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Letter

Nanogravimetric gauge for surface density measurements and deposition analysis of Langmuir–Blodgett films

Paolo Facci, Victor Erokhin and Claudio Nicolini*
Institute of Biophysics, University of Genova, Via Giotto, 2, 16153-Genova (Italy)

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Abstract

A simple tool for surface density measurements of deposited Langmuir–Blodgett films based on the principle of nanogravimetric piezoelectric resonators has been developed. This device can be very useful for monitoring the quality of the deposited films by checking the functional dependence of the surface density upon the number of deposited layers. Moreover, knowing the molecular weight and the area per molecule of the deposited compound, it is also possible to calculate the packing degree. This parameter can provide a direct method for comparing different techniques of deposition and film formation, taking into account the different behavior of the molecules at the air/water interface and on the solid substrate.

The developed tool can, from another point of view, provide information on the area-per-molecule of those samples whose properties do not allow its estimation by usual pressure/area isotherms.

1. Introduction

Any technique for directly monitoring the quality of the Langmuir–Blodgett (LB) film deposition onto solid substrates can be very useful in thin film science. Usually, the film quality check is performed by transfer ratio measurements or looking to the homogeneity of the colors resulting from the interference fringes in rather thick films [1]. These techniques can obviously give only rough ideas about the quality of the deposition; the transfer ratio being an indirect parameter and the optical check being applicable only on several layer films.

Therefore, a direct technique capable, even on a single deposited monolayer, of providing information about the surface density by measuring the amount of deposited mass within the accuracy of fractions of one nanogram [2], seems to be a tool which can guarantee a good control on the reliability of the depositions.

In this work, we applied quartz piezoelectric resonators, used as gravimetric gauges, to match these requirements developing a simple tool that, knowing the value of the area covered by the deposited film, allows simple estimation of the film surface density. Moreover, this device can also be used to obtain information on the packing degree (see later) of the deposited film as well as on the area-per-molecule over the solid substrate.

First of all the system was tested and calibrated with cadmium arachidate layers and then it was applied to check the deposition quality of antibody LB films with different substrate preparations [2].

2. Materials and methods

Quartz resonators with a resonance frequency of about 10 MHz were used. A simple circuit was developed to allow the quartz resonators to oscillate at their resonance frequency (Fig. 1).

The shift in the resonance frequency, induced on a quartz resonator by subsequent LB deposition, was monitored by a usual frequency meter. The frequency shift Δf owing to the amount of mass Δm attached to the resonator surface was expressed in mass units by applying the Sauerbrey equation [3]:

$$\frac{\Delta f}{f_0} = -\frac{\Delta m}{A\rho l} \tag{1}$$

where f_0 is the resonance frequency of the quartz, ρ its density, and l and A the thickness and the area covered by the deposited monolayer, respectively. The knowledge of the area of the resonator covered by the deposited layers (measured by an optical microscope) allowed us to convert these values to surface density units (ng mm^{-2}) [4].

To calculate the packing degree (the ratio between the film surface density at the air/water interface and that after deposition onto the solid substrate), the area per molecule from either the literature or from pressure/area isotherms was taken, as well as the molecular weight.

To calculate the area-per-molecule, data were used for the molecular weight and the resonator area.

A standard trough (MDT) was used for the monolayer formation and both Langmuir–Blodgett [5, 6] and Langmuir–Schaefer [7] techniques were applied for

*Author to whom correspondence should be addressed.

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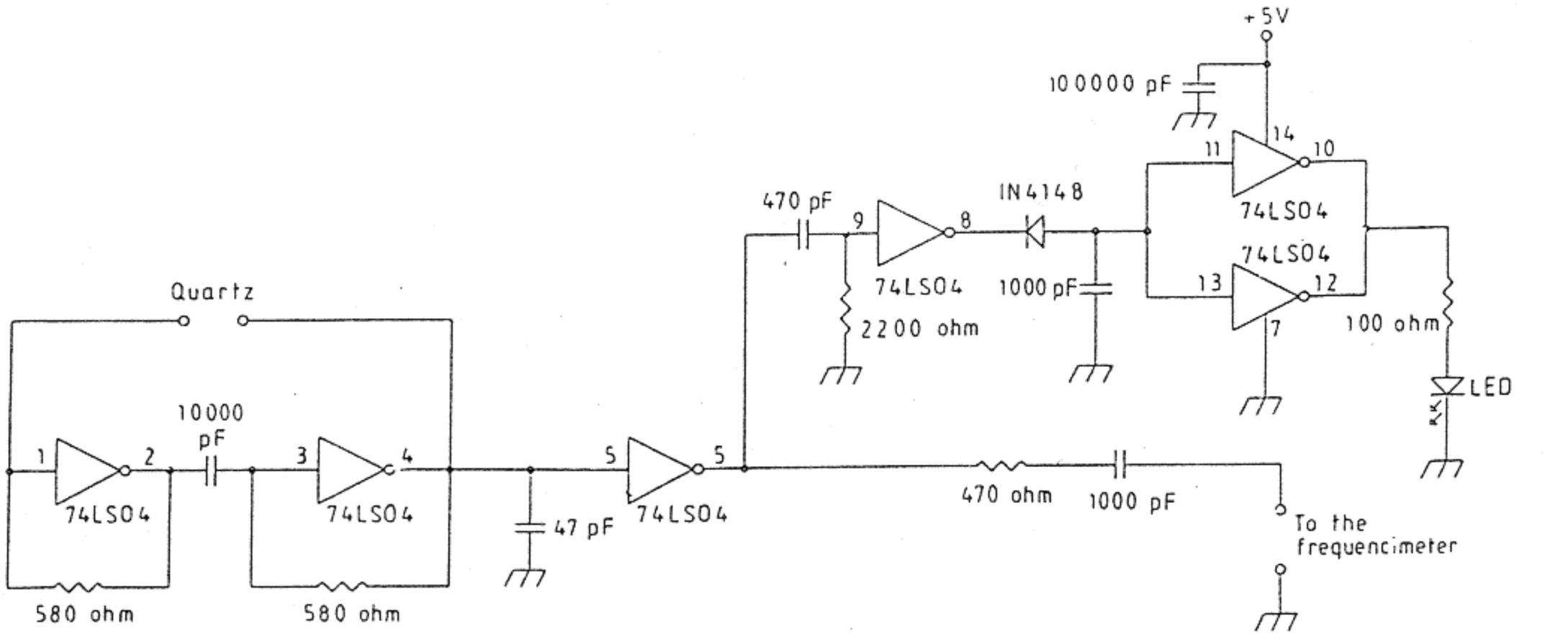

Fig. 1. Electric scheme of the circuit for driving the quartz resonators. The same circuit can also drive the quartz resonators with different resonance frequency.

deposition according to the properties of the different monolayers.

Experiments were performed on cadmium arachidate layers as a standard sample and on anti-insulin antibody films with different solid substrate preparations.

3. Results and discussion

As a first step, we carefully checked the properties of the technique [8] dealing with stability and reliability of the measurements as well as the effect of the humidity of the air and of the room temperature on the frequency shift. We found that it is more than adequate to put the resonator in a closed plastic chamber during measurements to prevent the resonator from any sudden change in the experimental conditions. Moreover, we noticed that it was sufficient to wait for 5 min after connecting the resonator to the driving circuit, to be able to neglect any thermal drift.

After these checks, we began to measure the frequency shift induced by the deposition of several bilayers of cadmium arachidate (Fig. 2). The layers were deposited by the Langmuir–Blodgett technique at a deposition pressure of 25 mN m^{-1}. Using data on the molecular weight of the compound and on the area per molecule from pressure/area isotherms at the trough surface, it was possible to calibrate the weight gauge finding a sensitivity of 42 ± 13 pg (Hz mm^2)$^{-1}$ and a minimum detectable mass of 22 ± 7 pg mm^{-2}. The behavior turned out to be linear up to several micrograms. From the same data, it was even possible to calculate the packing degree (Fig. 3). This parameter can be a useful one, as it can give information on the phenomena taking

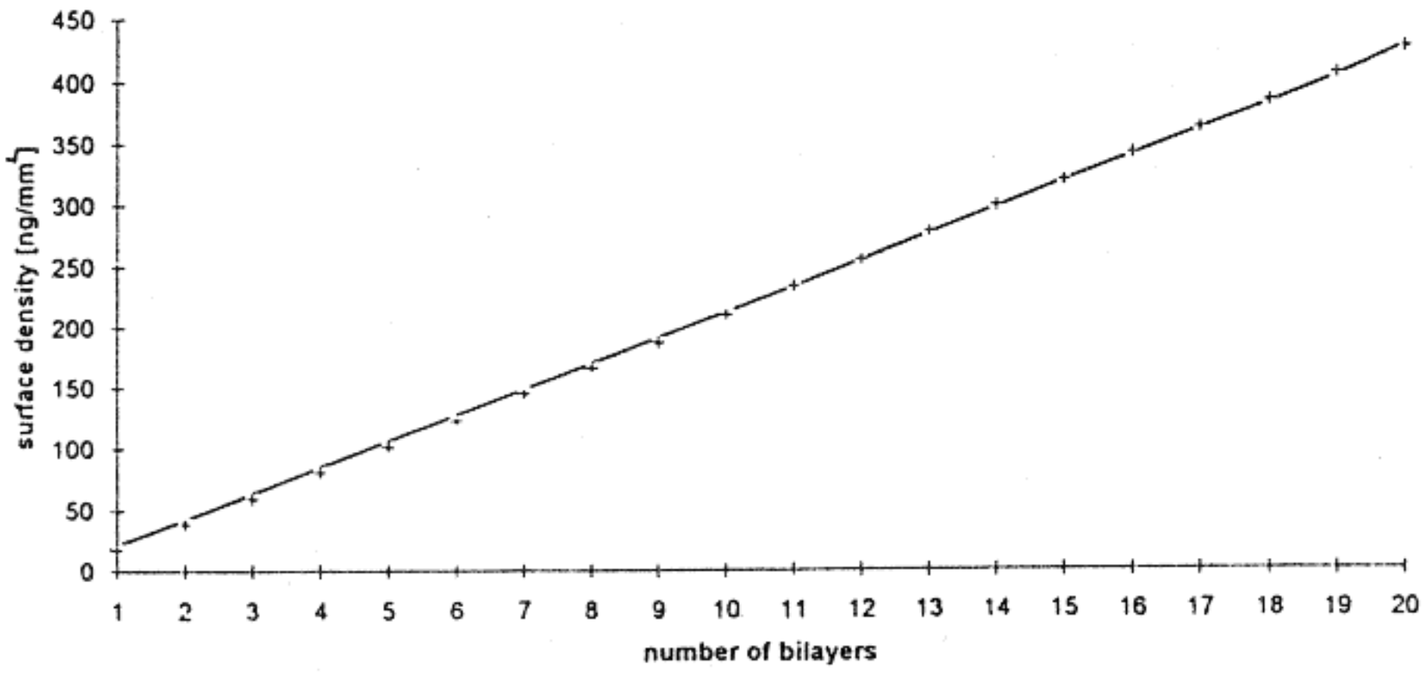

Fig. 2. Surface density of 20 bilayers of cadmium arachidate as a function of the number of deposited bilayers. The straight line is the best fit of the experimental data (+).

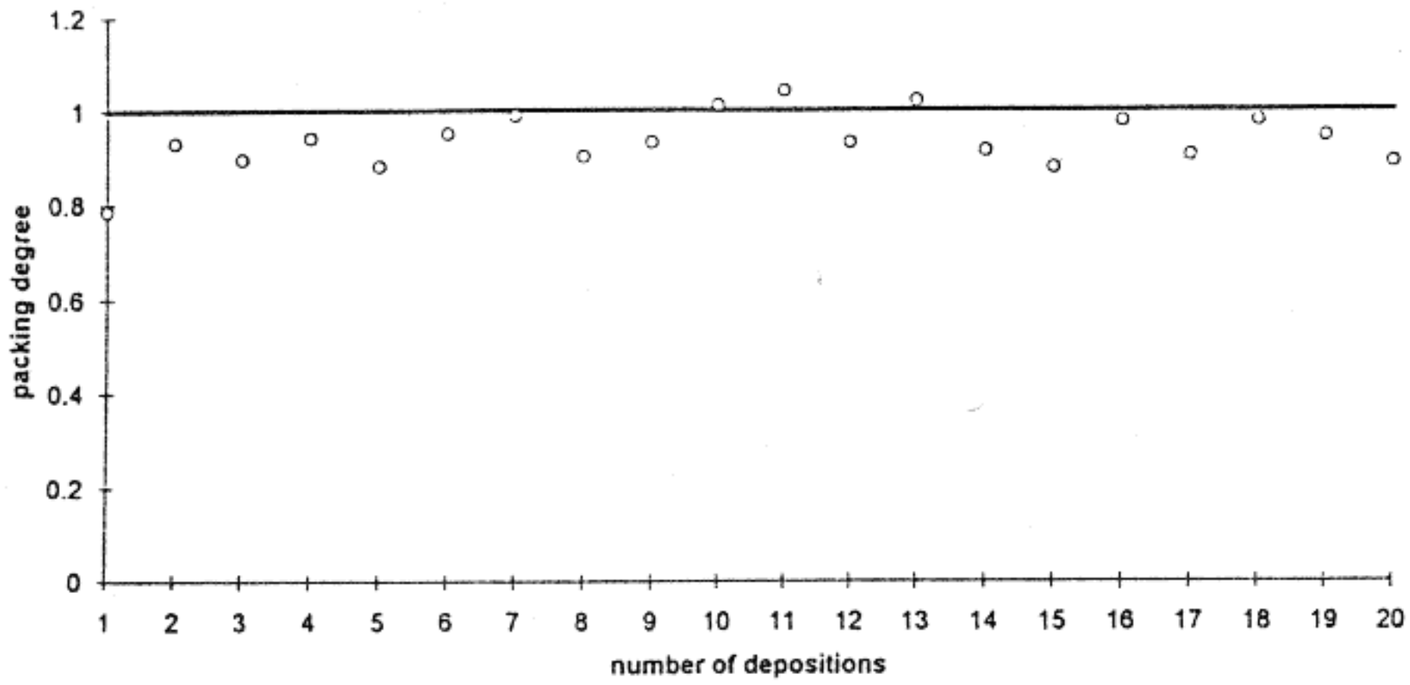

Fig. 3. Packing degree of the 20 deposited cadmium arachidate bilayers. The straight line is the ideal result corresponding to the film packed as if it was on the water surface at the deposition pressure.

place during film deposition, e.g. loss of order, bad adhesion to the substrate, formation of microcollapses, etc. In other words, this kind of analysis can provide information about the film organization on the solid substrate with respect to the air/water interface or as a function of the different deposition techniques or substrate preparations.

After this standard sample, we approached a more complicated system; antibody LB films. Water-soluble protein films are rather difficult to form and to transfer onto a solid substrate as they have weak amphiphilic properties [9]. Moreover, the film quality can easily deteriorate during the deposition process owing to the different amphiphilic properties of the environments (air/water interface and air/solid substrate) [2]. Therefore, it can be very useful to have data on the surface density of the deposited layers to obtain information on the reliability of the deposition. The water solubility of antibody molecules, moreover, does not enable the value of the area-per-molecule at the air/water interface to be known. Thus, the value of the area-per-molecule in the deposited layers can provide unique information about the packing behavior of these proteins.

We deposited 20 layers of mouse anti-insulin antibodies. The deposition pressure was 35 mN m^{-1}. Owing to the rigidity of the monolayer, it was necessary to use the Langmuir–Schaefer method of deposition [9] (horizontal lifting). The deposition curve is shown in Fig. 4. The linear behavior is worse than in the case of cadmium arachidate, pointing out less reliability of the deposition process. The variability of the area-per-molecule for these monolayers (Fig. 5), calculated using a protein molecular weight of 160 kDa [10], indicates that the deposition process perturbs rather strongly the original situation at the air/water interface. However, the average value suggests that the packing is rather close and, therefore, these molecules are likely to be placed in a vertical position.

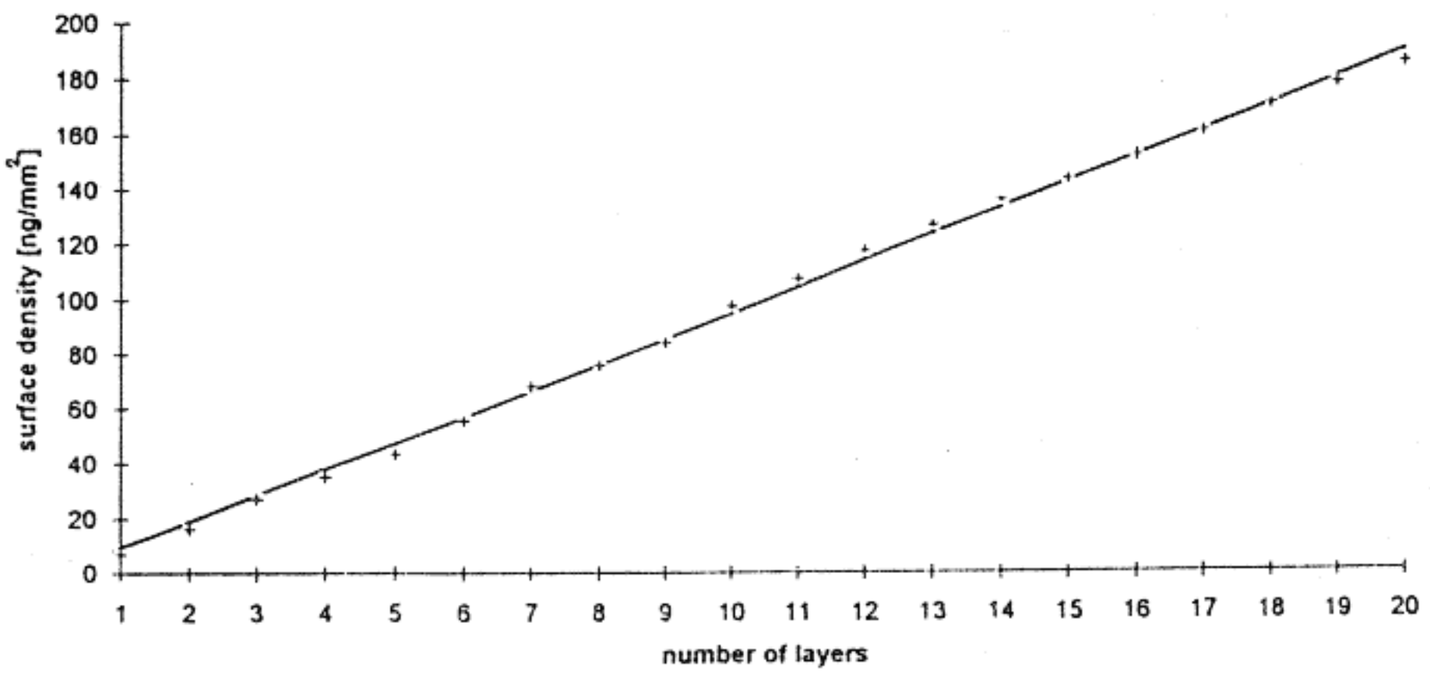

Fig. 4. Surface density of 20 layers of anti-insulin antibodies as a function of the number of deposited layers. The straight line is the best fit of the experimental data (+).

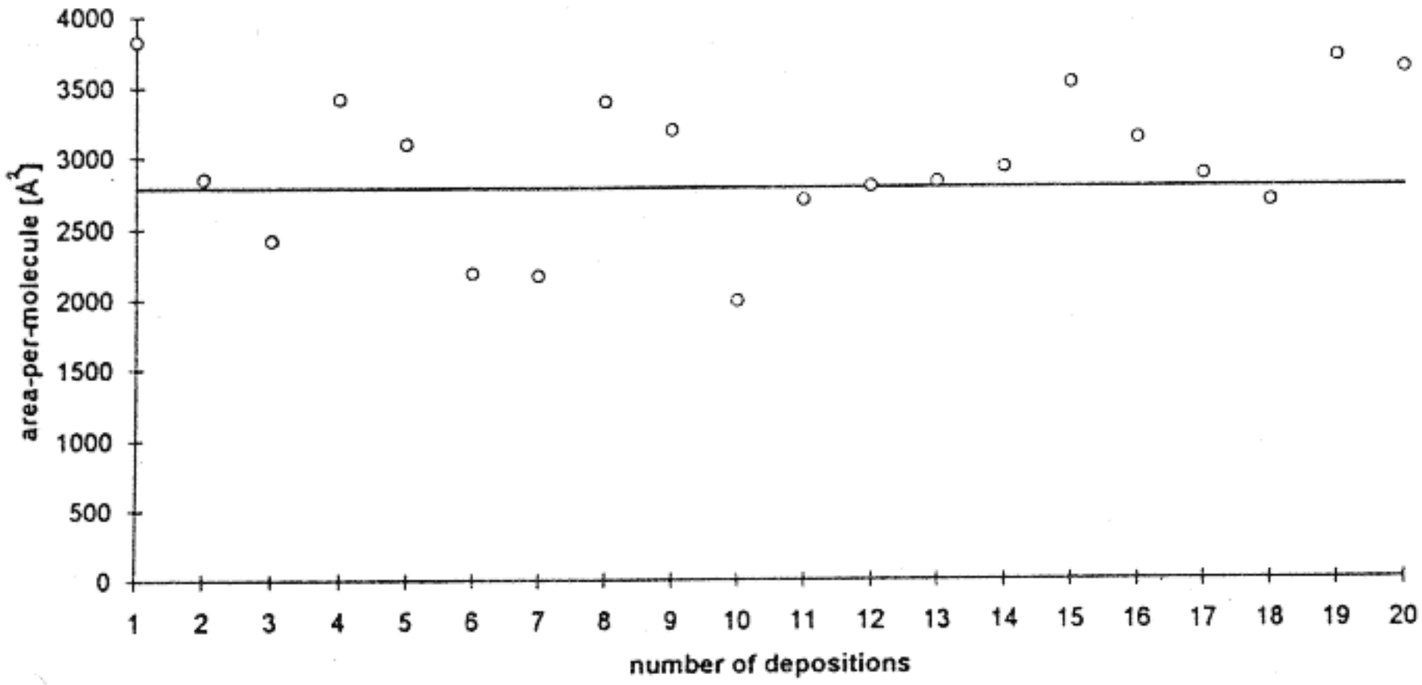

Fig. 5. Area-per-molecule of the 20 deposited antibody layers. Note the scattering in the experimental point suggesting the unreliability of the deposition.

TABLE 1. Comparison between the value of the area-per-molecule of four subsequent layers of antibodies (AB), treating and not treating the substrate with GA before each deposition

Number of AB monolayers	Area-per-molecule (Å²)[a]	
	Without GA	With GA
1	3824 ± 85	2477 ± 55
2	2850 ± 63	1853 ± 41
3	2421 ± 54	1802 ± 40
4	3418 ± 76	1879 ± 42

[a]Errors are mainly due to uncertainty on the surface area determination.

This type of analysis, in connection with the usual ones, can also be very useful in obtaining information on the deposited layers of less well known molecules as well as on different methods of substrate preparation and transfer of the LB sample. To give an example of this last statement, we deposited the same antibody after coating the substrate, before each deposition, with glutaraldehyde (GA), a fixative which binds the amino and hydroxyl groups [11]. The results, in comparison with the data for the unfixed layers, are reported in Table 1 in terms of area-per-molecule. In this case, the area-per-molecule for the fixed sample, which is dramatically constant after the first layer, suggests the idea that treating the substrate with GA can "freeze" the condition affecting the antibody monolayer at the water surface [2] providing a reliable film deposition with a close molecular arrangement which is likely to be similar to that at the water surface.

In conclusion, we can say that this simple technique can really become a useful tool for application to LB (and not only LB) science. It can be thought to be of use as a standard check on each monolayer deposition to control which deposition took place and that it is reliable. Moreover, this tool can also be applied to investigate the changes in the packing degree as well as to estimate the area-per-molecule in the deposited film, asserting itself as a new and easy-to-use device for early investigations in thin film science.

References

1 G. Roberts, *Langmuir–Blodgett Films*, Plenum Press, New York, 1990.
2 V. Erokhin, P. Facci, F. Antolini and C. Nicolini, *Nature* (1993), submitted for publication.
3 G. Z. Sauerbrey, *Z. Phys.*, *178* (1964) 457–462.
4 V. V. Erokhin, R. L. Kayushina, M. Yu. Lvov and L. A. Feigin, *Il Nuovo Cimento*, *12D* (1990) 1253–1258.
5 I. Langmuir, *Trans. Faraday Soc.*, *15* (1920) 62–74.
6 K. B. Blodgett, *J. Am. Chem. Soc.*, *57* (1935) 1007–1022.
7 I. Langmuir and V. J. Schaefer, *Am. Chem. Soc.*, *60* (1938) 1351–1360.
8 T. B. Dubrovsky, V. V. Erokhin and R. L. Kayushina, *Biol. Mem.* *6* (1) (1992) 130–137.
9 Yu. M. Lvov, V. V. Erokhin and S. Yu. Zaitsev, *Biol. Mem.* *4* (9) (1992) 1477–1513.
10 E. Harlow and D. Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, New York, 1988.
11 A. L. Lehninger, *Biochemistry*, Worth Publishers, New York, 1970, p. 59.