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Opto-electrophoretic detection of bio-molecules using conducting chalcogenide glass sensors

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Abstract: Novel telluride glasses with high electrical conductivity, wide infrared transparency and good resistance to crystallization are used to design an opto-electrophoretic sensor for detection and identification of hazardous microorganisms. The sensor is based on an attenuated total reflectance element made of Ge-As-Te glass that serves as both an optical sensing zone and an electrode for driving the migration of bio-molecules within the evanescent wave of the sensor. An electric field is applied between the optical element and a counter electrode in order to induce the migration of bio-molecules carrying surface charges. The effect of concentration and applied voltage is tested and the migration effect is shown to be reversible upon switching the electric field. The collected signal is of high quality and can be used to identify different bacterial genus through statistical spectral analysis. This technique therefore provides the ability to detect hazardous microorganisms with high specificity and high sensitivity in aqueous environments. This has great potential for online monitoring of water quality.

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References and links

1. Introduction

Infrared spectroscopy is a non-invasive and highly selective method for detecting microorganisms and identifying their strains [1–3]. The highly specific vibrational signature of biological molecules located in the fingerprint region between 5 and 12 µm permits to probe the identity of microorganisms and to distinguish potentially harmful species. Normal collection methods are based on traditional FTIR technology where a sample culture is introduced in a spectrometer for analysis [2]. However there is an increasing demand for new probing techniques that allows to test samples remotely, in situ, or in continuous flow configurations. These new techniques require the development of materials that can effectively collect and transmit the infrared signal but which can also be shaped into appropriate optical components. The recent development of infrared Fiber Evanescent Wave Spectroscopy (FEWS) is an example of such technologies [4–6].

While it is highly selective, infrared spectroscopy has a limited sensitivity in comparison to other analytical techniques. This can be a drawback when analyzing a sample in situ where the analyte might not be in sufficiently high concentration. External techniques such as filtration and culture growth are then required to enrich the sample up to the detection limit, but these methods do not lend themselves to in situ or continuous-flow detection configurations.

In this study, we report a new kind of sensor made of chalcogenide glass that can be used as both an infrared optical element and an electrode for electrophoretic collection and detection of biological molecules. Most bio-molecules and microorganisms such as proteins, bacterial spores and viruses carry a net surface charge that can be utilized to force their migration toward the optical sensor by simple application of an electric field. This approach therefore enables enrichment of bio-molecules on the optical sensing surface and provides both the selectivity and sensitivity for detection of bio-molecules. This is made possible through the use of a new sensor based on telluride glass that has an extended transparency in the infrared, possesses an unusually high electrical conductivity and is resilient towards crystallization therefore allowing fiber drawing and opening potentials for many new sensor configurations. Here we provide the proof of concept for this technology using an Attenuated Total Reflection (ATR) configuration.

2. Experimental

2.1 Glass synthesis

The ATR plate used in the experiments is made from Ge$_{10}$As$_{15}$Te$_{75}$ (GAT) glass. The glass was synthesized by melting the mixture of high purity (≥5N) constituent elements in evacuated (10$^{-5}$ torr) and flame-sealed silica tubes in a rocking furnace. In order to eliminate undesired impurities in the glasses, arsenic was treated in advance at 350°C under vacuum to remove high vapor pressure surface oxides. The mixture was also combined with aluminum oxygen-getter and distilled to remove remaining traces of low vapor pressure contamnants. The distilled mixture was finally re-homogenized in the rocking furnace at 800°C for 8 hours. Then the melt was quenched in air and the resulting glass was annealed around T$_g$ for 3 hours.

2.2 Sensor design

The GAT glass has a good resistance toward crystallization and can be produced in large bulk pieces in order to fabricate sizable optical elements such as ATR plates. Figure 1 shows a photograph of an ATR plate (80mmx10mmx3.9mm) made of GAT glass which was used in this study to build an opto-electrophoretic sensor. One of the unique features of the GAT glass is its unusually high conductivity ($\sigma = 7.8x10^{-5}\Omega^{-1}.cm^{-1}$) in addition to a wide transparency in the infrared region (see Fig. 1). The glass element is therefore used as an
electrode and is electrically connected to a layer of aluminum for applying the voltage connection (see Fig. 2). A counter electrode made of Indium Tin Oxide (ITO) is then placed on top of the solution reservoir at a distance of ~1 cm. The voltage is then applied with a conventional power source and is kept constant throughout the duration of the measurement. The device is subsequently set in a FTIR spectrometer for signal collection while the microorganisms introduced in the reservoir migrate toward the optical element. It is worth to mention that the GAT glass has excellent chemical resistance in aqueous solution [7] and it was shown that these glasses also have good biocompatibility [8,9].

Fig. 1. Transmission spectrum of Ge$_{10}$As$_{15}$Te$_{75}$ glass, a 2 mm polished disk is used for the measurement; the inset is a photograph of the ATR plate made from Ge$_{10}$As$_{15}$Te$_{75}$ glass

Fig. 2. (Color online) Schematic drawing of the electro-deposition device used for collection and analysis of charged biological molecules

3. Results and discussion

3.1 Demonstration of principle using BSA proteins

Most bio-molecules carry a net surface charge that is gauged by the isoelectric point (pI). The species have a net positive charge when the surrounding pH is below their pI, a neutral charge at the pI and a negative charge above it. The Bovine Serum Albumin (BSA, lyophilized powder from Sigma-Aldrich) protein, which is mainly used in our tests, has a pI of about 4.9 [10] and carry a net negative charge in normal drinking water having a neutral pH = 7. The opto-electrophoretic tests on the charged BSA protein using the GAT ATR sensor are illustrated in Figs. 3 and 4.

When an initial BSA aqueous solution of concentration 10mg/ml is introduced in the reservoir, no significant signal can be detected. But as soon as the voltage is applied the proteins migrate within the evanescent wave at the surface of the ATR plate and the signal grows rapidly into a fully developed spectrum of BSA. Figure 3 illustrates the effect of applied voltage on the kinetics of electrophoretic deposition. The intensity of the characteristic Amide II peak (~1548cm$^{-1}$) is used as a reference feature to monitor the spectral evolution. It is shown that the migration rate increases with voltage as expected from standard Coulombic principles. It is also shown that the entire population of BSA can be driven to the sensor surface within about 30 min at the higher voltage. This is an acceptable time scale for online water monitoring systems and it still has large potential for further optimization.
From these results it is debatable whether the protein migration is entirely due to the electric field or if they naturally settle down under gravitational forces. An experiment was then performed with no applied voltage for 30 min followed by an applied voltage of 1.6 V and finally a reversed applied voltage of –4 V. Figure 4 clearly indicates that no significant settling effect occurs within the first 30 min while the signal grows gradually as soon as the voltage is applied until it reaches saturation. More interestingly, it is shown that applying a reversed voltage permits to drive the protein off the surface and eventually would permit to replenish the sensing surface for a subsequent experiment. However, Fig. 4 indicates that the process is not entirely reversible and that some proteins cannot be driven off the surface. This is likely the effect of denaturation of the proteins contacting the electrically polarized and conducting sensor surface. An electrochemical process might induce and result in loss of surface charge thereby preventing electrophoretic protein removal. It is expected that these effects could be largely remedied through application of a protective layer on the glass surface and these options are currently being investigated.

Fig. 3. (Color Online) ATR spectra in a BSA electro-deposition experiment with an applied voltage of 2.0V, 10mg/ml BSA aqueous solution was used and the curves from the bottom to the top correspond to 0, 1, 2, 3, 4, 6, 8, 10, 13, 16, 22, 28, and 34 minutes, respectively; the inset shows the evolution of Amide II peak height as the applied voltages are 2.0V and 1.6V, respectively

Fig. 4. Evolution of the Amide II peak intensity in a BSA electro-deposition experiment, 15mg/ml BSA aqueous solution was used; no voltage was applied in the first 30 min, then 1.6V voltage was applied, after saturation, –4V reversed voltage was applied

It is not entirely clear from the data shown above whether the saturation observed after long collection times is an effect of having driven the entire population of BSA on the sensor surface or whether it is the result of saturating the evanescent wave at the surface of the ATR upon deposition of multiple layers of proteins. In the latter case, increasing the concentration of BSA would not improve the signal any further, while in the former, the signal intensity should be proportional to the BSA concentration. Figure 5 clearly indicates that the former
case applies and that the signal intensity at saturation increases linearly with concentration. This confirms that the entire population of bio-molecules within the reservoir volume can be driven onto the sensor surface.

### 3.2 Selective detection of bio-molecules

Bio-molecules have complex vibrational spectra which contain highly specific features in the fingerprint region between 5 and 12 µm. These features permit to selectively identify the bio-molecules, granted that the spectra can be consistently collected with sufficient quality. Here we test the potential of the electrophoretic sensor for selective detection using two types of bacteria and one protein. The two bacteria were *Escherichia coli* (*E. coli*) with a pI = 4.5 and *Staphylococcus aureus* (*S. aureus*) with a pI = 2 [11] and the protein was BSA purchased from Sigma-Aldrich. Overnight cultures of the bacteria were grown in Trypticase soy broth and were then centrifuged and washed in deionized water, finally the bacteria were resuspended in 100ml deionized water and titered. The solutions used for the spectroscopic test were aqueous solutions of 10mg/ml BSA, 1.2x10⁵cfu/ml *E. coli* and 1.0x10⁹cfu/ml *S. aureus*. Fig. 6 displays the ATR spectra measured with an applied voltage of 2V. The BSA protein shows very distinct spectral features in the 1300-1000cm⁻¹ region in comparison to the bacteria, therefore these two types of bio-molecules can be undoubtedly distinguished by their ATR spectra. On the other hand, the two bacteria spectra appear fairly similar.
When bio-molecules have very similar IR spectra which are not easy to distinguish by simple visual inspection, statistical spectral analysis approaches such as “Principal Component Analysis” (PCA) can help identify them [12]. In this technique, each spectrum is described as a n dimension vector and statistically compared to a group of similar spectra. Figure 7 shows PCA score maps of a group of 22 bacterial spectra including 12 of *E. coli* and 10 of *S. aureus* collected using the electrophoretic sensor. The maps indicate that the two types of bacteria can be distinguished based on statistical spectral features corresponding to the weight of a particular eigenvector called a score. The distinction between the two bacteria is illustrated based on the three most discriminating scores in the 3-D map of Fig. 7(a) (9 red dots are visible in the 3-D map because two of them superimpose), however Fig. 7(b) shows that a 2-D map based on two scores only also provides good selectivity.

It should be noted that the analysis of Fig. 7 was performed on the full spectrum 3000-800 cm\(^{-1}\) without preselecting any wavelength domain. It is therefore expected that the selectivity could be further refined upon prior selection of relevant spectral domain for performing the principal component analysis.

![Fig. 7. (Color online) Principal Component Analysis maps of 22 bacteria spectra showing the potential for distinguishing different bacterial strains (E. coli and S. aureus): (a) 3-D plot of the 2nd, 3rd and 4th PCA eigenvectors, (b) 2-D plot along the 2nd and 4th eigenvector axes.](image)

4. Conclusion

It is shown that the surface charges of bio-molecules can be utilized to drive them at the surface of an infrared sensor. This new opto-electrophoretic sensor is composed of an ATR plate fabricated from a novel conducting telluride glass that is used as both an optical sensor and an electrode for driving the migration of bio-molecules. It is shown that the entire population of bio-molecules can be driven on the sensor surface. We confirm that the effect results from the net surface charges of bio-molecules by reversing the migration direction with a negative voltage. This indicates that the sensor surface can potentially be replenished by repealing the molecules off the surface. The infrared signals collected using this sensor provides great specificity and it is shown that different bacterial genus can be selectively indentified in aqueous environment. This sensor design therefore has great potential for online monitoring of water safety.

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