of electricity passed in the electrochemical step.

a solvent for amidization, DMF was markedly superior to CH$_2$Cl$_2$ (runs 3 and 4). Since immersing a GC plate for more than 7 d seemed impractical as a modification method, further examinations were carried out using amidization in DMF for 7 d.

The results in runs 3 and 5—7 compare how the values of Γ_{TEMPO} were affected by the passed electricity as well as the identity of the supporting electrolyte used in the electrochemical treatment. Anodization with **2a** as a supporting electrolyte afforded a much better value of Γ_{TEMPO} than that with **2b** (runs 3, 5). This result suggested that anodization of a GC electrode in **1b** introduced carboxylate much more effectively on the surface when **2a** was used as a supporting electrolyte rather than **2b**, although almost no differences were previously observed in voltammetric performance of GC electrodes anodized in **1b** containing **2a** or **2b**.[12] Derivatization of a GC surface with 4-amino-TEMPO was achieved more effectively by passing 3C rather than 2C of electricity, and passing 4C resulted in a slight decrease in Γ_{TEMPO} although the reason for this is not clear at present (runs 6, 7).

As a tool for derivatization of GC surfaces with carboxylate, anodization in **1a** or **1c** instead of **1b** was also examined (runs 8, 9). However, electrochemical treatment in **1a** was of

no use for this purpose. A TEMPO-modified GC electrode was effectively prepared by anodization in **1c** followed by amidization. However, Γ_{TEMPO} was slightly lower than that obtained with anodization in **1b**. Thus, it was concluded that anodization in **1b** containing **2a** as a supporting electrolyte at the expense of 3C electricity works best as the initial derivatization tool for preparation of a TEMPO-modified GC electrode. It should be mentioned here that the procedure with the best conditions afforded a TEMPO-modified GC electrode with high reproducibility, *i.e.* a satisfactory relative standard deviation (RSD) such as 5.7%, regardless of the histories of GC plates.

It was previously reported that a self-assembly technique with a derivative of lipoic acid provided a TEMPO-modified gold electrode with Γ_{TEMPO} of 5.8×10^{-10} mol cm^{-2}.[16] The present method to immobilize a TEMPO molecule on electrodes is inferior to this self-assembly method. However, Γ_{TEMPO} of 1.36×10^{-10} mol cm^{-2} obtained in this study was still typical of redox species immobilized on electrodes as monolayers.[3] In addition, it seems rather tedious to prepare thiol compounds derivatized with TEMPO as a modifier in a self-assembly technique applicable only to surface modification of noble metals such as gold, which are less common electrode material than GC for various analytical purposes. Thus, the present covalent bonding method for modification of a GC surface with a TEMPO molecule is believed to have an advantage over the self-assembly technique.

The catalytic ability of a TEMPO-modified GC electrode obtained under the optimal conditions was evaluated by cyclic voltammetry in CH$_3$CN containing allyl alcohol and/or 2,6-lutidine. Allyl alcohol and 2,6-lutidine themselves exhibited no voltammetric responses at a bare GC electrode over the potential range between 0 and 0.9 V. The voltammogarm of the TEMPO-modified GC electrode was not affected by addition of the alcohol or the base alone. However, in the presence of both additives,[20] the modified electrode showed typical behavior for catalytic oxidation of alcohol[19,21—25]: as shown in Fig. 1, anodic peak current was enhanced more than two fold, while the cathodic wave became smaller to some extent, which is well in line with voltammetric results of electrodes modified with a TEMPO analogue, 2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl, by polymer film coating methods.[17,18] The catalytic response was quite reproducible, and almost no changes were observed in the enhanced anodic currents even after 50 successive voltammetric measurements. This was in marked contrast with the following observation that a GC electrode coated with a hydrophobic poly(phenylene oxide) film containing immobilized TEMPO exhibited good catalytic ability for electrochemical oxidation of hydrophobic alcohols such as allyl alcohol and alkanol, but gradual electrode passivation was observed during electrochemical measurements due to adsorption of oxidation products of hydrophobic alcohols through hydrophobic interaction with the polymer film.[26] Thus, the reproducible catalytic responses at the present TEMPO-modified GC electrode might have been due to a hydrophilic linker between the TEMPO molecule and the electrode surface.

In summary, our results demonstrated that anodization in **1b** containing **2a** works as an initial derivatization tool for preparing a GC electrode covalently modified with 4-amino-TEMPO. The TEMPO-modified GC electrode was shown to exhibit satisfactory and stable catalytic ability probably due to the hydrophilicity of its surface, and hence is expected to be useful for analytical applications requiring continuous, long-term operation in a flow system. Further studies of this point and of application of the present method to preparation of GC electrodes modified with other redox species are currently being carried out in our laboratory.

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