0273-2289/00/89/0209/$12.00

Sol-Gel-Derived Prussian Blue–Silicate Amperometric Glucose Biosensor

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Abstract

A new type of inorganic biosensor is introduced. The sensor comprises glucose oxidase enzymes encapsulated in a sol-gel-derived Prussian blue–silicate hybrid network. Glucose is detected by the biocatalytic reduction of oxygen followed by catalytic reduction of hydrogen peroxide by the Prussian blue catalyst. The sol-gel silicate entails a rigid encapsulating matrix, the Prussian blue provides chemical catalysis and charge mediation from the reduction site to the supporting electrode, and the enzyme is responsible for the biocatalysis. The feasibility of a dual optical/electrochemical mode of analysis is also demonstrated.

Index Entries: Sol-gel; biosensor; Prussian blue; modified electrodes.

Introduction

Redox polymers attract considerable scientific and technological attention in many branches of electrochemistry because of their ability to transport electric charge without long-range movement of redox mediators. This property, when combined with the ability to entrap sensitive compounds in the polymer network, is especially attractive for electrochemical biosensing *(1,2)*. Thus, electron transport from the active center of oxidoreductase enzymes to the electrode can be performed by an electron-hopping mechanism without the addition of leachable charge mediators. During the last decade, considerable scientific efforts were devoted to the development of electrochemical biosensors based on organic redox polymers. The evolution of sol-gel technology—a technology for the polymerization of inorganic porous networks—directed some of these scientific activities to the development of hybrid inorganic-organic redox matrices *(3–5)*. Indeed, other groups as well as ours have demonstrated that it is

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possible to entrap oxidoreductase enzymes in inorganic-organic hybrid networks that provide long-range charge transport by an electron-hopping mechanism *(6–11)*. For example, we have encapsulated glucose oxidase (GOD) in ferrocene-modified silicate networks *(9–11)*, and Pravda et al. *(7)* and Park et al. *(8)* described a sol-gel-based technique for the encapsulation of oxidoreductases (lactate oxidase and GOD) and redox polymer [(Os)(bpy)$_2$(poly(4-vinylpyridine))$_{10}$Cl]Cl in methyltrimethoxysilane (MTMOS)-based sol-gel films.

In the present study, we have taken this concept a step forward and demonstrated that it is also possible to entrap redox enzymes in pure inorganic redox networks that can provide both encapsulation and charge mediation. In this case, the protein is encapsulated in a copolymer comprising Prussian blue (PB) and silicate network. The protein is the only organic moiety in this sensing device. The electrocatalytic activity of metal hexacyanoferrates has been amply exploited before for the catalytic reduction of H_2O_2 and for biosensing *(12–15)*. Knocki and Wolfbeis *(16)* demonstrated the feasibility of an optical biosensor for determination of urea and acetylcholine by a composite material comprising covalently linked *N*-substituted polypyrrole-redox enzymes and PB. We have demonstrated the electrochemical and photochromic activity of sol-gel-derived silicate–PB composite films *(17)*, and, recently, Guo and Guadalupe *(18)* demonstrated another sol-gel route for the preparation of the PB-silicate network using bistrimethoxysilane tetrasulfide monomers.

Materials and Methods

Chemicals

Tetramethoxysilane was purchased from Aldrich. MTMOS was purchased from ABCR. Ferric sulfate, ferrous sulfate, potassium ferricyanide, potassium chloride, and potassium hydrogen phosphate were purchased from Merck (Darmstadt, Germany). GOD (50,000 U) was purchased from Sigma (St. Louis, MO). Indium tin oxide (ITO)-coated glass with resistivity R_s = 100 Ω/square was purchased from M/S Delta (Stillwater, MN).

Apparatus

A PC controlled EG&G PARC potentiostat model 273 was used for cyclic voltammetry studies. A single compartment three-electrode cell equipped with a platinum wire counterelectrode and saturated Ag/AgCl reference electrode was used. All potentials are referred to saturated Ag/AgCl reference, unless otherwise specified. A Varian Carry E1 spectrophotometer coupled to a potentiostat was used for the dual photometric-electrochemical studies.

Formation of PB-Modified Electrodes

PB-modified electrodes were prepared by cycling the potential of an ITO electrode between –0.2 and 1 V vs Ag/AgCl in a solution containing

2 m*M* ferric sulfate, 2 m*M* potassium ferricyanide, and 0.1 *M* KCl. Modified electrodes were tested in 0.1 *M* KCl or 0.1 *M* KH_2PO_4.

Formation of PB-Doped Silicate Films

One milliliter of MTMOS and 1 mL of methanol were mixed. Then, 0.1 mL of 0.1 *M* ferrous sulfate was added followed by the addition of 0.1 mL of 0.1 *M* HCl solution and 0.1 mL of distilled water. The solution was sonicated for 10 min, and the resulting sol was then used to dip coat the ITO-coated glass slides. PB was incorporated into the sol-gel film by cycling the electrode potential between –0.2 and 1.0 for 15 min in a solution containing 1 *M* KCl and 2 m*M* potassium ferricyanide. A similar procedure has been reported previously *(17)*.

Preparation of GOD-Doped PB-Silicate Hybrid Films

One milliliter of MTMOS and 0. 2 mL of GOD solution (from a 40 mg/mL stock solution, 50,000 U/mL) were mixed, followed by the addition of 0.1 mL of 0.1 *M* ferrous sulfate and 0.1 mL of 0.1 *M* HCl. The resulting sol was used to dip coat the ITO-coated substrate. PB was incorporated into the sol-gel film as described in the previous section.

Results and Discussion

Figure 1 compares the voltammetric response of PB-silicate film (Fig. 1A) with that of PB-modified electrode (Fig. 1B) at two scan rates (5 and 100 mV/s). The two anodic and cathodic peaks correspond to the following redox reactions *(19,20)*:

$$K_2Fe^{(II)}[Fe^{(II)}(CN)_6] \longleftrightarrow KFe^{(III)}[Fe^{(II)}(CN)_6] + K^+ + e- \quad (1)$$

$$KFe^{(III)}[Fe^{(II)}(CN)_6] \longleftrightarrow Fe^{(III)}[Fe^{(III)}(CN)_6] + K^+ + e- \quad (2)$$

The formal potentials of reactions 1 and 2 in 0.1 *M* KH_2PO_4 solution correspond to E_p = –50 and 680 mV/Ag/AgCl, respectively. The anodic and cathodic peak separation changed between 54 and 78 mV for the first oxidation step, and between 52 and 138 mV for the second oxidation step, when the scan rate was increased from 5 to 100 mV/s. The PB electrodes deposited on bare ITO exhibited the same formal potentials as those of the PB-silicate-modified electrodes. The reversibility manifested in the E_p values was quite similar for the two types of PB-modified electrodes. The similarity between the PB-coated ITO electrode and the PB-silicate hybrid film suggests that the PB dopant forms a continuum within the inorganic silicate matrix through the interconnected porous structure of the silicate film. The insulating silicate matrix had only a minor effect on the effective diffusion coefficient of charges in the redox polymer. Even at a scan rate of 100 mV/s, the peak separation was changed only by about 60 mV compared to pure PB film.

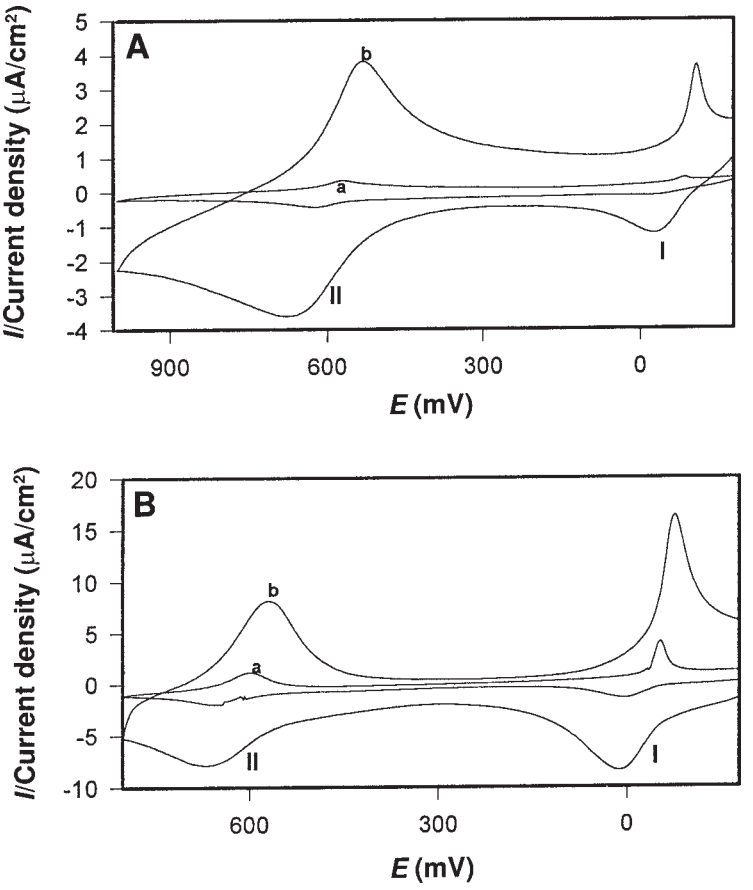

Fig. 1. Cyclic voltammograms of PB-silicate-coated ITO electrode **(A)** and PB-coated ITO electrode **(B)** in 0.1 *M* KH_2PO_4, pH 4.7. Scan rates: (a) 5 and (b) 100 mV/s.

Figure 2 shows the catalytic reduction in H_2O_2 by the PB-silicate composite. The catalytic wave started at about 0 mV/Ag/AgCl, and, as expected, it coincided with the onset of the first cathodic peak for PB reduction (Fig. 1). The reduction in H_2O_2 on bare ITO or on silicate-modified ITO started at about –600 mV (not shown). The catalytic wave for a reduction in H_2O_2 on PB-silicate was rather sluggish and saturation was not reached even at –200 mV/Ag/AgCl. A calibration plot for the reduction in H_2O_2 at –200 mV/Ag/AgCl is depicted in Fig. 3. A linear dependence up to about 10 m*M* was observed. The minimum detection limit of H_2O_2 is approx 0.05 m*M*, corresponding to three times the noise level.

Figure 4 gives the cyclic voltammetry response of the GOD-doped PB-silicate-modified ITO electrode in several glucose solutions. The voltammetric response was similar to the H_2O_2 cyclic voltammetries (Fig. 2), indicating that the mechanism does not involve direct charge transfer from the flavin prosthetic group of the GOD to the PB. Oxygen reduction followed by H_2O_2 reduction is the underlying mechanism. This, indeed, was verified

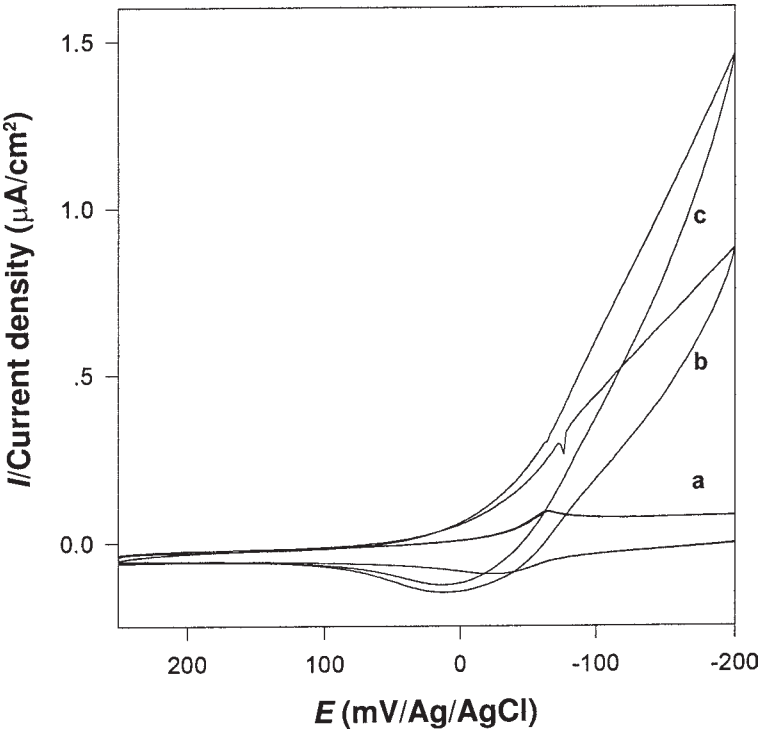

Fig. 2. Cyclic voltammograms of a PB-silicate electrode in the presence of (a) 0, (b) 1, and (c) 5 m*M* H_2O_2. Scan rate = 10 mV/s.

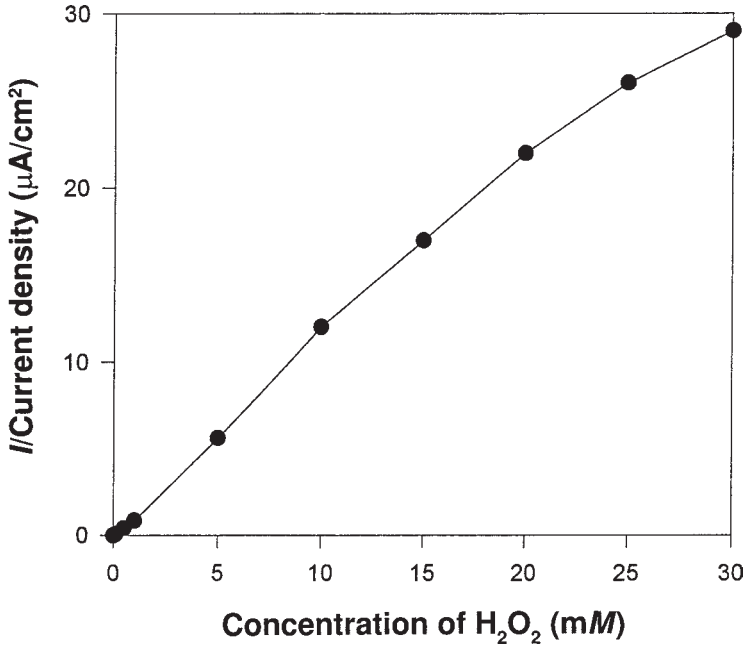

Fig. 3. H_2O_2 calibration curve for PB-silicate-modified ITO electrode. Applied potential = –200 mV/Ag/AgCl in 0.1 *M* KH_2PO_4, pH 4.7.

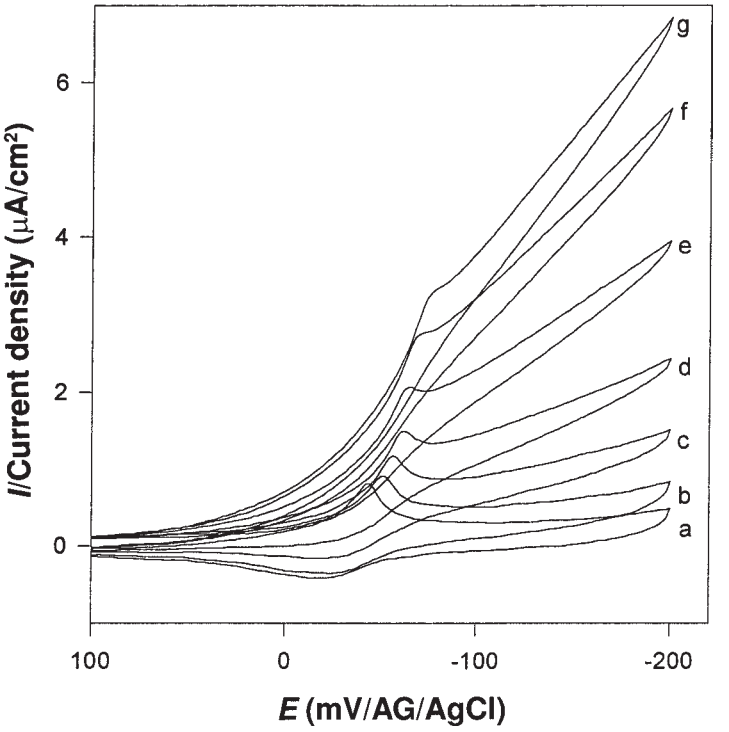

Fig. 4. Cyclic voltammogram of GOD-doped PB-silicate-modified ITO electrode in the presence of (a) 0, (b) 2, (c) 5, (d) 10, (e) 15, (f) 20, and (g) 23 m*M* glucose. Scan rate = 10 mV/s in 0.1 *M* KH_2PO_4, pH 4.7.

by bubbling nitrogen, which restored the blank (zero glucose) cyclic voltammetry curve. No response for glucose was noticed for the PB-silicate electrode without GOD, thereby confirming that the catalytic response is caused by the enzymatic reaction.

Figure 5 shows the amperometric response of the GOD-doped PB-silicate electrode at –200 mV / Ag / AgCl. Each addition corresponds to 5 m*M* glucose. The response was found to be linear between 0 and 20 m*M* with a minimum detection limit of approx 0.05 m*M* glucose. The response time ($T_{90\%}$) was <15 s, which agrees well with the expected behavior of submicrometer PB-silicate film. Shelf-life stability of the PB-silicate GOD biosensor was checked by repeated measurement of 10 m*M* glucose solutions, which showed that the electrode was stable to within 75% for at least 4 wk.

Figure 6 shows the electrochromic activity of the GOD-doped PB-silicate film in the absence and presence of 5 m*M* glucose. In the absence of glucose, the film was in the reduced form, as noted by the absence of a broad absorbance peak at about 600 nm. While in the presence of glucose, H_2O_2, generated by the enzymatic reaction, converted the PB to its oxidized form.

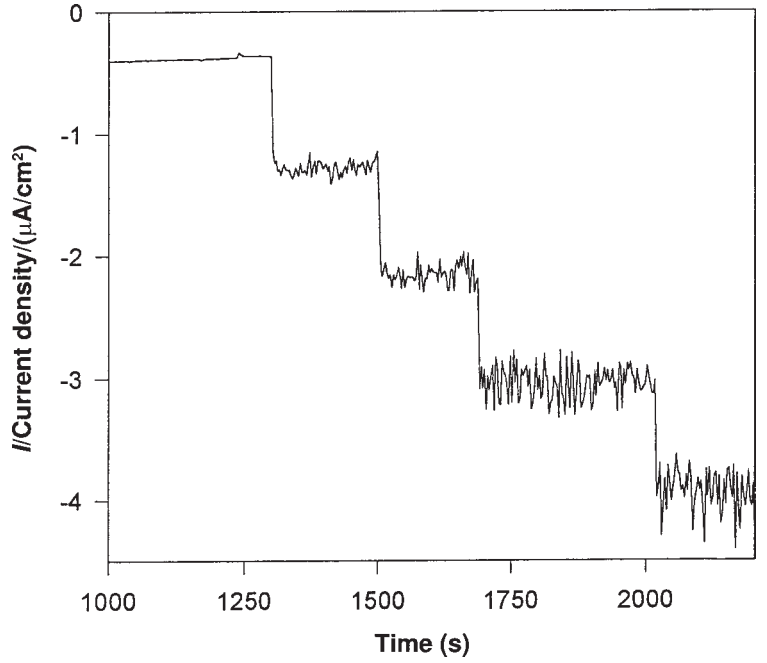

Fig. 5. Dynamic response of GOD-doped PB-silicate-modified ITO electrode for consecutive additions of 5 m*M* glucose. Applied potential = –200 mV/Ag/AgCl.

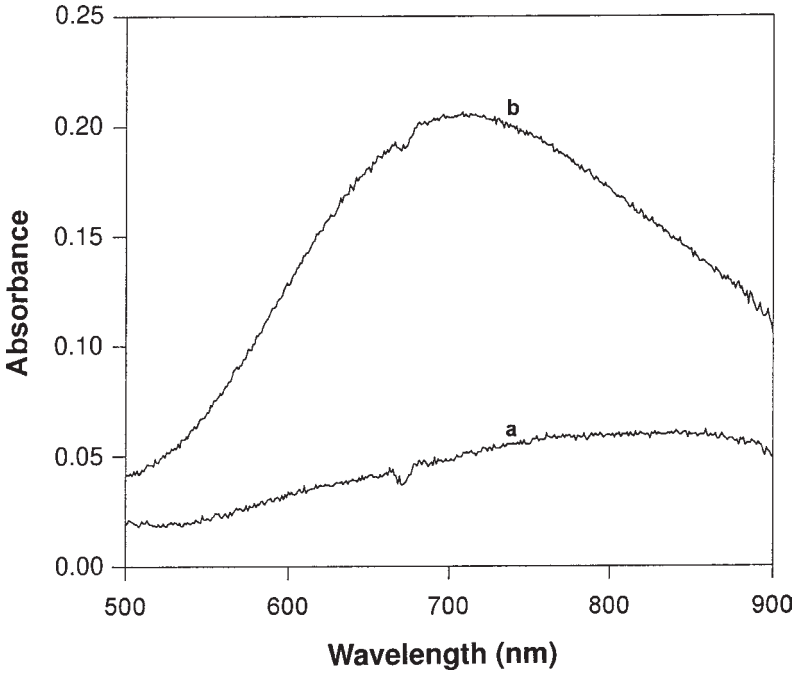

Fig. 6. Visible spectra of the GOD-doped PB-silicate film before (a) and after (b) the addition of 5 m*M* glucose (0.1 *M* KH_2PO_4, pH 4.7).

Conclusion

We have demonstrated, for the first time, the feasibility of the GOD-doped Prussian blue–silicate amperometric biosensor. The results suggest that dual-mode photometric-amperometric biosensing is feasible. The sensor exploits the rigid construction of the porous silicate, the hopping transport mechanism of the redox inorganic polymer, and the catalytic reduction activity of PB for H_2O_2. Additionally, the transparency of all the constituents of this biosensor (ITO, silicate, and PB film) and the electrochromic behavior of the PB provide the ability to conduct the quantification by a dual amperometric/photometric sensing mode.

Acknowledgments

We wish to thank Prof. S. Sampath for useful collaboration and the Indo-Israel Foundation for financial support.

References

1. Heller, A. (1990), *Accounts Chem. Res.* **23,** 128–134.
2. Hale, P. D., Bouguslavsky, L. I., Inagaki, T., Karan, H. I., Lee, S., and Skotheim, T. A. (1991), *Anal. Chem.* **63,** 677–682.
3. Lev, O., Wu, Z., Bharathi, S., Glezer, V., Modestov, A., Gun, J., Rabinovich, L., and Sampath, S. (1997), *Chem. Mater* **9,** 2354–2375.
4. Collinson, M. M. (1998), *Mikrochim. Acta* **129,** 149–165.
5. Walcarius, A. (1998), *Electroanalysis* **10,** 1217–1235.
6. Albery, W. J., Bartlett, P. N., and Craston, D. H. (1985), *J. Electrochem. Chem.* **194,** 223–242.
7. Pravda, M., Jungar, C. M., Iwuoha, E. I., Smyth, M. R., Vytras, R., and Ivaska, A. (1995), *Anal. Chim. Acta* **304,** 127–138.
8. Park, T. M., Iwuoha, E. I., Smyth, M. R., and MacCraith, B. D. (1996), *Anal. Commun.* **33,** 271–273.
9. Sampath, S. and Lev, O. (1996), *Electroanalysis* **8,** 1112–1116.
10. Gun, J. and Lev, O. (1996), *Anal. Chim. Acta* **336,** 95–106.
11. Gun, J. and Lev, O. (1996), *Anal. Lett.* **29,** 1933–1938.
12. Karyakin, A. A., Karyakina, E. E., and Gorton, L. (1996), *Talanta* **43,** 1597–1606.
13. Karyakin, A. A., Karyakina, E. E., and Gorton, L. (1998), *J. Electroanal. Chem.* **456,** 97–104.
14. Lu, W., Wallace, G. G., and Karyakin, A. A. (1998), *Electroanalysis* **10,** 472–476.
15. Karyakin, A. A., Gitelmacher, O., and Karyakina, E. E. (1995), *Anal. Chem.* **67,** 2419–2423.
16. Knocki, R. and Wolfbeis, O. S. (1999), *Biosens. Bioelectron.* **14,** 87–92.
17. Bharathi, S., Wu, Z., and Lev, O. (1997), in *SPIE, Sol Gel Optics IV*, Dunn, B. S., Mackenzie, J. D., Pope, E. J. A., Schmidt, H. K., and Yamane, M., eds., SPIE, Bellingham, Washington, pp. 127–132.
18. Guo, Y. Z., Guadalupe, A. R., Resto, O., Fonseca, L. F., and Weisz, S. Z. (1999), *Chem. Mater.* **11,** 135–140.
19. Itaya, K., Uchida, I., and Neff, V. D. (1986), *Accounts Chem. Res.* **19,** 162–167.
20. Ellis, D., Eckhoff, M., and Neff, V. D. (1981), *J. Phys. Chem.* **85,** 1225–1230.