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10. We used an ion chromatograph (Dionex model 10) with a 500-mm anion separator column with 1.2 m*M* Na_2CO_3–1.5 m*M* $NaHCO_3$ eluent at a 30 percent pump rate. Under these conditions, $CH_3OSO_3^-$ was well separated from NO_2^- (Fig. 1c); NO_2^- does not separate from monomethyl sulfate in this instrument with the usual anion eluent (*9*) which is twice as concentrated nor does it separate with Na_2CO_3-NaOH or borate eluents.
11. We used a gas chromatograph (Hewlett-Packard 5720A) equipped with an FID. The glass column (1.2 m long by 6.3 mm in inside diameter) was packed with 35/60 mesh Tenax. The carrier gas was helium, at a flow rate of 30 ml/min. The oven was run isothermally at 100°C.
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15. This work was supported by the Electric Power Research Institute under contract RP1154-1. We thank C. E. Chrisp and G. L. Fisher for many helpful discussions during the preparation of the manuscript. Contribution No. 182 from the Thermochemical Institute, Brigham Young University.

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A Palladium–Palladium Oxide Miniature *p*H Electrode

Abstract. *A wire-form miniature palladium-palladium oxide electrode has been fabricated for* p*H measurement. The electrode exhibits a super-Nernstian behavior and gives a mean* p*H response of 71.4 millivolts per* [p*H*] *(standard deviation, 5.2 millivolts). Uncorrected zero current potential values can be used to determine the* p*H value of the medium to within 0.012* p*H. The electrode should find applications in biological, medical, and clinical studies.*

Miniature or microminiature sensors are needed for *p*H measurements of blood and extracellular fluids as well as for laboratory animal micropuncture studies. Very small glass electrodes have been fabricated for these applications (*1*); however, the fragile nature and thrombogenic characteristics of the glass membrane have limited in vivo applications of this sensor. Antimony–antimony oxide and quinhydrone electrodes have also been used, but their stability is relatively poor (*2*). Coon and his coworkers have reported that palladium oxide (PdO) can be used for *p*H sensing in a pCO_2 sensor (pCO_2 is the partial pressure of CO_2) (*3*), but PdO has not been directly used as a miniature *p*H sensor.

In biological and medical investigations, there is a need for a continuously operating, miniature *p*H electrode with reasonable reliability, stability, sensitivity, and durability. We have fabricated a Pd-PdO wire-form electrode which is *p*H-sensitive both in various buffers and in whole blood. The sensor's diameter is either 0.25 or 0.5 mm. The zero current potential measured between the sensor and an Ag-AgCl reference electrode in a test medium exhibits a linear relation between the potential and the *p*H value of the medium. Its *p*H response is reproducible to within 0.25 *p*H without calibration, and it should find use in a wide range of biological and medical applications.

Betteridge and Rhys (*4*) have demonstrated that a thin PdO surface film can be formed when Pd metal is heated to 973 K in air. This film is too thin to form a stable electrode. We have taken an alternate electrochemical approach in which pure Pd wire (0.25 or 0.5 mm in diameter and approximately 90 mm long) served as the anode and pure Pt wire (0.25 mm in diameter) was used as the cathode. A mixture of a 98 percent $NaNO_3$ (by weight) and 2 percent LiCl was used as a molten-salt electrolyte (melting point, 583 K). Empirically, we found the best performing Pd-PdO *p*H electrode was formed with an anodic potential of 5.9 to 6.2 V, a current density of 20 mA/mm², and an oxidation time of 90 seconds. A well-oxidized Pd wire had a velvety black appearance. When a thinner oxide layer was formed, the oxide film was light gray or brown. After oxidation, the electrode was stored in a phosphate buffer (*p*H 7) for a minimum of 24 hours. This preconditioning of the electrode enhanced its performance.

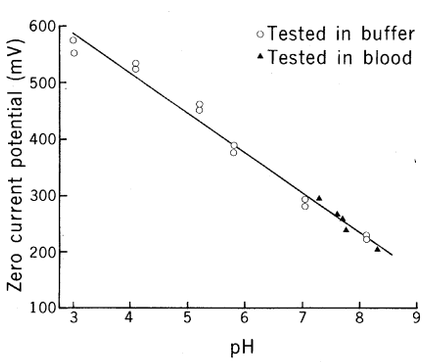

Fig. 1. Calibration curve of a Pd-PdO *p*H electrode in buffer solutions and whole blood at 310 K. The potential was measured versus an Ag-AgCl electrode.

The Pd-PdO electrode reacts with H^+ according to the reaction

$$PdO + 2H^+ + 2e^- \rightleftarrows Pd + H_2O \quad (1)$$

The activities of PdO and Pd for this electrode are unity; thus the *p*H response in the presence of a large quantity of water should be given by the Nernst equation

$$E = E^0 - \frac{RT}{F}[pH] \quad (2)$$

where E is the measured zero current potential, E^0 is the standard-state potential, R is the gas constant, T is the absolute temperature, and F is the Faraday constant.

Electrodes were calibrated in buffer solutions and in whole blood with a standard Ag-AgCl electrode as a reference. The electrical source impedances of the electrodes were nominally 600,000 ohm/mm²; thus for our sensors which had an active length of 38 mm, the source impedance was approximately 10,000 ohm. This meant that the zero current potential between the Pd-PdO and the reference electrodes could be measured with an ordinary electronic voltmeter or high-input impedance chart recorder.

Figure 1 shows the response of a Pd-PdO electrode in buffer solutions and blood, the latter over the physiological *p*H range of 7 to 8.2. The absolute values of the zero current potentials are reported. The reproducibility of the *p*H response taken at different times indicates that this electrode does not have an asymmetric potential such as the glass electrode and thus frequent recalibration is not necessary. The super-Nernstian behavior may indicate the interaction between H^+ and the electrode surface.

Ten electrodes gave a mean *p*H response of 71.4 mV/[*p*H] (the standard deviation is 5.2, and the coefficient of variation is 7.3 percent). Linear regression analysis gave a mean correlation coefficient of .995. At *p*H = 6.98, the ten electrodes gave a mean absolute potential of 313.2 mV (standard deviation, 3.7). This corresponded to a difference of 0.012 *p*H.

The time response of the electrode, determined from step changes of *p*H in phosphate buffer solutions, was on the order of 0.5 second. No observable hysteresis effects were seen throughout the test. It is difficult to say whether this response time was limited by solution mixing or the electrode.

The theoretical temperature effect on

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the sensor output is 0.02 mV per degree Kelvin, which is negligible. Our experimental measurements over the temperature range of 297 to 315 K and a *p*H range of nominally 6 to 8 show that the temperature effect on the sensor output is within experimental error and negligible.

In addition to the pure Pd wire used in this study, we also investigated a Pd-Ag alloy wire (60 percent Pd and 40 percent Ag). This alloy is stronger than pure Pd and might be more suitable for certain applications. Our results showed that the *p*H electrode characteristics of this alloy are identical to those of pure Pd.

Palladium oxide can dissolve in a medium containing Cl^-. Coating the electrode with a cation-permeable polymer material, Nafion latex, to minimize the dissolution of the oxide was investigated. The coating proved to be effective and did not alter the sensitivity of the electrode, but it did significantly increase the response time. Without this coating, the fabricated Pd-PdO electrode could be reused repeatedly in a normal saline solution over a period of 6 days without any deterioration in performance. Coated electrodes operated for as long as 10 days.

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Human Rotavirus Type 2: Cultivation in vitro

Abstract. *A strain of type 2 human rotavirus (Wa) was grown to relatively high titer through 14 passages in primary cultures of African green monkey kidney (AGMK) cells. This passage series was initiated with virus that had been passaged 11 times serially in newborn gnotobiotic piglets. In contrast, virus present in the stool of patient Wa as well as virus from the first, second, or third passage in piglets could not be propagated successfully in African green monkey kidney cells. Prior to each passage in cell culture, the virus was treated with trypsin and the inoculated cultures were centrifuged at low speed. Cultivation of a type 2 human rotavirus should aid attempts to characterize this virus and to develop a means of immunoprophylaxis for a serious diarrheal disease of human infants.*

Rotaviruses of the family Reoviridae are now recognized as major pathogens causing acute diarrheal disease in the young of a number of species including man (*1*, *2*). Only a few animal rotaviruses have been adapted to grow to high titer in cell culture (*1*–*3*). Numerous attempts to propagate human rotaviruses to high titer in vitro have failed (*4*–*8*). In this report we describe the successful adaptation of a human rotavirus strain that grows efficiently in primary cultures of African green monkey kidney (AGMK) cells.

We obtained 42 fecal specimens from pediatric patients with diarrheal disease; the patients were previously shown to be infected with rotavirus by electron microscopic examination of their feces (*9*–*11*). The specimens were tested for their efficiency in initiating infection in AGMK cells. A 0.5-ml portion of a 2 to 10 percent stool filtrate was mixed with 0.5 ml of trypsin (20 μg/ml) (Sigma) and incubated for 1 hour at 38°C. The mixture was then inoculated into a culture well containing approximately 10^6 AGMK cells. Plates containing six such wells were centrifuged at 1400*g* in an IEC centrifuge (model PRJ) for 1 hour at 37°C, after which the inoculum was removed. This technique of centrifugation had previously been shown to increase the efficiency with which various rotaviruses initiated infection of cells in culture (*5*, *12*, *13*). Plates were washed once and refed with L-15 medium containing a solution of sucrose, phosphate, and glutamate (*14*) as well as glutamine and antibiotics. The cells were incubated for 48 hours at 37°C and then fixed with cold (−20°C) methanol and stained for examination by direct fluorescence microscopy by using fluorescein-conjugated goat antiserum (immunoglobulin G fraction) to human rotavirus. The percentage of antigen-containing cells was estimated. Thirty-one of the fecal suspensions were positive by this technique, but the proportion of cells exhibiting viral antigens was low in most instances, 1 percent or less. Only five specimens induced antigens in 10 percent or more of cells. These five strains were previously determined to be serotype 2 by the enzyme-linked immunosorbent assay (ELISA) (*10*).

To promote the growth of viruses that induced antigens in 10 percent or more of cells and hence increase the probability of the emergence of a cell culture–adapted mutant, we administered orally 1 to 2 ml of a 2 percent bacteria-free fecal filtrate containing type 2 rotavirus from patients S or Wa to four 26-hour-old gnotobiotic piglets (*15*, *16*). The piglets developed mild, transient diarrhea. Intestinal contents for subsequent passage were collected from individual animals killed 46 to 48 hours after inoculation. Examination of intestinal contents from the passage in piglets revealed rotavirus by electron microscopy, ELISA, and quantitative fluorescence microscopy (*12*). A piglet inoculated with Wa strain of human rotavirus had a titer of $10^{5.7}$ fluorescent foci (FF) per milliliter of intestinal contents. Immunofluorescence staining of jejunal impressions (*15*) revealed that 2 to 3 percent of epithelial cells were infected with rotavirus. Because infection by the Wa strain was slightly more extensive than that seen with the S strain, the former was used for subsequent passages.

Ten additional passages in gnotobiotic piglets (6 to 144 hours old) were performed serially, 1 ml of a mixture of contents from the small and large intestine being used as the inoculum except during the second passage when mixed intestinal contents were diluted 1:5 and the third passage when an aspirate from the large intestine was used as the inoculum. At each passage the virus administered orally consisted of gut contents collected at necropsy from piglets killed 19 to 48 hours after they were inoculated with virus. At each passage, infection was documented by the detection of viral antigen in gut contents. During passage 2 in piglets the quantity of virus in intestinal contents was $10^{6.8}$ FF per milliliter and remained at approximately this level during the subsequent nine passages. Infection was also confirmed by detection of rotavirus by electron microscopy of intestinal contents and direct fluorescence microscopy of intestinal impressions. During passage

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