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Effects on phospholipid large unilamellar vesicles of two flavonoids: S-5682 and troxerutin

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ABSTRACT

The effects on phospholipid large unilamellar vesicles of two flavonoid derivatives: S-5682 (mixture of 90% diosmin and 10% hesperidin) and troxerutin (7,3',4'-tris(O-(2-hydroxyethyl)) rutin) have been studied by using quasielastic light scattering (QLS) and Fourier transform infrared spectroscopy (FT-IR) techniques. The results of QLS show a decrease of the Young elastic modulus of the phospholipid bilayer in the presence of these drugs and an effect on its phase transition by shifting the transition point and by inducing a second step in the transition. The results of FT-IR indicate an interaction of the drugs with the bilayer essentially at the aqueous interface. This interaction is consistent with the effects observed by QLS.

Keywords: Flavonids, Micronized Diosmin, Elasticity modulus, Quasielastic light scattering.

Abbreviations:
QLS: quasielastic light scattering
FT-IR: Fourier transform infrared spectroscopy
DMPC: Dimyristoylphosphatidylcholine
LUV: large unilamellar vesicles
REV: reverse phase evaporation
DSC: Differential Scanning Calorimetry
ESR: Electron Spin Resonance

INTRODUCTION

It is known that flavonoid compounds show many pharmacological, physiological and biochimecal activities [1-4], and are used as antioxidant [5] anti-inflammatory [6], hemorheologic [7] and phlebotonic drugs [8]. These compounds have a chemical structure derived from the parent compound flavone. One of the best known is diosmin. Among the properties of diosmin, the effects on the microcirculation [9] and the amelioration of the deformability and the stability of red cells are noteworthy [10]. There is some evidence that diosmin molecules are bound to phospholipid membranes. In order to understand the origin of the effects of diosmin on natural complex cell membranes, an alternative way is to study them on phospholipid model membranes.

The phospholipid model membranes used for this work were the large unilamellar vesicles prepared by the reverse phase evaporation method. For this purpose we used two physical techniques, quasielastic light scattering (QLS) and Fourier transform infrared spectroscopy (FT-IR). These techniques have been used to investigate the effects of several drugs on thermal behavior and to measure the elasticity modulus of phospholipid vesicles. The method

chosen for this work consists of using QLS to determine vesicle size in order to investigate thermal behavior and to measure the osmotic pressure-induced deformations. The change in size under the effect of osmotic pressure gradient allows determination of the elastic modulus and its modification in the presence of the drugs. By FT-IR spectroscopy we have located the interaction sites between the drugs and the lipid membranes.

MATERIALS AND METHODS

The drugs

We have studied the effects of two flavonoid compounds: S-5682 and troxerutin.

a) *S-5682* (Daflon 500®), was furnished by Servier (France), conditioned as a powder or an aqueous solution with cyclodextrin. It is a mixture of 90% diosmin and 10% hesperidin whose formulas are indicated in Figure 1b. There is only one difference between diosmin and hesperidin: a single bond in hesperidin instead of a double bond in diosmin between C2 and C3 of heterocycle. S-5682 is poorly soluble in water, so we have used it either in powder form or in aqueous solution containing cyclodextrin. The presence of cyclodextrin renders S-5682 more hydrosoluble. The drug shows two absorption bands in the spectral region 260-210 nm, which have been used to titrate the drug concentration after subtracting the contribution of cyclodextrin.

b) Troxerutin is a hemisynthetic derivative of rutin with ramifications in positions 3', 4', and 7 and another ramification at C3 of heterocycle (Fig. 1c). This is a slightly yellow powder, very soluble in water.

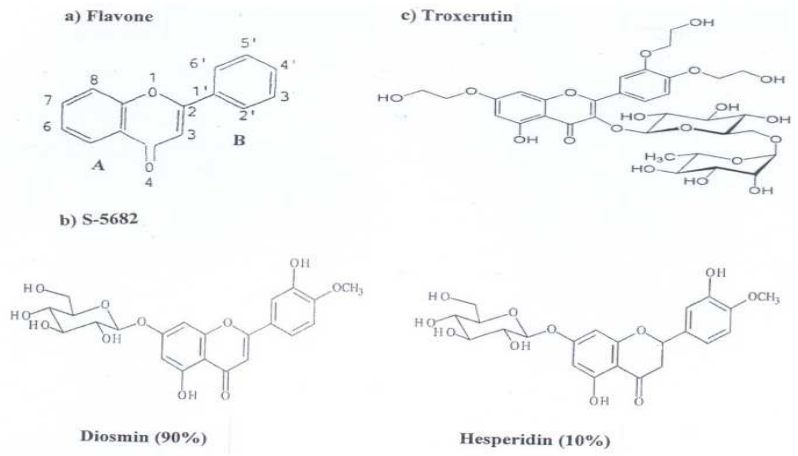

Figure 1: Molecular formula of the studied flavonoid derivatives:
a) The parent compound flavone; b) Diosmin and hesperidin. They differ by the single bond (for hesperidin) or the double bond (for diosmin) between C_2 and C_3 of the heterocycle; S-5682 is a mixture of 90% diosmin + 10% hesperidin; c) Troxerutin.

Lipids and reagents

Dimyristoylphosphatidylcholine (DMPC) was purchased from Sigma (St Louis, USA). The purity of the lipid, which was used without further purification, was checked by using thin layers chromatography. Other chemical agents used for the preparation of buffers were of analytical grade and purchased from Merck (Darmstadt, Germany).

Model membranes preparation

The model membranes used for this work are large unilamellar vesicles (LUV) of DMPC prepared in 150 mM NaCl, 10 mM tris, pH 8.3 aqueous buffers by the reverse phase evaporation (REV) method of Szoka and Papahadjopoulos as previously described [11]. This technique allows to obtain spherical unilamellar vesicles of about 130-160 nm in diameter as controlled by QLS and freeze fracture followed by electron microscopy. By successive centrifugations, a polydispersity factor of 0.10 to 0.20 was obtained. A low value polydispersity factor is necessary for a good determination of the elasticity modulus. Lipid concentrations were determined using colorimetric methods [12] and vesicles were stable within two weeks.

Quasielastic light scattering

Quasielastic light scattering (QLS) was performed on a photon-beating spectrometer operating at a 90° scattering angle. The correlation functions were obtained with a 128-channel Malven K7032 correlator interfaced with a calculator which allowed data analysis. Solutions were centrifuged and filled into cylindrical cells of 10 mm in diameter. Cells were fixed into a thermostated bath. The temperature of the samples was controlled to 0.1°C and

temperature measurements were obtained at discrete intervals with a waiting time of 20 mn. Each result is an average of 10 measurements. For QLS experiments the lipid concentration used was typically 0.3 $mg.mL^{-1}$ or 0.44 mM.

The measured correlation functions of the scattered intensities were analyzed using a second order cumulant development [13] which allowed determination of the average translational diffusion coefficient D and the polydispersity factor. The diffusion coefficient D is related to the hydrodynamic radius R_h via the Stokes-Einstein relation:

$$R_h = k_B T/6\pi\eta D \quad (1)$$

where η is the viscosity of the solvent at the temperature T. From the measurements of D or R_h, the phase transition of the membranes and their elasticity can be monitored.

a) Phase transition: It is well known that phospholipid vesicles undergo phase transitions when the temperature is changed. This transition point separates two phases, a liquid crystalline phase above the transition point and a gel-like phase below this temperature. The physical properties of membranes vary from one state to the other; in particular, the molecular arrangement of the hydrocarbon chains is modified. As a consequence, the area per head group changes too and thus the total area of the vesicles is altered depending on the temperature. By measuring the hydrodynamic radius R_h by QLS, one can monitor this phase transition. When cooling, the temperature-dependence plot of R_h shows a decrease of the size and the midpoint is defined as the transition point of the vesicles.

b) *Osmotic response and elastic modulus determination:* For this purpose, a swelling of vesicles was provoked by a gradient of osmotic pressure between the inside and the outside of the vesicles. Vesicle size before and after the swelling was measured by QLS. The pressure gradient was obtained by diluting the external medium by buffers without NaCl and it can be estimated in accordance with Van't Hoff's law. Once the swelling is observed and measured (expressed as the relative change $\Delta r/r$ of the vesicle radius r), the theory of thin spherical layers elasticity can be applied to determine the elastic modulus of the bilayers as outlined below.

When a NaCl concentration gradient C_{so}-C_{s2} is established between the inside and the outside of a vesicle, we assume that a) the osmotic response is a fast process so that the internal salt concentration immediately takes the corrected value $C_{sl0} = C_{so}(1 - 3\ \Delta r/r)$ and b) if there is leakage of NaCl, the leakage follows an exponential decay law, expressed by a decay rate k.

Under these conditions, the Young elastic modulus E can be calculated from the strain $\Delta r/r$ measured at time t using the formula:

$$E = [2RTr^2_0(1\text{-}2e/r_0)/4e\Delta r](C_{so}\text{-}\ C_S\ )[\exp(\text{-}kt)\ \text{-}3C_{s0}\Delta r/(C_{s0}\text{-}C_s)r_0] \quad (2)$$

where R is the ideal gas constant, T the absolute temperature, e the membrane thickness, r_o the initial external radius and Δr the change of r. The corrective term $-3C_{so}\Delta r/(C_{so}\ -C_s)r_o$ is due to the swelling and the exponential term describes the decay the salt concentration gradient due to the leakage.

FT-IR spectroscopy

Infrared spectra were obtained using a Perkin Elmer 1760 Fourier transform spectrophotometer with ZnSe as window material. Each spectrum is an average of several scans with a spectral path width of 1 cm^{-1}. DMPC unilamellar bilayers were prepared in 150 mM NaCl, 10 mM tris-HCl, pH 8.3, H_20 or $^2H_20$ buffers depending on the spectral region investigated. The lipid concentration used is typically 30-50 $mg.mL^{-1}$. The spectra were recorded at discrete temperatures with a waiting time of 15 mn between two subsequent spectra.

The peak heights and the wave numbers of some characteristic bands of LUV as well as their band widths were studied in order to locate the interaction with the drugs. The temperature-dependence of some characteristic wave numbers was used to monitor the effect on the phase transition of the bilayers.

RESULTS

Effect of the drugs on the elastic modulus of DMPC LUV

a) *The elastic modulus of* pure *DMPC LUV:* The effect of the osmotic pressure gradient on pure DMPC LUV is shown in Figure 2a. When the external salinity was diluted from the initial salt concentration of 150 mM NaCl, the hydrodynamic radius R_h increased, indicating a swelling of the vesicles. The salt was moderately diluted in order to

prevent the breakup of vesicles. At each state of the swelling, the Young elastic modulus was calculated according to the formula (2). In this calculation, the corrective term due to leakage has been estimated from the constant k deduced from the loss of the isotope ^{22}Na measured as described by Singer [14]. The effect of the ion permeability in the determination of the elastic modulus was also recognized by Rivers and Williams [15]. The Young modulus E is related to the stretching elastic modulus ks by the relation ks = eE, e being the bilayer thickness [16]. For pure lipid vesicles, the values of E (or ks) observed by QLS here are in the same order, although smaller, as those observed by micropipette aspiration experiments In the liquid crystalline phase, it is very close to that in DMPC vesicles having the same size observed by Sun [17] or to that of brush border vesicles in buffer containing 10 mM glucose.

b) *Effect* of the drugs: The swelling of vesicles in the presence of S-5682 is shown in Figure 2b. This figure, compared with Figure 2a, indicates unambiguously that in the presence of S-5682 the vesicles swell much more than in its absence. This swelling showed that the presence of the drug have decreased the elastic modulus or/and reduced the ion permeability of the bilayer. We have estimated the Young elastic modulus E in two extreme cases by using the formula (2). In the first case, we took exp(-kt) = 1, assuming a complete impermeability for the bilayer. In the second case, we took for k the value of pure lipid vesicles. The correction is very small for DMPC bilayers in the gel-like phase at 15°C. The values of E obtained by extrapolation to vesicles at osmotic equilibrium, in the absence and in the presence of the drug are given in Table 1. It show that in the presence of S5682 at drug/lipid molar ratio x = 0.1 the elastic modulus E of the DMPC bilayer, in the gel phase or in the crystalline liquid phase, is decreased.

Table 1- Effect of flavonoid derivatives on the elastic modulus of DMPC LUV membrane.
(Buffers : 10 mM tris, 150 mM NaCl, pH 8.3). x refers to the drug/lipid molar ratio.
(a) : Value obtained with k taken from pure lipid vesicles
(b) : Value taken with k = 0, in the hypothesis that the presence of the drug renders the the bilayers completely impermeable.

DMPC LUV	Young elastic modulus E (10^8 dyne/cm^2)	
	at 15°C	at 33°C
Pure lipid LUV	2,5	1,6 [(a)]
LUV + S-5682 (x = 0.1)	1,2	0,6[(a)] 1,05[(b)]
LUV + troxerutin (x = 0.1)	1,7	0,4[(a)] 0,72[(b)]

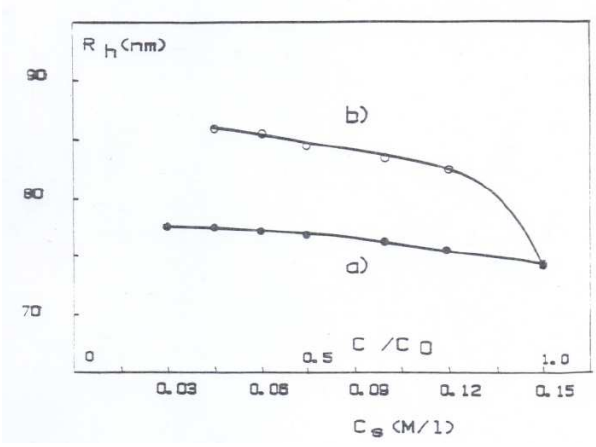

Figure 2: Effect of the osmotic pressure gradient on DMPC LUV a) in the absence and b) in the presence of S-5682 (drug/lipid molar ratio: x = 0.1).The plots represent the variation of vesicle radius when the external NaCl concentration C_s was diluted from the initial value 150 mM in 10mM tris, pH 8 aqueous buffer. This dilution of the salinity implies also a dilution of the vesicle concentration C which, in the studied range, did not affect the diffusion coeffident and the measured vesicle radius

Effect of the drugs on the phase transition of DMPC LUV

The phase transition of DMPC LUV is shown by the temperature-dependence of the hydrodynamic radius R_h (normalized to the value at 35°C of pure lipid LUV) (Fig. 3). In this figure, the plot (a) related to the pure lipid has been presented for reference. For DMPC LUV, a single transition is observed at 24°C with a decrease of R_h when the pure lipid bilayer undergoes the transition from the liquid crystalline state to the gel-like state. This is in good agreement with the transition point observed by different physical methods (DSC, ESR, IR, Raman spectroscopy, etc...) [18-21].

The effect of the studied drugs on DMPC LUV is depicted in Figures 3b-c. The plots correspond to DMPC LUV in the presence of S-5682 and troxerutin, respectively.

The effect of S-5682 is shown in Figure 3b. The plot represents the variation of R_h versus temperature at a concentration of this drug corresponding to a drug/lipid molar ratio x = 0.1. For this study the drug was inserted into the bilayer during the preparation by the REV procedure by mixing together the drug powder and the lipid in chloroform solution with the desired concentration (or molar ratio).

The drug S-5682 alters the phase transition of DMPC bilayers and induces a second step. By cooling a vesicle suspension with a lipid concentration of 0.3 mM, for a drug concentration of 0.02 mM, the first step of the transition occurs at 28°C. The size decrease in this step is 2%. The second step occurs at a lower temperature (22°C) and the vesicles undergo a transition to the gel-like state. When the drug/lipid molar ratio is increased, the transitions are widened so that at the drug/lipid molar ratio 1/3 there is only one very wide transition. With S-5682 a second step transition was observed. The first transition T_1 occurred at a temperature above 24°C and a second transition T_2 below 24°C.

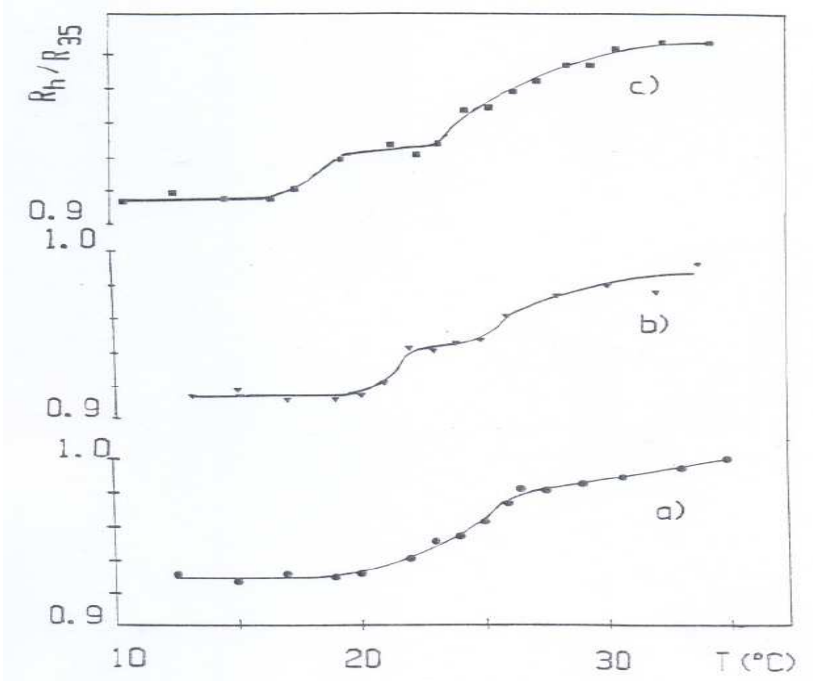

Figure 3: Comparative effect of S-5682 and troxerutin on the phase transition of DMPC LUV observed by QLS. The plots represent the temperature-dependence of the vesicle size in the absence of drug (a) or in the presence of: (b) S-5682: drug/lipid molar ratio x = 0.1; (c) Troxerutin: x = 0.2; lipid concentration: 0.3 3 mM

Infrared spectroscopy results

Fourier-transform infrared spectroscopy (FT -IR) was used in order to investigate the effect of S-5682 on vesicles at a molecular level. Three spectral regions of major interest are affected by the presence of the drug, corresponding to the vibration stretching modes of the ethylene CH_2 groups in the hydrophobe region, of the phosphoric PO_2^- groups in the polar heads and of the carbonyl C=O groups in the interface, respectively.

a) *Effect* on *the spectral region 3000-2800 cm^{-1}:* The wave numbers of the stretching vibrations of the ethylene groups in the spectral region from 3000 to 2800 cm^{-1} are very sensitive to conformational changes in the hydrocarbon chains. In particular, the strong absorption band at 2850 cm^{-1}, assigned to the symmetric stretching vibration of the CH_2 groups, shows temperature-induced shifts reflecting the change of the trans/gauche ratio of the acryl chain conformers. Temperature variation induces a shift of the CH_2 stretching band as shown in Figures 4a-c wave numbers near 2852 cm^{-1} are characteristic of conformationally disordered polyethylene chains with a high content of gauche conformers (generally observed in the liquid-crystalline state) while lower values are characteristic of ordered ethylene chains as found in the gel-like state[20,22]. For pure DMPC vesicles (Fig. 4a), the transition point near 24°C separates the two states, in agreement with the shift of this band.

In the presence of S-5682 this feature was changed (Figs. 4b and 4c). The liquid crystalline to gel-like phase transition was slightly shifted towards higher temperatures and a second step of the transition appeared. This is in agreement with the above transition curves for the temperature-dependence of the vesicle size observed by QLS.

It is remarkable that, in the presence of S-5682, a change in wave number was not observed at temperatures higher or lower than, but far from, the transition points (Fig. 4b). Concerning troxerutin, there is only a very weak

modification on the temperature-dependence of the absorption bands at 2852 cm^{-1} (Fig. 4c) and the second transition was practically unobservable. This indicated a weak effect on the inner region of the bilayer.

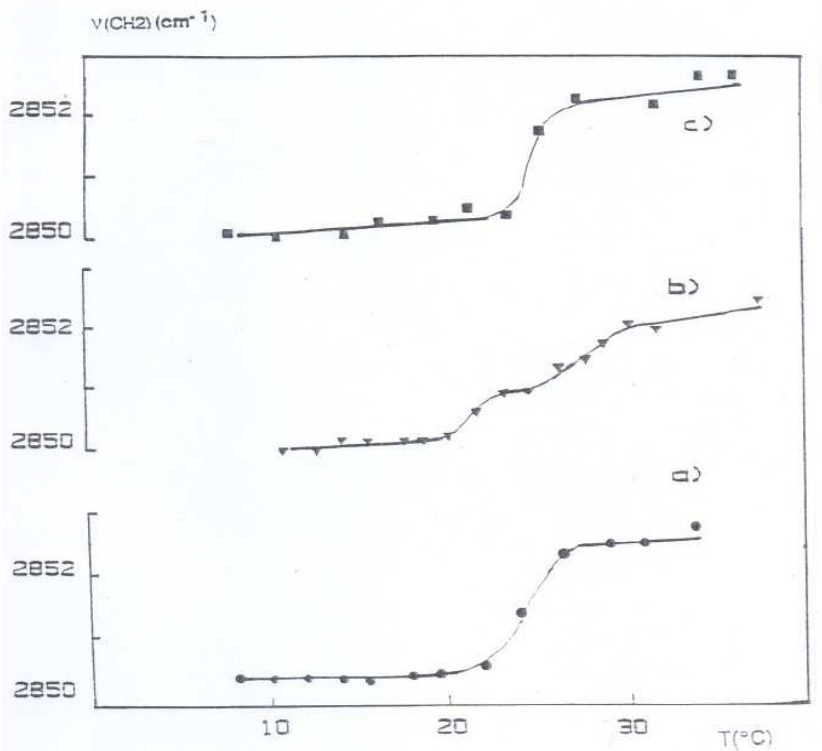

Figure 4: Effect on the phase transition of DMPC LUV of flavonoid derivatives observed by FT-IR spectroscopy. The plots represent the temperature-dependence of the wave nurnber of the symmetric CH_2 stretching mode

a) pure lipid LUV
b) LUV with S-5682 (drug/lipid molar ratio x = 0.17);
c) LUV with Troxerutin (x = 0.2).
Concentration of lipid: 30 $mg.mL^{-1}$ in 10 mM tris 150 mM NaCl, pH 8.3 aqueous buffer

b) Effect on the spectral region 1350-950 cm^{-1}: This spectral region was explored in order to investigate the effect of the drugs on the polar heads of the lipid. Figures 5a-c show infrared absorption bands of DMPC LUV in the absence and in the presence of S-5682 and troxerutin respectively. In the absence of the drugs (Fig. 5a) the spectrum is characterized by two strong bands at 1232 cm^{-1} and 1088 cm^{-1} corresponding to the antisymmetric and symmetric stretching modes of the PO_2^- groups of the polar heads. The band at 1088 cm^{-1} has a shoulder at 1068 cm^{-1} assigned to the coupling between the C-O groups and the adjacent C-C bonds.

The effect of S-5682 on DMPC LUV is shown in Figure 5b. The contribution of the drug cannot be entirely subtracted because of the shift in wave number of its bands (compare Figs. 5a and 5b). Taking into account this contribution, an alteration of the peak height ratio I(1232)/I(1088) from the value 0.77 of pure lipid LUV to that of 1.1 was evaluated. This shows an interaction between S-5682 and the bilayer at the $P0_2^-$ groups in the polar head group region.

Concerning troxerutin (Fig. 5c), the comparison between the spectra in the absence and in the presence of the drug shows practically no change either in the wave number or in the peak height ratio I(1232)/I(1088) (Table 2).

Table 2- Effect of flavonoïd derivatives on the PO_2^- groups of the lipid bilayer expressed by the change in the relative peak heights of the antisymmetric and the symmetric stretching bands at 1232 cm^{-1} and at 1088 cm^{-1}. x refers to the drug/lipid molar ratio.

DMPC LUV	Peak height ratio I(1232)/I(1088) at 36°C
Pure lipid LUV	0.80
LUV + S-5682 (x = 0.1)	0.56
LUV + troxerutin (x = 0.2)	0.81

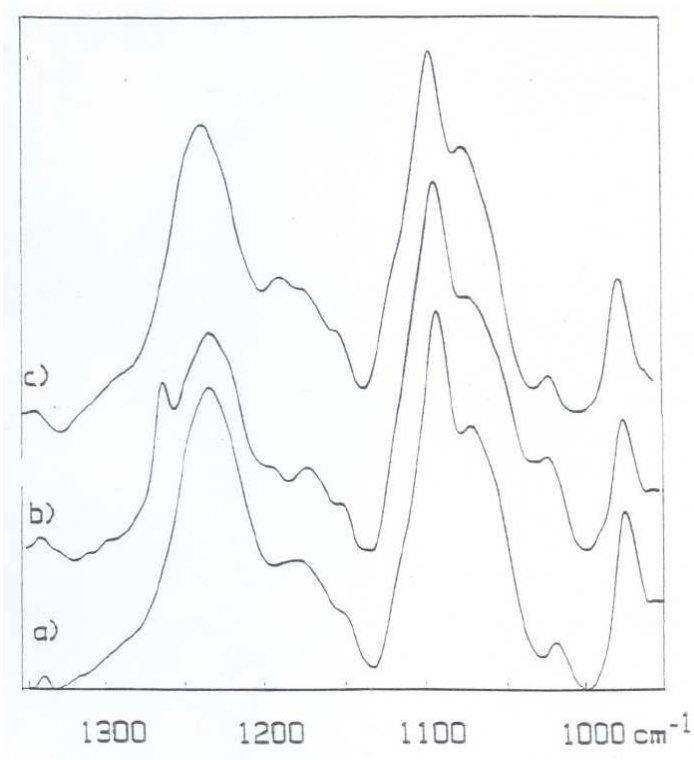

Figure 5: FT-IR spectra in the region of the $P0_2^-$ stretching bands:
a) pure DMPC LUV; b) DMPC LUV with S-5682 (drug/lipid molar ratio 0.17); c) DMPC LUV with troxerutin (x= 0.1);
Buffers : H_20, 10 mM tris, 150 mM NaCl, pH 8.3.

c) *Effect on the spectral region 1800-1500* cm^{-1}: The interface region is constituted by the carbonyl groups sn1-C=O and sn2-C=O which link the polar head to the acyl tails of each phospholipid molecule. For pure lipid LUV, the IR absorption band characteristic of these groups is a broad band at about 1735 cm^{-1} (Fig. 6a). This band can in fact be described by two components at 1742 cm^{-1} and 1725 cm^{-1} (Figs. $6b_1$ and $6b_2$) the sum of which gives Figure 6b. These components correspond respectively to the stretchings of nonhydrogen-bonded and hydrogen-bonded carbonyl groups [23]. Their peak height ratio varies with temperature so that the peak wave number of the overall band contour varies as a function of temperature, as shown in Figure 6 c.

When the drug S-5682 is present, at 8°C, below the transition, only one broad band without structure is observed but when the temperature is raised this band becomes split into two peaks, not well resolved but a study of the maximum of the whole band contour is no longer possible. Therefore we studied the components obtained by a band fitting protocol assuming a lorentzian shape for each of them. The peak wave numbers, the heights and the widths were adjustable parameters chosen in such a manner that the sum of two spectral components fit the experimental spectrum. The peak heights and the widths of both components obtained from the curve-fitting procedure are given above and below the transition points in Table 3. One can see from this Table the decrease of the bandwidths when the drug is present and this is more important when the temperature is increased. The change of the bandwidths and of the peak height ratio indicates that bath C=O groups may be affected by the presence of S-5682.

Concerning troxerutin, when it is present, only one wide band is observed whether the temperature was high or low. However, when the temperature varies, the wave number of the maximum of the band contour does not change (Fig. 6 d) as it does in the case of pure lipid vesicles (Fig. 6c). The results of the decomposition are given in Table 3 which shows an increase of the bandwidth of the component at 1742 cm^{-1} and a decrease of the bandwidth of the component at 1727 cm^{-1}. This would explain why the overall band contour seems not to show a temperature-dependence of its peak wave number.

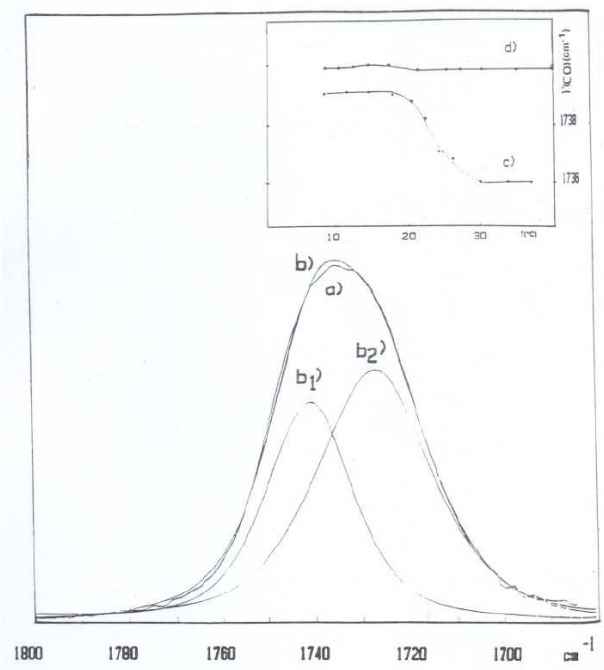

Figure 6: FT-IR spectra in the spectral region corresponding to the stretching mode of the C=O groups in the aqueous interface of the DMPC LUV at 35°C:
a) Unsplit experimental spectrum as an overall band contour; b) The reconstituted curve obtained by fitting with the spectrum a) in two components b1) and b2).
Buffers : 10 mM tris, 150 mM NaCl in $^2H_20$
c) and d) Temperature-dependence of the wave number of the peak of the C=O stretching band contour
c) pure DMPC LUV; d) DMPC LUV in the presence of troxerurin (x = 0.2).

Table 3 Variation of the relative peak heights I(1742)/I(1727) and bandwidths Γ(1742)/ Γ(1727) (in cm^{-1}) of the components (1) at 1742 cm^{-1} and (2) at 1727cm^{-1} of the band contour corresponding to C=O stretching vibrations for pure lipid DMPC LUV and LUV in the presence of S-5682 or troxerutin

Peak height ratio I(1742)/I(1727)	At 8°C	At 36°C
Pure lipid LUV	1.24	0.87
LUV + S-5682	1.29	1.06
LUV + troxerutin	1.24	0.96
Bandwidth Γ(cm^{-1}) Γ(1742)/Γ(1727)	At 8°C	At 36°C
Pure lipid LUV	19.5/25	22/29
LUV + S-5682	17.5/24	20/28
LUV + troxerutin	16/28	18/30

DISCUSSION

The above results concerning the effect of flavonoid derivatives on the physical state and on the thermal behavior of DMPC large unilamellar vesicles suggest a scheme for the location of the interactions with respect to the bilayer and a general survey of these interactions with lipidic model membranes. These drugs have the same parent compound, flavone, and possess ramifications at positions C7 of the A ring, and C3', C4' of the B ring.

At a macroscopic level, the QLS results show that S-5682 decreases the Young elastic modulus of DMPC membranes splits the transition by inducing another transition step reflecting an intermediate phase between the liquid crystalline phase and the gel-like phase. The first effect concerns the elastic modulus E. in the presence of S-5682 its value is clearly smaller than that of pure lipid LUV indicating that the rigidity of the DMPC bilayer is decreased in the presence of the drug.

The second effect of S-5682 is to alter the liquid crystalline to gel-like phase transition. When comparing the temperature-dependence plots of the vesicle hydrodynamic radius R_h in the presence and in the absence of S-5682 the same ratio between the vesicle radius at 35°C and at 12°C is observed. However, the single transition at 24°C of the pure lipid vesicles splits into two steps, one at a higher temperature, about 26°C, and another at a lower temperature, about 20°C. The splitting into two transition steps is commonly observed when amphiphilic drugs are added to DMPC LUV. At least we have observed the same phenomenon for Pentoxifylline and D-propranolol

[24,25]. Between the two transition temperatures, the vesicle radius variation indicates an intermediate state of the bilayer. The main effect of S-5682 is to lower the transition point but this lowering is preceded by a rearrangement of the lipid molecules in the bilayer. Such a rearrangement has been observed in DMPC multilayered vesicles in the presence of some antibiotic peptides such as valinomycin [26]. The latter induces a lowering of the transition point but this transition is preceded by a rearrangement occurring at a temperature higher than the melting point of the pure lipid vesicles.

At a molecular level, the stabilization of a lipid phase in vesicles is ensured by equilibrium between the electrostatic interaction of the polar heads and the hydrophobic interaction of the acyl chains [27]. The presence of amphiphilic molecules may contribute to altering both effects and modifying the transition. Although FT-IR data were obtained only with a drug/lipid molar ratio higher than in the case of the elastic measurements, the information about the interactions is significant. The change in the relative peak height ratio I(1232)/I(1088) (Figs. 5a-c and Table 2) appears to reflect an interaction with the polar PO_2^- groups. This is an important effect, taking into account the fact that the PO_2^- stretching bands are generally not easily affected. Moreover, the change of the relative peak height ratios I(1742)/I(1725) of the components at 1742 cm^{-1} and 1725 cm^{-1} (Table 3) reveals an interaction with the C=O groups in the interface region. Another interesting result is the decrease of the bandwidths of these components under the effect of both drugs. It may be that both drug molecules interact with the C=O groups, altering the glycerol moiety in such a manner as to change the shape of the potential well from which the C=O stretching vibrations are derived. An interaction between guest molecules such as salicylic acid or phenol with DPPC bilayers at the interface region has been recognized [28]. The change of the relative peak heights of the absorption bands corresponding to C=O stretchings has been interpreted as due to a change in hydration and head group volume.

We think that these interactions of S-5682 with the bilayer contribute to the modification of the phase transition and the decrease of the elastic modulus of the bilayer. It is interesting to notice that in the liquid crystalline phase an interaction in the hydrophobic region by cholesterol [29] or by amiodarone [30] increases the rigidity of the bilayers while an interaction essentially in the external interface as in the present case decreases its rigidity.

Concerning troxerutin, the situation is different because of its chemical formula. It contains also a rhamnoglucosyl (rutinose) radical but this radical is attached to the A ring at the position C3 instead of C7 in diosmin while all the radicals at C7, C3', and C4', possess alcohol functions. Troxerutin is very hydrosoluble. The results by FT-IR spectroscopy show that this drug has only a weak effect on the main transition of the hydrophobic region. No alteration is observed in the stretching vibrations of polar PO_2^- groups. However, the drug induced a weak 2^{nd} step of the transition, not observable by FT-IR but detectable by QLS, and decreased the elastic modulus. Moreover, FT-IR revealed (Table 3) an effect on the absorption bands characteristic of the C=O groups of the interface region of the bilayer. The decomposition of the broad band contour indicates that the bandwidth of the component at 1742 cm^{-1} was broadened while that of the component at 1725 cm^{-1} was narrowed. It is clear that troxerutin interacts with the external region of the membrane but this interaction is not sufficiently strong to alter the stretching vibrations of the PO_2^- groups. Moreover, it apparently does not reach the inner region of the bilayer as indicated by the temperature-dependence profile of $\nu s(CH_2)$. Because the QLS measurements concern the vesicles in the whole, this may explain the discrepancy between the transitions observed by FT-IR and QLS comparison of the results concerning troxerutin and S-5682, lead us to the following conclusions:

i) flavonoid derivatives S-5682 and troxerutin interact with phospholipid bilayers. This seems to be independent of the state of the drugs, In other words, the flavone compound plays an important role in this interaction.

ii) flavonoid derivatives S-5682 and troxerutin decrease the rigidity of phospholipid bilayers.

CONCLUSION

Using two optical techniques, quasielastic light scattering (QLS) and Fourier transform infrared spectroscopy (FT-IR), we have studied the effects of two flavonoid derivatives on phospholipid bilayers. The results show that S-5682 and troxerutin interact with the bilayers, essentially at the outer surface. In the case of S-5682 whose chemical formula is very close to that of diosmin, there is a more important alteration of the phase transition of the phospholipid bilayer. In all cases, there is a decrease in membrane rigidity in the presence of these two flavonoids.

These findings therefore lead us to think that flavonoids interact with the phospholipids of biological membranes and that this interaction might be related to some of their pharmacological properties such as hemorheologic effects, inhibition of leukocyte diapedesis, protection of membranes against oxidation, and permeability of capillaries.

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