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CHAPTER 2

Problem 2.1: In a person with active sickle-cell anemia, the concentration of hemoglobin in their whole blood is ~70 g/L. What is the effective molar concentration of hemoglobin in the average tissue in that case?

Answer: 43 μM.

Solution:
The molar concentration of Hb in the blood of this person is:

$$C_{\text{Hb-blood}} = \frac{70\ \text{g/L}}{64500\ \text{g/mol}} = 1.085 \times 10^{-3}\ \text{M}$$

where M is the shorthand unit symbol for mol/L.

Then, assuming the blood-volume-fraction in average tissue to be 4% = 0.04:

$$C_{\text{Hb-tissue}} = 0.04 \times C_{\text{Hb-blood}} = 43\ \mu\text{M}$$

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Problem 2.2: Based on an assumed tissue molar concentration for hemoglobin of 100 μM, and for the case of a hemoglobin oxygen saturation of 85% averaged over the capillary bed, find the absorption coefficient, μ_a, of tissue due to total hemoglobin at a wavelength of 700 nm. (Use the extinction coefficients of Hb and HbO in Table 2.A.1). What would be the mean-free-path for absorption?

Answer: $\mu_a = 0.1257\ \text{cm}^{-1}$; $\text{mfp}_a = 7.96$ cm.

Solution:
85% of the hemoglobin is oxy-hemoglobin, and 15% is deoxy-hemoglobin. At 700 nm, in Table 2.A.1 we find $\varepsilon_{\text{Hb}} = 4206\ \text{cm}^{-1}/\text{M}$ and $\varepsilon_{\text{HbO}} = 736.4\ \text{cm}^{-1}/\text{M}$. (Remember that if you want to compare these values with those in Fig. 2.4, the extinction coefficients in Fig. 2.4 must be multiplied by a factor 2.3 to determine values in base-*e*, to enable calculation of μ_a.)

Then:

$$\begin{aligned}\mu_a &= C_{\text{Hb}}\varepsilon_{\text{Hb}} + C_{\text{HbO}}\varepsilon_{\text{HbO}} \\ &= 15 \times 10^{-6}\ \text{M}\ (4206\ \text{cm}^{-1}/\text{M}) + 85 \times 10^{-6}\ \text{M}(736.4\ \text{cm}^{-1}/\text{M}) \\ &= 0.1257\ \text{cm}^{-1}\end{aligned}$$

and, $\text{mfp}_a = 1/\mu_a = 7.96$ cm.

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Problem 2.3: For the conditions of Problem 2.2, what would be the absorption coefficient and mean-free-path for absorption at 420 nm?

Answer: $\mu_a \approx 92\ \text{cm}^{-1}$; $\text{mfp}_a \approx 108\ \mu\text{m}$.

Solution:
At 420 nm (an isosbestic point), from Fig. 2.4 we estimate

$$\varepsilon_{\text{Hb}} \cong \varepsilon_{\text{HbO}} \cong (4.0 \times 10^5\ \text{cm}^{-1}/\text{M}) \times\ 2.3 = 9.2 \times 10^5\ \text{cm}^{-1}/\text{M}$$

where the factor 2.3 is introduced to convert the molar extinction coefficient in base-10 of Fig. 2.4 to that in base-*e* needed here to calculate μ_a.

Then use $C_{\text{Hb+HbO}} = 1 \times 10^{-4}$ M to find the absorption coefficient:

$$\mu_a = C_{\text{Hb+HbO}}\varepsilon \cong 10^{-4}\ \text{M} \times 9.2 \times 10^5\ \text{cm}^{-1}/\text{M} = 92\ \text{cm}^{-1}$$

Finally, the mean-free-path for absorption is:

$$\text{mfp}_a = \frac{1}{\mu_a} \cong \frac{1}{92\ \text{cm}^{-1}} \cong 108\ \mu\text{M}$$

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Problem 2.4: The absorption spectra of HbO and Hb are equal at ~800 nm, which is referred to as an *isosbestic point*, a wavelength at which the extinction coefficients of the two forms of hemoglobin (oxy- and deoxy-) are equal, such that the total absorption by hemoglobin is independent of the redox stoichiometry. Explain why it is useful to make spectroscopic measurements at both 800 nm and another wavelength, say 650 nm, when determining the oxygen saturation of a blood sample.

Answer: Since the extinction coefficient at an isosbestic point is independent of the stoichiometry, the absorption coefficient will also be independent of oxygenation, regardless of the total concentration of hemoglobin. Thus, a measurement of absorbance at that point serves as a good reference point for normalization of the response of an absorbance measuring system. Then, at a wavelength such as 650 nm, where the difference between the extinction coefficients of Hb and HbO is large, the absorbance can be directly compared to that at 800 nm, and the oxygen-saturation (stoichiometry) can be determined without knowledge of the individual or overall concentrations. In short, determination of the oxygen saturation of hemoglobin only requires measurement of the ratio of the absorption coefficients at the two wavelengths, and not of their individual values (see Eq. (15.10)).

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Problem 2.5: A *pulse oximeter* (see Section 15.1.2) is a device that attempts to determine the oxygen saturation of arterial blood, by measuring the intensity of light that diffuses across peripheral tissue like a fingertip or earlobe. For simplicity, assume that the oxygen saturation of the blood on the arterial side of the capillaries is 100%, and for the venous side is 60%. Also assume that the blood-volume fractions of both sides of the capillaries are equal. If the blood-volume fraction of the arterial side fluctuates by ±5% from its mean value as a result of blood-pressure variations with the pulse (and the blood-volume fraction of the venous side is constant), what will be the percent size of the measured fluctuations detected in diffusely transmitted light at 800 nm, if the average effective photon pathlength is 2 cm?

Answer: 1.9%.

Solution:
As posed, this problem provides more information than is necessary to determine the answer. The extinction coefficients for Hb and HbO are equal at ~800 nm, and the blood-volume fractions (BVF) of the arterial and venous sides are stated to be equal, so the saturation of the venous side does not matter, because the arterial fluctuations simply represent fluctuations in half of the total BVF. (The capillary volume also contributes to both sides of the BVF, adding a baseline but not exhibiting pulsatile volume changes, and we ignore that contribution in this simplified discussion.) Thus, the resulting fluctuations in total BVF will be half of the variation of the arterial side (or 5% deduced from ±2.5%): $\Delta C_{\mathrm{Hb+HbO}} = 0.05 C_{\mathrm{Hb+HbO}}$. Again, we assume average tissue BVF of 4%, such that the average tissue concentration of hemoglobin is $C_{\mathrm{Hb+HbO}} \cong 100\ \mu\mathrm{M}$; then the high and low values of the fluctuating concentration will be 102.5 and 97.5 μM, respectively.

From Table 2A.1 we find the isosbestic point, more precisely, at 798 nm, where $\varepsilon_{\mathrm{Hb}} \cong \varepsilon_{\mathrm{HbO}} \cong 1.96 \times 10^3\ \mathrm{cm^{-1}/M}$. The resulting absorption coefficient for the *average* concentration is $\mu_a = 100\ \mu\mathrm{M} \times 1.96 \times 10^3\ \mathrm{cm^{-1}/M} = 0.196\ \mathrm{cm^{-1}}$. The resulting absorption coefficients, μ_{a2} and μ_{a1}, for the high and low values of the hemoglobin concentration, respectively, will be

$$\mu_{a2} = 1.025 \times 10^{-4}\ \mathrm{M}\ (1.96 \times 10^3\ \mathrm{cm^{-1}/M}) = 0.201\ \mathrm{cm^{-1}}$$

$$\mu_{a1} = 0.975 \times 10^{-4}\ \mathrm{M}\ (1.96 \times 10^3\ \mathrm{cm^{-1}/M}) = 0.191\ \mathrm{cm^{-1}}$$

Then, the Beer-Lambert law tells us how to calculate the attenuated light intensity I, relative to the incident intensity, I_0, after propagating through the distance $\langle L \rangle$, the average effective photon pathlength (= 2 cm):

$$I = I_0 e^{-\mu_a \langle L \rangle},$$

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assuming the scattering effects lead to the same effective pathlength for the high and low concentrations. Then, we can calculate the ratio of the transmitted light intensities I_2 and I_1 , corresponding to the high and low concentrations:

$$\frac{I_2}{I_1} = \frac{e^{-\mu_{a2}\langle L\rangle}}{e^{-\mu_{a1}\langle L\rangle}} = 0.981\,.$$

Thus, the percent fluctuation in the transmitted intensity is:

$$\left|\frac{I_2 - I_1}{I_1}\right| = \left|\frac{I_2}{I_1} - 1\right| = 0.019 = 1.9\%$$

We note that this problem deals with an extension of the Beer-Lambert law to highly scattering media (where the sample thickness is replaced by an average photon pathlength, which, in general, depends on both the absorption and scattering properties of the medium), leading to the so-called *modified Beer-Lambert law*. Examples and more details are provided by Eq. (8.27) (and the following text), and in Section 10.3.

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Problem 2.6: A turbid medium used for a tissue phantom is made of an aqueous suspension of scattering microparticles with an average particle diameter of 1.5 μm.

a) If the value of the scattering efficiency for the particles is 0.5, what will be the needed density of the particles (in number of particles per cm^3) to achieve a scattering coefficient, μ_s , of 50 cm^{-1}?
b) What would need to be the effective anisotropy factor, g, of the particles to result in a reduced scattering coefficient, μ_s' , of 10 cm^{-1} ?

Answer: (a) 5.7×10^9 cm^{-3}; (b) 0.8.

Solution:

(a) The particle physical area is $A_s = \pi r^2$, where r is the particle radius, 0.75 μm; thus, $A_s = 1.767 \times 10^{-8}$ cm^2 Then, from Eq. (2.5) we have

$$N = \frac{\mu_s}{Q_s A_s} = \frac{50\ \text{cm}^{-1}}{0.5(1.767 \times 10^{-8}\ \text{cm}^2)} = 5.7 \times 10^9\ \text{cm}^{-3}$$

(b) Solve Eq. (1.30) for g:

$$g = 1 - \frac{\mu_s'}{\mu_s} = 1 - \frac{10\ \text{cm}^{-1}}{50\ \text{cm}^{-1}} = 0.8$$

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