Optical sensor for the quantification of monoclonal antibodies

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An optical sensor for the quantification of a monoclonal antibody (MoAb) in solution by the reflectometry technique is shown in this work. A silicon wafer was chosen as a reflecting surface. An amount of AB blood group antibody (anti-AB) is added to the sample when the pseudo-Brewster angle of incidence has been fixed. The reflected laser intensity is registered in real time as the protein is being adsorbed onto the wafer. The mathematical analysis of the results verifies that the antibody adsorption follows Langmuir's kinetics. From the curve analysis, the parameters related to the anti-AB concentration are extracted and the calibration curve is constructed. This curve allows us to obtain the desired commercial antiserum quantification.

Keywords: Laser reflectometry; immunosensor; Langmuir adsorption.

Se presenta un sensor óptico basado en la técnica de reflectometría láser, para la cuantificación de anticuerpos monoclonales (MoAb) anti-AB en solución. Se eligió como superficie reflectante una oblea de silicio. Fijado el ángulo de pseudo Brewster, se agrega una cantidad de anti-AB. Se registra la intensidad láser reflejada en función del tiempo a medida que la proteína es adsorbida. El análisis matemático de los resultados verifica que la adsorción de anticuerpos sigue la cinética de Langmuir. Se calculan parámetros relacionados con la concentración de anti-AB con los que se construye una curva de calibración que permite cuantificar anticuerpos monoclonales en solución.

Descriptores: Reflectometría láser; inmunosenor; adsorción de Langmuir.

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1. Introduction

Antibodies [1] (Ab) are macromolecules that recognize foreign substances (Antigen Ag) as invaders in an organism and stick to them through their epitopes in many ways in order to help eliminate the Ag. In contrast with conventional antiserum, the monoclonal antibodies (MoAb) are biological reagents with homogeneous activity. They are prepared from monoclonal antibodies secreted by cellular lines of mouse hybridoma, through a careful production process. MoAb are generally used in the recognition and quantification of biological substances present in very small amounts (hormones, enzymes), Ag identification, blood group determination, oncology, organ transplants, etc.

MoAb can be characterized by its specificity and affinity. Affinity may be expressed as the equilibrium association constant (K). Several techniques are available for determining the equilibrium constant of the Ag-Ab interaction. Three methods are widely used (dialysis, precipitation with ammonium sulfate, and ultra-centrifugation) [2], which are based on the separation of bound and free reactants. Values of K can also be obtained by the biosensor technique, which is the most reliable way to record binding kinetics [3], as it happens with the surface plasmon resonance technique (SPR) [4,5]. The laser reflectometry technique (null ellipsometry technique) [6,7], as well as the surface plasmon resonance technique, give us information about the kinetics of the interactions, stoichiometry of molecular binding and the concentration of molecules in a solution, and also offer detailed and accurate determinations of real-time adsorption kinetics of proteins without labeling [8,9].

In this work, we present an optical sensor with the characteristics mentioned above, thus obtaining a calibration curve which not only makes the determination of the association and dissociation constants of MoAb to the silicon wafer possible, but its affinity constant as well.

2. Theory

The simplest superficial adsorption model is the Langmuir's classical model, which is based on the supposition that all the sites of adsorption are equivalent, and that the binding capacity of the molecules to bind is independent of whether the neighboring sites are occupied or not. The surface coating θ is usually expressed as the relationship between the number of occupied adsorption sites and the number of available adsorption sites. The resulting velocity at which the coating is performed, is provided by [10]:

$$\frac{d\theta}{dt} = k_a \cdot C \cdot (1-\theta) - k_d.\theta, \tag{1}$$

where k_a and k_d are the adsorption and desorption constants, and C is the molecule concentration. The solution of this equation is:

$$\theta(t) = \frac{k_a \cdot C}{k_a \cdot C + k_d}\left[1 - \exp^{-(k_a \cdot C + k_d)t}\right], \tag{2}$$

which corresponds to the Langmuir limit (small surface/ volume ratio) [11]. It is always possible to work experimentally in the vicinity of this limiting situation by choosing sufficiently low bulk concentrations.

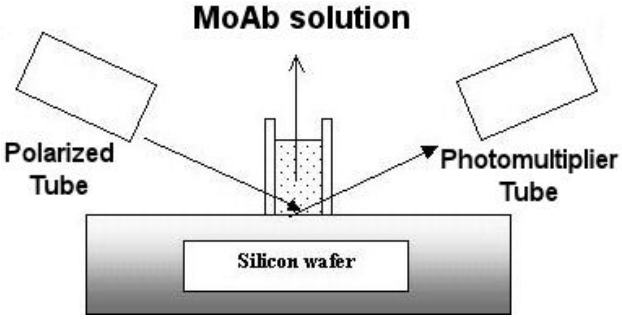

FIGURE 1. Schematic representation of the setup.

3. Materials

Monoclonal Anti-AB: IgM antibodies from Wiener Lab. (Cod: 1443153). The Ab concentration was determined by the Gornald method. Ab was dissolved in a physiological solution to obtain the desired concentration.

Experimental setup: Measurements were carried out with an Ellipsometer (Rudolf Instruments) with a polarized laser beam (630 nm) and a photomultiplier tube (Fig. 1). The cell was made of glass and acrylic pressed against a piece of silicon wafer as a base (liquid/solid interface area of 25 mm^2). The polarized laser beam is reflected by the liquid-solid interface. A photomultiplier tube detects the reflected laser intensity. This signal is digitized and stored in a computer file.

4. Method

Reflectometry is one of the methods used for the detection and quantification of biomolecules in solution. It is based on measurements of reflectance changes, owing to superficial adsorption of the biomolecules present in the solution [6,7]. When polarized light (linear polarization parallel to the incidence plane) is reflected on a solid surface (silicon wafer), the reflectance has a minimum value at the pseudo-Brewster angle (θ_P). As the proteins adhere to the surface, the reflected intensity (at θ_P) increases. Therefore, the temporal register of reflected intensity gives a binding kinetics curve

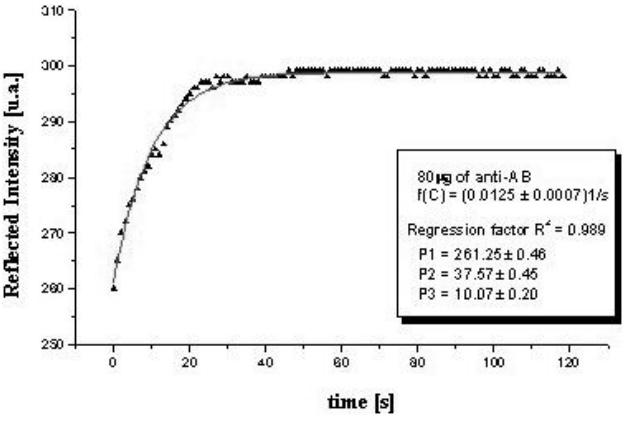

FIGURE 2. Reflected intensity vs. time for 80μg of anti-AB monoclonal antibody into the cell.

in real-time. In this work, the measured angle of pseudo-Brewster for the silicon/physiologic solution interface was θ_P = (64,2 $\pm$ 0,4)°. Fixing this incidence angle, the solution containing MoAb was poured into the cell. The intensity of the reflected light changed as the antibodies adhered to the silicon surface. About 1 μg/cm^2 of adsorbed protein can be detected because of the significant difference between the refractive index of silicon wafer and that of the adhered organic material [8].The intensity was registered (Fig. 2) for 30 seconds before and 120 seconds after the MoAb addition for different MoAb concentrations. The equation that describes the reflected intensity in the first steps of the antibody adhesion to the silicon wafer according to Langmuir's classical superficial adsorption model is:

$$I(t) = A + \frac{k_a \cdot C \cdot I_{max}}{k_a \cdot C + k_d} \left[1 - e^{-[k_a \cdot C + K_d] \cdot t}\right], \quad (3)$$

where A is a constant depending on the instrumental setup and I(t) is the reflected intensity related to the adhesion of proteins on the silicon wafer. I_{max} is the maximum value of reflected intensity. The kinetic constant of association k_a and the kinetic constant of dissociation k_d can be obtained from this curve of intensity versus time.

The graph of the reflected intensity as a time function can be fitted as

$$I(t) = P_1 + P_2 \cdot \left[1 - e^{\frac{-t}{P_3}}\right], \quad (4)$$

where

$$P_1{=}A; \;\; P_2{=}\frac{k_a \cdot C \cdot I_{max}}{k_a \cdot C + k_d} \;\text{ and }\; P_3{=}\frac{1}{[k_a \cdot C{+}k_d]}. \quad (5)$$

Then $P_2/P_3 = k_a C I_{max}$, and considering $t \to \infty$, the Eq. (3) results in:

$$I(t) = P_1 + P_2 \cdot \left[1 - \left(1 - \frac{t}{P_3}\right)\right] = P_q - \frac{P_2}{P_3} \cdot t \quad (6)$$

When we consider the same equation when t $\to \infty$ the intensity tends to I_{max}, we obtain that: I_{max}= P_1+ P_2.

From these expressions, we obtain:

$$\frac{P2}{P3(P1 + P2)} = k_a C = f(C), \quad (7)$$

where we introduced the novel parameter f(C). Then, as $(1/P_3) = k_a C + k_d$ by definition, the association constant is given by the equation:

$$K = \frac{k_a}{k_d} = \frac{P_2}{P_1 C} \quad (8)$$

The parameters P_1, P_2 and P_3 are obtained from the curves corresponding to the reflected intensity as a time function for different concentrations of monoclonal anti-AB. From these curves, f(C) values are obtained and the calibration curve is plotted. This curve allows us to obtain the desired MoAb quantification in a sample of unknown concentration.

TABLE I. Typical parameters for different amounts of MoAb added to the cell

Anti-AB	$\mathbf{P_1}$	$\mathbf{P_2}$	$\mathbf{P_3}$	**f(C)**
[μg]	**[u.a.]**	**[u.a.]**	**[s]**	**[1/s]**
20	279.8 ± 0.7	22.6 ± 0.7	8.9 ± 0.5	0.0083 ± 0.0007
33.3	256.7 ± 0.1	46.2 ± 0.5	16.5 ± 0.3	0.0091 ± 0.0003
40	267.9 ± 0.5	34.3 ± 0.5	11.3 ± 0.3	0.0100 ± 0.0004
80	259.7 ± 0.6	39.1 ± 0.6	9.7 ± 0.3	0.0125 ± 0.0007
100	219.0 ± 0.9	76.5 ± 0.9	14.4 ± 0.3	0.0179 ± 0.0007
180	253.9 ± 0.6	40.1 ± 0.6	7.0 ± 0.2	0.019 ± 0.001
200	255.8 ± 0.9	49.1 ± 0.9	7.1 ± 0.2	0.023 ± 0.002
300	246 ± 1	54 ± 1	6.2 ± 0.3	0.028 ± 0.003
400	202 ± 3	116 ± 2	10.0 ± 0.4	0.036 ± 0.003

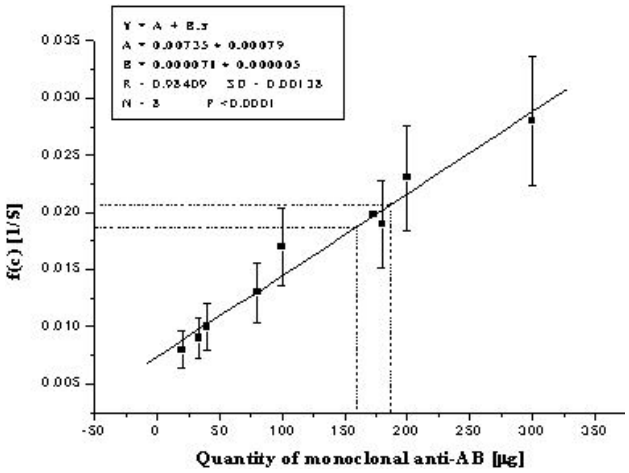

FIGURE 3. "Calibration curve" consisting of the graph f(C) vs. the anti-AB concentration. From interpolation on the calibration curve, the unknown concentration results: C* = (173 ± 9) μg for f**(C*)** = (0.0198 ± 0.0007) 1/s.

5. Results

Figure 2 shows the typical curve obtained for the light intensity reflected by the wafer for one anti-AB concentration. The curves were fitted using Eq. (4) for the different concentrations of monoclonal anti-AB. The P_1, P_2, and P_3 values obtained from the different curves are shown in Table I, which also shows the mean values of f(C) for each anti-AB quantity. Figure 3, or the "calibration curve" shows the plots of f(C) versus Ab concentration. This plot results in a straight line whose slope is the adsorption kinetics constant: $k_a = (7.2 \pm 0.5).\ 10^{-5}$ 1/μg.s. The adsorption kinetics constant is obtained from this value by using (4), and the result is $k_d = (0.09 \pm 0.03)$ 1/s. To determine the equilibrium constant K, Eq. (8) was used, obtaining the following mean value: $K = k_a / k_d = (80 \pm 30)$ 1/μg. P_1, P_2, P_3 and f(C) were determined for an unknown sample, with the following results: f * **(C***) = (0.0198 ± 0.0007) 1/s. The amount of MoAb adsorbed onto the wafer is inferred by interpolation on the calibration curve (Fig. 3), resulting in C* = (173 ± 9) μg, which agrees with the results obtained by the colorimetric method: (180 ± 10) μg (taking the uncertainty gap into account).

6. Discussion and conclusions

The technique developed proves to be sensitive and precise. As the values of f(C) and P_3 are independent of the incident intensity on the wafer/sample interface, this method is independent of the light source characteristics. The graph obtained (see Fig. 2) properly fitted (regression factor R>0,94) shows that the monoclonal anti-AB association occurs according to the classical Langmuir surface adsorption model [8]. The experimental points (see Fig. 3) are adjusted by a linear regression (R>0,99), which allows us to obtain k_a, k_d and K values for the MoAb adsorption onto the silicon wafer.

The advantages of this method are: its simple and fast assembly, its precise determination of the total amount of protein in the sample without labeling purification or destroying, measurements of the active concentration of the analyte in real time. The determination requires very little sample volume.

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