Review for Exam II – Chem 370

1. Spectroscopy / Fluorescence

$E = h\nu = hc/\lambda$ (different names depending on energy or wavelength: for λ in cm)

Interaction of Light with Matter (induce oscillating dipoles in matter)

a) Scattered – ($\sim 10^{-16}$ sec)

b) Absorption - ($\sim 10^{-15}$ sec) – due to "transition" from one energy level to another

Beer-Lambert Law: Absorbance (A); Intensity (I, I_o); Transmittance ($T = I / I_o$)

$A = \log (I_o / I) = \log (1/T)$

Extinction Coefficient – E (1%), ε_M = Molar extinction coeff.

$$A = O.D. = \varepsilon \bullet c \bullet \ell \quad \text{also} \quad [E^{1\%} \bullet MW = 10 \bullet \varepsilon_M]$$

Proteins: A_{280} ; E (1%) ~ 10 (or O.D. of 1 for 1 mg/mL)

Nucleic Acids: A_{260} ; E (1%) ~ 200 (or O.D. of 1 for 50 μg/mL)

Instrumentation

Light Source - Monochromator (filter, prism, grating) - Slit - Cuvette -Detector (PM tube)

Fluorescence / Phosphorescence

Fluorescence ($\sim 10^{-4}$ sec to 10^{-9} sec) / Phosphorescence ($> 10^{-3}$ sec)

Resonance Energy Transfer – needs "spectral overlap"

$R = Ro[(1-\varepsilon)/\varepsilon]^{1/6}$ or FRET (Fluor. Res. Energy Transfer) Eff. = $1/[1 + (R/Ro)^6]$

2. Chromatography

"Column" chromatography - separate by absorptive property / collect fractions

- ion exchange chromatography (separate by charge / pH / pI)
- affinity chromatography (use ligand / His tag; IMAC)
- gel exclusion chromatography (zonal technique / size)

Gradient methods – role of salt or pH gradients

3. Electrophoresis – transport of charged particle in an electric field.

Theory: $F_{tot} = qE - fv = ma = m(dv/dt) = 0$; $v = (qE/f)$

$f = 6\pi\eta R$ for spheres; η = Viscosity ~ 0.01g/(cm-sec)

Ferguson Plots: electrophoretic mobility reflects both charge and size/shape

Methods: slab / tube / seq. gels / (native; denatured) / Disc. Gel / PAGE / PFGE / IEF / CE

a) PAGE – polyacrylamide gel electrophoresis; concentration and cross-linking

b) SDS-PAGE (subunit MW) / role of buffer system / use of stains

c) IEF gels / 2D-PAGE

4. Radioactivity and Counting

Types of radioactive decay processes (α / β+ / β- / E.C.); Radioactivity rays (γ-rays)

Activity Units: Curies (Ci); 1 Ci = 3.7×10^{10} disintegrations per sec;

1 becquerel = 1 radioactive decay per second

Math: Half life: $A = Ao \exp(-kt)$ where $k = \ln 2/\text{half-life}$

Measurement of Radioactivity

Geiger Counter – counts ions

Film / PhosphorImagers – "reusable film" – BaRBr:Eu+2 crystals / traps electrons

Liquid Scintillation Counting: Excited solvent / 1^o "fluor" / 2^o "fluor" / PM

Exposure: (Rad / Gray = 1J/kg) (Rem / Sievert)

5. Centrifugation

$F_{tot} = ma = m(dv/dt) = m_{eff}\omega^2 r - fv = m\omega^2 r(1 - \nu'\rho) - fv = 0$ at steady state; (ν' is "v bar")

Preparative Methods: RCF / Rotors / Density Gradient: Zonal vs. Isopycnic Methods

Analytical Methods / Modern Analytical Ultracentrifuge

a) Optics / Rotors

Schlieren optics ($a = aK(\mathbf{dc/dx})$

Interference optics ($DJ = (aKDc)/\lambda$)

Absorption optics ($A \sim \mathbf{c}$)

b) Sedimentation Velocity: $s = v/\omega^2 r$ → plot **(ln r) vs. t → slope =** sw^2

$s = v/\omega^2 r = (m\omega^2 r (1 - \nu'\rho)/f) /\omega^2 r$ or $\mathbf{s = M(1 - n'r)/N^o f}$

s is a function of size and shape! s had units of sec ($1S = 10^{-13}$ sec)

c) Sedimentation Velocity plus Diffusion

Diffusion Coefficient $\mathbf{D = (kT/f) = (RT/ N^o f)}$

$s = v/\omega^2 r = (m(1 - \nu'\rho)/f)$ → $\mathbf{s = MD(1 - n'r)/RT}$

d) Sedimentation Equilibrium: Diffusion vs. Centrifugation

Flow due to diffusion = $- D (dc_r/dr)$

Net flow in centrifuge = $s\omega^2 r c_r - D (dc_r/dr) = 0$ at equilibrium

$\mathbf{(1/c_r)(dc_r/dr) = Mw^2 r(1 - n'r)/RT}$

or at equilibrium

$\mathbf{\ln c_r - \ln c_{rm} = [Mw^2(1 - n'r)/(2RT)](r^2 - r_m^2)}$ → plot **ln c vs.** $\mathbf{r^2}$

6. Light Scattering: "Static" vs. "Dynamic"

Rayleigh (Static) Scattering – scattering from *N* identical particles, each much smaller than λ

$i/I_o = N[8\pi^4\alpha^2 / r^2\lambda^4](1 + \cos^2\theta)$ for unpolarized radiation.

Substituting α in terms of dn/dC (spec. refractive index increment) and noting that N is equal to $[C(g/mL)/M]N^o$.

$i/I_o = [2\pi^2 n_o^2 (dn/dC)^2 / r^2\lambda^4 N_o^2] CM(1 + \cos^2\theta)$.

Intensity falls off with $\mathbf{r^2}$ **/ Intensity decr. with incr. wavelength** λ^4

Intensity depends on scattering angle

Define Raleigh Ratio as $R_\theta = (i_\theta / I_\theta)(r^2 / (1 + \cos^2\theta))$ and thus

$R_\theta = [2\pi^2 n_o^2 (dn/dC)^2 / \lambda^4 N_o^2] CM$ or $\mathbf{R_q = KCM}$.

$\mathbf{KC/ R_q = 1/M}$ **(ideal) or** $\mathbf{KC/ R_q = 1/(M*P(q)) + 2 A_2C}$ **(real soln.)**

Mean Square Radius (**Rg) 10 nm to 150 nm**

Dynamic Light Scattering – Measurement of Diffusion from fluctuations with time

The movement of molecules is related to their **diffusion constants** or frictional coeff.

Hydrodynamic (Stokes) Radius ($\mathbf{R_h}$) **1.5 to 1000 nm** $D = (RT)/(N_o f)$

Experimental (Use 2-detector method / LS and RI)

Light Scattering: $\mathbf{LS = K_{LS} CM(dn/dC)^2}$

Refractive Index: $\mathbf{RI = K_{RI} C(dn/dC)}$

or $\mathbf{LS/RI = M[(K_{LS}/K_{RI})(dn/dC)]}$ **or** $\mathbf{M = K'(LS)/(RI)}$

Polydispersity ($\boldsymbol{Mw/Mn}$ **);** If normalized, LS = RI for monomer but LS = 2*RI for dimer

Weight Average Molecular Weight $M_w = \Sigma N_i M_i^2 / \Sigma N_i M_i$

7. CD

The instrument: Need to have an absorption band to have a CD spectrum.

Typical measurements for proteins are in far UV 170-240 nm.

CD – measures the difference in left and right handed absorbance **A(l)- A(r).** The CD is a function of wavelength. CD spectra can distinguish different types of secondary structure (helix vs. sheet vs, random coil / B-DNA vs. A-DNA, etc.).

Applications: Folding / Secondary Structure / Denaturation / Thermal Stability