GENERAL PHYSICS

I. MOLECULE MICROSCOPY

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A. SUMMARY OF RESEARCH PROGRESS

JSEP

Joint Services Electronics Program (Contract DAAB07-75-C-1346)
National Institutes of Health (Grants 5 SO5 RR07047-10, 5 PO1 HL14322-05, and 1 RO1 GM22633-01)

John G. King, James C. Weaver

We are continuing the development and application of molecule microscopy which makes possible the mapping of spatial variations of such sample properties as permeability, diffusion, binding of neutral molecules, and/or temporal distribution of enzymes.

1. Scanning Pinhole Molecule Microscopy (SPMM)

During the past six months much time has been spent studying various aspects of the SPMM system which are common to both the vascular smooth muscle experiments and any form of in vitro molecule microscopy. Our objective is to measure small concentrations of dissolved molecules such as oxygen or carbon dioxide. Even with semipermeable membranes as a vacuum/sample interface, however, there is a large flux of water into the vacuum system. These water molecules may undergo a variety of reactions in the electron bombardment ionizer, depending on the material and temperature of the filaments, and the potentials of the ionizer electrodes. Thus, for example, when using thoria-coated iridium filaments, we observed a false oxygen signal, approximately 6% of the water signal. The exact mechanism is not understood, but this effect does not take place to any measurable degree with tungsten filaments, although the well-known "water cycle" does. Preliminary results also lead us to suspect that atomic oxygen from the water molecules combines with either carbon impurities in the tungsten or residual carbon on the chamber walls to give carbon dioxide.

Another problem that has been studied is the variation of the electron multiplier gain with differing species and input current. These effects are particularly important when precise quantitative measurements of rates of change of several species are wanted simultaneously.

We have done some work on trying to understand the basic signal-to-noise limitations of quadrupole mass spectrometers. We have designed improved driver circuitry for the

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JSEP scanning stage of SPMM that should substantially reduce vibrations and allow minimum scanning increments of 6×10^{-7} cm.

2. Scanning Desorption Molecule Microscopy (SDMM)

H. F. Dylla (now at the Princeton Plasma Physics Laboratory), M. J. Cardillo (now at Bell Laboratories) and J. G. King are completing the analysis of the experiments carried out last year by H. F. Dylla, and are preparing a paper for publication. The work is best summarized by the draft abstract.

The adsorption of CO on highly polished (planar) surfaces and oxygen-etched Si(111) and Si(100) surfaces has been studied by a combination of Scanning Electron Microscopy (SEM), Auger Electron Spectroscopy (AES), Electron Stimulated Desorption (ESD), and flash desorption mass spectrometry techniques. Average sticking probabilities, saturation coverages, Arrhenius parameters for desorption, and the effect of reaction etch pits on each of these have been determined. In contrast to previous findings in other laboratories, we find sticking probabilities of CO on planar Si surfaces and high saturation coverages. The presence of pits dramatically increases the sticking probabilities, as well as the characteristic desorption temperatures. The low preexponential factor in the Arrhenius equation for CO desorbing from the planar and pitted surfaces, as well as the anomalous lack of either AES or ESD signals for all CO coverages (up to 1 monolayer), is discussed in terms of CO penetration below the surface.

3. Desorption Experiments Related to the Scanning Desorption Molecule Microscope (SDMM)

B. R. Silver has investigated electron-stimulated desorption of such molecules as D_2O and ethanol from surfaces such as platinum and platinum coated with glycogen and BSA. He has found cross sections as large as 10^{-15} cm^2 and investigated their energy dependence. These cross sections (as much as 10^4 times larger than cross sections for more strongly bound molecules) are very encouraging from the standpoint of resolution and sensitivity in the application of the SDMM.

D. G. Lysy has continued his studies of the thermal desorption of water from platinum and platinum coated with BSA, glycogen, poly-L-Alanine, poly-L-Lysine Hydrobromide, and Tristearin. It is encouraging to see that the flash desorption spectra vary considerably and we can foresee preparation of an extensive catalogue of spectra of combinations of staining molecules and biological materials and interpretation of the results at various levels of detail and rigor. The apparatus used by Lysy is well suited also for studies of denaturation. We are now almost ready to introduce biological material into the SDMM developed by Dylla, using a suitable sample holder that is still to be designed and to observe the ESD and/or thermal desorption of sample molecules or JSEP staining molecules applied to the sample.

4. Molecule Fluxes in Tissue

We have also been exploring the application of mass spectrometry methods to the study of fluxes of volatile molecules through biological tissues and membranes. This work is now being performed by S. J. Rosenthal in the laboratory of Dr. Alvin Essig at Boston University Medical School. Recent experimental results show that essentially simultaneous measurement of O_2 consumption and CO_2 productions are feasible; this approach can be extended to the nonvolatile species, lactate, by use of the VEP method.

5. Volatile Enzyme Product Method

We have completed an initial VEP apparatus and have begun to characterize it. Preliminary data on passive, continuous removal of preexisting volatile contaminants and on enzyme assays are in fair agreement with experiment, and therefore very encouraging. Specifically, with a nonoptimal arrangement, we have been able to detect and measure 7×10^9 enzyme molecules (urease) in $\sim 10^2$ seconds. A more detailed discussion of this technique follows in Section I-B.

B. PROGRESS WITH THE VOLATILE ENZYME PRODUCT (VEP) METHOD

National Institutes of Health (Grants 1 RO1 GM22633-01 and 5 SO5 RR07047-10)

Jerome H. Abrams, James C. Weaver, Frederick Villars, Augustine J-Y. Chong

Recent work on the first apparatus devoted to the volatile enzyme product (VEP) method is described in this report. This method, which combines the specificity of enzymes with the sensitivity of mass spectrometry, offers a potential for rapid assays of substrates, cofactors, enzymes, and inhibitors for many enzyme-catalyzed reactions.[1, 2] The method interfaces a solubilized enzyme or an immobilized enzyme reaction to a mass spectrometer by means of a synthetic semipermeable membrane, at present, dimethyl silicone. Depending on the exact configuration, much or most of the volatile product of the reaction enters a region of relative vacuum where a mass spectrometer counts individual, volatile-product molecules with an efficiency $\epsilon_T \approx 3 \times 10^{-5}$.

During the past year we have focused our work on the design and construction of an apparatus that can provide a low background at the mass peak or peaks of interest (e. g., CO_2, ethanol, and acetic acid), that will have a high total counting efficiency, ϵ_T, for rapid counting of individual molecules and the flexibility to accommodate many anticipated design changes. The apparatus based on these considerations is shown in Fig. I-1. This system features an approximately 50-liter stainless-steel vacuum system pumped

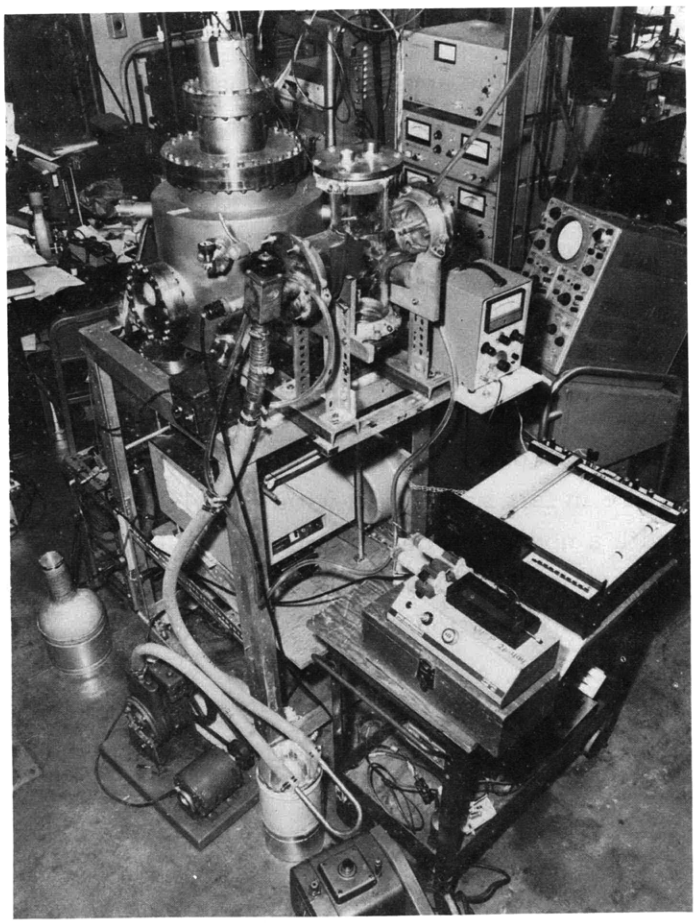

Fig. I-1. First apparatus devoted to the VEP method.

by an oil diffusion pump (1200 l/s nominal pumping speed). The main pump is backed with a second oil diffusion pump and forepump; all pumps are separated from the main vacuum system by a liquid nitrogen trap. The mass spectrometer, which is suspended from the central top flange, is an Extranuclear quadrupole mass filter spectrometer (QMS) supplied by Extranuclear Laboratories, Pittsburgh, Pennsylvania. It has an axial ionizer with a stated efficiency of approximately 10^{-3}. The membrane, and a small channel that brings a liquid sample to the membrane, are located on a flange at the bottom of the apparatus (Fig. I-2). Most of our recent work has been concerned with this sample-handling region.

The vacuum system has not yet been thoroughly baked at 400°C, a procedure that should provide an essentially permanent reduction by a factor of approximately 30 in the CO_2 background. Other features, such as continuous passive degassing of pre-existing volatile contaminants (e. g., CO_2), have been investigated but have not yet been used in the present apparatus. Our results are therefore subject to these limitations.

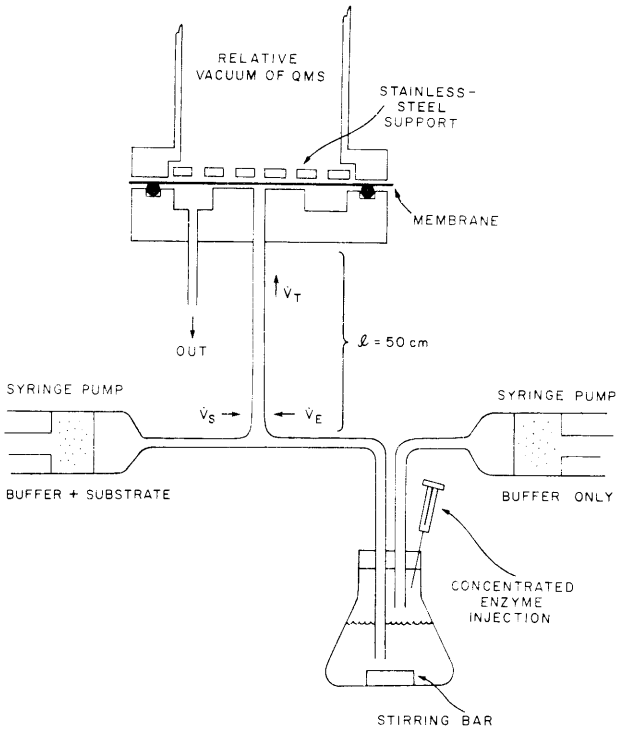

Fig. I-2. Sample-handling portion of the apparatus.

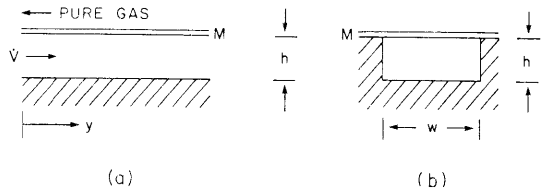

Fig. I-3. Configuration for continuous, passive degassing of a sample tested (see Fig. I-4a) by substitution of the relative vacuum of the QMS for the pure gas region. $\dot{V}$ is the total volume flow rate (ml-sec^{-1}) of the sample, h is the thickness of the degassing channel, w is its width, and M is a dimethyl silicone membrane. (a) Side view. (b) End view.

Much effort has been directed toward testing and characterizing the apparatus. For example, the total counting efficiency ϵ_T was determined to be $\sim 3 \times 10^{-5}$ by direct injection of a steady flux of CO_2 just above the membrane in the sample region (Fig. I-2). This efficiency is somewhat greater than two orders of magnitude better than that obtained in the first prototype apparatus[2, 3] but still less than that obtainable in an optimal configuration. We have also tested our ability to recover volatiles from a

flowing stream in a thin channel (Fig. I-3). A simple model suggests that if $\dot{V}$ is the volume flow rate, and D_V the diffusion constant of the volatile molecule in water, then the concentration of a preexisting volatile $C_V(0)$, should fall off approximately exponentially as

$$C_V(y) \approx C_V(0) \exp(-y/\lambda), \tag{1}$$

where $\lambda = h\dot{V}/wD_V$. If $f(\dot{V})$ is the fraction of the preexisting volatile that leaves the flowing sample stream by permeating the membrane and escaping into the counterflow of pure gas, and if Δy = total membrane-channel length, then

$$f(\dot{V}) \approx 1 - \exp(-wD_V\Delta y/h\dot{V}). \tag{2}$$

The prediction of this simple model has been tested for CO_2. In these experiments, a vacuum and the QMS have been substituted for the pure gas counterflow region to measure directly the flux of CO_2 across the membrane. We expect the steady-state count rate in this case to be

$$\dot{n}_s = \epsilon_T f(\dot{V})\, \dot{V} C_V(0). \tag{3}$$

Several experiments have been performed on the membrane-channel configurations shown in Fig. I-4; the experimental results are shown in the curves. As expected, the simple calculation does not hold for large $\dot{V}$, but in the small $\dot{V}$ region agreement with theory is good. Both membrane-channel configurations show a region at low $\dot{V}$ where $\dot{n}_s$ is nearly proportional to $\dot{V}$, which indicates that in this region $f(\dot{V}) \approx 1$. This result confirms our expectations that we ought to be able to degas an incoming sample stream efficiently by using a continuous and essentially passive method and, after passing the sample past an immobilized enzyme region or introducing a solubilized enzyme, recover most of the volatile product into the mass spectrometer in a second thin-channel membrane section.

We have also made some tests by varying the concentration of the preexisting volatile, in this case, dissolved CO_2 and bicarbonate. Although a multiple equilibrium exists, we may calibrate the apparatus by varying the concentration of HCO_3 and using Eq. 3.

We have also observed a transient response correlated with injection of the sample. This effect seems to be attributable to the transient increase in pressure occurring during injection which is such that $\dot{V}$ increases and, by Eq. 3, the count rate also increases. Recent sample-handling improvements have reduced but not eliminated this artifactual, transient response.

Finally, using urease, we have recently obtained some preliminary results on

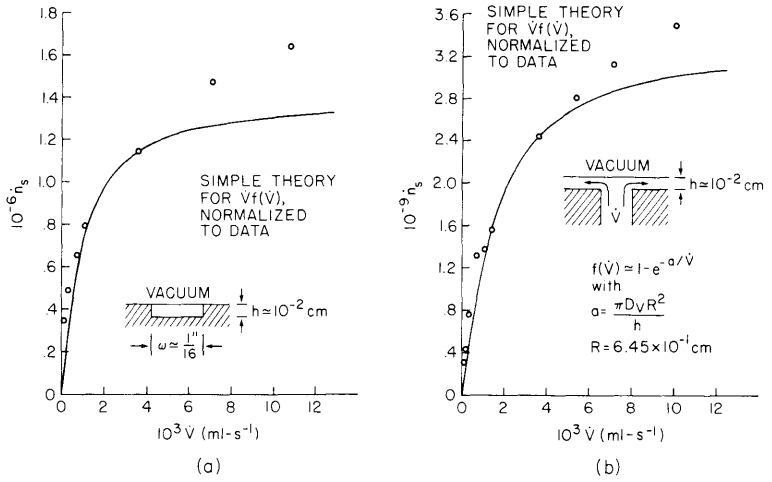

Fig. I-4. (a) Comparison of experiment and theory (Eqs. 2 and 3) for the channel shown. A .001 inch thick dimethyl silicone membrane was used, and CO_2 ($D_V = 2 \times 10^{-5}$ $cm^2\text{-}s^{-1}$) in a solution buffered at pH 7.0 was the test volatile molecule. In Eq. 2, $\Delta y = 5$ cm.

(b) Similar comparison for a related, radial flow geometry. CO_2 was the test volatile, but with pH 5.8, a larger membrane area, and different QMS tuning than in (a).

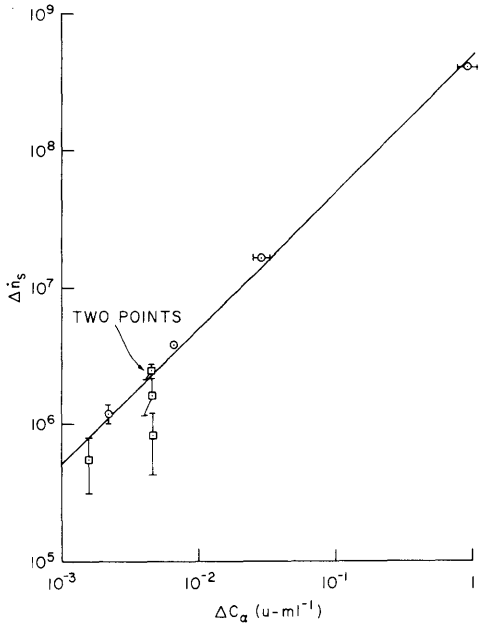

Fig. I-5. Preliminary data for enzyme assay using urease (EC 3.5.1.5) and CO_2 as the volatile product molecule. ⊙ is from the run of 4/29/76, and ⊡ from 5/27/76, both with pH 7.0.

enzyme assay. A typical response curve is shown in Fig. I-5. Note that these data were obtained with a fundamental time response that is obscured by the artifactual transient associated with injection of the sample. With this limitation, an interval of approximately 10^2 s was required to measure the change in count rate, $\Delta\dot{n}_s$, following an enzyme injection that produced a change, ΔC_a, in enzyme activity concentration. At the lower levels, the measured response can be attributed to the detection of $N_E = \dot{V}_T \Delta C_a \nu_T^{-1} t_R \approx 7 \times 10^9$ urease molecules ($\nu_T \approx 3 \times 10^2$ molecules-s^{-1} is the turnover number for urease, and $t_R \approx 10^2$ is the longer approximate response time caused by the artifactual response) in a solution volume of $\Delta_V = \dot{V} t_R = 10^{-1}$ ml, i.e., a minimum concentration detection of enzyme of $\sim 10^{-10}$ M. The observed sensitivity, $\sim 5 \times 10^8$ cps-ml-u^{-1}, is in reasonable agreement with a simple calculation, which predicts[4] a sensitivity of $\sim 2 \times 10^8$ cps-ml-u^{-1}.

As this work continues, attention will be directed toward obtaining thinner membranes so that other volatile products, such as acetic acid and ethanol, can be used more effectively. For such exploratory work on different membranes and channels, we have constructed a simple, separate test vacuum system, so that when a membrane bursts or leaks, no damage to the QMS will occur. After this has been accomplished, we shall turn our attention to other enzyme systems such as DOPA decarboxylase (EC 4.1.1.26), isocitrate dehydrogenase (EC 1.1.1.42), carbonic anhydrase (EC 4.2.1.1), pyruvate decarboxylase (EC 4.1.1.1), lactate dehydrogenase (EC 1.1.1.27), and acetylcholinesterase (EC 3.1.1.7).

During the course of this work we have enjoyed many stimulating discussions with other members of our group, particularly with J. G. King, J. A. Jarrell, A. M. Razdow and J. W. Peterson, and also with C. L. Cooney of the M. I. T. Nutrition and Food Science Department. This work was supported in part by the Francis Friedman Chair of Physics.

References

1. J. C. Weaver, Quarterly Progress Report No. 114, Research Laboratory of Electronics, M.I.T., July 15, 1974, pp. 8-10.
2. J. C. Weaver, M. K. Mason, J. A. Jarrell, and J. W. Peterson, "Biochemical Assay by Immobilized Enzymes and a Mass Spectrometer," Biochim. Biophys. Acta 438, 296-303 (1976).
3. M. K. Mason, S.B. Thesis, Department of Physics, M.I.T., June 1974.
4. 1 Unit = 1 u = 1 International Unit of Enzyme Activity = 1 μmol-min^{-1} = 10^{16} molecules-sec^{-1} of product by the enzyme catalyzed reaction; see T. E. Barman, Enzyme Handbook, Vol. 1 (Springer-Verlag, New York, 1969).