Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Langmuir Films of Pure Antibiotic Ionophores and of Mixtures with Phospholipid at Different Temperatures

C. Betrencourt [a], A. Hochapfel [a], A. Tahir [a] & P. Peretti [a]

[a] Groupe de recherche en Physique et Biophysique, Universite' Paris V 45, rue des Saints Pkres, 75270, Paris, Cedex 06, France

Version of record first published: 24 Sep 2006

To cite this article: C. Betrencourt, A. Hochapfel, A. Tahir & P. Peretti (1999): Langmuir Films of Pure Antibiotic Ionophores and of Mixtures with Phospholipid at Different Temperatures, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 332:1, 513-522

To link to this article: http://dx.doi.org/10.1080/10587259908023797

Mol. Cryst. Liq. Cryst., 1999, Vol. 332, pp. 513-522
Reprints available directly from the publisher
Photocopying permitted by license only

Langmuir Films of Pure Antibiotic Ionophores and of Mixtures with Phospholipid at Different Temperatures

C. BETRENCOURT, A. HOCHAPFEL, A. TAHIR and P. PERETTI

Groupe de recherche en Physique et Biophysique, Université Paris V 45, rue des Saints Pères, 75270 Paris Cedex 06, France

New results are presented concerning insertion of two antibiotic ionophores Lasalocid and Monensin as sodium salts (LAS-Na, MON-Na) in biomimetic membranes. Langmuir films of Dipalmitoylphosphatidylcholine (DPPC) have been used as model systems. Evolution of the compression isotherms has been investigated below the gel – liquid crystal phase transition, between the ambiant and temperatures approching physiological conditions, not only for the pure antibiotics but also for the antibiotic – lipid mixtures, the molecular ratio ρ antibiotic/lipid equal to 0.1. In the mixed films with LAS-Na, the antibiotic molecule is well maintained by the film also when raising the temperature, whereas MON-Na is expelled above the low pressure region. The results are discussed and compared with effects produced in model systems of bilayers submitted to similar temperature variations.

Keywords: Langmuir film; Antibiotic; Ionophore

INTRODUCTION

Several antibiotic ionophores such as Lasalocid and Monensin have amphiphilic properties in the biomembrane. The polar inside of their cyclic configuration forms cation complexes and their hydrophobic exterior enables the complexes to move across the lipid barrier (1,2,3).

The fact that some molecules belonging to the family of antibiotic ionophores can be spread on the water surface and have reproducible π - A isotherms (surface pressure versus molecular area), is also a consequence of amphiphilic behavior (4,5,6).

Measurements at room temperature of the compression isotherms of Lasalocid and Monensin when they are mixed with Dipalmitoylphosphatidylcholine (DPPC), have provided information on interaction and implantation modes in monolayers (7,8). Strong repulsion takes place between the antibiotics and the lipid molecules at the interface where there is evidence of aggregation of the antibiotic molecules when the concentration increases. This is the dominating insertion mode for Monensin whereas for Lasalocid, lower values of the aggregation number can be explained by a tendency to penetrate into the lipid monolayer competeing with the aggregation mechanism. The results seem to be confirmed by differential scanning microcalorimetry measurements on multilamellar bilayer systems which show that Lasalocid interacts with the bilayer while Monensin does not (9).

With the purpose of approaching physiological conditions, raising the temperature and using a buffered subphase, we have investigated the influence on monolayers of Lasalocid, Monensin and their lipid mixtures. We have moreover looked for conditions where correlations are possible between mono- and bilayers.

MATERIALS AND METHODS

The antibiotics Lasalocid and Monensin as sodium salts were purchased from Sigma Chimie and recrystallized, Lasalocid from aqueous methanol and acetone, m.p. 169-172°C and Monensin from aqueous methanol and diethyloxyde, m.p. 260-264°C. Dipalmitoylphosphatidylcholine (DPPC) was of the purest available quality from Sigma Chimie.

Stock solutions of antibiotics 2.49×10^{-4} M or DPPC, 5×10^{-4} M, were prepared in chloroform-hexane 2:3 v/v and spread, separetely or mixed, in the wanted molar ratios. The solvents were of analytical grade from Prolabo. They were distilled on molecular sieve for moisture removal.

The monolayers were spread at different temperatures on a subphase of buffer Hepes 10 mM, sodium chloride 145 mM, pH 7.4. The compounds were purchased from Sigma and the subphase solution was prepared in water treated on an Elgastat UHQ 2 system. The films were spread with a Hamilton

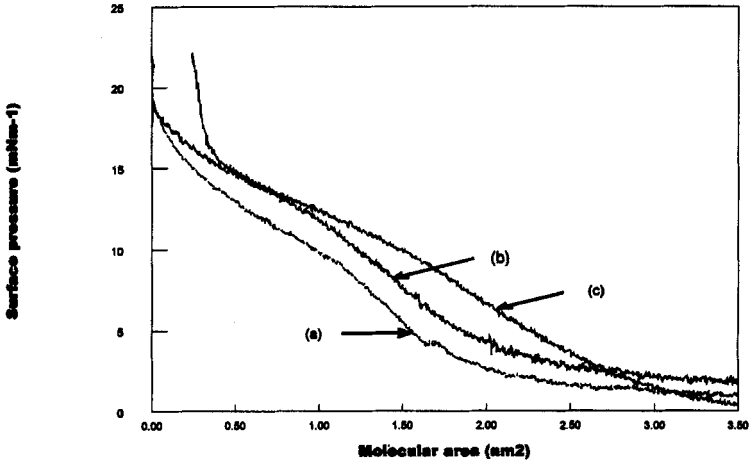

FIGURE 1 Compression isotherms of LAS-Na on Hepes buffer (pH 7.4) (a) 22 °C; (b) 30 °C; (c) 40 °C.

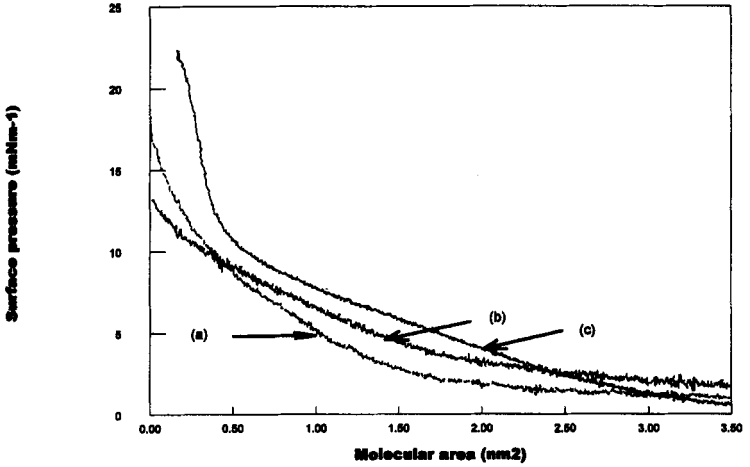

FIGURE 2 Compression isotherms of MON-Na on Hepes buffer (pH 7.4) (a) 22 °C; (b) 30 °C; (c) 40 °C.

CR-700-200 syringe (50µl corresponding to 1 $nm^2molecule^{-1}$ at 150 cm^2 for the lipid and to 2 $nm^2molecule^{-1}$ for the pure antibiotics). The isotherms were measured with a Krüss film balance using a pendulum 100 and recorded with an IBM-AT computer. The compressions were performed at a speed of 0.15 $nm^2min^{-1}molecule^{-1}$. The trough was thermostated with a precision of ±0.5°C.

RESULTS AND DISCUSSION

Two kinds of monolayer studies at 22°C, 30°C and 40°C on Hepes buffer at pH 7.4 have been performed, one of the pure antibiotics and the other of antibiotic-lipid mixtures. Only π-A isotherms obtained at least twice in succession have been taken into account. The higher the temperature, the more difficult to reproduce these pressure-area diagrams. This is particularily the case at 40°C. The selected results represent however the behavior most frequently encountered.

a) Pure antibiotics.

Compression isotherms of the pure antibiotics are shown in Figure 1 for LAS-Na and in Figure 2 for MON-Na.

As seen, the molecular areas at given pressures become in general lower when the temperature is increased. A possible explanation is that the organization of the molecules at the interface varies, but loss of molecules cannot be excluded due to very slight water solubility (10,11).

There is some incertainty in the measurements of these isotherms at low molecular area where the films likely collapse and some molecules might go into the subphase, since there are few molecules spread on the surface and the trough area then becomes small. The measurements are then easily contaminated by traces of impurities resulting in a sudden raise in the surface pressure on some of the compression isotherms of the pure antibiotics.

b) Antibiotic-lipid mixtures.

Figures 3 a,b,c and 4 a,b,c represent the compression isotherms for films of DPPC respectively containing LAS-Na and MON-Na. In the experiments shown the value of the molar ratio ρ = antibiotic /lipid, is equal to 0.1.

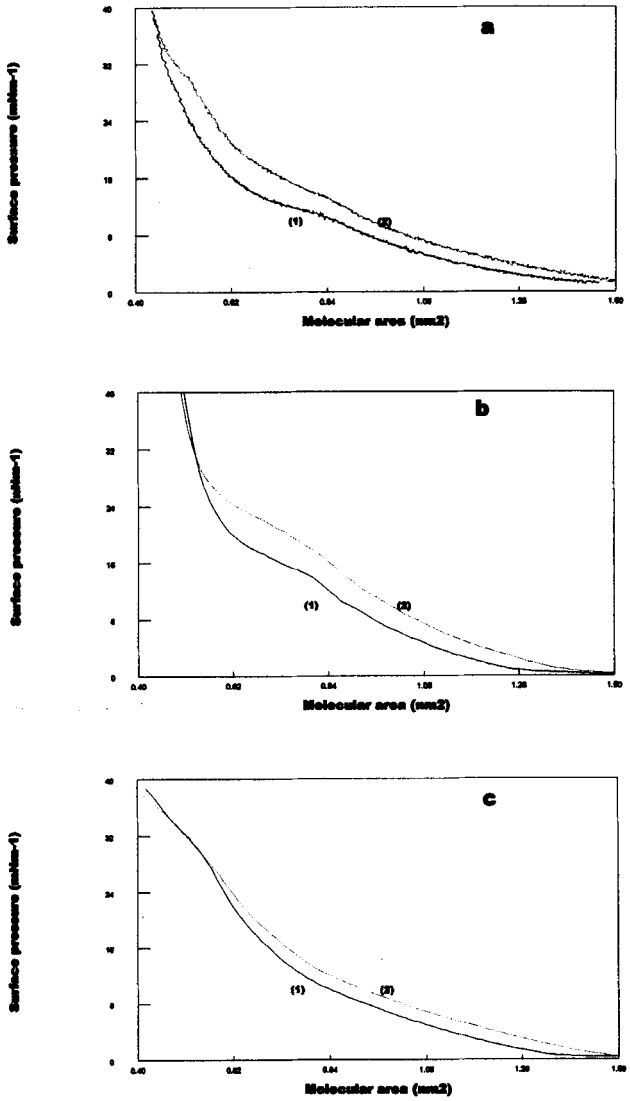

FIGURE 3 Compression isotherms on Hepes buffer (pH 7.4) of pure DPPC (1), mixed film LAS-Na-DPPC, $\rho = 0.1$ (2) ; **a** 22 °C, **b** 30 °C, **c** 40 °C.

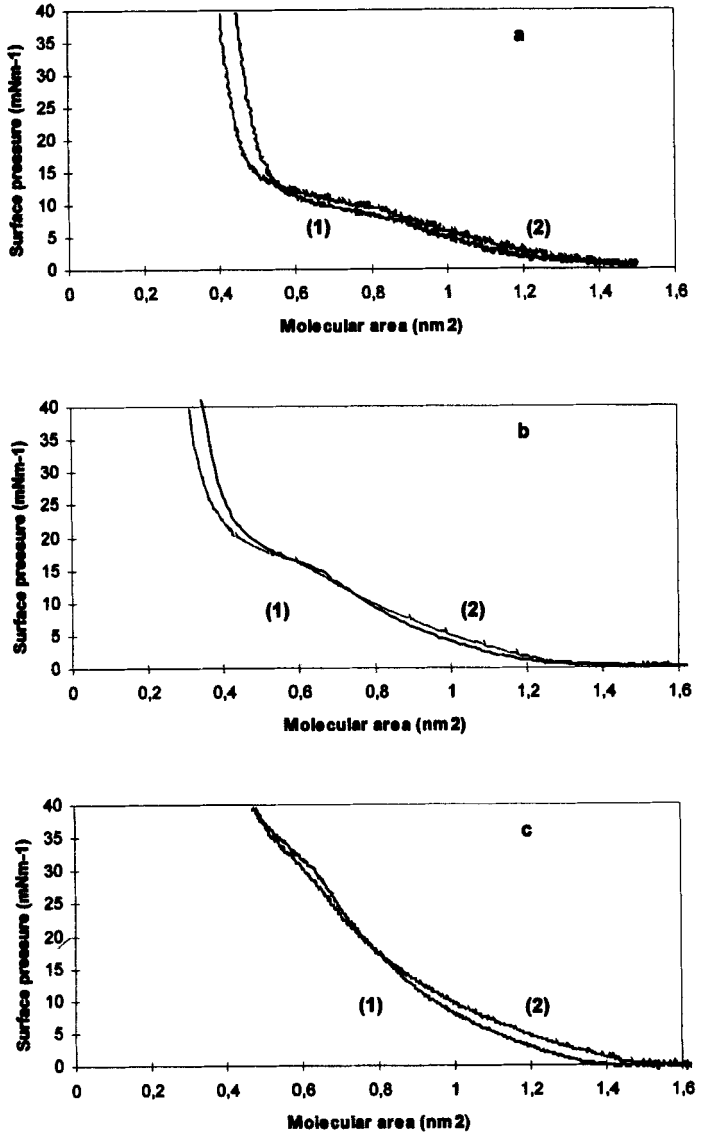

FIGURE 4 Compression isotherms on Hepes buffer (pH 7.4) of pure DPPC (1), mixed film MON-Na-DPPC, $\rho = 0.1$ (2) ; **a** 22 °C, **b** 30 °C, **c** 40 °C.

Each π-A diagram also contains the corresponding compression isotherm of pure DPPC since slight variations easily occur due to uncontrollable experimental variations. Therefore lipid and antibiotic/lipid mixtures have to be compared under conditions as close as possible.

The compression isotherm of pure DPPC moves to higher surface pressure for given molecular areas as the temperature is raised. This is a behavior encountered on other aqueous subphases (12).

The main purpose of this study was not only to investigate the temperature and buffer effects, but also to relate the results to bilayers under packing conditions as close as possible.

There are efforts in the literature to define an equivalent surface pressure where the molecule areas at the interface are similar in mono- and bilayers. Some authors report a value of 30 mNm^{-1} (13,14) and others 20 - 25 mNm^{-1} (15,16). It therefore seems likely that the part of the isotherm suitable for comparison with bilayer behavior is situated in the high pressure region.

When LAS-Na is incorporated in the DPPC monolayer, shown in Figure 3, the compression isotherms move to higher molecular areas for given surface pressures up to above 30 mNm^{-1} where they join the isotherm of the pure lipid and this is generally considered to represent a stage where the host molecule is expelled from the monolayer. The results in Figure 1 show that pure LAS-Na collapses at surface pressures below 20 mNm^{-1}. The lipid film thus contributes to maintain LAS-Na at the interface up to considerably higher surface pressures.

The results have been expressed quantitatively as the area occupied by the antibiotic molecule at given surface pressure as follows:

$$S_A^* = \frac{S_{LA}(N_A + N_L) - S_L N_L}{N_A} = S_{LA}(1 + \frac{1}{\rho}) - \frac{S_L}{\rho}$$

In this relation, N_A is the number of antibiotic molecules and N_L the number of lipid molecules. S_{LA} is the mean molecular area of the antibiotic/lipid mixture and S_L the molecular area of the lipid. The S_L value is

obtained directly from the compression isotherm of the pure lipid and S_{LA} after correction of the values from the experimental plot antibiotic/lipid which is a mixture with the same number of lipid molecules as the reference isotherm (ex. for $\rho = 0.1$, S_{LA} = molecular area of the mixture on the experimental plot divided by 1.1). S_A is the molecular area of the antibiotic obtained from the compression isotherms of the pure antibiotic in Figure 1.

The numerical values for the evolution of the molecular areas when the temperature is raised are given for 14 mNm^{-1}, chosen to represent the high surface pressure domain and given in table 1. At this level the value of S_A^*/S_A ratio can still be expressed whereas at 20 mNm^{-1} and above, the pure antibiotic film is collapsed so that no value for S_A is available.

TABLE 1 At 14 mNm^{-1}, occupied areas S_A^* of LAS-Na in mixed films with DPPC for $\rho = 0.1$ and molecular areas S_A of pure LAS-Na. Subphase: Hepes buffer 10 mM, sodium chloride 145 mM, pH 7.4.

Température (°C)	S_A^* (nm^2)	S_A (nm^2)	S_A^*/S_A
22	1.34	0.70	1.90
30	0.90	0.74	1.2
40	0.50	0.40	1.2

At 14 mNm^{-1} the molecular areas of LAS-Na in the lipid film mixture S_A^* are above the values of the areas of the pure antibiotic molecule S_A. This has been shown as due to repulsion between guest and host molecules at room temperature (7).

As seen, both S_A^* and S_A tend to become lower when the temperature is increased. However, the relative value S_A^*/S_A also decreases. This may indicate that the molecules become closer to each other as a consequence of deeper penetration into the monolayer.

Our conclusion is that LAS-Na is well maintained in the monolayer up to the region where the molecular packing is similar to the one in bilayers. The molecules remain in the film up to surface pressures well above those at

which the pure compound can exist as a monolayer and within the range of temperature concerned in our study. The raise in temperature might also contribute to better penetration of the drug into the membrane.

As seen from Figure 4, MON-Na affects the DPPC monolayer at low surface pressures, more at room temperature than above. At surface pressure up to about 10 mNm^{-1}, which is also the pressure limit for the film of pure Monensin, the host molecule is expelled. After expulsion the molecular areas become lower than the areas of the reference isotherm of pure lipid and this part of the isotherm vary from one experiment to another. We believe that there is simultaneous and uncontrollable loss of lipid molecules.

The results show that LAS-Na and MON-Na have similar effects on the DPPC monolayer in the low pressure region. The important difference in action on the lipid film takes place only above a certain pressure level. Since there is absence of effect of MON-Na on bilayer systems (17,18), we feel that the low pressure region cannot be compared to bilayer systems and that there is little sense in dicussing values from this part of the compression isotherms.

Moreover, these experiments show the importance of selecting packing situations as close as possible when comparing results from monolayers and bilayers.

References

[1] B.C. Pressman, *Fed. Proc.*, **27,** 1283–1288 (1968).
[2] J. Juillard, C.Tissier and G. Jeminet, *J. Chem. Soc. Faraday Trans. I,* **84** 951–958 (1988).
[3] F.G. Riddell and M.K. Hayer, *Biochimica et Biophysica Acta.* **817,** 313–317 (1985).
[4] N. Davion Van Mau and G. Amblard, *J. Colloid Interface Sci.*, **91,** 138–150 (1983).
[5] Jr. Ries, H. E., *Langmuir,* **6,** 883–885 (1990).
[6] H. Hasmonay, A. Hochapfel, A. Hadj-Sahraoui, M. Jaffrain and P. Peretti, *Thin Solid Films,* **210/211,** 747–749 (1992).
[7] H. Hasmonay, A. Hochapfel, C. Betrencourt, A. Tahir and P. Peretti, *Biochimica et Biophysica Acta,* **1193,** 287–292 (1994).
[8] A. Hochapfel, H. Hasmonay and P. Peretti, *Mol. Cryst. Liq. Cryst.,* **270,** 23–35 (1995).
[9] C. Betrencourt, A. Hochapfel, H. Hasmonay, P. Peretti, M. Ollivon and C. Madelmont, *Mol. Cryst. Liq. Cryst.,* **262**, 179–190 (1995).
[10] R. Lyazghi, Y. Pointud and J. Julliard, *J. Chem. Soc. Faraday Trans.* **88,**1017–1021 (1992).
[11] M. Hebrant, Y. Pointud and J. Juillard, *J. Phys. Chem.*, **95,** 3653–3662 (1991).
[12] M.C. Phillips and D. Chapman, *Biochimica et Biophysica Acta,* **163,** 301(1968).
[13] A. Blume, *Biochimica et Biophysica Acta* **557,** 32 (1979).
[14] R.A. Demel, W.S.M. Geurts van Kessel, R.F.A. Zwaal, B. Roelofsen and L.L.M. Van Deenen, *Biochimica et Biophysica Acta,* **406,** 97 (1975).
[15] D.O. Shah and J. H. Schulman, *J. Lipid Res.*, **6**, 341 (1965).
[16] D. Papahadjopoulos, *Biochimica et Biophysica Acta.* **163**, 240 (1968).

[17] X.F. Aranda and J.C. Gomez-Fernandez, *Biochimica et Biophysica Acta,* **860,** 125–130 (1986).
[18] M.K. Jain and N.M. Wu, *J. Membrane Biol.,* **34,** 157–201 (1977).