This article was downloaded by: [Tomsk State University of Control Systems and Radio]
On: 18 February 2013, At: 11:24
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

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

Lasalocid and Monensin: Aggregation at the Lipid-Water Interface in Mixed Films

Ambjörg Hochapfel [a], Hans Hasmonay [a] & Pierre Peretti [a]
[a] Groupe de Recherche en Physique et Biophysique (EA 228), Université Paris V, 45 rue des Saints Pères, 75270, Paris, France
Version of record first published: 24 Sep 2006.

To cite this article: Ambjörg Hochapfel , Hans Hasmonay & Pierre Peretti (1995): Lasalocid and Monensin: Aggregation at the Lipid-Water Interface in Mixed Films, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 270:1, 23-35

To link to this article: http://dx.doi.org/10.1080/10587259508031012

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Mol. Cryst. Liq. Cryst., 1995, Vol. 270, pp. 23–35
Reprints available directly from the publisher
Photocopying permitted by license only

© 1995 OPA (Overseas Publishers Association)
Amsterdam B.V. Published under license by
Gordon and Breach Science Publishers SA
Printed in Malaysia

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

Lasalocid and Monensin: Aggregation at the Lipid-Water Interface in Mixed Films

AMBJÖRG HOCHAPFEL, HANS HASMONAY and PIERRE PERETTI

Groupe de Recherche en Physique et Biophysique (EA 228), Université Paris V, 45 rue des Saints Pères, 75270 Paris, France

(Received December 22, 1994; in final form February 6, 1995)

Insertion properties of the antibiotics Lasalocid and Monensin sodium salts (LAS-Na and MON-Na) in Langmuir monolayers of two different lipid molecules, dipalmitoylphosphatidylcholine (DPPC) and octadecanol (C18OH), have been investigated. Results from compression isotherms of the films on pure water and on a biologically buffered subphase have been compared. Values of the molecular areas of mixed films of antibiotic/lipid and of the molecular areas of the pure lipids, at a given surface pressure, have been used to calculate the aggregation number k of the solute molecules in a concentration range of ρ (moles of antibiotic/moles of lipid) from 0.005 to 0.1. The k number in the film of DPPC increases when ρ increases, it is enhanced on the buffered subphase compared to the subphase of water and it is more important for MON-Na than for LAS-Na. There is hardly any evidence of aggregation in the films of C18OH. The two lipid systems both have in common that the mixed films with LAS-Na on the buffered subphase are the most pressure resistant and the ones from which the antibiotic is the least easily expelled.

Keywords: Lasalocid, Monensin, aggregration, Lupid-water interface

INTRODUCTION

Monomolecular lipid films spread on the water surface provide simple models of the half biomembrane. Compression isotherms of Langmuir films (surface pressure Π versus molecular area A) characterize the physical state of the molecules in these models.

The function as cation carriers of Lasalocid and Monensin, antibiotics from the Nigericin family, is well known.[1,2]

In previous papers,[3–5] we studied the effect of the two antibiotics as sodium salts (LAS-Na and MON-Na), on the compression isotherm of dipalmitoylphosphatidylcholine (DPPC), one of the main lipids present in the membrane. Some results were also given in one of these papers for octadecanol (C18OH) as the lipid component of the mixed film.[3]

We focused these studies on effects arising from variations in the antibiotic concentration (expressed as the ratio ρ = moles antibiotic/moles lipid) going from the lowest possible ρ values limited by the precision of the method of measurements. These effects were expressed as the modifications of the area occupied by each antibiotic molecule in the mixed film at a given surface pressure where the molecules undergo the same physical strain, chosen in a range where the molecular areas of the mixed films deviated the most from that of the pure lipid. We observed a decrease in the value of this area at

the DPPC/water interface as the ρ values increased, the magnitude of the variations depended on the subphase and they were different for the two antibiotics.[4,5] Possible insertion mechanisms were suggested and discussed in relation to microcalorimetric measurements on similar bilayer systems.

In the present investigation, we have used measurements from the former studies together with new results in order to examine the possibility of an aggregation process for the antibiotic molecules as one insertion mechanism in the monolayer. The effects have been expressed through calculation of the aggregation number.

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

EXPERIMENTAL PART

The antibiotics as sodium salts were purchased from Sigma Chimie. Lasalocid (LAS-Na) was recrystallized in aqueous methanol and acetone, m.p. 169–172 °C. Monensin (MON-Na) was recrystallized in aqueous methanol and diethyloxyde, m.p. 260–264 °C. *L*-α-Dipalmitoylphosphatidylcholine (DPPC) was of the purest available quality from Sigma Chimie. Octadecanol was purchased from Merck and recrystallized twice in acetone. The solvents were of analytical grade from Prolabo. They were distilled on molecular sieve for moisture removal. The subphase was water treated on an Elgastat UHQ 2 system (resistivity of 18 MΩ cm), pH 5.7, or water containing HEPES buffer 10 mM, sodium chloride 145 mM, pH 7.4, compounds purchased from Sigma Chimie.

Stock solutions of LAS-Na, MON-Na and DPPC, $5\,10^{-4}$ M, were prepared in chloroform-hexane 2:3 *v/v* and mixed in the different molar ratios. Solutions containing the antibiotics were kept in silanized glass vessels. Volumes representing a molecular area for the phospholipid of 1 nm^2 at a film area of 150 cm^2, were spread on the subphase. The compression was performed at a barrier speed representing 0.15 $nm^2\,min^{-1}$ of the molecular area.

Stock solutions at a concentration of 10^{-3} M of the antibiotics and C18OH were prepared and spread as above in volumes representing a molecular area of 0.5 nm^2 for the alcohol at a film area of 150 cm^2. The compressions were performed at the same barrier speed as for the phospholipid mixtures representing 0.075 $nm^2\,min^{-1}$ of the molecular area.

The experiments were performed at 22°C using a Lauda RM6 thermostat. The solutions were spread with a Hamilton CR-700-200 syringe. The isotherms were measured with a Krüss film balance using a pendulum 100 and recorded with an IBM-AT computer.

Only experiments resulting in exactly the same isotherms at least twice, were taken into consideration. The reproducibility of the reference isotherms of DPPC was checked before and after having recorded the isotherm of each mixed film.

RESULTS

The compression isotherms of the mixed films have been measured for the following concentrations: $\rho = 0.005$, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.1 of LAS-Na and

MON-Na in DPPC and $\rho = 0.02, 0.04, 0.06$ and 0.1 in C18OH. The experiments were performed on pure water (pH 5.7) and on HEPES buffer (pH 7.4). These isotherms were compared to the isotherms of the pure lipid.

The Π-A isotherm of DPPC depends very strictly on the experimental conditions. It is therefore necessary to compare the specific reference isotherm of the lipid with the isotherm of the mixed film in each experiment (see Experimental part).

Some of the compression isotherms have been published in the previous papers. Therefore, and for comparison purposes, we have chosen to present the isotherms at the medium concentration of $\rho = 0.06$ in DPPC as examples, LAS-Na and MON-Na on the water subphase in Figures 1 and 2, and on a subphase containing HEPES buffer in Figures 3 and 4.

The Π-A isotherm of C18OH is much less subject to fluctuations than the isotherm of DPPC. Therefore, all the studied concentrations within the same series have been presented together, since the reference isotherm remained identical for all ρ values. Figures 5 and 5 represent LAS-Na and MON-Na on the water subphase and Figures 7 and 8 on the HEPES buffer for these mixed films.

The area S_A^* occupied by each antibiotic molecule in the mixed film has been calculated according to:

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

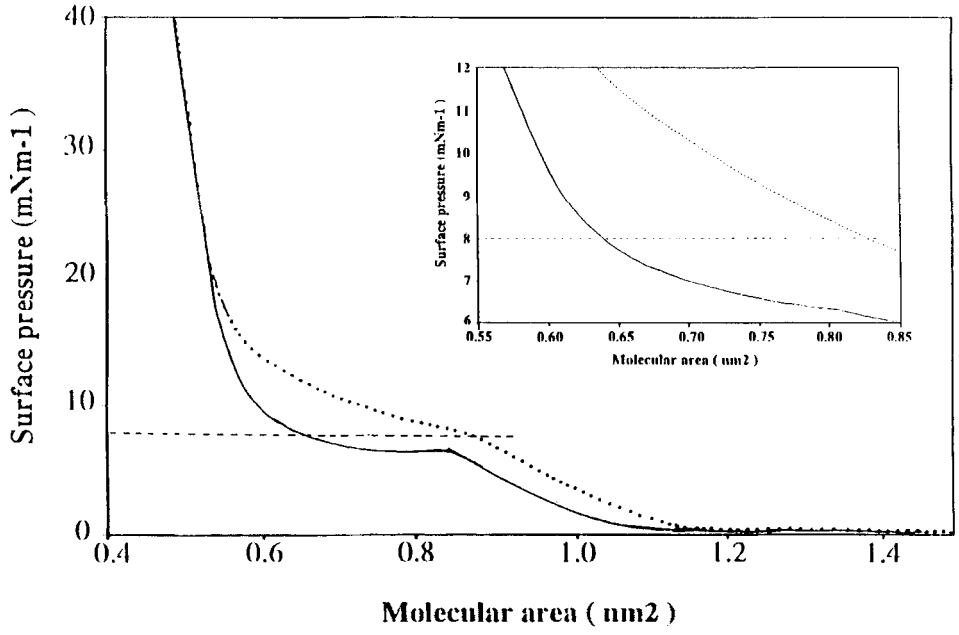

FIGURE 1 Compression isotherms of pure DPPC —— and of LAS-Na/DPPC, $\rho = 0.06$....., at 22 °C and at pH 5.7 on water. Insert: compression isotherm of pure LAS-Na under the same conditions. Dotted lines: surface pressure level used to calculate S_A^*.

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

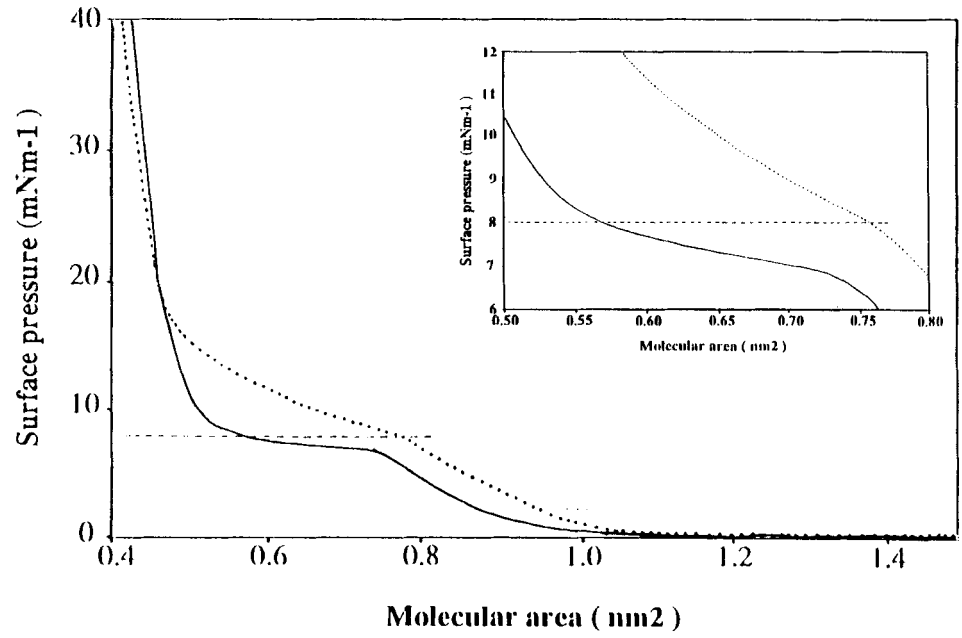

FIGURE 2 Compression isotherms of pure DPPC —— and of MON-Na/DPPC, $\rho = 0.06$....., at 22 °C and at pH 5.7 on water. Insert: compression isotherm of pure MON-Na under the same conditions. Dotted lines: surface pressure level used to calculate S_A^*.

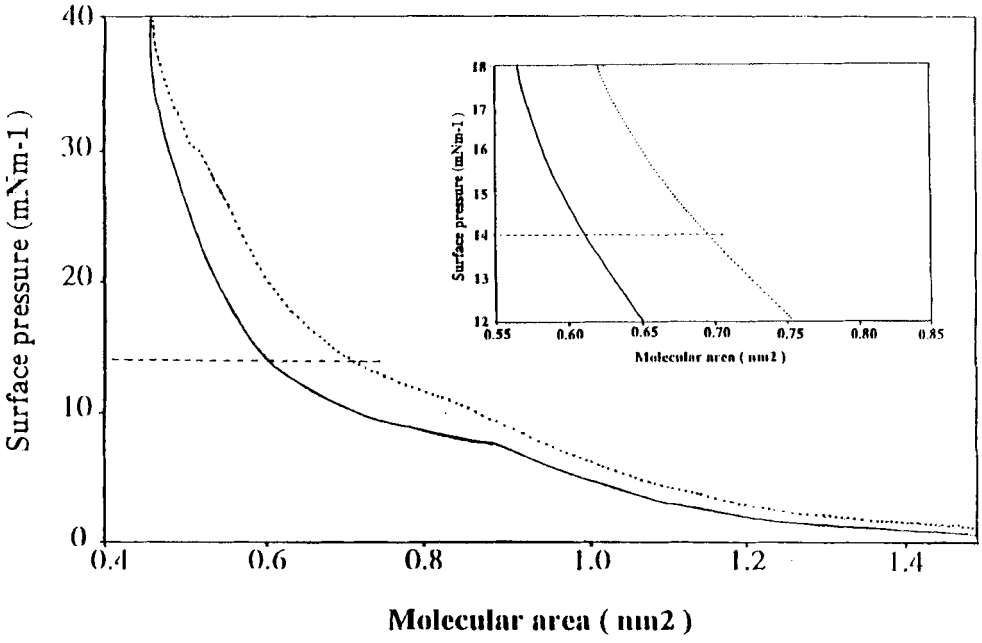

FIGURE 3 Compression isotherms of pure DPPC —— and of LAS-Na/DPPC, $\rho = 0.06$....., at 22 °C and at pH 7.4 on buffer HEPES 10 mM, NaCl 145 mM. Insert: compression isotherm of pure LAS-Na under the same conditions. Dotted lines: surface pressure level used to calculate S_A^*.

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

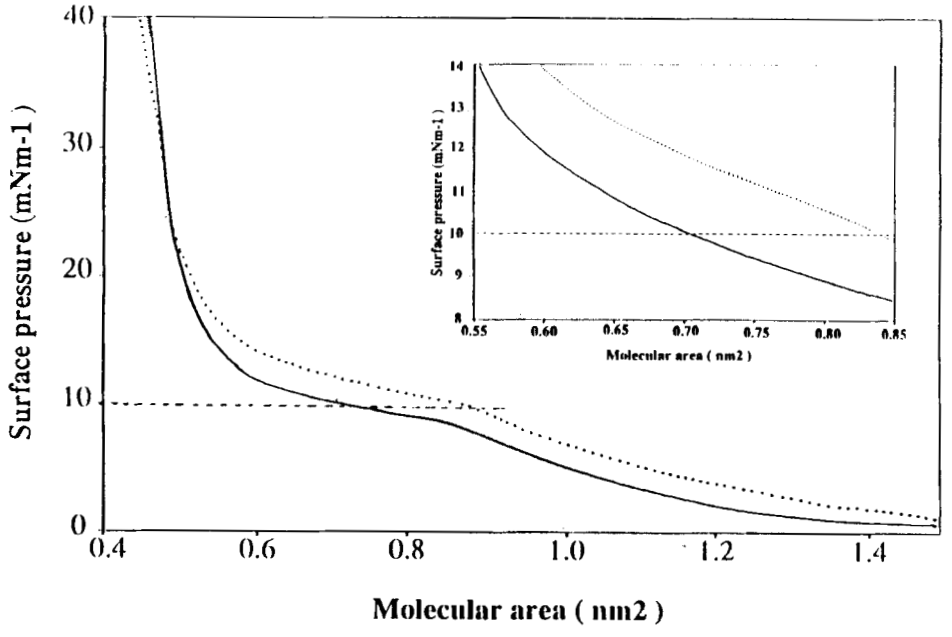

FIGURE 4 Compression isotherms of pure DPPC —— and of MON-Na/DPPC, $\rho = 0.06$....., at 22 °C and pH 7.4 on buffer HEPES 10 mM, NaCl 145 mM. Insert: compression isotherm of pure MON-Na under the same conditions. Dotted lines: surface pressure level used to calculate S_A^*.

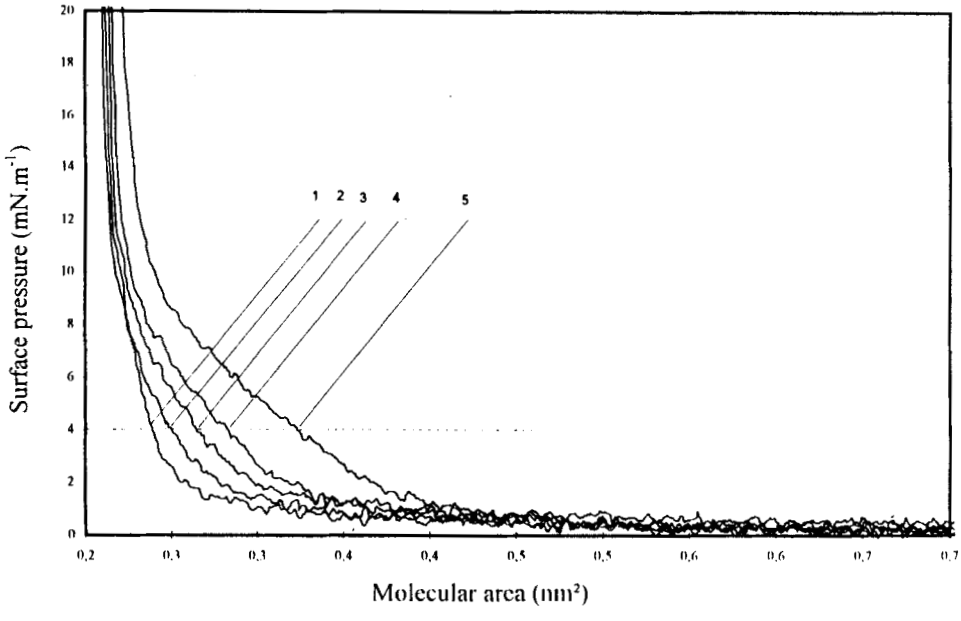

FIGURE 5 Compression isotherms of pure C18OH and of LAS-Na/C18OH, at different values of ρ, at 22 °C and at pH 5.7 on water. Curve 1: $\rho = 0$, curve 2: $\rho = 0.02$, curve 3: $\rho = 0.04$, curve 4: $\rho = 0.06$, curve 5: $\rho = 0.1$. Dotted line: surface pressure level used to calculate S_A^*.

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

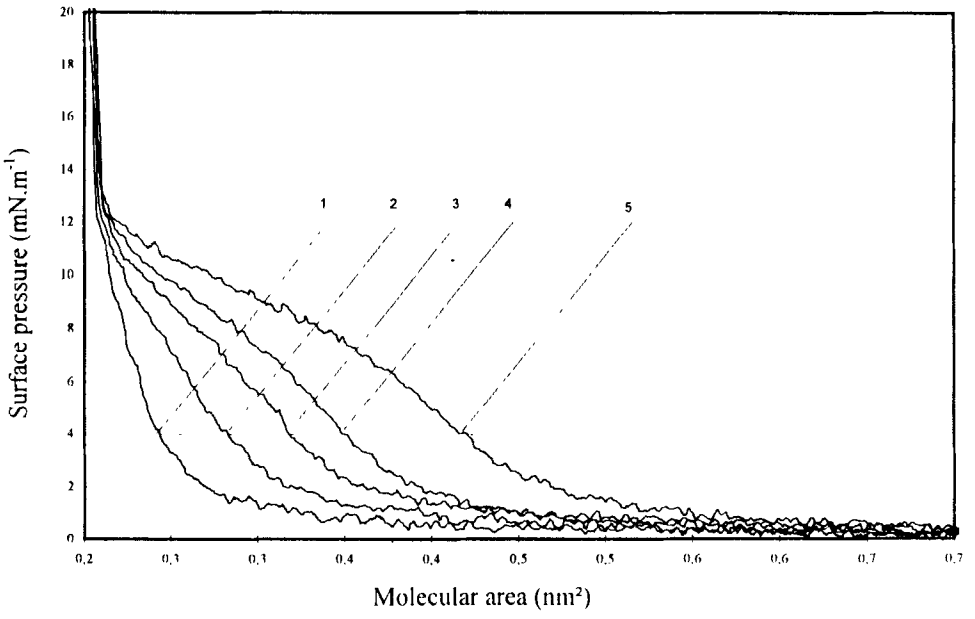

FIGURE 6 Compression isotherms of pure C18OH and of MON-Na/C18OH, at different values of ρ, at 22°C and at pH 5,7 on water. Curve 1: $\rho = 0$, curve 2: $\rho = 0.02$, curve 3: $\rho = 0.04$, curve 4: $\rho = 0.06$, curve 5: $\rho = 0.1$. Dotted line: surface pressure level used to calculate S_A^*.

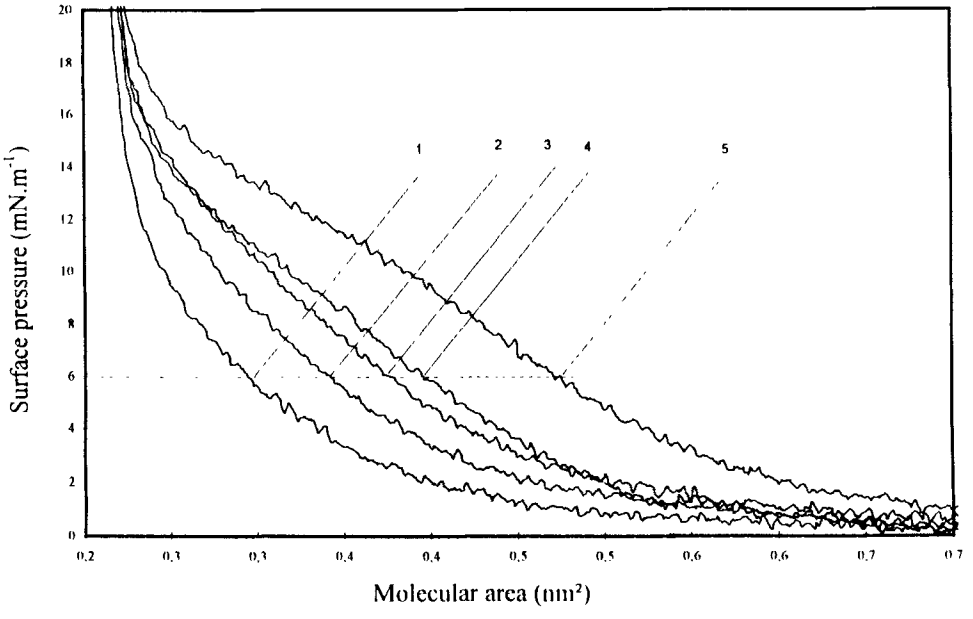

FIGURE 7 Compression isotherms of pure C18OH and of LAS-Na/C18OH, at different values of ρ, at 22°C and pH 7.4 on buffer HEPES 10mM, NaCl 145mM. Curve 1: $\rho = 0$, curve 2: $\rho = 0.02$, curve 3: $\rho = 0.04$, curve 4: $\rho = 0.06$, curve 5: $\rho = 0.1$. Dotted line: surface pressure level used to calculate S_A^*.

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

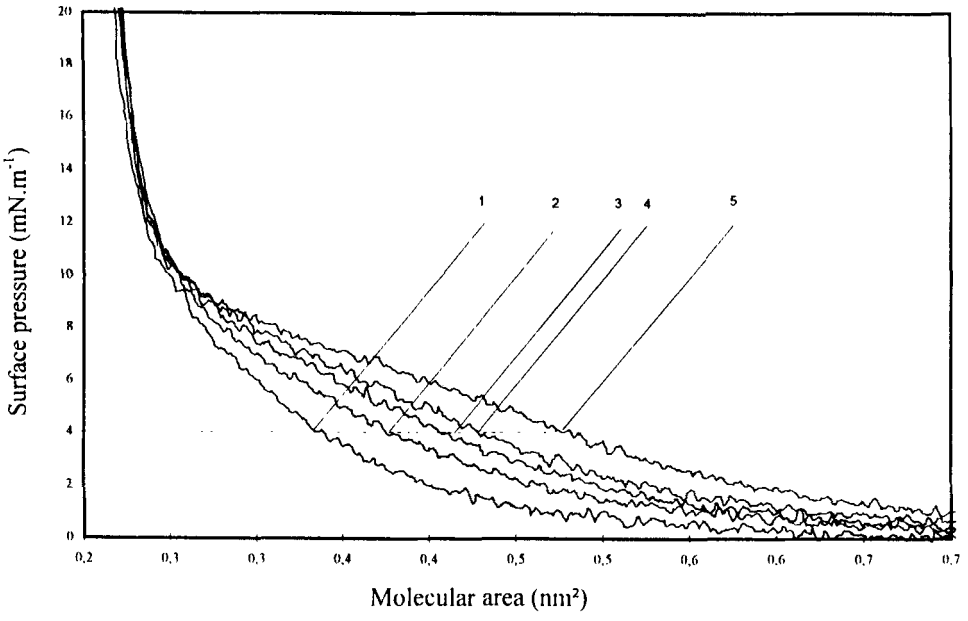

FIGURE 8 Compression isotherms of pure C18OH and of MON-Na/C18OH, at different values of ρ, at 22 °C and at pH 7.4 on buffer HEPES 10 mM, NaCl 145 mM. Curve 1: $\rho = 0$, curve 2: $\rho = 0.02$, curve 3: $\rho = 0.04$, curve 4: $\rho = 0.06$, curve 5: $\rho = 0.1$. Dotted line: surface pressure level used to calculate S_A^*.

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. S_L 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 represents a mixture with the same number of lipid molecules as the reference isotherm (ex. for $\rho = 0.06$, $S_{LA} = S_{LA}$ observed/1.06). S_A is the molecular area of the antibiotic obtained from the compression isotherms of the pure antibiotics in Figure 9a, b, c, d.

The surface pressure[5] at which S_A^* has been determined, are indicated as dotted lines on the compression isotherms of the figures.

The molecular areas used to calculate S_A^* for the different mixtures antibiotic/DPPC have been taken from enlarged domains of the compression isotherms, seen as inserted parts in Figures 1, 2, 3 and 4. The results are shown in Table I.

Graphs of S_A^* as a function of ρ were presented in the previous communications.[2,3] The calculations were made with values of molecular areas taken directly from the standard scale isotherms. The magnitude of S_A^* in Table I is in some cases not exactly the same as earlier published. We believe that the values used in this study are more accurate, since they have been obtained from magnified parts of the graphs. However, the trend of variations is the same as before.

The values of S_A^* for the antibiotic/C18OH mixture are shown in Table II.

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

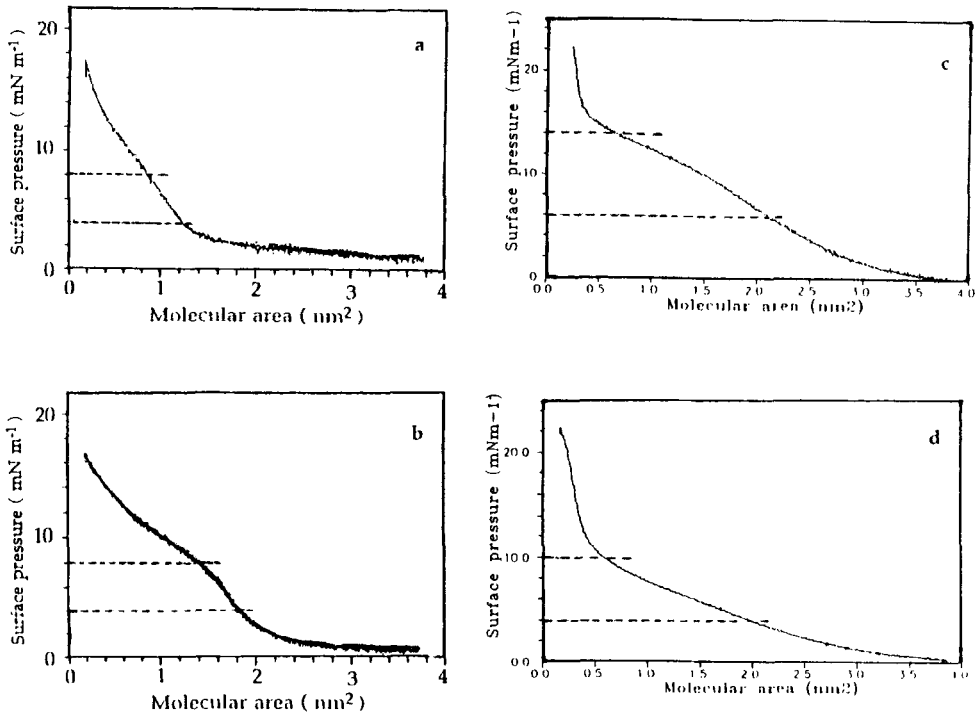

FIGURE 9 Compression isotherms, on water pH 5.7 a) of pure LAS-Na, b) of pure MON-Na and on buffer HEPES 10 mM, NaCl 145 mM pH 7.4 c) of pure LAS-Na, d) of pure MON-Na, at 22 °C. Dotted lines: surface pressure levels used to calculate S_A.

DISCUSSION

If each antibiotic molecule were surrounded by lipid molecules, which would be likely in the situation of great lipid excess, S_A^* would not change with ρ when the molecules underwent the same external strain from surface pressure and temperature.

The experimental results show that this is not true for the mixed films antibiotic/DPPC, since S_A^* decreases considerably as ρ increases. Repulsive forces must be responsible for the high values of S_A^*.[2]

There are several possible reasons for the decrease in the area occupied by the antibiotics at the interface when the concentration increases. Part of the molecules might penetrate the lipid layer and change their orientation, or the molecules might form aggregates from which the distance to the lipid is nearly the same as from the isolated antibiotic molecule.

We have made the assumption that the antibiotic molecules from aggregates at the lipid-water interface and we have calculated the mean number k of molecules in each aggregate based on two hypotheses:

- $k = 1$ at the lowest antibiotic concentration, $\rho = 0.005$.
- the distance d due to repulsion between the two different molecules is the same from

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

TABLE I

Aggregation number and molecular areas (in brackets) occupied by LAS-Na and MON-Na in mixed films antibiotic/DPPC, at various concentrations

Mixed film	Surface pressure	S_A	k $(S_A^*)\,nm^2$							
Subphase	mNm^{-1}	nm^2	$\rho = 0.005$	$\rho = 0.01$	$\rho = 0.02$	$\rho = 0.03$	$\rho = 0.04$	$\rho = 0.05$	$\rho = 0.06$	$\rho = 0.1$
LAS-Na DPPC Water	8	0.9	1 (4.44)	1.2 (4.06)		1.1 (4.23)	1.6 (3.50)	1.9 (3.23)	2.0 (3.12)	3.5 (2.46)
MON-Na DPPC Water	8	1.4	1 (9.48)	1.2 (8.52)	1.7 (7.05)	3.0 (5.23)	6.0 (3.80)	5.1 (4.11)	10.6 (3.13)	54 (2.09)
LAS-Na DPPC HEPES	14	0.65	1 (4.48)	2.0 (2.98)	2.6 (2.61)	10.6 (1.44)	8.9 (1.53)	8.9 (1.53)	11.1 (1.42)	13 (1.35)
MON-Na DPPC HEPES	10	0.6	1 (8.13)	1.9 (5.27)	8.0 (2.29)	13.0 (1.83)	8.3 (2.24)	12.5 (1.87)	10.1 (2.05)	102 (0.97)

TABLE II

Molecular areas occupied by LAS-Na and MON-Na in mixed films antibiotic/C18OH, at various concentrations

Mixed film / Subphase	Surface pressure mNm^{-1}	S_A nm^2	S_A^* nm^2			
			$\rho = 0.02$	$\rho = 0.04$	$\rho = 0.06$	$\rho = 0.1$
LAS-Na / C18OH / Water	4	1.2	0.8	0.6	0.8	0.9
MON-Na / C18OH / Water	4	1.8	2.0	1.9	1.8	1.8
LAS-Na / C18OH / HEPES	6	2.1	2.5	2.1	1.9	1.8
MON-Na / C18OH / HEPES	4	2.0	1.3	1.4	1.2	1.2

the surrounding lipid domain to the isolated antibiotic molecules as to the aggregate as indicated in Figure 10 where r is the radius of the antibiotic molecule, r_k the radius of the aggregate and S_{Aq} and S_{Aq}^* respectively the area of the aggregate unit and the area occupied by the aggregate unit in the DPPC film.

As a matter of fact, in the case of aggregate formation, the relation (1) can be written as:

$$\frac{S_{Aq}^*}{k} = \frac{S_{LA}(N_A + N_L) - N_L S_L}{N_A} = c \tag{2}$$

As seen in Figure 10, we can write the following expression:

$$S_{Aq} = \pi r_k^2 = k S_A = k\pi r^2, \quad \text{thus: } r_k = r\sqrt{k}$$

and

$$S_{Aq}^* = \pi(r_k + d)^2 = \pi(r\sqrt{k} + d)^2 \tag{3}$$

Elimination of S_{Aq}^* between (2) and (3), results in:

$$k = \frac{d^2}{\left(\sqrt{\frac{c}{\pi}} - r\right)^2}$$

The expression of c is the same as for S_A^* and the experimental values are those listed in Table I and II. Since there are different conceptions of the way the antibiotic molecules are implanted in the interface area, we have named c the mean area occupied

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

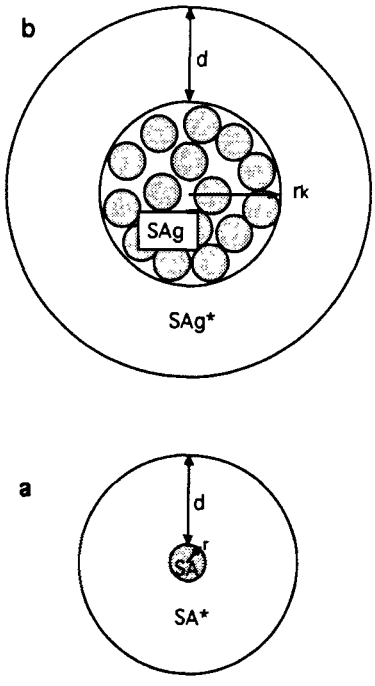

FIGURE 10 Models used to calculate the aggregation number k for the antibiotics in mixed films at the lipid-water interface. a) representation of the isolated antibiotic molecule for $k = 1$ in the mixed monolayer where S_A is the molecular area of the pure antibiotic and S_A^* the area this molecule occupies in the mixed film; d is the distance from the antibiotic to the surrounding lipid region. b) representation of aggregated antibiotic molecules for $k > 1$ in the mixed monolayer where S_{Ag} is the aggregate area and S_{Ag}^* the area occupied by the aggregate in the mixed film; d is the distance from the aggregate region to the surrounding lipid region. d is assumed the same in the two situations.

by molecules belonging to the aggregate and S_A^* the area occupied by isolated molecules in the mixed film. The antibiotic molecule radius r is calculated from S_A obtained from the compression isotherms of the pure antibiotics in Figure 9 and d is calculated from expression (3) for $k = 1$ ($\rho = 0.005$).

The experimental values used to calculate k at the selected surface pressure are the following on the pure water subphase: for LAS-Na at ($\Pi = 8\,\mathrm{mNm}^{-1}$) $r = 0.53\,\mathrm{nm}$, $d = 0.66\,\mathrm{nm}$ and for MON-Na ($\Pi = 8\,\mathrm{mNm}^{-1}$) $r = 0.67\,\mathrm{nm}$, $d = 1.07\,\mathrm{nm}$. On the subphase containing HEPES buffer, these values are: for LAS-Na ($\Pi = 14\,\mathrm{mNm}^{-1}$) $r = 0.45\,\mathrm{nm}$, $d = 0.74\,\mathrm{nm}$ and for MON-Na ($\Pi = 10\,\mathrm{mNm}^{-1}$) $r = 0.44\,\mathrm{nm}$, $d = 1.17\,\mathrm{nm}$.

The calculated values of k for the antibiotics in the DPPC monolayer are given in Table I. They are in good agreement with the hypothesis of aggregation. We do not pretend to have determined the number of molecules in the aggregate with precision, but we believe that they disclose an aggregation tendency, more important for MON-Na than for LAS-Na, and that the tendency is enhanced in the buffered subphase compared to the subphase of water. As seen from Table I, the k values at $\rho = 0.1$ for LAS-Na and MON-Na are respectively: 3.5 and 54 on water, 13 and 102 on buffer HEPES.

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013

The results agree with our DSC measurements on multilamellar bilayer suspensions of the antibiotic-DPPC mixtures[5] and also with results from the literature.[6,7] MON-Na does not affect the enthalpy and the temperature onset of the gel-liquid crystal phase transition. This must signify that the molecules remain at the polar extremity of the lipid layer where they form aggregates according to our results. LAS-Na, on the contrary, lowers the transition temperature and broadens the enthalpy peak of the lamellar phase, this means that the antibiotic molecules must be situated to some extent inside the lipid bilayer. Fewer molecules capable of forming aggregates must then remain at the interface. This is an explanation for the much lower values of the aggregation number k for LAS-Na than for MON-Na as shown in the present study.

The results given in Table II do not indicate significant aggregation of the antibiotic molecules in the monolayers of C18OH. Comparable behavior is observed for the two different systems of mixed films: MON-Na/C18OH on water and LAS-Na/C18OH on HEPES buffer. The S_A^* values are close to those of S_A which might signify that the molecules are oriented in the same way in these monolayers as in the films of the pure antibiotics and that only weak interactions exist with the surrounding lipid molecules. There is, however, a small decrease of the S_A^* values as the ratio ρ increases.

In the two other film mixtures, LAS-Na/C18OH on water and MON-Na/C18OH on HEPES buffer, the values of S_A^* are inferior to those of S_A, so these molecules must expose smaller areas to the interface in these mixed films that they do in the films of the pure antibiotics. Moreover, the slight variations with ρ are not systematic.

As seen from Figures 6 and 7, these two systems have bulkier isotherms thus more pressure resistant mixed films, than the two others in Figures 5 and 8.

When the isotherm of the antibiotic/lipid mixture joins the reference isotherm of the pure lipid as the surface pressure increases, the antibiotic is considered being squeezed out of the layer. The Π value at this point represents therefore the expulsion pressure.

The antibiotic is expelled from the C18OH monolayer at 16 mNm-1 in the system of LAS-Na on HEPES buffer, whereas the surface pressure of expulsion is at about $10\,mNm^{-1}$ in the case of MON-Na on the buffered subphase and of both antibiotics on water. This point is common with the behavior of LAS-Na in the DPPC monolayer on the buffered subphase where the expulsion pressure is much higher than in the other systems.[5]

CONCLUSIONS

This study indicates that the antibiotic molecules LAS-Na and MON-Na aggregate at the lipid-water interface of DPPC monolayers. This is likely the insertion mechanism for MON-Na. For LAS-Na it might be part of the mechanism only, since the values of the aggregation number are lower than for MON-Na and since microcalorimetric measurements have shown that the molecules interact with the lipid chains in the case.

Our results disclose that the aggregation is specifically related to the nature of the lipid, because it seems to be absent in monolayers of C18OH. They further suggest that the extent of aggregation depends on the antibiotic molecule and on the subphase quality such as pH and ionic strength.

Behavior independent of aggregation is seen from this study. The system containing LAS-Na on the biologically buffered subphase is the one which is the most pressure resistant and from which the antibiotic molecule is the least subject to expulsion from the mixed film.

Acknowledgments

This work was supported by DRET (DGA) France.

References

1. B. C. Pressman, E. J. Harris, W. S. Jagger and J. H. Johnson , *Biochemistry* **58**, 1949 (1967).
2. H. H. Mollenhauer, D. J. Morré and L. D. Rowe, *Biochim. Biophys. Acta* **1031**, 225 (1990).
3. A. Hochapfel, H. Hasmonay, M. Jaffrain and P. Peretti, *Mol. Cryst. Liq. Cryst.* **215**, 221 (1992).
4. H. Hasmonay, A. Hochapfel, C. Betrencourt, A. Tahir and P. Peretti, *Biochim. Biophys. Acta* **1193**, 287 (1994).
5. C. Betrencourt, A. Hochapfel, H. Hasmonay, P. Peretti, M. Ollivon and C. Madelmont, *Mol. Cryst. Liq. Cryst.* (in press).
6. X. F. J. Aranda and J. C. Gomez-Fernandez, *Biochim. Biophys. Acta* **860**, 125 (1986).
7. M. K. Jain and N. M. Wu, *J. Membrane Biol.* **34**, 157 (1977).

Downloaded by [Tomsk State University of Control Systems and Radio] at 11:24 18 February 2013