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EQUILIBRIUM BINDING ISOTHERMS USING CONCENTRATION GRADIENTS. Donald B. Mountcastle* (SPON:R.Rubio). Depts. Biochemistry & Pharmacology, Univ. of Va. School of Medicine, Charlottesville, Virginia 22903

With a simple constant volume mixing apparatus, an exponential concentration gradient may be generated as a function of time, by continuous dilution of any specific solution component(X). Thus by recording the signal from a suitable monitor, the degree of saturation($\alpha$) of available binding sites in the solution may be measured as a continuous function of log[X], yielding the experimental data directly as the familiar sigmoidal binding isotherm, $\alpha$ vs. log[X]. Analysis of such continuous data then does not require any curve fitting or the prior assumption of any mathematical model. With one such experiment one can easily identify the dissociation constant(s) of the binding equilibrium, along with any interaction between sites;i.e.,cooperativity. Preliminary experiments using both a flow microcalorimeter and spectrophotometer with model systems such as divalent cation indicators have been carried out successfully. It is proposed that this technique will be quite useful in characterizing a broad range of molecular systems of biological interest;e.g., the binding of proteins with ligands, enzymes with substrates or inhibitors, antibodies and haptens, etc.(Supported by NIH No.GM20637-02).

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MEASUREMENT OF ENZYME KINETICS BY A DILUTION GRADIENT - FLOW MICROCALORIMETRY TECHNIQUE. Robert E. Johnson* (SPON: R.C. Haynes, Jr.). Dept. of Biochemistry, Univ. of Va. School of Medicine, Charlottesville, Virginia 22903

When seperate enzyme and substrate solutions are continuously pumped into the mixing chamber of a flow microcalorimeter, the signal produced is directly proportional to enzyme velocity. If the incoming substrate solution is then diluted exponentially, the recorder trace of the signal becomes a direct plot of the velocity versus the log of the substrate concentration. Some of the advantages of this method are: The entire apparatus is commercially available. Several hundred data points are produced instead of ten or twenty, making it especially good for computer analysis of kinetic models. The entire experiment is done with only one substrate solution and one enzyme solution. The enzyme is easily recovered. Temperature control is not a problem. The solution does not have to be optically clear. The enzyme solution can be kept at 0°C. during the experiment. Studies with chymotrypsin and ribonuclease indicate that results compare well with the literature and that this method can be used even when initial velocities are not measured.
(Supported by NIH Grant No.GM-00184-15).

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MAGNETIC OSMOMETRY: THERMODYNAMICS OF VIRUS SOLUTIONS. D. W. Kupke, J. W. Beams* and M. G. Hodgins*. Dept. of Biochemistry and Dept. of Physics, Univ. of Virginia, Charlottesville, Virginia, 22901

Osmotic pressure, though capable of yielding unambiguous molecular weights (M) in multicomponent systems, is much maligned because the method, as conventionally practiced, is restricted to relatively high molal (m) concentrations of macromolecules ($> 10^{-4}$ m). Hence, M for very large macromolecules is usually not obtainable, and the deviation from ideal behavior, reflecting excluded volume, self association and other interactions, is often missed. By a magnetic suspension technique, utilizing a pressure-sensitive buoy, it has been possible to obtain data to $\sim 10^{-6}$ m concentrations easily. Measurements to 0.1 mm $H_2O$ are obtained with a precision of $\sim$ 0.01 mm $H_2O$. Turnip Yellow Mosaic virus has been studied down to $\sim$ 4 mg/ml; a particle weight of $\sim 5.4 \times 10^6$ daltons was calculated. The second virial coefficient was determinable. The deviation may be interpreted via the Scatchard & Linderstrøm-Lang equations as largely an excluded volume effect with allowances for interior solvent space. The latter was estimated from magnetic density-viscosity determinations.

Supported by grants from the National Science Foundation.

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OSMOTIC PRESSURE MEASUREMENTS ON DILUTE AQUEOUS SOLUTIONS OF ALBUMIN, CHONDROITIN SULFATE AND DEXTRAN: EXCLUDED VOLUMES. Marion L. Shaw,* (SPON: Arthur C. Brown). University of Washington, Seattle, Wa. 98195.

The objective is to interpret osmotic pressure data from solutions of one and two nondiffusible components - globular proteins and mucopolysaccharides extracted from the interstitium - with a model based on the solution theory of McMillan and Mayer (J. Chem. Phys. 13:276, 1945). Osmotic pressure data was collected with a membrane osmometer from solutions of (I) bovine serum albumin (BSA), Dextran T110, and chondroitin sulfate (99% pure prep. of mixed isomers), respectively, and (II) BSA with Dextran T110, and with chondroitin sulfate, (.15 M NaCl, pH 7.2, 23°C). For each solution, data could be fit (by stepwise regression) with a 3-4 term polynomial in integral powers of total nondiffusible solute concentration. From "first virial coefficients," no. average mol. wts. were calculated: BSA, 68,000 ± 10%; Dextran T110, 76,000 ± 11%; chondroitin sulfate, 37,000 ± 7% (agreeing with an ultracentrifugation determination). From "second virial coefficients," expressed as sums of cluster integrals, excluded volumes were obtained: BSA (.49 ± 30%) x $10^{-18}$ $cm^3$/molecule; Dextran T110 (1.5 ± 30%) x $10^{-18}$; chondroitin sulfate (9.0 ± 24%) x $10^{-18}$; BSA-Dextran T110 (7.3 ± 30%) x $10^{-18}$; BSA-chondroitin sulfate (16.7 ± 50%) x $10^{-18}$. It appears that chondroitin sulfate is the "largest" of the three molecules in terms of exclusion effects. (Supported by NIH Grant DE-02600 and Wash. St. Heart Assn.)

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ULTRACENTRIFUGAL ANALYSIS OF THE SELF-ASSOCIATION OF TRITON X-100. Marc S. Lewis and William D. Kirk*. Lab. of Vision Research, Nat. Eye Inst., N. I. H., Bethesda, Md. 20014.

Triton X-100 is a non-ionic detergent of the octylphenol-polyoxyethylene type which is widely used for solubilizing membrane proteins, and thus its self-association is significant to its association with detergent-solubilized proteins. The ultracentrifugal experiments were performed on a sample of Triton X-100 with a mean chain length of 9.5, giving a mean monomer molecular weight of 624. Initial concentrations, solution column lengths, and rotor speeds were selected so that the meniscus was always depleted of n-mer and thus was always just slightly below critical micelle concentration. The solute gradient distributions were analyzed as represented by a monomer-n-mer system and described by the equations: $c_r = c_{1,r} + c_{n,r}$, $c_{1,r} = c_{1,m}\exp(AM_1(r^2 - m^2))$, and $c_{n,r} = Kc_{1,r}^n$. The analysis used a non-linear curve-fitting computer program to obtain the values of the parameters $c_{1,m}$, n, and K; the very large value of K made it necessary to fit for ln K. At 5°, CMC = $1.23 \times 10^{-3}$ M, n = 105, $nM_1$ = 65520, and ln K = 683.5, giving $\Delta G^\circ$ = -369.7 kcal/mole of n-mer or -3.52 kcal/mole of monomer. This may be compared to a value of $\Delta G^\circ$ = -4.52 kcal per mole of monomer which we have recently obtained for the binding of Triton X-100 to rhodopsin when this was analyzed on the basis of a model having a single class of identical independent non-specific sites.

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