Features and application of a microcontroller-driven autosampler applied to a surface plasmon resonance biosensor platform

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Abstract: A microcontroller-driven autosampler, based on simple and low cost components, has been developed and connected to a surface plasmon resonance (SPR) sensor. The device has been applied for determination of the mutual diffusion coefficient of the protein bovine serum albumin (BSA) in aqueous solution. The theoretical background of SPR sensors is briefly outlined and technical alternatives typically used for automatic sample manipulation described. In general, their large device size, difficult handling and high costs are severe disadvantages. Here, a rotating carousel-type storage apparatus for up to 20 sample vials, a vertical Z-drive needle sampler to take out analyte solutions from the vials has been combined with a peristaltic pumping system. All the actuating devices, as stepper motors for the carousel and z-drive, and the pump are controlled by a microcontroller, while an attached bar-code device is used for vial / sample identification. The system is easily programmable via a user friendly interface.

Keywords: autosampler, surface plasmon resonance, biosensor.

1. INTRODUCTION

Biosensors, based on surface plasmon resonance (SPR) phenomenon, are analytical tools and commonly used for monitoring of strength and kinetics of biomolecular interactions. They already substitute conventional methods due to their fast response, high sensitivity and selectivity, ease of integration, portability and absence of labels [1]. Basically, SPR biosensors integrate an optical apparatus with a microfluidic system. The liquid sample (or analyte) is admitted to the sensor by a pump. Typically, among others, refractive output signal or film thickness variations are obtained and processed by appropriately designed hardware.

In most applications, variations of admitted solutions and analyte changes are required, either due to a prior surface cleaning process, or to generate specific conditions for surface functionalization. This is essential for the detection of immune reactions or, likewise, antibody-antigen interactions. Manual sample handling by change of vials has been frequently used to vary admitted analyte solutions, which is acceptable for simple tasks. However, when the amount of vials is high and time is an issue, the use of automatic sample control (the so called auto-sampler) is essential. Fully automatically operating SPR instruments [2] are already available, and a large fraction of biochemical publications, which commonly comprise the SPR biosensing principle, has been based on this device [3]. However, they are quite bulky, very expensive and thus not suitable for on-line and portable applications. Although multi-valve set-ups have a minor volume, they are still expensive and have a limited number of input channels [4].

Here, an autosampler apparatus has been developed and attached to a SPREETA evaluation kit [5]. It is considered as a suited alternative to the aforementioned technical approaches. The proposed auto-sampler utilizes a microcontroller, and is connected to a PC. It comprises all necessary control signals to the remaining parts of the apparatus. Simple and relatively low-cost actuators (two stepper motors and a peristaltic pump) are used for the vertical movement of a needle solution sampler, mounted onto a Z-drive, and for the rotating carousel-type sample holder of the vials, and transport of the analyte to the microfluidic cell of the SPR biosensor. A user-friendly interface, using Java programming, has been developed in order to control the autosampler. Moreover, a bar code device is also integrated for vial/analyte identification. Eventually, function of the experimental laboratory set-up is demonstrated by measurements of the protein diffusivity in water.

2. THEORY

2.1. Surface plasmon resonance biosensing

Surface plasmons are collective longitudinal (electronic) charge density oscillations at the metal-dielectric interface. Their dispersion relation is highly sensitive to the optical

boundary conditions. With total internal reflection (TIR) and under energy and momentum conservation conditions, when a p-polarized light beam hits the metal surface (Fig. 1a)-and for a certain range of incidence angle and light wavelength- the radiation is partially or totally absorbed at metal surface by resonant excitation of a surface plasmon at the metal-dielectric interface. This is called the surface plasmon resonance phenomenon. A dip in the reflectance (Fig. 1b) curve thus can be observed by a suitable light detector. All, optical coupling prisms, waveguides, optical fibers or diffraction grating support the interaction between surface plasmons and incident photons [6].

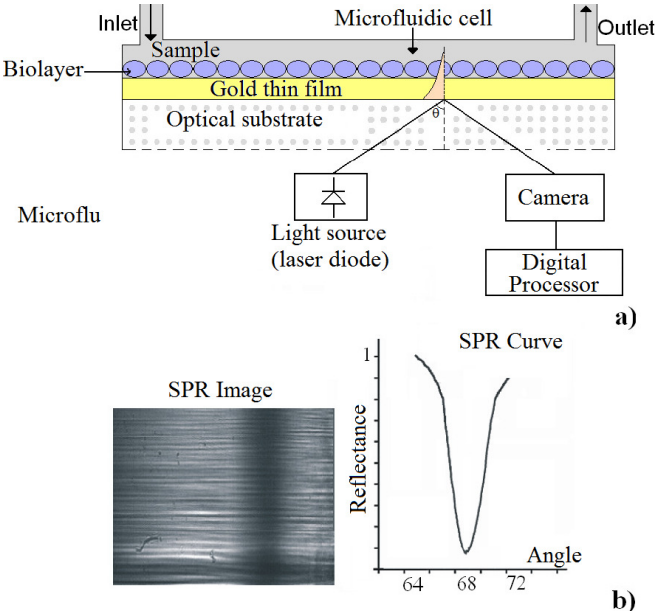

Fig. 1. SPR biosensor set-up (a), with all components and the (b) SPR image detected by camera and the SPR curve obtained from signal processing.

Although the SPR phenomenon has been first observed in 1900 with Wood's experiments, only after Otto and Kretschmann's studies (1968) and the proposed experimental set-up, a practical solution to excite accurately surface plasmon oscillations at a metal-dielectric interface was provided [1,6]. The first SPR sensor was proposed in 1983 for detection of halogenated gases [7]. Due to its unusually high surface sensitivity and selectivity, and absence of need for radioactive, fluorescence and other labeling methods, the SPR sensing principle found many applications in biochemical research. Since the first proposal, applications like microorganism monitoring in water [8], bio-warfare and anti-terror detection agents [9], and biomolecular interaction studies [10] have attracted attention.

A SPR biosensor, based on Kretschmann's configuration (as is frequently used in the majority of the articles related to the SPR phenomenon [3]), is composed by the layers arrangement, shown in Fig. 1. A glass or a polymeric prism is coated with a thin gold film, where its thickness (50 nm thick) is much smaller than the input wavelength. Although not shown in Fig. 1, there is frequently a very thin addition chromium layer (2-4 nm thick) needed, to reliably attach the gold layer to the substrate.

When an aqueous solution circulates through the microfluidic cell, the analyte of interest may bind to a functionalized protein biolayer beneath and the refractive index, which is being monitored, varies.

Consequently, the resonance angle and associated reflectance minimum varies, and thus is easily detected by the camera. The resulting electric signal is fed to a quite complex signal conditioning and processing system, where the output can be, e.g., a reflectance curve (Fig. 1), or a sensorgram. This is a graphic representation that exhibits the analyte concentration as refractive index variation as a function of time. Generally, the reflected light can be monitored by either a fixed photo-detector array in the angular interrogation mode with varying input angles (AIM), or by use of an optical spectrometer in the wavelength interrogation mode (WIM), employing a broadband input beam at fixed input angle [1,6]. The processing core is normally a DSP, integrated to a RISC or ARM microcontroller [1]. Furthermore, the performance of a SPR sensor is crucially affected by environmental influences, and recent theoretical and experimental investigations have shown the importance of temperature control and monitoring system [11].

2.2. Alternatives for analytes automatic sampling for SPR biosensors

Due to its simplicity, the non-automatic sampling of analytes is generally used in experimental procedures with SPR biosensors; however it is appropriate method only when the number of analytes is low and the time is not restrictive. When a cleaning process or the need of more steps to the experiment is necessary, the quantity of samples increases and an automatic method is beneficial. An immediate solution is the use of a commercial auto-sampler. The available configurations are based either on a robotic arm or a carousel set-up. In the former case, a large quantity of analyte vials is handled, and a 3-dimensional displacement operation needs to be accomplished by the robotic system, in order to extract or supply the analyte from / into the correspondent vials. Obviously, this approach is bulky and needs complex associated electronics for signal control and processing. On the other hand, although the latter case has minor volume and more simple control and processing hardware, the number of vials will be much smaller than in the robotic approach. Both alternatives are very expensive and are applied to different fields, like chromatography and biotechnology.

Another alternative is the multi-valve set-up, which is less expensive than commercial autosampler; however it has a limited quantity of inputs (around 10) [4], which leads to cost per input almost similar or higher than commercial auto-samplers.

The third alternative refers to a totally automatic SPR biosensor, which has been manufactured by a Swedish factory since 1990 and is already a good reference for laboratorial analysis in medicine, food and environmental analysis, substituting conventional methods [2]. However, it is bulky and very expensive, being used commonly for routine operation in large laboratories and universities.

3. PROPOSED SET-UP

The SPR biosensor platform connected to the proposed autosampler is shown in Fig. 2. The SPREETA evaluation kit operates exclusively in the angular interrogation mode. The sensor element is integrated with a LED light source with emission at a wavelength of 830 nm, where a polarizer, the sensing surface, a reflecting mirror and a one dimensional array light detector are contained into a single, molded polymer structure. The compact device of 3x4x1 cm is externally controlled by a DSP-based system and a friendly-user interface shows the resulting graphs and permits also configure the processing algorithms and other conditions used in the experiments.

A carousel-type autosampler (Fig. 2) was implemented, where the carousel contains the sample vials, which are rotated by a stepper motor when an extraction process occurs. The analytical probe is attached to a Z-drive, where another stepper motor permits the vertical movement of the probe. A peristaltic pump with Teflon tubes transports the analyte by microfluidic channel of the SPR biosensor. For all these actuating devices the microcontroller (ADUC842) controls the signals and communicates with a PC for programming and configuration. The 8-bit architecture of the microcontroller was adequate for our experiments, which also diminished the total cost of our device.

Furthermore, a USB bar-code device has been also included for samples identification and its programming is made also by microcontroller.

Below, the characteristics of these subsystems and some discussions about their choices are presented.

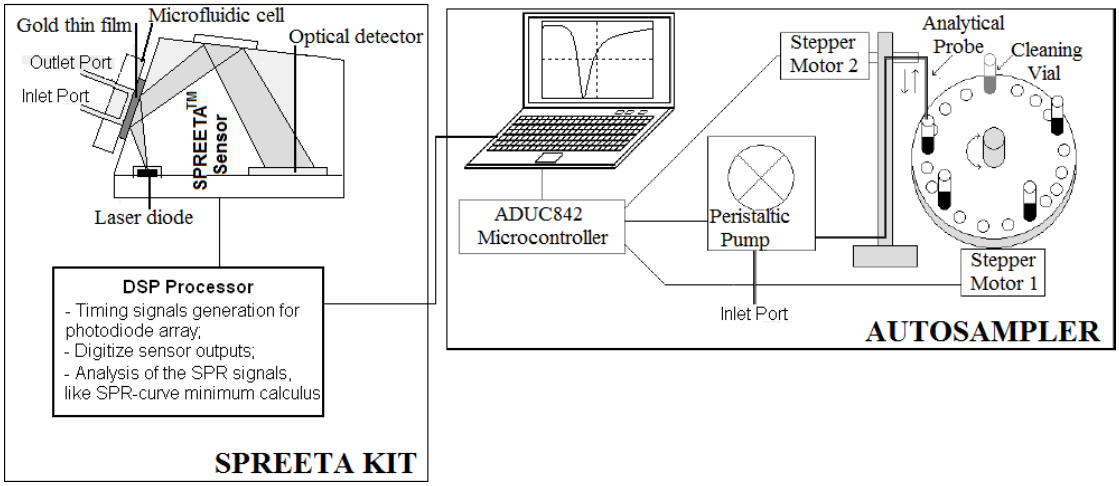

Fig. 2 - The SPR biosensor platform connected to our autosampler, where the details of the SPREETA and the autosampler have been illustrated.

3.1. Carousel

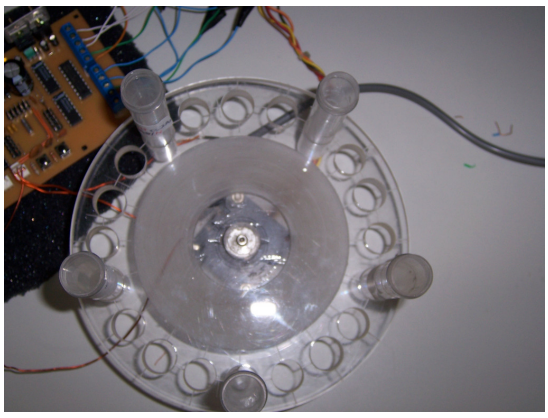

Fig. 3 – The carousel set-up with 20 vials, being commanded by stepper motor 1

Fig. 3 illustrates the carrousel set-up, where there are 20 holes for samples vials. The rotating movement is made by single stepper motor (SM1), which has a unipolar operation and a step angle of 7.5°, i.e., a total of 48 rotations per cycle. It is found commonly into printers and the simplicity and low-cost were mandatory for its choice.

A Darlington transistor-based integrated circuit (IC) has been used for driving at full step SM1, where the control signals were furnished by microcontroller. For an input voltage of 10 V, approximately, a soft and adequate rotating of the carousel has been noted with a consumed current of 400 mA. A soft rotating is necessary to provide correct sample identification by bar-code device.

One of the vials contains a cleaning solution for the microfluidic channel, where normally is used sodium hypochlorite at low molar concentration [12]. However, this cleaning solution can change according the samples over utilization.

3.2. Z-drive

Fig. 4 illustrates a vertical arm with an attached analytical probe, which extract the samples from vials. Another stepper motor (SM2), with similar characteristics to the SM1, moves vertically the probe. Its driving is also made by a Darlington transistor IC, where a vertical displacement of 10 cm was reached, with a consumed current of approximately 700 mA. The torque and power of SM2 is a little higher than SM1, however is also normally found into printers.

3.3. Analyte pumping

A peristaltic pump with Teflon tubes (Fig. 5) has been used to transport the analyte by microfluidic channel of the SPR biosensor. All its functions can be controlled by microcontroller using a RS-232 communication protocol, where three ASCII characters must be sent, at 9600 baud, in order to make any command, like turn-on or turn-off.

Fig. 4 – Z-drive set-up with analytical probe and stepper motor 2

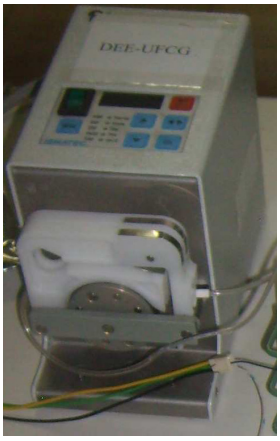

Fig. 5 – Illustration of the peristaltic pump and Teflon tubes used to transport analyte by microfluidic channel of the SPR biosensor

3.4. A friendly-user interface

A java code, using the open-source Eclipse platform [13], has been developed to create a friendly-user interface for our system. The fluxogram, illustrated in the Fig. 6, indicates the procedures to configure the system.

Firstly, a screen (Fig. 7) is shown where the user enters with the number of analytes that will be used in the experiment. After that information, a database is accessible and the user can to choose the desired analyte. For each analyte, there is a respective code that will be read by bar-code device. It is also possible to indicate the pumping time of the analyte. After the adjusting the instrument settings, the procedures are automatic and the experiment takes place.

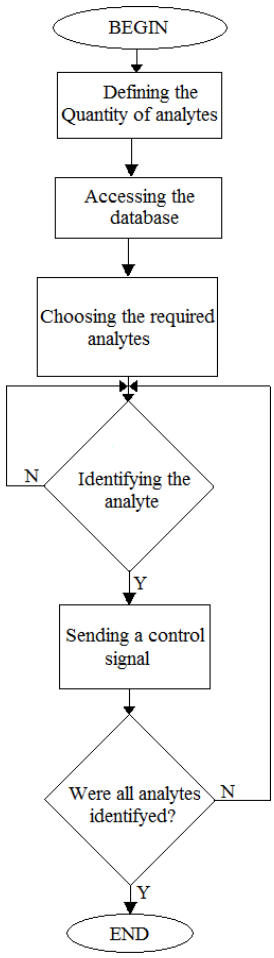

Fig. 6 –Java code fluxogram used for implementing user-friendly interface

Fig. 7 – A screenshot of the friendly-user interface

3.4. Autosampler operating

When a sample is required by a user, the microcontroller send control signals to turning on the stepper motor 1 and also starting the bar-code device. With the sample has been correctly identified, the stepper motor 2 is turned on and the analytical probe is displaced, which starts the extraction process. The protocol continues with the driving of the peristaltic pump and after the required time of sample extraction, the process is reseted and the system is ready for

a new sample. Depending on the application, the cleaning steps can be executed at this moment.

4. EXPERIMENTAL SET-UP AND DISCUSSIONS

For verification of the proposed system, the solute diffusivity calculation procedure based on SPR spectrometry, which was developed by our group [14], has been used. This application was chosen by its timing restrictions and by associated difficulty to be manually implemented. A Bovin Serum Albumin (BSA)-water solution was chosen, where BSA is a protein largely used as a fixing layer in SPR experiments.

Here, two aqueous solutions of BSA, with 1% and 10%, were used to calculate the diffusion coefficient. More information about the applied method can be found in [13]. Fig. 8 illustrates the sensorgram used for the experiment, i.e., the tasks that must be executed:

1. Adsorption of the 1% BSA-water solution around 5 minutes;
2. Adsorption of the 10% BSA-water solution around 5 seconds;
3. Adsorption of the 1% BSA-water solution around 5 minutes;
4. End of the experiment.

In order to make the described procedures, the following operations were made by our system:

1. Check the default state of the autosampler, i.e., actuating devices turned off;
2. Movement of the carousel, using stepper motor 1, to find the vial containing the 1% BSA-water solution by using bar-code device;
3. After sample identification, the analytical probe must be vertically displaced by stepper motor 2, thus starting the extraction process;
4. The peristaltic pump is turned on and pumps the analyte along the specified time (Fig. 8);
5. After the desired time, the peristaltic pump is turned off and the analytical probe goes up;
6. The system is reseted and the adsorption of a new analyte can be done.

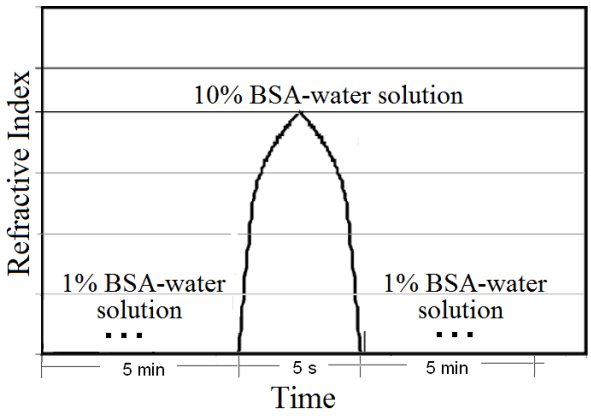

Fig. 8 – Sensorgram illustrating the tasks executed by our system

The experiments have indicated a good agreement with manual procedures already done, which indicates our system is a good alternative for biosensor experiments. More experiments have been developed to test the proposed set-up.

5. CONCLUSION

A SPR biosensor platform using a microcontroller-driven autosampler was presented. A description of all components of the proposed set-up and the calculation of the diffusion coefficient at a BSA-water solution were also illustrated. The proposed platform is compact, has a simple and a relatively low-cost hardware, a simple programming and a friendly-user interface, which becomes a good alternative for portable and on-line applications.

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