Problem 1 16.7% of total	Question	1-1	1-2	1-3	1-4	1-5	1-6	1-7	1-8	Total
	Points	1	3	1	3	2	1	3	3	17

Problem 4: Metals in our body

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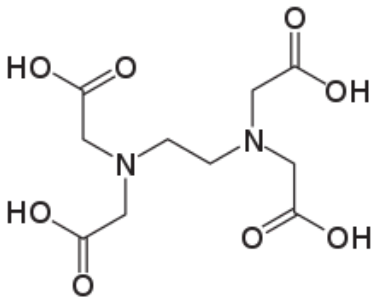
Both Ca and Zn play an important role biologically in the body system. Vanadium ions are poisonous as they can cause bone porosity by replacing the calcium ions in bones.

One treatment for vanadium poisoning involves administering a solution of ethylenediaminetetraacetic acid (edta, picture shown below). Edta forms hexacoordinate complexes with many divalent metal ions using the 4 donor oxygen and two donor nitrogen atoms.



1.1) **Draw** a diagram of how edta might bond with a V^{2+} ion.

For the general reaction:

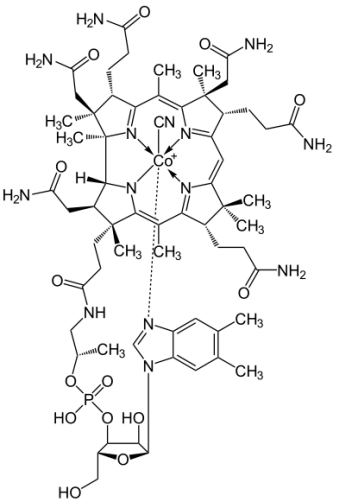
$$M^{2+} + edta^{4-} \rightleftharpoons [M(edta)]^{2-},$$

The equilibrium constants of the reaction can be determined by experimentation. The following K_c values have been computed for the various ions below.

Metal ion	K_c value
Ca^{2+}	5×10^{10}
Zn^{2+}	3×10^{16}
V^{2+}	4×10^{16}

1.2) **Explain** what problem might arise during the treatment, and **suggest** two methods to overcome the problem mentioned.

Vitamin B12 (structure on right) has a cobalt ion centre. This cobalt ion centre can undergo substitution with a zinc ion. The zinc derivative, **Znby**, is fluorescent.



A study was conducted to determine the details of its fluorescence.

The natural fluorescence lifetime is the characteristic time that a molecule remains in an excited state prior to returning to the ground state, **only** due to fluorescence. The decay of fluorescence intensity as a function of time in a uniform population of molecules excited with a brief pulse of light is described by an exponential function:

$$I(t) = I_0 e^{-t/\tau_f},$$

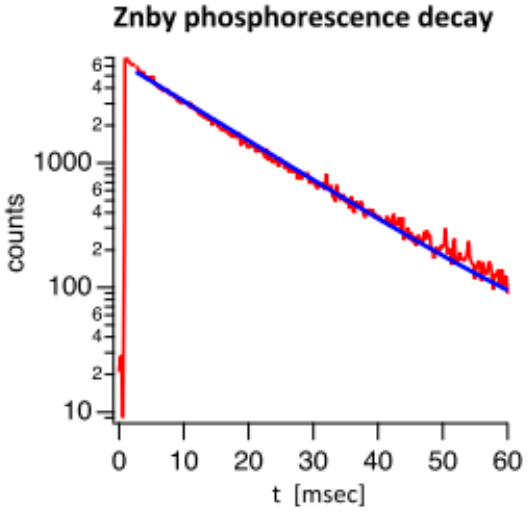
where:

- $I(t)$ is the fluorescence intensity measured at time t
- I_0 is the initial intensity observed immediately after excitation
- τ_f is the natural fluorescence lifetime

After 1 fluorescence lifetime, the initial fluorescence intensity of a fluorophore decays to a smaller intensity. If the original intensity is I_0, and the new intensity is I_1.

1.3) **Calculate** the number of times weaker the decayed intensity is after 1 natural fluorescence lifetime, the ratio I_1/I_0.

We can then record the number of photons measured after **Znby** is excited. The results are shown below, on a logarithmic axis.



1.4) **Calculate** the natural fluorescence lifetime, τ_f, of **Znby**.

However, in fluorescence, there are often other pathways in which a fluorophore can be deactivated. The quantum yield of fluorescence, Φ_f, is the rate of fluorescence, k_f, divided by the sum of the rates of all of the deactivation processes (including fluorescence). The unquenched fluorescence lifetime, τ_0, is the lifetime when the fluorophore is deactivated by all possible deactivation processes.

It is known that the emission quantum yield, Φ_f, of **Znby** is 0.70, and the unquenched intensity of emission is 24355.

1.5) By denoting the rate of all the other deactivation processes as k_{other}, **derive** the relationship between the unquenched fluorescence lifetime, τ_0, the natural fluorescence lifetime, τ_f, and the quantum yield of fluorescence, Φ_f.

1.6) Hence, **calculate** the unquenched fluorescence lifetime for **Znby**.

In an experiment, 50 μM of a quencher quenches the emission of Znby dissolved in methanol (η = 0.594 mPa s) at 20°C.

The quenched fluorescence lifetime is often denoted as τ. It is known that the ratio of the quenched, I, and unquenched emission, I_0, intensity is equal to the ratio of the quenched and unquenched fluorescence lifetimes. The concentration of the quencher is denoted [Q].

1.7) By denoting the quencher rate constant as k_q, **show** that $\frac{\tau_0}{\tau} = \frac{I_0}{I} = 1 + k_q \tau_0 [Q]$.

The rate of diffusion (k_d) in solution is

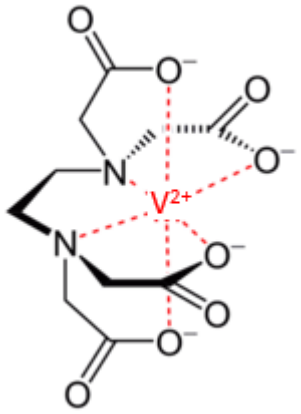
$$k_d = \frac{8RT}{3\eta}$$

where η is the viscosity of the solution, R is the ideal gas constant, and T is the temperature of the solution. **Znby** exhibits what is known as diffusion-limited quenching, where the time for quencher particles to diffuse toward and collide with excited particles is the rate-determining step for quenching.

1.8) **Calculate** the emission intensity of **Znby** after quenching. **State** any simplifying assumptions made.

Problem 1: Solution

1.1) **Draw** a diagram of how edta might bond with a V^{2+} ion.



1 point for a correct diagram.

1.2) **Explain** what problem might arise during the treatment, and **suggest** two methods to overcome the problem mentioned.

Due to the close proximity in K_c between $[V(edta)]^{2-}$ and $[Zn(edta)]^{2-}$, Zn^{2+}, which is essential for health, will also be removed in addition to V^{2+}.

Therefore, to counteract this problem:

- Limited amount of edta should be administered to prevent the loss of Ca^{2+}
- Zn^{2+} supplements can be used to counteract the removal of Zn^{2+}
- Or any other reasonable methods

1 point for a correct explanation.
1 point for each reasonable method. (Maximum 2 points)

1.3) **Calculate** the number of times weaker the decayed intensity is after 1 natural fluorescence lifetime, the ratio I_1/I_0.

$I(\tau_f) = I_1 = I_0 e^{-\tau f/\tau f}$
Hence, $I_1/I_0 = e^{-1}$ = 1/e times, or 0.368 (3 s.f.) times. Hence, it is 2.71 times weaker.

1 point for correct answer.

1.4) **<u>Calculate</u>** the natural fluorescence lifetime, τ_f, of **Znby**.

$-1/\tau_f$ is the gradient of the log graph shown.
$I = I_0 e^{-t/\tau f}$ means that $\log(I) = -1/\tau_f (t) + \log(I_0)$. Count rate of photons is directly proportional to the intensity of the photons.

Since the graph given is plotted on a logarithmic scale, simply read off the graph, taking the log of the y-values of any 2 points, and calculate the corresponding gradient.

For example, taking the points (26, 1000) and (58, 100), we can compute the gradient:
Gradient = (log(1000) - log(100))/(26-58) = –1/32.

Hence, τ_f = 32 ms.

1 point for linearising the equation.
1 point for correctly computing the gradient. (-0.5 points if the points taken are too close, covering less than ⅔ of the graph) → No points given if a single point is taken
1 point for τ_f from [10, 50] ms.

1.5) By denoting the rate of all the other deactivation processes as k_{other}, **<u>derive</u>** the relationship between the unquenched fluorescence lifetime, τ_0, the natural fluorescence lifetime, τ_f, and the quantum yield of fluorescence, Φ_f.

Since $\Phi_f = k_f / (k_f + k_{other})$, and $\tau_0 = 1 / (k_f + k_{other})$, and $\tau_f = 1 / k_f$.
Then, it is obvious that $\tau_0 = \Phi_f \tau_f$.

1 point for writing out the expression for all 3 variables.
1 point for correct relationship.

1.6) Hence, **<u>calculate</u>** the unquenched fluorescence lifetime for **Znby**.

$\tau_0 = \Phi_f \tau_f = 0.70 \times 32$ ms = 22.4 ms

1 point for correct answer.

1.7) By denoting the quencher rate constant as k_q, **<u>show</u>** that $\frac{\tau_0}{\tau} = \frac{I_0}{I} = 1 + k_q \tau_0 [Q]$.

Since $\tau_0 = 1 / (k_f + k_{other})$, $\tau = 1 / (k_f + k_{other} + k_q[Q])$, then
$\tau_0 / \tau = (k_f + k_{other} + k_q[Q]) / (k_f + k_{other}) = 1 + k_q / (k_f + k_{other}) [Q] = 1 + k_q \tau_0 [Q]$.
Since $\tau_0 / \tau = I_0 / I$, $\tau_0 / \tau = I_0 / I = 1 + k_q \tau_0 [Q]$.

1 point for relating τ_0 / τ to the rate constants.
1 point for writing the quenching rate as $k_q[Q]$, since it is a first order reaction.
1 point for simplifying showing the relationship stated in the question.

1.8) **Calculate** the emission intensity of **Znby** after quenching. **State** any simplifying assumptions made.

Assume that almost all collisions of quencher particles and excited particles are effective. This makes it such that $k_q = k_d$.

$k_d = (8 \times 8.3145 \times 293)/(3 \times 0.594 \times 10^{-3}) = 1.09 \times 10^{10}$ mol^{-1} dm^3 s^{-1}.
Rearranging the equation we got above, $I = I_0/(1+ k_d\tau_0 [Q]) = 24355/(1 + 1.09 \times 10^{10} \times 22.4 \times 10^{-3} \times 50 \times 10^{-6}) = 24355/13.208 = 1840$.

1 point for correct assumption.
1 point for correct k_d value.
1 point for correct computation of final answer.