Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl17

Thermodynamical and Structural Effects of Diltiazem on Lecithin Liposomes

A. Ambrosini [a], B. Dubini [a], L. Leone [b], M. G. Ponzi Bossi [a] & P. Russo [c]

[a] ISTITUTO DI FISICA MEDICA

[b] ISTITUTO DI MEDICINA SPERIMENTALE E CLINICA

[c] ISTITUTO DI CLINICA MEDICA UNIVERSITA' DI ANCONA

Version of record first published: 20 Apr 2011.

To cite this article: A. Ambrosini , B. Dubini , L. Leone , M. G. Ponzi Bossi & P. Russo (1990): Thermodynamical and Structural Effects of Diltiazem on Lecithin Liposomes, Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics, 179:1, 317-334

To link to this article: http://dx.doi.org/10.1080/00268949008055378

Mol. Cryst. Liq. Cryst., 1990, Vol. 179, pp. 317–334
Reprints available directly from the publisher
Photocopying permitted by license only

Printed in the United States of America

THERMODYNAMICAL AND STRUCTURAL EFFECTS OF DILTIAZEM ON LECITHIN LIPOSOMES

A.AMBROSINI[1], B.DUBINI[1], L.LEONE[2], M.G.PONZI BOSSI[1], P.RUSSO[3].

1 ISTITUTO DI FISICA MEDICA
2 ISTITUTO DI MEDICINA SPERIMENTALE E CLINICA
3 ISTITUTO DI CLINICA MEDICA
UNIVERSITA' DI ANCONA

Abstract In the present work the structural and thermodynamical modifications induced by the calcium antagonist drug diltiazem, on DPPC liposomes, considered as model of the lipid matrix of the cell membrane, have been studied, by using differential scanning calorimetry, x-ray diffraction and optical microscopy techniques.
Calorimetric scans and X-ray diffraction patterns show that, at higher concentration of diltiazem, the pretransition peak disappears, the main transition temperature decreases, the lamellar thickness increases and the chains, in the β conformation, are packed in a hexagonal undistorted lattice.
Finally for higher concentrations of diltiazem the liquid crystalline phase with melted chains seems to become hexagonal.

INTRODUCTION

Calcium antagonists have provided a new approach to the management of a wide spectrum of cardiovascular disorders. They all share a common mode of action: that of inhibiting calcium influx. However, the various calcium antagonists

differ from one another in terms of their chemistry, bioavailability, tissue specificity, potency and side effects[1]. Diltiazem (fig.1) is an active calcium channel blocking agent shown to be an effective and well-tolerated treatment for angina, hypertension and supraventricular tachy-arrhytmias[2].

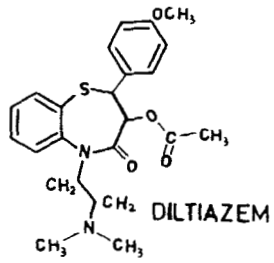

Figure 1. Chemical structure of diltiazem

It exerts other effects in addition to that of blocking slow channels. For example, it has local anaesthetic-like effects[3]. At high doses this drug suppresses the fast sodium inward current as well as the slow calcium inward current. Both of these properties could contribute to the ability of diltiazem to slow atrio-ventricular conduction.

Moreover, a local anaesthetic-like effect of this drug may mediate the inhibition of platelet aggregation and this effect may mediate part of the therapeutic efficacy in patients with coronary artery disease.

Lastly, the membrane activity of diltiazem may be important to determine its toxicity following overdose[4].

For these reasons we have investigated diltiazem-DPPC mixtures in excess water by differential scanning calorimetry, in order to obtain information on the thermodynamical behaviour, and by x-ray diffraction, in order to find the structural modifications induced by diltiazem on the lamellar organization of DPPC.

MATERIALS AND METHODS.

Sample preparation

1-2-dipalmitoyl-3-sn-phopsphatidylcholine (DPPC) and diltiazem · HCl were obtained from SIGMA and used without further purification.

The lipid-drug mixtures were made in a chloroform solution, dried in a nitrogen flow and lyophilized. To calculate the molar ratio R (moles of diltiazem/moles of DPPC) we have taken into account the n-octanol/water partition coefficient of diltiazem · HCl, which is 19.4[5]: in fact, considering the solubility properties, n-octanol is commonly accepted as the best model of the biological membranes.

Distilled water was added in a water-DPPC weight ratio 3:1.

The liposomes were obtained by heating the aqueous mixtures above the chain melting temperature and by vortexing several times during some hours.

Calorimetry

A DSC2 Perkin Elmer calorimeter with related processor was used to investigate the thermodynamical properties of samples versus the molar ratio of the drug in DPPC. All the scans were obtained using a heating rate of 2.5 °K/min and for each molar ratio were made seven samples and each sample was run two or three times.

X-ray diffraction

X-ray diffraction profiles were obtained using a vertical powder diffractometer and some diffraction pictures of aligned and not aligned samples were obtained by using an Hentschel low angle camera. The x-ray source was a rotating anode generator Rigaku Denki RU300, and Ni-filtered Cu k_α radiation (λ =1.54 Å) was used. The sample temperature was controlled by an electronic device.

Optical microscopy

Optical observations were performed using a LEITZ ORTOLUX 2POL polarizing microscope, equipped with a Mettler FP52 hot-stage, to determine if the drug was all solubilized and to see if the mixtures were organized in a liposome system, or in stacks of lamellae, or in a hexagonal phase.

RESULTS

Differential scanning calorimetry.

Calorimetric heating scans were started at 200°K and stopped at 330°K. An endothermic ice melting

peak ensures that the samples are in excess water. The pure DPPC liposomes show two endothermic peaks (fig. 2a): one at 36°C, the so-called pretransition peak, due to an L_β, to P_β, phase transition, the other, the main transition peak at 41.5°C, due to the P_β, to L_α phase transition.

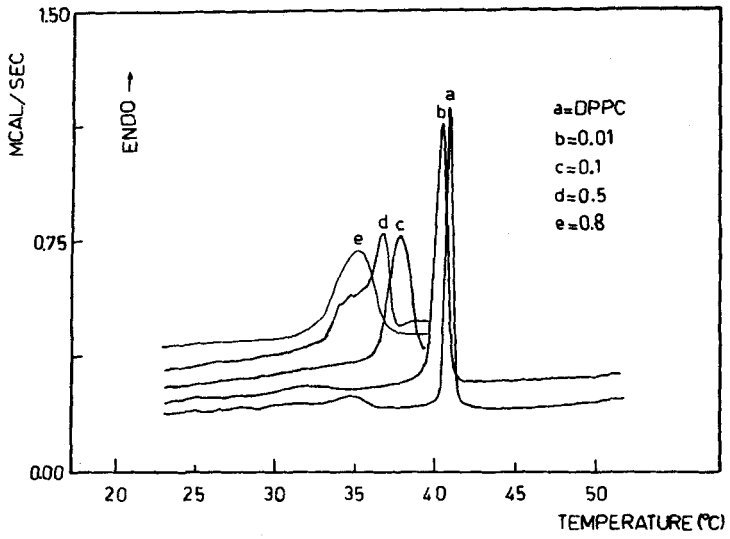

Fig.2 Calorimetric scans of DPPC-diltiazem mixtures, scan rate 2.5°C/min.

The heating scans of DPPC-diltiazem mixtures show, at molar ratio $R \leq 10^{-2}$, the same two peaks (fig.2b $R=10^{-2}$). The main transition temperature is almost constant, but the half height width shows a small increase; the former peak temperature decreases to 33°C ($R=10^{-2}$) and for molar ratio $R>10^{-2}$, only the chain melting peak appears, which becomes larger and shifts toward lower temperatures as the molar ratio increases (see an example in fig.2c for R=0.1). A shoulder

seems to appear on the main transition peak toward lower temperatures, at molar ratios between 0.4 and 0.5 (fig.2d R=0.5) . Figure 3 shows the behaviour of the transition temperature as a function of the diltiazem molar ratio.

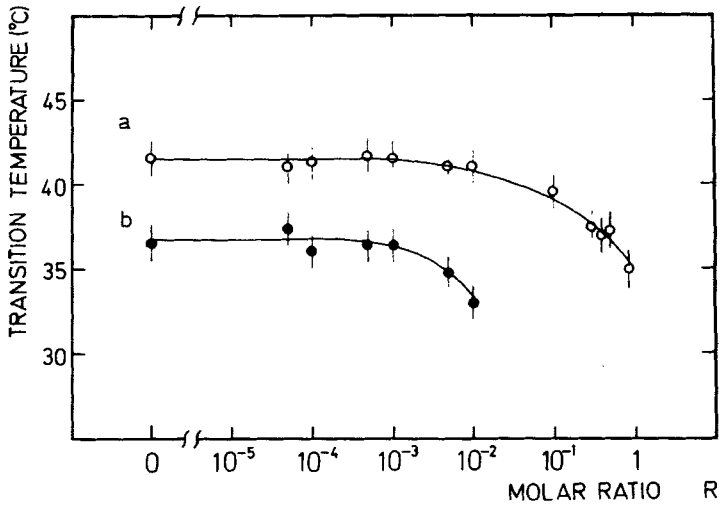

Figure 3. Transition temperature versus diltiazem molar ratio "R": a) melting chain transition, b) L_β, to P_β, phase transition.

The enthalpy of the main transition peak referred to the lipid plus drug weight is constant within the experimental uncertainty. The cooperative unit (CU) of the chain melting transition was calculated by using the relation[6]:

$$CU = \frac{4 \cdot R \cdot T^2}{\Delta T \cdot \Delta H}$$

where R is the gas constant, T is the transition temperature, ΔT is the peak width and ΔH the molar transition enthalpy, considered as the enthalpy of the lecithin alone. Figure 4 shows that the cooperative unit decreases from 120 (pure DPPC) to 55 (R=0.8), as the diltiazem molar ratio increases. The cooling scans show two additional peaks only for the saturated solution, R=0.8.

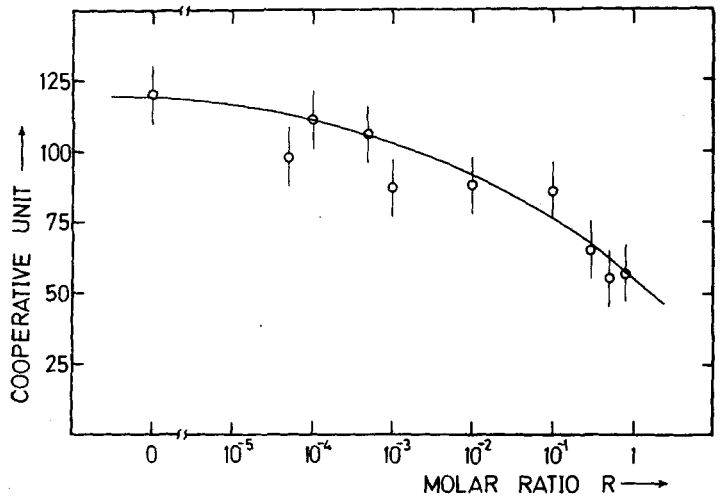

Figure 4. Cooperative unit CU versus diltiazem molar ratio "R".

X-ray diffraction

Vertical goniometer profiles and low angle pictures show that the phases of the lipid mixtures at room temperature are lamellar for any concentration of diltiazem (fig. 5 a,b,c).

In particular low angle pictures were taken to determine if the phase was lamellar or hexagonal,

namely to detect the diffraction first order of the lamellar phase.

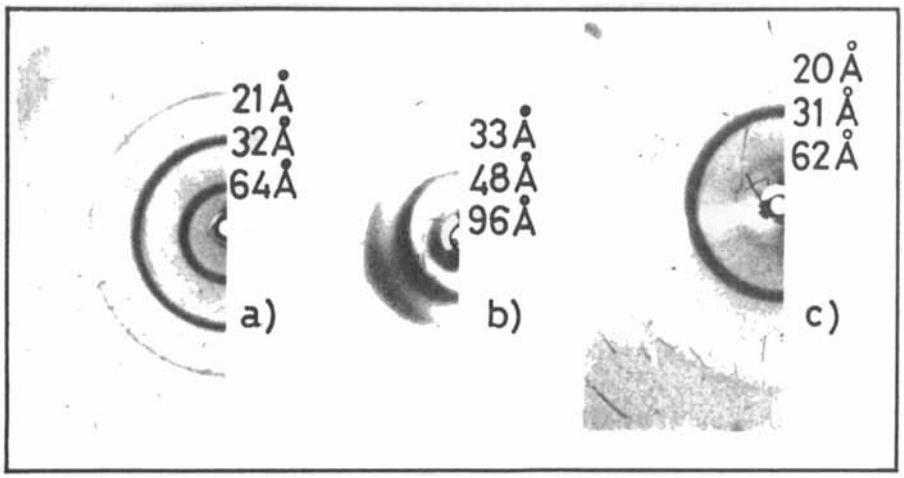

Figure 5. X-ray low angle pictures (enlargement 2 x 1):
a) R=0.001, b) R=0.3, c) R=0.8. T=25°C

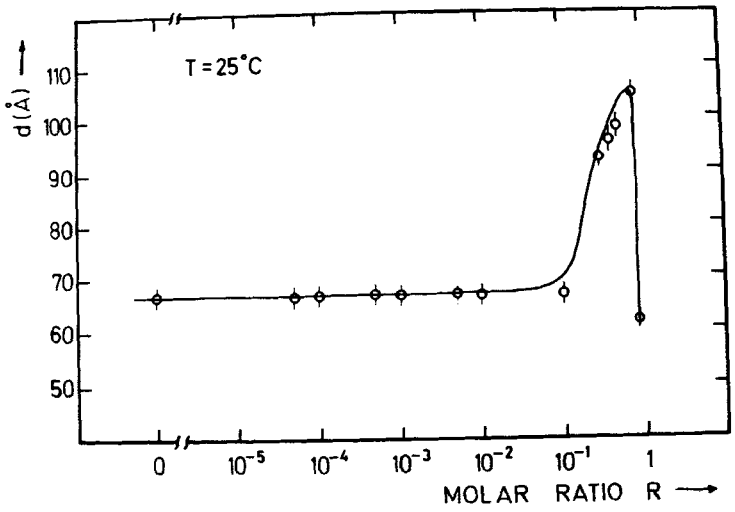

Figure 6. Lamellar repeat spacing, d, vs diltiazem molar ratio.

Diffraction profiles, from 2θ =1 to 2θ =35, were used to calculate the lamellar repeat

spacing and to investigate the high angle scattering.

For diltiazem molar ratios $0<R\leq10^{-2}$ (fig.5a and fig.6), the lamellar repeat spacing appears to be very similar to that of pure DPPC L_β, phase (d=64A); when $10^{-2}<R\leq0.5$, the repeat spacing strongly increases to a value of about 100Å (fig.5b and fig.6); whilst, at higher concentrations, it suddenly decreases almost to the normal value, (fig.5c and fig.6).

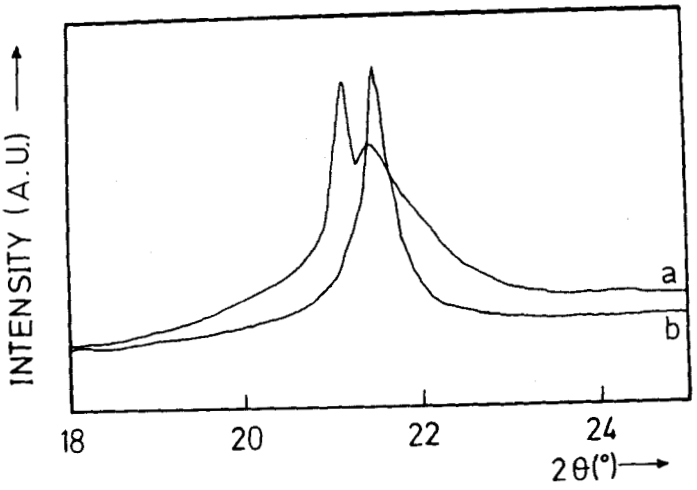

Figure 7. High angle x-ray diffraction:
a) R=0.01, b) R=0.5. T=25°C.

High angle patterns show that, for low molar ratios ($R\leq10^{-2}$), the phase is L_β,: in fact (fig.7a) two peaks appear, the one sharp and the other broader, corresponding to a distorted hexagonal arrangement of hydrocarbon chains as in pure DPPC L_β, phase. If $R>10^{-2}$ (fig.7b) only a

strong and sharp peak appears, shifted toward higher angles.

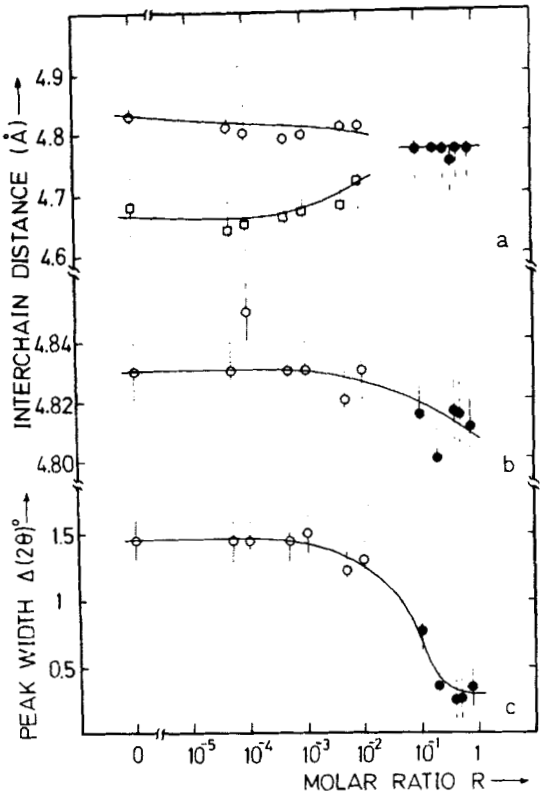

Figure 8. Interchain distances vs diltiazem molar ratio: a) T=25°C, b) the temperature is 2°C below the chain melting transition, c) full width at half maximum of the same high angle peaks reported in curve b.

Figure 8a reports the interchain distances ℓ_1 (empty circles), ℓ_2 (squares) and ℓ (filled circles) at T=25°C; ℓ_1 and ℓ_2 were calculated from the d_1 and d_2 Bragg values of the high angle peaks in the L_β, phase, by using the relations[7]:

$$d_2 / d_1 = 2\cos\alpha$$
$$\ell_1 = d_1 / \sin\alpha$$
$$\ell_2 = 2d_1 / \tan\alpha.$$

where α is the angle between ℓ_1 and ℓ_2.

Moreover ℓ was calculated by the equation $\ell = 2d/\sqrt{3}$ according to a regular hexagonal bidimensional lattice, as in pure DPPC P_β, phase. Figure 8b shows the behaviour of the interchain distances at a temperature 2°C below the chain melting transition, corresponding to each concentration and reported in Figure 3. The empty circles refer to the P_β, phase and the filled circles refer to the new phase. At lower concentrations the interchain distance is constant and at higher drug molar ratios it slightly decreases.

Figure 8c reports the full width at half maximum of the same peaks reported in Figure 8b: it appears that the high angle peak becomes sharper as the molar ratio increases from $R=10^{-2}$ to $R=0.8$.

The figure 9 shows some low angle pictures of the melted chain phases : $R=10^{-3}$ (a), $R=0.1$ (b), $R=0.5$ (c), and in figure 10 are reported the lamellar repeat spacings as a function of the diltiazem/DPPC molar ratio.

Low concentration samples ($R\leq10^{-2}$) adopt a lamellar phase similar to that of pure DPPC L_α phase, as shown in fig.9a ($d=72\text{Å}$, $R=0.01$). Intermediate concentration samples, $10^{-2}<R\leq0.3$, show a lamellar phase with a periodicity almost

twice as compared to the one of pure DPPC (fig.9b d=112 Å).

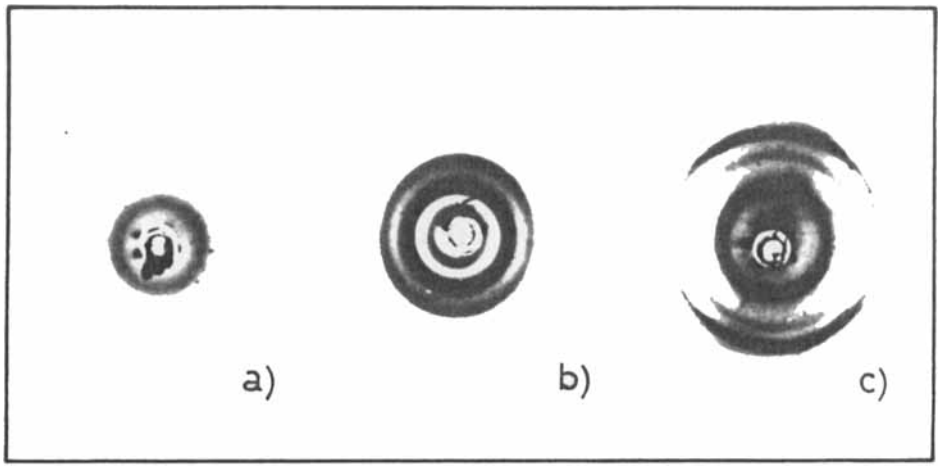

Figure 9. Low angle pictures at T=50°C (enlargement 2 x 1):
a) R=0.01, b) R=0.1, c) R=0.5.

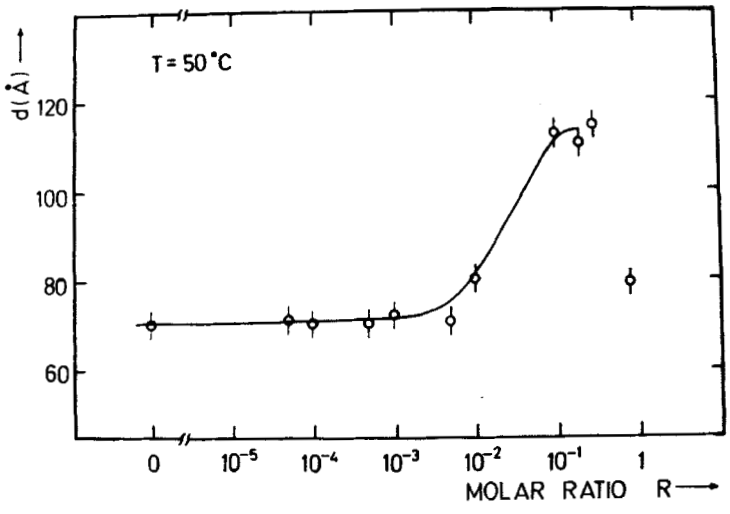

Figure 10. Lamellar repeat spacing, d, vs diltiazem molar ratio. T=50°C

Two higher concentration samples, R=0.4 and R=0.5, adopt a hexagonal phase (fig.9c). The three peaks shown have Bragg distances 58 Å, 34 Å and 29 Å, with the ratios $1:\sqrt{3}:2$.
Finally, at R=0.8, the phase seems to be lamellar with a periodicity of 79 Å, but with a very weak first order peak and a very strong and broad second order peak.
At all concentrations a diffuse wide angle peak indicates that the chains are melted.

Optical microscopy

Polarized light observations show that, for lower drug concentration samples, the textures suggest a liposome lamellar phase, while at $R \geq 0.1$ the textures are similar to those obtained with lamellar planar stacks[8], both at room and at high temperatures. The R=0.4 and R=0.5 samples show lamellar textures at room temperature but the phase with fluid chains appears almost pseudoisotropic with some clusters in a lamellar planar phase. The R=0.8 sample shows a texture of lamellar planar phase at any temperature, whereas some diltiazem crystals are present, at $R>0.8$, indicating that the saturated solution was reached in agreement with Cassidy et al[5].

DISCUSSION

The calorimetric results (fig 2 and 3), as in particular the decrease of transition

temperatures , the disappearance of the former peak and the decrease of the cooperative unit, suggest that diltiazem fluidises the bilayer. A relationship between the type of the transition profile and the drug localization in the bilayer was presented by Jain et al[9]. In our case the main peak behaviour can be referred to the so-called type C profile at lower drug molar ratios and to type A, then to type B at higher concentrations. Therefore, as indicated by Jain's results, the drug molecules at lower concentration would be localized in the region of the chain end, below the 9^{th} carbon atom, while at higher concentration they can be also localized in the C_1-C_8 region. At still higher concentrations, the presence of the shoulder suggests that diltiazem, localized also in the glycerol region, induces the appearance of another phase which coexists with the normal phase. It is interesting to observe a coherence in the data: in fact all changes in calorimetric and in structural curves appear at the same concentration, between 10^{-2} and 10^{-1}.

From the structural point of view, Figures 6 and 8a,b,c show that for $0.1 \leq R \leq 0.5$ the lamellar repeat spacing of the new gel phase, strongly increases as a function of drug concentration, the interchain distance slightly decreases and the full width at half maximum of the high angle peak strongly decreases. These facts suggest that the distorted hexagonal lattice transforms into an undistorted one, while the molecular tilt

angle disappears when more than 1 drug molecule is present every 10 lecithin molecules. Low angle pictures obtained with aligned samples show that in all the phases characterized by a single sharp high angle peak, the chains are perpendicular to the layers and fully elongated in the β conformation[10]. The loss of the chain tilt cannot be the only cause of the observed interlayer distance strong increase. In fact the saturated solution, R=0.8, adopts a lamellar phase with about the pure DPPC interlayer distance, even if with very different relative peak intensities. Also in this case only one sharp wide angle peak is present, indicating an ordered β conformation of the chains packed in a hexagonal lattice. However a still more important increase of the lamellar repeat spacing is observed in the melted chain lamellar phase L_α for the R=0.1 to R=0.3 concentration range (fig.10): the spacing appears to be almost twice as compared to the one of pure DPPC. Of course the effect of tilt removal cannot be claimed as the chains are melted. The increase of the lamellar repeat distances, induced by the drug presence, can be then related to a corresponding increase of the trapped water content.

Considering the melted chain phases observed at higher drug content, it is important to note that, by increasing the molar ratio above R=0.3, the lamellar phase Lα transforms to a hexagonal phase, whose unit cell parameter[11] is a=68 Å.

A possible explanation of this phase transition can be that the relatively large and short molecules of diltiazem are located as a wedge in the glicerol region (as confirmed by DSC data), in such a way to produce a layer curvature. For still higher concentration (R=0.8), the calorimetric cooling scan presents two new peaks, which suggest a phase coexistence. The X-ray diffraction patterns of the melted chain phase can be indexed as a one-dimensional lamellar lattice[11], but, unusually, the second order appears to be very broad and strong as compared to the first and to the third one.

The sequence lamellar-hexagonal-lamellar, observed at high temperature, with increasing diltiazem ratio, can be explained considering that, for very large diltiazem molar ratios, the drug concentration gradient along the chain direction, existing when the hexagonal phase occurs, vanishes or is strongly reduced. The behaviour of the diltiazem DPPC mixtures is similar to that obtained in our laboratory by using other drugs like propranolol and vitamin E. We are now studying the lamellar phase electron density profile of DPPC doped with diltiazem or propranolol in order to measure the trapped water content and the lipid layer thickness and to determine the position of the drug molecules inside the bilayer. A preliminary diltiazem-DPPC electron density profile for R=0.3 (d=114 A) suggests that the swelling of the bilayer is due to a strong trapped water thickness increase and

to a smaller lipid thickness increase. We are also planning to use neutron diffraction to obtain further information on this quite complicated system.

CONCLUSIONS

At higher molar ratio, diltiazem has a fluidising effect, lowering the main transition temperature. In the gel phase the lamellar repeat spacing increases and the chains are stiff and parallel, in the β conformation, and packed into an undistorted hexagonal lattice. In the L_α phase the repeat spacing is almost twice as large as that of pure DPPC L_α phase. The fluid phase becomes hexagonal, at R=0.4 and R=0.5, and it is again lamellar, at R=0.8.

For the sequence lamellar-hexagonal-lamellar, observed at high temperatures, with increasing diltiazem ratio, a possible explanation was given, in term of drug concentration gradients along the chain direction.

ACKNOWLEDGEMENTS

We would like to thank Mr. M.Pergolini, Mr. S.Polenta and Mr. E.Mosca for valuable technical assistance.

This work was supported by Regione Marche.

The necessary equipment was obtained on the basis of financial help of C.N.R. and M.P.I.

REFERENCES

1. T.T.ZSOTER, J.G.CHURCH, Drugs, 25, 93 (1983).
2. T.NAGAO, M.SATO, H.NAKAJIMA, A.KIYOMOTO, Jap. J. Pharmacol., 22,1 (1972).
3. M.CHAFFMAN, R.N.BRODGEN, Drugs, 29, 387 (1985).
4. J.A.HENRY, S.L.CASSIDY, Lancet, 1, 1414 (1986).
5. S.L.CASSIDY, P.A.LYMPANY, J.A.HENRY, J. Pharm. Pharmacol., 40, 130 (1988).
6. R.N.Mc ELHANEY, Chem. Phys. Lipids, 1, 445 (1982).
7. G.ALBERTINI, E.FANELLI, L.GUIDONI, F. IANZINI, P.MARIANI, F.RUSTICHELLI, V,VITI, Int. J. Radiat. Biol. 48, 785 (1985).
8. M.KLEMAN, C.COLLIEX, M.VEYSSIE, Lyotropic Liquid Crystals (Friberg, Washington, 1976), p. 71.
9. M.K.JAIN, N.M.WU, J. Membrane Biol., 34, 157 (1977)
10 A.TARDIEU, V.LUZZATI,F.C. REMAN, J. Mol. Biol. 75, 711 (1973).
11. V.LUZZATI, Biological Membranes (Chapman and Wallach, London and New York, 1968), p. 71