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On the other hand, the optimal dye concentration adsorbed on the alumina clad of the optode was tested and its influence on the sensitivity was observed too. Alumina-coated fibers were immersed in the N719-ethanol solution (0.3 mM) at 30 °C at different interval times: 5, 30, 60 and 90 minutes. Mercury titrations were performed in order to determine the sensitisation conditions. Similarly to the alumina mesoporous films previously described at Chapter 4, in order to obtain a stable response, each optode was immersed in pure water during 45 minutes before the measurement process. Hence, any excess of the adsorbed molecular indicator in the sensitive medium was removed. By observing the spectral mercury titrations of the optode with a sensitised time of 90 minutes, it appears several spectral features at  $\lambda=800$  nm that should be constant, see the spectral scope represented in Figure 5.8a. This behaviour suggests that an excess of dye is present onto the alumina

cladding and a de-sorption process occurred, which makes difficult the signal stabilization.

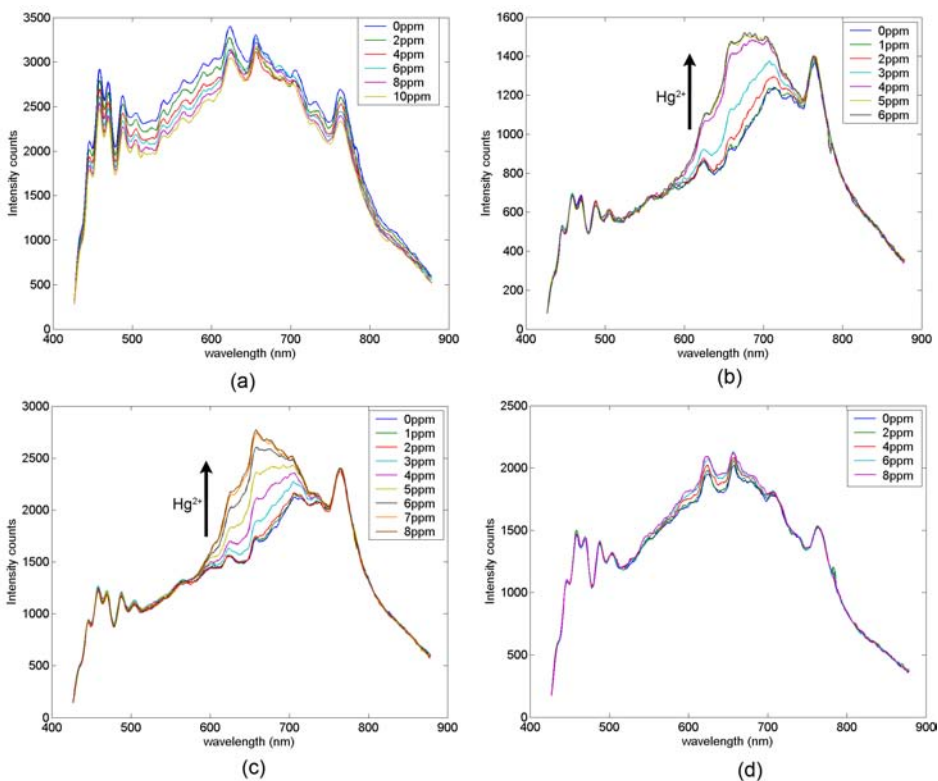


Figure 5.8. Intensity counts of the optical fiber probe with different sensitization times in N719 ethanol solution: a) 90 minutes, b) 60 minutes, c) 30 minutes, and d) 5 minutes. The reflected light is registered after a conditioning time of 45 minutes whereby the optodes has been immersed in buffered water solution at ph 7. After that, titrations were performed by adding Hg<sup>2+</sup> drops with an interval time between measurements of five minutes.

Sensitisation times of 60 and 30 minutes provided a clearly spectral signal correlated with mercury additions, being the optode slightly more stable in the later case as illustrated in Figure 5.8b and Figure 5.8c

respectively. Whereas, low immersion times produce poor spectral changes because the poor coloration of the alumina cladding hardly absorbs the corresponding fraction of light that emerges from the core to the dyed alumina cladding, see Figure 5.8d. Note the attenuation on the spectral region from the 450 to 720 nm of the blank when the proper concentration of the reagent is present onto the alumina cladding (see Figure 5.8b and Figure 5.8c). Meanwhile, if the alumina cladding of the optode has an excess or insufficient dye concentration, the final spectrum is remarkable comparable to the original spectrum of the lamp. Thus, the proper working conditions of the optode are also controlled by the dye concentration adsorbed onto the alumina cladding. Additionally, we must to consider a variance in the light intensity counts, which is dependant of the fabrication process for each optical fiber probe. Representing the scope of the spectral signal from Figure 5.8c in terms of absolute absorbance, as can be seen in Figure 5.9, the maximum peak of the observed spectral changes are localised at  $\lambda \sim 680$  nm. This result notably differs from mercury titrations performed either in liquid or alumina film measurements with the N719, wherein the maximum peak was localised around  $\lambda = 530$  nm.

In order to check the origin of this mismatch, the direct absorbance measurements of mercury titrations with N719-ethanol solution were carried out. In this experiment, an adapted cuvette holder for optical fiber measurements connected between the lamp and the spectrophotometer was employed.

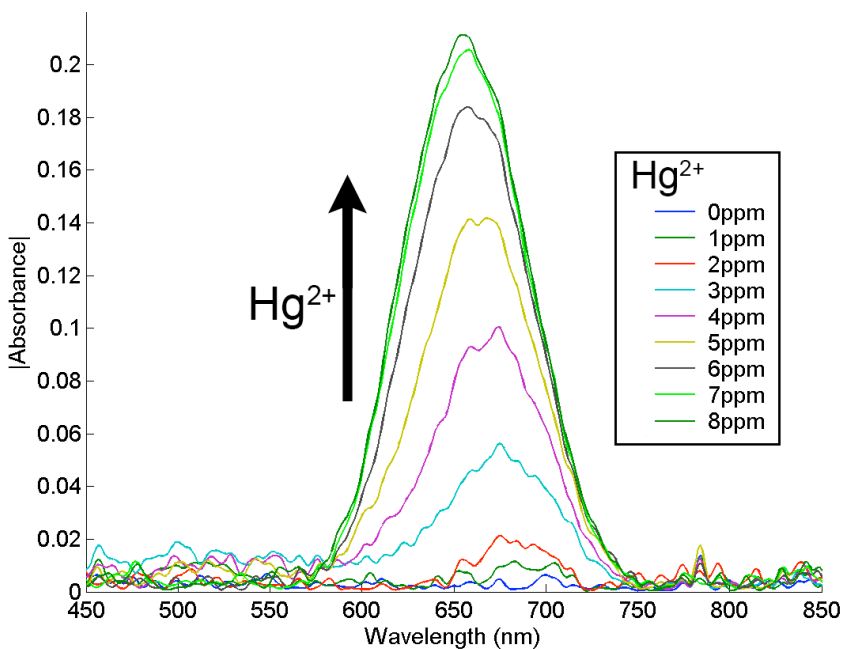


Figure 5.9. Absolute spectral absorbance of  $\text{Hg}^{2+}$  titrations corresponding to an optical alumina cladding-based fiber with a sensitization time in N719 ethanolic solution of 30 minutes. The absorbance is related to the blank of the optode immersed in pure water.

As it was expected, from Figure 5.10a, it can be observed that spectral changes, induced by the mercury addition, appear in a wide range from  $\lambda=450$  nm to 750 nm, with a maximum peak localised at  $\lambda=530$  nm. After all, regarding Figure 5.10b represents the absolute value of absorbance. Therefore, the observed spectral absorbance changes are in agreement with previous measurements realised by using conventional equipment, and thus the optical fiber measurement system is correctly working.

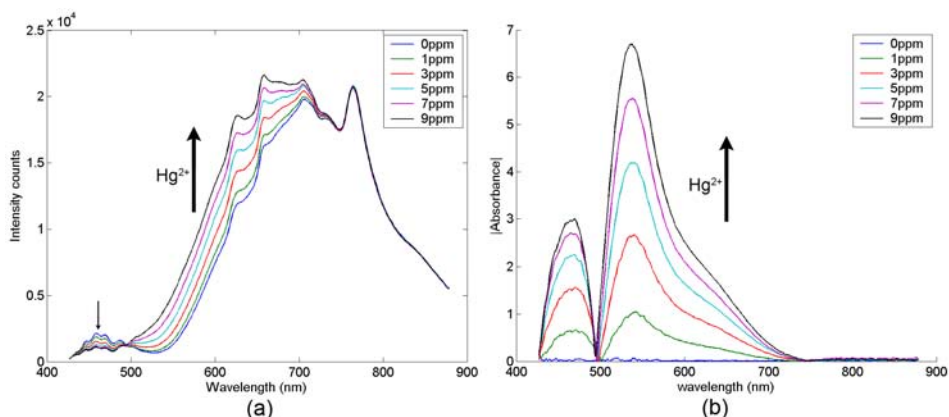


Figure 5.10. Mercury titrations in N719-ethanol liquid measurements registered by the optical fiber system using a cuvette holder: a) intensity counts, and b) its absolute absorbance representation.

Moreover, one of the aspects that may influence the spectral shift of the fabricated optode can be extracted by observing the light transmitted by the direct measurement of the reagent (Figure 5.10a). As can be seen, a great attenuation of light is produced in the region of  $\lambda=430\text{-}550$  nm and it is progressively less remarked as the wavelength increases. Obviously, this effect is inherent to the N719 transmittance because this is the spectral region wherein the more dye absorbs. In contrast, this effect may be responsible of the major attenuation of this part of spectrum light once this is refracted through the alumina cladding. Therefore, the dye may attenuate the spectral region of the around  $\lambda=530$  nm in which the the sensitivity is larger. This fact reduces the features of the optode in order to improve the sensibility in solid state, but anyway a high level of integration is achieved with this optode configuration. It would be expected that augmenting the power of the light source it could observe

changes in the  $\lambda \sim 530$  nm region, but an excess of light may produce photodegradation problem in the molecule.

## 5.2 Reproducibility and multivariate calibration

The reproducibility is a lack of most of this kind of optodes. According to Alabbas<sup>14</sup> et al. sometimes the major source of non-uniformity of the response is generated by the sensor construction rather than sensor operation. We tested the mercury response with diverse optodes. Figure 5.11 shows the response in both intensity counts and absolute spectral absorbance corresponding to mercury titrations of three optodes with the same fabrication conditions: alumina cladding length of 2 cm, thick of 5  $\mu\text{m}$ , and a N719 sensitisation time of 30 minutes at 30°C. The optodes were immersed in the cuvette with 3 ml of HEPES water solution at pH 7 for a conditioning time of 45 minutes. Thereafter the mercury drops were added and the optical reflectance intensity were registered. The amplitude of the optical signal varies significantly from each optode. At a first time, this fact may suppose an inconvenient so as to predict mercury levels at a fixed wavelength. But observing the spectral intensity of the optode response, some useful information may be extracted from comparing different parts of the spectrum. This can be a good challenge to be solved by multivariate linear regression, wherein spectral synergies can contribute to find linear relationships for predictions of mercury levels.

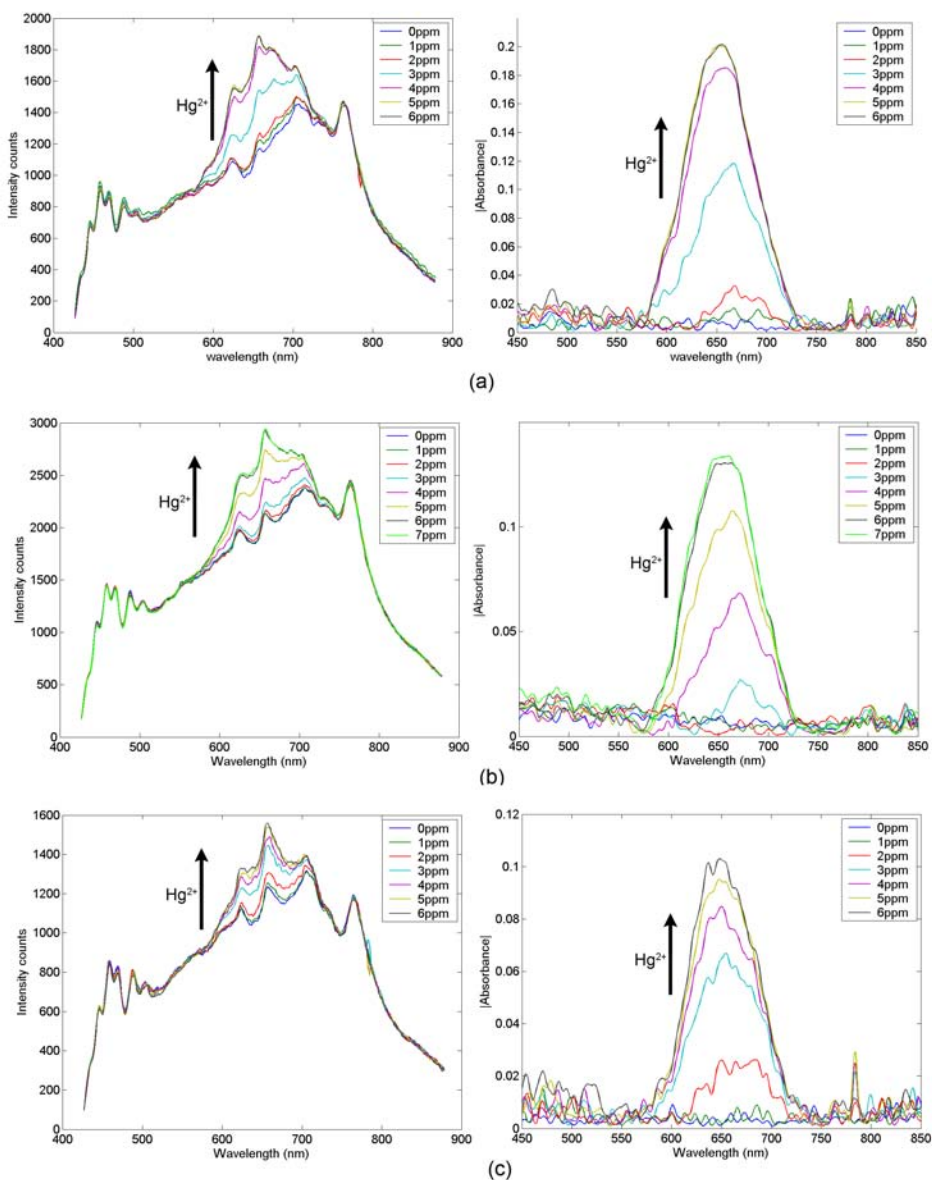


Figure 5.11. a), b) and c) represents the intensity counts (on the left) and their absolute spectra absorbance (on the right) corresponding to three different mercury probes with the same fabrication conditions. After the conditioning time of 45 minutes immersed in buffered water (pH 7) for each optode, the mercury additions have been carried out with an interval time between measurements of five minutes.

Therefore, in order to calibrate the mercury probes PLS processing was carried out. As the analytical signal, the intensity counts response was selected because it contains more relevant information, which can serve to predict mercury concentrations. Precisely, the spectral variables arises from 525 to 780 nm, which contains the most significant optical changes when mercury is present, and also it includes invariant regions that can serve as reference signal for the PLS model. A moving average filter was applied in order to smooth out the high frequency fluctuations. The moving average is similar to the low pass filter whereby each element is replaced by the average of a certain number  $N$  of neighbouring elements.

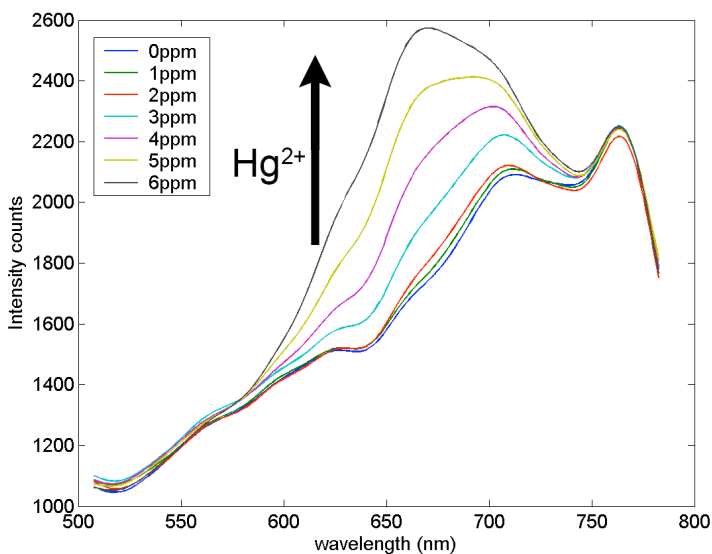


Figure 5.12. Smoothed spectral mercury response of an optode used in the calibration of the mercury probes.

The difference equation of this process is defined by

$$y(k) = \frac{1}{2N+1} [y(i+N) + y(i+N-1) + \dots + y(i-N)] \quad (5.5)$$

where  $y(k)$  is the smoothed value for element  $k$  corresponding to the whole elements within the span  $2N+1$ . In practice, this difference equation is easily programable with MATLAB as a convolution with a finite impulse response filter (FIR), wherein the vectors are ones. Figure 5.12 shows the smoothed spectral response of a mercury probe obtained considering a filter span of 20 elements.

The construction of the PLS model was carried out with the mercury responses from six optical fiber mercury probes. Mercury titrations were performed for each optode in a concentration range from 0 to 6 ppm of  $\text{Hg}^{2+}$ . The whole calibration data was composed by 44 measurements and 291 spectral variables, which resulted after decimating by a factor of 5.

The behaviour of the PLS model was observed with other 3 N719/ $\text{Al}_2\text{O}_3$  optical fiber probes which had not involved in the calibration process. One of these probes was used for training the model and choosing the correct number of PLS factors. The other two probes were used to validate the PLS model with new mercury samples. Herein, the adjustment and evaluation criterion applied was similar to PLS modelling in previous chapters. Figure 5.13 shows the mercury predictions of the PLS model corresponding to training and validation measurements. A

good fitting was achieved for the training probe response considering 4 PLS factors. The root mean square error of prediction percent (% RMSEP) for the other two mercury probes was 11.56 (%) was achieved, considering a mercury measurement range of 0-6 ppm. Moreover, statistical feature selection pre-treatment was also applied. The discrimination factor plot showed a unique peak localized at 665 nm. Several Gaussian kernels with different  $\sigma$  were applied, but any reduction of the spectral variable set assisted by SFS did not improve the performance of the PLS model. As in Chapter 4, the performance of the direct PLS model was more robust when the variance was mainly caused by the fabrication procedure.

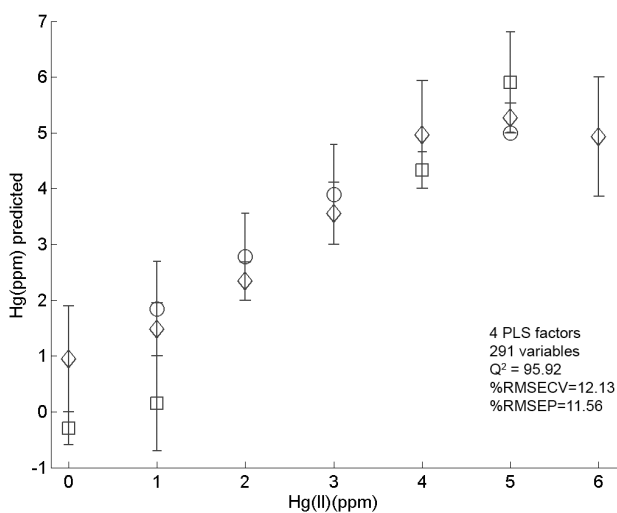


Figure 5.13. Mercury predictions of the PLS model with 4 PLS factors from three different optical fiber probes: ( $\diamond$ ) training data set, ( $\circ$ ) validation data set 1, ( $\square$ ) validation data set 2.

As discussed above, the optical response of this kind of sensors show a remarked dependence on the cladding homogeneity. Any variation regarding thickness, particle distribution, porosity, humidity,

contamination due to dust particles, etc, may cause variations in the response among probes. Therefore, it is of utmost importance that the manufacture process be directed to reducing all these factors that may introduce changes in the sensitive layer. Many of these factors are not considered in the usual laboratory conditions, unless all steps of the manufacture process are performed inside a special room that can guarantee a clean and controlled environment. Moreover, the fact that the probe does not show an optimal regeneration, forces a single use of the probes, thus making it necessary to obtain a generic model with a greater error instead of a tailored individual calibration model for each probe, which could be obtained from repetitive measures. Even though, the mercury predictions yielded by the PLS model present a good linearity regarding the real mercury values, making it possible to obtain a general calibration model that allows to quantify the response of new probes. Future improvements of this system, aimed to broaden its applicability, should be directed to enhance the system sensitivity or to increase the dynamic range. These factors are directly related to the concentration of the adsorbed indicator. However, this parameter cannot be increased to a considerable extent, as there is a balance between the dye concentration and the stability of the probe response. It still remains to explore the film thickness and the nanoparticle diameter, which parameters are more closely related to the analyte diffusion through the film, and which could have impact over the sensitivity and measurement range.

## 5.5 References

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DEVELOPMENT OF AN OPTICAL FIBER PROBE FOR MERCURY DETECTION

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# 6 Conclusions

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## 6. Conclusions

Nowadays, much increasing research efforts are devoted on the chemical sensor field. Although chromogenic probes have been popular in last decades, the molecular design tends to use fluorescence detection systems, often combined with recent advanced materials, such as quantum dots. In general, the fluorimetric detection provides higher sensitivities. However, the use of this type of molecules involves certain working constrictions; for example, working in aqueous solution may not be feasible because the fluorescence of some molecules is only possible with organic solvents. Moreover, the attachment of the molecule to an optical support may change its emission properties. In contrast to this kind of sensors, colorimetric sensors are widely used in combination with supporting matrices and with simpler and low cost measurement instrumentation. Accordingly, N719 dye has been selected because of its suitable affinity to mercury, its wide spectral response and the possibility of being anchored onto metal oxide films, making its integration into an optical device feasible.

An important part of this thesis has been dedicated to multivariate analysis of spectral data. A number of pre-processing techniques coupled to a defined calibration method have been applied, showing that it is feasible to reduce the effect of chemical interferences present when using a molecular probe. In this context, concentrations of mercury from 0 to 2

mg L<sup>-1</sup> have been quantified even in the presence of 19.5 mg L<sup>-1</sup> of interferential metals. In order to improve the prediction ability of PLS calibration models, a comparison of several pre-treatment techniques has been carried out, all these methods being tested and validated on the same data sets. The results showed that the lower prediction error was obtained by employing variable selection techniques. Indeed, we have demonstrated that the variable selection can overcome interference effects in the mercury quantification. Initially, the genetic algorithm was expected to be the best technique of this group. However, the adjustment of the algorithm towards an optimal solution with less over-fitting was tedious and entailed long computation times (around 20 minutes) if compared to the above-mentioned SFS adjustment (0.26 seconds). On the other hand, the use of statistical feature selection to identify important spectral features provided for the best performance of the PLS calibration model. Moreover, the approach of using Gaussian kernels, together with the SFS algorithm, allowed a considerable reduction of the number of variables to be selected, having a crucial impact on the quality of the final PLS model. Similarly, the extension of the SFS to bi-variate analysis enabled the variable selection technique to identify synergic variables, which also resulted in a low prediction error. A further advantage, of the statistical feature selection approach, relies in its simplicity and very low computational cost. The results of this work can be extended to a wide variety of chemical probes, improving their robustness in quantification tasks. The results also show the potential use

of this particular chemical probe for the production of a chemosensor that would be highly selective to mercury.

On the other hand, great advances have been achieved by immobilizing the indicator on a solid support. Although N719 is susceptible of being anchored onto TiO<sub>2</sub> films, this support is not suitable for measuring mercury in aqueous solution because leaching problems occur upon exposure to water. Herein, we have demonstrated a great improvement by immobilizing the indicator onto Al<sub>2</sub>O<sub>3</sub> nanoparticles films. This material is optically more transparent than titania films, and on the other hand its high surface area allows a better control of the dye concentration in the mesoporous films. Higher mercury levels could be detected by increasing the concentration of indicator adsorbed onto the films. The stability in water has been improved by changing the acidity conditions of the film. Among the several acid treatments tested, the choice of sulphuric acid showed a superior performance in terms of reagent leakage, while maintaining the sensitivity performance of the N719/Al<sub>2</sub>O<sub>3</sub> films. However, the lack of reversibility reduces the possibility of online mercury monitoring and forces to make a single use of the sensitive film. Nevertheless, the dye of the alumina support can be renewed by a first immersion of the film in a basic solution (for example NaOH solution at pH 10), and a subsequent immersion in a N719 ethanol solution. The calibration of the Al<sub>2</sub>O<sub>3</sub> films showed a good correlation with mercury concentrations, even in the presence of most important interferential metal ions. These results corroborate a similar behaviour as

the experiments in liquid solutions, and thus the adsorption onto alumina films is innocuous to the mercury binding. In this set of experiments, there is a great variance introduced by the error of reproducibility of the films. Under these circumstances, the direct PLS solution has provided for the best fitting to mercury predictions. The better performance of the PLS model with the whole spectral variables concludes that a higher variable number shall be recommended when a great signal variance is present. Furthermore, several deposition techniques are compatible with this alumina paste, such as screen-printing, spin coating and dip coating, what makes the production of different types of optical sensors feasible.

This is the first time that an optical fiber probe based on a functionalized cladding for mercury(II) determination in water samples has been obtained. This novel type of optical probe allows a high level of integration of the molecular indicator, whereby the measurement process is further simplified. A simple and low cost fabrication procedure makes the N719/ $\text{Al}_2\text{O}_3$  films suitable to be sensitive towards mercury. By using the alumina cladding, we have observed an unusual situation in the literature, related to the fact that the outer cladding has a refractive index greater than the optical core, what may give rise to the combination of evanescent field and radiative mode. A similar response time has been observed regarding the sensitive films supported onto glass, but with a different spectral response when compared to the direct absorbance measurements of the sensitised films. The narrower spectral range and the peak shift in the optical fiber implementation may be due to an

attenuation of the most significant spectral regions of the N719. In this sense, the sensitivity is not enhanced as it was expected with this type of optical fiber design. The mercury response between different sensors have significant variance, but applying PLS calibration the reproducibility error is reduced, allowing a mercury prediction error of 11.5 per cent. Some problems are still to be solved regarding the sensitivity and reproducibility. There are many variables involved in the optical fiber probe design, which will require to set some of those parameters in order to discern the principle-of-proof in the experiment. Moreover, further studies can be performed in order to observe the particle size and thickness of the alumina thin film and its role regarding sensitivity. Finally, the application of this optical fiber implementation to mercury screening in food, wherein the sensitivity requirements are minor, is feasible.