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ORIGINAL ARTICLE

Multi-wavelength thermal-lens spectrometry for high-accuracy measurements of absorptivities and quantum yields of photodegradation of a hemoprotein–lipid complex

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Absorptivity

Abstract Accurate measurements of absorptivities and quantum yields of biochemical species under various conditions are an important task of applied photonics and analytical chemistry. In this work, we provide a comparison of the capabilities of thermal-lens spectrometry to measure these parameters of various samples. Measurements of relevant model substances, biologically active substituted 2-thiohydantoins and their complexes of copper(I,II) and heme proteins (forms of hemoglobin and cytochrome *c*), showed negligible differences in apparent molar absorptivities for thermal-lens spectrometry and optical-absorption (spectrophotometric) data. The values for tabletop and microscale thermal-lens measurements under batch conditions differ insignificantly. The precision of measurements of molar absorptivities by thermal-lens spectrometry is no less than in the case of spectrophotometry or the precision is even higher in the cases of low absorptivities. For cardiolipin–cytochrome *c*-NO complex, the difference between absorptivity values calculated from thermal-lens data and acquired by spectrophotometry is significant due to complex photodegradation. The quantum yield of its photolysis reaction calculated from optical absorption and thermal-lens data altogether at two wavelengths for 0.4–360 s of the reaction, 0.46 ± 0.04, was estimated.

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1. Introduction

Photothermal spectroscopy (**PTS**) has an increasingly high impact on applied photonics, especially in biology and medicine and in materials science (Nedosekin et al., 2013a; Vidovic et al., 2013; Bagheri et al., 2014; Cassano et al., 2014; Guo et al., 2014). Photothermal spectroscopy and thermal-lens spectrometry (**TLS**) as a most widespread photothermal method have two advantages over conventional (transmission) methods of molecular absorption spectroscopy (spectrophotometry and IR-spectroscopy). The first of them is considerably higher sensitivity because TLS is based on direct measurements of the nonradiative energy transfer from excited chromophore molecules (Bialkowski, 1996). Thermal relaxation in the sample results in a nonuniform refractive-index spatial profile (a *thermal lens* effect). Its optical strength is proportional to the sample absorbance and, thus, to the absorber concentration and its absorptivity. As a result, TLS provides detection limits of 10^{-9}–10^{-6} abs. units or *ca.* 10^{-11} mol L^{-1} (Bialkowski, 1996; Proskurnin et al., 2015) e.g. the sensitivity that is comparable to laser-induced fluorimetry but for non-fluorescing chromophores and with a low impact from light scattering (Fischer and Georges, 1996).

The second advantage of PTS, and TLS in particular, is the possibility of measurements of chromophore absorption in complex (even living) systems and *in vivo* studies (Liu and Franko, 2014b; Nedosekin et al., 2014) and also with a low impact from scattering backgrounds (Georges, 1999).

These two advantages provide an important feature of TLS. In many applications of laser photonics, it is important to know the absorption-band parameters of the species in question—and simultaneously with high sensitivity and high precision. Especially important is the determination of absorptivities at low concentrations and in low volumes of real samples as a basis of elucidation of reaction or bioprocess mechanisms. However, at this level, the possibilities of conventional transmission measurements become limited (Luk'yanov and Novikov, 2000; Proskurnin et al., 2003; Skvortsov, 2013), while indirect methods sometimes lead to spurious results. Thus, PTS, together with photoacoustic techniques (Galanzha and Zharov, 2012; Haisch, 2012; Wang and Hu, 2012), becomes the tool in demand. This was used for measuring absorbances at a very low level such as pure water (Cruz et al., 2009), stable species (Cruz et al., 2011, 2013), and disperse solutions (Ryasnyanskiy et al., 2009; Marcano et al., 2011). Certainly, TLS and other photothermal methods are used for optical materials (Wu et al., 1997; Faycel, 1999; Ono et al., 2000; Commandre et al., 2008; Proskurnin, 2014). However, especially for complex systems, the correctness and accuracy of photothermal measurements require both the trueness and precision of measurements (ISO, 1994); thus, the accuracy verification of these values by the comparison with convention methods is required.

Next, if the absorption-band parameters are known with high accuracy, the conditions of photothermal measurements, especially in the case of laser-induced modalities, can be used for experiments that are more sophisticated. Thus, if there are processes competing with thermal relaxation of the laser-excited samples (e.g. luminescence or photochemistry), the thermal-lens effect strength decreases, which can be used for elucidating such processes and estimating their yields (Fischer and Georges, 1996; Lapotko et al., 2002), especially for fluorescence (Fischer and Georges, 1997; Isak et al., 2000; Yamaji et al., 2001; Santhi et al., 2004a,b; Tanaka et al., 2004; Cruz et al., 2010; Estupinan-Lopez et al., 2013; Würth et al., 2015). Photochemical reaction yields for fast processes were also measured by thermal-lens spectrometry from time-resolved experiments (Pedreira et al., 2004, 2006; Astrath et al., 2009, 2010a). On the other hand, TLS and more advanced techniques such as thermal-lens microscopy (Chen et al., 2011; Dudko et al., 2012; Cassano et al., 2014; Liu and Franko, 2014a) are used in complex biomedical applications (Brusnichkin et al., 2010; Nedosekin et al., 2013a, 2014; Cassano et al., 2014; Vidovic et al., 2014), which also require a high level of accuracy of the primary results on absorption of chromophores and for the whole system.

Thus, the aim of this study was dual. The first one was to evaluate the figures of merit of TLS for accurate determination of absorption-band parameters taking into account both their trueness and precision. We selected the examples of biologically active complexes (2-thiohydantoins) and relevant heme proteins (hemoglobin and oxidized and reduced cytochrome *c*) which are extensively measured by TLS *in vitro* and *in vivo*.

The second aim was to compare photochemically stable and unstable species to elucidate the possibilities of measuring quantum yields of slow photochemical reactions by TLS. It was done by the example of a slowly and irreversibly photodegradable nitrosyl complex of cytochrome *c* in the presence of cardiolipin (Osipov et al., 2007; Brusnichkin et al., 2008) to estimate the quantum yield by the comparison of molecular absorption data obtained from conventional transmission measurements and thermal-lens spectrometry.

2. Material and methods

2.1. Reagents and solvents

Water from a Milli Q water purification system (Millipore, France) was used: specific resistance 18.2 MΩ cm, Fe, 2 ppt; dissolved SiO_2, 3 ppb; total ion amount, < 0.2 ppb; TOC, < 10 ppb; the own thermal-lens signal, 0.004 ± 0.001. The glassware was washed with acetone followed by conc. nitric acid. 69% nitric and 37% hydrochloric acids (PA-ACS-ISO grade, Panreac, Spain) and acetonitrile (CH_3CN, Merck, gradient grade for HPLC) were used throughout. Aqueous solutions of hemoglobin (RENAM, Russia, 159 g L^{-1}) and cytochrome *c* from equine heart, 99% (Sigma, USA, $M = 12383$ Da) for biochemistry were used. The reagents: 1,10-phenanthroline iron(II) sulfate complex (ferroin, 0.025 mol L^{-1}, CAS No. 14634-91-4, Sigma–Aldrich) and phosphoric acid (H_3PO_4, 85%, Riedel–de Haën) were used throughout. All other reagents and solvents used in this study were of chemically pure grade or higher and were used without further purification.

Complex of iron(II) with 1,10-phenanthroline was synthesized as described previously (Filichkina et al., 1992a). The synthesis of thiohydantoins: 3-phenyl-5-(pyridylmethylene)-2-thiohydantoin (**I**), 3-allyl-5-(pyridylmethylene)-2-thiohydantoin (**II**) and 3-(propen-2-yl)-5-(pyridine-2-ylmethylene)-3,5-dihydro-4H-4-oxoimidazol-2-yl)-thio]-ethane (**III**) (Majouga et al., 2004) and their copper complexes with **I** (Beloglazkina et al., 2005) and **II** mono- and di-complexes (Beloglazkina et al., 2014; Majouga et al., 2014) were discussed in detail in previous studies. TLS procedures for hemoglobin and cytochrome *c* are described previously (Brusnichkin et al., 2008, 2009).

2.2. Auxiliary measurements

Spectrophotometric measurements in batch and flow modes were made using an Agilent Cary 60 spectrophotometer (USA) with $l = 10$ mm, 0.3 cm^3. The pH values were measured by an inoLab pH Level 1 pH-meter (Germany) with a glass pH-selective electrode (precision ± 5%). Solutions were mixed with a Biosan MMS 3000 automixer (Latvia) and a micro-stirrer.

2.3. Tabletop thermal-lens measurements

The scheme of the tabletop thermal-lens spectrometer (Tsar'kov et al., 2011; Volkov et al., 2012) is shown in Fig. 1. The key parameters are summarized in Table 1 (from

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