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Original article

Formation of micelles and liposomes from carnitine amphiphiles

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Abstract – Esterification of the carboxy and/or the hydroxy groups of (*R*)-carnitine (3-hydroxy-4-trimethylammonium butanoate) produces interesting classes of (cationic or zwitterionic) surfactants whose CMC values are in general predictable from their molecular structure. In fact similar relationships between CMC and the number of carbon atoms, C_n, have been found for three classes of such surfactants. However the sensitivity of CMC to C_n for the diesters is considerably lower than that calculated from literature values for the monoesters (either in their cationic or zwitterionic forms). The CMC values for the diesters have been determined by tensiometric, conductimetric and spectrophotometric methods, both in pure water and in 0.154 M NaCl solutions, at 25 °C. In particular the tensiometric results provide evidence that double-chain diesters undergo self assembly into structures more complex than simple micelles if the two chains are of comparable length. EPR and electron microscopy experiments show that the aggregates spontaneously formed by these surfactants are a mixture of multilamellar vescicles. © 2000 Éditions scientifiques et médicales Elsevier SAS

carnitine / micelles / CMC / liposomes / amphipiles

1. Introduction

In a previous paper [1] the partition coefficients, activity coefficients and thermodynamic acid dissociation constants of (*R*)-carnitine (3-hydroxy-4-trimethylammoniumbutanoate) and its acetyl and propionyl derivatives were measured and the effect of the addition of several electrolytes on these parameters was studied.

As long-chain acylcarnitine esters salts of the following general structure (I) (*figure 1*) are cationic surfactants of biochemical importance [2], we thought it interesting to measure the critical micelle concentration (CMC) values of some representative compounds of this class of surfactants, both in pure water and in the presence of 0.154 M NaCl at 25 °C. Solubilisation, conductivity and surface tension methods [3] for measuring the CMC values were used and the obtained results were critically compared.

As far as we are aware, there are only two publications [4, 5] concerning micelle formation by similar zwitterionic carnitine derivatives of the following general structure (II) (*figure 2*).

Recent pharmacological studies [6] have shown that the esters object of the present work (**1–14**, for their structures see *table I*) possess antimicrobial activity [6a], particularly toward gram-positive bacteria and *Filamentous Fungii* (including *Dermatophytes*) and biological activity apparently depends upon the length of the alkyl chains on the ester groups of carnitine [6b].

Resemblance to lysolecithins in the tendency of forming aggregates and the importance of (*R*)-carnitine as a cofactor for the transport of fatty acids

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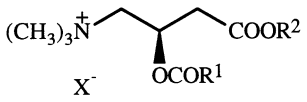
across the inner mitochondrial membrane [6c] makes these materials very attractive for possible pharmacological or cosmetic applications. In addition it should be considered that the chirality of the formed aggregates might be exploited in molecular recognition and/or asymmetrical catalysis in the hydrolysis reactions of suitable chiral substrates. The liquid crystalline phases formed by **1–9** at higher concentration are fully described elsewhere [7, 8].

2. Results

The CMC values determined at 25 °C in water and in physiological solution (0.154 M NaCl) by the three different techniques (see Section 4) are collected in *table I*. It is well known [9] that each of these techniques has advantages and limitations. In particular the use of a water-insoluble dye molecule can influence the formation of micelles thereby affecting the spectrophotometrically determined CMC values. In the conductivity measurements the surfactant behaves as a conventional electrolyte. Beyond the CMC there is a large decrease in equivalent conductivity which makes the use of the conductivity technique quite precise for ionic surfactants, but the sensitivity becomes too low when excess electrolytes are present (i.e. the conductivity method could not be used for measurements in 0.154 M NaCl solutions).

The technique used to measure the surface tension of the surfactant solutions is usually sensitive and reproducible. However, the presence of impurities can seriously affect [9] the shape of the plot of surface tension against the logarithm of concentration. As the absence of a well-defined break point in the surface tension versus molar concentration curves for compounds **4**, **7**, **8** and **9** (see for example *figure 3* for

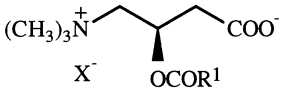


(**1-9, 11-14**) (I)

$X^- = Cl^-, CH_3SO_3^-$

R^1 and R^2 = alkyl chains as in the Table

Figure 1. General structure for long-chain acylcarnitine esters salts.



(II)

R^1 = alkyl chains C_7-C_{15}

Figure 2. General structure for Zwitterionic long-chain acylcarnitine monoesters salts.

compound **7**) is usually taken as an indication of the formation of aggregates other than simple micelles, we decided to further investigate the behaviour of compound **9** by EPR and of compounds **7–9** by electron microscopy.

The EPR technique of spin labelling [10] has been recently used to measure bioenergetic parameters such as volume changes, pH gradients and electrical potentials [11] in cells and vesicles. The effect of exchange broadening has been employed to differentiate between the signal of a suitable spin probe on the two sides of the membrane. The broadening of the EPR signal of the spin label occurs when paramagnetic species collide with a high frequency to allow significant exchange of unpaired electrons between paramagnetic molecules, thus quenching the EPR signal of the spin label. The phenomenon of quenching can be used to observe either intracellular or extracellular EPR spectrum. The intracellular signal can be observed by quenching the extracellular signal with transition metal complexes that are impermeable to the membrane. The intracellular signal is proportional to the total number of unquenched spins in the sample. The probes of choice for measuring bioenergetic parameters and volume changes are nitroxide radicals having narrow intrinsic line widths, since a given line broadening causes relatively more signal decrease than that observed with probes having a broader intrinsic line width. The most important feature of useful quenching agents is impermeability into the heterogeneous system under investigation. In fact the diffusion of the quenching agent into the vesicle or cell could cause broadening effects on the EPR signal of the spin label inside the system being studied. A convenient quenching agent is potassium ferricyanide which is quite impermeable to membranes and its EPR signal does not interfere with that of the nitroxide radical. Line broadening of nitroxide radical induced by the ferricyanide ion is proportional to the square of the quenching agent concentration [12]. TEMPO choline was chosen as the paramagnetic probe in the present work in view of its low partition coefficient between *n*-octanol and water [10].

If the carnitine derivative forms aggregates like liposomes, only the intravesicular EPR signal of TEMPO choline should be observed, the extravesicular signal being quenched by $K_3Fe(CN)_6$. In the absence of potassium ferricyanide the EPR signal, shown in *figure 4a* for compound **9**, consists of three lines of intensity 1:1:1, due to the coupling of the unpaired electron with the nitrogen atom. In the presence of ferricyanide the EPR spectrum looks very similar, due to TEMPO choline located in the vesicle inner aqueous phase (see *figure 4b*) thus suggesting the formation of liposomes by compound **9**.

The transmission electron microscopy observations fully confirm the spontaneous formation of multilamellar liposomes in water by compounds **7–9** as is shown in *figure 5* for compound **9**. The lamellar distance measured from the electron micrographs is about 120 Å.

3. Discussion

Inspection of the results reported in *table I* shows that the CMC values obtained by the different methods agree satisfactorily being the values from surface tension measurements [13] probably the most accurate ones as mentioned in Section 2.

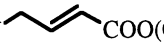
The effect of the addition of 0.154 M NaCl produces, as expected [14], a decrease in CMC due to the fact that, for ionic surfactants, in solutions of increasing ionic strength, the forces of electrostatic repulsion between head groups in a micelle are considerably reduced, enabling micelles to form more easily (i.e. at lower concentration). The effect of changing the counterion on CMC appears to be quite small (see compounds **1** and **14**).

Generally the longer the lengths of the carbon chains, R^1 and R^2, of the monomeric surfactant, the lower the CMC in pure water becomes, due to hydrophobic bonding [14]. This can be seen in *table I* by comparing, for example, compounds **11** and **12** which bear the same R^1 chain but have the R^2 group of different length (C_8 and C_{13}, respectively), and compounds **5** and **6** which vice versa bear the same R^2 but have the R^1 groups of 4 and 3 carbon atoms, respectively. Accordingly the highest CMC value was found for compound **10** which has the lowest total carbon atom number. On the other hand chain branching tends to increase the CMC as it can be seen in *table I* for compounds **1** and **3**.

It can be concluded that most of the carnitine derivatives (I) studied in the present work behave as single chain surfactants forming micelles at low concentration. However, it is known [14] that double

Table I. CMC values of acylcarnitines (I) obtained by different techniques (counterion is Cl^- if not stated otherwise).

	R^1	R^2	CMC_{H_2O} (10^{-3} M) dye solubilisation	CMC_{H_2O} (10^{-3} M) equivalent conductivity	CMC_{H_2O} (10^{-3} M) surface tension	CMC_{NaCl} (10^{-3} M) dye solubilisation	CMC_{H_2O} (10^{-3} M) surface tension
1	$CH_2CH(CH_3)_2$	$(CH_2)_{10}CH_3$	1.18	1.18	1.70	0.122	[a]
2	$CH_2CH(CH_3)_2$	$(CH_2)_9CH{=}CH_2$	3.47	3.40	3.49	0.628	0.914
3	$CH_2CH(CH_3)_2$	$CH[(CH_2)_4CH_3]_2$	5.46	5.33	6.71	1.05	1.08
4	$(CH_2)_5CH_3$	$(CH_2)_{10}CH_3$	0.774	0.720	1.16	0.0940	[b]
5	$CH_2CH(CH_3)_2$	$(CH_2)_{11}CH_3$	0.823	0.810	1.33	0.271	[a]
6	$CH(CH_3)_2$	$(CH_2)_{11}CH_3$	1.17	1.15	1.88	0.108	0.985
7	$(CH_2)_5CH_3$	$(CH_2)_{12}CH_3$	0.294	0.380	[b]	0.0392	[b]
8	$(CH_2)_6CH_3$	$(CH_2)_{12}CH_3$	0.403	0.270	[b]	0.0566	[b]
9	$(CH_2)_5CH_3$	$(CH_2)_{11}CH_3$	0.622	0.970	[b]	0.0102	[b]
10[c]		$(CH_2)_{10}CH_3$	6.26	7.14	7.00	0.870	1.21
11	$CH_2CH(CH_3)_2$	$(CH_2)_7CH_3$	13.1	12.7	9.75	4.18	3.51
12	$CH_2CH(CH_3)_2$	$(CH_2)_{12}CH_3$	0.373	[a]	0.637	0.126	[a]
13	$(CH_2)_2CH(CH_3)_2$	$(CH_2)_{10}CH_3$	1.13	0.845	1.62	0.0613	[a]
14[d]	$CH_2CH(CH_3)_2$	$(CH_2)_{10}CH_3$	1.34	1.50	1.85	0.219	0.150

[a] Irreproducible results.
[b] Anomalous behaviour attributable to aggregates different from simple micelles.
[c] The molecular structure is: $Cl^-(CH_3)_3\overset{+}{N}$  $COO(CH_2)_{10}CH_3$. [d] $X^- = CH_3SO_3^-$. .[d] $X^- = CH_3SO_3^-$.

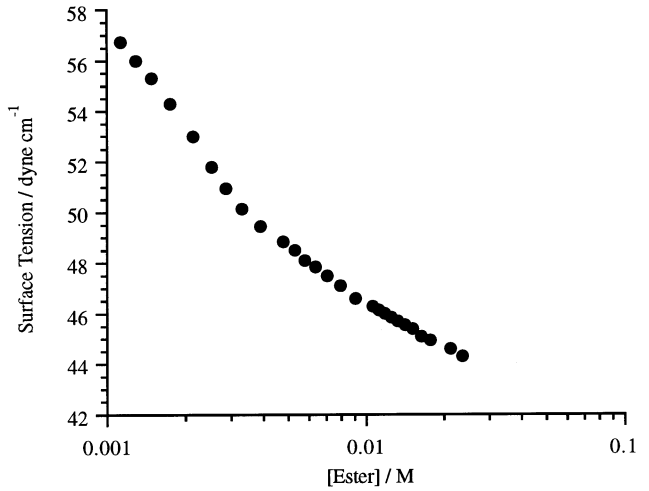


Figure 3. Variation of the surface tension determined for compound **7** as a function of ester concentration (logarithmic abscissa).

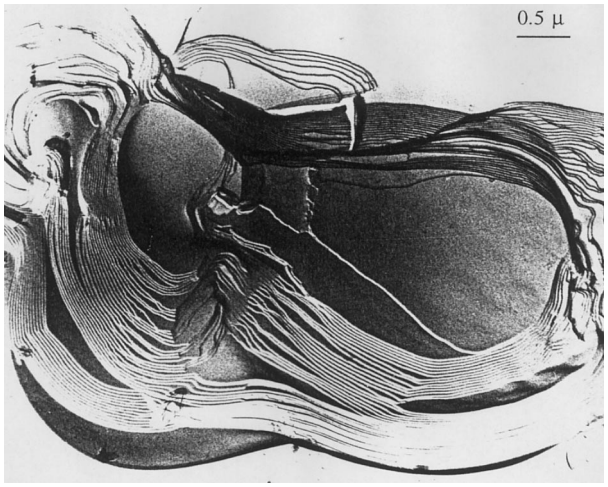


Figure 5. Compound **9**. Multilamellar liposome in water observed by transmission electron microscopy.

chain surfactants in dilute aqueous solution can form flexible bilayers and vesicles. A bilayer can take on a completely closed configuration because it then contains no enthalpically unfavoured edges that would have regions of hydrocarbon/water contact. In addition a vesicle is a smaller entity than an 'infinite' bilayer so it is entropically favoured as well. It has been recently shown [7] that derivatives **7–9**, bearing a relatively long alkyl chain R^1 of six or seven carbon atoms, behave as effective double chain surfactants in forming liquid crystalline phases at high concentration. Accordingly the tensiometric method did not provide a well-defined CMC value for the same compounds (**7–9**) in dilute solution. The EPR (for compound **9**) and the electron microscopy (for

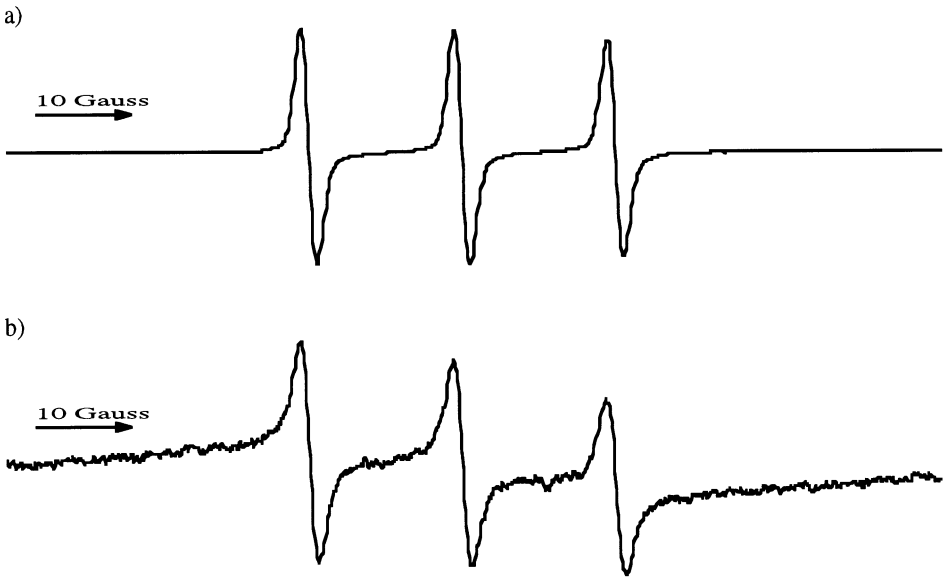


Figure 4. EPR signal of TEMPO choline in an aqueous suspension (10% w/v) of (*R*)-eptanoylcarnitine dodecyl ester chloride **9** in the absence (a) and in presence (b) of ferricyanide ion (100 mM). The EPR spectrum was recorded at room temperature by using the settings reported in Section 4.

compounds **7–9**) results strongly suggest that the aggregates spontaneously formed upon dissolution of these double chain surfactants in water are multilamellar vesicles rather than simple micelles beyond the (apparent) CMC value determined by the spectrophotometric method. However any attempt of size measurements by laser light scattering and determination of the encapsulation volume failed probably due to the fact that compounds **7–9** form in solution a system that is not homogeneous but consists instead of a mixture of multilamellar vesicles of different size.

Finally a brief comparison between the present results for acylcarnitine esters salts (I) and those previously reported [4, 5] for acylcarnitines chlorides and zwitterions (II) seems appropriate. It has long been observed that the number of carbon atoms, C_n, in a given family of surfactants is linearly related to the logarithm of the CMC. Indeed by plotting the CMC values [4, 5] reported by Yalkowsky and Zografi for both the zwitterionic and the protonated forms of the acylcarnitines (II) against C_n, excellent linear correlations (Eqs. (1) and (2)) can be obtained.

Zwitterionic form

$$\log \text{CMC (II)} = 3.09(\pm 0.09) - 0.504\ (\pm 0.008)\ C_n \quad (r = 0.9997;\ n = 4) \qquad (1)$$

Cationic form

$$\log \text{CMC (II)} = 3.22\ (\pm 0.02) - 0.503\ (\pm 0.002)\ C_n \quad (r = 0.9999;\ n = 5) \qquad (2)$$

Bearing in mind the results of Eqs. (1) and (2) we have similarly plotted the CMC values of those compounds that form simple micelles (**1**, **2**, **4–6**, **11–14**) against the total number, C_n, of carbon atoms of the alkyl chains (R^1 and R^2). Notwithstanding the high variability in the structure of the considered compounds also the CMC values of these acylcarnitine esters salts (I) show a linear correlation (Eq. (3)).

$$\log \text{CMC (I)} = 0.46\ (\pm 0.38) - 0.21\ (\pm 0.02)\ C_n \quad (r = 0.94;\ n = 9) \qquad (3)$$

Compounds **7–9**, which form aggregates different from simple micelles, have of course been excluded from the correlation of Eq. (3) as well as compounds **3** and **10** because of their different chemical structure.

It is not surprising that the correlation of Eq. (3) is less satisfactory than those of Eqs. (1) and (2), due to the higher variability in the structure of the considered compounds. Interestingly compound **10** which is closer in structure to acylcarnitines (II) falls exactly on the straight line of Eq. (2).

The slope values of Eqs. (1–3) indicate that carnitine monoesters (II) are more sensitive to C_n than the diesters (I). It should be noticed that the carnitine monoesters (II) display essentially the same sensitivity to C_n, independently of their zwitterionic (Eq. (1)) or (protonated) cationic (Eq. (2)) forms and that their CMC values can easily be fitted into a single linear free energy correlation (Eq. (4)).

$$\log \text{CMC (II)} = 3.10\ (\pm 0.12) - 0.498\ (\pm 0.010)\ C_n \quad (r = 0.9985;\ n = 9) \qquad (4)$$

The diesters (I) show higher CMC values than the monoesters (II) for $C_n > 9$, probably due to their more branched molecular structure. However at $C_n = 9$ the CMC value (3×10^{-2} M) is the same for both classes (I and II) of surfactants as the two straight lines of Eqs. (3) and (4) intersect at this point.

4. Experimental protocols

4.1. Materials

Compounds **1–14** were prepared from the corresponding (*R*)-acylcarnitine chlorides and the appropriate long-chain alcohols. (*R*)-Acylcarnitine chlorides, $(CH_3)_3N^+$–$CH_2CH(OCOR)$–CH_2–$COOH$ Cl^-, were synthesised according to Bohmer and Bremer [15]; long-chain alcohols, R′OH, from Aldrich were used without further purification.

*4.2. General procedure [7] for the synthesis of compounds **1–14***

The (*R*)-acylcarnitine chloride (0.1 mol) was suspended in dry methylene chloride (100 mL). The mixture was cooled at 0 °C and oxalyl chloride (13 mL; 0.15 mol) in anhydrous CH_2Cl_2 (15 mL) was slowly added under stirring. After 30 min at room temperature, a further amount of oxalyl chloride (19 mL; 0.22 mol) in anhydrous CH_2Cl_2 (10 mL) was added. The resulting

solution was stirred for 2 h at room temperature, then the solvent was removed under reduced pressure. The residue thus obtained was washed twice with anhydrous CH_2Cl_2 and the solvent removed again under reduced pressure. The obtained acyl chloride was used as such in the next reaction. 0.1 mol of crude acyl chloride was dissolved in anhydrous CH_2Cl_2 (40 mL). The solution was cooled at 0 °C and the appropriate long chain alcohol (0.168 mol) dissolved in CH_2Cl_2 (35 mL) was added under a nitrogen atmosphere. The solution was stirred at room temperature for 2 h and then concentrated under reduced pressure until an oily residue was obtained. The reaction mixture was chromatographed on a silica gel column buffered with 2% Na_2HPO_4. CH_2Cl_2 was used as the eluent till the long chain alcohol was eluted, then CH_2Cl_2/MeOH 9:1 v/v was used to complete elution of the desired compound. The solvent was evaporated from the collected fractions and the desired (*R*)-acylcarnitine esters salts were obtained. Physical constants, NMR spectra as well as elemental analyses for compounds **1–9** are reported in ref. [7].

4.3. Characterisation of compounds **10–14**

All the synthesised compounds contain significant amounts of water (0.7–1.9%) which affect the results of elemental analyses.

4.4. Crotonoylbetaine undecyl ester chloride **10**

Anal. calcd. for $C_{18}H_{36}ClNO_2$: C, 64.74; H, 10.87; Cl, 10.62; N, 4.19; found: C, 62.43; H, 11.26; Cl, 11.08; N, 4.06. H_2O% 1.7. HPLC Column: nucleosil-SA (5 μm) 200 mm, i.d. 4.0 mm. *T*: 30 °C. Mobile phase: CH_3CN/$(NH_4)_2HPO_4$ 50 mM 50/50 pH 4.0 with H_3PO_4. Flow rate: 0.75 mL/min; R_t: 13.33 min. ^{1}H-NMR (D_2O) δ: 7.1 (m, 1H, C*H*=CH); 6.4 (d, 1H, CH=C*H*); 4.3 (d, 2H, CH_2N^+); 4.1 (t, 2H, $COOCH_2$); 3.2 [s, 9H, $(CH_3)_3N^+$]; 1.7 (m, 2H, $COOCH_2CH_2$); 1.4 [m, 16H, $(CH_2)_8$]; 0.9 (t, 3H, CH_3); oil b.p. not determined.

4.5. Isovaleryl-R-carnitine octyl ester chloride **11**

$[\alpha]_D^{25} = -17.4$ ($c = 1\%$ H_2O). Anal. calcd. for $C_{20}H_{40}ClNO_4$: C, 60.97; H, 10.23; Cl, 9.00; N, 3.56; found: C, 59.98; H, 11.66; Cl, 8.64; N, 3.55. H_2O% 1.7. HPLC Column: Spherisorb-C1 (5 μm) 200 mm, i.d. 4.6 mm. *T*: 40°C. Mobile phase: CH_3OH/KH_2PO_4 50 mM 65/35 pH 4.5 with H_3PO_4. Flow rate: 1 mL/min; R_t: 5.75 min. ^{1}H-NMR (D_2O) δ: 5.7 (m, 1H, CHOCO); 4.1 (m, 2H, $COOCH_2$); 4.0–3.7 (m, 2H, CH_2N^+); 3.2 [s, 9H, $(CH_3)_3N^+$]; 3.0–2.7 (m, 2H, CH_2COO); 2.3 (m, 2H, $OCOCH_2$); 2.1 [m, 1H, C*H*$(CH_3)_2$]; 1.6 (m, 2H, $COOCH_2CH_2$); 1.3 [broad, 10H; $(CH_2)_5$]; 1.0 [d, 6H, $CH(CH_3)_2$]; 0.8 (t, 3H, CH_3); oil b.p. not determined.

4.6. Isovaleryl-R-carnitine tridecyl ester chloride **12**

$[\alpha]_D^{25} = -11.8$ ($c = 1\%$ H_2O). Anal. calcd. for $C_{25}H_{50}ClNO_4$: C, 64.70; H, 10.86; Cl, 7.64; N, 3.02; found: C, 63.73; H, 12.50; Cl, 7.03; N, 3.17. H_2O% 1.2. HPLC Column: Spherisorb-C1 (5 μm) 200 mm, i.d. 4.6 mm. *T*: 40 °C. Mobile phase: CH_3OH/KH_2PO_4 50 mM 65/35 pH 4.5 with H_3PO_4. Flow rate: 0.5 mL/min; R_t: 9.39 min. ^{1}H-NMR (D_2O) δ: 5.7 (m, 1H, CHOCO); 4.1 (m, 2H, $COOCH_2$); 4.0–3.7 (m, 2H, CH_2N^+); 3.2 [s, 9H, $(CH_3)_3N^+$]; 3.0–2.7 (m, 2H, CH_2COO); 2.3 (m, 2H, $OCOCH_2$); 2.1 [m, 1H, C*H*$(CH_3)_2$]; 1.6 (m, 2H, $COOCH_2CH_2$); 1.3 [broad, 20H, $(CH_2)_{10}$]; 0.9 [d, 6H, $CH(CH_3)_2$]; 0.8 (t, 3H, CH_3); m.p. dec. 150 °C.

4.7. Isocaproyl-R-carnitine undecyl ester chloride **13**

$[\alpha]_D^{25} = -13.12$ ($c = 0.8\%$ H_2O). Anal. calcd. for $C_{24}H_{48}ClNO_4$: C, 64.04; H, 10.75; Cl, 7.88; N, 3.11; found: C, 61.28; H, 11.10; Cl, 9.01; N, 3.12. H_2O% 1.9. HPLC Column: Spherisorb-C1 (5 μm) 200 mm, i.d. 4.6 mm. *T*: 40 °C. Mobile phase: CH_3OH/KH_2PO_4 50 mM 65/35 pH 4.5 with H_3PO_4. Flow rate: 0.5 mL/min; R_t: 8.45 min. ^{1}H-NMR (D_2O) δ: 5.7 (m, 1H, CHOCO); 4.1 (t, 2H, $COOCH_2$); 4.0–3.7 (m, 2H, CH_2N^+); 3.2 [s, 9H, $(CH_3)_3N^+$]; 3.0–2.7 (m, 2H, CH_2COO); 2.6–2.3 (m, 2H, $OCOCH_2$); 1.7–1.4 [m, 5H, $2CH_2$, C*H*$(CH_3)_2$]; 1.3 [broad, 16H, $(CH_2)_8$]; 0.9 [d, 6H, $CH(CH_3)_2$]; 0.8 (t, 3H, CH_3); oil b.p. not determined.

4.8. Isovaleryl-R-carnitine undecyl ester methanesulfonate **14**

A solution of sodium methanesulfonate (0.01 mol) in H_2O (2 mL), was added dropwise to a solution of the compound **1** (0.01 mol) in acetonitrile (20 mL) cooled at 4 °C. The mixture was stirred at room temperature for 2 h, then dried under reduced pressure at 60 °C. Acetonitrile was added to the residual solid and the NaCl formed was filtered off. The solution was dried under reduced pressure and the residual solid was suspended in diethylether and then filtered off. Yield 80%. $[\alpha]_D^{25} = -11.9$ ($c = 1\%$ H_2O); m.p. 115–119 °C Mettler (DSC 30).

Anal. calcd. for $C_{24}H_{49}NO_7S$: C, 58.15; H, 9.96; N, 2.83; S, 6.47; found: C, 57.69; H, 9.69; N, 2.79; S, 6.28. H_2O% 0.7. HPLC Column: Nucleosil-SA (5 μm) 200 mm, i.d. 4.0 mm. *T*: 30°C. Mobile phase: CH_3CN/KH_2PO_4 50 mM 65/35 pH 3.5 with H_3PO_4. Flow rate: 0.75 mL/min; R_t: 12.7 min. ^{1}H-NMR (D_2O) δ: 5.6 (m, 1H, CHOCO); 4.0 (m, 2H, $COOCH_2$); 3.8–3.6 (m, 2H, CH_2N^+); 3.1 [s, 9H, $(CH_3)_3N^+$]; 2.8–2.6 (dd + s, 5H, CH_2COO; $CH_3SO_3^-$); 2.2 (m, 2H, $OCOCH_2$); 1.9 [m, 1H, $CH(CH_3)_2$]; 1.5 [m, 2H, $COOCH_2CH_2$]; 1.2 [m, 16H, $(CH_2)_8CH_3$]; 0.8 [d, 6H,$CH(CH_3)_2$]; 0.7 (t, 3H, CH_3).

4.9. Orange OT (o-tolyl-azo-2-naphthol)

This compound was synthesised [15] by diazocoupling reaction between *o*-toluidine (Carlo Erba, purified by distillation under reduced pressure), and 2-naphthol (Carlo Erba, purified by vacuum sublimation). The obtained dye was crystallised from ethanol: m.p. 136–138 °C (lit. 136–138 °C).

4.10. Physical determinations

NMR spectra were recorded with a Varian VXR 300 MHz. NMR data are reported with respect to tetramethyl silane as a reference. Optical rotation was measured with a Perkin Elmer 241 MC polarimeter. HPLC chromatograms were obtained with a Waters chromatograph: pump 600 MS, injection autosample 715, detector UV 484 $\lambda = 205$ nm, R.I. 410, (Software Millenium). Melting points were measured with Mettler FP 80 apparatus. The water content was determined by a Karl Fisher apparatus.

4.11. Methods of CMC determination [3]

The CMC values were determined by the following methods.

4.12. Solubilisation

A few crystals of Orange OT were added to ten solutions at different concentrations of acylcarnitine esters chlorides (**1–14**). After at least a day of gentle shaking, the solutions were filtered through a 0.22 μm Millipore filter. The absorbance values, at $\lambda_{max} = 492$ nm of the dye, were plotted against the concentration of the ester. A typical example is shown in *figure 6.*

4.13. Electrical conductivity

The conductivities of the acylcarnitine esters chlorides (**1–14**) in redistilled water solution were measured at different concentrations with an Orion Conductivity meter mod.101 using a platinum electrode cell (cell constant 0.90 cm^{-1}). Equivalent conductivity values determined at each concentration, were plotted versus the square root of molar concentration. A typical example is shown in *figure 7.*

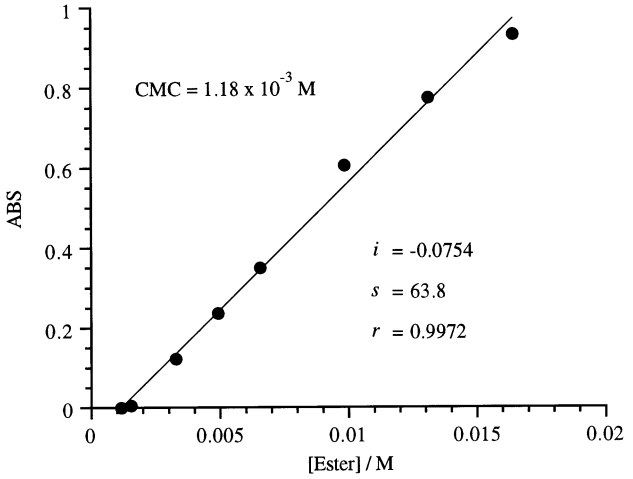


Figure 6. Variation of the absorbance determined for compound **1** as a function of ester concentration.

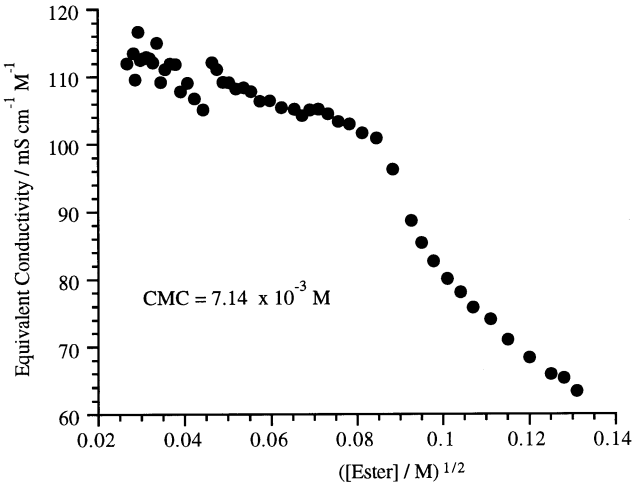


Figure 7. Variation of the equivalent conductivity determined for compound **10** as a function of the square root of ester concentration.

4.14. Surface tension

The surface tensions of the solutions of the acylcarnitine esters chlorides (**1–14**), at different concentrations, were measured with a SensaDyne 6000 Surface Tensiometer by the maximum bubble pressure method [13]. Dry nitrogen flows into a capillary immersed into the surfactant solution: if the pressure in the capillary is gradually increased, the bubble increases in size and curvature until it becomes hemispherical. Beyond this point the bubble becomes unstable and eventually escapes. Surface tension values (dyne cm^{-1}) were plotted versus the decimal logarithm of molar concentration. A typical example is shown in *figure 8*.

4.15. EPR measurements

The sample was prepared by adding 40 mg of carnitine ester chloride **9** to 400 μL of an aqueous 1.2×10^{-3} M solution of TEMPO choline (*figure 9*).

The obtained milky suspension was stored at room temperature for 24 h; oxygen was removed from the suspension by bubbling nitrogen then the solution was introduced in a capillary tube placed into the EPR cavity. The EPR spectra were recorded at room temperature by a Bruker ESP 300 spectrometer under the following settings: power 2 mW, modulation amplitude 1 G, sweep time 164 s, time constant 164 ms, centre field 3316 G, attenuation 20 dB. The same settings were used for the sample in the presence of potassium ferricyanide.

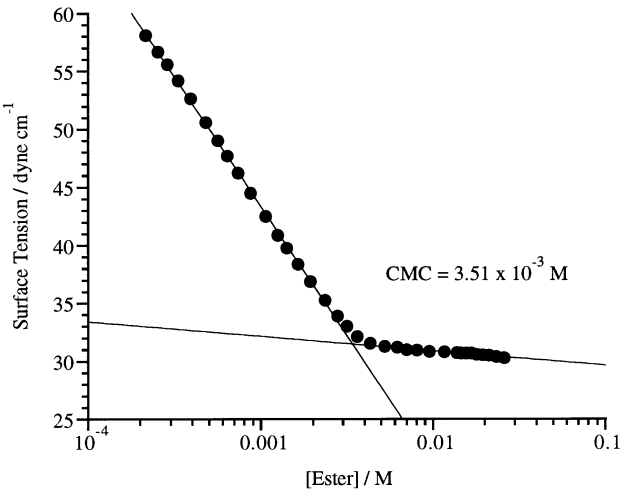


Figure 8. Variation of the surface tension determined for compound **11** in 0.154 M NaCl solution, as a function of ester concentration (logarithmic abscissa).

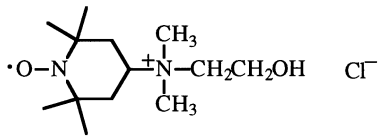


Figure 9. TEMPO choline.

4.16. Freeze-fracture electron microscopy

The fracturing of a rapidly frozen 10% w/w aqueous solution of compounds **7–9** and the platinum–carbon replication were carried out at a temperature of −100 °C and a pressure of 10^{-6} Torr in a Balzer BAF 301 freeze-etching apparatus, equipped with an electron gun.

Replicas were cleaned in methanol. The electron micrographs were taken in a Zeiss EM 902 transmission electron microscope, operating at 80 kV.

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