CALCULATION OF DIELECTRIC PARAMETERS FROM TIME DOMAIN SPECTROSCOPY DATA

W. D. MOORHEAD, *Physics Department, Youngstown State University, Youngstown, Ohio 44555 U.S.A.*

To obtain accuracy in the determination of the dynamic dielectric constant with time domain spectroscopy (TDS) equipment, detector face reference voltages must be corrected for the effective source impedance and must include the phase correction due to the delay line between the detector and sample (1, 2). We report the corresponding algorithm for σ, ϵ_s, the conductivity and static dielectric constant. For sample termination, one may write:

$$\sqrt{\epsilon}\coth\lambda\frac{l}{c}\sqrt{\epsilon} = \epsilon\frac{V + R_sX + e^{2\gamma L}(X - V)}{V + R_sX - e^{2\gamma L}(X - V)},$$

$$V = A\frac{(1 - R_se^{-2\gamma L_A})}{(1 + e^{-2\gamma L_A})};\ \gamma = \lambda/c,$$

where ϵ, λ, l, L_A, A, L, X, and R_s, are respectively: dielectric constant, transform parameter, sample length, tube lengths and transformed voltages for sample out and in place, and effective source reflection coefficient. At low frequency, the effective source reflection coefficient may be expanded as:

$$R_s = R_0 + \lambda R_1.$$

The result of the calculation is:

$$\sigma/e_0 = [C(A_\infty - X_\infty)(1 - R_0)]/[lX_\infty(1 + R_0)],$$

$$\epsilon_s(1 + R_0)X_\infty + \frac{\sigma}{\epsilon_0}\left[A_\infty\frac{L}{C}(R_0 - 1) + \Delta X(R_0 + 1)\right.$$

$$\left. + X_\infty\left(R_1 + \frac{L_A - 2L - R_0L_A}{C}\right)\right] = \frac{C}{l}\left[(\Delta A - \Delta X)(1 - R_0)\right.$$

$$\left. + A_\infty\left(\frac{2L_A - L}{C}R_0 + \frac{L}{C} - R_1\right) + X_\infty\left(R_1 + \frac{L_A - 2L - R_0L_A}{C}\right)\right]$$

$$+ \frac{1}{3}\frac{l}{C}\frac{\sigma}{\epsilon_0}[A_\infty - X_\infty](1 - R_0).$$

Where $\Delta X = \int_0^\infty [X(t) - X_\infty]\,dt$, $\Delta A = \int_0^\infty [A(t) - A_\infty]\,dt$ and X_∞, A_∞ are the

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observed voltages at long times. The parameters R_0, R_1 are obtained from measurement of standard samples and then applied as corrections to unknowns.

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LIGHT-JUMP PERTURBATION OF CARBON MONOXIDE BINDING BY VARIOUS HEME PROTEINS

EMILIA R. PANDOLFELLI, CELIA BONAVENTURA, *and* JOSEPH BONAVENTURA,
Duke University Marine Laboratory, Beaufort, North Carolina 28516 U.S.A. and
MAURIZIO BRUNORI, *Istituto di Chimica Biologica, Centro di Biologia Molecolare, Citta Universitaria, Rome, Italy*

Relaxation spectroscopy, a technique which measures the relaxation of a system back to equilibrium after a small perturbation, has found useful application in the kinetic analysis of hemoglobin function. One type of relaxation method, the light jump, has been employed in the present study as a means of investigating the extreme pH dependence of ligand binding by the hemoglobin of a marine teleost, *Leiostomus xanthurus*, commonly known as the spot. The light-jump method is in principle similar to the more familiar technique of temperature-jump spectroscopy, except that light of sufficient intensity to partially dissociate CO from hemoglobin is used to perturb the equilibrium between free and bound ligand. The measurements consequently involve analysis of the approach to a steady state in the light and the decay to the dark equilibrium when the photodissociating light is turned off. Variable parameters include the intensity and duration of the photodissociating beam. A comparison between the ligand affinity of the protein under photodissociating conditions and that measured in the absence of photodissociating illumination provides information on the net rate of light-induced ligand dissociation and thereby permits calculation of the quantum yield for photodissociation characteristic of the protein under investigation. Light-jump perturbations have also been used to investigate the contributions of the "on" and "off" kinetic rate constants to the homotropic and heterotropic interactions of the Root effect hemoglobin of the spot. The method is illustrated by presentation of results from simple heme proteins and various fish hemoglobins where more complex kinetics are observed. The pH sensitivity of Root effect fish hemoglobins makes these molecules particularly interesting cases to investigate by the light jump method.