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Interactions of α-tocopherol with biomembrane models: Binding to dry lecithin reversed micelles

D. Bongiorno [b], L. Ceraulo [b], M. Ferrugia [b], F. Filizzola [b], A. Longo [c], A. Mele [d], A. Ruggirello [a], V. Turco Liveri [a,*]

[a] *Dipartimento di Chimica-Fisica "F. Accascina", Università degli Studi di Palermo, Viale delle Scienze Parco d'Orleans II, 90128 Palermo, Italy*
[b] *Dipartimento di Chimica e Tecnologie Farmaceutiche, Università di Palermo, Via Archirafi n.32, 90123 Palermo, Italy*
[c] *ISMN, Istituto per lo Studio dei Materiali Nanostrutturati, Via U. La Malfa n.153, 90146 Palermo, Italy*
[d] *Dipartimento di Chimica, Materiali ed Ingegneria Chimica "G. Natta" del Politecnico, Via L. Mancinelli n.7, 2 0131 Milan, Italy*

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Abstract

The state of α-tocopherol (Vitamin E) in solutions of dry lecithin reversed micelles dispersed in an apolar medium has been investigated as a function of the Vitamin E to surfactant molar ratio (R_{VE}) at fixed surfactant concentration by FT-IR, ^{1}H NMR and SAXS with the aim to emphasize the role played by anisotropic intermolecular interactions and confinement effects as driving forces of its partitioning between apolar bulk solvent and polar nanodomains and of mutual Vitamin E/reversed micelle effects. It has been found that its binding strength to reversed micelles, triggered by steric and orientational constrains, is mainly regulated by specific interactions between the hydrophilic groups both of Vitamin E and surfactant. Moreover, the R_{VE} dependence of the Vitamin E distribution constant and of the micellar size suggest that the inclusion of increasing amounts of Vitamin E in reversed micelles involves substantial changes in the structural and dynamical properties of the micellar aggregates.

The occurrence of mutual effects and the partitioning of Vitamin E between hydrophilic/hydrophobic interfaces and apolar domains allow to infer some important biological implications concerning the capacity of Vitamin E to scavenge free radicals arising from hydrophilic and/or hydrophobic domains, possible variations of its local reactivity respect to that observed in bulk as well as its significant influence on the stability of biomembranes.

Keywords: α-Tocopherol; Vitamin E; Lecithin; Reversed micelles; Intermolecular interactions; Solubilization

1. Introduction

Vitamin E is a term used to indicate a group of some closely related compounds (tocopherols and tocotrienols) whose molecules are constituted by two spatially distinct regions: a chromanol nucleus terminated with a hydroxyl group and a long hydrophobic phytyl side chain. These structural peculiarities are responsible of the chemical and physical properties of Vitamin E and, as a consequence, of its biological role in living systems.

First of all, the phenolic hydroxyl group of Vitamin E is able to donate an electron to free radicals, mainly peroxyl radicals, acting as chain-breaking of peroxidation reactions of polyunsaturated fatty acyl chains of membrane lipids. Besides, tocopherols react with other oxidant species as singlet oxygen, alkoxy radical, peroxynitrite, nitrogen dioxide, ozone, and superoxide (Burton and Ingold, 1986; Niki, 1989; Wang and Quinn, 1999). For this reason, Vitamin E contributes significantly in preserving the integrity of biomembranes from free radical attack. Secondly, the amphiphilic nature of Vitamin E molecules involves that they are partially located, opportunely oriented, at the lipid/water interface where in addition to the antioxidant function they can perturb significantly the biomembrane structure and dynamics (Quinn, 2004). Thirdly, the presence of a long hydrophobic phytyl chain suggests that Vitamin E can frequently visit entirely hydrophobic domains preserving also these districts from free radical-induced damages. Finally, it can be expected that local physico-chemical properties of Vitamin E molecules can be more or less affected by the specific nature of the various explored solubilization sites. However, in addition to these qualitative statements based on

* Corresponding author. Tel.: +39 091 6459844; fax: +39 091 590015.
E-mail address: turco@unipa.it (V. Turco Liveri).

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simple molecular considerations, quantitative information on the state of Vitamin E in biological environments are advisable and certainly more useful in order to rationalize its behavior in living systems.

The role of tocopherols in living systems strongly depends on their location in cell membrane. To date there is not direct information arising from studies on biomembranes, mainly because the binding of biomolecules is accompanied by a variety of side processes which make the interpretation of the experimental data difficult. For this reason, several investigations have been carried out on phospholipid model systems in aqueous media with the purpose to obtain piece of information on location and interactions of Vitamin E in biological environments (Wang and Quinn, 1999). However, scarce efforts have been devoted to extend these studies to its partitioning between phospholipid model systems and quite apolar bioenvironments.

With the aim to shed some light on this subject, using reversed micelles as membrane models, the partitioning of Vitamin E in various solutions of water-containing reversed micelles at a maximum Vitamin E to surfactant molar ratio (R_{VE}) of 0.001 was previously investigated by a spectrophotometric method (Avellone et al., 2002). It was found that, in the presence of domains from apolar bulk solvent to surfactant palisade layer and to aqueous micellar core, Vitamin E is preferentially confined in the apolar region of reversed micelles with the OH group of the chromanol nucleus soaked among the hydrated polar or ionic head groups of the surfactant. Moreover, from an analysis of the distribution coefficients of Vitamin E between apolar solvent and reversed micelles of various surfactants, it was argued that its binding strength to micellar aggregates depends mainly by specific interactions between the hydrophilic portions of Vitamin E and surfactant molecules.

In order to extend our previous investigations on the intermolecular interactions responsible of the local properties and partitioning of Vitamin E in membrane models, we have undertaken an FT-IR, ^{1}H NMR and SAXS study of the state of Vitamin E in solutions of lecithin reversed micelles as a function of R_{VE} and at fixed surfactant concentration ([lecithin] = 0.0474 mol kg^{-1}). These techniques have been employed because it has been proven that they are well suited to gain a lot of structural information on solubilizate-containing reversed micelles (Bongiorno et al., 2004).

On the other hand, solutions of L-α-phosphatidylcholine (lecithin) reversed micelles in apolar media were chosen because they are certainly the most suitable to build up realistic models of biomembranes. This because lecithin is the main constituent of the natural membranes, so that the intermolecular interactions between Vitamin E and lecithin are similar to those operating in vivo. It is a zwitterionic phospholipid characterized by a positively charged choline and a negatively charged phosphate group which, together with the glycerol moiety, form the polar head while two long hydrocarbon chains of fatty acids constitute the apolar region of the molecule. By small-angle neutron scattering of solutions of dry lecithin in deuterated cyclohexane, it has been found that lecithin self-assembles as quite spherical reversed micelles with a mean radius of about 25 Å (Aliotta et al., 1996).

2. Materials and methods

Vitamin E (α-tocopherol, Sigma, 95% HPLC) and soybean phosphatidylcholine (lecithin, Degussa, Epikuron 200, 98%, generous gift of Degussa Texturan Systems) were maintained for several days under vacuum before use. Carbon tetrachloride (Sigma, 99.97%) and cyclohexane (Aldrich, 99+%) were used without further purification.

Samples at various R_{VE} were prepared by adding appropriate quantities of a 0.0474 mol kg^{-1} lecithin/CCl_4 solution to a weighted amount of Vitamin E.

FT-IR spectra were recorded with solvent compensation in the spectral region 900–4000 cm^{-1} using a Perkin Elmer (Spectrum BX) spectrometer and a fixed-path cell equipped with CaF_2 windows. All measurements were collected at 25 °C with a spectral resolution of 0.5 cm^{-1}.

^{1}H NMR spectra of all the samples were recorded at 25 °C with a Bruker AC250E spectrometer operating at a frequency of 250 MHz. Spectra calibration was performed using DMSO-d_6 as an external standard.

SAXS measurements have been performed by a laboratory instrument consisting of a Philips PW 1830 X-ray generator providing Cu Kα, Ni-filtered (λ = 1.5418 Å) radiation with a Kratky small-angle camera in the "finite slit height" geometry equipped with a step scanning motor and scintillation counter. Each scattering spectrum was subtracted by the cell + solvent contribution. Best-fit analysis were performed by the CERN minimization program called MINUITS. Since the SAXS study of Vitamin E/lecithin aggregates in CCl_4 is prevented by the scarce contrast between the solvent and reversed micelles, to overcome this drawback, surfactant solutions were prepared using cyclohexane as apolar medium. This substitution should involve some changes in the structural properties of reversed micelles; but it can be reasonably expected that, at least qualitatively, the R_{VE} dependence of the micellar size is similar.

3. Results and discussion

3.1. FT-IR spectra

A typical spectrum of Vitamin E/lecithin/CCl_4 system (R_{VE} = 0.995, [lecithin] = 0.0474 mol kg^{-1}) in the frequency range 900–4000 cm^{-1} is shown in Fig. 1. For comparison, the spectra of lecithin/CCl_4 system ([lecithin] = 0.0474 mol kg^{-1}), a dilute Vitamin E/CCl_4 solution ([VE] = 0.0273 mol kg^{-1}) and pure Vitamin E are also shown.

Taking into account the transparency of CCl_4 in the IR window of interest, all the observed bands can be attributed to the functional groups of Vitamin E and lecithin and their assignments, made according to the literature, are reported in Table 1.

3.1.1. Vitamin E OH stretching band

An inspection of Fig. 1 indicates that only a sharp absorption occurring at about 3630 cm^{-1} is observed when Vitamin E is monomerically solubilized in CCl_4 ([VE] < 0.03 mol kg^{-1}). On the other hand, in addition to this peak, a broad band in the 3000–3600 cm^{-1} frequency range is observed when lecithin is

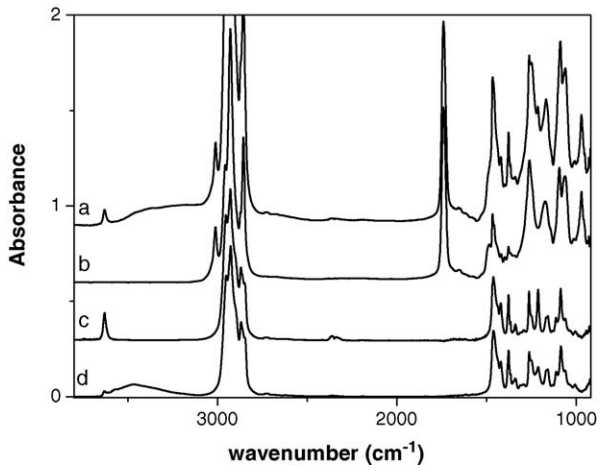

Fig. 1. Infrared spectra of Vitamin E/lecithin/CCl_4 system (a, $R_{VE} = 0.995$, [lecithin] = 0.0474 mol kg^{-1}), lecithin/CCl_4 system (b, [lecithin] = 0.0474 mol kg^{-1}), dilute Vitamin E/CCl_4 solution (c, [VE] = 0.0273 mol kg^{-1}) and pure Vitamin E (d).

also present in the system. Considering that H-bonding broadens the OH stretching bands and shifts them toward lower frequencies, this finding is consistent with the hypothesis that, in such condition, the Vitamin E OH group is dynamically partitioned between the apolar bulk solvent and the hydrophilic micellar cores constituted by the polar surfactant heads. It can be also noted that the position of H-bonded OH band in Vitamin E/lecithin/CCl_4 systems is centered at lower wavenumbers with respect to that of the pure vitamin, thus indicating that Vitamin E/lecithin interactions are, on the average, stronger than Vitamin E/Vitamin E ones.

More detailed information on the state of Vitamin E were achieved by deconvolution of its OH band in terms of Gaussian components. Some representative examples are shown in Fig. 2 while the fitting parameters of all the investigated samples are collected in Table 2.

It was found that the peak due to monomeric Vitamin E is always well-described by two sharp Gaussian components whereas two and four Gaussian components are required to fit the contribution due to H-bonded molecules in lecithin solution and in pure Vitamin E, respectively. It must be stressed that the need of a more reduced set of Gaussian components to fit the OH bonded band of Vitamin E confined in lecithin reversed micelles suggests the existence of a narrower spectrum of differently H-bonded populations with respect to those in the pure state. This can be taken as an indication that the ordered structure of lecithin reversed micelles imposes a well-defined orientation of Vitamin E molecules within the micellar palisade layer thus significantly reducing the number of differently H-bonded accessible states. Moreover, the observed R_{VE} dependence of the ratio between the area of the two H-bonded components suggests that the fraction of the most strongly H-bonded Vitamin E populations increases with the mean number of Vitamin E molecules solubilized in a micelle. This means that the increase of the amount of confined Vitamin E is accompanied by a parallel structural rearrangement of the reversed micelles leading to a more tight Vitamin E/lecithin H-bonding.

According to the literature, the two components due to Vitamin E monomers can be attributed to two different conformers arising from the sterically hampered rotation of the OH group around the CO axis of the phenolic bond while the system-dependent values of the ratio between their area suggest subtle effects of surrounding molecules on the conformers energy (Sassi et al., 2002). Besides, the possibility to directly monitor the R_{VE} dependence of the OH band due to Vitamin E monomers allowed us to evaluate the distribution coefficient K of Vitamin E between bulk solvent and reversed micelles. In particular, from the values of the band area it was calculated the monomer concentration (n_0) and then K considering that (Avellone et al., 2002):

$$K = \frac{n_m}{n_0 m_s P_A} \quad (1)$$

where n_m is the concentration of Vitamin E in the micelles ($n_m = [VE] - n_0$), m_s the surfactant molal concentration and P_A is the surfactant molecular weight expressed in kg mol^{-1}. The results of these calculations are summarized in Table 3.

It is worth to note the significant initial decrease of the binding constant with R_{VE} trending to a plateau value at higher R_{VE}, shown in Fig. 3. This finding indicates that, by progres-

Table 1
Infrared frequencies and assignments of functional groups of Vitamin E and lecithin in the 1000–4000 cm^{-1} range

Wavenumber (cm^{-1})			Group assignment
Vitamin E/CCl_4 [VE] = 0.0273 mol kg^{-1}	Pure Vitamin E	Vitamin E/lecithin/CCl_4 [lecithin] = 0.0474 mol kg^{-1}	
3629	3630	3629	ν_s(OH) free
	3000–3600	3000–3600	ν_s(OH) H-bonded
		3011	ν(HC=)
2954	2952	2958	$\nu_{as}(CH_3)$
2928	2925	2929	$\nu_{as}(CH_2)$
2870	2868	2873	$\nu_s(CH_3)$
2849	2847	2855	$\nu_s(CH_2)$
		1741	ν(CO)
1378	1377	1378	$\delta_s(CH_3) + \delta_s(CH_2)$
		1251	$\nu_{as}(PO_4^-)$
		1092	CH_2 wag, CH_2 twist
		1058	δ(P–O–C), $\nu_s(PO_4^-)$

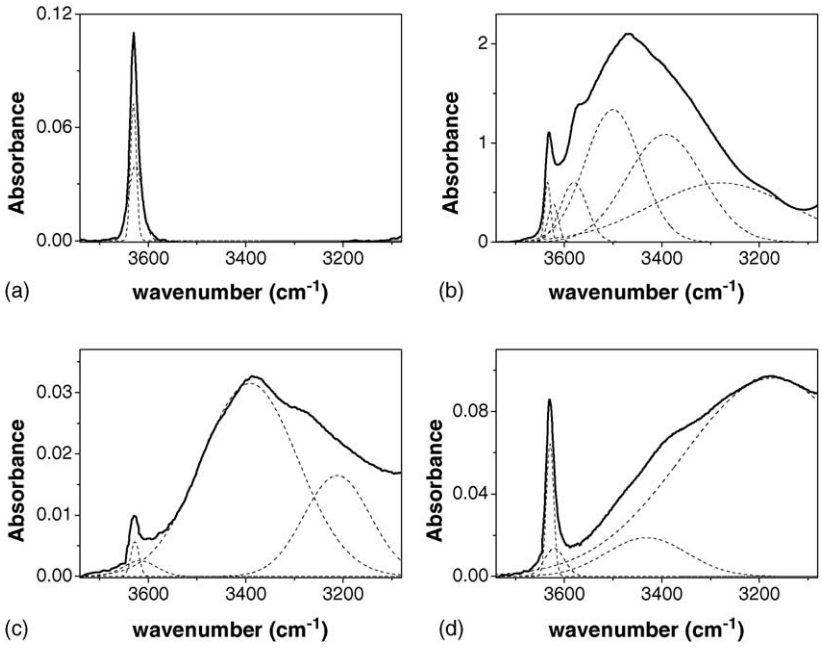

Fig. 2. OH stretching band fitting results for dilute Vitamin E/CCl_4 solution (a, [VE] = 0.0273 mol kg^{-1}) pure Vitamin E (b), Vitamin E/lecithin/CCl_4 (c, R_{VE} = 0.101, [lecithin] = 0.0474 mol kg^{-1}) and Vitamin E/lecithin/CCl_4 systems (d, R_{VE} = 0.995, [lecithin] = 0.0474 mol kg^{-1}).

Table 2
Single component parameters obtained by deconvolution of the OH stretching band of Vitamin E-containing systems in the 3000–3700 cm^{-1} frequency region

Sample	Free OH position (cm^{-1})/width (cm^{-1})/area (a.u.)	Bonded OH position (cm^{-1})/width (cm^{-1})/area (a.u.)
Vitamin E/CCl_4 ([VE] = 0.0273 mol kg^{-1})	3630/12/1.05 3626/28/1.40	
Pure Vitamin E	3634/12/9.2 3623/21/10.1	3580/54/41 3500/115/192 3393/155/211 3279/290/217
Vitamin E/lecithin/CCl_4 R_{VE} = 0.101	3630/15/0.10 3617/31/0.04	3391/198/7.8 3212/136/2.8
Vitamin E/lecithin/CCl_4 R_{VE} = 0.289	3630/15/0.28 3620/31/0.17	3418/161/3.4 3206/360/17
Vitamin E/lecithin/CCl_4 R_{VE} = 0.601	3630/14/0.60 3622/32/0.40	3429/145/2.1 3167/377/30
Vitamin E/lecithin/CCl_4 R_{VE} = 0.995	3630/14/0.98 3623/32/0.83	3431/166/3.9 3172/371/45

sively increasing the mean number of Vitamin E molecules per micelle, the propensity of the micellar aggregates to host additional molecules displays an initial decrease followed by less marked changes. The *K* decrease seems to be in contrast with the observation that the increase of the amount of confined Vitamin E is accompanied by more tight Vitamin E/lecithin H-bonding. However, this apparent contradiction can be solved by postulating the concurrence of unfavourable entropic effects attributable to a parallel structural rearrangement of the reversed micelles imposed by the Vitamin E presence.

Table 3
Binding constants of Vitamin E in lecithin/CCl_4 solutions at various R_{VE}

R_{VE}	*K*
0.101	55.6
0.289	46.6
0.601	41.9
0.995	36.9

3.1.2. Lecithin CO stretching band

The CO stretching bands of the lecithin/CCl_4 and Vitamin E/lecithin/CCl_4 systems at various R_{VE} are shown in Fig. 4. As it can be observed, quite independently of the Vitamin E concentration, its presence in the micellar system does not perturb significantly the position and shape of the surfactant CO band even at the higher R_{VE} values investigated. This finding indicates the absence of specific interactions between the surfactant

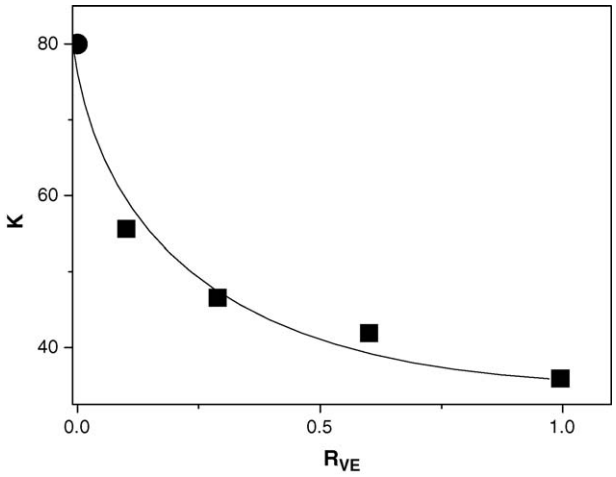

Fig. 3. R_{VE} dependence of the binding constant K of Vitamin E to lecithin reversed micelles ((■) present work; (●) Avellone et al., 2002).

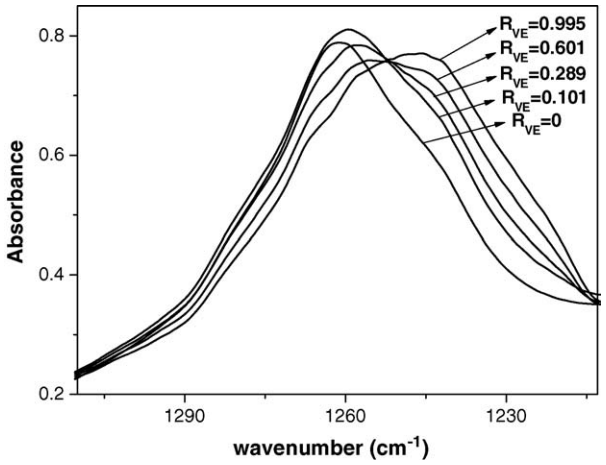

Fig. 5. Lecithin PO_4^- stretching band of Vitamin E/lecithin/CCl_4 solutions at various R_{VE}.

CO and Vitamin E OH groups suggesting a deep insertion of this moiety in the micellar core.

3.1.3. Lecithin PO_4^- stretching band

Lecithin PO_4^- antisymmetric stretching bands of samples at various R_{VE} are reported in Fig. 5. Apart the band of the sample at $R_{VE} = 0$, all the other bands have been obtained by subtracting to the spectra of Vitamin E/lecithin/CCl_4 systems that of Vitamin E/CCl_4 solution to eliminate the small contribution due to Vitamin E occurring in the same spectral region.

The observed shift of the band maximum (f^*) towards lower wavenumbers suggests that the binding of Vitamin E to lecithin reversed micelles is mainly driven by the establishment of specific interactions between surfactant PO_4^- and Vitamin E OH groups. In particular, it indicates that, increasing the Vitamin E to surfactant molar ratio, a linear increase of the fraction of lecithin PO_4^- groups engaged in H-bonds with the OH chromanol group of Vitamin E occurs (see Fig. 6).

3.2. ^1H NMR spectra

The comparison between a typical ^1H NMR spectrum of Vitamin E/lecithin/CCl_4 system ($R_{VE} = 0.995$, [lecithin] = 0.0474 mol kg^{-1}) with those of lecithin/CCl_4 ([lecithin] = 0.0474 mol kg^{-1}) and Vitamin E/CCl_4 ([VE] = 0.0273 mol kg^{-1}) solutions is shown in Fig. 7. Peak assignments to Vitamin E and lecithin protons, made according to the literature, are also shown in the same figure (Bongiorno et al., 2004).

Besides, the chemical shifts (δ) of the most significant protons of Vitamin E and lecithin, detectable in the ^1H NMR spectra, are collected in Table 4.

It is worth to note the marked variation of the $\delta_{(OH)}$ values of the Vitamin E hydroxyl proton caused by changing R_{VE}. In order to test if this finding can be mainly attributed to the correlated changes of the fraction X_m ($X_m = n_m/[VE]$) of Vitamin E molecules bonded to micelles and of that ($1 - X_m$) monomerically dispersed in the apolar media, these chemical shifts have been reported in Fig. 8 as a function of X_m together with the

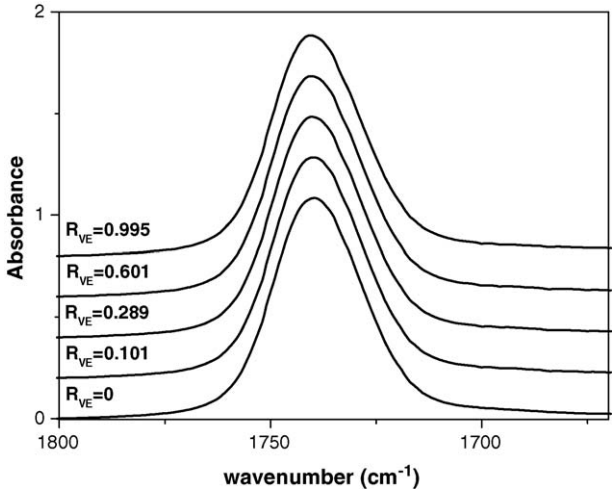

Fig. 4. Lecithin CO stretching band of Vitamin E/lecithin/CCl_4 solutions at various R_{VE}.

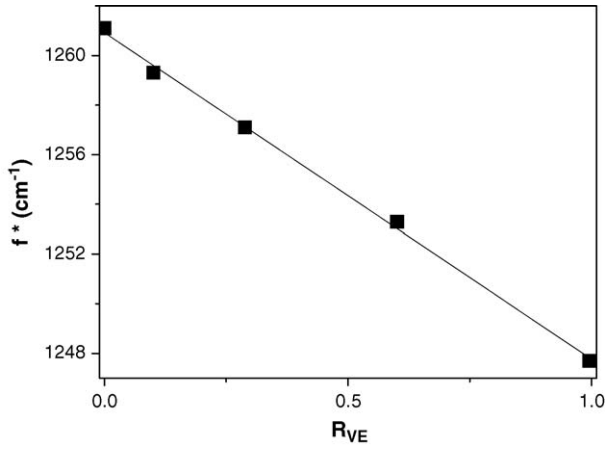

Fig. 6. Peak wavenumber (f^*) of the lecithin PO_4^- antisymmetric stretching band as a function of R_{VE}.

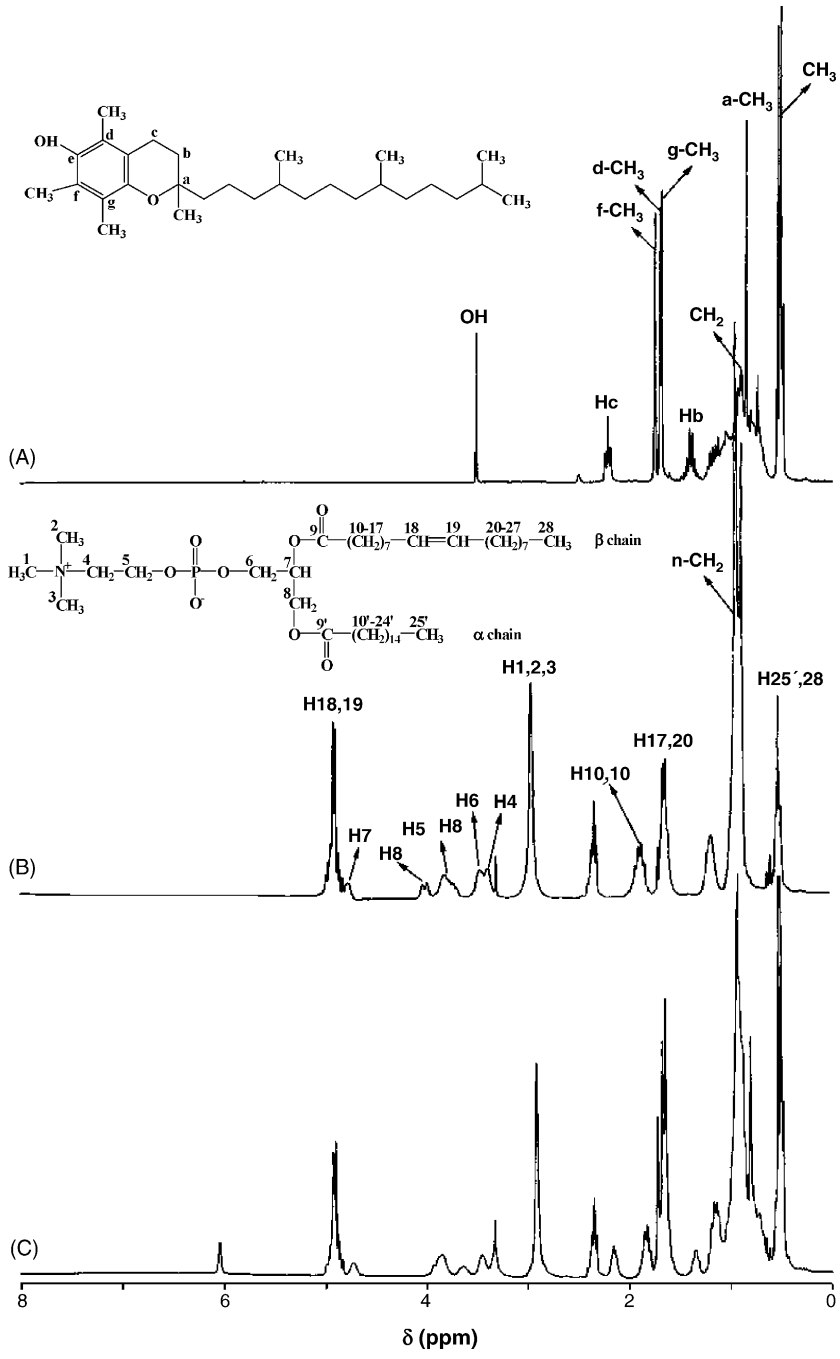

Fig. 7. Comparison between the ^{1}H NMR (CCl_4) spectra of Vitamin E (A), lecithin (B) and Vitamin E/lecithin (C, $R_{VE} = 0.995$).

Table 4
Chemical shifts of the most significant protons of Vitamin E and lecithin

Sample	$\delta_{(OH)}$	$\delta_{(Hc)}$	$\delta_{(Hb)}$	$\delta_{(H7)}$	$\delta_{(H8)}$	$\delta_{(H6)}$	$\delta_{(H4)}$	$\delta_{(H1,2,3)}$	$\delta_{(H10,10')}$
Vitamin E/CCl_4 ([VE] = 0.0273 mol kg^{-1})	3.55	2.24	1.44						
Lecithin/CCl_4 ([lecithin] = 0.0474 mol kg^{-1})				4.85	4.05	3.54	3.47	3.04	1.97
Vitamin E/lecithin/CCl_4, $R_{VE} = 0.101$	6.73	2.22	1.40	4.83	4.04	3.53	3.45	3.02	1.96
Vitamin E/lecithin/CCl_4, $R_{VE} = 0.289$	6.53	2.22	1.40	4.82	4.02	3.52	3.44	3.01	1.94
Vitamin E/lecithin/CCl_4, $R_{VE} = 0.601$	6.30	2.22	1.40	4.80	3.99	3.52	3.42	2.99	1.93
Vitamin E/lecithin/CCl_4, $R_{VE} = 0.995$	6.10	2.21	1.40	4.78	3.98	3.51	3.39	2.97	1.90

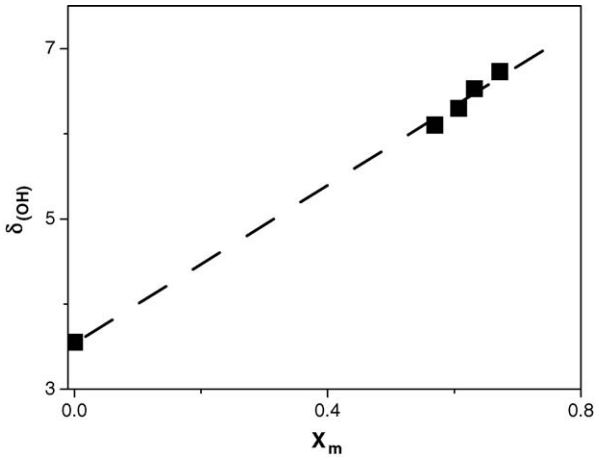

Fig. 8. Chemical shift values of the Vitamin E hydroxyl proton as a function of X_m.

straight line representing the equation:

$$\delta_{(OH)} = X_m\delta_{(OH,bonded)} + (1 - X_m)\delta_{(OH,free)} \quad (2)$$

where $\delta_{(OH,bonded)}$ and $\delta_{(OH,free)}$ indicate the chemical shift of hydroxyl protons of bonded and monomerically dispersed Vitamin E molecules. X_m values were calculated using the K values obtained throughout the IR spectra analysis.

The consistency of the experimental data with Eq. (2) supports the above reported hypothesis and allows to estimate the chemical shift of hydroxyl protons of Vitamin E bonded to lecithin reversed micelles (8.2 ppm). The strong difference between this value and that (3.55 ppm) of monomeric Vitamin E emphasizes that its solubilization in lecithin reversed micelles is caused by the formation of strong hydrogen bonds with the surfactant head groups.

Concerning the Hc and Hb Vitamin E protons (see Fig. 9), it can be noted that their chemical shifts show a modest variation passing from Vitamin E/CCl_4 to Vitamin E/lecithin/CCl_4 solutions while they are practically R_{VE} independent.

This indicates that the corresponding molecular region of the vitamin experiences quite similar and apolar environment independently if it is within or outside the micelle. On the other hand, it can be noted that the lecithin protons labelled H1,2,3, H4 and H10,10′ show a progressive upfield shift with R_{VE}. This behavior emphasizes that the insertion of Vitamin E in the surfactant palisade layer leads to a progressive variation of the chemical environment of the lecithin head group protons and suggests the occurrence of some structural rearrangement of lecithin reversed micelles.

3.3. SAXS spectra

Information on the size of Vitamin E/lecithin reversed micelles were achieved by SAXS measurements of samples at fixed lecithin concentration ([lecithin] = 0.0517 mol kg^{-1}) and various R_{VE}. Typical X-ray scattering profiles ($I(q)$, q) are shown in Fig. 10.

All SAXS spectra were found to be well-fitted by a model of not-interacting polydisperse homogenous scattering spheres.

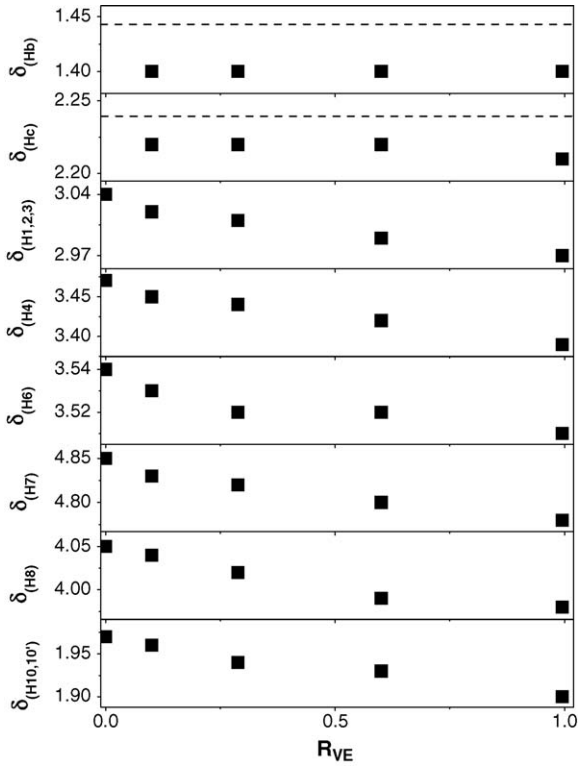

Fig. 9. Chemical shift values (ppm) of some significant Vitamin E and lecithin protons as a function of R_{VE} (the δ values of Hc and Hb protons of Vitamin E in pure CCl_4 are represented as dashed lines in the first two panels).

The fitting parameters, the mean micellare core radius r_m and the polydispersity parameter b, of all samples are collected in Table 5 and the maximum error on these quantities estimated from a contour plot analysis are 0.1 and 0.3, respectively. It must be pointed out that the mean micellar core radius of the bare lecithin reversed micelles (19.1 Å) can be considered in good agreement with the micellar radius previously found (25 Å) by the small-angle neutron scattering technique, if one takes into account the contribution given by the surfactant alkyl chains to the micellar size (Aliotta et al., 1996).

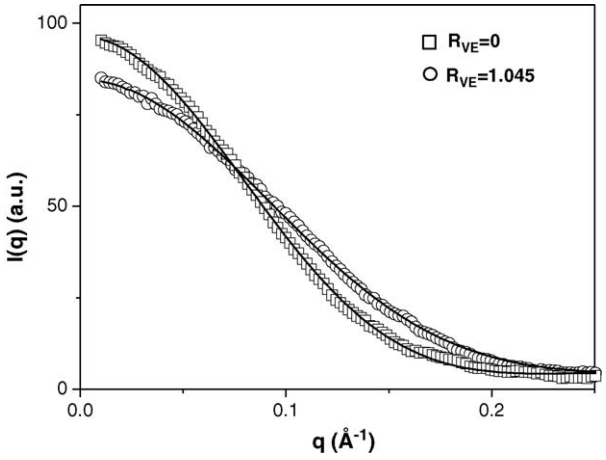

Fig. 10. Typical smeared scattering profiles of Vitamin E/lecithin/cyclohexane solutions at the R_{VE} value shown.

Table 5
Fitting parameters derived from the least-squares analysis of the SAXS data of the Vitamin E/lecithin/cyclohexane systems at various R_{VE}

R_{VE}	r_m	b
0.0	19.1	15.0
0.099	18.6	13.0
0.578	16.6	9.3
1.045	14.7	6.3

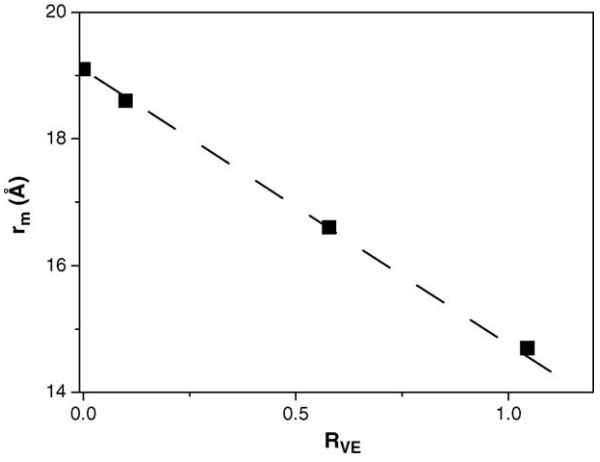

Fig. 11. Micellar core radius r_m as a function of R_{VE}.

The R_{VE} dependence of r_m and b is illustrated in Figs. 11 and 12, respectively. It is worth to note that, by increasing R_{VE}, the micellar core radius decreases and the polydispersity increases, monotonously. Both these effects emphasize that, in addition to specific Vitamin E OH/lecithin head group attractive interactions, the insertion of Vitamin E in the micellar palisade layer involves steric and dynamic constrains leading to smaller and less stable aggregates and consequently more polydisperse reversed micelle populations.

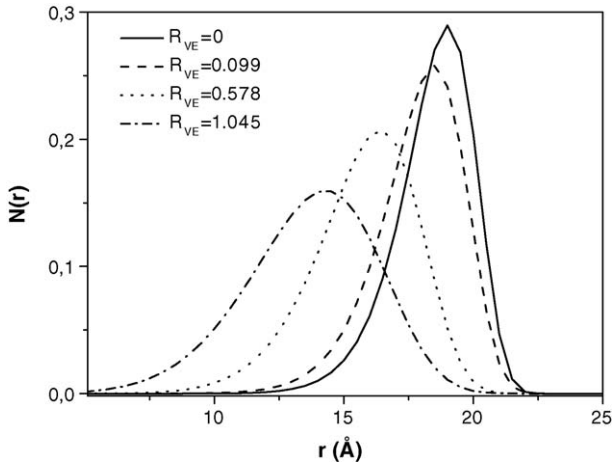

Fig. 12. Distribution functions $N(r)$ of the core radius of Vitamin E/lecithin reversed micelles at various R_{VE}.

4. Conclusions

Interesting information concerning the binding strength, mutual effects, location and distribution constants of Vitamin E in solutions of dry lecithin reversed micelles were achieved by suitable physico-chemical techniques. The results suggest that Vitamin E is partitioned between the bulk organic phase and the reversed micelles and that the binding of Vitamin E to reversed micelles depends on the Vitamin E to surfactant molar ratio. It has been also found that the driving force of the Vitamin E binding is mainly the interaction between the OH group of the nucleus and the surfactant PO_4^- group. Interestingly, H-bonding of the phenoxyl hydroxyl group to either the carbonyl or phosphate oxygen of phospholipid molecules has been previously claimed (Salgado et al., 1993). On the other hand, on the ground of permeability experiments, H-bonding of the phenoxyl hydroxyl group to the carbonyl one of the ester bond has been indicated for phospholipids arranged in a bilayer configuration (Urano et al., 1990). Even if the investigated models are quite different, our FT-IR data seem to role out the participation of the carbonyl group of the ester moiety to the H-bond.

The incorporation of Vitamin E molecules involves a structural rearrangement of lecithin reversed micelles leading to smaller and less stable aggregates. It is to note that contrasting data on the effect of the Vitamin E on the cell membrane stabilities have been reported (Wang and Quinn, 1999). Our data suggest a destabilizing effect, even if the concentration of α-tocopherol in our experiments is noticeably higher than that in cell membranes.

From a biological and pharmacological point of view, all these findings should be considered in order to rationalize the ability of Vitamin E to scavenge free radicals in hydrophilic, hydrophobic and interfacial nanodomains of biological environments as well as to hypothesize further effects on biomembrane structure and dynamics.

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