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Experimental Determination of the Far-Infrared Optical Properties of Biological Matter in Aqueous Solution

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Abstract. Experimental details are given of how to reliably measure the optical properties of aqueous solutions in the far-infrared spectral range using a Fourier transform spectrometer. We discuss the analysis of reflection and transmission data in order to obtain the refractive index and the absorption coefficient. Some results on water and biological systems are presented.

Key words: blood serum, far-infrared properties, Fourier-transform spectrometer, interference pattern, measurement cell, reflection measurements, silicon windows, water

1. Introduction

Optical spectroscopy is among the most important and fruitful methods which can be used in order to characterize and understand the basic properties of matter. Following the large impact on atomic and molecular physics, for the last hundred years optical methods have been developed up to an extremely high level of accuracy and sensitivity and applied to a large variety of problems in condensed matter physics. Needless to say that also the frequency range was expanded far beyond the visible, in particular down to the infrared. Nowadays optical methods (defined by the free-field propagation of light) cover the spectral range from the vacuum ultraviolet of frequency 10^5 cm^{-1} (corresponding to a quantum energy of 10 eV) down to the very far-infrared (FIR, approximately 1 cm^{-1} or 0.1 meV), below which microwave and coaxial techniques have to be utilized to probe the electrodynamic properties [3]. In particular the infrared spectral range turns out to be extremely important to study structural and vibrational properties of molecules and solids. Already the simplest ball and spring model tells us that heavy entities will have their vibrational modes at lower frequencies compared to light ones; this becomes of particular importance for the investigation of large biological molecules. In the last decade, commercial applications in communication technology, sensors, imaging, and medicine, for instance, approach the upper GHz and the THz range of

frequency (1 THz corresponds to 30 cm^{-1}). Hence the question of possible side effects of the radiation in this spectral range becomes of increasing importance.

Nowadays optical spectroscopy is widely used to study biological problems [1, 2]. However, it seems to be confined to the visible and near-infrared range of frequency and almost no data are available in the FIR. The reasons for that seem to be mainly technical: (i) Fourier transform spectrometers working in the FIR range are cumbersome and expensive. (ii) They have to be operated in dry atmosphere but require vacuum condition in order to achieve superior performance. (iii) Since the radiation sources of traditional FIR spectrometers are very weak in the FIR range, helium cooled bolometers are required to detect the signal; a troublesome and expensive venture. (iv) Due to Abbe's diffraction limit the samples have to be rather large at the lower frequency end. (v) In general the material is prepared in aqueous solution, but water is highly absorbing in the FIR spectral region.

In response to the problems (iii) and (iv), we recently presented a novel THz near-field spectrometer which allows to perform biological and medical studies with a spatial resolution down to several microns [4]. Monochromatic sources provide powerful coherent cw radiation tunable from 50 GHz up to 1.5 THz. Transmission and reflection experiments can be performed, which enable us to study solids and molecules in aqueous solution. In the following we will discuss our approach to deal with problems (ii) and (v) using a commercial Fourier transform spectrometer. Examples for spectroscopic investigations on water and biological samples are presented.

2. Experimental Details

A modified Bruker IFS 113v Fourier transform spectrometer is utilized in the frequency range from 10 cm^{-1} to 700 cm^{-1} with a resolution of up to 0.03 cm^{-1}. The radiation is generated by a mercury arc lamp and detected by helium cooled Si bolometers, one of which can be pumped to operate at $T = 1.2$ K. The complete frequency range is covered by only two or three Mylar beamsplitters (6 μm, 23 μm, and 75 μm). The design of the experimental setup is governed by the fact that a meaningful measurement of any biological system has to be performed in aqueous solution. Figure 1 gives an idea of the optical parameters of water, the refractive index n and the extinction coefficient k as tabulated in the *Handbook of Optical Constants of Solids* [5]. Also shown is the power transmission through a water layer of different thicknesses as indicated (reflection losses were not included since they depend on the adjacent material). From this rough estimate, it becomes obvious that in the spectral range between 50 cm^{-1} and 1000 cm^{-1} extremely thin layers of water have to be prepared in order to perform measurements in transmission configuration.

In order to obtain quantitative data, a cuvette has been prepared which is transparent in the FIR range; it has an open diameter of about 10 mm and a sample-space thickness of approximately 10 to 20 μm. Typical window materials like poly-

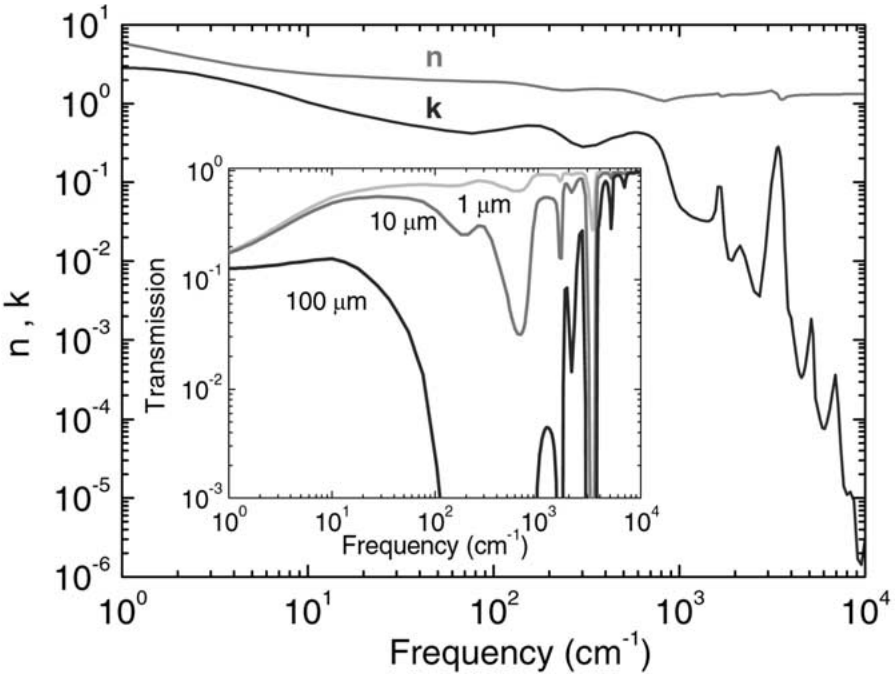

Figure 1. Frequency dependence of the refractive index n and the extinction coefficient k of water at room temperature (data taken from [5]). The inset shows the transmission through a water layer of different thicknesses; the reflection is not taken into account.

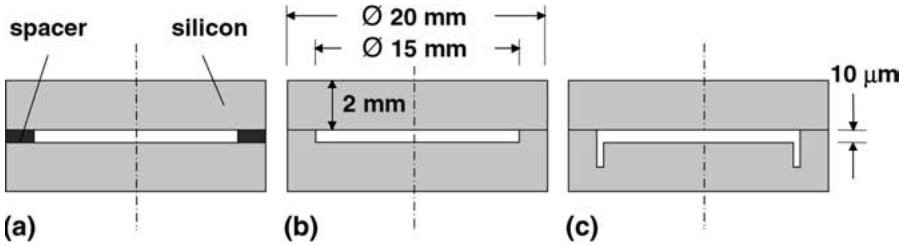

Figure 2. Design of cuvette to measure aqueous solutions in the FIR spectral range: (a) variable spacers allow to easily vary the thickness; (b) lower central part is manufactured by precise polishing to the desired shape and thickness; (c) the exceeding liquid can collect in a circular groove which also prevents the outflow due to capillary forces.

ethylene or Mylar are too soft to be manufactured precisely enough to meet the requirements of plane parallelism and roughness of approximately 1 μm. Silicon with low doping concentration seems to be the best choice although the large refractive index of $n = 3.42$ causes reflection losses of up to 75%. In order to prevent significant bending when exposed to vacuum, the Si windows have to be at least 2 mm thick. The simplest and most flexible way to generate the required sample volume is by a spacer (e.g. Mylar foil) between two windows (Figure 2a). Alternatively, a circular part of one of the windows can be polished down to the

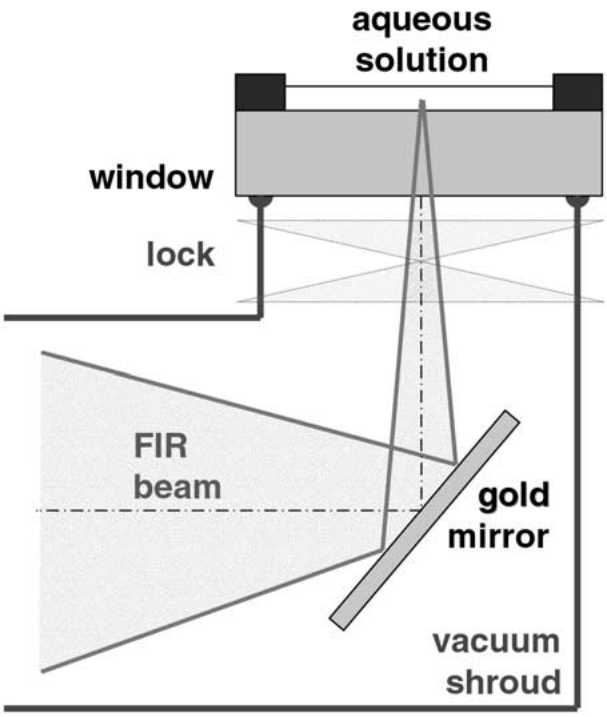

Figure 3. Reflection setup for measurements of liquid samples employing a spectrometer with the optical path and components in vacuum. The use a lock allows for easy replacement of the window without venting the entire interferometer.

appropriate thickness (Figure 2b). In order to give surplus of liquid some extra volume and to prevent the liquid to flow out of the cuvette due to surface adhesion, a circular groove can be cut as shown in Figure 2c. Multireflections within the two windows and (less pronounced due to the large absorption) aqueous solution lead to an interference pattern which can be rigorously modelled by the Fresnel equations of a three-layer system of vacuum, the Si window, and the liquid [3]. For data analysis we utilized a home-made fitting software package which meets all our requirements.

Although the FIR spectrometer operates in vacuum, the solution has to remain at ambient pressure. It turns out to be a technical challenge to fill an extremely thin cuvette without air bubbles and seal it vacuum tight. An inverted pot (where beam is guided from vacuum through windows to normal pressure and back to the vacuum chamber) allows to keep the cuvette at ambient atmosphere, but requires two additional windows. The problem can be circumvented by performing reflection measurements. For this case the bottom part of the cuvette also serves as the vacuum window (Figure 2). The alignment can be done by adjusting the mirror and placing the cuvette on a flexible bellow. Within the reflection setup, the liquid must be thick enough to prevent any light to be transmitted, or the upper window of the

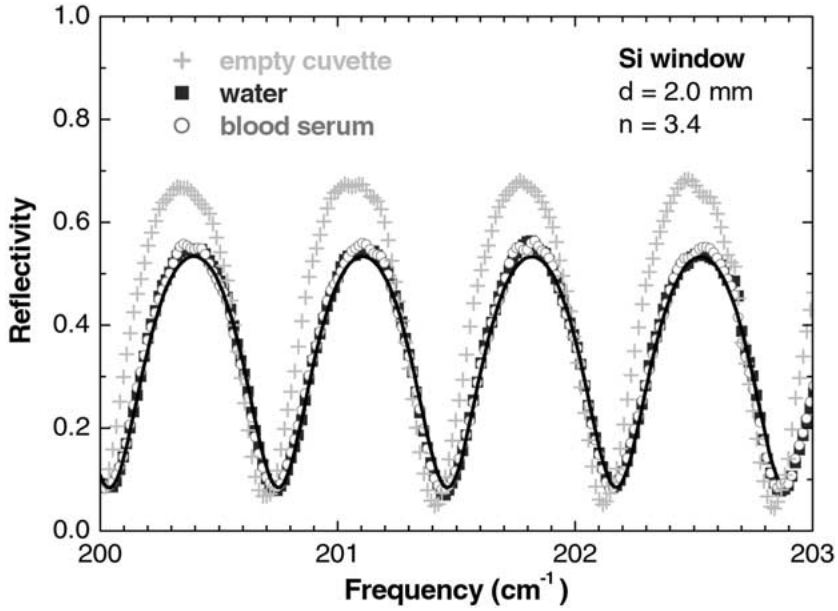

Figure 4. Portion of the reflection spectra of an empty Si cuvette (+), cuvette filled with water (filled squares), and cuvette filled with H5422 blood serum (open circles); the line corresponds to a fit of the spectrum of the cuvette filled with water. The interference pattern is caused by the multireflections within the Si window of thickness 2 mm.

cuvette has to be replaced by a mirror in order to reflect the radiation back (which then passes the liquid twice). A good mirror can be obtained by evaporating gold on the surface of a silicon disk placed on top of the liquid. Again, the multilayer arrangement has to be modelled by Fresnel's equations in order to obtain the optical properties of the liquid. In general, the disadvantage of the reflection setup is the considerable background signal; due to the large difference in the refractive indices of silicon on one side and air or liquid on the other side, a big part of the radiation is reflected before interacting with the sample. The interference within silicon may be avoided by using wedged windows; an accurate fit of both the amplitude and the position of the fringes allows us to obtain information on the refractive index n and the extinction coefficient k of the liquid sample under test [3].

3. Results and Discussion

Figure 2 shows a small part of the reflectivity spectrum of our cuvette (similar to the sketch of Figure 2) taken with 0.03 cm^{-1} resolution; the film is thick enough to prevent the radiation to interact with the covering window. The fringes are caused by the interference of the light reflected at the front and back surfaces of the Si window; the periodicity $\Delta\nu \approx 0.73$ cm^{-1} depends on the thickness $d = 2$ mm and refractive index $n = 3.42$ of Si: $\Delta\nu = 1/2nd$. If the cuvette is filled by water we see a shift of the interference pattern and a change of the amplitude. With the

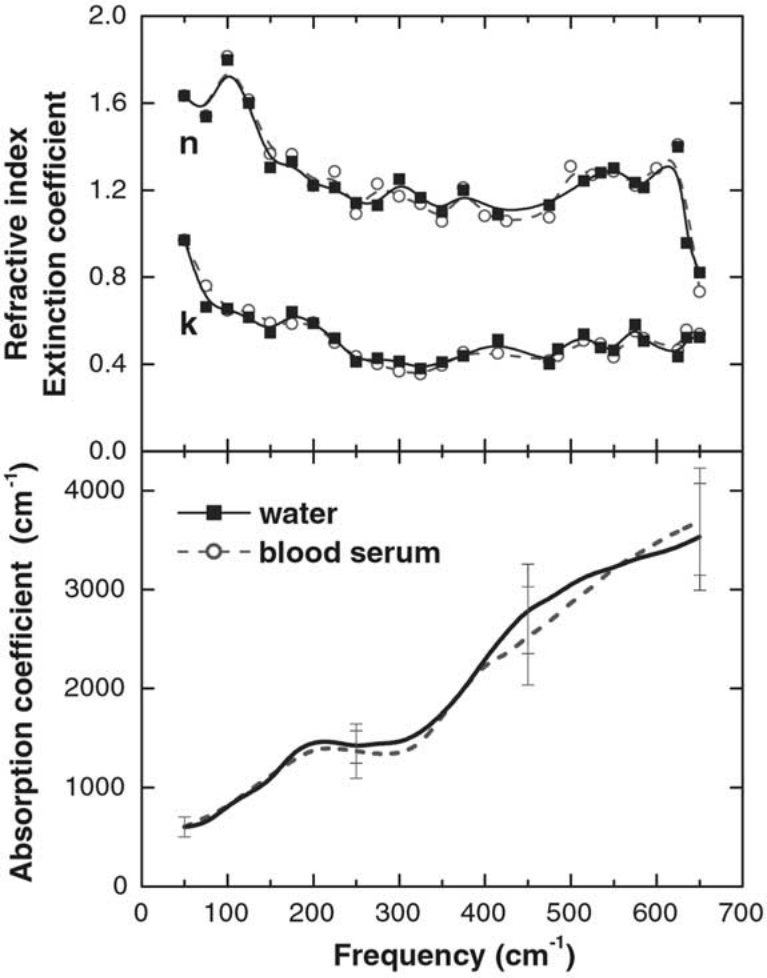

Figure 5. Frequency dependence of the optical properties of water and blood serum in the far-infrared spectral range. The upper frame shows the refractive index n and the extinction coefficient; the lower frame shows the absorption coefficient α.

help of our software package the data can be fitted by means of Fresnel's equation of a three-layer system (vacuum/Si/liquid) [3, 6] as demonstrated by the solid line. We first constructed a vacuum/silicon model, to determine the values for eps1 and eps2 for silicon over the entire frequency range. In the next step we introduced these values in the three layered system and tuned the eps1 and eps2 for the sample so that the fitting curve describes the behavior of the reflectivity. This allows us to unequivocally determine both components of the optical properties of the liquid without using the Kramers-Kronig analysis. Also plotted is the reflectivity spectrum of blood serum (human serum, male, type AB, from Sigma Aldrich, H5422). Due to the high water content ($> 90\%$), the differences between water and serum

spectra are extremely small. This is basically true in the entire spectral range which we have examined (10 cm^{-1} - 700 cm^{-1}). Thus, we can immediately conclude that the optical properties of blood in the FIR spectral range are governed by water. We do no find any large deviations from the water spectrum and no strong features.

In Figure 2a we plot our results on the optical properties of water and the blood serum in the FIR frequency range; the lower frame shows the absorption coefficient α which is calculated by $\alpha = 2k\omega/c$, where ω is the angular frequency and c is the speed of light in vacuum [3]. As a matter of fact, sharp absorption lines are not expected in a liquid. While in the gases the interaction of the molecules can be neglected leading to very sharp intramolecular vibrational lines, this is not the case in a liquid. In contrast to a crystal, each molecule sees a different environment which causes inhomogeneous broadening of the absorption lines; in general they show up only as broad bands. However, it is known from the mid-infrared spectral range that these bands can be distinguished and identified, giving valuable information on the structural and functional properties of the macromolecules [7].

4. Conclusion and Outlook

We have developed an experimental setup in order to measure the optical properties of aqueous solutions in the far-infrared frequency range using a Fourier transform spectrometer. Due to its high water content the far-infrared properties of blood serum do not exhibit any sharp features or strong deviations compared to H_2O. Thus, any side effect of the far-infrared radiation on blood will be governed by the effects on water.

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