COMPACT OPTOCHEMICAL ANALYSER BASED ON INTEGRATED WAVEGUIDE ABSORBANCE OPTODES

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Abstract: A rugged, compact, mass produced, portable and easily automated optochemical measurement system has been developed for the feasible application of Integrated Waveguide Absorbance Optodes (IWAOs) in field. All the optochemical components are integrated in one module. To evaluate the analyser operation, a calcium selective optode has been employed as the sensing region. It contains a ketocyanine dye, which shows a narrow absorption band located in the working wavelength, and no absorption bands located in the reference wavelength.

Keywords: optode, ketocyanine dye, IWAO

INTRODUCTION

Trends in the optical sensor field are the joining of traditional bulk optodes with fibre optics and planar waveguides to exploit the technological improvements attained by the telecommunication industry and the implementation of automated mass fabrication methodologies.

Recently some Integrated Waveguide absorbance optodes (IWAOs) have been proposed by our research group [1-3]. Such devices consists of the same chemically active membrane as those of the conventional configuration, but deposited over a microfabricated planar waveguide circuit constructed by IC technology. The optical membrane works as the selective recognition region while acting as part of the light guiding planar structure. As guided light path is transverse to the diffusion direction of the analyte through the membrane, sensitivity is enhanced while it does not compromise the response time. IWAOs are sensing platforms, which confer versatility, robustness and mass production capabilities besides high sensitivity on conventional bulk optodes.

However, for the feasible application of such sensors in field, a more compact, portable and automated system is needed, as well as mass production exchangeable transducers.

In this work we report a novel compact optochemical analyser, which uses new IWAOs constructed with silicon micromachining for their easy exchangeability. The set-up includes two different light sources, one being used as a reference signal to correct any deviation due to unspecific optical interferences.

In order to optimise the system performance, a calcium-selective IWAO has been proposed as a potential example for the determination of calcium ion in biomedical, environmental fields or industrial processes. In this way, we have developed a calcium-selective membrane combining a previously characterized ketocyanine dye with a commercially available calcium ionophore. The absorbance maxima of the ketocyanine dye matches the wavelength of the working LED (780 nm), provides a high capacity to differentiate between little concentration variations as a result of its high molar absorptivity and shows no absorption bands at the wavelength of the reference LED.

EXPERIMENTAL SECTION

Reagents. Calcium chloride solutions have been prepared by dilution of a 1 M stock solution. The buffer solution is 0.05 M Tris adjusted to a selected pH using concentrated hydrochloric acid. 0.05 M EDTA adjusted at pH=5.5 has been used to force the recovery of the dye. For membrane preparation, the following components have been obtained from Fluka (Buchs, Switzerland): poly(vinyl chloride) (PVC high molecular weight) as the polymer, Tris(2-ethylhexyl)phosphate (TOPH) as the plasticizer and tetrahydrofuran (THF) as the solvent; potassium tetrakis(4-clorophenyl)borate (KTpClPB) has been used as the lipophilic anionic additive and calcium bis[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate as the calcium ionophore. The ketocyanine 5ee used as the chromoionophore has been synthesized in our laboratory [4] (Figure 1).

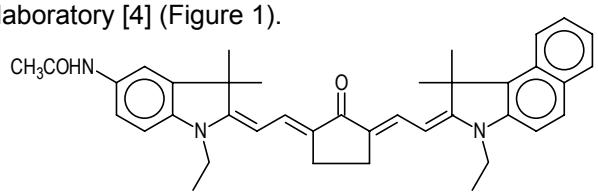

Fig. 1. Chemical structure of the ketocyanine dye.

Apparatus. Spectra of the PVC bulk optode have been done between 1000 and 400 nm using a double-beam UV-vis-NIR scanning spectrophotometer (Shimadzu UV-310PC).

The continuous flow system and the injection flow system used for the determination of calcium in

water consists of a GILSON Minipuls 3 peristaltic pump, equipped with PVC pump tubing (Elkay, Boston, MA), a six-way selection valve (Hamilton MVP) or an injection valve (Hamilton MVP) and a flow cell similar to a wall-jet layout. An IWAO based on a 500-μm-long cavity for the recognition region has been used.

RESULTS

The main innovation of the measurement system is the introduction of a reference wavelength (λ_2) into the optical sensor, along with the sensing wavelength (λ_1). This reference wavelength is located out of the main absorption peak of the dye, thus providing a signal, which value depends only on physical changes of the system (such as fibre bending, membrane hydration or refractive index changes) and not on the presence or absence of the analyte. This reference can be used to correct the output signal of the sensor, avoiding new calibrations, by removing the changes in absorbance due to causes other than variations on the analyte concentration, using the following equation:

$$A = -\log\left[\left(P / P_o\right) / \left(P_{ref} / P_{oref}\right)\right] \qquad \text{Eq. 1}$$

In the case of cyanine 5ee, as the absorbance spectrum has its maximum around 780 nm, the selected reference wavelength is 850 nm. Even if the optical source is a Light Emitting Diode (LED), with a several nanometres wide spectrum, the reference source is not significantly affected by changes in the absorption spectrum of the dye. The sensing wavelength λ_1 is 780 nm. (Figure 2).

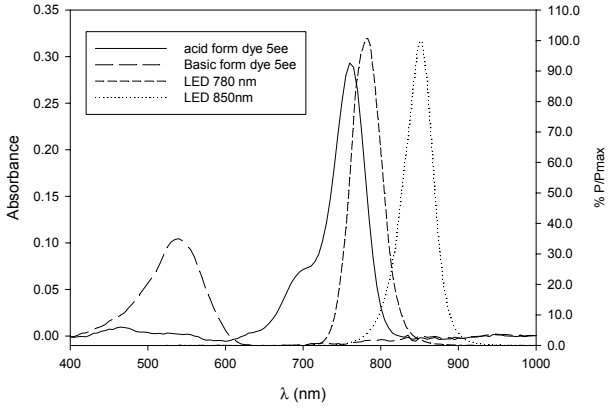

Fig 2. Localisation of the absorption bands of the dye and the emission LEDs.

The use of LEDs instead of laser diodes as optical sources has great advantages: firstly, the use of lower optical power prevents the membrane from decomposing, thus extending the longevity of the sensor. Secondly, as both the optical fibres and the waveguides used are multimode, the use of lasers would result in a severe modal noise and reduce the performance of the system. Finally, LEDs are cheaper and can be found in a wider range of wavelengths than diode lasers.

Figure 3 schematically shows all the components that are enclosed in the measurement equipment, including the IWAO.

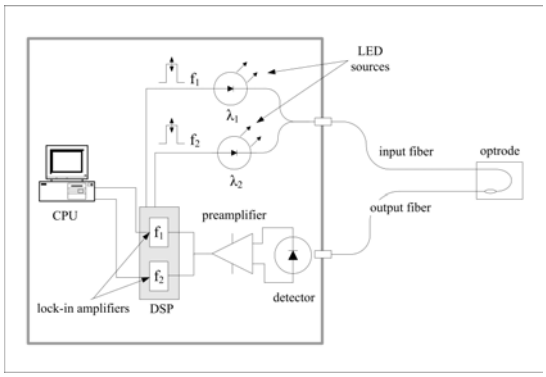

Fig. 3. Scheme of the compact optochemical analyser.

However, the lower optical power of LEDs requires also an improvement of the detection system in order to maintain a good dynamical range. This is achieved by the use of a lock-in amplifier, designed by our research group, which filters the signal leaving only the portion modulated at a given frequency, improving the signal to noise ratio and, as a consequence, the dynamic range of the instrument.

Moreover, if the interaction wavelength λ_1 is modulated at f_1 (480 Hz) and the reference λ_2 at another frequency f_2 (1200 Hz), the use of two different lock-in amplifiers allows also the detection of both signals in only one optical detector, and its separation without the need of optical filters or a demultiplexer.

The lock-in amplifiers and the two modulation signals can be implemented using the same DSP (Digital Signal Processing), reducing costs and space. In fact, the whole detection system has been included in a small PC case along with a mini-ITX main board, which controls it via RS232. This system results in a portable instrument containing the optical sources, the detector, the lock-in amplifiers and a computer which performs the data processing associated to absorbance measurements and which also provides a handy interface to save and export data.

Other instrumental improvement is the fabrication of the transducer and its support in order that it can be easily exchangeable. The use of silicon micromachining can assure the size of the transducer with high precision. Figure 4 shows how a transducer is obtained by simply pressing it from the silicon wafer.

Three main parts of the sensor can be distinguished (Figure 5): (1) the transducer based on a curved planar waveguide, where the optical sensing membrane is deposited on, (2) the V-grooved auxiliary support, where the optical fibres are fixed, and (3) the connection platform, which permits the alignment of the transducer waveguides to the optical fibres, by simply laying and clicking a new transducer.

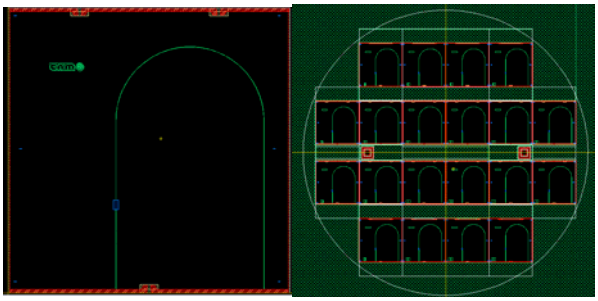

Fig. 4. On the left, individual transducer. On the right, general view of the silicon wafer.

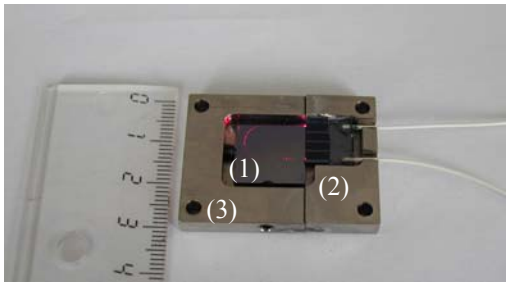

Fig. 5. New improved IWAOs. (1) Waveguide sensor; (2) V-grooved platform; (3) connexion platform.

To demonstrate the possibilities offered by the use of the compact optochemical sensor, an IWAO has been activated with a calcium bulk optode. Optical membranes have been previously characterized with a conventional absorbance flow cell in a Flow Injection Analysis (FIA) system to firstly optimise the buffer composition and to perform a comparative study of the analytical response.
Since ion-selective optodes based on ion exchange mediated by an ionophore and an indicator sense the ratio of the analyte and hydrogen ions in the sample solution, the pH must be kept constant.
The FIA technique is based on the injection of a certain sample volume in a carrier solution, which is continuously directed to the detector. It easily allows adding a conditioning channel that buffers the inserted sample before the detection, even though a sample dilution occurs. Figure 6 shows the simple FIA manifold used. Since the blank signal is taken at the base line generated by the buffer solution half diluted (fully protonated chromoionophore) absorbance decreasing is recorded when calcium samples reach the optode.

- Optimization of the flow system

The equilibrium between the bulk of the membrane and the sample must be reached for the bulk optode signal generation, and response time is often determined by the time to attain a uniform concentration of the components in the membrane. Hence, diffusion within the polymeric membrane is time limiting. Since the FIA technique produces a non-equilibrated transitory signal, hydrodynamic parameters must be kept under control to allow signal reproducibility.
The measuring ranges of these optodes typically cover from two to four concentration decades and depend on the membrane composition and the buffer pH.

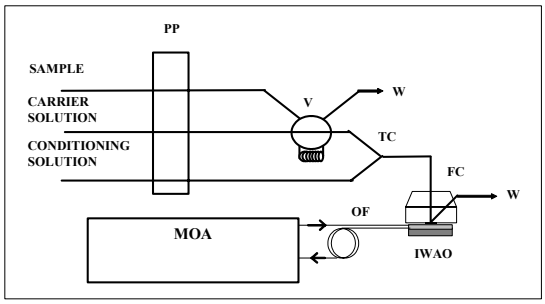

Fig. 6. FIA system used for the determination of calcium in water samples. PP, peristaltic pump; V, six-way injection valve with a determined injection volume; W, waste; TC, tee connector; FC, flow cell; IWAO, integrated waveguide absorbance optode incorporating the calcium-selective bulk optode; OF, optical fibres; MOA, miniaturised optical analyser. Sample, $CaCl_2$; Carrier solution, distilled water; conditioning solution, 0.05M Tris buffer at pH=6.5.

Initially, the influence of the buffer pH on sensitivity and linear range is studied. The buffer solution concentration chosen was 0.05 M. As it is known, the measurement concentration range can be shifted within a wide range by changing the buffer pH, as it determines the quantity of available protons (Figure 7). In our study, the selected pH is 6.5 to cover the range from $1x10^{-4}$ to $1x10^{-1}$ M calcium concentrations with the maximum sensitivity.

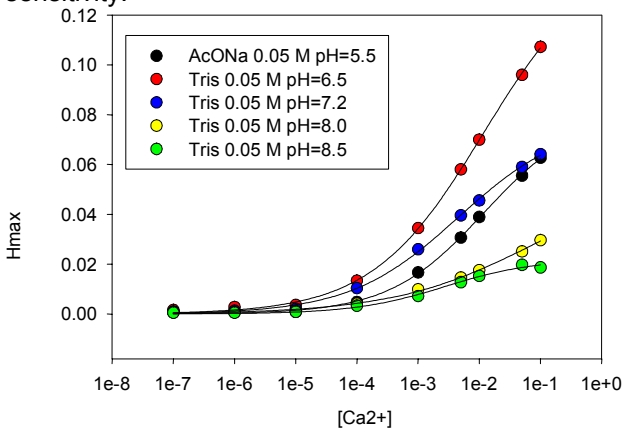

Fig. 7 Sensitivity and linear range dependence on the pH of the buffer solution. Best results are obtained at pH=6.5.

After that, the influence of the flow rate and injection volume on sensitivity and response time is studied (Figure 8). In these experiments a $1x10^{-3}$ M calcium solution is injected in triplicate in the carrier solution.
The analytical signal is proportional to the time interval in which the membrane is in contact with the sample, until the steady-state signal is reached, but ion diffusion on the membrane and effects as dispersion of the sample into de carrier solution, or sample renewal after each peak, must be taken into account. Five different injection volumes have been tested (500, 600, 850, and 1000 μL) at different flow rates (3.2, 4.6, 7.1 and 8 mL/min). As Figure 8 shows, each sample volume has an optimum flow rate, in which a maximum analytical

signal is obtained. As a compromise between sampling rate and analytical signal, an injection volume of 850 μL and a flow rate of 4.6 mL/min are chosen.

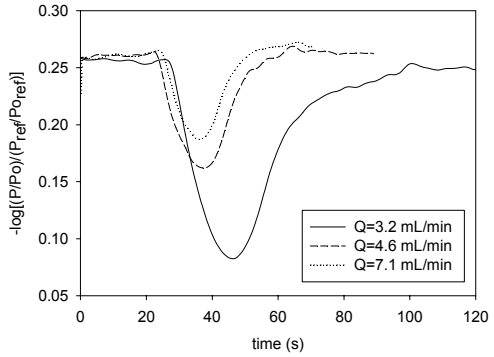

Fig. 8 Recorder signals for the hydrodynamic parameter optimisation of the FIA system. V_{inj}=500 μL.

- Signal correction

Although it was previously demonstrated that using the planar configuration it is possible to avoid interferences from the solution matrix, it has been noticed that the refractive index of the membrane changes while it is hydrated at the beginning of the experiments. This optical interference can be corrected using the new miniaturised analyser as the guiding properties of both wavelengths vary in the same manner due to changes of the refractive index. Figure 9 shows an example of the correction comparing the obtained direct signal with the corrected one by using the reference wavelength at 850 nm.

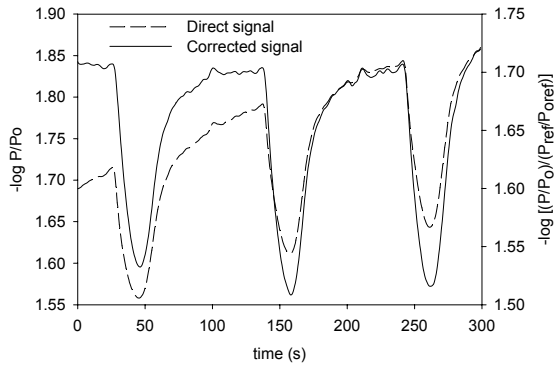

Fig. 9 Recorder signals at the beginning of the membrane hydration. A sample of $1x10^{-3}$ M $CaCl_2$ is injected in triplicate at the optimised hydrodynamic parameters.

- Sensitivity, detection limit and repeatability

Sensitivity is calculated as the slope of the linear range of the calibration curve but the peak height of the injected samples at a fixed concentration level must be also determined. To demonstrate the advantages of using the planar configuration of the optical sensor, results obtained with the IWAO are compared to those acquired in a conventional flow-cell configuration, using the same membrane with the same thickness (4 μm).

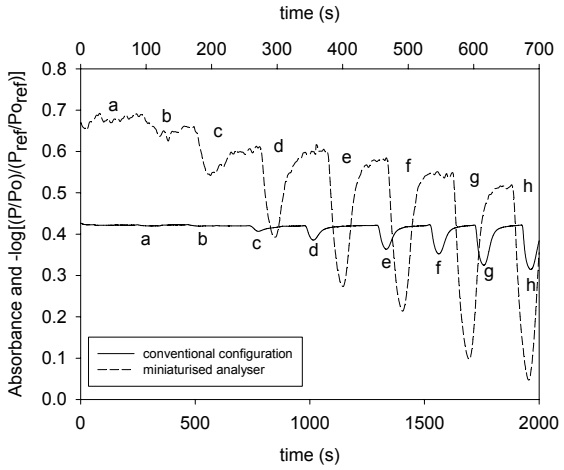

Fig. 10 Recorder output for the optimised FIA systems for the detection of calcium in water samples using a conventional spectrophotometer and the miniaturised analyser. a) $1x10^{-6}$, b) $1x10^{-5}$, c) $1x10^{-4}$, d) $1x10^{-3}$, e) $5x10^{-3}$, f) $1x10^{-2}$ M, g) $5x10^{-2}$, h) $1x10^{-1}$ M.

The achieved absorbance change with the conventional configuration (transmission mode) is multiplied using the miniaturised analyser for every analyte concentration injection, even though the injection volume employed (850 μL) and the flow rate (4.6 mL/min) are lower. In order to obtain significant changes with the conventional configuration it is necessary to use an injection volume of 1000 μL and a flow rate of 2 mL/min.

Therefore, the sample throughput and the sensitivity are improved using the proposed miniaturised analyser. The detection limit with the proposed system is $1.41x10^{-4}$ M and using a conventional flow cell is $5.99x10^{-4}$ M.

The short time repeatability of the analytical signal is evaluated by successive repeated injections of the same solution ($5x10^{-3}$ M calcium). The signal is repeatable in a short time scale, but the peak height diminishes due to the sample renewal.

CONCLUSIONS

We present fast and sensitive integrated optical sensors, composed by a versatile general platform of easily disposable or regenerable transducers, which can be chemically activated with some types of new Vis NIR acidochromic dyes, of appropriate characteristics, and which employ a compact instrumentation with a reference signal for avoiding calibration of the system when the optical characteristics vary.

REFERENCES

1. M. Puyol, M. del Valle, I. Garcés, F. Villuendas, C. Domínguez, J. Alonso. *Analytical Chemistry*, 71, (1999), pp 5037-5044

2. M. Puyol, S. Miltsov, I. Salinas, J. Alonso. *Analytical Chemistry*, 74 (2002) pp 570-576

3. M. Puyol, I. Salinas, I. Garcés, F. Villuendas, A. Llobera, C. Domínguez, J. Alonso. *Analytical Chemistry*, 74 (2002) pp 3354-3361

4. S. Miltsov, C. Encinas, J. Alonso. *Tetrahedron Letters*, 42 (2001) pp 6129-6131.