Infrared optical fiber as evanescent wave bio-sensors

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ABSTRACT

Chalcogenide glass optical fibers exhibit a large optical transparency in the mid-IR extending typically from 2 to 22 µm for the best compositions. Moreover most of these glasses possess unique thermo-mechanical properties that enable to shape them into optical fibers exhibiting low optical losses from 2 to about 12 µm. Due to their properties, such fibers can be used to implement remote infrared spectroscopy, known as Fiber Evanescent Wave Spectroscopy (FEWS). The glasses and ceramics Laboratory of Rennes have an active research group on this topic since about 4 years leading to interesting results in several fields of application : environment, biology, medicine… In this contribution we would like to focus our attention mainly in the technical choices that have been done to obtain these results, for instance the glass composition, the shape of the optical fiber and the abilities of the sensor.

Keywords : chalcogenide glass, optical fiber, infrared spectroscopy, PCA, medicine, bacterial culture

1. INTRODUCTION

Due to a technical interest which was not depending on telecom technology the research on chalcogen-based glasses have not been suffering from the recent recession in the field. On the contrary, new emerging technologies related to thermal imaging as well as infrared sensors have nucleated new projects involving infrared transmitting materials including chalcogenide glasses. The need for optical sensors operating in the mid IR region where are located the main IR signatures of molecules and biomolecules is playing an important role in the development of analytical techniques giving in-situ information on metabolism mechanisms.

Fiber Evanescent Wave Spectroscopy is an efficient and easy way to record such infrared spectra that enable in situ and in real time studies with no sampling. As represented in figure 1, the experimental set up consists in a Fourier Transform InfraRed (FTIR) spectrophotometer coupled with a chalcogenide glass fiber and a mercury cadnium tellurium (MCT) detector. The principle of evanescent wave spectroscopy is based on the fact that the light propagates in the optical fiber by total reflections at the interface between the glass and the air. At each reflection, a part of the energy is absorbed by any chemical or biological species having absorption bands in the IR spectral zones. Thus, the signal picked up at the fiber output corresponds to the initial signal minus the absorbed signal giving rise to the spectrum. The glasses and ceramics Laboratory of Rennes have an active research group on this topic since about 4 years[1-6]. At this time numerous pioneer works have been carried out in Rennes in the following domains of application : detection of pollutant in waste water, following of chemical reactions in microwave oven or of alcoholic fermentation process, monitoring of bacterial biofilm spreading, study of tumorous tissues, and of biological liquid such as serum, blood or plasma. Most of this work have been lead in close collaboration with O. Loreal from Inserm, French medical research agency, and O. sire from the University of South Brittany, for respectively medicine and micro-biology applications. More recently an efficient collaboration with Pierre Lucas from the University of Arizona has also given interesting results on the effects of toxic agents on lung cells. All these studies prove the efficiency of chalcogenide glass fiber to record IR spectra by FEWS with a great sensitivity.

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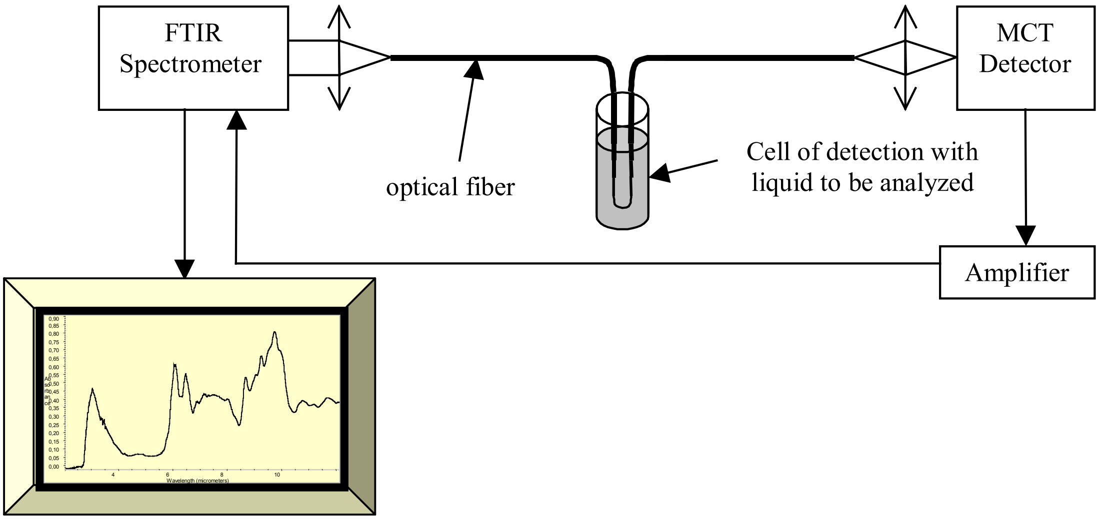

Figure 1 : Schematic representation of the experimental set up used for FEWS experiments. It is composed of an IR source, a chalcogenide glass fiber and a MCT detector.

In this contribution we would like to focus our attention mainly in the technical choices that have been done to obtain these results, for instance the glass composition, the shape of the optical fiber and the abilities of the sensor.

2. MATERIALS

The glass-forming ability of chalcogens or pseudo-chalcogens combinations has been known for several decades but compared to oxide glasses, especially silicates, this class of vitreous materials is just emerging from their infancy. The main attention paid to these materials relies on their large optical window extending in the mid-infrared and covering usually the two atmospheric windows lying from 3 to 5 and 8 to 12µm. These low-phonon materials have to be considered as heavy-anion glasses since sulfur, S, selenium, Se and even tellurium, Te, are the main constituents of their compositions. This situation leads to fundamental vibrational modes shifted far in the IR, and rending these glasses interesting for the fabrication of thermal-imaging systems. This exceptional transparency, associated with a suitable viscosity/temperature dependence, creates a good opportunity for the development of molded optics for low-cost infrared cameras.

Vitreous Systems	**Young Modulus (GPa)**	**Vitreous Transition (°C)**	**Optical Windows (µm)**
TeX glasses[7] Te and Se, X=Cl, I, Br	8	60	2 - 20
TAS glasses[8] Te, As, Se	18	140	2 - 16
GasIR[9] Ge, As or Sb, Se or Te	24	280	2 - 12

Table 1 : Three examples of glass compositions synthetized in Rennes. The optical and thermo mechanical properties of the glass strongly depend on the chemical composition of the glass. The TeX glass are constituted of chains which are not at all reticulated and so their mechanical properties are poor with a vitreous transition temperature above 100°C, but the optical windows of transparency is wide from 2 to 20 µm. At the opposite, the Ge based compositions give rise to 3 dimensional structural network with much higher Tg up to 300°C but with a narrow transparency which is stopped at about 12 µm on a bulk of glass with a 1mm thickness. These types of compositions are suitable for lenses of night vision camera.
The TAS glass appears as the best compromise between these both behavior exhibiting a nice transparency from 2 to 16 µm, on bulk, and keeping correct mechanical properties. Inside this ternary diagram, the $Te_2As_3Se_5$ composition has been choosen to implement FEWS due to its good stability against recrystallisation during the drawing process.

A good knowledge of the glass structure is essential to understand some mechanical, thermodynamical and chemical properties of such materials and the difficulties associated with the disordered nature of the glassy state is a real challenge. A convenient way to classify the numerous glass compositions which are based on chalcogen combinations is by reference to their structural organization, which also reflects the thermodynamical and mechanical properties of the materials (see Table 1). Glasses such as vitreous selenium Se or the TeX glasses (X= Cl, Br, I), are chain-like materials with a one-dimensional (1D) framework. Their transparency range is very large (until 20 µm) but they have a poor network rigidity and consequently possess a low glass-transition temperature T_g, usually below 100°C ; for instance T_g for Se is 40°C. At the opposite the Ga and Ge based selenide glasses possess a highly reticulated vitreous network (3D) which lead to much better thermo-mechanical properties with Tg up to 300°C. Unfortunately their transparency is narrower and multi-phonon absorbency appears right from 12 µm. Between these both extreme behavior, the TeAsSe ternary system (TAS glass) is an interesting compromise keeping correct mechanical properties associated with Tg = 137°C, which enables to implement experiment at room temperature. This glass offer exceptional spectral windows, typically lying from 2 to 16 µm for a bulk with a thickness of 1 mm (see figure 4b below). Moreover, This glass composition exhibits an excellent resistance to devitrification permitting to shape it into optical fiber.

Figure 2 : Bulks of selenide glasses in various sizes photographied with optical fibers.

To obtain a perfect optical transparency, we have to paid a special attention on the purification of the glass. Thus TAS glasses are synthesized under vaccum in silica vessels from high pure single elements (Te, As and Se) with high purity. To avoid any contamination by hydrogen and oxygen impurities, the elements are repurified by heating for arsenic and selenium and by chemical reaction for tellurium. After that, they are brought together in the reactional silica tube which is sealed. After this step, a distillation is operated to eliminate carbon and silica impurities.
The tube is sealed and the glass is homogenized in a rocking furnace at 650 °C for 12 h. The tube is then quenched in water and the glass is annealed near Tg to reduce the mechanical stresses. Such bulks of selenide glass have been photographied in figure 2 together with optical fibers.

3. OPTICAL FIBERS PROPERTIES

The TAS glass fiber preparation is detailed in ref. 10,11. The drawing process is carried out in the glass and ceramics Laboratory thanks to an home made fibering tower specially adapted for chalcogenide glasses (figure 3). The attenuation curve of the fiber is given in figure 4. The minimum of attenuation is less than 1 dB.m-1 and is located between 6.5 and 9 µm. Obviously, this value is far from the one obtained with silica glass fiber, but they are suitable for such range application such as remote spectroscopy. Overall, the fiber spectral window encompasses the MIR domain, since transparency is observed from 800 cm^{-1} to 4000 cm^{-1} on FEWS spectra.

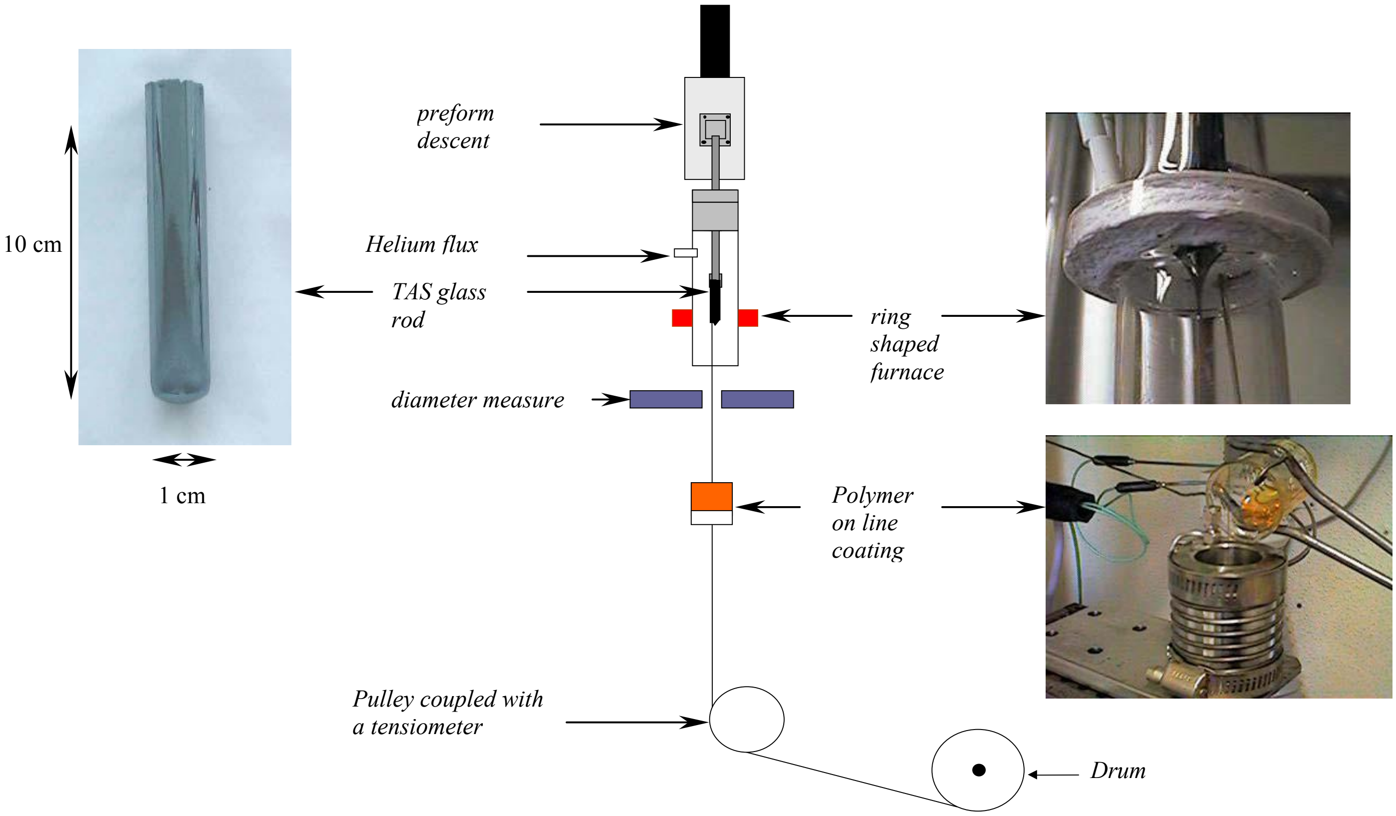

Figure 3 : Schematic representation of the drawing tower built in our Laboratory. From a selenide glass rod photographied on the left, we obtain typically 25 m of fiber with a 400 µm diameter. Note that we can applied a coating polymer on-line to improve the mechanical behavior of the fibers.

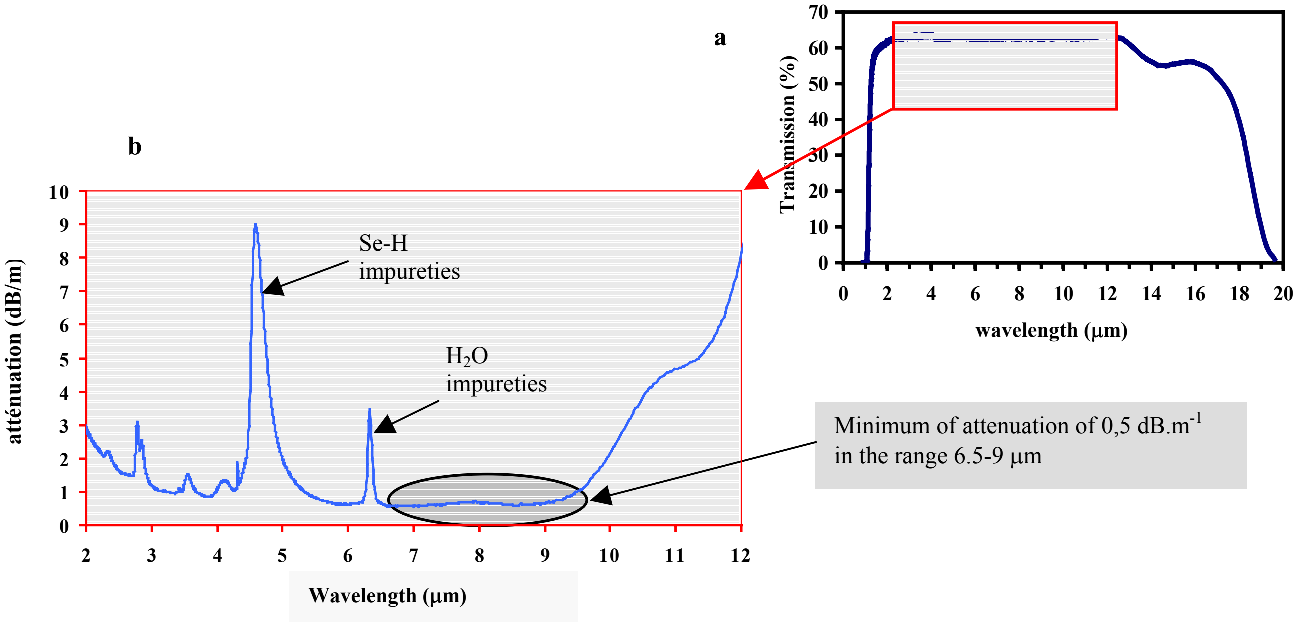

Figure 4 : (a) transmission windows for a bulk of TAS glass (thickness 1 mm) together with the attenuation curve of the TAS glass fiber (b) in the range where the transmission is perfectly flat. Although the minimum of attenuation (0,5 dB.m^{-1} in the range 6.5-9 µm) is much higher than for silica glass fibers, the attenuation remains low enough in a much wider range towards mid IR (2-12 µm) permitting to record FEWS spectra encompassing most of the vibration modes of biomolecules.

4. DESIGN AND PRINCIPLE OF THE SENSOR

According to the Snell-Descartes law, a Total Internal Reflection (TIR)[12] occurs at the interface between the fiber and the air, when :

$$\theta \geq \theta_c = \mathrm{Sin}^{-1}\left(\frac{n_2}{n_l}\right)$$

where n_l is the refractive index of the fiber, n_2, the index of the air (n_2=1), θ_c, the critical angle from normal for TIR and, θ the angle of incidence from normal. In the case of TAS glass fiber (n_l=2.8), the index difference is high, and the condition for TIR is fulfilled for all optical rays entering the TAS glass fiber. The number of bound modes M for a circular fiber is estimated by the following equation,

$$M(\lambda) = \frac{2\pi^2 r^2 (n_l^2 - n_2^2)}{\lambda^2}$$

with r being the radius of the fiber, n_l the index of the fiber core and, n_2 the index of the cladding. The number of modes is therefore dependent on both the index of the core, index of cladding, diameter of the fiber and the wavelength. With a diameter of 400 µm, a fiber index of 2.8 and the index of the air of 1, the number of modes can be estimated approximately between 37 500 at 12 µm (833 cm^{-1}) and 135 000 at 2 µm (5 000 cm^{-1}). Due to the multi-mode characteristic and the high refractive index of the TAS glass fiber, the number of TIR inside the fiber is very high. Thus, the light propagation in a multi-mode TAS glass fiber is complex. To cope with this complexity, a background spectrum is collected before each experiment. So, many effects can be neglected : the entrance and exit conditions of the infrared beam, the interaction and attenuation along the optical signal transportation section, the transition of the modes during the taper to the sensing zone, the absorption due to the fiber and any effect related to fiber bending or surface roughness.

As explained in the introduction (figure 1), the experimental set-up consists in a Vector 22 FTIR spectrometer (BRUCKER), coupled with a tapered fiber, and a Hg-Cd-Te (MCT) detector. A single fiber is used both as a waveguide and sensing element. The sample to analyze is just put into contact with tapered part of the fiber. This way to collect the infrared spectra is called FEWS for Fiber Evanescent Wave Spectroscopy because it is generally assumed that the principle of the measurement is based on the presence of evanescent wave around the fiber during the propagation of light into the fiber.

In this experimental configuration, the evanescent wave penetration depth allows probing of only the very first microns of the sample. Classically, for our experiments, the length and surface of fiber in contact with the sample is about 10 mm +/- 0.1 and 1.4 mm^2 respectively, but can be easily decreased down to 1 mm. The total length of the fiber was 1 m, but can be increase or decrease if necessary. Spectral resolution is set to 4 cm^{-1} and spectra resulted from the co-addition of 100 scans, corresponding to a time sampling frequency of 0.016 Hz.

The number of reflections over length L of a fiber with a diameter of d is dependent upon the following,

$$N(\theta, d, L) = L * \frac{\tan(90 - \theta)}{d}$$

with θ, being the angle of incidence from normal. In the present situation, it is known that propagation within waveguides can be efficiently described by classical geometric optics. With these considerations a model of the fiber optic probe's response was presented to aid in predictions and to simulate data[13]. It was shown that to improve the sensitivity of the sensor, the diameter of the fiber should be locally reduced to create a tapered sensing zone which will be brought into contact with the sample to be analyzed (figure 5a).

Two routes are used for tapering locally the fiber. The first one consists in a strong increase of the fibering speed during the drawing process which enable to reach a diameter between 100 to 200 µm in the sensing zone. The second is a chemical etching process using a special solution (95% concentrated H_2SO_4 + 5%H_2O_2) which enable to dissolve congruently the glass reducing the diameter down to about 50 µm. The dissolution mechanism, in these very strong oxidizing conditions, corresponds to the transformation of the elements present in the glass into arseniate, tellurate and seleniate anions. The second benefit of this etching process results in a chemical polishing of the glass leading to high optical quality, shiny surfaces. The order of magnitude of the dissolution kinetic corresponds to a diameter decrease of about 5µm.min^{-1}. Finally, before the etching treatment, the fiber shape is 400/200/400 µm for the inpout transportation section / the sensing zone / outpout section, while after the congruent dissolution, the diameter have change to 400/50/400 µm for a polishing time of half an hour. The corresponding increasing of the sensitivity is illustrated by figure 5b for a $CHCl_3$ absorption band.

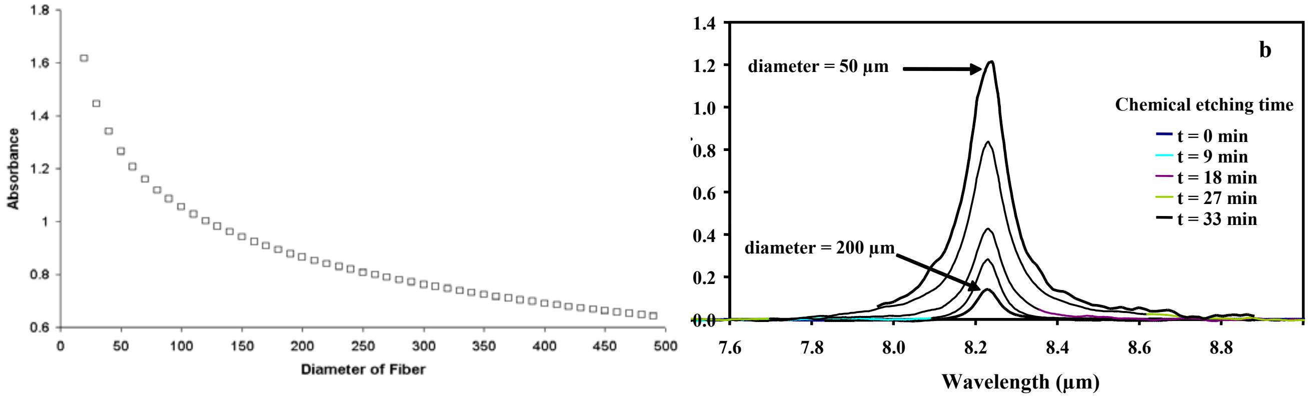

Figure 5: (a) Calculated evolution of the absorbency versus sensing zone diameters (b) $CHCl_3$ absorbency spectra versus chemical etching time (t), i-e, fiber diameter.

5. ABILITIES FOR APPLICATIONS IN BIOLOGY

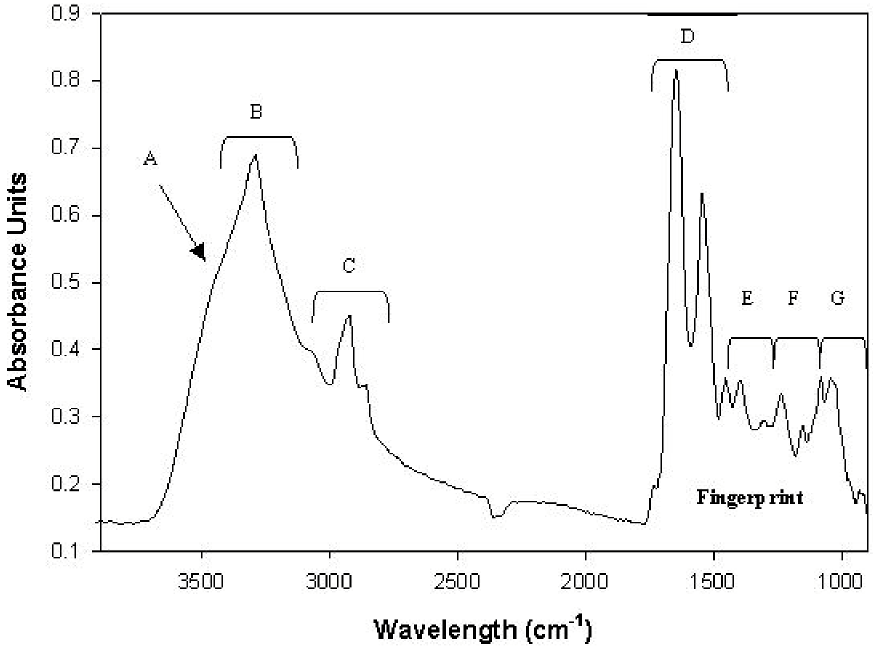

Figure 6: Typical FEWS spectrum of a liver cell. The figure displays the various frequency domains of absorbance of biomolecules. Schematically, it is possible to identify distinct spectral regions : region A : O-H and N-H elongation bands; region B : proteins Amide A and B bands; region C : CH_2, CH_3 of lipids; region D : proteins Amide I and II bands; region E : fatty acids and amino acids; region F : phospholipids and nucleic acids; region G : polysaccharides

The Figure 6 displays a typical FEWS spectrum of a liver sample. The unique difference observed in figure 6, compared to the same IR spectra recorded in the classical transmission mode, the sample being deposited on a CaF_2 window, deals with the bands intensities which arise from penetration depth of the evanescent wave in the low refractive index medium. Otherwise, it clearly appears that the transmission windows of the TAS glass fiber is well fitted for biological studies. Typically, this window extends from 2 µm (5000 cm^{-1}) to 12 µm (830 cm^{-1}). Most of the absorption bands have been assigned as explained in figure 6 caption by reference to 14.

As well as the optical window, the other key point to discuss concerns the sensitivity of such a sensor. In biology or medicine, it is quite impossible to define a systematic critical concentration below which the sensors should not be sensitive enough. Nevertheless, the limit of detection was evaluated using a simpler model : tetrachloroethylene (C_2Cl_4) in low concentration in water. In figure 7a, one can check that the absorbency of this absorption line increases linearly with the concentration of pollutant in agreement with the pseudo Beer-Lambert law, $A = \varepsilon(\lambda).l.c$ where $\varepsilon(\lambda)$ is the extinction coefficient, l the length of immersion of the fiber and c the concentration. For low concentration in aqueous solution, the Beer-Lambert Law remains a good approximation.

Nevertheless, note that this correlation can not be generalized. Indeed, some experiments lead in the laboratory on ethanol/water mixtures showed that the increase of the ethanol line absorbency is not linear in the whole range of concentration (figure 7b). This behavior is attributed to the hydrophobic property of the TAS glass which enhances the ethanol absorption lines to the detriment of the water ones. This property makes chalcogenide glass sensor particularly suitable for application in biology where water is pervasive and much of the time saturates the spectra recorded with the classical transmission mode.

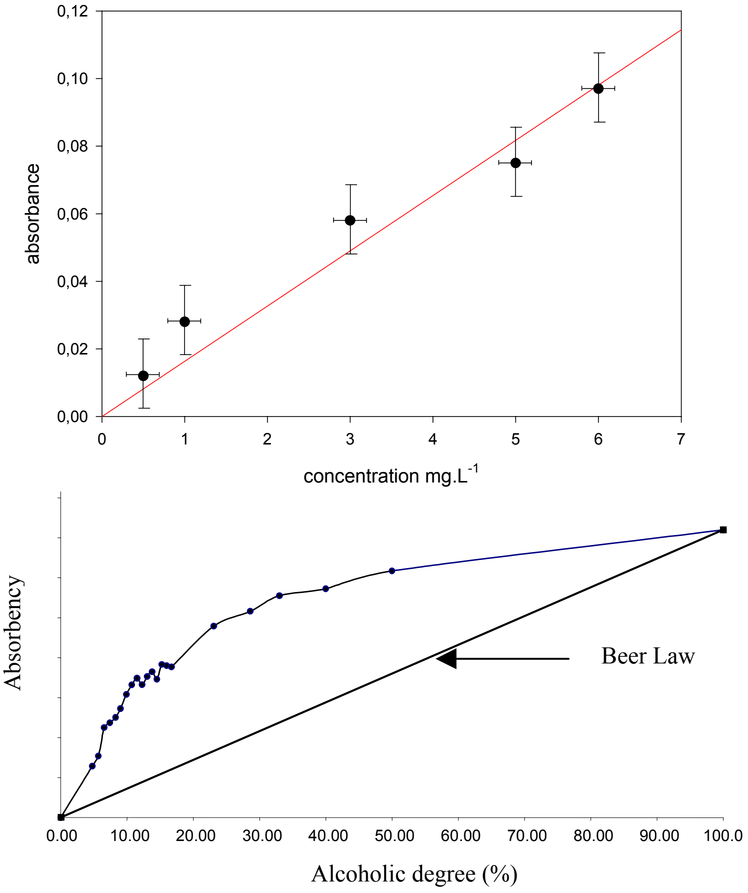

Figure 7 : (a) Intensity of the C-Cl absorption band versus the concentration of C_2Cl_4 in water. The limit of detection for this molecule is evaluated at 1 mg.L^{-1} (1 ppm).
(b) Increase of the intensity of the ethanol line at 1050 cm^{-1} in water/ethanol mixtures, where the rate of alcohol varies from 0 to 100%. The straight line depicts the expected Beer-Lambert Law. The large discrepancy observed between both lines is attributed to the TAS glass fiber hydrophobic behavior which enhance the signal of the ethanol to the detriment of the water absorption lines for higher concentration.

6. CONCLUSION

To conclude this overview on this bio-sensor properties, note that modern techniques of spectral analysis, such as Principle Component Analysis (PCA), are very useful to extract information on analyzed samples from their infrared spectra. PCA is an unsupervised method which becomes essential when the absorption bands can not be explicitly attributed to vibrationnal bonds because of overlapping for example. This point have already been discussed in a previous paper 15.

From our point of view, FEWS associated with PCA constitutes a protocole that could permit early medical diagnosis. That the reason why we are working with our colleagues from Inserm on human biologycal liquid (serum, plasma, blood) in order to distinguish at the earliest stage possible some metabolic deregulation associated with cirrhoses for example.

Research program has also begun this year on one hand to study the biofilm spreading for food security, and in the other hand to detect and warn against sea pollution.

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