XIX IMEKO World Congress
Fundamental and Applied Metrology
September 6−11, 2009, Lisbon, Portugal

A LOW-COST AUTOSAMPLER FOR SURFACE PLASMON RESONANCE BIOSENSOR PLATFORMS

C. S. Moreira [1,2], *A. G. S. Barreto Neto* [1,3], L. C. Oliveira[1], *A. M. N. Lima*[1] *and F. C. C. Loureiro*[1] and H. Neff[1,4]

[1]Department of Electrical Engineering
Center for Electrical Engineering and Informatics
Universidade Federal de Campina Grande
Rua Aprígio Veloso, 882, 58429-900 - Caixa Postal 10.105, Campina Grande, Paraiba – BRAZIL
e-mails: cleumar@ee.ufcg.edu.br, amnlima@dee.ufcg.edu.br
[2]Department of Electronics – IF-AL
[3]Department of Electromechanics – IF-/PB
[4]CETENE/LINCS

Abstract − A low-cost autosampler for surface plasmon resonance (SPR) biosensor platforms has been designed and applied for the determination of the mutual diffusion coefficient of ethanol in water. Its configuration is simple and based on a motorized carousel-type set-up. The sample loading process comprises a Z-drive, along with a syringe type analyte extractor. Furthermore, a commercial peristaltic pump is used to circulate the analyte through the microfluidic cell of the biosensor. A microcontroller connected to a PC provides control of the set-up. Theoretical aspects and experimental results are presented.

Keywords: autosampler, surface plasmon resonance, microcontroller, biosensor

1. BASIC INFORMATION

Surface plasmon resonance (SPR) sensors, due to their fast response, high sensitivity and selectivity and non-labelling requirements, are capable to determine bio-molecular interactions [1], detect biological and chemical contaminants for food safety [2] or environmental monitoring [3] and pathogens (like bacterias and viruses). The method is thus widely used in the pharmaceutical and medical industry as a reliable research tool [4]. Since 1983, when the first commercial SPR biosensor has been presented, the number of non-commercial or commercial instruments has increased substantially and some devices already substitute conventional analytical tools for specific applications [5]. SPR sensors are composed basically as optical multilayer devices, where on a thin layer metal-dielectric interface, a so-called surface plasmon resonance occurs under specific conditions of total internal reflection. The associated optical apparatus houses equipment for generation of the incoming and detection of the reflected light beam, a microfluidic system with a pump, which provides the sample (or analyte) circulation to the interface, and a signal conditioning and processing system.

For the majority of SPR biosensing applications, it is usually necessary to change the admitted liquid to the interface several times during an experiment. This can be either due to the need for a surface / interface cleaning process or in order to apply a specific and desired condition to the system. This can be established in a laboratory simply by manually exchanging vials with the solutions. However, when the amount of samples is high and time requirements are crucial, the use of an automatic device becomes necessary. A fully automatic SPR biosensor is commercially available. However, it is bulky (non-portable) and quite expensive [6]. Use of commercial autosamplers [7] or multi-valve set-ups [8] are also possible, but are also no low cost alternatives.

A carousel-type auto-sampler, largely constructed with low cost, easy to find and simple components and easily adapted for SPR biosensor platforms is presented. Two stepper motors were installed: one to generate the vertical movement of the syringe extractor, the other to rotate the carousel containing the sample vials. Also included is a peristaltic pump, which admits the sample liquid to the microfluidic cell of the SPR sensor. All components are controlled by a micro-controller attached to a PC. An investigation of the mutual diffusion coefficient of an ethanol-water solution has been used to characterize functionality of our set-up.

2. THEORETICAL ASPECTS

The SPR phenomenon is known since 1902 through experimental and theoretical investigations, and related to appearance of unusual optical properties of very thin metal films [5]. However, only after Otto's and Kretschmann's seminal work in 1968, the SPR phenomenon could be used practically for sensor applications [5].

ISBN 978-963-88410-0-1

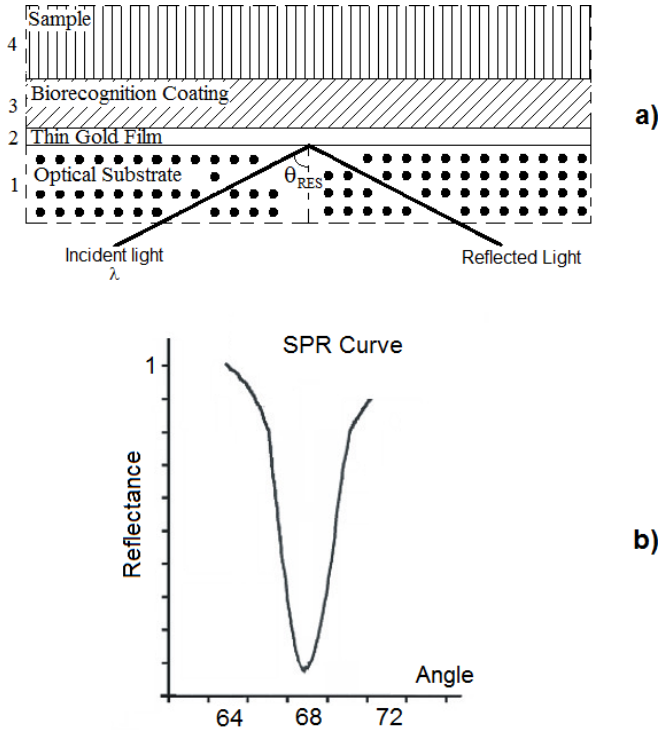

Fig. 1. (a) The multilayer SPR biosensor structure showing all elements, where the SPR curve (b) results from resonant absorption of an incoming light beam at the dielectric-metal interface under specific boundary conditions.

Surface plasmons are longitudinal electromagnetic charge density oscillations at the metal-dielectric interface. They are excited by a resonant interaction of incoming photons with the plasma oscillations of the thin metal film and are highly sensitive to interfacial variations of the optical boundary conditions. We refer the reader to [5,9] for more details about the physical aspects of the SPR phenomenon.

For an optical excitation at a metal-dielectric interface, composed of the thin gold film layer, the dielectric, and the sampling layer, the total internal reflection (TIR) and energy and momentum conservation conditions are required. Therefore, when a p-polarized light beam hits the metal surface, for a certain range of incidence angle and light wavelength, an evanescent electromagnetic field is partially or totally absorbed at the metal surface, due to an excitation of the so-called surface plasmon-polariton. This condition corresponds to a matching of energy and momentum of both waves. It is clearly identified by a dip in the reflectance curve, as is depicted in Fig. 1b [5,9]. Furthermore, both Otto's and Kretschmann's approaches showed the necessity of a coupling element, which is necessary for the propagation constants matching of the metal and dielectric.

The first use of the SPR phenomenon for sensor applications was initially presented in 1983 for detection of halogenated gases [10] and quickly extended to the detection of protein layer systems in biochemistry. Due to its characteristics, like high sensibility and selectivity, non-label method and fast response-time, SPR bio-sensors became the dominant analytical tool for the study of bio-molecular interactions and eventually was applied to other areas as environment monitoring, quality control in the food and drink industries and pathogens, as well as bio- and chemical warfare detection [5].

The typical SPR biosensor set-up is shown in Fig. 1a. It comprises basically a four layer configuration [9]: an optical coupling substrate (prism, waveguide, optical fibre, diffraction grating), a thin metal (gold is preferred due to its chemical stability) film, a bio-recognition coating which interacts with target molecules present in the fluidic sample and provides a horizontal displacement of the resonance angle or wavelength (Fig. 1b). This change is monitored usually by two optical detection methods: either using a fixed photo-detector array in the angular interrogation mode with varying input angles (AIM), or an optical spectrometer in the wavelength interrogation mode (WIM), employing a broadband input beam at fixed input angle [7,8,9]. The desired reflectivity and parameters of interest (refractive index, concentration or mass of the analyte) are calculated by a digital processor, like a DSP which are normally attached to a micro-controller, or a PC [11,12]. Nowadays, the majority of the commercial instruments employ the angular interrogation method, originally based on the Kretschmann's configuration in a prism-based coupling set-up [9].

3. PROPOSED AUTOSAMPLER SET-UP

3.1. Alternatives to analyte sampling in a SPR biosensor

As mentioned before, the non-automatic sampling of analytes is used in experimental procedures with SPR bio-sensors for applications where the number of target substances is low and time is not restrictive. Otherwise, the use of process automation is required.

An immediate solution is the use of a commercial auto-sampler [7]. Available technical configurations are generally based on a robotic arm, or a carousel type set-up. In the former case, a high quantity of analyte vials is mapped and a 3-dimensional movement is performed by the robotic system in order to extract from, or put back analyte solutions into a selected vial. Obviously, this approach suffers from high complexity for system control and processing, along with relatively large dimensions. The latter approach features smaller volume and more simple control and processing hardware, the quantity of vials is smaller than robotic approach. Again, both alternatives are expensive.

Another possibility is the use of a multi-valve set-up. It is less expensive than a commercial auto-sampler, but it has a limited quantity of inputs (around 10) [8], which provides a cost per input almost similar or higher than commercial autosamplers.

The third alternative involves a SPR biosensor, which is totally automatic. It has been manufactured by a Swedish factory since 1990, and became in recent years a reference analytical tool for study of bio-molecular interactions and chemical kinetics investigation in medicine, food and environmental analysis [6]. Nevertheless, it is bulky and very expensive, which restricts its utilization to large companies and financially well equipped universities.

3.2. Description of the autosampler

Low cost, easy to find and simple elements have been used to implement the here presented auto-sampler. Attached to a SPR biosensor platform, the overall system has been applied to the determination of solute diffusivity in water solutions, which will be more described in more detail in the next section.

Fig. 2 illustrates the autosampler set-up with its three elements, i.e.: a carrousel-based sample holder; a Z-drive with an analytical probe; and an analyte pumping mechanism.

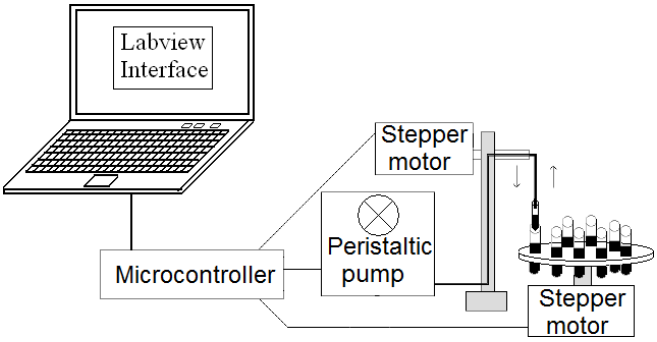

Fig. 2. The autosampler set-up with all elements connected to a microcontroller and a PC, which is used for programming and user interface.

The carousel contains 20 openings for sample vials. The rotating movement is provided by the stepper motor 1, which has unipolar operation at full step and a step angle of 7.5^{o} or, equivalently, a total of 48 rotations per cycle. At each coil, one Darlington transistor and one optical coupler have been used to command the stepper motor and for power isolation. The ADUC 842 micro-controller has been used to generate the unipolar operation driving signals for stepper motor 1. Soft rotating is necessary for correct samples identification by an attached bar-code device. The unit operates at a supply voltage of 10 V and a current of 400 mA.

The used Z-drive consists of a vertical arm attached to an analytical probe. Its function is to extract the analyte liquid sample from the selected vial through operation of stepper motor 2. It has identical electrical characteristics as stepper motor 1, using both driving and power isolation circuits. The vertical movement of 10 cm, approximately, is adequate for our set-up.

A commercial peristaltic pump conveys the analytes into the microfluidic channel of the SPR bio-sensor. It is fully controlled by software, using a RS-232 protocol. A word of 3 ASCII numbers must be sent, at 9600 baud, to drive the peristaltic pump.

Furthermore, a friendly-user interface has been developed, using Java programming. Fig. 3 depicts a screenshot of the Java interface, where the user enters with the number of analyte vials required for experiment. Therefore the specific analytes can be chosen from database and the circulation time by microfluidic channel can be configured. With these instrumental settings, the operation procedures are automatic and the experiment can be initiated.

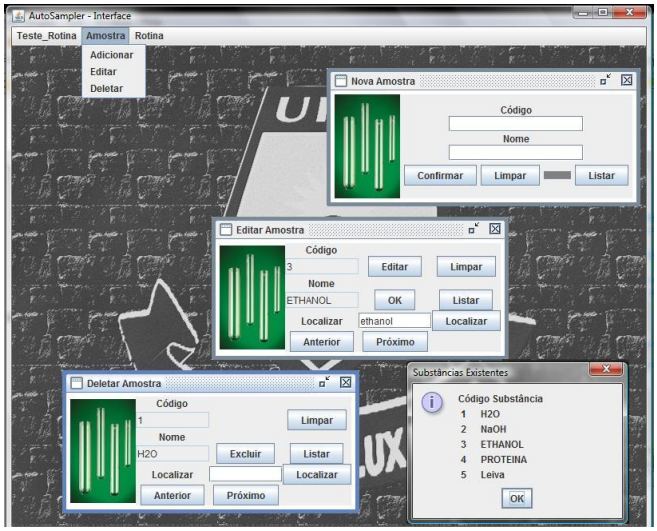

Fig. 3. A screenshot of the Java interface.

3.3. Autosampler operation

When a sample is required by a user, using the Java interface, the micro-controller sends the ASCII word to switch-on on the stepper motor 1. The bar-code device searches for the desired sample. When identified, the stepper motor 2 is turned on and the analytical probe is vertically displaced, which starts the extraction process. The peristaltic pump is also turned on. After the required time for sample extraction, the system is reinitialized for a new sample. Depending on the application, cleaning of the probe and micro-fluidic channel can be performed after these steps.

For micro-fluidic channel cleaning, normally is a highly oxidizing solvent with low molar concentration, like sodium hypochloride is used. However, this cleaning solution can change, according to the actual protocol. For syringe probe cleaning a specially designed vial can be employed.

4. EXPERIMENTAL RESULTS

4.1. Calculus of the molecular diffusivity at the ethanol-water solution

In order to illustrate the practical application of the proposed system, an experiment for determination of the mutual molecular diffusion coefficient of ethanol in water has been performed. Its severe time restriction and the utilization of three different substances make this experiment a reasonable test for the present auto-sampler, because a manual fluidics handling method is somewhat tricky.

Molecular diffusivity is a relevant subject for many physico-chemical and physiological processes, as well as for molecular weight determination [13]. Diffusion coefficients D of solutes in aqueous solution vary widely over orders of magnitude and are affected by the chemical composition of the solvent, temperature and solute concentrations. For many materials, reliable quantitative data are not available, since measurements are technically demanding.

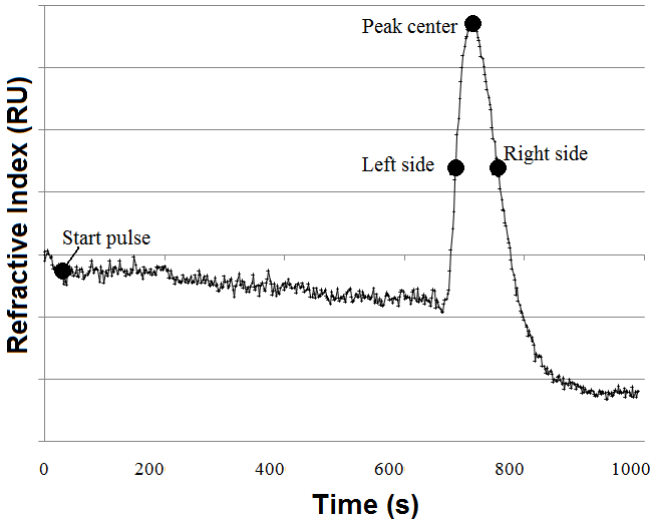

Fig. 4. The sensorgram used for the calculation of the molar diffusivity at the 5% ethanol-water solution.

The present label-free method relies on monitoring the dynamic evolution of the refractive index of an aqueous solution, while transported to and passing through microfluidic cell. The readers are referred to [13], in order to find more details about the employed methodology and other theoretical and experimental considerations.

Through the temporal data evolution, shown in the sensorgram (graph which represents the temporal evolution of the analyte refractive index or concentration) of Fig. 4, the diffusivity D can be determined using the following relation:

$$D \approx \frac{R_0^2 \cdot t}{24 \cdot \tau_B^2} \qquad (1)$$

where R_0 is radius of the tube used to transport analyte, t is the dispersive transport time taken from start pulse until peak center and τ_B is the line broadening at FWHM (full width at half maximum), as taken by the distance between the left side point until right side one.

4.2. Experimental results and discussion

A sketch of the experimental set-up is shown in Fig. 5, with the detailed flow cell, with the diffusive and convective involved processes, of the SPR biosensor and the attached auto-sampler. An initial 5% ethanol-water solution pulse, revealing a sharp 3 seconds long concentration profile, has been injected at the tube entrance and transported to the micro-fluidic sensing channel through a long (3.4m). By diffusion of the ethanol molecules into the surrounding pure water environment during the transport time, the sharp pulse broadens up significantly, according to (1). The temporal evolution of the incoming analytes refractive indices (Fig. 4) shows three different states for two different concentrations at the ethanol-water solutions.

In order to perform the described tasks, the following operations must be executed by our system:

1. Checking the default state of the auto-sampler, i.e., actuating devices turned off;

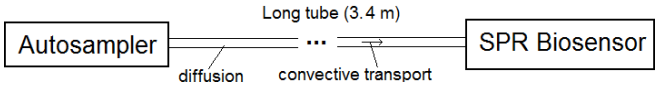

Fig. 5. The experimental sketch used, where the diffusion and convective effects along the tube are detailed.

2. Turning-on stepper motor 1 for carousel movement;
3. Finding the vial containing the 1% ethanol-water solution, utilizing the bar-code device;
4. After sample identification, the analytical probe must be vertically displaced through stepper motor 2, which starts the extraction process;
5. The peristaltic pump is then turned on, according the specified time;
6. After the desired time, the peristaltic pump is turned off and the analytical probe goes up;
7. Restart the procedures for admission of a new analyte.

Although the operation of the auto-sampler is relatively simple and provides good results, special care has been taken, when the peristaltic pump is turned on. Arising of air bubbles has been noted when temporal differences from that specified in Fig. 4 occur. It has been corrected, using a closed-loop control algorithm which can be implemented on the microcontroller.

Using higher capacity processors like DSP or FPGA, to control and process the optical components of a SPR bio-sensor platform, including the auto-sampler features, appears as a good possibility to realize a portable, small and fully controllable system, at reasonable cost.

5. CONCLUSIONS

A low cost auto-sampler applied in connection with a SPR biosensor platform is presented. A brief theoretical view of the SPR phenomenon and a description of the components and the operation of the proposed set-up are also presented. Our set-up has been tested by molar diffusivity determination of ethanol in water, where sharp initial concentration profiles are required. The experiment constitutes a good example to prove the capabilities of our arrangement. The proposed auto-sampler relies on simple, low-cost hardware elements, attached to a user-friendly interface. When connected to a SPR biosensor platform, it becomes a good alternative to other analytical tools already proposed in the literature.

ACKNOWLEDGMENTS

The authors would like to thank CNPq, FAPESQ and CETENE for the research grant, financial support and study fellowship.

REFERENCES

[1] Myszka, D.G., “Kinetic analysis of macromolecular interactions using surface plasmon resonance biosensors”, *Current Opinion in Biotechnology*, vol.8, pp. 50-57, February 1997.

[2] Bokken, G.C.A.M., Corbee R.J., van Knapen F. and Bergwerff A.A., "Immunochemical detection of *Salmonella* group B,D and E using a optical surface plasmon resonance biosensor", *Microbiology Letters,* vol. 222, pp. 75-82, January 2006.

[3] Lin, W.B., Lacroix, M., Chovelon, J.M., Jaffrezic-Renault, N. and Gagnaire, H., "Development of a fiber-optic sensor based on surface plasmon resonance on silver film for monitoring aqueous media", *Sensors and Actuators B, Chemical*, pp. 203-209, May 2001.

[4] Myszka, D.G. and Rich, R. L., "Implementing surface plasmon resonance biosensors in drug discovery", *Pharmaceutical Science & Technology Today*, vol. 3, pp. 310-317, Sept. 2000.

[5] Homola, J. "Surface Plasmon Resonance Based Sensors", *Springer*, New York, 2006.

[6] Biacore Life Sciences, website: http://www.biacore.com.

[7] HT800L HPLC Autosampler, website: http://www.labhut.com/products/autosamplers/autosampler_ht800l.php}

[8] Tecan Systems. Cavro Smart Valve, website: http://tecan.com/platform/apps/product/index.asp?MenuID=1291&ID=761&Menu=1&Item=21.3.1.6

[9] Maier, S. A. "Plasmonics: Fundamentals and Applications", Springer, New York, 2007.

[10] Liedberg, B., Nylander, C. and Lundström, I., "Surface Plasmon Resonance for Gas Detection and Biosensing", *Sensors and Actuators*, vol. 14, pp. 299—304, 1983.

[11] Naimushin, A.N., Soelberg, S.D., Bartholomew, D.U. Bartholomew, Elkind, J.L. and Furlong, C.E., "A portable Surface Plasmon Resonance (SPR) Sensor System with Temperature Regulation", *Sensors and Actuators B*, vol. 96, pp. 253—260, 2003.

[12] Thirstrup, C., Zong, W., Borre, M., Neff, H., Pedersen, H.C. and Holzhueter, G. "Diffractice optical coupling element for surface plasmon resonance sensors", *Sensors and Actuators B*, vol.100, pp. 298-308, May 2004.

[13] Neff, H., Lima, A.M.N. Lima, Loureiro, F.C.C.L. and Almeida, L.A.L., "Transient response analysis and modeling of near wall flow conditions in a micro channel: evidence of slip flow", Microfluidics & Nanofluidics 3, (2007) 591–602.