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Simultaneous study of mechanical and electrical properties of a self assembled insoluble monolayer by axisymmetric drop shape analysis (ADSA)†

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A novel technique has been devised to measure mechanical and electrical properties of a lipid monolayer in contact with an electrolytic solution and supported by a metal electrode. The technique is based on a sessile drop apparatus in which the surface of a mercury drop, covered with the lipid film, can be alternately increased or decreased, while optically measuring the drop shape, hence the interfacial tension, by the ADSA technique. At the same time the film polarization state is controlled by a voltage clamp circuit and differential capacitance is measured by a lock-in amplifier. By this technique it is possible to obtain, *e.g.* the relation between surface pressure and differential capacitance, or surface pressure and polarization, that cannot be obtained with any other technique in a single experiment and on a single film. Films formed on the drop surface have been shown to be stable for many hours.

1 Introduction

Lipid monolayers and bilayers have been widely studied for about a century because of their importance as models for biological membranes and for industrial applications.[1] Most of this work has been performed on monolayers spread at the air/water interface in a Langmuir trough[2,3] and on black lipid membranes (BLM), formed between two liquid phases.[4,5]

The former technique is suitable for accurate measurements of the mechanical properties of the film, *e.g.* compression isotherms. Electrical properties of the assembly, often as important as mechanical ones, such as the surface dipole potential, that can give an estimate of the average molecular orientation, have been studied by providing a Langmuir trough with either a Kelvin vibrating electrode or a radioactive ionizing electrode.[3] These arrangements however result in poor sensitivity and do not allow one to modify the state of electrical polarization of the film, *i.e.* the potential difference across it, or to measure its electrical capacitance, which is an important property for predicting the monolayer thickness.[5]

The latter technique, on the other hand, developed in the sixties, allows very accurate measurements of the electrical properties of bilayer films, mainly capacitance and conductance as a function of electrical polarization.[5]

The BLM, formed on a small hole in the wall separating two compartments of a cell filled with electrolyte solution, effectively closes the hole between the two compartments and behaves as a dielectric between the two electrically conducting solutions. Film capacitance and conductance can be measured with good accuracy by dipping two electrodes connected to a capacitance bridge (usually silver/silver chloride electrodes) into the solution compartments.

It is very difficult to gain information about the mechanical state of the film: neither surface pressure in the bilayer, nor the area per molecule can be determined. The effective area of the film is also not accurately known as usually near the Plateau border the film is more than two molecules thick. The interfacial tension of the film can be varied by bulging the BLM surface,[5] but the range of surface pressure that can be covered is very small. Interesting results on electro-mechanical properties of bilayers in lipid vesicles have been obtained by the micropipette technique.[6] In this study attention was focused on the role of membrane tension and compressibility for membrane breakdown.

Both electrical and mechanical properties of films have been studied by transferring the film from the water/air interface to the surface of a supporting metal electrode and applying standard electrochemical techniques[3,7] but by this method, measurements refer to different films, even if their composition is similar. In another approach, films have been formed directly in the Langmuir trough at the interface between two conducting liquid phases in order to measure both electrical and mechanical properties on the same film.[8]

More recently, a variant has been proposed to the Langmuir technique, using the surface of a liquid pendant or sessile drop to support a monomolecular film.[9] Surface pressure is given by the lowering of interfacial tension γ at the drop surface in the presence of the film, with respect to the value γ_0 obtained with a clean surface and can be measured by well-known drop shape techniques;[10–12] expansion–compression cycles can be performed, changing the drop surface. On the other hand, the electrical properties of the interface between two immiscible electrically conducting liquids can be studied by well established electrochemical techniques. Combining both techniques, we have implemented a drop shape apparatus in which a mercury drop, immersed in an electrolyte solution, supports a monolayer film, enabling us to measure at

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one time both mechanical and electrical properties: the drop shape technique yields surface pressure and area while a voltage clamp circuit connected to a lock-in amplifier allows us to polarize the film while measuring the capacitance.

Hence, with this apparatus it is possible to study properties of the film such as capacitance as a function of the compression state, or the influence of electrical polarization on compression–expansion cycles. The possibility of determining simultaneously both electrical and mechanical properties on the same monomolecular film can be considered a very powerful tool, since it allows us to detect which property, electrical or mechanical, plays the critical role in a particular membrane function. In fact, it is well known that in some cases mechanical properties are preponderant—lung membranes[13]—while in other circumstances—neuronal cells, neuro-muscle junctions[14]—the electrical behaviour is fundamental to determining the biological functions. Therefore, this new technique can be very useful for understanding the complex phenomenology in the physical chemistry of cellular membranes.

2 Experimental

We have used a sessile mercury drop as the supporting surface for the film. The choice was guided by both chemical and physical considerations: mercury is a semi-noble metal, inert enough with most compounds of interest and it accepts a wide range of polarization potential; it is a good conductor and, as a metal, avoids the complexities due to the presence in the system of a second electrochemical interface; it is opaque, giving optimum contrast with back illuminated drop profile analysis; the surface tension of mercury is an order of magnitude higher than the greatest surface pressure values needed in the measurements, so that high compression states are attainable for the film.

Sessile drop geometry has been preferred to pendant drop in this study, as it yields a more stable system from a mechanical point of view; however the technique can also be applied to pendant drop with minor modifications.

2.1 Reagents

Mercury was purified by repeated prolonged bubbling in HNO_3 and NaOH solutions. No significant difference was found in the interfacial behaviour with respect to mercury that was distilled after this washing.

Electrolyte solutions were prepared using NaCl Suprapur grade from Merck and water purified through a Millipore Milli-Q apparatus fed with spring water. Mono-oleilglycerol (GMO) was supplied by Fluka, and propan-1-ol and ethanol by Merck.

2.2 The drop-shape apparatus

The apparatus is derived from a previous drop shape apparatus described in ref. 12. The imaging system is composed of a 1024×1024 pixel CCD video camera (MX12P + PSU120 ADC by Adimec, Holland), a frame grabber (IT-AM-DIG by Imaging Technology, USA) and a PC equipped with a 450 MHz Pentium processor. The optical system is a Zeiss Tessovar objective (Zeiss, Germany).

The sessile drop electrode has been described in detail in ref. 12.

The measuring cell, manufactured by Hellma (Germany), is made of Special Optical Glass slides 2.5 mm thick, with external dimensions $33 \times 43 \times 51.5$ mm^3. An aluminium jacket is wrapped around the cell (apart from the light path windows and the passages for electrodes and tubing) for temperature control.

The measuring procedure from frame grabbing to interfacial tension value is described in detail elsewhere.[12,15]

2.3 Control of the drop surface

The measurement and control of the drop surface is critical in the technique. The value of the surface area is obtained through numerical integration of the drop profile extracted from the drop image and the computer that controls the apparatus also controls the drop surface, either by increasing or decreasing the drop volume: water, as the piston liquid, is pushed into or pulled from the mercury reservoir that feeds the electrode, by two automatic burettes (Dosimat 665 by Metrohm, CH), that work in opposition to each other. The burette that works in pull mode has been custom-modified by Metrohm Italiana srl, Italy. The mercury reservoir and pipes are at constant volume, so a mercury volume equal to the volume of the water pushed or pulled goes to the drop on the electrode. Burette resolution is ± 0.05 μl; the volume of the drop is in the range 40–70 μl, so the volume is controlled with $\pm 0.1\%$ accuracy. It can be shown that under normal working conditions the dependence of surface area on volume is fairly linear,[16] hence the same control tolerance of $\pm 0.1\%$ also applies to the surface area.

A software feedback loop controls the drop surface as required by the measurement procedure, either keeping it constant as interfacial tension varies as a function of other parameters (*e.g.*, polarization potential or time) or varying it at constant speed when performing expansion–compression cycles. The control software has been designed for a quiet and regular operation and even if each change in volume is actually applied in discrete 0.1 μl steps, the resulting behaviour is smoothed by a certain degree of intrinsic sluggishness in the system response due to the presence of some elasticity in the water and mercury tubings. This lack of stiffness, beneficial for smooth operation, nevertheless does not hamper surface area accuracy that is guaranteed to the level allowed by resolution ($\pm 0.1\%$) by the high sensitivity and accuracy that the feedback loop is endowed with by the optical measuring system.

The iterative surface management software extracts from the grabbed image the drop profile and then calculates the area. By comparison with previous values, it computes the speed of variation of the surface: if this value is greater than a given threshold, then the drop is still changing because of former corrections and the program waits for a short time (0.4 s). If the drop is stable, however, a correction proportional to the difference between the actual and the requested surface is applied to the drop volume. The proportionality factor between volume corrections and area variations is obtained from analysis of previous operations.

Once the actual and requested area differ by less than the resolution, the surface management process pauses and the measuring process gets and stores valid drop data. After a 5 s stand-by (or less if the measuring process calls for a new measure), the surface management process repeats the cycle. It takes no more than 3 s to attain a given surface value and less than 1 s for smaller corrections.

2.4 Capacity measurements

The polarization of the mercury-film–solution system and the measurement of capacitance is accomplished through a 3-electrode potentiostatic circuit, as shown schematically in Fig. 1.

The AC component from the lock-in reference oscillator, added to the DC polarization potential from a digital to analog converter, is about 1.5 mV of amplitude at 1000 Hz frequency. The amplitude is low enough to avoid interference with the DC polarization signal; the frequency is high enough to avoid setting up resonant oscillations of the drop; the resonance frequency for mercury drop we currently use (40/70 μl) falls around 15 Hz for the fundamental oscillation mode and can reach 100 Hz for superior modes. At 1000 Hz it became

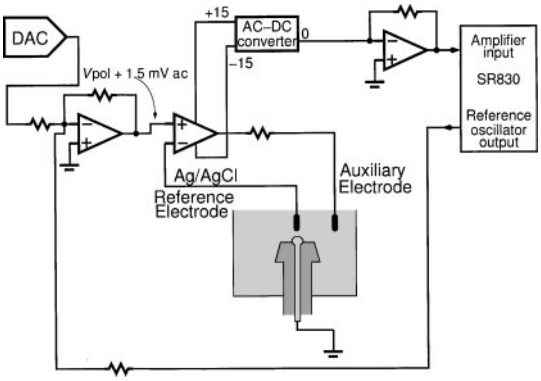

Fig. 1 Block diagram of the interphase polarization circuit and connections with the lock-in amplifier for capacitance measurements.

impossible to excite any mechanical oscillation, yet according to current opinion in the literature the measured capacitance should coincide with the zero frequency value.

The reference electrode is an Ag/AgCl pellet electrode, the auxiliary electrode is a platinum wire.

2.5 Formation of the film

The lipid GMO was dissolved in a suitable solvent at a concentration of 0.24 mg ml^{-1}; the solvents selected were propan-1-ol and ethanol since these short chain aliphatic alcohols are both soluble in water and good solvents for GMO. This enabled us to spread the lipid molecules at the Hg/water interface by injecting a droplet ($\sim$0.7 μl) of the GMO–alcohol solution with a microsyringe (Hamilton 87919, resolution 0.1 μl) underneath the surface of the mercury drop (Fig. 2a). When the droplet, pushed by hydrostatic buoyancy, reaches the mercury/solution interface, the solvent dissolves into the electrolyte solution (Fig. 2b), while the lipid mostly remains trapped at the surface and arranges to form the film. After deposition, 2 min are allowed to elapse so as to ensure complete dissolution of the spreading solvent in the bulk electrolyte solution before the experiment is started.

3 Results

The new technique has been tested by determining the electrical and mechanical properties of a GMO monolayer at the interface between Hg and aqueous 0.1 M NaCl.

Fig. 3 shows film capacitance as a function of surface pressure. The plot has been obtained by combining several experiments that differ in the compression–expansion rate, in the solvent used (propan-1-ol or ethanol) and in the amount of substance deposited, but all were performed with the mercury electrode kept at a potential of -500 mV *vs.* Ag/AgCl (see Fig. 1), that is, very close to the potential of zero surface charge density of mercury in NaCl 0.1 M solution. While only

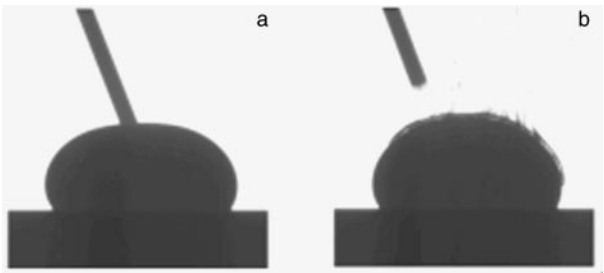

Fig. 2 Deposition of the film (a) The lipid, dissolved in alcohol, is injected by a microsyringe underneath the surface of the mercury drop; (b) alcohol dissolves into the electrolyte solution; the process is made visible by refractive index gradient.

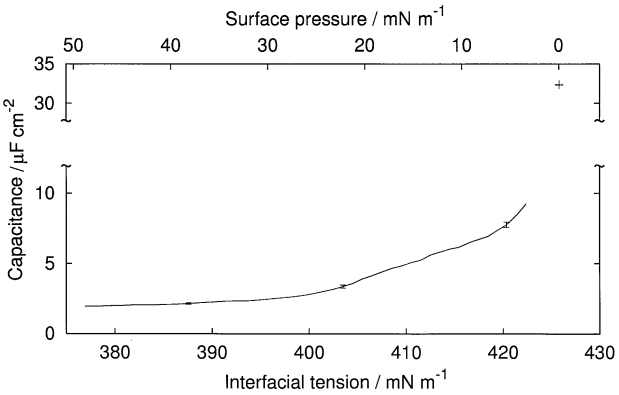

Fig. 3 Capacity of the mercury/GMO monolayer/electrolyte solution interphase at constant polarization potential. The curve has been obtained by combining results from different experiments, performed on several different films. The spread in the experimental data, shown by the three markers at 388, 403 and 420 mN m^{-1} indicates a good reproducibility, even when data refer to a different film deposition. The cross in the top right corner corresponds to the clean mercury surface in contact with the electrolytical solution.

a limited range of surface pressure is covered by a single experiment (about 12 mN m^{-1}), all data sets overlap quite reproducibly and together span the full range of interest. As the drop area is decreased, the surface pressure increases, molecules become more closely packed and tend to align normal to the surface. Hence the film thickness increases and the capacitance decreases. An asymptotic trend is clearly visible for a capacitance with limiting value $C_1 = 1.9$ μF cm^{-2}, as surface pressure increases. It is worthwhile to mention that the behaviour shown in Fig. 3 ensures that the Hg surface is completely covered by a homogeneous lipid monolayer. It is known that reproducibility in the decrease of specific capacitance as a function of monolayer formation can be considered a first criterion for the formation of compact films without defects.[17] For example, it has been shown that defects of self-assembled monolayers on gold cause variations of the capacitance between 50 and 90% of the theoretical value expected for a compact film.[18] In contrast, when the monolayers have very low defect density the capacitance is very reproducible and the values are 95–100% of the theoretical estimate for a perfectly compact film.[17] Concerning the expected value of the specific capacitance of GMO monolayers at the Hg/solution interface, it is interesting to compare our results both with BLM formed with the same compound and with monolayers of hexadecanethiol at the same interface.

According to the plane plate capacitor model, the capacitance value for a monolayer should be twice that for a bilayer. In ref. 19 the value $C = 0.79$ μF cm^{-2} has been reported, hence a value of 1.58 μF cm^{-2} was expected in our measurements. The discrepancy is not very large, indicating that studying membrane models at the Hg/electrolyte solution interface can be useful, despite the different interactions expected for the Hg/lipid membrane interface with respect to the lipid–lipid interactions in the bilayers. However, we can invoke several reasons that could explain such small but significant discrepancy. The hypothesis that a monolayer is just one half of a bilayer may be at fault: in BLM the hydrophobic tails of one layer are in contact with their counterparts in the other layer, while in our experiment they are in contact with the metal; the resulting arrangement can be different, giving a not quite perpendicular orientation. Alternatively, some solvent molecules may remain trapped in the monolayer, hindering the full packing that corresponds to greatest layer thickness. It is well known that solvent incorporation occurs with BLMs (mainly with hydrophobic chain length greater than C_{10}),[20,21] but in a BLM the solvent would remain trapped between the two layers, so effectively increasing the

membrane thickness and decreasing the measured capacitance. In monolayers at the Hg/water interface we expect that solvent adsorption at the interface will not increase the film thickness, due to the very short chain length of propan-1-ol, as compared to the hydrophobic chains of the lipid GMO. Instead, adsorbed propan-1-ol can prevent molecular packing of the GMO molecules leading to a tilted orientation of the lipid hydrocarbon chains which results in a hydrophobic layer thinner than half that of the fully packed GMO bilayer in BLMs, with consequent increase in the measured specific capacitance. Furthermore, propan-1-ol adsorption at the Hg/water interface tends to increase the relative permittivity of the lipid film, further increasing the capacitance. Finally, in BLM the measure of capacitance per surface unit depends upon the measure of the surface of the truly bimolecular part of the membrane, that does not coincide with the supporting hole section, because of the presence of a thicker rim on the border. This leads to an overestimate of membrane surface, hence an underestimate in specific capacitance that could explain, at least partially, the discrepancy. On the other hand, if we compare our capacitance results with those reported in ref. 17 for hexadecanethiol at the Hg/NaF solution interface, some interesting considerations can be deduced. Using the same estimate for the relative permittivity of 2.3, from our results of $C = 1.9$ μF cm^{-2} we can calculate a thickness of the hydrocarbon region of GMO of *ca.* 11 Å. The hydrocarbon chain of GMO differs from that of hexadecanethiol by (i) one $-CH_2$ group and (ii) the presence of a double bond in the middle of the chain. This means that, if we assume a thickness of 20 Å for hexadecanethiol as in ref. 17, then the estimated theoretical value for GMO should be *ca.* 21.3 Å, due to the presence of one $-CH_2$ group, giving a contribution of about 20/16 = 1.25 Å. Considering that the BLM data[19] infer a bent conformation for the GMO hydrophobic chain, we can estimate a GMO hydrophobic core thickness of *ca.* 11 Å, in very good agreement with our capacitance results. This confirms our arguments given above to explain the discrepancy between our results and BLM measurements.

It is to be underlined that the high reproducibility of the measurements and the agreement between the results and the expected theoretical values cannot be considered a complete proof of maximally dense film, since they are based on an estimate of the relative permittivity and on the use of a technique that does not allow the direct measurement of Hg area,[17] an additional source of error that should be considered. Taking into account that our method permits a very accurate determination of Hg area, we can conclude that GMO monolayers at the Hg/NaCl solution interface form with high reproducibility and with low defect density. Further studies are in progress to measure the conductance of the system in order to quantify the defect density.

In Fig. 4 film capacitance is shown as a function of the mercury polarization potential at constant drop surface area; the large flat pit in the curve in the range −300 to −900 mV is suggestive of the presence of a surface film of roughly constant composition. When the potential scan goes beyond these limits the film dissolves, as shown by the steep rise in the capacitance. However the lipid molecules, insoluble in the electrolyte solution, remain trapped near the electrode surface, and when the potential reverts to values inside the pit range they reassemble into the film, as shown by the two overlapping curves, obtained by scanning the potential back and forth. If the potential is brought beyond −1300 mV, the film molecules become fully desorbed and no film develops again on reverting to lower potential values (data not shown). This demonstrates that we have a true insoluble film and not an adsorption equilibrium from bulk solution.

A subset of data shown in Fig. 3 is reported in Fig. 5 and 6, plotting separately interfacial tension (Fig. 5) and capacitance (Fig. 6) as a function of drop surface at constant potential. The

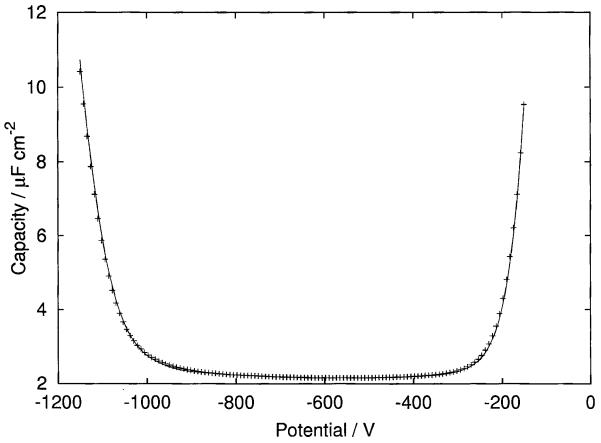

Fig. 4 The film capacitance as a function of polarization potential at constant drop surface. The solid line has been obtained by scanning potential from −150 to −1150 mV; crosses refer to the scan in the opposite direction. The large flat pit in the capacitance is indicative of the presence of a film of constant composition that, at extreme potential, disassembles and reassembles. (Data are not corrected for the capacitance of the Gouy–Chapman diffuse layer; the correction is a few percent in the pit region).

data are samples taken at the beginning, middle and end, of successive compression–expansion cycles, as obtained by cycling the drop area back and forth for about 26 h, in the range 40–70 mm^2 at a speed of 1 mm^2 min^{-1}. The curves do

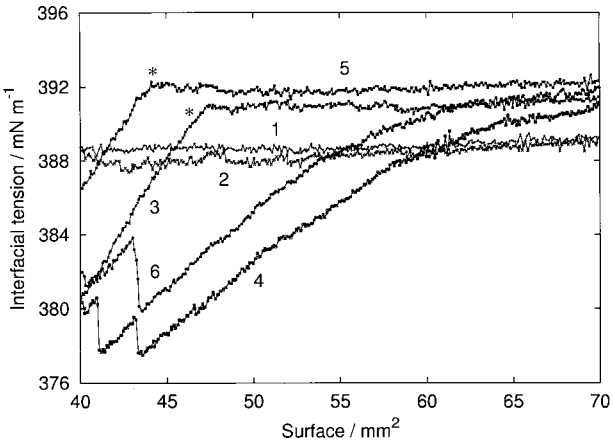

Fig. 5 Interfacial tension per unit surface as a function of drop surface, at constant potential. Scanning directions are: curves 1,3,5: expansion; curves 2,4,6: compression. Scanning speed is 1 mm^2 min^{-1}. Curves 1 and 2 were taken at the beginning of a 26 h run of expansion–compression cycles; curves 5 and 6 were taken at the end of the run; curves 3 and 4 are intermediate.

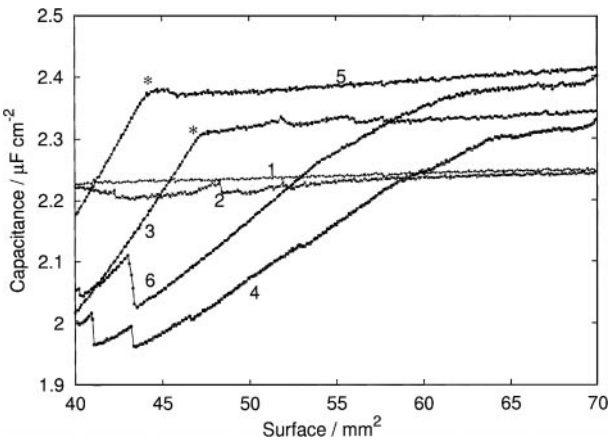

Fig. 6 Capacitance per unit surface as a function of drop surface, at constant potential. Details as for Fig. 5.

not overlap and show a marked hysteresis. The lack of overlap can be explained by considering that a small amount of the lipid gets lost on every cycle, probably because some of the film molecules upon compression condense on the flat glass of the electrode and lose contact with the drop border. As for the hysteresis, values of capacitance and surface pressure obtained at the same surface value are sensibly different depending upon the scan direction; however, when data are plotted as capacitance *vs.* pressure, as in Fig. 3, they coincide, indicating that the arrangement of molecules in the film at the same pressure does not depend upon scan direction.

The evolution in the expansion–compression cycle behaviour can be explained by supposing the presence of a tridimensional condensed phase of the lipid in contact with the bidimensional film. Upon compression lipid condenses into the tridimensional phase in greater quantity, whereas upon expansion it moves back into the film. These two processes (lipid condensation from the film to the tridimensional phase and lipid withdrawal in the opposite direction) have different kinetics. Initially (curves 1 and 2) the available condensed phase is large enough to plentifully supply the film on expansion and to draw rapidly excess lipid during compression, so that only small variations in C and γ can be observed; under these conditions the chemical potential in the film is roughly constant. As cycling proceeds (curves 3–6), the available tridimensional phase progressively decreases, due to irreversible loss of lipid molecules. This hinders lipid exchange between the film and the tridimensional phase. Therefore, the first part of the increasing area curves (curves 3 and 5, up to the asterisk), involves only monolayer expansion without any withdrawal of lipid molecules from the tridimensional phase in contact with the film. This is deduced from the proportional behaviour of C as a function of A. Accordingly, during compression (curves 4 and 6) excess lipid in the film condenses into the tridimensional phase only when higher values of surface pressure are attained. Note that there is an asymptotic value of surface pressure (*ca.* 46 mN m^{-1}) where the film begins to collapse suddenly releasing excess pressure into the formation of the condensed phase as shown in curves 4 and 6 by fluctuations of both interfacial tension and capacitance as a function of area.[22] This collapse pressure is comparable with the value of 42 mN m^{-1} found for GMO monolayers at the water/air interface.[23]

4 Conclusions

The reported results show that the new technique is viable to study the correlation between the mechanical state of a monomolecular film and its electrical properties. In particular, we succeeded in determining simultaneously surface pressure and specific capacitance of a GMO monolayer spread at the Hg/NaCl solution interface. The results achieved are complementary to those that can be obtained by the Kelvin probe technique. In fact, the latter technique allows one to determine surface potential, and consequently average molecular orientation, while our method permits one to attain a correlation between surface pressure and specific capacitance, that is, film thickness. Hence this technique can be useful in the study of the interactions between organic molecules of biological interest and the electric field at the surface of a biomimetic membrane.

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