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Near Infrared Spectroscopy

By Clare Elwell and Jem Hebden

Introduction

All of us are exposed to optical (i.e. visible and near-infrared) radiation from the sun and other sources throughout our lives. Assuming our eyes are shielded from excessive intensity, and our skin is protected from the ultraviolet content of sunlight, we accept this exposure in the knowledge that it is perfectly safe. Unlike x-rays, optical photons are insufficiently energetic to produce ionisation, and unless light is concentrated to such a high degree that it causes burning to the skin, optical radiation offers no significant hazard. The diagnostic potential of optical methods has been widely known since Jöbsis [1] first demonstrated that transmittance measurements of near-infrared (NIR) radiation could be used to monitor the degree of oxygenation of certain metabolites. This led to the development and increasingly widespread use of clinical near-infrared spectroscopy (NIRS), which offers a safe, non-invasive means of monitoring cerebral function at the bedside without the use of radioisotopes or other contrast agents [2].

Human tissues contain a variety of substances whose absorption spectra at NIR wavelengths are well defined, and which are present in sufficient quantities to contribute significant attenuation to measurements of transmitted light. The concentration of some absorbers, such as water, melanin, and bilirubin, remain virtually constant with time. However, some absorbing compounds, such as oxygenated haemoglobin (HbO_2), deoxyhaemoglobin (Hb), and oxidised cytochrome oxidase (CtOx), have concentrations in tissue which are strongly linked to tissue oxygenation and metabolism. Increasingly dominant absorption by water at longer wavelengths limits spectroscopic studies to less than about 1000 nm. The lower limit on wavelength is dictated by the overwhelming absorption of Hb below about 650 nm. However, within the 650-1000 nm window, it is possible with sensitive instrumentation to detect light which has traversed up to 8 cm of tissue.

Absorption

The absorption of light intensity in a non-scattering medium is described by the Beer-Lambert Law. This law states that for an absorbing compound dissolved in a non-absorbing medium, the attenuation (A) is proportional to the concentration of the compound in the solution (c) and the optical pathlength (d):

$A = \log_{10}[I_0/I] = \varepsilon c d$

where *A* is the attenuation measured in optical densities, I_0 is the light intensity incident on the medium, I is the light intensity transmitted through the medium, *a* is the specific extinction coefficient of the absorbing compound measured in micromolar per cm, *c* is the concentration of the absorbing compound in the solution measured in micromolar, and *d* is the distance between the points where the light enters and leaves the medium. The product *ac* is known as the *absorption coefficient* of the medium μ_a. In a medium containing several different absorbing compounds (except at very high concentrations not usually met in biological media) the overall extinction coefficient is simply the linear sum of the contributions of each compound:

$$A = \log_{10} [I_0/I] = [a_1.c_1 + a_2.c_2 + a_3.c_3 + ... + a_n.c_n] d \quad .$$

A compound which absorbs light in the spectral region of interest is known as a *chromophore*. Each chromophore has its own particular absorption spectrum which describes the level of absorption at each wavelength. The principle chromophores in tissue are as follows:

i) Water

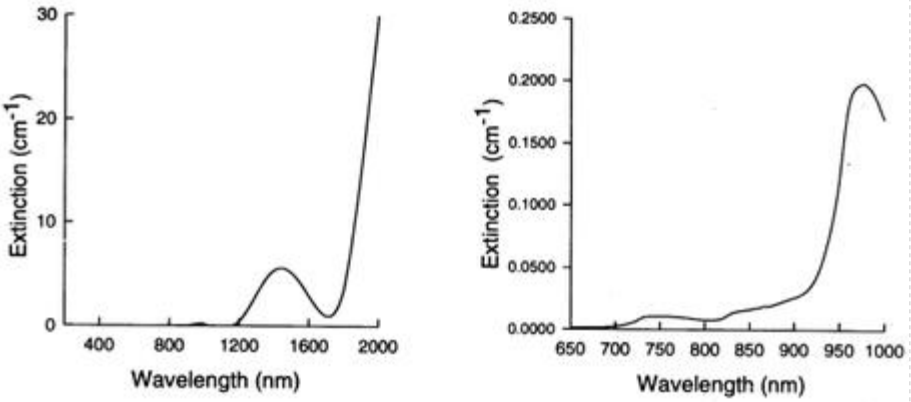

Figure 1: The absorption spectra of pure water. As shown above in figure 1, the absorption of light by water is relatively low between 200 - 900 nm. Beyond 900 nm absorption starts to rise with increasing wavelength, a spectral peak being visible at 970 nm. The high concentration of water in living tissue, typically 80% in adult brain tissue [3], (equivalent to 56 molar) determines the wavelength region in which spectroscopic interrogation of tissue is possible by strongly limiting the tissue thickness through which light can penetrate. For this reason, the water spectrum is said to demonstrate a "window" of transparency between 200 and 900 nm within which spectroscopic measurements can be made. For the purposes of most clinical measurements the water concentration in tissue can be thought of as constant, and as such water acts as a fixed constant absorber.

ii) Lipids

Although the distribution of lipid in tissue is dependent upon tissue type, it can also be thought of as a constant absorber with changes in its concentration throughout the course of a clinical measurement being unlikely. The absorption spectrum of lipid is approximately the same as that of water and it can comprise 10 - 40 % (i.e. several molar) of tissue.

iii) Melanin

especially in the ultraviolet region of the spectrum. Although this absorption can be considered to be constant and oxygen independent, the concentration of melanin in tissue will directly effect the reflectance of light from the skin and therefore the transmission of light into the tissue below.

iv) Haemoglobin

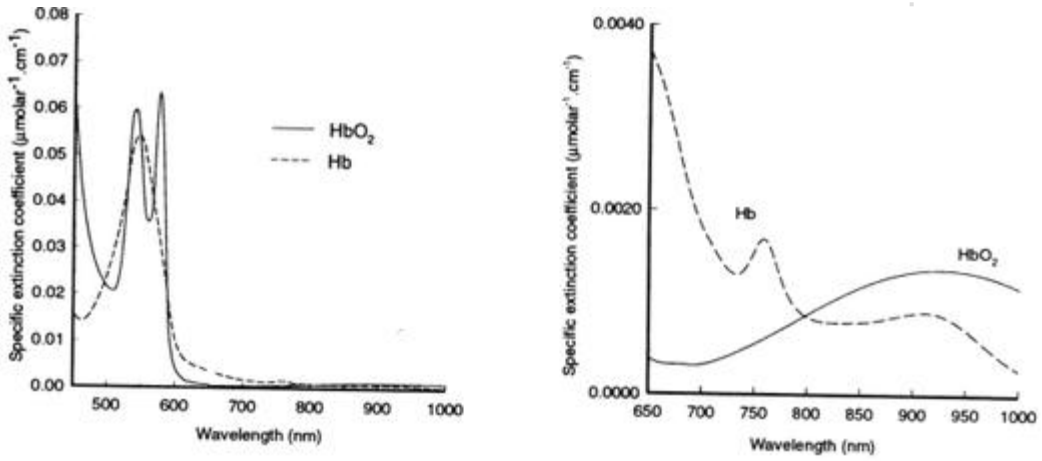

Figure 2: The absorption spectra of HbO2 and Hb. Figure 2 above shows the specific extinction coefficients of oxygenated haemoglobin (HbO_2) and deoxyhaemoglobin (Hb) in the wavelength range 450 - 1000 nm [4]. The difference in the absorption spectra explains the well recognised phenomena of arterial blood (containing approximately 98% HbO_2) having a bright red appearance while venous or deoxygenated blood appears more blue. In the NIR region of the spectrum the absorption of both chromophores decreases significantly compared to that observed in the visible region. However the absorption spectra of Hb and HbO_2 remain significantly different in this region allowing spectroscopic separation of the compounds to be possible using only a few sample wavelengths. An isobestic point where the specific extinction coefficients of the two compounds are equal can be seen at around 800 nm, which can be used to calculate haemoglobin concentration independent of oxygen saturation. The typical value for haemoglobin concentration in, for example, adult brain tissue is 84 micromolar.

There are other haemoglobin compounds which have a characteristic absorption in the near infrared, although their concentrations in tissue are low and in many cases almost non existent in normal blood. These compounds include carboxyhaemoglobin, (HbCO), which may be present in significant quantities in the tissue in some subjects, but has a low specific extinction coefficient in the NIR rendering its effect on most in-vivo measurements negligible. Haemiglobin (Hi) is present in very low concentrations and sulfhaemoglobin (SHb) is not present at all in normal blood. The combined error in ignoring these compounds in the measurement of the total haemoglobin signal is probably less than 1% in normal blood and in the majority of clinical conditions encountered. However it is worth remembering that some of these forms of haemoglobin, especially Hi, may become significantly raised in some diseases of the liver or in malaria.

v) Cytochrome c oxidase

Cytochrome oxidase (CtOx) is the terminal enzyme in the cellular respiratory chain, and is located in the mitochondrial membrane. The enzyme contains four redox active groups, two haem iron (*a* and a_3) and two copper (Cu_A and Cu_B) centres.

during electron turnover of the enzyme. The oxygen binding site of the enzyme is the binuclear unit which is formed of the Cu_B and haem a_3. It is the donation of electrons from this unit to oxygen which accounts for the great majority of oxygen consumption in biological tissue. The Cu_A and haem *a* centres donate electrons to this binuclear unit and are therefore not directly involved in reduction of oxygen. However absorption of NIR radiation by cytochrome oxidase occurs primarily at the Cu_A centre, the oxidised spectrum having a characteristic shape, with a broad peak centred around 830 nm which is missing in the reduced enzyme. In the short term the total tissue CtOx concentration does not vary and NIRS measurements of changes in CtOx thus measure alterations in the redox state concentration of Cu_A within cytochrome oxidase.

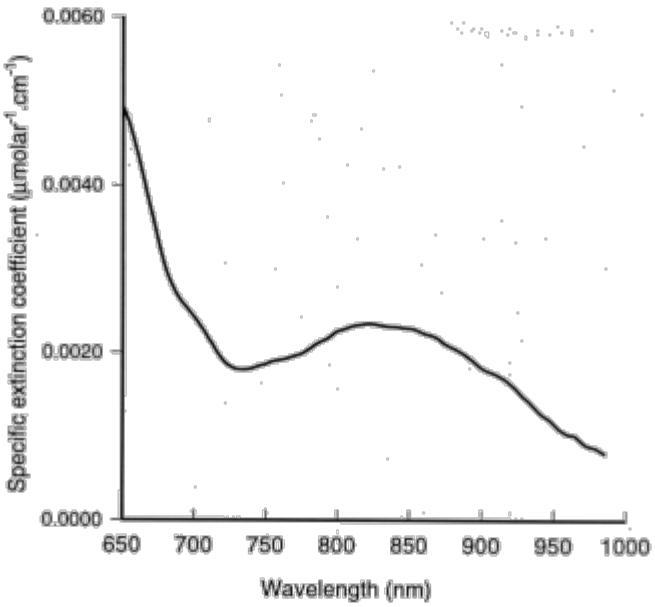

Figure 3: The difference absorption spectrum between the oxidised and reduced forms of CtOx.

Since the total CtOx concentration does not alter, NIRS measurements need only be made of the *change* in redox state, so it is only necessary to know the *difference* spectrum between the oxidised and reduced forms of the enzyme. This difference spectrum is shown in figure 3. It can be seen that the magnitude of the specific extinction coefficients are similar to those of haemoglobin, but since the concentration of cytochrome oxidase in living tissue is usually at least an order of magnitude below that of haemoglobin [5], the measurement of cytochrome oxidase with optical techniques is by no means as easy as that of haemoglobin. When oxygen limits the rate of oxygen consumption by cytochrome oxidase, the Cu_A centre becomes more reduced. Therefore the absorbance of NIR light by cytochrome oxidase may be used as an indicator of oxygen availability at a cellular level and ultimately of cell metabolism.

Scattering

Scatter of light in tissue is due to the chaotic variation in refractive index at a microscopic and macroscopic scale. This occurs at membrane boundaries of the cells themselves as well as at boundaries between various organelles inside the cell. Index mismatching will occur between intra and extracelluar fluid, or

such as mitochondria, ribosomes, fat globules, glycogen and secretory globules. As with absorption, the volume of a particular scatterer within the tissue is as important as its scattering ability. Evidence suggests that cell membranes are the most important source of scattering in brain tissue since they account for a large proportion of the solid content of the tissue.

Scatter is by far the most dominant tissue-photon interaction at NIR wavelengths. The effect of scattering is to substantially increase the pathlength travelled by photons within tissue, and therefore significantly increase the probability of absorption occurring. When NIR radiation is scattered in tissue virtually all the collisions are elastic, and the direction in which the scattered photon travels is dependent upon the size of the scattering particle, the wavelength of the light, and the refractive indices of the scattering media through which it is travelling.

The attenuation (A) due to *single* scattering is proportional to the number density of the scattering particles (N), the scattering cross section of the particles (s) and the optical pathlength (d):

$$A = \log_{10} [I_0/I] = N.s.d \quad .$$

The product Ns is known as the *scattering coefficient* of the medium (μ_S), and is the probability per unit length of a photon being scattered. The reciprocal is the mean free path between scattering events. The scattering coefficients of human tissues are generally within the range 10 - 100 mm^{-1}, roughly one hundred times greater than those for absorption [6]. The most highly scattering tissues include bone, cerebral white matter, and skin dermis.

For multiply scattering media such as tissue, the simple formula given above no longer applies. In order to fully describe scatter of light in tissue, it is necessary to consider the probability of a photon being scattered in a given direction at each interaction. The probability of a photon, incident along a unit vector **p** being scattered into a direction **q** is described by the phase function $f(\mathbf{p},\mathbf{q})$. For a random medium it can be assumed that this probability is independent of **p** and only depends on the angle between the incident and scattered directions, e. Thus the phase function can be conveniently expressed as a function of the scalar product of the unit vectors in the initial and final directions, which is equal to the cosine of the scattering angle $\cos(e)$. The anisotropy in the probability distribution is commonly characterised in terms of the mean cosine of the scattering angle g.

In biological tissues, scatter occurs principally in a forward direction, corresponding to an anisotropy in the range $0.69 > g > 0.99$ [6]. Despite the forward scatter, typical values of scattering coefficient ensure that light travelling through more than a few millimetres of tissue loses all of its original directionality, and can be treated as effectively isotropically distributed. Thus it is convenient to express the characteristic scatter of tissues in terms of a transport scatter coefficient:

$$\mu_S' = \mu_S (1 - g) \quad ,$$

which represents the effective number of isotropic scatters per unit length, and is a fundamental parameter in diffusion theory.

The Modified Beer-Lambert Law

When a highly scattering medium is considered, the Beer-Lambert relationship

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