# Lincoln College Biochemistry Tutorials

# Dr. Mark C. Leake (m.leake1@physics.ox.ac.uk)


## 1st years, Hilary Term, Tutorial 3

**Title: Biophysical Chemistry – EM radiation and matter (Lincoln)**

You should ensure you have notes on the following areas:
This week will provide an introduction to how atoms and molecules interact with electromagnetic radiation.
You should cover (make notes on) the following areas --

1. Energy levels in atoms and molecules :-
Translational, rotational, vibrational and electronic energy levels. Relative separations of energy levels, occupancies (Boltzmann equation). Zero-point vibrational energy and isotope effects.
2. EM radiation and matter :-
Nature of electromagnetic radiation. Interaction of EM radiation with matter, resonance. Relationship between absorption, elastic scattering and inelastic scattering.
3. Atomic Spectroscopy :-
Absorption and emission of radiation by atoms (changes in electron energy levels).
Relationship between intensity of radiation absorbed and concentration. UV and X-ray spectroscopy, use for identification of atoms and elemental composition.
4. Molecular Spectroscopy :-
Types of transitions that can be excited by UV, visible and IR irradiation. Fates of excited molecules. Intensity and Beer-Lambert Law. [Selection rules for transitions a bit more advanced].
5. Interaction of electromagnetic radiation with collections of molecules :-
Diffraction and Bragg's Law. Microscopy, resolving power (also a little on electron microscopy).

**References**


Standard general texts.
P.W. Atkins, (1974) "Quanta: a handbook of concepts", OCS, pub. Clarendon.
I.D. Campbell and R.A. Dwek, (1984) "Biological Spectroscopy", pub. Benjamin-Cummings.
W.G. Richards and P.R. Scott, (1976) "Structure and Spectra of Atoms", pub. Wiley.
R. McWeeny, (1979) "Coulson's Valence", pub. Oxford -- *updated version of "Valence" by Coulson*.


**Problems**

All submitted material to be attached as one bundle from each separate student, to be clearly marked with the title of the tutorial, the date, the name of the

student, and to clearly display “FAO Dr. Mark Leake, Clarendon Lab” on the first page. To be handed in to either the receptionist, or placed in the “L” pigeon-hole, of the Clarendon Laboratory, Dept of Physics by *12 noon* the day before the tutorial.

1) State the equation that relates the intensity (I) of light transmitted at a specific frequency by a solution containing a single absorbing solute to the concentration of that solute, explaining the terms involved in the equation. [4]

How is this equation modified if the solution contains two different absorbing solutes? [2]

For a compound that undergoes acid dissociation

$$HX + H_20 \leftrightarrow H_30^+ + X^-$$

$K_a$ association constant, where both HX and $X^-$ absorb visible radiation, show that in a solution containing c mol $dm^{-3}$ HX and d mol $dm^{-3}$ $X^-$, the ratio of concentrations of $X^-$ to HX is:

$$[X^-]/[HX]=(A-A_{HX})/(A_X-A)$$

[8]

where A is the absorbance of this solution, $A_{HX}$ is the absorbance of a solution of (c+d) mol $dm^{-3}$ $X^-$.

For a solution of phenolphthalein, the absorbance varies with pH as:

| **pH** | **A** |
|---|---|
| 5.0 | 0.052 |
| 8.7 | 0.076 |
| 9.3 | 0.125 |
| 9.9 | 0.272 |
| 10.3 | 0.315 |
| 10.8 | 0.368 |
| 13.0 | 0.402 |

Determine the $pK_a$ of this compound. [11]

2) The rate of decay of a radioactive species with $N_0$ atoms at time $t=t_0$ and N atoms at time t is:

$$dN/dt=-\lambda N$$

a) Derive an expression for N as a function of $N_0$, $\lambda$, t and $t_0$. [7]

b) What is meant by the “half-life”, “lifetime” and “decay constant” of the species and how are they related? Sketch a graph of N vs t and mark on it the half-time and lifetime. [6]

c) The following measurements were recorded for a radioisotope. By plotting a suitable graph, determine the decay constant and calculate the half-life of the species:

| **time (days)** | **activity (disintegrations/min)** |
|---|---|
| 0 | 10000 |
| 2 | 9841 |
| 4 | 9682 |
| 6 | 9536 |
| 8 | 9423 |
| 10 | 9235 |

d) $^{40}K$ (half-life=$1.3 \times 10^{10}$ years) constitutes 0.012% of the potassium in nature. The human body contain about 0.03% potassium by weight. Calculate the radioactivity (in disintegrations/min) resulting from the $^{40}K$ in a 75kg person.

3) The enzyme glucose-6-phosphate dehydrogenase (G6PDH) catalyses the conversion of glucose-6-phosphate (G6P) to 6-phosphogluconolactone, using $NADP^+$ as an electron acceptor. The reaction goes to completion in the presence of excess $NADP^+$.

The reaction taking place is:
G6P + $NADP^+ \rightarrow$6-phosphogluconolactone + NADPH + $H^+$

0.5ml of an unknown solution of G6P was added to 2.4ml of a solution containing excess $NADP^+$, and 0.1ml G6PdH added to start the reaction. Absorbance at 340nm ($A^{340}$) was monitored over the course of the reaction until no further change was observed. The final level of $A^{340}$ was recorded as 0.61. Assume that, in the mixture, NADPH is the only absorbing species at 340nm and the optical path length was 1cm.

Also:
$\varepsilon_{260}$ (extinction coefficient at 260nm) for $NADP^+ = 1.84 \times 10^4 M^{-1}cm^{-1}$; $\varepsilon_{260}$ for $NADPH = 1.5 \times 10^4 M^{-1}cm^{-1}$; $\varepsilon_{340}$ for $NADPH = 6.22 \times 10^3 M^{-1}cm^{-1}$.

a) Sketch a graph to show the change in $A^{340}$ with time in this experiment. On the same axes, sketch a graph showing the change if the amount of enzyme added was doubled. [4]

b) Calculate the concentration of G6P in the original solution. [5]

After completion of the reaction, the reaction mixture was diluted 10fold (1ml in 9ml water) and $A^{260}$ measured on the dilution. The value obtained was 0.94.

c) Explain why the mixture was diluted before $A^{260}$ was measured. [2]

d) Assuming that the only absorbing species at 260nm are $NADP^+$ and NADPH, calculate the concentration of $NADP^+$ that was present in the original mixture. [7]

e) Calculate the mass of $NADP^+$ that was dissolved in the original reaction mixture to produce this concentration. The relative molecular mass of $NADP^+$ is 765. [4]

f) The experiment was repeated on a new spectrophotometer with a lamp of 3x the intensity of the first model. How would this affect your results? Explain your answer. [3]