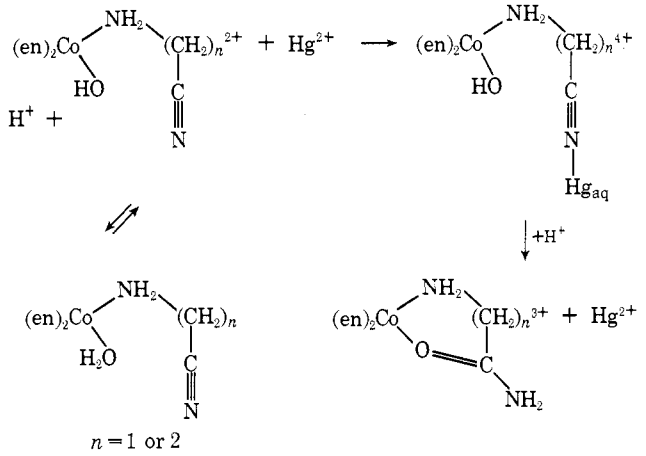
Calcd: C, 17.13; H, 4.93; N, 22.84; Cl, 7.22. Found: C, 16.79; H, 4.99; N, 22.71; Cl, 7.47.

The results are rationalized in the following mechanism where the electrophile Hg^{2+} adds to the nitrile N atom and thereby assists the attack of the nucleophile–Co–OH.



$n = 1$ or 2

From published data[5] for propionitrile the specific rates for base-catalyzed hydration of free NH_2CH_2CN and $NH_2CH_2CH_2CN$ are estimated as $\sim 10^{-6}$ M^{-1} sec^{-1}; the specific rates for the corresponding hydroxocobalt(III) complexes assisted by Hg^{2+} (0.1 M) are estimated as $>10^5$ M^{-1} sec^{-1} and 10^2 M^{-1} sec^{-1} at 25°, respectively.[6] Since there is a linear dependence upon Hg^{2+} up to at least 0.5 M Hg^{2+}, the $[Co(en)_2(OH)(RCNHg]^{4+}$ species is far from saturation and its specific rate must therefore be greater than the estimates above. This implies that there is a rate enhancement of $>10^{18}$ and $>10^{15}$ for the complexed aminonitriles at pH 7 relative to the uncoordinated nitriles. These enhancements might be compared with those in previous studies ($\sim 10^8$) using labile metal ion complexes.[7-9] Part of this acceleration is attributed to the Hg^{2+} addition, which enhances the attack of the nucleophile, and part must be attributed to the "intramolecular chelation." Factors of 10^8 or more have been observed for the latter effect relative to uncoordinated substrates. Moreover the formation of five-membered rings appears to be generally faster than their six-membered counterparts in analogous circumstances.[1,2,10] This last pattern is also preserved in this instance.

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D. A. Buckingham, A. M. Sargeson,* A. Zanella
Research School of Chemistry, Australian National University Canberra, A.C.T. 2600, Australia
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Hollow Fiber Enzyme Reactors

Sir:

Enzymes have been physically adsorbed on surfaces by electrostatic interactions, cross-linked around a carrier, entrapped within a gel lattice, covalently attached to porous and nonporous solids,[1-4] and encapsulated within semipermeable microcapsules.[5,6] Such procedures facilitate enzyme separation from products, both in batch and continuous operation, and in certain cases partially stabilize the enzyme against thermal denaturation. We wish to report what are apparently the first successful experiments in which enzymes are contained in (and, in effect, "insolubilized" within) semipermeable hollow fibers.[7,8]

In experiments designed to demonstrate the feasibility of the concept, the fiber bundle within a Dow Chemical Co. beaker dialyzer b/HFD-1 was filled with a 0.1 mg/ml of solution of alkaline phosphatase (Worthington Biochemical Corp. BAPC) in 0.05 M Tris at pH 8.0 and then sealed with a pair of eyedropper bulbs. A solution of disodium 4-nitrophenyl phosphate (100 ml) in 0.05 M Tris at pH 8.0 was then rapidly added to the well-stirred dialyzer. The initial rate of reaction was monitored at 410 nm with the aid of a Beckman DB-G uv–visible spectrophotometer and a Technicon pump that recirculated the external solution through a 1-cm flow-through cell. After the initial rate of reaction was determined, the 100 ml of solution external to the fiber bundle was rapidly poured into a separate 150-ml beaker and continuously monitored at 410 nm for indications of enzyme leakage. At the completion of each experimental run, the hollow fiber bundle was dialyzed with copious amounts of deionized water and 4 × 100 ml of 0.05 M Tris at pH 8.0. In separate experiments, the activity of the alkaline phosphatase was determined to be 5.4 μmol of 4-nitrophenol liberated per minute per milligram of enzyme for a solution that was 1.06×10^{-3} M in substrate and 0.90 M in Tris at pH 8.0 (approximately 5.4 units/mg according to the Worthington assay).

The initial reaction rate was directly proportional to the substrate concentration over the entire range of substrate concentrations studied—36–640 μM. The pseudo-first-order "rate constant" determined from a plot of initial rates *vs.* substrate concentration was 1.19×10^{-2} min^{-1}. This result is in distinct contrast to the behavior of alkaline phosphatase in the absence of hollow fibers, under which conditions the reaction is normally zero order at substrate concentration levels above 180 μM.

The limiting rate of permeation of the substrate–product system through the fiber walls was determined by increasing the amount of alkaline phosphatase within the fiber bundle to 20 mg. At such high enzyme concentrations, the hollow fiber beaker functioned as a *reactor dialyzer*, with the measured pseudo-first-order rate constant being approximately[9] equal to the permeation rate of the substrate under normal dialysis condi-

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tions. The characteristic constant, β, in the dialysis equation,[7] $\ln c/c_0 = -\beta t$, where c_0 is the substrate concentration at $t = 0$ and c is the concentration at time t, was determined to be 2.1×10^{-2} min^{-1} for a substrate solution containing 0.05 *M* Tris at pH 8.0 and 25°. To ensure that the disodium salt of the substrate was the diffusing species, 0.85 *M* NaCl was also present. When 0.85 *M* tetra-*n*-butylammonium bromide was substituted for the NaCl, the activity of the free alkaline phosphatase, in the absence of hollow fibers, was inhibited by a factor of 3.5, whereas the value of β decreased by a factor of seven to 0.3×10^{-2} min^{-1}, a value consistent with the increased bulkiness and decreased solubility in the membrane of the diffusing salt. We wish to emphasize that, when the hollow fiber reactor is operated in the diffusion-controlled regime, *a modest change either in the concentration or activity of the contained enzyme will have a negligible influence upon the measured value of* β.[7] The above values of β compare favorably with 3.9×10^{-2} min^{-1} for 4-nitrophenol dissolved in 0.05 *M* Tris at pH 8.0 and with the following data given in the Dow brochure on the b/HFD-1 beaker dialyzer: 12.5×10^{-2} min^{-1} for NaCl, 9.0×10^{-2} min^{-1} for creatinine, 4.8×10^{-2} min^{-1} for sucrose, 2.2×10^{-2} min^{-1} for raffinose, and 1.1×10^{-2} min^{-1} for vitamin B_{12}.

Experiments with chymotrypsin were similarly successful. Two milligrams of chymotrypsin (Worthington) were dissolved in 2 ml of 0.05 *N* HCl and placed in the interior of the hollow fibers of the same beaker dialyzer as used above. One hundred milliliters of a solution containing 0.07 *M* $CaCl_2$, 23.3% methanol, 0.035 *M* Tris at pH 7.80, and 5.0×10^{-4} *M* benzoyl-L-tyrosine ethyl ester (BTEE) was rapidly added to the dialyzer and the initial rate monitored at 256 nm and 25°. The measured value of β was 1.1×10^{-2} min^{-1}. When the amount of chymotrypsin was increased to *ca.* 20 mg, the value of β became 2.0×10^{-2} min^{-1}. As an upper limit for enzyme leakage, less than one part in 12,000 leaked out of the fibers during the course of 2 hr of experimentation with the more concentrated enzyme system. With hydrolytically stable substrates, such an enzyme leakage test is extremely sensitive. The activity of the chymotrypsin used in these experiments was, in the absence of the fibers, 31 μmol of substrate hydrolyzed per minute at 25° and the above solution conditions.

Less detailed exploratory experiments, which will not be discussed here, with urease and catalase also demonstrated the value of the hollow fiber reactor approach. The activity of the catalase was followed by winding 12–36 b/HFO-1 beaker osmolyzer strands containing the enzyme around a small magnetic stirrer present in a Yellow Springs Instrument Co. Model 53 biological oxygen monitor. The enzyme-containing sealed hollow fibers were also wound around a small stirring rod, which provided a convenient enzyme "tea bag" for conducting small batch reactions.

Our experiments clearly demonstrate that cellulose and cellulose acetate hollow fiber membranes can readily contain any individual enzyme, and presumably any mixture of enzymes as well, without the use of chemical reactions. *No enzyme leakage was detected with any of the four enzymes studied in this investigation.* The commercial and laboratory advantages of hollow fiber enzyme reactors are readily apparent. Contained enzymes can be re-used in different experimental runs and then recovered at the end of a series of runs by flushing the interior of the fiber bundles with deionized water. The ability to flush the fibers also permits the recirculation of an enzyme solution as well as the re-use of the fibers for different enzymes. Quantitation of such procedures for a variety of enzymes has yet to be performed, however.

Finally, we make no claims for enzyme stabilization within the hollow fibers; in fact, some enzymes may be destabilized owing to the large internal surface area. Enzyme stabilization must be treated as a topic that is separate from that of insolubilization or containment.[10]

(10) O. Zaborsky, private communication.

(11) Address correspondence to: Department of Chemical Engineering, Virginia Polytechnic Institute and State University, Blacksburg, Va.

Peter R. Rony[11]

Corporate Research Laboratories
Esso Research & Engineering Company
Linden, New Jersey 07036

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Measurement of Decay Rates of Triplet Excited States Using Time-Correlated Single Photon Counting. Benzophenone Triplets in Aromatic Solvents[1,2]

Sir:

The absolute measurement of excited state lifetimes in the submicrosecond time range was made possible recently by the development of the laser flash photolysis technique, which provides flash lifetimes of about 20 nsec.[3,4] Initial studies using the technique were directed toward determination of the absorption spectra and lifetimes of excited singlet states.[3,4] An extension of the method to determination of short triplet state lifetimes has been reported[5] in a case where the conventional microsecond flash technique was either inapplicable or gave results accompanied by large errors because the measured lifetimes were shorter than or similar to the flash decay times. Use of the nanosecond laser flash technique is, however, limited by the relatively high cost and complexity of the apparatus, and it is perhaps not surprising that the method is in use in a limited number of laboratories.

We have been engaged in studies designed to determine the factors responsible for the short lifetime (10 μsec) of benzophenone triplets in benzene solution, where simultaneously the quantum efficiency for disappearance of benzophenone is very small ($\sim 10^{-3}$).[6-9] Our initial approach involved determination of the

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