

Near infrared diode laser spectroscopy of organic compounds in turbid aqueous solutions

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Introduction

Accurate quantitative analysis of absorption and scattering properties in turbid media is an important task in many optical-based sensing techniques in biomedicine,¹ biotechnology² and chemical production processes.³ Due to light scattering in turbid media, determination of absolute concentrations of absorbing species can, in most cases, not easily be achieved without extensive calibration, either because light scattering prevents simple Lambert–Beer’s law analysis or the absorption coefficient is not known to a sufficient certainty. Scattering properties are of relevance, for example, for the determination of biomass in fermentation processes.² The objective of the current study is the elucidation of experimental methods for determining absorption and scattering coefficients and species concentrations in highly scattering media. While light absorption of organic compounds in the infrared (IR) spectral region is generally high, molecular light scattering in this spectral region is usually weak due to the dependence of light scattering processes on wavelength λ , for example, simple Rayleigh light scattering intensity scales with λ^{-4} . On the other hand, high absorption of visible radiation, which is more effectively scattered than IR radiation, is restricted to compounds containing extended π -electron systems. In the near infrared (NIR) spectral region, both sufficient light absorption of most organic compounds and reasonable intense light scattering can be observed, which, in principle, allows simultaneous analysis of absorption and scattering properties. The concentrations of a wide range of absorbing and scattering species can, therefore, be detected in the NIR spectral region.

Prominent NIR absorption bands are associated with the overtone and combination bands of the fundamental mid-IR vibrations of CH, NH, and OH functional groups. Since the intensity of an NIR absorption band is typically 10–100 times weaker than its comparable mid-IR band, sensitive absorption measurements in this spectral region may require sophisticated noise cancellation schemes. Recent improvements in room temperature diode laser technology for communications and data storage applications have given rise to a new class of optical NIR sensors with improved sensitivity. Compared to traditional lasers, diode lasers are generally compact, reliable, easy to operate, amenable to electronic high frequency modulation and temperature tuning and are, in most cases, of low cost. Diode lasers recently enabled significant progress in ultrasensitive gas analysis in both research and industrial applications (for example, see References 4 and 5 and references cited therein). High quality room temperature diode laser devices are now available in a number of discrete spectral windows between 410 nm and 2000 nm.

In the current study, two experimental procedures using intensity modulated NIR diode lasers were employed to analyse light absorption and scattering properties of turbid solutions. In the first procedure employed, diffusely reflected light is analysed, while in the second procedure, light inten-

sity, due to photon migration inside the sample, is detected. The applicability of theoretical models used to evaluate the measurements was established by investigation of simple light scattering and absorbing model systems.

The employed techniques allow analysis of any light-scattering sample containing a compound with selective absorption of radiation. However, we concentrated, in the current work, on aqueous solutions because of their outstanding role in environmental analysis, biotechnology, and biomedicine. The organic compounds selected include chlorinated hydrocarbons, which are of particular relevance as ground and waste water pollutants⁶ and various sugars, which are important analytes in fermentation processes⁷ and human blood glucose analysis,⁸ respectively.

Experimental

Absorption and total light attenuation measurements were carried out on a conventional Varian Cary 500 UV/VIS-NIR absorption spectrometer, if not otherwise stated. Fibre-optic diffuse reflectance measurements and photon migration studies of selected organic compounds in turbid aqueous solutions were performed with an intensity-modulated laser diode spectrometer (IMLDS). The IMLDS consisted of a home-built array of intensity-modulated diode lasers as light sources. Photodiodes, amplifier and a lock-in amplifier (EG&G 7260) set to the modulation frequency, were employed for phase-sensitive detection of scattered light intensity. Current laser diode wavelengths included $\lambda = 678, 790, 1544$ nm (from standard Fabry–Perot laser diodes) and the wavelength regions 763–780 and 1360–1420 nm (from tunable external cavity diode lasers). Additionally, a Helium–Neon laser (633 nm) was employed. The laser diodes and the detectors were coupled to fibre-optics which allow flexible illumination and detection geometries. For diffuse reflectance measurements, the sample surface was illuminated under 60 degrees, while the detection fibre-optics were placed at 0 degrees with respect to the axis perpendicular to the sample surface. A reflectance Spectralon standard with 98% average reflectance was used as the white standard (Labsphere). For the photon migration studies, both the illumination and the detection fibre-optics were immersed in the medium at 0 degrees angle with respect to the axis perpendicular to the sample surface, the two fibres being separated by the distance r . Ultrafiltrated, deionised, non-degassed water and commercially available milk powder (Nestlé) were used. All other chemicals were purchased from Aldrich Co. and used without purification.

Results and discussion

Detailed understanding of the water NIR absorption spectrum, which is known to crucially depend on temperature and solutes,^{9–11} is the first step of spectroscopic investigations of aqueous solutions in the NIR spectral region. Figure 1 shows the absorption spectrum of water between *c.* 900 and 1900 nm in the temperature range 10–70°C and (inset) in selected organic solvents.

Following the assignment of Luck,¹⁰ the spectrum consists of four main maxima located at *c.* 970 nm (10300 cm⁻¹), *c.* 1190 nm

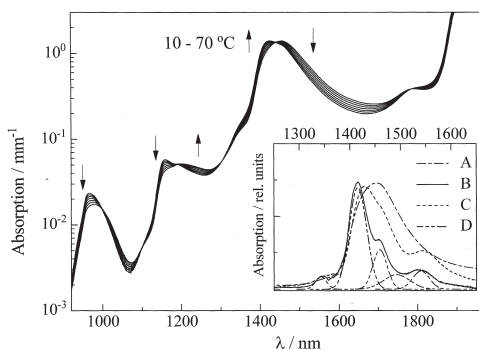


Figure 1. Absorption spectrum of water in the temperature range 10–70°C. The arrows indicate increasing or decreasing absorption with increasing temperature. Inset: water absorption spectrum between 1250 and 1650 nm in water (A), in acetone (B, *c.* 2 vol.%), and in *p*-dioxane (C, *c.* 2 vol.%) at room temperature. D: Exemplary fitting of the water absorption spectrum in acetone by five gaussians.

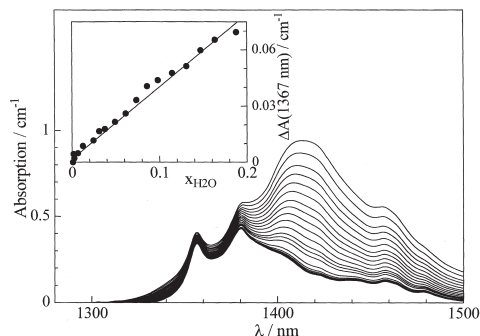


Figure 2. Absorption spectrum of acetone containing various concentrations of water. Inset: plot of the absorption changes of acetone/water mixtures at 1367 nm ($\Delta A(1367 \text{ nm})$) detected with a diode laser v. mole fraction of water.

has been a field of intensive research and discussion during the last decades. Different models describing the water structure may be divided into two main classes: mixture and continuum models.¹² The former indicates an equilibrium mixture of discrete species, differing according to their numbers of hydrogen bonds. The latter suggests almost completely hydrogen-bonded molecules in a continuous network where distortion of the H-bonds results in a continuous distribution of the H-bond distances, angles and energies.

The water absorption spectrum in the spectral region between 1350 and 1650 nm can tentatively be separated into five or six gaussian-like bands (Figure 1, inset). Spectral variations can be analysed using derivative spectroscopy to locate band positions and by curve fitting procedures.^{9,11} In this way, the changes of the water spectrum by solutes can be systematically studied and corrections for water background absorptions can be achieved. Furthermore, understanding of spectral variations of the water absorption spectrum is necessary for NIR optical determination of water contents in organic solvents. Figure 2 shows the absorption spectrum of acetone/water mixtures containing between 0 and c. 0.2 mole fraction of water and (inset) the absorption changes at 1367 nm as measured with the IMLDS (intensity-modulated laser diode spectrometer). At 1367 nm the absorption of the solution increases linearly with increasing water mole fraction.

Up to this point we were concerned with clear solutions. For analysis of absorption and scattering properties of turbid samples, diffuse reflectance and photon migration studies with NIR diode lasers were applied. The former can simply be described by the Kubelka-Munk law:¹³

$$\frac{K}{S} = \frac{(1 - R')^2}{2R'} = F(R') \quad (1)$$

(K denotes the absorption and S the scattering parameter which are in first approximation proportional to absorber and scatterer concentration, respectively. $R' = R/R_0$ is the relative reflectance with R being the reflectance of the sample and R_0 the reflectance of the white standard.)

The photon migration studies, in which light intensity after migration through turbid solution is measured, are analysed in terms of simplified photon diffusion theory.¹³ In the framework of this theory, light transport through the solution is described by a transport equation derived from a particle dif-

(8400 cm^{-1}), c. 1450 nm (6900 cm^{-1}) and c. 1940 nm (5160 cm^{-1}) due to the second overtone of the O–H stretching band ($3\nu_{1,3}$), the combination of the first overtone of the O–H stretching and the O–H bending band ($2\nu_{1,3} + \nu_2$), first overtone of the OH-stretching band ($2\nu_{1,3}$), and combination of the O–H stretching band and the O–H bending band ($2\nu_{1,3} + \nu_2$), respectively. Closer inspection of the water spectrum, for example, by calculating second derivative spectra, reveals even more local maxima, which indicates contributions of rotational and/or more vibrational modes than have been accounted for in the qualitative analysis above.

The large spectral variations of the water NIR absorption spectrum induced by temperature and solvents are interpreted in the literature as being due to changes of the hydrogen-bonded water structure. The hydrogen-bonded structure of water and its temperature-dependent variation

fusion approach of photon migration through random media. Solving the transport equation for appropriate boundary conditions, yields for the dependence of (intensity-modulated) light intensity on the separation distance between light source and detection point r :¹⁴

$$I_{ac} = \frac{SA}{4\pi v D r} \exp \left[-r \left(\frac{v^2 \mu_a^2 + \omega^2}{v^2 D^2} \right)^{1/4} \cos \left\{ \frac{1}{2} \tan^{-1} \left(\frac{\omega}{v \mu_a} \right) \right\} \right] \quad (2)$$

Equation 2 yields

$$\ln(I_{ac} r) = -r \left(\frac{v^2 \mu_a^2 + \omega^2}{v^2 D^2} \right)^{1/4} \cos \left[\frac{1}{2} \tan^{-1} \left(\frac{\omega}{v \mu_a} \right) \right] + \ln \left(\frac{SA}{4\pi v D} \right) \quad (3)$$

(I_{ac} —ac light intensity; S —fluence of photon source; A —modulation depth of source; v —speed of light in the medium; $D = [3\{\mu_a + \mu_s\}(1 - g)]^{-1}$ diffusion coefficient with absorption coefficient μ_a , scattering coefficient μ_s , and the average of the cosine of the scattering angle g ; ω —angular modulation frequency).

Assuming $\omega < 1$ MHz and $\mu_a < \mu_s$, the slope m of $\ln(I_{ac} r)$ v. r simplifies to:

$$m = \frac{\partial(I_{ac} r)}{\partial r} \approx \sqrt{\mu_a / D} \approx \sqrt{3(1 - g)\mu_s \mu_a} \quad (4)$$

The term $(1 - g)\mu_s$ is constant for a species of given concentrations at a given wavelength and called effective scattering coefficient. In the first approximation, m^2 is thus proportional to the absorption and to the effective scattering coefficient.

Because the theoretical approaches of Equations 1 and 2 assume a number of boundary conditions which may be difficult to achieve experimentally, we established the applicability of Equations 1 and 2, by investigating simple model systems. A typical model system consisted of an absorber (for example, malachite green, black ink) and scatterer (for example, milk powder, caolinit), which exhibit negligible absorption in the spectral region of interest, in aqueous solution. Figure 3 shows exemplary results for diffuse reflectance measurements of milk powder solutions containing various concentrations of malachite green. A plot of $F(R')$ v. malachite green concentration ([MG]) exhibited good linearity, which indicates that the absorption parameter K is proportional to the absorber concentration. Equation 1 provides a good basis for the determination of absorber concentration in a turbid sample, but absolute determination of absorber and scatterer concentrations without calibration procedures is not trivial, because (i) experimental conditions to meet the assumptions of Equation 1 are not easy to employ practically and (ii) there is no unambiguous description of the physical meaning of parameters K and S in the literature. Although considerable efforts have been undertaken in the literature to relate the parameters K and S to molecular (Lambert–Beer) absorption and scattering coefficients, it still seems that calibration procedures are necessary to employ diffuse

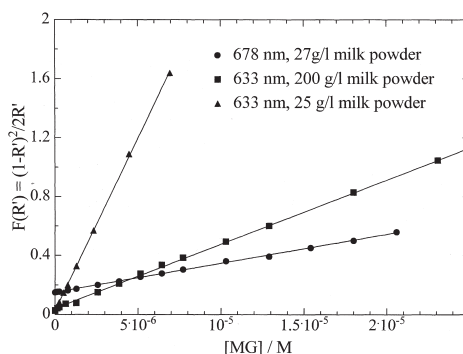


Figure 3. Diffuse reflectance measurements of milk powder solutions in water containing various concentrations of malachite green (MG). The diffuse reflectance in terms of $F(R')$ is plotted v. MG concentration.

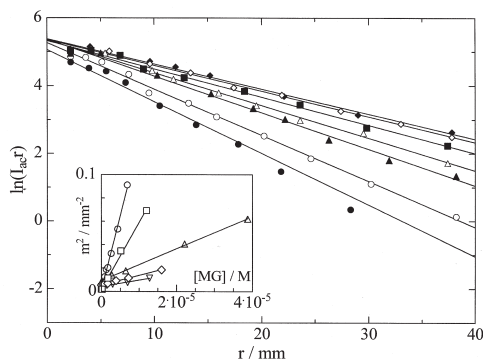


Figure 4. Photon migration studies of malachite green solutions ($[MG] = 0 - 2.86 \times 10^{-5} M$) in water containing 15 g l^{-1} milk powder (MP). Logarithmic plot according to Equation 3 of the light intensity v. source/detector separation r . Inset: squares of the slope of analogous plots for various milk powder concentrations and wavelengths vs. MG concentration. (c.: $[MP] = 27 \text{ g l}^{-1}$, 633 nm; | $[MP] = 14 \text{ g l}^{-1}$, 633 nm; Δ : $[MP] = 25 \text{ g l}^{-1}$, 678 nm; ∇ : $[MP] = 12 \text{ g l}^{-1}$, 678 nm; $\diamond$: $[MP] = 15 \text{ g l}^{-1}$, 678 nm).

reflectance measurements for determination of, for example, absolute absorber concentrations in turbid media.

Figure 4 shows the results of photon migration studies of milk powder solutions containing various malachite green concentrations ($[MG] = 0 - 2.86 \times 10^{-5} M$). The following conclusions can be drawn: (i) A plot of $\ln(I_{ac}r)$ v. r exhibits good linearity in accordance with Equation 3 (ii) the amount of the slope of the plot (m) is increasing with increasing absorber concentrations in accordance with Equation 4. In the inset of Figure 4, results obtained from analogous measurements of m^2 are plotted v. the absorber concentration according to Equation 4. The good linearity of the plots for various milk powder concentrations and wavelengths is clearly discernible.

Equation 4 provides a good basis for the easy determination of the product of the absorption and the effective scattering coefficient of the sample. Knowledge of one parameter allows quantitative determination of the other. For example, we used Lambert–Beer coefficients of the absorber for evaluation of the photon migration studies and estimated g factors (average of the

cosine of the scattering angle) from our results. Employing Lambert–Beer absorption coefficients (ϵ_a) of the absorber, for example, malachite green (MG), $\mu_a (= \epsilon_a [MG])$ can be calculated and the effective scattering parameter of, for example, milk powder is obtained from Equation 4. From simple light attenuation measurements with low scatterer concentration and short optical pathlength (to avoid multiple scattering), the total attenuation coefficient μ_t of scatterer solutions containing no absorber can be determined. Since the absorption coefficient μ_a of the scatterer is negligible, μ_s can be estimated from μ_t :

$$\mu_t = \mu_a + \mu_s ; \mu_s \quad (5)$$

Finally, g can be estimated from μ_s and the effective scattering coefficient obtained from photon migration studies. $g = 0.69 \pm 0.02$ and 0.80 ± 0.02 was obtained at 633 nm for milk powder samples containing 1% fat and 17.5% fat, respectively. For caolinit, the evaluation yields $g = 0.95 \pm 0.02$ at 678 nm. The results are in good agreement with literature values. $g = 0.79 \pm 0.01$ for milk powder, $g = 0.60 \pm 0.02$ for caolinit obtained from narrow beam and angle-dependent scattering experiments are given in the literature.¹⁵ We conclude that the Equation 2 provides an excellent basis for quantitative determination of absorption and scattering coefficients of turbid solutions. In distinction to Equation 1, Equation 2 allows determination of absorber (or scatterer) concentration or absolute (Lambert–Beer) absorption (or scattering) coefficients under the conditions of the turbid solution.

Optical analysis, based on light absorption, is in aqueous solutions generally restricted to wavelength regions between c. 1500 and 1900 nm and below approximately 1400 nm, where water absorption is negligible or relatively weak. Figure 5 shows the NIR absorption spectra of organic compounds

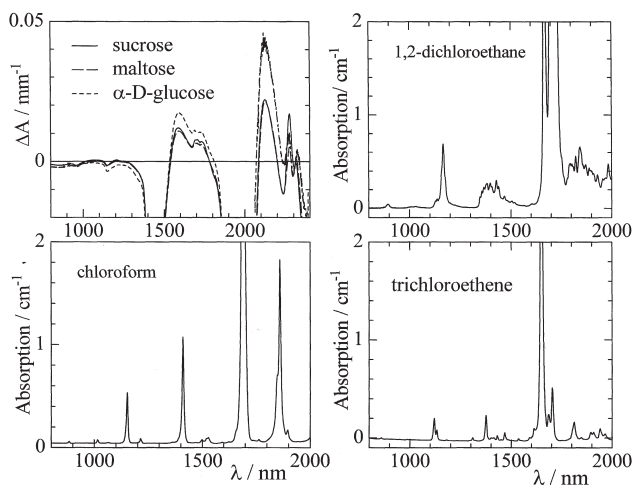


Figure 5. Absorption spectra of 1,2-dichloroethane, chloroform, and trichloroethene in the NIR spectral region at room temperature. Difference absorption spectra of α -D-glucose, maltose and sucrose (100 g l^{-1}) in aqueous solution at room temperature.

selected for the current study. The chlorinated hydrocarbons (CHC) chloroform, 1,2-dichloroethane and trichloroethene, exhibit strong absorption in the 1600 and 1750 nm region due to first overtones of CH-stretching vibrations. In this spectral region, the absorption coefficient of the CHC selected is larger than the one of water leading to a positive netto change in the absorption of aqueous solutions with increasing CHC concentration. Inspection of difference absorption spectra is instructive in the case of overlapping absorption bands of water and the solute. Figure 5 also shows the difference absorption spectra of selected sugars, which exhibit absorption features around 1600 (due to overtone bands), 2150 and 2220 nm (due to combination bands). Absorption-based detection of both substance classes in aqueous solution is thus feasible. Currently, work is in progress to detect the organic compounds selected in turbid solutions with photon migration studies and NIR diode lasers.

Conclusions

The current study demonstrates the enormous potential of NIR spectroscopy with intensity-modulated diode lasers for analytical chemistry and sensor technology. Possible sensor and *in-situ* monitoring applications of NIR diode lasers demonstrated in this work range from simple determination of water contents in organic solvents to absolute determinations of absorption and scattering coefficients of species concentrations in turbid solutions.

Work is in progress to develop evaluation methods of photon migration studies performed under different boundary conditions as the investigations described above and to relate parameters derived from diffuse reflectance measurements with molecular absorption and scattering coefficients.

Furthermore, the employed diode laser array is currently extended to wavelength regions not previously available for NIR laser studies in this laboratory. This will allow quantitative determination of a variety of organic compounds in the turbid media.

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