1. **13.11(b)** Which of the following transitions are electric-dipole allowed?
   
   (a) \( ^1\Sigma_u^+ \rightarrow ^3\Sigma_u^+ \)  
   (b) \( ^3\Sigma_u^+ \rightarrow ^1\Sigma_u^+ \)  
   (c) \( t_u \rightarrow e_u \)  
   (d) \( n^* \rightarrow n \).

   The electronic spectrum selection rules concerned with changes in angular momentum are (Section 13.2(b)): \( \Delta A = 0, \Delta S = 0, \Delta \Omega = 0, \pm \), where \( A + \Sigma A \) gives the total orbital angular momentum about the internuclear axis and \( \Sigma \) gives the total spin angular momentum about the internuclear axis. The superscript selection rule for reflection in the plane along the internuclear axis is \(+ \leftrightarrow -\) (i.e., \( ++ \leftrightarrow -\) is forbidden). The Laporte selection rule states that for a centrosymmetric molecule (those with a centre of inversion) the only allowed transitions are those that are accompanied by a change of parity: \( u \leftrightarrow g \).

   (a) The changes in the transition \( ^1\Sigma_u^+ \leftrightarrow ^3\Sigma_u^+ \) are \( \Delta A = 0, \Delta S = 0, \Delta \Omega = 0, u \leftrightarrow g \), and \(+ \leftrightarrow -\) so the transition is **allowed**.

   (b) The changes in the transition \( ^3\Sigma_u^+ \leftrightarrow ^1\Sigma_u^+ \) are \( \Delta A = 0, \Delta S = 0, \Delta \Omega = 0, u \leftrightarrow g \), and \(+ \leftrightarrow -\) so the transition is **allowed**.

   (c) Parity does not change in the transition \( t_u \leftrightarrow e_u \) so the transition is **forbidden**. However, this transition is often observed because of either the presence of asymmetric vibrations or the Jahn–Teller effect.

   (d) The transition \( n^* \leftrightarrow n \) is **forbidden** for example in a carbonyl group, because the non-bonding orbital of the lone pair on the oxygen does not change sign (1) under reflection in the plane that contains the \( n \) bond, and the \( n^* \) orbital does change sign (−). The \(+ \leftrightarrow -\) transition is forbidden.

2. **13.12(b)** The ground-state wavefunction of a certain molecule is described by the vibrational wavefunction \( \psi_0 = N_0 e^{-ax^2} \). Calculate the Franck–Condon factor for a transition to a vibrational state described by the wavefunction

\[
\psi'_1 = N'_1 e^{b(x-x_0)^2},
\]

with \( b = a/2 \).

We begin by evaluating the normalization constants \( N_0 \) and \( N'_1 \).

\[
N_0^2 = \frac{1}{\pi^{1/4}} \int_{-\infty}^{\infty} e^{-\frac{2x^2}{\pi}} dx
\]

Likewise,

\[
N'_1^2 = \frac{1}{\pi^{1/4}} \int_{-\infty}^{\infty} e^{-\frac{2x^2}{\pi}} dx
\]

Furthermore, we can easily check that

\[\alpha x^2 + b(x-x_0)^2 = \frac{a+b}{a+b} x_0^2 \]

Where \( x = (a+b)x - \frac{b}{(a+b)^2} x_0 \) and \( dx = \frac{1}{(a+b)^2} dz \)

Then, the vibration overlap integral between the vibrational wavefunction in the upper and lower electronic states is:

\[
S(1',0) = \langle 1' | 0 \rangle = N_0 N'_1 \int_{-\infty}^{\infty} e^{-\frac{\alpha x^2}{(a+b)^2}} e^{-\beta x^2} dx
\]

\[
= N_0 N'_1 \int_{-\infty}^{\infty} e^{-\frac{\alpha x^2}{(a+b)^2}} e^{-\beta x^2} dx
\]

\[
= \frac{N_0 N'_1}{a+b} \int_{-\infty}^{\infty} e^{-\frac{\alpha x^2}{(a+b)^2}} \left[ e^{-\beta z^2} \right] dz
\]

\[
= \frac{N_0 N'_1}{a+b} \left[ e^{-\frac{\alpha x^2}{(a+b)^2}} \right]_{-\infty}^{\infty} + \int_{-\infty}^{\infty} e^{-\beta z^2} dz
\]

\[
= \frac{N_0 N'_1}{a+b} \left[ e^{-\frac{\alpha x^2}{(a+b)^2}} \right]_{-\infty}^{\infty} + \int_{-\infty}^{\infty} e^{-z^2} dz
\]

The integral of the above expression is necessarily zero because on the \( z \)-axis the function \( z \) has an odd-parity symmetry while the function \( e^{-z^2} \) has an even-parity symmetry. Thus, \( u \times g = u \) and the integral over the complete \( z \)-axis of an ungerade function equals zero.

\[
S(1',0) = \frac{N_0 N'_1}{a+b} \left[ e^{-\frac{\alpha x^2}{(a+b)^2}} \right]_{-\infty}^{\infty} + \int_{-\infty}^{\infty} e^{-z^2} dz
\]

For the case \( b = 0 \), this simplifies to:

\[
S(1',0) = \frac{4}{3a} \int_{-\infty}^{\infty} e^{-\frac{\alpha x^2}{(a+b)^2}} e^{-\beta x^2} dx
\]

The Franck–Condon factor is

\[
|S(1',0)|^2 = \frac{4}{3a} \int_{-\infty}^{\infty} e^{-\frac{\alpha x^2}{(a+b)^2}} e^{-\beta x^2} dx
\]
3. 13.15(b) 1,3,5-hexatriene (a kind of 'linear' benzene) was converted into benzene itself. On the basis of a free-electron molecular orbital model (in which hexatriene is treated as a linear box and benzene as a ring), would you expect the lowest energy absorption to rise or fall in energy?

Modelling the π electrons of 1,3,5-hexatriene as free electrons in a linear box yields non-degenerate energy levels of

\[ E_n = \frac{n^2 \hbar^2}{8mL^2} \]  [8.4a]

The molecule has six π electrons, so the lowest-energy transition is from \( n = 3 \) to \( n = 4 \). Including half a bond length at each end of the molecule, the length of the box is six times the C-C bond distance, \( L \), so

\[ \Delta E_{\text{hex}} = \frac{(4^2 - 3^2)\hbar^2}{8m(6d)^2} = \frac{7\hbar^2}{288m_d^2} \]

Modelling the π electrons of benzene as free electrons on a ring of circumference equal to six times the C-C bond distance, \( d \), and radius \( R \) equal to \( 3d \), yields energy levels of

\[ E_n = \frac{m_d^2 \hbar^2}{2L} \]  [8.38a]

where \( m_d \) is the moment of inertia: \( I = mL^2 \). These energy levels are doubly degenerate, except for the non-degenerate \( m_l = 0 \). The six π electrons fill the \( m_l = 0 \) and \( \pm 1 \) levels, so the lowest-energy transition is from \( m_l = 1 \) to \( m_l = 2 \):

\[ \Delta E_{\text{benz}} = \frac{(2^2 - 1^2)\hbar^2}{2mL^2} = \frac{(2^2 - 1^2)\hbar^2}{2m(3d)^2} = \frac{\hbar^2}{24m_d^2} \]

Comparing the two shows

\[ \Delta E_{\text{hex}} = \frac{7\hbar^2}{288m_d^2} < \Delta E_{\text{benz}} = \frac{\hbar^2}{24m_d^2} \]

Therefore, the lowest-energy absorption will rise in energy on conversion of 1,3,5-hexatriene to benzene.

4. 13.16(b) 3-Buten-2-one (9) has a strong absorption at 213 nm and a weaker absorption at 320 nm. Justify these features and assign the ultraviolet absorption transitions.

9 3-Butene-2-one

The weak absorption at 320 nm is typical of a carbonyl chromophore of an enol. The assignment is \( \pi^* \rightarrow \pi \), where a non-bonding electron comes from one of the two lone pairs of the oxygen valence. The two lone pairs of oxygen are in sp³ hybrid orbitals, which define the xy plane that contains the o bond of the carbonyl. The π† molecular orbital is perpendicular to this plane. There is little overlap between the \( \pi \) and \( \pi^* \) orbitals, producing a low value for the dipole transition integral and a low molar absorption coefficient.

The strong absorption at 213 nm has the \( \pi^* \rightarrow \pi \) assignment. The conjugation of the π bonds of the ethenic chromophore and the carbonyl chromophore causes this transition to be shifted to lower energies with respect to both the \( \pi^* \rightarrow \pi \) transition of ethene (165 nm) and the \( \pi^* \rightarrow \pi \) transition of propanone (190 nm). This shift can be understood in terms of the simple Hückel theory of π molecular orbitals using the butadiene π energy model shown in text Figure 10.43 and Figure 13.1 below. Figure 13.1 demonstrates a broad principle: the difference between neighbouring energy levels becomes smaller as the number of adjacent, overlapping orbitals becomes larger.

[Diagram of Figure 13.1 showing energy levels and transitions]
This answer goes beyond the course, uses group theory. I think that both $\Sigma_u \rightarrow \Sigma_g$ and $\Sigma_u \rightarrow \Pi_g$ are allowed in a simpler picture fitting your experience. The simpler picture works for hetero atom diatomics.
6. **13.16 Spin angular momentum is conserved when a molecule dissociates into atoms. What atom multiplicities are permitted when (a) an O₂ molecule, (b) an N₂ molecule dissociates into atoms?**

Use the Clebsch-Gordan series (Chapter 10) to compound the two resultant angular momenta, and impose the conservation of angular momentum on the composite system.

(a) O₂ has S = 1 (it is a spin triplet). The configuration of an O atom is [He]2s²2p⁶, which is equivalent to a He atom with two electron-like ‘holes’. The atom may therefore exist as a spin singlet or as a spin triplet. Since S₁ = 1 and S₂ = 0 or S₁ = 1 and S₂ = 1 may each combine to give a resultant with S = 1, both may be the products of the reaction. Hence multiplicities \( \frac{3}{2}+\frac{1}{2} \) and \( 3+3 \) may be expected.

(b) N₂ has S = 0. The configuration of an N atom is [He]2s²2p³. The atoms may have \( S = \frac{3}{2} \) or \( \frac{1}{2} \). Then we note that \( S₁ = \frac{3}{2} \) and \( S₂ = \frac{1}{2} \) can combine to give \( S = 0 \); \( S₁ = \frac{3}{2} \) and \( S₂ = \frac{3}{2} \) can also combine to give \( S = 0 \) (but \( S₁ = \frac{3}{2} \) and \( S₂ = \frac{3}{2} \) cannot). Hence, the multiplicities \( \frac{3}{2}+\frac{3}{2} \) and \( 3+2 \) may be expected.

---

7. **13.20 The Beer–Lambert law states that the absorbance of a sample at a wavenumber \( \nu \) is proportional to the molar concentration \( C \) of the absorbing species \( J \) and to the length \( L \) of the sample (eqn 13.4). In this problem you will show that the intensity of fluorescence emission from a sample is also proportional to \( C \) and \( L \). Consider a sample of \( J \) that is illuminated with a beam of intensity \( I(\nu) \) at the wavenumber \( \nu \). Before fluorescence can occur, a fraction of \( J \) must be excited and an intensity \( I(\nu) \) will be transmitted. However, not all of the absorbed intensity is emitted and the intensity of fluorescence depends on the fluorescence quantum yield, \( \phi_\text{f} \), the efficiency of photon emission. The fluorescence quantum yield ranges from 0 to 1 and is proportional to the ratio of the integral of the fluorescence spectrum over the integrated absorption coefficient. Because of a Stokes shift, fluorescence occurs at a wavenumber \( \nu_f \), with \( \nu_f + \Delta \nu \text{Stokes} = \nu \).

(a) Use the Beer–Lambert law to express \( I_\text{abs}(\nu) \) in terms of \( I(\nu), [J], L \), and \( \epsilon(\nu) \), the molar absorption coefficient of \( J \) at \( \nu \). (b) Use your result from part (a) to show that \( I_\text{f}(\nu_f) = I(\nu) \epsilon(\nu) \phi_\text{f}[\nu]L \).

(a) The Beer–Lambert law is:

\[
A = \log \frac{I_0}{I} = \epsilon(J)L
\]

The absorbed intensity is:

\[
I_\text{abs} = I_0 - I \
\]

So \( I_0 = I + I_\text{abs} \)

Substitute this expression into the Beer–Lambert law and solve for \( I_\text{abs} \):

\[
\log \frac{I}{I_0 - I_\text{abs}} = \epsilon(J)L \\
\]

So \( I_0 - I_\text{abs} = I_0 \times 10^{-\epsilon(J)L} \)

and \( I_\text{abs} = \left[I_0 \times (1 - 10^{-\epsilon(J)L})\right] \)

(b) The problem states that \( I_\text{abs}(\nu) \) is proportional to \( \phi_\text{f} \) and to \( I_\text{abs}(\nu) \), so:

\[
I_\text{f}(\nu_f) = \phi_\text{f} I_\text{abs}(\nu) \times (1 - 10^{-\epsilon(J)L})
\]

If the exponent is small, we can expand \( 1 - 10^{-\epsilon(J)L} \) in a power series:

\[
10^{-\epsilon(J)L} = (\epsilon(J)L)^{\epsilon(J)L} \approx 1 - \epsilon(J)L \ln 10 + \ldots
\]

and \( I_\text{f}(\nu_f) = \phi_\text{f} I(\nu) \epsilon(\nu) \phi_\text{f}[\nu]L \)

---

Here it is important to take into account that the next expansion term is \( (\epsilon(J)L)^2 \) and it must be small compared to \( \epsilon(J)L \). For this to happen, need \( \epsilon(J) < 0.1 \) for 1% accuracy or \( \epsilon(J) < 0.01 \) for 0.01% accuracy.
8. P19.6) Calculate the transition dipole moment. \( \mu_{zm}^{mn} = \int \psi_{m}^{*}(r) \mu_{z} \psi_{n}(r) \, d\tau \), where \( \mu_{z} = -er \cos \theta \) for a transition from the 1s level to the 2p, level in H. Show that this transition is allowed. The integration is over \( r \), \( \theta \) and \( \phi \). Use \( \psi_{210}(r, \theta, \phi) = \frac{1}{\sqrt{32\pi}} \left( \frac{1}{a_{0}} \right)^{\frac{3}{2}} \frac{r}{a_{0}} e^{-r/a_{0}} \cos \theta \) for the 2p, wave function.

The transition is allowed if the transition dipole moment is non-zero:

\[ \mu_{zm}^{mn} = \int \psi_{m}^{*}(\tau) \mu_{z} \psi_{n}^{*}(\tau) \neq 0 \]

We use the wave functions for a hydrogen 1s orbital:

\[ \psi_{100} = \frac{1}{\sqrt{\pi}} \left( \frac{1}{a_{0}} \right)^{\frac{3}{2}} e^{-r/a_{0}} \]

and the wave function for the 2p, orbital given above. The transition dipole moment is then:

\[ \mu_{zm}^{mn} = \int \psi_{210}(r, \theta, \phi) \psi_{100}(r) \, d\tau \]

Doing the integrals one at a time:

\[ \int_{0}^{2\pi} \, d\phi = 2\pi ; \]

\[ \int_{0}^{\pi} \cos^{2}[\theta] \sin[\theta] d\theta = \frac{2}{3} ; \]

\[ \int_{0}^{\pi} (-e \cos[\theta]) r^{2} \left( \frac{r}{a_{0}} e^{-r/a_{0}} \right) \left( e^{-r/a_{0}} \right) \, dr = -e \int_{0}^{\infty} \left( \frac{r^{4} e^{-r/a_{0}}}{a_{0}} \right) \left( e^{-r/a_{0}} \right) \, dr = -e \int_{0}^{\infty} r^{4} \left( e^{-r/a_{0}} \right) \left( e^{-r/a_{0}} \right) \, dr \]

\[ = -e \int_{0}^{\infty} r^{4} \left( e^{-r/a_{0}} \right) \left( e^{-r/a_{0}} \right) \, dr = e \int_{0}^{\infty} r^{4} \left( 1 + \frac{1}{2a_{0}} \right) \, dr = e \int_{0}^{\infty} r^{4} \left( 1 + \frac{1}{2a_{0}} \right) \, dr = e \frac{24 \times 32 a_{0}^{5}}{243} = -\frac{24 \times 32 a_{0}^{5}}{243} \]

\[ \mu_{zm}^{mn} = \left( \frac{1}{\sqrt{32\pi}} \left( \frac{1}{a_{0}} \right)^{\frac{3}{2}} \right) \left( \frac{1}{\sqrt{\pi}} \left( \frac{1}{a_{0}} \right)^{\frac{3}{2}} \right) \frac{2}{3} \times 2\pi \times \frac{24 \times 32 a_{0}^{5}}{243} = -\frac{4\pi}{\sqrt{32\pi a_{0}^{3}}} \times \frac{24 \sqrt{32} \sqrt{32} a_{0}^{4}}{3 \times 243} \]

\[ = \frac{4 \times 8 \times 4 \sqrt{2}}{243} e a_{0} = -\frac{128}{243} \sqrt{2} e a_{0} \]
9. **P19.9)** The absorbance of a nucleic acid solution at 260 nm is called $A_{260}$. The OD (i.e., optical density) is the amount of nucleic acid in a volume of 1.00 mL in a 1.00-cm path length cell for which $A_{260} = 1.00$. How many moles of nucleotide are contained in a 1.00 mL solution of a double-stranded nucleotide for which $A_{260} = 2.50$, assuming the extinction coefficient per nucleotide is $7.00 \times 10^3$ M$^{-1}$ cm$^{-1}$. Express this quantity in OD’s. Assume a 1.00 cm path length.

Solving $A_{260} = \log \left[ \frac{I_0}{I} \right] = \varepsilon \ell c$ for the concentration, $c$, gives:

$$c = \frac{A_{260}}{\varepsilon \ell}$$

Therefore the concentration of nucleic acid in the solution is:

$$c = \frac{2.5}{7 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1} \times 1 \text{ cm}} = 3.57 \times 10^{-4} \text{ mol l}^{-1}$$

Consequently, 1 ml of nucleic acid solution contains $3.57 \times 10^{-7}$ moles. This concentration corresponds to an $A_{260} = 2.5$.

10. **P19.10)** Because of interactions between transition dipoles of the constituent nucleotides, the extinction coefficient for a single strand polynucleotide is not simply the sum of the extinction coefficients for the individual nucleotides. These dipole-dipole interactions depend on $1/r^3$ where $r$ is the distance between bases, so for the purpose of calculating the extinction coefficient for a single-stranded polynucleotide, only nearest neighbor interactions need be considered. For a hypothetical polynucleotide strand $GpCpUp...ApG$ the extinction coefficient is

$$\varepsilon (GpCpUp...ApG) = 2 \left[ \varepsilon (GpC) + \varepsilon (CpU) + \varepsilon (ApG) \right] - \left[ \varepsilon (Cp) + \varepsilon (Up) + ... + \varepsilon (Ap) \right]$$

Interacting nucleotides pairs in a single strand are indicated by $XpY$, where $p$ represents the phosphate group that joins the nucleotides $X$ and $Y$. In the equation above $\varepsilon (ApG)$, $\varepsilon (ApC)$, etc., are extinction coefficients for component dinucleotide phosphates per mole of nucleotide. Hence they are counted twice, which accounts for the 2 in the expression above. To correct for this fact, the extinction coefficients of the individual nucleotides, except for the terminal nucleotides, are subtracted. The preceding equation gives good agreement with experimental extinction coefficients for DNA and RNA single strands. Consider the tables of extinction coefficients per nucleotide (M$^{-1}$ cm$^{-1}$) at 260 nm, 298K, and pH 7 for RNA nucleotides.

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**b.** Calculate $A_{260}$ for a $1.50 \times 10^{-4}$ M solution of the RNA in part a for a path length of 1.00 cm.

**c.** Repeat the calculations in part a and b for the single strand RNA $GpCpUpUpApA$. Assume a path length of 1.00 cm.
\[ e[ApCpGpUpUAppGp] = 2(10.67 + 9.39 + 10.96 + 11.1 + 12.1 + 12.4 + 12.9 + 10.96) \times 10^3 \text{M}^{-1} \text{cm}^{-1} \]
\[ - (7.60 + 12.16 + 10.21 + 10.21 + 15.34 + 12.16) \times 10^3 \text{M}^{-1} \text{cm}^{-1} \]
\[ e[ApCpGpUpUAppGp] = 8.712 \times 10^{-4} \text{M}^{-1} \text{cm}^{-1} \]

b. As in P19.9, solving \( A_{260} = e\ell \) for the concentration, c, gives:

\[ A_{260} = e\ell = 8.712 \times 10^{-4} \text{M}^{-1} \text{cm}^{-1} \times 1.5 \times 10^{-4} \text{M} \times 1.00\text{cm} = 13.068 \]

\[ e[GpCpUpUAppA] = 2(9.19 + 8.37 + 10.11 + 12.52 + 13.65) \times 10^3 \text{M}^{-1} \text{cm}^{-1} \]
\[ = (7.60 + 8.70 + 8.70 + 15.34) \times 10^3 \text{M}^{-1} \text{cm}^{-1} \]
\[ = 67.4 \times 10^3 \text{M}^{-1} \text{cm}^{-1} \]
\[ A_{260} = e\ell = 67.4 \times 10^3 \text{M}^{-1} \text{cm}^{-1} \times 1.5 \times 10^{-4} \text{M} \times 1.00\text{cm} = 10.11 \text{ (still out of range of spectrometer!!)} \]

11. P19.12) For solutions composed of more than one absorbing species the absorbances at a given wavelength \( \lambda \) are additive. For two absorbing species M and N the absorbances of the individual species at \( \lambda \) are additive:

\[ A_\lambda = A_\lambda^M + A_\lambda^N = e_\lambda^M \ell c_M + e_\lambda^N \ell c_N = \ell \left( e_\lambda^M c_M + e_\lambda^N c_N \right) \]

Therefore, the concentrations of the two species can be obtained by making absorbance measurements at two different wavelengths \( \lambda_1 \) and \( \lambda_2 \), and using known values of the four extinction coefficients.

a. Derive expressions for the concentrations \( c_M \) and \( c_N \) in terms of the extinction coefficients \( e_{\lambda_1}^M, e_{\lambda_2}^M, e_{\lambda_1}^N, e_{\lambda_2}^N \), the path length \( l \), and the absorbances \( A_{\lambda_1} \) and \( A_{\lambda_2} \).

b. For tyrosine (Y) the extinction coefficients at 240 and 280 nm are \( e_{240}^Y = 11.300 \text{ M}^{-1} \text{ cm}^{-1} \) and \( e_{280}^Y = 1500 \text{ M}^{-1} \text{ cm}^{-1} \). For tryptophan (W) the corresponding extinction coefficients are \( e_{240}^W = 1960 \text{ M}^{-1} \text{ cm}^{-1} \) and \( e_{280}^W = 5380 \text{ M}^{-1} \text{ cm}^{-1} \). A solution of tyrosine and tryptophan has absorbances of \( A_{260} = 0.350 \) and \( A_{280} = 0.226 \). Calculate the concentrations of tyrosine and tryptophan. Assume a path length of 1.00 cm.

a) From: \( A_1 = A_1^M + A_1^N = e_1^M \ell c_M + e_1^N \ell c_N = \ell \left( e_1^M c_M + e_1^N c_N \right) \)

follows: \( A_1 = A_1^M + A_1^N = \ell \left( e_1^M c_M + e_1^N c_N \right) \); \( A_2 = A_2^M + A_2^N = \ell \left( e_2^M c_M + e_2^N c_N \right) \).

where 1 and 2 depict two different wave lengths, and N and M designate two different absorbing species. Solving for \( c_N \) and \( c_M \) yields:

\[ c_N = \frac{A_2 e_1^N}{e_2^N} - \frac{A_1 e_2^M}{e_2^N} c_M \quad \text{and} \quad c_M = \frac{A_1 e_1^N}{e_1^M} - \frac{A_2 e_1^N}{e_1^M} c_N \]
combining these two equations gives:

$$
\begin{align*}
\frac{A_N}{\ell e_N} = & \frac{A_2}{\ell e_N} - \frac{e_2^m}{e_2^N} \left( \frac{A_1}{\ell e_1^M} - \frac{e_1^N}{e_1^M} c_N \right) \\
\frac{A_N}{\ell e_N} = & \frac{A_2}{\ell e_N} - \frac{e_2^m}{e_2^N} \frac{A_1}{\ell e_1^M} + \frac{e_2^m}{e_2^N} e_1^N c_N \\
\frac{A_N}{\ell e_N} = & \frac{A_2}{\ell e_N} - \frac{e_2^m}{e_2^N} e_1^N c_N \\
\frac{A_N}{\ell e_N} \left( 1 - \frac{e_2^m}{e_2^N} e_1^N \right) = & \frac{A_2}{\ell e_2^N} - \frac{e_2^m}{e_2^N} e_1^M \\
\frac{A_N}{\ell e_N} = & \frac{1}{\ell e_2^N} \left( \frac{A_2}{\ell e_2^M} - \frac{e_2^m}{e_2^N} e_1^M \right) \\
\frac{A_N}{\ell e_N} = & \left( 1 - \frac{e_2^m}{e_2^N} e_1^N \right) \left( 1 - \frac{e_2^m}{e_2^N} e_1^M \right) \\
\end{align*}
$$

b) With: tyrosine (Y) → species N with:  
$$
e_1^N \rightarrow e_{240}^Y = 11300 \text{ M}^{-1}\text{cm}^{-1}
$$

$$
e_2^N \rightarrow e_{280}^Y = 1500 M^{-1} \text{cm}^{-1}
$$

tryptophan (W) → species M with:  
$$
e_1^M \rightarrow e_{240}^W = 1960 M^{-1} \text{cm}^{-1}
$$

$$
e_2^M \rightarrow e_{280}^W = 5380 M^{-1} \text{cm}^{-1}
$$

$$
A_1 = A_{240} = 0.350 \quad \text{and} \quad A_2 = A_{280} = 0.226,
$$

the concentration of tyrosine (Y) can be calculated as:

$$
\begin{align*}
A_N = & \frac{1}{\ell e_2^N} \left( \frac{A_2}{\ell e_2^M} - \frac{e_2^m}{e_2^N} e_1^M \right) \\
= & \frac{1}{\ell e_2^N} \left( \frac{0.226}{1500} - \frac{5380 \times 0.350}{1500 \times 1960} \right) M^{-1} \text{cm}^{-1}
\end{align*}
$$

$$
c_N = \frac{-4.898 \times 10^{-4}}{-19.678}
$$

$$
c_N = 2.489 \times 10^{-5} M
$$

and:

$$
\begin{align*}
A_M = & \frac{A_1}{\ell e_1^M} - \frac{e_1^N}{e_1^M} c_N = \frac{0.350}{1960} - \frac{11300}{1960} - 2.489 \times 10^{-5} M
\end{align*}
$$

$$
c_M = 3.507 \times 10^{-5} M
$$

12. P19.15) As explained in Section 19.11, FRET can be used to determine distances between fluorescent chromophores in macromolecules, thus providing information on macromolecular conformation. The efficiency of energy transfer $E_i$ in a FRET experiment is given by $E_i = \frac{r_i^6}{R_0^6 + r_i^6}$ where $r$ is the distance between the donor (D) and acceptor (A) chromophores. The excitation transfer can be determined from fluorescent life times $E_i = 1 - \frac{\tau_{D+A}}{\tau_D}$ where $\tau_0$ is the fluorescent life time of the donor D in the absence of the acceptor A and $\tau_{D+A}$ is the life time of the donor in the presence of the acceptor.
Consider a protein labeled with a donor naphthyl group and an acceptor dansyl group. The fluorescence lifetime for the naphthyl group in the protein is 23 ns. When dansyl is added to the protein the lifetime of the naphthyl group decreases to 18 ns. Calculate the distance $r$ between the naphthyl and dansyl chromophores assuming the Förster radius $R_0 = 34 \, \text{Å}$.

Using:

$$E_t = 1 - \frac{\tau_{A+D}}{\tau_D} = \frac{R_0^6}{(R_0^6 + r^6)}$$

and solving for $r$ gives:

$$\left( R_0^6 + r^6 \right) = \frac{R_0^6}{1 - \frac{\tau_{A+D}}{\tau_D}}$$

$$r^6 = R_0^6 \left( 1 - \frac{\tau_{A+D}}{\tau_D} \right) R_0^6 - R_0^6 = R_0^6 \left( \frac{1}{1 - \frac{\tau_{A+D}}{\tau_D}} \right)^6$$

$$r = \sqrt[6]{\frac{1}{1 - \frac{\tau_{A+D}}{\tau_D}}} R_0 \quad r = \sqrt[6]{\frac{1}{1 - \frac{18 \, \text{ns}}{23 \, \text{ns}}}} R_0 = 42.09 \, \text{Å}$$

**Extra Problems**

1. **13.2(b)** When light of wavelength 400 nm passes through 3.5 mm of a solution of an absorbing substance at a concentration 0.667 mmol dm$^{-3}$, the transmission is 65.5 per cent. Calculate the molar absorption coefficient of the solute at this wavelength and express the answer in cm$^2$ mol$^{-1}$.

   
   \[ \varepsilon = \frac{1}{[J]} \log \frac{I_0}{I} \quad \text{[13.2, 13.3]} \]

   \[ = \frac{-1}{(6.67 \times 10^{-4} \, \text{mol dm}^{-3}) \times (0.35 \, \text{cm})} \log 0.655 = 787 \, \text{dm}^3 \, \text{mol}^{-1} \, \text{cm}^{-1} \]

   \[ = 787 \times 10^3 \, \text{cm}^3 \, \text{mol}^{-1} \, \text{cm}^{-1} \quad [1 \, \text{dm} = 10 \, \text{cm}] \]

   \[ = 7.9 \times 10^5 \, \text{cm}^2 \, \text{mol}^{-1} \]

2. **13.5(b)** The following data were obtained for the absorption by a dye dissolved in methylbenzene using a 2.50 mm cell. Calculate the molar absorption coefficient of the dye at the wavelength employed:

   \[
   [\text{dye}] / (\text{mol dm}^{-3}) \quad 0.0010 \quad 0.0050 \quad 0.0100 \quad 0.0500
   \]

   \[
   T/(\text{per cent}) \quad 73 \quad 21 \quad 4.2 \quad 1.33 \times 10^{-5}
   \]

   The Beer–Lambert law is

   \[
   \log \frac{I_0}{I} = -\varepsilon [J] = \log T
   \]

   so a plot (Figure 14.1) of $\log T$ versus $[J]$ should give a straight line through the origin with a slope $m$ of $-\varepsilon l$. So $\varepsilon = -m/l$. 


The data follow

<table>
<thead>
<tr>
<th>[dye]/(mol dm$^{-3}$)</th>
<th>$T$</th>
<th>log $T$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0010</td>
<td>0.73</td>
<td>-0.1367</td>
</tr>
<tr>
<td>0.0050</td>
<td>0.21</td>
<td>-0.6778</td>
</tr>
<tr>
<td>0.0100</td>
<td>0.042</td>
<td>-1.3768</td>
</tr>
<tr>
<td>0.0500</td>
<td>$1.33 \times 10^{-7}$</td>
<td>-6.8761</td>
</tr>
</tbody>
</table>

![Graph showing a linear relationship between log T and [dye]/(mol dm$^{-3}$)](image)

Figure 14.1

The molar absorptivity is

$$
\varepsilon = \frac{-138 \text{ dm}^3 \text{ mol}^{-1}}{0.250 \text{ cm}} = 522 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}
$$

3. **13.1(b)** The molar absorption coefficient of a substance dissolved in hexane is known to be 327 dm$^3$ mol$^{-1}$ cm$^{-1}$ at 300 nm. Calculate the percentage reduction in intensity when light of that wavelength passes through 1.50 mm of a solution of concentration 2.22 mmol dm$^{-3}$.

Use the Beer–Lambert law

$$
\log \frac{I}{I_0} = -\varepsilon \tau J
$$

$$
\tau = \frac{	ext{path length}}{2.22 \times 10^{-3} \text{ mmol dm}^{-3} \times 0.15 \text{ cm}}
$$

$$
\frac{I}{I_0} = 10^{-0.10889} \approx 0.778
$$

The reduction in intensity is 22.2 percent.

4. **13.3(b)** The molar absorption coefficient of a solute at 440 nm is 323 dm$^3$ mol$^{-1}$ cm$^{-1}$. When light of that wavelength passes through a 7.50 mm cell containing a solution of the solute, 52.3 per cent of the light was absorbed.

What is the concentration of the solution?

$$
\log T = -\varepsilon \tau J
$$

$$
\tau = \frac{1}{\varepsilon \log(1 - 0.523)} = \frac{1}{(323 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}) \times 0.750 \text{ cm}}
$$

$$
[1.33 \text{ mmol dm}^{-3}]
$$
5. 13.6(b) A 2.50-mm cell was filled with a solution of a dye. The concentration of the dye was 15.5 mmol dm$^{-2}$. Calculate the molar absorption coefficient of benzene at this wavelength given that the transmission was 32 per cent. What will the transmittance be in a 4.50-mm cell at the same wavelength?

The Beer-Lambert law is

$$\log T = -\varepsilon |I|$$

$$\varepsilon = \frac{-1}{|I| \log T}$$

Now that we have $\varepsilon$, we can compute $T$ of this solution with any size of cell:

$$T = 10^{-\varepsilon |I|} = 10^{-\left(\frac{128\text{ dm}^3\text{mol}^{-1}\text{cm}^{-1}}{0.0155\text{ mol dm}^{-3} \times 0.250\text{ cm}}\right)} = 0.13$$

6. 13.18(a) The line marked A in Fig. 13.41 is the fluorescence spectrum of benzophenone in solid solution in ethanol at low temperatures observed when the sample is illuminated with 360 nm light. What can be said about the vibrational energy levels of the carbonyl group in (a) its ground electronic state and (b) its excited electronic state?

(a) Vibrational energy spacings of the lower state are determined by the spacing of the peaks of fluorescence spectrum A of benzophenone ($\nu = 1800\text{ cm}^{-1}$).

(b) The peaks of A give no information about the spacing of the upper vibrational levels (without a detailed analysis of the intensities of the lines).

7. 13.18(b) When naphthalene is illuminated with 360 nm light it does not absorb, but the line marked B in Fig. 13.41 is the phosphorescence spectrum of a solid solution of a mixture of naphthalene and benzophenone in ethanol.

![Fig. 13.41](image)

Now a component of fluorescence from naphthalene can be detected. Account for this observation. After some vibrational decay the benzophenone (which does absorb near 360 nm) can transfer its energy to naphthalene. The latter then emits the energy radiatively.

8. 13.4 In many cases it is possible to assume that an absorption band has a Gaussian lineshape (one proportional to $e^{-x^2}$) centred on the band maximum. Assume such a lineshape, and show that $A = 1.064E_{\text{max}} \Delta \nu_{1/2}$, where $\Delta \nu_{1/2}$ is the width at half-height. The absorption spectrum of azoethane (CH$_3$CH$_2$N$_2$) between 24 000 cm$^{-1}$ and 34 000 cm$^{-1}$ is shown in Fig. 13.42. First, estimate $A$ for the band by assuming that it is Gaussian. Then integrate the absorption band graphically. The latter can be done either by ruling and counting squares, or by tracing the line shape on to paper and weighing. A more sophisticated procedure would be to use mathematical software to fit a polynomial to the absorption band (or a Gaussian), and then to integrate the result analytically.
The absorption band of Figure 13.42 appears to be a positively skewed Gaussian with a peak at the point (28 000 cm⁻¹, 9.6 dm³ mol⁻¹ cm⁻¹) and half-heights at the points (26 300 cm⁻¹, 4.8 dm³ mol⁻¹ cm⁻¹) and (30 200 cm⁻¹, 4.8 dm³ mol⁻¹ cm⁻¹). When skew is considered later in this solution, the point (34 000 cm⁻¹, 0.7 dm³ mol⁻¹ cm⁻¹) will also be used, but first we note that the low side of the peak appears to have the normal Gaussian shape so we use the half-height on the low side and the peak to estimate the area with a normal Gaussian lineshape having the form

\[ A = A_{\text{max}} \pi e^{-\left(\frac{2\Delta \nu}{a}\right)^2}, \]

where \( a \) is a constant related to the half-width

\[ \Delta \nu = 2 \times (28 000 - 26 300) \text{ cm}^{-1} = 3400 \text{ cm}^{-1} \]

\[ A = A_{\text{max}} \pi e^{-\left(\frac{2\Delta \nu}{a}\right)^2} \]

The relationship between the half-width and \( a \) is found by evaluation of the lineshape at \( e(\nu_{\text{off}}) = e_{\text{max}}/2 \):

\[ e_{\text{max}}/2 = e_{\text{off}} e^{-\left(\nu_{\text{off}} - \nu_{\text{peak}}\right)^2/a^2} \]

\[ \ln(1/2) = -\left(\nu_{\text{off}} - \nu_{\text{peak}}\right)^2/a^2 \]

\[ a^2 = \frac{\left(\nu_{\text{off}} - \nu_{\text{peak}}\right)^2}{\ln(2)} \]

\[ a = \frac{\Delta \nu}{2 \sqrt{\ln 2}} \]

Fig. 13.42
\[
A = \frac{1}{2} \Delta v_0 \sqrt{\frac{\Delta v_0}{\ln(2)}} = 1.6645 \Delta v_0 \sqrt{\ln(2)}
\]

\[
x = \frac{1}{2} (340 \text{ cm}^{-1}) \times (5.6 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}) = 5.5 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}
\]

The above calculation underestimates the value of \(A\) because the assumption of a normal Gaussian line shape neglects the fact that this band is obviously skewed towards the higher energies. This increases the area under the curve. In fact, the value of \(\Delta v_0\) with the value of \(B_{00}\) that comes from the low-energy side of the band because the band appears to be Gaussian when \(P < P_{\text{meq}}\). To account for the tail at higher energy the normal Gaussian line shape can be multiplied by the function \(1 + \text{erf} \left( \frac{P - C}{B} \right) \), where \(\text{erf}(x)\) is the error function (see a mathematics handbook). The parameters \(A_{\text{meq}}\) and \(B\) of the normal Gaussian function are treated as adjustable parameters, as are \(a\) and \(c\) within the error function, that is, the four parameters are adjusted in the sense of a least-squares fit of square error (SSE) so as to fit the experimental data. The following Mathcad worksheet, which uses the symbol \(V\) to represent wavenumber, determines the four parameters.

Estimates of parameters in following equations:

\[
\begin{align*}
egpr & = 0.6 \text{ cm}^{-1}, \quad 10^3 \text{ mol}^{-1} \text{ cm}^{-1} \\
\psi & = 26000 \text{ cm}^{-1} \\
b & = 2000 \text{ cm}^{-1} \\
c & = 28000 \text{ cm}^{-1}
\end{align*}
\]

**Experimental Data:**

<table>
<thead>
<tr>
<th>(v_{\text{exp}})</th>
<th>(\Delta v_{\text{exp}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>26000</td>
<td>28000</td>
</tr>
<tr>
<td>32000</td>
<td>34000</td>
</tr>
<tr>
<td>38000</td>
<td>40000</td>
</tr>
</tbody>
</table>

**Gaussian Distribution of \(v\):**

\[
\delta_{\text{Gaussian}}(v - \bar{v}) : \Delta v_{\text{exp}} \times \frac{1}{\sqrt{2\pi}} e^{-\left(\frac{(v - \bar{v})^2}{2\Delta v_{\text{exp}}^2}\right)}
\]

**Distribution of \(v\) with skewed Gaussian:**

\[
\delta_{\text{Skewed Gaussian}}(v, \bar{v}, a, b, c) = \delta_{\text{Gaussian}}(v - \bar{v}) \times \left(1 + \text{erf} \left( \frac{v - C}{B} \right) \right)
\]

**Sum of Square Error function for which constants are adjusted to minimize SSE:**

\[
\text{SSE}(\bar{v}, \psi, a, b, c) = \sum_{i=1}^{n} \left( V_{\text{obs}} - \psi \left(1 + \text{erf} \left( \frac{v - C}{B} \right) \right) \right)^2
\]

As expected, the integrated absorption coefficient is larger than the value predicted by the normal Gaussian estimate. The data fit of the skewed Gaussian, shown in the Mathcad worksheet, is very good.
9. **13.10** A certain molecule fluoresces at a wavelength of 400 nm with a half-life of 1.0 ms. It phosphoresces at 500 nm. If the ratio of the transition probabilities for stimulated emission for the S$^*$ $\rightarrow$ S to the T $\rightarrow$ S transitions is $1.0 \times 10^6$, what is the half-life of the phosphorescent state?

The ratio of the transition probabilities of spontaneous emission to stimulated emission at a frequency $\nu$ is given by

$$A = \frac{8\pi \hbar \nu^2}{c^3} B \left[ \frac{k}{\lambda^3} \right] B,$$

where $k$ is a constant and we have $\nu = \frac{C}{\lambda}$.

Thus at 400 nm  $A(400) = \frac{k}{(400)^3} B(400)$

and at 500 nm  $A(500) = \frac{k}{(500)^3} B(500)$

Then, \[
\frac{A(500)}{A(400)} \left( \frac{400}{500} \right)^3 \times \frac{B(500)}{B(400)} = \left( \frac{64}{125} \right) \times 10^{-6} = 5 \times 10^{-6}
\]

Lifetimes and half-lives are inversely proportional to transition probabilities (rate constants) and hence

$$t_{\text{t}(T \rightarrow S)} = \frac{1}{5 \times 10^{-6}} t_{\text{t}(S^* \rightarrow S)} = (2 \times 10^4) \times (1.0 \times 10^{-4}) = 2 \times 10^{-4} s$$

10. **13.12** Consider some of the precautions that must be taken when conducting fluorescence microscopy experiments with the aim of detecting single molecules. (a) What is the molar concentration of a solution in which there is, on average, one solute molecule in 1.0 μm$^3$ (1.0 fl.) of solution? (b) It is important to use pure solvents in single-molecule spectroscopy because optical signals from fluorescent impurities in the solvent may mask optical signals from the solute. Suppose that water containing a fluorescent impurity of molar mass 100 g mol$^{-1}$ is used as solvent and that analysis indicates the presence of 0.10 mg of impurity per 1.0 kg of solvent. On average, how many impurity molecules will be present in 1.0 μm$^3$ of solution? You may take the density of water as 1.0 g cm$^{-3}$. Comment on the suitability of this solvent for single-molecule spectroscopy experiments.

(a) The molar concentration corresponding to 1 molecule per cubic μm is:

$$n = \frac{1}{6.022 \times 10^{23} \text{ mol}^{-1}} \times \frac{(10^2 \text{ μm m}^{-1})^3}{(1.0 \mu \text{m}^3)(10 \text{ dm m}^{-1})^3} = 1.7 \times 10^{-9} \text{ mol dm}^{-3}$$

i.e. nanomolar concentrations.

(b) An impurity of a compound of molar mass 100 g mol$^{-1}$ present at $1.0 \times 10^{-7}$ kg per 1.00 kg water can be expected to be present at a level of $N$ molecules per cubic μm where $N$ is

$$N = \frac{1.0 \times 10^{-7} \text{ kg impurity}}{1.00 \text{ kg water}} \times \frac{6.022 \times 10^{23} \text{ mol}^{-1}}{100 \times 10^{-3} \text{ kg impurity mol}^{-1}} \times (1.0 \times 10^3 \text{ kg water m}^{-3}) \times (10^{-6} \text{m})^3,$$

$$N = 6.0 \times 10^2$$

Pure as it seems, the solvent is much too contaminated for single-molecule spectroscopy.
11. **P19.8** An important biological application of absorption spectroscopy is the determination of the concentrations of solutions of nucleic acids. The $\pi \rightarrow \pi^*$ electronic transitions within the purine and pyrimidine bases of nucleic acids have absorption maxima near a wavelength of 260 nm. Assume the extinction coefficient of a nucleic acid is $1.00 \times 10^4 M^{-1} \text{cm}^{-1}$ at 260 nm. If the concentration of a nucleic acid solution is $5.00 \times 10^{-4} M$, calculate the absorbance of this solution in a 1.00 cm cell at 260 nm.

$$A = \frac{\varepsilon A_1 L [A] + \varepsilon B_1 L [B]}{\varepsilon A_1 L}$$

where $A_1$ and $A_2$ are absorbances of the mixture at wavelengths $\lambda_1$ and $\lambda_2$, and the molar extinction coefficients of A (and B) at these wavelengths are $\varepsilon A_1$ and $\varepsilon A_2$ (and $B_1$ and $B_2$).

The absorbances $A_1$ and $A_2$ at wavelengths $\lambda_1$ and $\lambda_2$ are the sum of the individual absorbances in the mixture of A and B.

$$A_1 = \varepsilon A_1 L [A] + \varepsilon B_1 L [B] \quad (i)$$

$$A_2 = \varepsilon A_2 L [A] + \varepsilon B_2 L [B] \quad (ii)$$

Solving (i) for [A] gives

$$[A] = \frac{A_1 - \varepsilon B_1 L [B]}{\varepsilon A_1 L} \quad (iii)$$

Substitution of (iii) into (ii) and solving for [B] gives

$$A_2 = \varepsilon A_1 \left( \frac{A_1 - \varepsilon B_1 L [B]}{\varepsilon A_1 L} \right) + \varepsilon B_2 L [B]$$

$$\varepsilon A_2 A_1 = \varepsilon A_1 A_1 - \varepsilon A_2 B_1 L [B] + \varepsilon A_2 B_2 L [B]$$

$$[B] = \frac{\varepsilon A_2 A_1 - \varepsilon A_2 A_1}{(\varepsilon A_2 B_2 - \varepsilon A_2 B_1) L} \quad (iv)$$

Substitution of (iv) into (iii) and simplifying gives

$$\varepsilon A_1 L [A] = A_1 - \varepsilon B_1 L \left( \frac{\varepsilon A_2 A_1 - \varepsilon A_2 A_1}{(\varepsilon A_2 B_2 - \varepsilon A_2 B_1) L} \right)$$

$$= \frac{(\varepsilon A_2 B_2 - \varepsilon A_2 B_1) A_1 - \varepsilon B_1 (\varepsilon A_2 A_1 - \varepsilon A_2 A_1)}{(\varepsilon A_2 B_2 - \varepsilon A_2 B_1) L}$$

$$= \frac{\varepsilon A_1 A_2 B_2 - \varepsilon A_1 A_2 B_1}{(\varepsilon A_2 B_2 - \varepsilon A_2 B_1) L}$$

$$[A] = \frac{\varepsilon A_1 A_2 - \varepsilon A_2 A_2}{(\varepsilon A_1 A_2 - \varepsilon A_2 B_1) L} \quad (v)$$

Equations (iv) and (v) are the desired results.

12. **P19.8** An important biological application of absorption spectroscopy is the determination of the concentrations of solutions of nucleic acids. The $\pi \rightarrow \pi^*$ electronic transitions within the purine and pyrimidine bases of nucleic acids have absorption maxima near a wavelength of 260 nm. Assume the extinction coefficient of a nucleic acid is $1.00 \times 10^4 M^{-1} \text{cm}^{-1}$ at 260 nm. If the concentration of a nucleic acid solution is $5.00 \times 10^{-4} M$, calculate the absorbance of this solution in a 1.00 cm cell at 260 nm.

$$A_1 = \log \left( \frac{I_0}{I} \right) = \varepsilon C \ell$$

$$A_{260} = 1 \times 10^4 \frac{1}{M \times \text{cm}} \times 5 \times 10^{-4} M \times 1.00 \text{cm}$$

$$A_{260} = 5$$