

Copper-Coated Cylindrical Internal Reflection Elements for Investigating Interfacial Phenomena

TEIKI IWAOKA, PETER R. GRIFFITHS,* JOHN T. KITASAKO, and GILL G. GEESEY

Department of Chemistry, University of California, Riverside, California 92521 (T.I., P.R.G.); Department of Plant Pathology, University of California, Riverside, California 92521 (J.T.K.); and Department of Microbiology, California State University, Long Beach, California 90840 (G.G.G.)

Techniques for coating thin copper films on the surface of cylindrical germanium internal reflection elements are described. These films were then characterized in an aqueous environment. The expected exponential relationship between the depth of penetration of the evanescent wave into water and the thickness of the copper film was verified experimentally. The stability of vacuum-deposited copper coatings was strong enough that the internal reflection element could be exposed to an aqueous solution of a polysaccharide for more than 40 h. The weak adhesion of polysaccharides to copper surfaces was studied spectroscopically.

Index Headings: Cylindrical internal reflection; Surface analysis; Metal coating techniques; Spectroscopic techniques.

INTRODUCTION

The observation of biological phenomena at the interface between aqueous solutions and solid surfaces is best investigated *in situ* by attenuated total reflection (ATR) spectrometry. Measurements of this type have been reported for the adsorption of proteins from aqueous solutions (including whole blood) onto uncoat-

ed germanium internal reflection elements (IREs) and IREs coated with thin polymer layers.¹⁻⁶ Adsorption of stearic acid onto an IRE coated with a thin layer of iron has been mentioned in one review paper,⁶ but few experimental details or spectra of adsorbed species were shown. In this paper we describe techniques for vacuum depositing a thin layer of copper onto a germanium cylindrical internal reflection (CIR) element and the results of several experiments designed to characterize this layer.

For ATR measurements, the effective depth of penetration, d_p , of the evanescent wave from an IRE of refractive index, n_1 , into an optically rare medium of refractive index, n_2 , is given by the equation:

$$d_p = \frac{\lambda}{2\pi n_1 \{\sin^2\theta - (n_2/n_1)^2\}^{1/2}} \quad (1)$$

where λ is the wavelength of the radiation in air and θ is the angle of incidence. For a polymer with $n_2 = 1.5$ deposited on a germanium IRE ($n_1 = 4.0$), the effective penetration depth of 10 μm radiation is 0.66 μm , assuming an incidence angle of 45°. Provided that a polymer film is deposited on the surface of a germanium IRE

Received 4 February 1986.

* Author to whom correspondence should be sent.

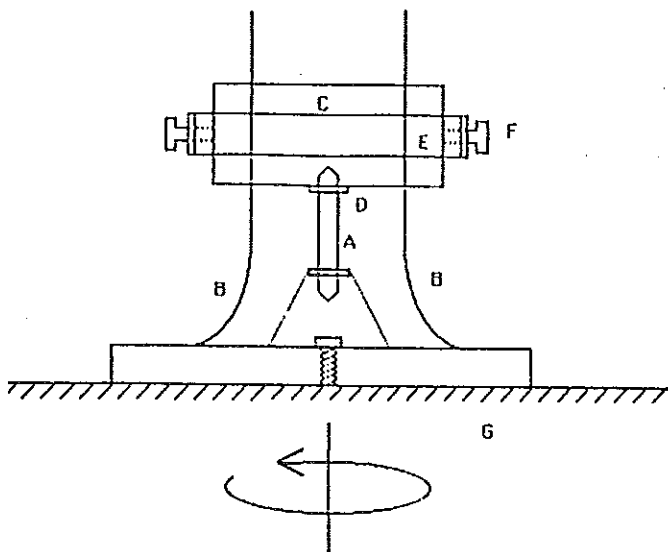


FIG. 1. Side view of the device used to hold an IRE for vacuum coating of metal. A: CIR crystal; B: piano wire; C: Teflon[®] crown; D: O-ring; E: crown collar; F: setting screw; G: turntable.

with a thickness significantly less than this value, the evanescent wave will interact with an absorbing medium in contact with the outer surface of the polymer. Thus, for example, Jakobsen *et al.*^{4,5} have been able to monitor the adsorption of proteins from blood onto polymers of the type used for artificial implants. Since the film thickness is small and the absorptivities of most polymer bands are relatively weak, the absorbance of the film is low enough that, even in multiple internal reflection measurements, sufficient energy is transmitted through the IRE that a good spectrum of both the bulk solution in contact with the polymer surface and any species adsorbed on the surface can be measured.

The situation for metal coatings is somewhat different since both the refractive index and absorptivity of most metals are not only much higher than those of polymers but the refractive index is often higher than n_1 , the refractive index of the IRE. The refractive index of most metals is between 2 and 20, so that the penetration depth through a metal deposit is often much smaller than the value calculated above for a polymer film. The absorptivity of metal films is of the order of the 10^6 cm^{-1} , two orders of magnitude greater than the absorptivity of intense bands in the vibrational spectra of most organic compounds. Nevertheless, layer thicknesses of a few nanometers can be tolerated. For metal films which are only a few atomic layers in thickness, the transmittance of metal-coated multiple internal reflection elements can still be sufficiently high to allow the measurement of ATR spectra of species near the metal surface.

We have investigated methods of achieving such measurements with a CIR cell using a Fourier transform infrared (FT-IR) spectrometer. CIR elements are well suited for installation on FT-IR spectrometers because of their circular cross section. In addition the absence of sharp edges makes them easier to seal with O-rings than are rectangular IREs. Finally, an unexpected benefit is that by the rotating of the IRE as the coating is being laid down, metals can be uniformly vacuum deposited to provide a well-defined surface coverage.

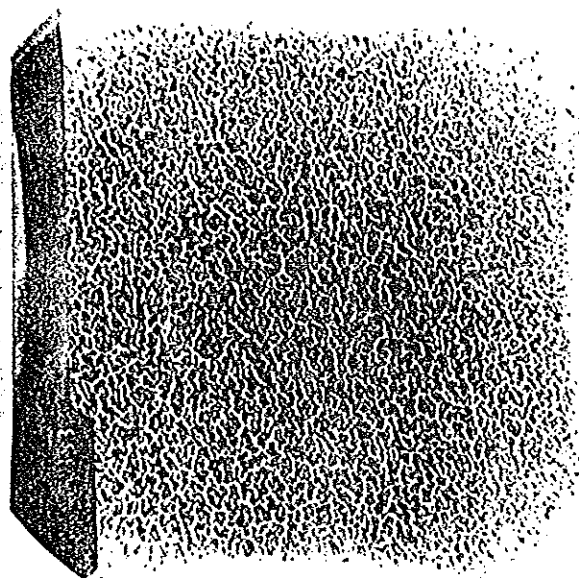


FIG. 2. Transmission electron micrograph of a carbon-coated copper grid which was subjected to the same copper-coating procedure as the Ge IRE. Magnification = $100,000\times$.

EXPERIMENTAL

Copper wire of 0.1 mm diameter (99.999%, Puratronic Grade, Alfa Products, Danvers, MA) was used as the source of copper used to coat a germanium CIR element (Spectra-Tech, Stamford, CT). The element was 3.2 mm in diameter and 38 mm in length, and was mounted in a stainless-steel "open boat" cell in a Spectra-Tech CIR accessory. A ZnSe CIR element of the same dimensions was also used for certain measurements. Spectra were measured at 4 cm^{-1} resolution with the use of a Nicolet 60-SX FT-IR spectrometer equipped with a medium-range mercury-cadmium-telluride detector.

Vacuum deposition of the copper coating was effected in an Edwards Model 306 Coater. The copper wire was wrapped around the apex of a V-shaped polished tungsten filament which was mounted 38 mm from the CIR element. When an adequate vacuum (10^{-6} Torr) had been obtained, the tungsten filament was heated so that the copper wire melted into a single droplet. Copper was then gradually evaporated onto the surface of the CIR element by further increasing the current through the filament.

To obtain a homogeneous coating, we designed a device which enabled the CIR crystal to be spun at about 120 rpm during the deposition. To prevent the ends of the crystal from being coated, we protected them by Teflon[®] "crowns," which were supported by 0.3-mm-diameter piano wire. This holder is shown schematically in Fig. 1. Before the crystal was mounted in this holder, it was necessary to polish it to remove any contaminants from its surface. This was achieved with the use of a slurry of $0.05 \mu\text{m}$ alumina powder (Buehler Ltd., Evanston, IL).

The amount of copper deposited on the germanium

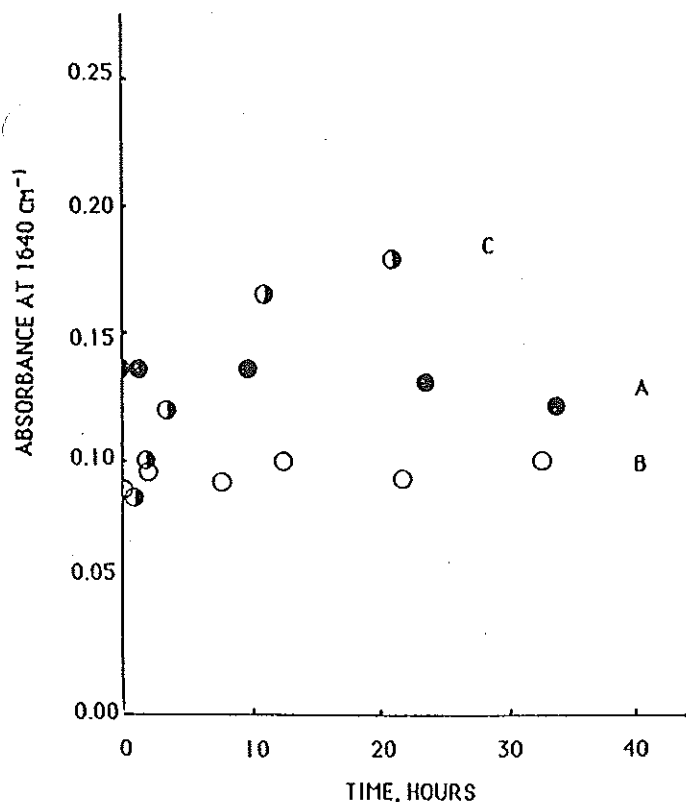


FIG. 3. Tolerance of deposited copper film against distilled water and polysaccharide solution. Absorbance of water band (1640 cm^{-1}) as a function of immersion time in distilled water: copper film thickness (A) $\sim 10\text{ Å}$ thickness; (B) $\sim 20\text{ Å}$; (C) absorbance of water band as a function of time for Gum Arabic solution in contact with Ge IRE coated with a $\sim 20\text{ Å}$ thick copper layer.

surface was determined spectrophotometrically as the neocuproin complex, after dissolving the metal in 10 mL of 0.05 N HNO_3 , reducing the dissolved copper to Cu(I) with hydroxylamine hydrochloride, forming the neocuproin complex at pH 4, and extraction into chloroform.

A Gum Arabic solution was prepared at 10% concentration (w/v) and filtered with a Millipore filter of $0.45\text{ }\mu\text{m}$ pore size to remove any microbiological contaminants. After preparation this solution was sealed in a glass vial under a gas flame and stored in a refrigerator.

RESULTS AND DISCUSSION

The homogeneity of the copper coating was checked at several points on the surface of the IRE by electron microscopy. Electron micrographs of the copper grains on the surface are shown in Fig. 2. The coating appears to be uniform at each position studied, and only minor irregularities were noted.

The stability of the copper layer was tested by immersing the coated IRE in water and monitoring the intensity of the water absorption band at 1640 cm^{-1} as a function of time. The absorbance of this band remained approximately constant over a period of 40 h, as shown in Fig. 3A and B, indicating good integrity of the copper layer over this period of time. No evidence of dissolved copper in the deionized water with which the layer was in contact was observed after spectrophotometric analysis with neocuproin.

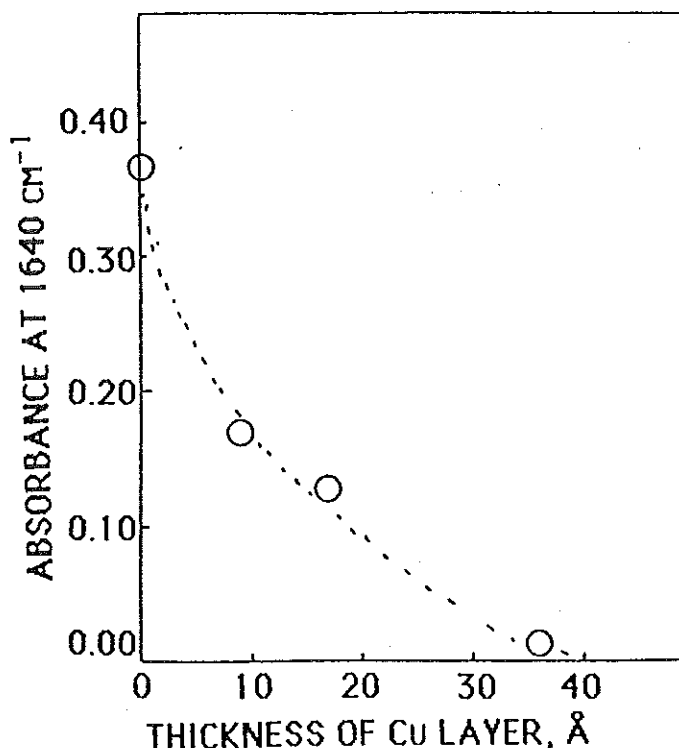


FIG. 4. Absorbance of the water band (1640 cm^{-1}) as a function of the thickness of copper film on Ge IRE.

An estimate of the absorption of radiation by the copper layer and concomitant decrease in the effective penetration depth was made by observing the decrease in the absorbance of the 1640 cm^{-1} water band as the thickness of the copper layer was increased. For coatings thicker than 50 nm of copper, too little energy was transmitted through the IRE to permit observation of the 1640 cm^{-1} water band with an adequate signal-to-noise ratio.

The extent of penetration of the evanescent wave into the bulk aqueous phase was evaluated by monitoring the absorbance of the 1640 cm^{-1} water band as a function of film thickness. Results are shown in Fig. 4. The value of the peak absorptivity of this band has been estimated to be $1 \times 10^3\text{ cm}^{-1}$ from a measurement made with the use of a transmission cell. From the measured absorbance of the 1640 cm^{-1} band using an uncoated Ge crystal, the effective pathlength may be calculated to be $3.6\text{ }\mu\text{m}$. This value is in good agreement with the value of $3.9\text{ }\mu\text{m}$ calculated from Eq. 1, assuming that the number of reflections in this element given by the manufacturer, 10, is correct. The decrease in d_p on coating the germanium surface can be inferred readily from Fig. 4.

Certain bacteria are known to promote corrosion of metals. It may be presumed that the first step of most biologically mediated corrosion processes involves the adhesion of bacteria to the metal surface. This project was initiated to allow the investigation of the attachment of bacteria to metal surfaces submerged in aqueous medium and, possibly, subsequent corrosion reactions.

Thin fibers, morphologically known as the glycocalyx, frequently extend from sessile bacteria to the surfaces to which the cells are attached.⁷ The glycocalyx is be-

TABLE I. Observed ratio of polysaccharide absorption band (1050 cm^{-1}) to water absorption band (1640 cm^{-1}) on uncoated ZnSe and Ge IREs and on copper-coated Ge IRE.

IRE	Absorbance		Ratio
	Water	Polysaccharide	
ZnSe (uncoated)	0.74	0.14	0.20
Ge (uncoated)	0.37	0.08	0.21
Ge (copper-coated)	0.20	0.08	0.40
	0.15	0.05	0.33
	0.09	0.02	0.22*
	0.06	0.04	0.67
	0.04	0.02	0.50
	0.01	0.01	1.00

* Third use of Cu-coated Ge IRE.

lieved to be a polysaccharide that contains various uronic acids and neutral sugars. In the present study we used an aqueous solution of the plant polysaccharide, Gum Arabic, which is a branched polymer of galactose, rhamnose, arabinose, and glucuronic acid (sodium salt form), to simulate the interaction of a bacterial glycocalyx with a metal surface.

Contact of a 10% (wt/wt) solution of Gum Arabic onto a copper film deposited on a germanium cylindrical IRE led to a slow change in IR spectrum, primarily manifested as a gradual increase in the intensity of the 1640 cm^{-1} water band with time of incubation (Fig. 3C). Adjustment of the polysaccharide solution from pH = 4 to neutral pH did not affect the result. The presence of the polysaccharide is therefore believed to promote the dissolution or peeling of the copper film from the IRE.

That Gum Arabic became intimately associated with the copper film on the germanium IRE was demonstrated by the appearance of an absorption band centered at 1050 cm^{-1} , due to the stretching mode of the C-O bond in the sugars of the polysaccharide. Evidence that the polysaccharide was concentrated on the copper film was provided by the relative intensity of the 1050 cm^{-1} polysaccharide absorption band and the 1640 cm^{-1} water band. For uncoated germanium and zinc selenide IREs, neither of which appeared to absorb the polysaccharide, the intensity ratio of the polysaccharide/water bands was the same, even though the sampling depths for these two crystals were quite different (see Table I). An intensity ratio of approximately 0.20 indicates that no polysaccharide is adsorbed to the crystal surface. Any increase in this value is indicative of an increase in

polysaccharide concentration, since water acts as an internal standard near the surface of the IRE. Such an increase was observed experimentally when a copper-coated, germanium IRE was submerged in the polysaccharide solution (Table I). Intensity ratios greater than 0.20 were obtained over a range of water band intensities, reflecting different copper film thicknesses (Table I).

After long (>60 h) periods of exposure of the copper-coated Ge IRE to Gum Arabic solution, the intensity of the polysaccharide absorption band gradually increased. Thus, as the dissolving copper film exposed the underlying Ge surface, the spectrum began to assume that of an uncoated Ge IRE. A more detailed discussion of the interactions between biopolymers and metal surfaces submerged in an aqueous environment as studied in our laboratories by ATR spectrometry will appear in the near future.⁸

The use of metal-coated internal reflection elements appears to have great potential for the investigation of chemical and biological phenomena at metal surfaces. Careful control of the thickness and homogeneity of the coating is required for these measurements. It is anticipated that the techniques described in this paper will become useful for measurements of phenomena occurring at both gas-solid and liquid-solid interfaces.

ACKNOWLEDGMENTS

Fruitful discussions with Robert J. Jakobsen of the National Center for Biomedical Infrared Spectroscopy are gratefully acknowledged. We also wish to thank Professor William Thompson and Dr. Kathryn Platt of the Botany Department, UCR for the electron micrographs.

1. R. M. Gendreau, R. I. Leininger, S. Winters, and R. J. Jakobsen, in "Morphology, Structure, and Interactions of Biomaterials," S. L. Cooper, A. S. Hoffman, N. A. Peppas, and B. D. Ratner, Eds., ACS Advances in Chemistry Series 199 (ACS, Washington, D.C., 1982).
2. R. J. Jakobsen, L. L. Brown, S. Winters, and R. M. Gendreau, *J. Biomaterials Res.* 16, 199 (1983).
3. R. J. Jakobsen, S. Winters, and R. M. Gendreau, *Proc. Soc. Photo-Opt. Instrum. Eng.* 289, 469 (1981).
4. R. M. Gendreau, S. Winters, R. I. Leininger, D. Fink, C. R. Hassler, and R. J. Jakobsen, *Appl. Spectrosc.* 35, 353 (1981).
5. S. Winters, R. M. Gendreau, R. I. Leininger, and R. J. Jakobsen, *Appl. Spectrosc.* 36, 404 (1982).
6. R. J. Jakobsen, "Application of FT-IR to Surface Studies," in *Fourier Transform Infrared Spectroscopy*, J. H. Ferraro and L. J. Basile, Eds. (Academic Press, New York, 1979).
7. J. W. Costerton, G. G. Geesey, and K.-J. Cheng, *Scientific American* 238, 86 (1978).
8. G. G. Geesey, T. Iwaoka, and P. R. Griffiths, *Anal. Biochem.*, submitted for publication (1986).