APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 6, 309–318 (1981)

Analysis of a Theoretical Model for Anisotropic Enzyme Membranes

Application to Enzyme Electrodes

HENRIK PEDERSEN* AND GOPAL K. CHOTANI

Department of Chemical and Biochemical Engineering, College of Engineering, Rutgers—The State University, PO Box 909, Piscataway, New Jersey 08854

Received May 26, 1981; Accepted August 31, 1981

A theoretical model of diffusion and reaction in an anisotropic enzyme membrane is presented with particular emphasis on the application of such membranes in enzyme electrodes. The dynamic response of systems in which the kinetics are linear, which comprises the practical operating regime for enzyme electrodes in analysis, is investigated via an analytic solution of the governing differential equations. The response is presented as a function of a single dimensionless group, μ, that is the membrane modulus.

Index Entries: Anisotropic membrane electrodes, theoretical model for; membrane electrode, theoretical model for; electrode, theoretical model for; enzyme electrode, theoretical model for.

Introduction

The immobilization of enzymes in a membrane structure that is coupled to an electrochemical sensor has led to the development of a variety of so-called enzyme electrodes (*1–3*). In most instances, the enzyme is distributed uniformly throughout the membrane support (*4–8*) and catalyzes the reaction of the analyte (substrate) material as the analyte diffuses through the membrane. Detection of any electroactive species takes place on the membrane face that is not exposed to the bulk solution by suitable potentiometric or amperometric methods (*3*). Modifications on this basic idea, such as introducing "air gaps," or immobilizing a number

0273–2289/81/1200–0309$02.00

of enzymes together that are each responsible for catalyzing an individual step in an overall reaction scheme, makes the technique extremely versatile and flexible.

Recently, Thévenot et al. (*9*) and Coulet et al. (*10*) have developed enzyme electrodes that have an enzyme immobilized as a *surface* layer on a highly polymerized collagen film. In particular, immobilized glucose oxidase was employed for the conversion of glucose (analyte) to hydrogen peroxide that was subsequently detected by a platinum electrode. The system provides a sensitive and stable assay for glucose (*11*) as well as a simple method for the investigation of the interplay of reaction and diffusion in a heterogeneous enzyme system (*10, 12–14*).

In this report, a theoretical model is developed to predict the dynamic response of anisotropic enzyme membranes that have catalyst immobilized at one of the faces of a thin membrane or inert film. The kinetics are restricted to linear reaction rate expressions, and the dynamics are thereby completely characterized by a single dimensionless group that is a Damkoehler number.

Theory

The physical model and coordinate system for an anisotropic enzymic membrane are shown in Fig. 1. Reactant diffusing through the supporting membrane from the bulk solution is converted by an interfacial enzymatic reaction to a product that is subsequently detected by an appropriate sensor. For example, an amperometric peroxide-sensing electrode can be used to detect H_2O_2 formed from the glucose oxidase-catalyzed reaction involving glucose and oxygen (*9*). The local reactant concentration at the catalyst surface is low so that the reaction kinetics are adequately described by a first-order rate law. This latter assumption assures that the electrode response is proportional to the analyte concentration in the absence of nonlinearities in the transport phenomena, consistent with what is usually observed and desired in practice.

The governing dimensionless equation describing reactant transport within the membrane is

$$\frac{\partial C}{\partial \xi} = \frac{\partial^2 C}{\partial \zeta^2} \tag{1}$$

where the dimensionless time ξ and penetration ζ variables are defined as

$$\xi = Dt/\delta^2 \tag{2a}$$

$$\zeta = x/\delta \tag{2b}$$

The membrane thickness is δ and D is the diffusion coefficient. Equation (1) is used with the boundary and initial conditions

$$C = 1 \qquad \xi > 0 \qquad \zeta = 0 \tag{3a}$$

$$\frac{\partial C}{\partial \zeta} = -\mu C \qquad \xi > 0 \qquad \zeta = 1 \tag{3b}$$

$$C = 0 \qquad \xi = 0 \qquad 0 \leq \zeta \leq 1 \tag{3c}$$

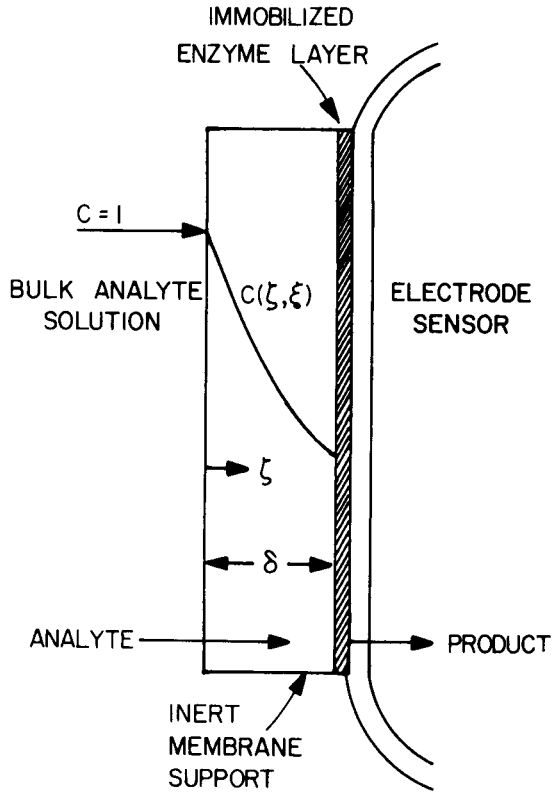

Fig. 1. Schematic description of an anisotropic enzyme electrode. The membrane (exaggerated) has active enzyme deposited as a surface layer at the electrode sensor interface. The product flux is the result of the reaction involving analyte diffusing through the membrane.

where a reaction modulus μ that is a Damkoehler number has been introduced and is defined as

$$\mu = \frac{k''\delta}{D} \tag{4}$$

The surface rate constant k'' is related to the "surface concentration" of the immobilized enzyme $[E'']$, the "turnover number" k_{cat}, and the intrinsic Michaelis constant for the immobilized enzyme K_m simply as $k'' = k''_{cat}[E'']/K_m$.
The solution to Eqs. (1)–(3) is expressed in terms of a "stationary" solution $C_s(\zeta)$ that depends only on the penetration distance and a "developing" solution $C_d(\xi, \zeta)$ where

$$C(\xi, \zeta) = C_s(\zeta) + C_d(\xi, \zeta) \tag{5}$$

Using standard techniques, the following expressions can be obtained,

$$C_s(\zeta) = 1 - \eta\mu\zeta \tag{6a}$$

where $\eta = (1 + \mu)^{-1}$ and

$$C_d(\xi, \zeta) = 2 \sum_{n=1}^{\infty} \frac{\sin \lambda_n \zeta}{\cos \lambda_n \sin \lambda_n - \lambda_n} \exp(-\lambda_n^2 \xi) \tag{6b}$$

The λ_n are found as the roots of $\lambda_n = -\mu \tan \lambda_n$. Also, of practical interest is the "downstream" product flux $m(\xi) = \mu C(\xi, 1)$ that is assumed to be a direct measure of the sensor response; i.e., amperometric detection is used. An integrated product flux, $m(\xi)$, defined as

$$m(\xi) = \mu \int_0^{\xi} C(\xi', 1) d\xi' \tag{7}$$

is related to the dimensionless "time lag" of the electrode ξ_{lag} by the expression

$$\xi_{lag} = -\frac{m_d(\infty)}{m_s} \tag{8}$$

From the solution, the time lag can be evaluated as

$$\xi_{lag} = 2(1 + \mu) \sum_{n=1}^{\infty} \frac{\sin \lambda_n}{\lambda_n^2 (\lambda_n - \cos \lambda_n \sin \lambda_n)} \tag{9}$$

However, it was found from numerically evaluating the sum in Eq. (9) that the following approximate relationship holds with a high degree of accuracy

$$\xi_{lag} = \frac{3 + \mu}{6(1 + \mu)} \tag{10}$$

Results and Discussion

The analytical solution given by Eqs. (6a) and (6b) was used to investigate the dynamic response of the membrane system for a step change in the bulk substrate concentration. The response corresponds to the use of an enzyme electrode for the measurement of an analyte concentration by immersing the electrode in a solution that is well mixed with the analayte. The accumulation of analyte in the membrane is depicted in Fig. 2, where a normalized *average* concentration β is plotted as a function of the dimensionless response time ξ. The variable β is defined as

$$\beta(\xi) = \int_0^1 C(\xi, \zeta) d\zeta / \int_0^1 C_s(\zeta) d\zeta \tag{11}$$

and will, in general, have values between zero and unity. For large values of the modulus, μ, the stationary concentration is obtained at ξ values close to 0.5. Much longer times are required to reach the stationary solution, however, when the modulus is small. Since the modulus represents the relative ratio of diffusion and reaction times, the former situation corresponds closely to the so-called diffusion-controlled response of the system, whereas the latter situation is usually referred to as a reaction-controlled response (*15*).

The response times for the membrane may also be interpreted by means of the expression for the time-lag. From Eq. (10), it is apparent that as μ increases, the time-lag approaches the minimum value $\xi_{lag} = 1/6$ that is predicted for a purely diffusion-controlled response (*16*). As μ decreases, the time-lag increases to values approach $\xi_{lag} = 1/2$ in contradistinction to what has been observed for iso-

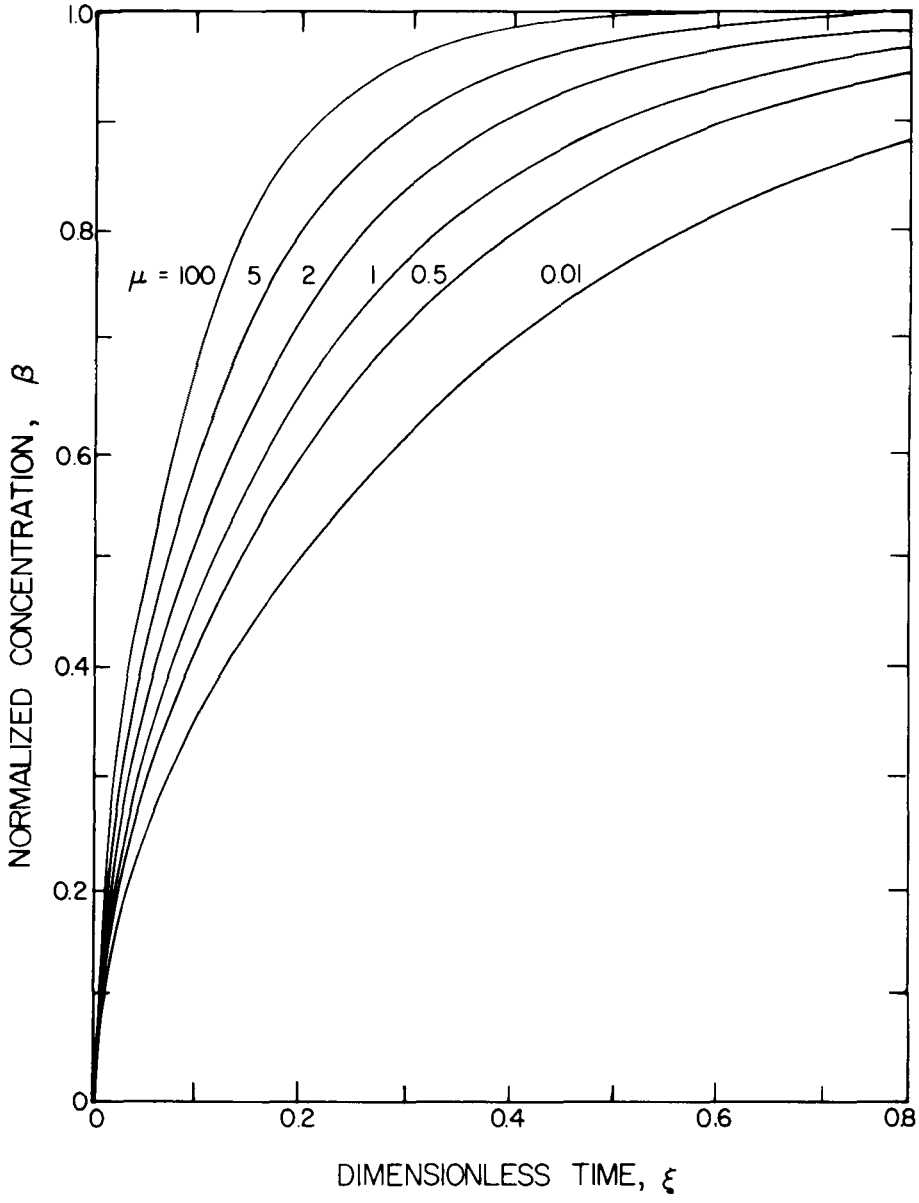

Fig. 2. Normalized average concentration of the analyte in the membrane as a function of dimensionless time ξ with the modulus μ as a parameter. The steady-state average concentration corresponds to $\beta = 1$.

tropic membrane reactors (*17*). The signal will also, of course, be much weaker on an absolute scale (e.g., milliamps) as the modulus decreases.

The predicted electrode response is shown in Figs. 3 and 4 as a function of the modulus μ. The values in Fig. 3 show the dimensionless product flux, from Eq. (3b), normalized to the steady state value, $\eta\mu$. In practice, the sensor output reading is followed until the steady state value is obtained. At that time, the electrode

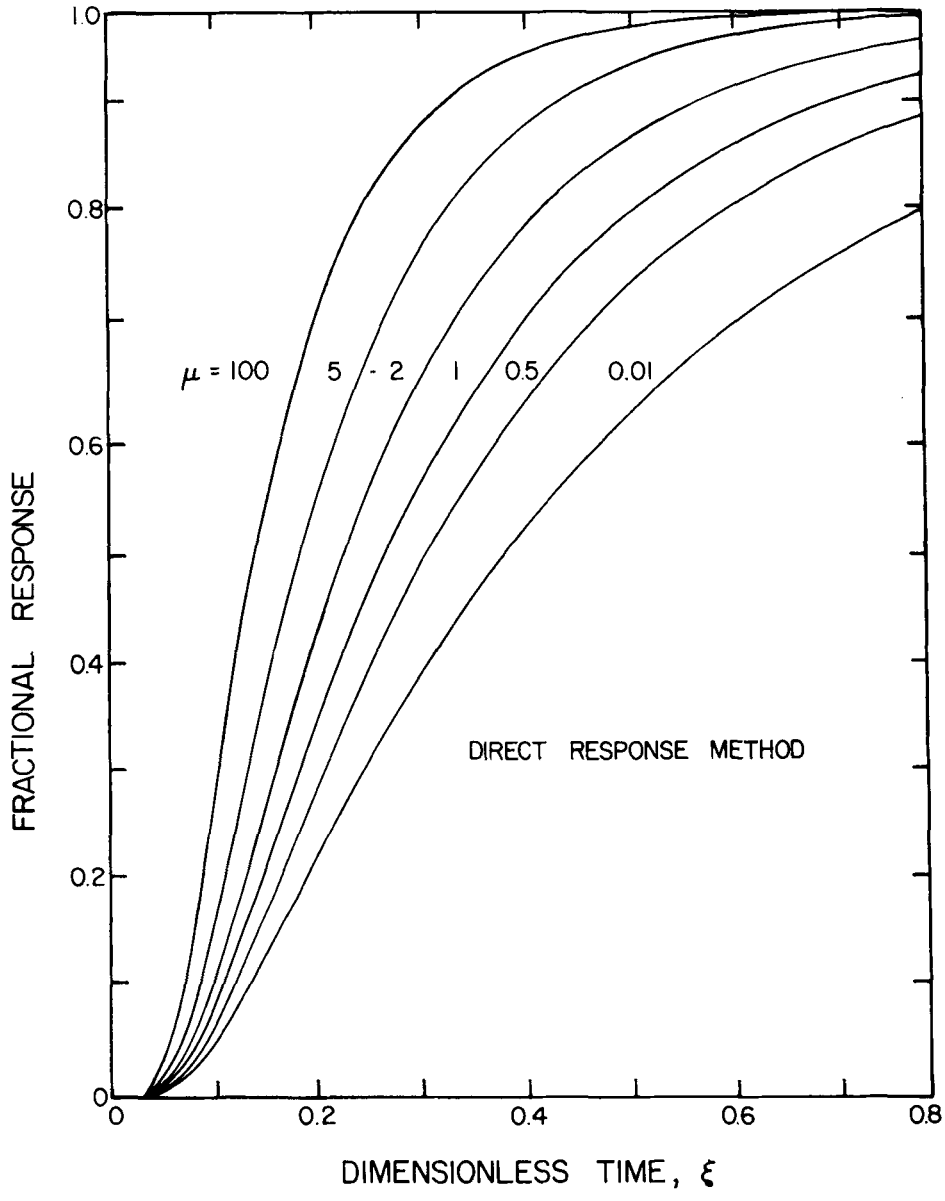

Fig. 3. Electrode response as a function of the dimensionless time ξ with the modulus μ as a parameter. The direct response method is depicted for amperometric detection.

response is calibrated with the analyte concentration. As seen in Fig. 3, however, for low enzyme loadings, the time to reach a steady state value may be extremely large. Rather, it is more expeditious to follow the *derivative* of the sensor response and make use of the sensor reading at the peak value of the derivative; i.e., at the inflection point of the original response curve. The predicted values obtained by this derivative method are shown in Fig. 4 for the corresponding curves of Fig. 3. All the curves have been normalized to the maximum response value. The time required to carry out a single analysis is considerably reduced, at least fivefold. Fur-

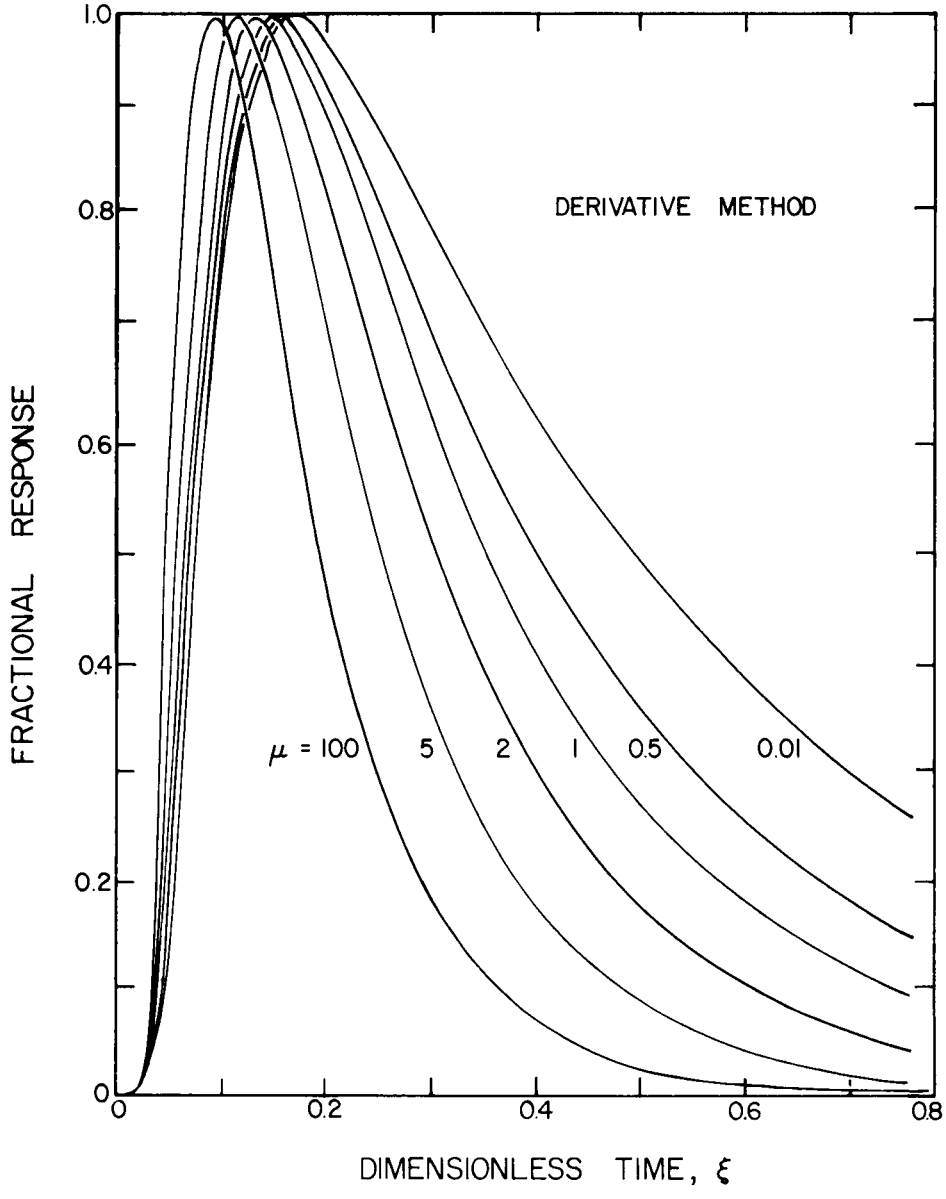

Fig. 4. Electrode response measured by the derivative method as a function of the dimensionless time ξ with the modulus μ as a parameter. The curves correspond to the derivative function of the corresponding curves in Fig. 3 when normalized to the peak derivative value.

thermore, from the location of the peak heights, it is also seen in Fig. 4 that the time necessary to reach the response signal recording value is less sensitive to the values of the modulus, relative to what is observed in Fig. 3. This implies that the derivative method is not only a more rapid procedure for carrying out assays with enzyme electrodes than the direct response method, but is also less sensitive to loss of enzyme activity as regards the assay time.

The derivative response method has been exploited recently in connection with an anisotropic membrane for a glucose sensing electrode (*9, 10*). The experimental data obtained are in qualitative agreement with the curves shown as Figs. 3 and 4. For example, membranes 0.3 mm thick with an analyte diffusion coefficient given as $2(10^{-6})$ cm^2/s would be expected to have a dynamic response time between 30 and 50 s, according to Fig. 4. This corresponds to the experimental values reported by Thévenot et al. (*9*).

Conclusion

The analysis presented here details the operation of anisotropic enzyme electrodes when the reaction kinetics can be described by the simple linear relationship given in Eq. (3b). Enzyme systems that deviate from this type of rate law, either because of product inhibition phenomena or because the analyte concentration in the vicinity of the enzyme is not sufficiently low to create an effectively first-order regime, are not amenable to our simple analytical treatment. However, such systems will also not find wide applicability as enzyme electrodes because the resulting sensor response will not be proportional to the analyte concentration, i.e., the calibration curves will not be linear. Furthermore, the boundary condition given in Eq. (3c) restricts the model results to systems that operate with the membrane initially "analyte free."

In view of the recent successful use of anisotropic enzyme electrodes for glucose detection (*9, 10*), the model may be of value in optimizing the electrode performance as well as for the design of other electrode systems employing amperometric detection. Furthermore, the theoretical results are of interest to other researchers working on model membrane systems where heterogeneous enzymic reactions take place.

Acknowledgment

This work was supported by a grant from the Donors of the Petroleum Research Fund, administered by The American Chemical Society.

References

1. Guilbault, G. G. (1975), "Enzyme Electrode Probes," in *Immobilized Enzymes, Antigens, Antibodies, and Peptides*, Weetall, H. H., ed., Marcel Dekker, New York, p. 366.
2. Guilbault, G. G., and Sadar, M. H. (1979), *Acc. Chem. Res.* **12,** 344.
3. Carr, P. W., and Bowers, L. D. (1980), *Immobilized Enzymes in Analytical and Clinical Chemistry*, Wiley-Interscience, New York.
4. Guilbault, G. G., and Mantalvo, J. G. (1970), *J. Am. Chem. Soc.* **92,** 2533.
5. Mell, L. D., and Maloy, J. T. (1975), *Anal. Chem.* **47,** 299.
6. Updike, S. J., and Hicks, G. P. (1967), *Nature* 214, 986.

7. Blaedel, W. J., and Jenkins, R. A. (1976), *Anal. Chem.* **48,** 1240.
8. Carr, P. W. (1977), *Anal. Chem.* **49,** 799.
9. Thévenot, D. R., Sternberg, R., Coulet, P. R., Laurent, J., and Gautheron, D. C. (1979), *Anal. Chem.* **51,** 96.
10. Coulet, P. R., Sternberg, R., and Thévenot, D. R. (1980), *Biochim. Biophys. Acta* **612,** 317.
11. Coulet, P. R., and Gautheron, D. C. (1976), in *Analysis and Control of Immobilized Enzyme Systems,* Thomas, D., and Kernevez, J. P., eds., North-Holland, Amsterdam, pp. 165–177.
12. Horváth, C., and Engasser, J.-M. (1974), *Biotechnol. Bioeng.* **16,** 909.
13. Engasser, J.-M., and Horváth, C. (1973), *J. Theor. Biol.* **42,** 137.
14. Engasser, J.-M., Coulet, P. R., and Gautheron, D. C. (1977), *J. Biol. Chem.* **252,** 7919.
15. Engasser, J.-M., and Horváth, C. (1976), in *Applied Biochemistry and Biotechnology,* **1,** Wingard, L., Katchalski, E., and Goldstein, L., eds., Academic Press, New York, pp. 127–220.
16. Frisch, H. L. (1957), *J. Phys. Chem.* **61,** 93.
17. Leypoldt, J. K., and Gough, D. A. (1980), *J. Phys. Chem.* **84,** 1058.