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Aggregation behaviour and copper-binding properties of surfactants containing imidazole and pyrazole ligands

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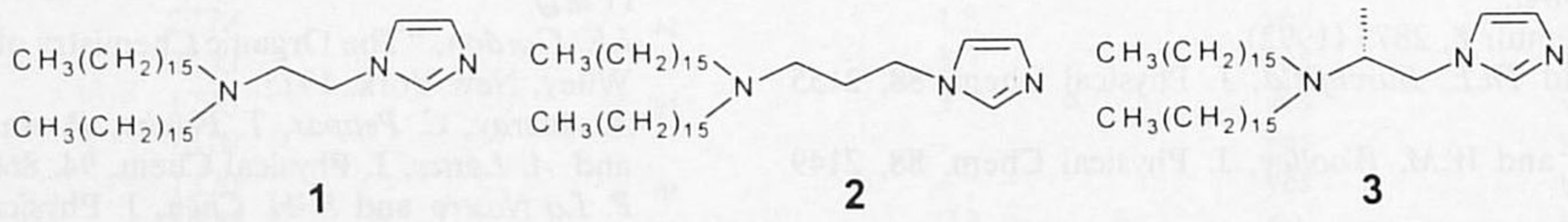
Abstract. The synthesis and aggregation behaviour of simple dialkylamine-imidazole and dialkylamine-pyrazole surfactants is described. In the presence of one equivalent of HCl the imidazole surfactants form stable vesicular dispersions. Addition of copper(II) dichloride to these dispersions leads to formation of metallo-vesicles in which four imidazole groups are coordinated to one copper(II) ion. These metallo-vesicles are unstable and are slowly converted into stacked bilayers, which precipitate. The pyrazole surfactants on the other hand do not form vesicles but give, with one molar equivalent of HCl, water-insoluble stacked bilayers. The pyrazole moieties in these stacked bilayers do not coordinate copper(II) ions. This behaviour is most probably due to the formation of an intramolecular hydrogen bond in the mono-protonated pyrazole surfactant molecule. The imidazole surfactants cannot form such a hydrogen bond.

Introduction

Metallo-micelles and metallo-vesicles are currently receiving interest as novel catalytic systems[1–3]. These aggregates possess catalytic or redox-active centres positioned at a lipid–water interface. Amphiphilic substrates may be bound at the surface or in the interior of such aggregates and may be converted selectively at the metal centres. The reactivity of the metal centres towards aqueous reactants can in principle be controlled via the properties of the electric double layer. Examples of metallo-micelles and metallo-vesicles include micelle- or bilayer-forming zinc and copper complexes which have been applied to achieve enantioselective hydrolysis of esters[1]. Micelles of cobalt-containing crown ethers have been shown to bind molecular oxygen[2]. Bilayer-forming copper chelates have been described which contain two-dimensional arrays of copper ions located at the bilayer–water interface[3]. Such ordered arrays have been used for the amplification of chemical signals[4].

The design of self-assembling systems with defined properties is often complicated by the complex aggregation behaviour of the surfactants, especially when interaction with ions can occur. Complexation of alkali-metal ions to crown-ether micelles has been shown to alter the CMC (critical miceller concentration) and aggregation number of the aggregates[5]. Binding of transition-metal ions has been reported to reduce the length of helices formed by peptide amphiphiles and other bilayer-forming surfactants[6]. We have previously[7] described the spontaneous formation of vesicles by ammonium surfactants containing imidazole groups, induced by binding of copper(II) ions. Remarkably, binding of zinc(II) ions resulted in the formation of stacked bilayer structures[7].

In order to attain a better understanding of the properties of metallo-surfactants we synthesised a series of simple ligand surfactants and studied their morphologies and metal-binding properties (Chart 1). The main features of these surfactants are an amine group, which makes it possible to vary the charge of the headgroup by protona-



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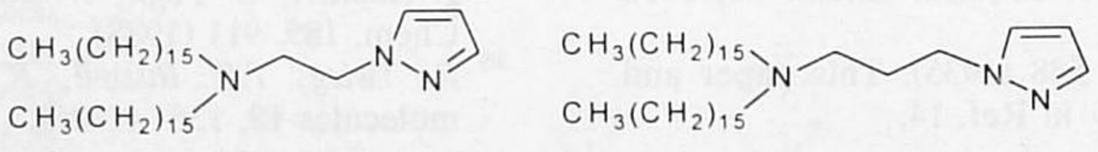

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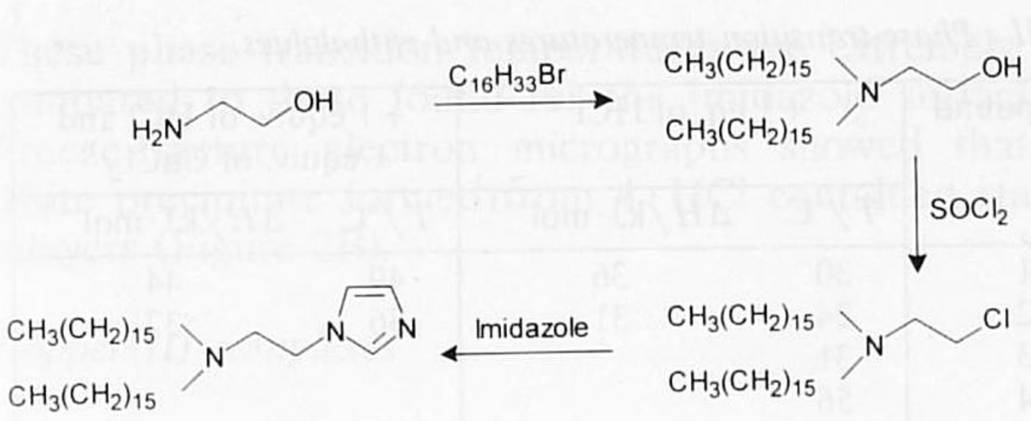

Scheme 1.

tion and deprotonation, a heterocyclic nitrogen ligand (pyrazole or imidazole) capable of coordinating transition metals, and an alkyl spacer.

Results

Synthesis of the surfactants

The surfactants were synthesised starting from amino alcohols according to the reaction sequence given for compound **1** (Scheme 1). Double alkylation on the amine function followed by reaction with thionyl chloride converted these amino alcohols into the amine chlorides (overall yields ~90%). Reaction of the latter chlorides with excess of imidazole in refluxing acetonitrile gave the imidazole surfactants **1** and **2** in 65–70% yields.

Similar reaction conditions applied to (*R*)-1-chloro-*N*,*N*-dihexadecyl-2-propanamine gave two products in equal amounts. One of them was the target product **3** but this compound appeared to be a racemic mixture. The other product was the isomeric *N*,*N*-dihexadecyl-β-methyl-1*H*-imidazole-1-ethanamine (**3a**), which was also racemic. The formation of the latter product together with **3** suggest that the intermediate of the reaction is an aziridinium ring[8]. The racemisation of both products is due to the apparent instability, under the conditions used, of the aziridinium intermediate, which can open and close with racemisation. When the much more nucleophilic sodium imidazolate was used as the reagent at room temperature in DMF, optically active **3** was obtained in 63% yield. Under these experimental conditions only 10% of **3a** was formed, which was not optically active. ^{1}H-NMR measurements carried out with **3** and the chiral shift reagent (+)-α-methoxy-α-(trifluoromethyl)benzeneacetic acid revealed that the optical purity of **3** was greater than 90%. The pyrazole surfactants **4** and **5** were synthesised by treating the corresponding dialkylamine chlorides with sodium pyrazolate, with yields of 43 and 30%, respectively.

Aggregation behaviour in water

Imidazole surfactants. Dispersion of the di-HCl salts of the imidazole surfactants **1** and **2** in water at 40°C resulted in the formation of stable opalescent solutions. Upon cooling to room temperature, a gel was obtained when the surfactant concentration was high (0.1 M). Titration of dilute solutions (0.001 M) of the di-HCl salts of **1** or **2** with an aqueous sodium hydroxide solution did not change the turbidity if less than one equivalent of base was added. However, above one equivalent of base the solution became turbid and the surfactant started to precipitate (Figure 1). Apparently, protonation of the imidazole surfactants is required for the formation of stable solutions. Weaker acids such as acetic acid were not able to solubilise compounds **1** or **2**. This is remarkable since a tertiary-amine group should be sufficiently basic to become protonated by weak acids. A possible explanation is that the surfactant is protonated, but that the protonated surfactant with acetate counter-ions is not soluble.

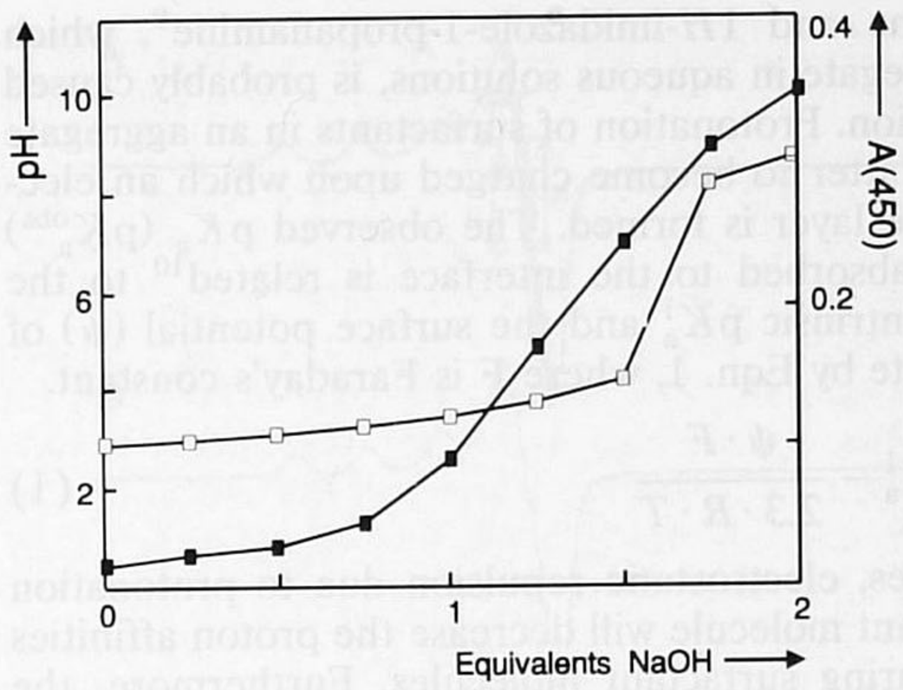

Figure 1. Change of the pH (□) and turbidity (■) of a 1 mM aqueous dispersion of **1** *in 2 mM HCl upon titration with an aqueous solution of sodium hydroxide at 20°C. The turbidity was measured as the absorbance at 450 nm.*

Acid–base titrations of the imidazole surfactants in methanol revealed two distinct protonation equilibria (Table 1). From the data in Table I it is clear that the inductive and/or field effects of the protonated amine function cause a substantial decrease of the basicity of the imidazole group. Similar effects of the imidazole substituent at the β or γ position relative to the amine function lower the basicity of the latter function. Although the observed effects are considerable they would not account for the absence of any protonation of **1** and **2** by weak acids in water.

Acid–base titrations in water were complicated by the fact that precipitation of surfactants **1** and **2** occurred if less than one equivalent of strong acid was present. Inhomogeneous solutions and fouling of the electrode may give rise to substantial errors in the determined pH values. Despite this, the resulting titration curves were very reproducible (Figure 1). The degree of protonation ($\beta = ([H^+_{total}] - [H^+_{free}])/[surfactant]$) can easily be calculated from the titration curves. Maximum values of β amounted to 0.74 and 0.8 for surfactants **1** and **2**, respectively. Apparently, in water only one protonation step occurs, most probably involving the amine function. Addition of excess HCl did not result in protonation of the imidazole group of the surfactants. The titration curves for **1** and **2** did not show clear end points, but approximate pK_a^{obs} values can be calculated from the pH values at $\beta = 0.5$ (Table I). The decrease of the pK_a^{obs} of surfactants **1** and **2** in water compared to the pK_a values of 1*H*-imidazole-

Table I Acid–base dissociation constants [a].

Compound	Methanol (20°C)		Water (20°C)	
	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}
1	6.7	8.5	– [b]	~4.0
2	7.2	9.5	– [b]	~4.4
3	6.7	8.5		
4	– [b]	8.7		
5	– [b]	9.8		
H_2N-$(CH_2)_2$-Im [c]			5.90	8.45
H_2N-$(CH_2)_3$-Im [c]			6.50	9.63
Imidazole	8.20		6.95 [d]	
Pyrazole	– [b]		2.5 [d]	
Ethylamine				10.8 [d]
Triethylamine		10.6		11.0 [d]

[a] The dissociation constants pK_{a1} and pK_{a2} correspond to the equilibria (Im is imidazole and Pyr is pyrazole):
Im-H^+ (or Pyr-H^+) + $H_2O \rightleftharpoons H_3O^+$ + Im (or Pyr)
Amine-H^+ + $H_2O \rightleftharpoons$ Amine + H_3O^+, respectively
[b] Not observed. [c] From Ref. 9. [d] From Ref. 29.

1-ethanamine and 1*H*-imidazole-1-propanamine[9], which do not aggregate in aqueous solutions, is probably caused by aggregation. Protonation of surfactants in an aggregate causes the latter to become charged upon which an electrical double layer is formed. The observed pK_a (pK_a^{obs}) of species absorbed to the interface is related[10] to the surface or intrinsic pK_a^i and the surface potential (ψ) of the aggregate by Eqn. 1, where F is Faraday's constant.

$$pK_a^{obs} = pK_a^i - \frac{\psi \cdot F}{2.3 \cdot R \cdot T} \quad (1)$$

In aggregates, electrostatic repulsion due to protonation of a surfactant molecule will decrease the proton affinities of neighbouring surfactant molecules. Furthermore, the dielectric constant of the double layer is less than the dielectric constant of the bulk aqueous phase[11]. This may also decrease the stability of the charged species. It is clear that both factors will cause the pK_a^i values of the surfactants to be different from the pK_a values of model compounds in water. Taking the $pK_{a,2}$ values of 2-(1-1*H*-imidazole-1-ethanamine and 1*H*-imidazole-1-propanamine from Table I as approximations for the pK_a^i values of surfactants **1** and **2**, one obtains values of 240 and 310 mV for the surface potentials of aggregates of **1** and **2**, respectively. Surface potentials of other cationic surfactant assemblies, such as micelles of *N*-dodecyltrimethylammonium chloride[12] and vesicles of *N*,*N*-dioctadecyldimethylammonium chloride[13] have been reported in the literature as 129 mV and 154 mV, respectively. These potentials are considerably smaller than the calculated surface potentials of our aggregates. It is therefore likely that intersite repulsions and the dielectric constant of the double layer also contribute to the decrease of the pK_a^i of the amine functions in aggregates of **1** and **2**.

The fact that protonated **1**, **2** and **3** form opalescent solutions suggests that large aggregates are present, *e.g.* vesicles, lamellae or tubular structures, but not micelles. DSC showed for aqueous dispersions of **1** · HCl a sharp phase transition at 30°C which is indicative of the presence of an ordered bilayer structure (Table II). A similar behaviour was observed for the mono-HCl salts of compounds **2** and **3**. For these surfactant aggregates the phase transitions occurred at 24 and 31°C, respectively. Freeze-fracture electron micrographs revealed that in the opalescent solutions of **1** · HCl unilamellar vesicles were present with diameters ranging from 60 to 2000 nm (Figure 2A). Similar structures were observed in solutions of **2** · HCl. Addition of excess HCl did not alter the phase-transition temperature of the imidazole surfactants **1**–**3**, nor did it change the turbidity of the solutions.

Table II Phase-transition temperatures and enthalphies.

Compound	+1 eq. of HCl [a]		+1 equiv. of HCl and 1 equiv. of $CuCl_2$ [b]	
	T/°C	ΔH/kJ·mol^{-1}	T/°C	ΔH/kJ·mol^{-1}
1	30	36	49	44
2	24	31	36	37
3	31			
4	56			
5	62			

[a] Phase-transition temperatures and enthalpies were taken from the heating scans of 0.05 M dispersions of the compounds in 0.05 M HCl. [b] Idem in 0.05 M HCl with 0.05 M $CuCl_2$ present. Scan rate 2°C/min.

Pyrazole surfactants. Compounds **4** and **5** did not give clear homogeneous solutions in the presence of one equivalent of HCl, not even after heating to 80°C and sonication of the suspensions. Instead, white precipitates were formed. Addition of more HCl did not solubilise the precipitates. Compounds **4** and **5** were only found to form stable opalescent solutions in concentrated HCl. Titration of the pyrazole surfactants in methanol revealed that these compounds undergo only one protonation step (Table I). The pK_a values measured in this solvent were similar to those of the amine functions of the imidazole surfactants **1** and **2**. In a separate experiment we tested whether the model compound 1*H*-pyrazole could be protonated in methanol. This appeared not to be the case. The pK_a values are therefore assigned to the amine functions of the pyrazole surfactants.

DSC (differential-scanning calorimetry) experiments indicated that the white precipitates of compounds **4** · HCl and **5** · HCl had an ordered structure: phase transitions were observed at 56°C and 62°C, respectively (Table II).

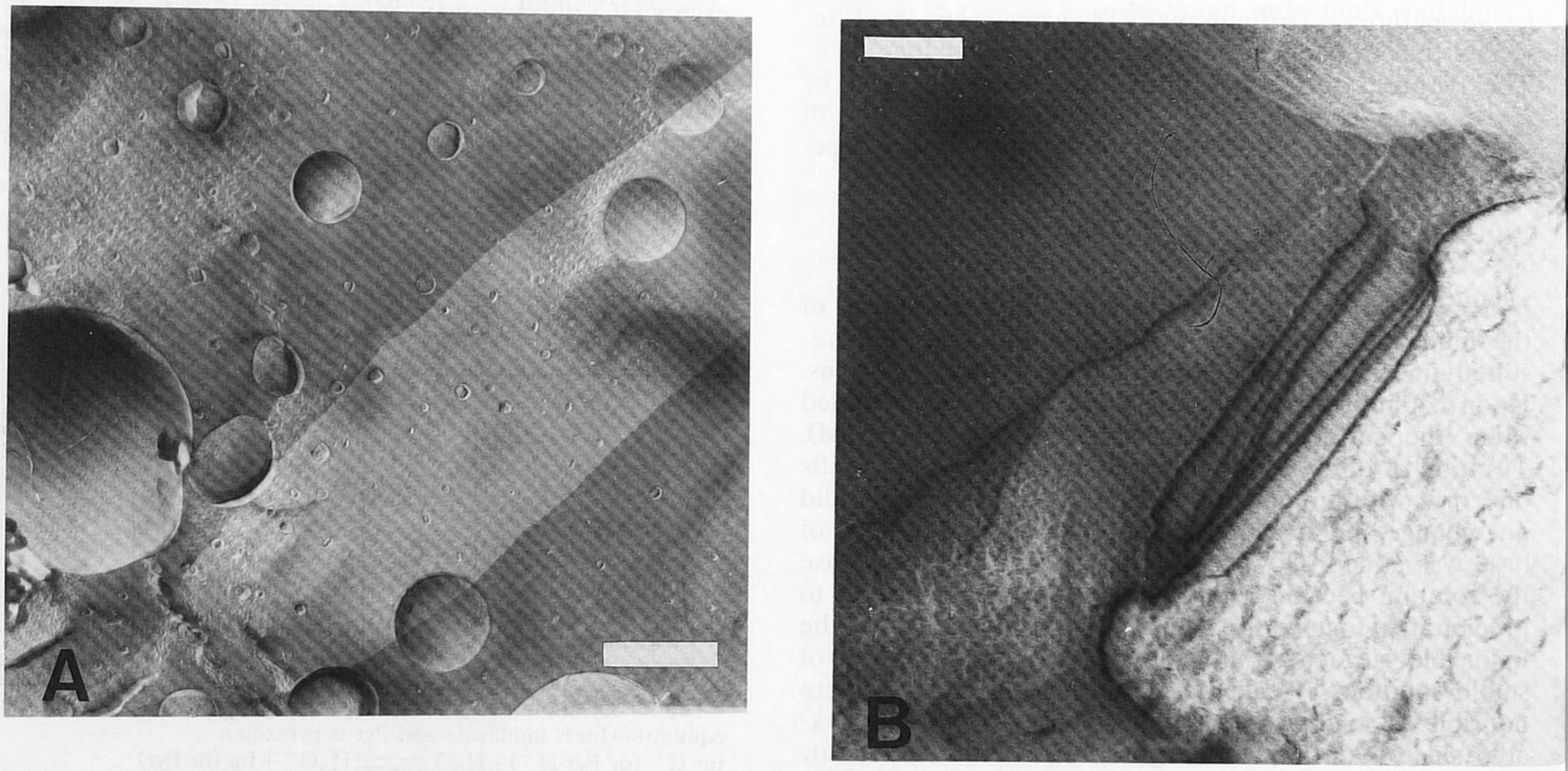

Figure 2. Freeze-fracture electron micrographs of 0.02 M dispersions of **1** *(A, magnification 14000 ×, bar represents 1 μm) and of* **4** *(B, magn. 61000 ×, bar represents 200 nm) in 0.02 M HCl.*

These phase transition temperatures are extremely high compared to those found for the imidazole surfactants. Freeze-fracture electron micrographs showed that the white precipitate formed from **4** · HCl contained stacked bilayers (Figure 2B).

Copper(II) complexes

Imidazole surfactants. In the presence of one equivalent of HCl only the amine functions of surfactants **1–5** are protonated, leaving the imidazole and pyrazole groups available for coordination to a metal ion. Addition of copper(II) chloride to a sonicated dispersion of the mono-HCl salts of the imidazole surfactants in water resulted in the formation of a blue-coloured solution. UV-Vis spectroscopy showed a broad band at 590 nm (ϵ 49 $M^{-1} \cdot cm^{-1}$), indicative of the presence of a complex of the type Cu(imidazole)$_4^{2+}$ (Figure 3A)[14,15]. Remarkably, the band at 590 nm already appeared at equimolar ratios of imidazole surfactant and copper. This indicates that complexes with a lower stoichiometry than four imidazole surfactant molecules per copper ion are not formed. On the contrary, titration of $Cu(ClO_4)_2$ with **1** · $HClO_4$ in ethanol/chloroform[16] showed a gradual shift of the absorption maximum from 820 nm ([imidazole]/[Cu^{2+}] < 1) to 610 nm ([imidazole]/[Cu^{2+}] > 4) (Figure 3B). This phenomenom points to the formation of complexes of the type Cu(imidazole)$_n^{2+}$, with n gradually increasing from 1 to 4. Such a behaviour is to be expected when the solution is completely homogeneous. This condition is met in the ethanol/chloroform solvent mixture but not in water. In water, compound **1** · HCl is aggregated, involving pre-organisation of the ligands at the aggregate–water interface. Because of this pre-organisation, binding of copper can now be described by a single-step equilibrium (Eqn. 2)[17].

$$Cu^{2+} + nL \rightleftharpoons CuL_n^{2+} \qquad (2)$$

From the titration curves the association constant K_a for the equilibrium given by Eqn. 2 and the stoichiometry n were calculated by non-linear regression analysis. Best fits were obtained with $n = 4.7 \pm 0.3$ and $K_a = (7.7 \pm 1.1) \cdot 10^3$ M^{-1} for compound **1** and $n = 3.4 \pm 0.3$ and $K_a = (5.4 \pm 1.4) \cdot 10^3$ M^{-1} for compound **2**. These calculated stoichiometries are in reasonable agreement with the formation of complexes of the type Cu(imidazole)$_4^{2+}$.

EPR (electron paramagnetic resonance) spectra were recorded of aqueous dispersions of the imidazole surfactants **1** · HCl, **2** · HCl, and **3** · HCl to which 0.02 equivalents of copper(II) chloride had been added. These spectra showed the characteristic features of four coordinated copper(II) complexes with axial ligand symmetry (Figure 4A)[18]. The $g_\parallel$ signal is split into four lines due to coupling of the unpaired electron with the copper nucleus ($I = 3/2$). The $g_\perp$ signal is superimposed on the extreme right line of $g_\parallel$ ($m_s = 3/2$). A hyperfine structure due to coupling of the unpaired electron with the nitrogen atoms ($I = 1$) of the imidazole ligands is resolved both on $g_\perp$ and on the extreme right line of $g_\parallel$. The spectral parameters agree very well with those observed for Cu[1-butylimidazole]$_4Cl_2$ (Table III). The resolution of the hyperfine structure on the lines on the extreme right in Figure 4A showed a strong dependence on both the surfactant to copper ratio and the temperature. Increasing the copper(II) chloride concentration to 0.1 equivalents per imidazole surfactant caused the nitrogen hyperfine splitting to disappear (Figure 4B). Most probably this splitting becomes less well resolved due to dipolar broadening of the lines at small distances between two close copper nuclei[19]. Increasing the temperature also decreased the hyperfine splitting, although it did not disappear completely. This decrease did not take place gradually, but rather suddenly at 29 and 23°C for compounds **1** and **2**, respectively (Figure 5)[20]. These temperatures are rather similar to the gel to liquid–crystalline-phase transition temperatures measured by DSC for aqueous dispersions of **1** · HCl and **2** · HCl without copper(II) ions present (Table II). Apparently, the presence of small amounts of copper(II)–surfactant complexes in bilayers of **1** · HCl and **2** · HCl does not have a large influence on the phase-transition temperatures.

At low concentrations (5 mM) the solutions of the copper(II) complexes of **1** · HCl, **2** · HCl, and **3** · HCl were stable for hours but at high concentrations (0.1 M) they became turbid and precipitates were formed within one hour. DSC showed that these precipitates still had an ordered structure, as was indicated by the presence of a phase transition, see Table II. As can be seen in Table II both the temperature and the enthalpy of the transition

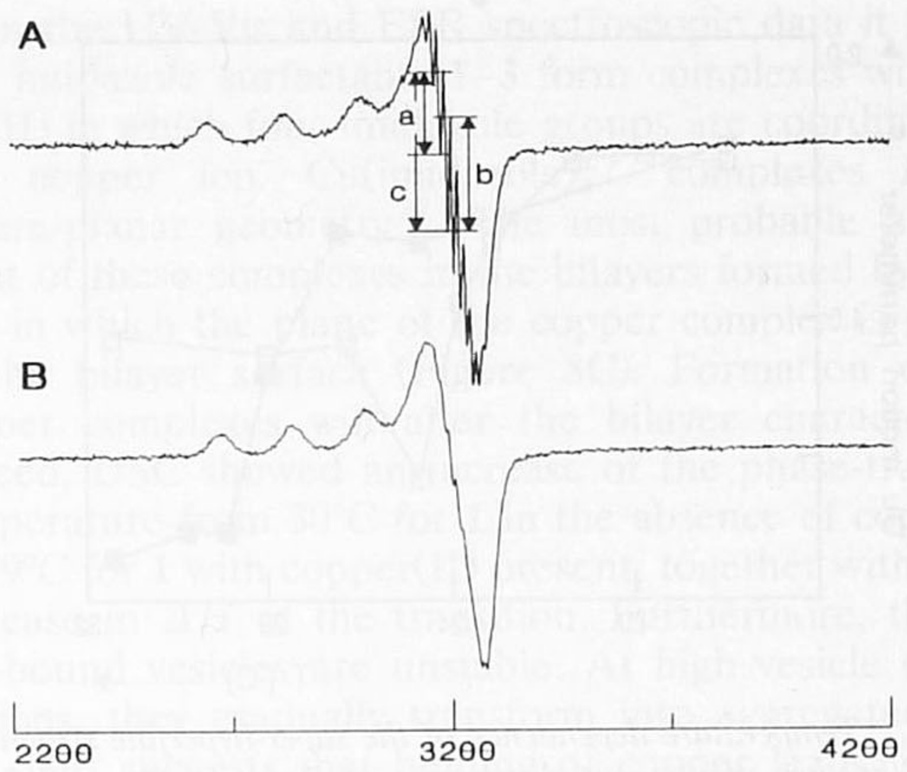

Figure 4. EPR spectra of aqueous dispersions of **1** · *HCl with 0.02 equivalent (A) and with 0.1 equivalent (B) of* $CuCl_2$ *present at 20°C.*

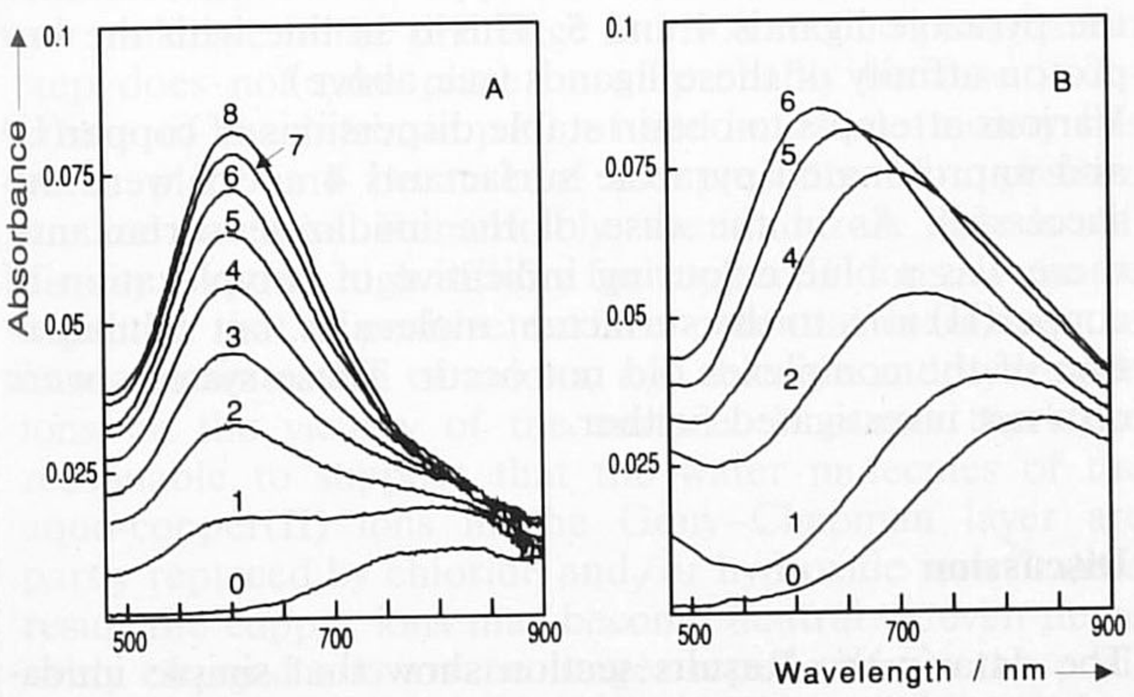

Figure 3. UV-Vis spectra of 1 mM aqueous $CuCl_2$ *in the presence of increasing concentrations of* **1** · *HCl (A). UV-Vis spectra of 1 mM* $Cu(ClO_4)_2$ *ethanol/chloroform (1/3, v/v) in the presence of increasing concentrations of* **1** · $HClO_4$ *(B). The numbers in the figures denote the surfactant to metal ion molar ratio, T 20°C.*

Table III EPR spectroscopic data [a].

Compound	$g_\parallel$	$g_\perp$	$A_\parallel^{Cu}$/G	A^N/G
1 · HCl + 0.02 eq. of $CuCl_2$	2.25	2.05	181	15.0
2 · HCl + 0.02 eq. of $CuCl_2$	2.26	2.05	176	15.5
Cu[1-butylimidazole]$_4(ClO_4)_2$ [b]	2.24	2.04	185	

[a] The spectral parameters were determined from spectra of 0.1 M aqueous dispersions of the surfactants in 0.1 M HCl and 0.002 M $CuCl_2$ at 20°C. [b] From Ref. 18.

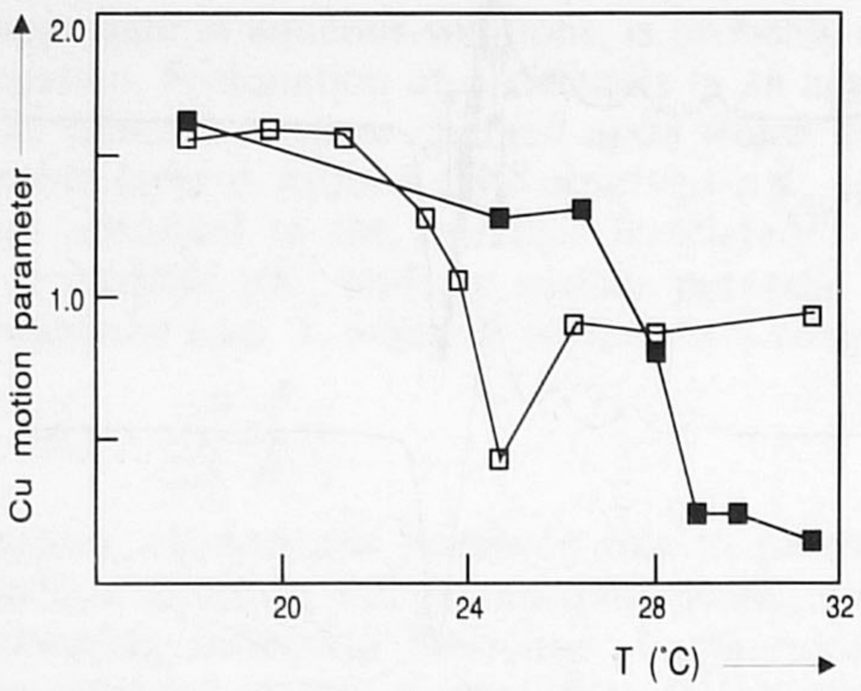

Figure 5. Temperature dependence of the super-hyperfine structure in the EPR spectra of aqueous dispersions of **1**·*HCl* (■) *and* **2**·*HCl* (□) *containing 0.02 equivalents of* $CuCl_2$. *The super-hyperfine structure is described by the Cu-motion parameter* $(a + b)/c$ *(Ref. 20). The variables a, b, and c are defined in Figure 4.*

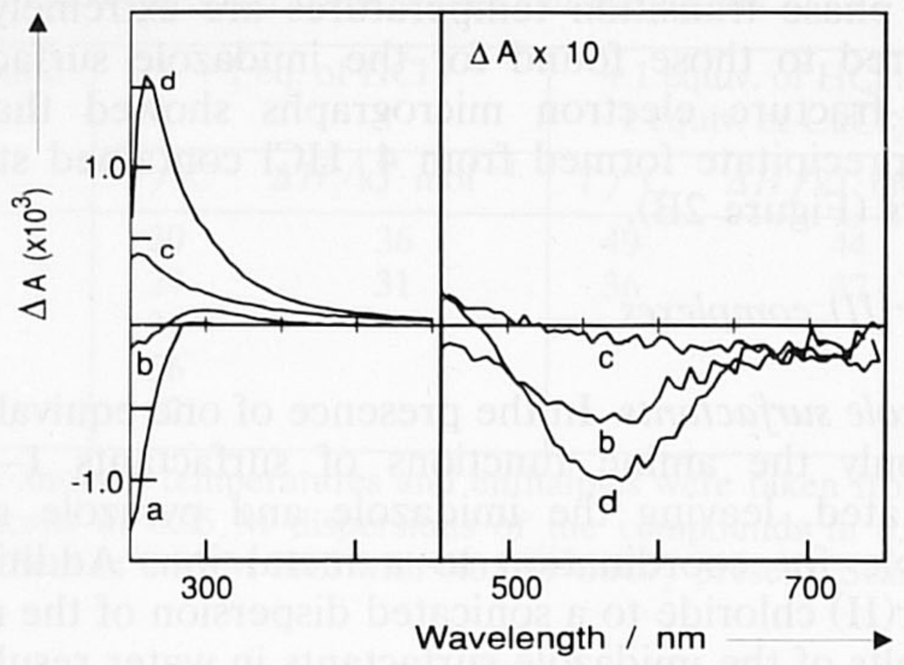

Figure 7. CD spectra of 3.5 mM solutions of **3**·$HClO_4$ *in ethanol / chloroform, 3 / 1 (v / v) (a) without and (b) with 0.65 mM* $Cu(ClO_4)_2$ *present. CD spectra of 3.5 mM aqueous dispersions of* **3**·*HCl (c) without and (d) with 0.65 mM* $CuCl_2$ *(d) T 20°C.*

have increased considerably upon addition of $CuCl_2$ compared to the metal-free dispersions. Freeze-fracture electron micrographs revealed that the precipitate of the copper complex of **1** · HCl consisted of aggregated bilayers (Figure 6).

The chiral imidazole surfactant **3** showed copper(II)-complexing properties similar to surfactants **1** and **2**. The UV-Vis absorbance spectrum of a 5 mM aqueous dispersion of **3** · HCl and 1 mM of $CuCl_2$ displayed a maximum at 590 nm. The EPR spectrum of this dispersion resembled that of the copper(II) complexes of **1** · HCl and **2** · HCl. In the circular-dichroism (CD) spectrum of **3** · HCl in water, a positive band with a maximum at 260 nm was visible (Figure 7). At wavelengths below 245 nm the CD spectrum became negative (not shown). Comparison with the UV spectra was not possible due to scattering of the solutions at low wavelengths. Remarkably, solutions of the $HClO_4$ salt of **3** in ethanol/chloroform (3:1 v/v) displayed a negative CD spectrum. Apparently, the CD spectrum is very sensitive to changes in the environment of the chiral head group, such as occur when the surfactant molecules become packed in a bilayer structure. Addition of $Cu(ClO_4)_2$ to a solution of **3**.$HClO_4$ in ethanol/chloroform (3:1 v/v) resulted in the formation of a positive band with a maximum at 300 nm and a negative band with a minimum at 570 nm. The former band probably originates from a metal-to-ligand charge transition in the chiral copper complex and the latter band from a *d–d* transition[15]. Upon addition of $CuCl_2$ to the aqueous vesicular dispersions of **3**.HCl a red shift of 5 nm and a threefold increase in the intensity of the band at 260 nm was observed. At the same time a negative band with a minimum at 570 nm appeared. The higher intensity of the band at 570 nm in water (Figure 7d) than in ethanol/chloroform solution (Figure 7b) is in line with the fact that the chiral copper complexes of **3** are more organised in the former solvent than in the latter.

Attempts were made to obtain stable dispersions of copper(II) and unprotonated imidazole surfactants **1–3**. Although the copper(II) ions were bound to the surfactant molecules, as could be detected by the blue colouring, solubilisation of the complexes did not occur. We did not therefore investigate these systems further.

Pyrazole surfactants. Addition of a copper(II) chloride solution to a suspension of the pyrazole compounds **4** · HCl and **5** · HCl did not result in any visible changes in the morphology or the colour of the precipitates. At temperatures above 60°C the precipitates became blue, indicating that coordination of copper(II) had occurred, but they did not solubilise. Upon cooling the blue colour disappeared, leaving only white solids. Separate titrations of $Cu(ClO_4)_2$ in ethanol/chloroform mixtures with compound **4** · $HClO_4$ revealed that in homogeneous solution the pyrazole moiety does not coordinate to the copper ion. EPR spectra of compound **4** · HCl recorded in the presence of 0.02 equivalents of copper(II) chloride in water displayed a single broad line, without any hyperfine structure. Similar spectra were observed for copper(II) chloride alone in aqueous solution. Apparently, the copper ions do not bind to the pyrazole ligands **4** and **5**. This is in line with the low proton affinity of these ligands (see above).

Various attempts to obtain stable dispersions of copper(II) and unprotonated pyrazole surfactants **4** and **5** were unsuccessful. As in the case of the imidazole surfactants, there was a blue colouring indicative of complexation of copper(II) ions to the surfactant molecules, but solubilisation of the complexes did not occur. These systems were also not investigated further.

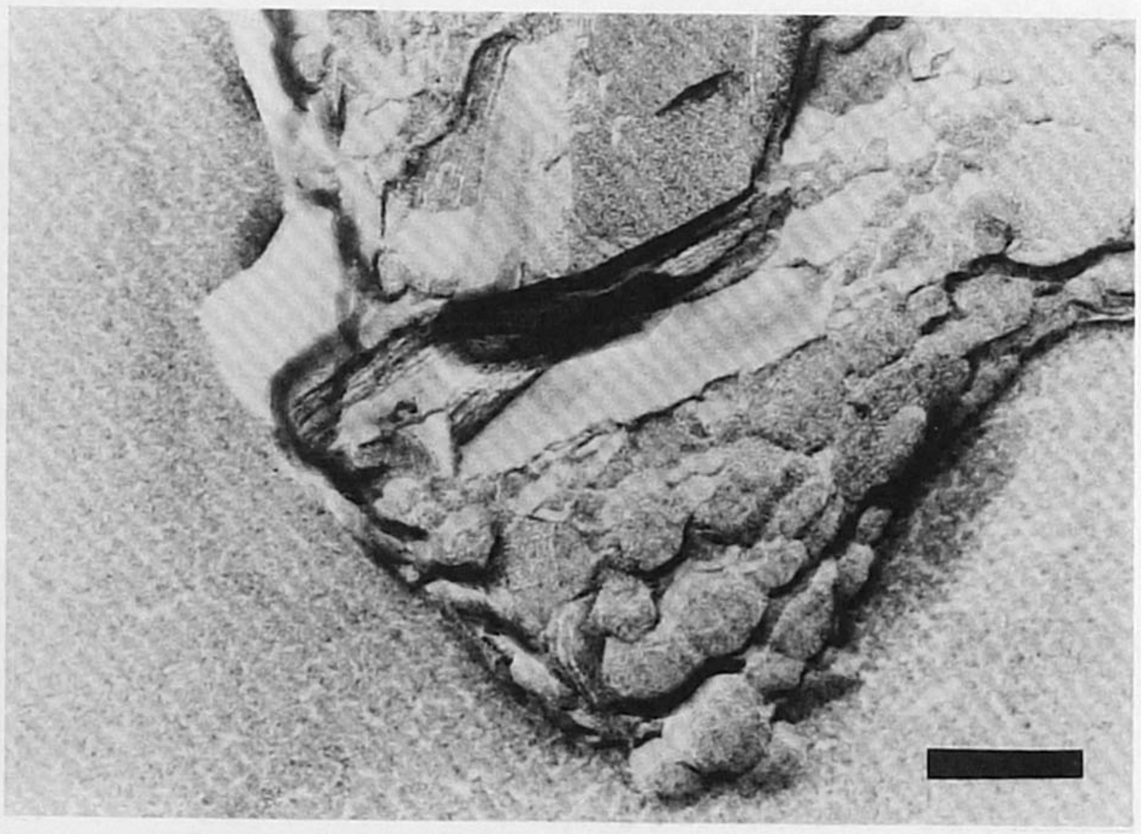

Figure 6. Freeze-fracture electron micrograph of a 0.05 M aqueous dispersion of **1**·*HCl in 0.05 M* $CuCl_2$ *(magn. 76000 ×, bar represents 200 nm).*

Discussion

The data in the Results section show that simple imidazole-containing surfactants such as **1–3** can form vesicles in aqueous solutions and can bind copper(II) ions to give complexes in which four imidazole ligands are coordinated to one copper ion. In contrast, the pyrazole surfactants **4** and **5** do not form vesicles and do not bind

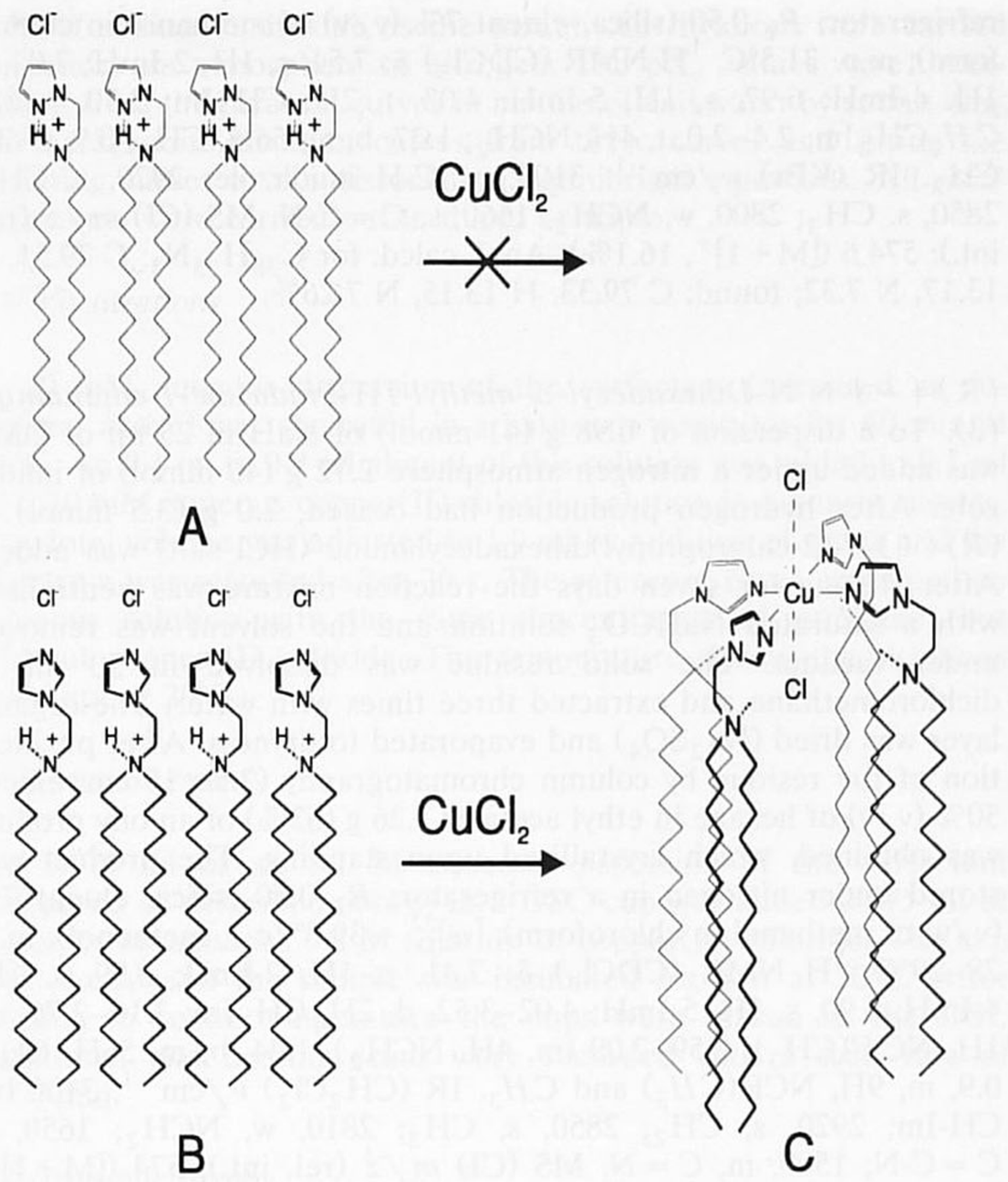

Figure 8. Possible arrangements of **4**·*HCl (A),* **1**·*HCl (B), and of copper(II) complexes of* **1**·*HCl (C) in bilayer aggregates.*

copper(II) ions. Instead, upon protonation in water, these surfactants give white precipitates which consists of stacked bilayers. We ascribe this different behaviour to the fact that compounds **4** and **5** can easily form intramolecular hydrogen bonds which prevents coordination of the pyrazole moieties to copper (Figure 8A). Intramolecular hydrogen bonds also lead to a dispersion of the positive charge, which most probably results in a less hydrated headgroup. As a result the packing parameters for **4** and **5** will be smaller than the packing parameters for **1–3**, causing the formation of planar bilayers instead of vesicles[21,22]. Studies reported in the literature on phosphatidylcholines and synthetic cationic surfactants have shown that incomplete hydration generally leads to higher phase-transition temperatures[23–25]. The much higher phase-transition temperatures of the pyrazole surfactant aggregates compared to those of the imidazole surfactant aggregates are in line with this.

The imidazole surfactants cannot form intramolecular hydrogen bonds, leaving the charge concentrated on the amine function which will become fully hydrated (Figure 8B). Protonation of surfactants **1–3** is limited to one H^+ per molecule in the pH range 3–11. A second protonation step does not take place, most probably because a decrease of the intrinsic pK_a^i of the imidazole moiety has taken place and because of the presence of a positive surface potential. Remarkably, the imidazole ligands still display a rather high affinity for copper(II) ions. It can be expected that a positive surface potential increases the local concentration of anions, *e.g.* chloride and hydroxide ions, in the vicinity of the surface. It seems therefore reasonable to suppose that the water molecules of the aquo-copper(II) ions in the Gouy–Chapman layer are partly replaced by chloride and/or hydroxide ions[26]. As a result the copper ions may become neutral or even negatively charged and are no longer repelled from the positively charged surface. It is also possible that ion exchange between the positively charged copper ions and protons bound to the surfactant molecules occurs. Such an exchange would be expected to affect both surface potential and pH, but this has not been further investigated.

From the UV-Vis and EPR spectroscopic data it is clear that imidazole surfactants **1–3** form complexes with copper(II) in which four imidazole groups are coordinated to one copper ion. Cu(imidazole)$_4^{2+}$ complexes have a square-planar geometry[27]. The most probable arrangement of these complexes in the bilayers formed by **1–3** is one in which the plane of the copper complex is parallel to the bilayer surface (Figure 8C). Formation of such copper complexes will alter the bilayer characteristics. Indeed, DSC showed an increase of the phase-transition temperature from 30°C for **1** in the absence of copper(II) to 49°C for **1** with copper(II) present, together with a 20% increase in ΔH of the transition. Furthermore, the copper-bound vesicles are unstable. At high vesicle concentrations, they gradually transform into aggregated bilayers. This suggests that binding of copper leads to a decrease of the surface charges and to a dehydration of the headgroups, as discussed above for the pyrazole surfactants.

The metallo-aggregates presented here are interesting systems, giving the possibility to perform enantio-selective catalysis. Work along this line is in progress.

Experimental

General

^{1}H-NMR spectra were recorded on a Bruker WH 90 instrument (90 MHz). Chemical shifts are given in ppm downfield from tetramethylsilane. Abbreviations used are s = singlet, d = doublet, t = triplet, m = multiplet, b = broad. The proton to which the signal is assigned is given in italics, when necessary. Transmission-electron microscopy was carried out with a Philips EM 201 instrument. A Branson 2200 sonication bath was used for the preparation of the vesicles by the sonication method. Differential-scanning calorimetry (DSC) measurements were performed on a Setaram DSC 111 instrument (Dr. *J.C. van Miltenburg*, Algemene Chemie, University of Utrecht). EPR (electron paramagnetic resonance) spectra were recorded with a Bruker ESP 300 spectrometer. Infrared and UV-Vis spectra were recorded on Perkin Elmer 298 and Perkin Elmer Lambda 5 spectrophotometers, respectively. CD (circular dichroism) spectra were recorded on an Auto-dichrograph Mark V (Jobin Yvon) apparatus (Department of Biochemistry, University of Wageningen). The cuvettes for UV-Vis and CD measurements were thermostatted with an accuracy of 0.1°C.

Compounds

Technical grade solvents were used, unless otherwise indicated. DMF (dimethylformamide) and THF (tetrahydrofuran) were distilled before use. Column chromatography and TLC (thin-layer chromatography) were performed on silica (Merck, silica gel 60H or precoated F-254 plates). For the preparation of the bilayer and vesicles dispersions de-ionised and triple-distilled water was used.

2-(Dihexadecylamino)ethanol. A solution of 3.06 g (50 mmol) of 2-aminoethanol, 36.6 ml (120 mmol) of hexadecyl bromide, and 21 ml (120 mmol) of N,N-diisopropylethylamine in 150 ml of ethanol was refluxed for 72 h. The reaction mixture was cooled to room temperature and the solvent was removed under vacuum. The resulting oily residue was dissolved in 100 ml of dichloromethane and washed three times with aqueous 2N HCl. The organic layer was dried (Na_2SO_4) and the solvent was removed under vacuum. The crude product was isolated from the residue by addition of 50 ml of ice-cold ethylacetate. After two recrystallisations from ethyl acetate the product was obtained as the HCl-salt; yield 26.4 g (49 mmol, 98%); R_f 0.37 (silica, eluent 7% (v/v) of methanol in chloroform). ^{1}H NMR ($CDCl_3$) δ: 4.0, t, 2H, CH_2OH; 3.21–3.0, b m, 6H, NCH_2; 2.1–1.6, b s, ~3H, H_2O; 1.30, b, m, 56H, CH_2; 0.9, t, 6H, CH_3.

3-(Dihexadecylamino)-1-propanol. This compound was synthesised as described for 2-(dihexadecylamino)ethanol, starting from 1.88 g (0.025 mol) of 3-amino-1-propanol, 18.3 ml (60 mmol) of 1-bromohexadecane, and 10.5 ml (60 mmol) of *N,N*-diisopropylethylamine in 100 ml of ethanol. After recrystallisation from ethyl acetate the product

still contained impurities. The product was dissolved in 100 ml of dichloromethane and washed twice with an aqueous 2N sodium hydroxide solution. The organic layer was dried (Na_2SO_4) and concentrated under vacuum. The pure product was obtained by flash chromatography (silica, 15×5 cm column, eluent 20% (v/v) of hexane in ethyl acetate); yield 6.8 g (0.013 mol, 53%) of a white solid; R_f 0.37 (silica, eluent 7 vol.% of methanol in chloroform). ^{1}H NMR (HCl salt) ($CDCl_3$) δ: 3.81, t, 2H, CH_2OH; 3.30–3.0, b, m, 7H, NCH_2 and OH; 1.30, b, m, 58H, CH_2 and CH_2CH_2OH; 0.9, t, 6H, CH_3.

(R)-(−)-2-(Dihexadecylamino)-1-propanol. This compound was synthesised as described for 2-(dihexadecylamino)-ethanol, starting from 2.05 g (27.3 mmol) of (R)-(−)-2-amino-1-propanol, 24 ml (79 mmol) of 1-bromohexadecane, and 14 ml (80 mmol) of *N,N*-diisopropylethylamine in 100 ml of ethanol; yield 14.0 g (92%) of the products as the HCl salt; R_f 0.39 (silica, eluent 7% (v/v) of methanol in chloroform); $[\alpha]_D^{22}$ −9.6° (c 1, methanol). ^{1}H NMR ($CDCl_3$) δ: 3.81, d, 2H, CH_2OH); 3.48–3.30, m, 1H, NCH(CH_3); 3.24–2.85, b m, 4H, NCH_2; 1.40, d, 3H, NCH(CH_3); 1.30, b m, 58H, CH_2 and CH_2CH_2OH; 0.9, t, 6H, CH_2CH_3; 2.1–1.6, b s, ~2H, H_2O.

N-*(2-Chloroethyl)dihexadecylamine.* To 5 g (9.15 mmol) of 2-(dihexadecylamino)ethanol (HCl salt) in 100 ml of dichloromethane was slowly added 3 ml of thionyl chloride at 0°C. Hereafter the reaction mixture was allowed to warm up to room temperature. After stirring for 2 h, the mixture was poured into 100 ml of ice–water. The organic layer was extracted twice with water and dried over anhydrous Na_2SO_4. After removal of the solvent under vacuum, the product was obtained as the HCl salt; yield 5.03 g (97%); R_f 0.87 (silica, eluent 7% (v/v) of methanol in chloroform). ^{1}H NMR ($CDCl_3$) δ: 4.04, t, 2H, CH_2Cl; 3.34, t, 2H, NCH_2CH_2Cl; 3.13–2.95, m, 4H, NCH_2; 1.30, b, 56H, CH_2; 0.9, t, 6H, CH_3; 2.1–1.6, b s, ~2H, H_2O.

N-*(3-Chloropropyl)dihexadecylamine.* This compound was synthesised as described for *N*-(2-chloroethyl)dihexadecylamine, starting from 5.96 g (0.011 mmol) of 3-(dihexadecylamino)-1-propanol; yield 6.09 g (92.5%) of a white solid product as the HCl-salt; R_f 0.73 (7% (v/v) of methanol in chloroform). ^{1}H NMR ($CDCl_3$) δ: 3.68, t, 2H, CH_2Cl; 3.10–2.90, b, m, 6H, NCH_2; 2.54–2.27, b, m, 2H, CH_2CH_2Cl; 1.26, b, 58H, CH_2; 0.9, t, 6H, CH_3; 2.1–1.6, b s, ~3H, H_2O.

(R)-(+)-N-2-(1-Chloropropyl)dihexadecylamine. This compound was synthesised as described for *N*-(2-chloroethyl)-dihexadecylamine, starting from 5.0 g (8.9 mmol) of (R)-(−)-2-(dihexadecylamino)-1-propanol; yield 4.5 g (83%) of the product as the HCl salt; R_f 0.81 (silica, eluent 7% (v/v) of methanol in chloroform); $[\alpha]_D^{22}$ +10.4° (c 1, methanol). ^{1}H NMR ($CDCl_3$) δ: 3.59–3.40, m, 1H, NCH(CH_3); 3.36–3.23, d, 2H, CH_2Cl; 3.19–2.98, b, m, 4H, NCH_2; 1.66, d, 3H, NCH(CH_3); 1.26, b, m, 56H, CH_2; 0.9, t, 6H, CH_3; 2.1–1.6, b s, ~2H, H_2O.

N,N-*Dihexadecyl-1*H-*imidazole-1-ethanamine* (**1**). A solution of 2.03 g (3.6 mmol) of N-(2-chloroethyl)dihexadecylamine (HCl salt) and 1.23 g (18 mmol) of imidazole in 70 ml of acetonitrile was refluxed for 3 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica, 4×10-cm column, eluent 20% (v/v) of hexane in ethyl acetate) to give 1.42 g (70%) of an oily product which became solid upon standing; R_f 0.50 (silica, eluent 7% (v/v) of methanol in chloroform); m.p. 40–41°C. ^{1}H NMR ($CDCl_3$) δ: 7.54, s, 1H, 2-ImH; 7.03, s, 1H, 5-ImH; 4.03, t, 2H, CH_2Im; 2.70, t, 2H, CH_2CH_2Im; 2.4–2.0, t, 4H, NCH_2; 1.27, b, m, 56H, CH_2; 0.9, t, 6H, $-CH_3$. IR (KBr) ν/cm^{-1}: 3100, w, CH-Im; 2920, s, CH_2; 2850, s, CH_3; 2800, w, NCH_2; 1660, s, C=C-N. MS (CI) m/z (rel. int.): 560.4 ([M+1]$^+$, 16.1%). Anal. calcd. for $C_{37}H_{73}N_3$: C 79.36, H 13.14, N 7.50; found: C 78.89, H 13.17, N 7.26%.

N,N-*Dihexadecyl-1*H-*imidazole-1-propanamine* (**2**). A solution of 8.7 g (15 mmol) of N-(3-chloropropyl)dihexadecylamine, 5.1 g (75 mmol) of imidazole, and 5.3 g of sodium iodide in 70 ml of acetonitrile were refluxed for 24 h. The solvent was removed under vacuum and the residue was dissolved in 100 ml of dichloromethane. After extraction with 2N aqueous HCl the organic layer was dried (Na_2SO_4) and the solvent was removed under vacuum. The oily residue was purified by column chromatography (5×10-cm column, eluent 50% (v/v) hexane in ethyl acetate) to give 3.4 g (41%) of an oily product which became solid upon standing. The product was stored under nitrogen in a refrigerator; R_f 0.50 (silica, eluent 7% (v/v) of methanol in chloroform); m.p. 31.5°C. ^{1}H NMR ($CDCl_3$) δ: 7.54, s, 1H, 2-ImH; 7.03, s, 1H, 4-ImH; 6.97, s, 1H, 5-ImH; 4.03, t, 2H, CH_2Im; 2.70, t, 2H, CH_2CH_2Im; 2.4–2.0, t, 4H, NCH_2; 1.27, b, m, 56H, CH_2; 0.9, t, 6H, CH_3. IR (KBr) ν/cm^{-1}: 3100, w, C-H imidazole; 2920, s, CH_2; 2850, s, CH_3; 2800, w, NCH_2; 1660, s, C=C-N. MS (CI) m/z (rel. int.): 574.6 ([M+1]$^+$, 16.1%). Anal. calcd. for $C_{38}H_{75}N_3$: C 79.51, H 13.17, N 7.32; found: C 79.33, H 13.15, N 7.26%.

*(R)-(−)-*N,N-*Dihexadecyl-α-methyl-1*H-*imidazole-1-ethanamine* (**3**). To a dispersion of 0.98 g (41 mmol) of NaH in 25 ml of DMF was added under a nitrogen atmosphere 2.72 g (40 mmol) of imidazole. After hydrogen production had ceased, 2.0 g (3.5 mmol) of (*R*)-(+)-*N*-(2-chloropropyl)dihexadecylamine (HCl salt) was added. After stirring for seven days the reaction mixture was neutralised with a saturated $NaHCO_3$ solution and the solvent was removed under vacuum. The solid residue was dissolved in 50 ml of dichloromethane and extracted three times with water. The organic layer was dried (Na_2SO_4) and evaporated to dryness. After purification of the residue by column chromatography (2.5×15 cm, eluent 50% (v/v) of hexane in ethyl acetate) 1.26 g (63%) of an oily product was obtained, which crystallised upon standing. The product was stored under nitrogen in a refrigerator; R_f 0.50 (silica, eluent 7% (v/v) of methanol in chloroform); $[\alpha]_D^{22}$ −39.6° (c 1, methanol); m.p. 28–29°C. ^{1}H NMR ($CDCl_3$) δ: 7.41, s, 1H, 2-ImH; 6.99, s, 1H, 4-ImH; 6.90, s, 1H, 5-ImH; 4.02–3.52, d, 2H, CH_2Im; 3.16–2.76, m, 1H, NCH(CH_3); 2.59–2.09 (m, 4H, NCH_2), 1.24, b, m, 56H, CH_2; 0.9, m, 9H, NCH(CH_3) and CH_3. IR (CH_2Cl_2) ν/cm^{-1}: 3100 (w, CH-Im; 2920, s, CH_2; 2850, s, CH_3; 2810, w, NCH_2; 1650, s, C=C-N; 1500, m, C=N. MS (CI) m/z (rel. int.): 574 ([M+1]$^+$, 2.93%). Anal. calcd. for $C_{38}H_{75}N_3$: C 79.51, H 13.17, N 7.32; found: C 79.53, H 13.04, N 7.34%.

N,N-*Dihexadecyl-1*H-*pyrazole-1-ethanamine* (**4**). This compound was synthesized as described for **3**, starting from 2.04 g (30 mmol) of pyrazole and 1.5 g (2.7 mmol) of *N*-(2-chloroethyl)dihexadecylamine (HCl salt): yield after a reaction time of 3 days 0.64 g (43%) of an oily product; R_f 0.55 (silica, eluent 7% (v/v) of methanol in chloroform). ^{1}H NMR ($CDCl_3$) δ: 7.48, d, 1H, 5-PzH; 7.42, d, 1H, 3-PzH; 6.19, s, 1H, 4-PzH; 4.16, t, 2H, CH_2-Pz; 2.76, t, 2H, CH_2CH_2-Pz; 2.4, b, 4H, NCH_2; 1.27, b, m, 56H, CH_2; 0.9, t, 6H, CH_3. IR (CH_2Cl_2) ν/cm^{-1}: 3115, w, CH-Pz; 2920, s, CH_2; 2855, s, CH_3; 2800, w, NCH_2; 1690, s, C=C-N; 1510, m, C=N. MS (CI) m/z (rel. int.): 560.0 ([M+1]$^+$, 14.35%). Anal. calcd. for $C_{37}H_{73}N_3$: C 79.36, H 13.14, N 7.50; found C 79.20, H 13.68, N 7.51%.

N,N-*Dihexadecyl-1*H-*pyrazole-1-propanamine* (**5**). This compound was synthesized as described for **3**, starting from 2.04 g of pyrazole (30 mmol) and 1.48 g (2.57 mmol) of *N*-(3-chloropropyl)dihexadecylamine: yield after a reaction time of 3 days 0.42 g (43%) of a white solid product; R_f 0.55 (7% (v/v) of methanol in chloroform); m.p. 33°C. ^{1}H NMR ($CDCl_3$) δ: 7.51, s, 1H, 5-PzH; 7.24, s, 1H, 3-PzH; 6.23, s, 1H, 4-PzH; 4.19, t, 2H, CH_2-Pz; 2.70, t, 2H, CH_2CH_2-Pz; 2.47–2.23, m, 6H, NCH_2; 1.27, b, m, 56H, CH_2; 0.9, t, 6H, CH_3. IR (KBr) ν/cm^{-1}: 3115, w, CH-Pz; 2917, s, CH_2; 2855, s, CH_3; 2790, w, NCH_2; 1631, s, C=C-N; 1513, m, C=N. MS (CI) m/z (rel. int.): 574.1 ([M+1]$^+$, 22.28%). Anal. calcd. for $C_{38}H_{75}N_3$: C 79.51, H 13.17, N 7.32; found: C 79.49, H 13.23, N 7.32%.

Preparation of vesicle dispersions

In a typical preparation 11.2 mg (0.02 mmol) of **1** was added to 0.2 ml of an aqueous 0.1N HCl solution. An opalescent dispersion was obtained after heating the solution to 50°C and vortexing for 1 min. This dispersion was diluted with water to the desired concentration of the surfactant.

Acid–base titrations

For measurements of the pH in methanol (pH*) a combined glass reference electrode was used from which the reference compartment was filled with a saturated solution of lithium chloride in methanol. The electrode was calibrated with buffer solutions of 4-toluenesulphonic acid in methanol (pH 3.0) and of benzene acetic acid in methanol (pH 8.0)[28]. The titrations in methanol were carried out on 40-ml samples containing 2.5 mM of the surfactant, 9.6 mM HCl, and 0.05 M $(CH_3)_4NCl$, using 0.085 M sodium methanolate in methanol as the titrant. Titrations in water were performed on 40 ml samples containing 1 mM of the surfactant and 3 mM HCl, using aqueous

0.01 M sodium hydroxide as the titrant. All titrations were carried out under an atmosphere of nitrogen. The pK_a values were determined from the titration curves in methanol and water by subtracting the background titration curves from these curves and fitting the resulting curves to the dissociation equilibrium equations. All measurements were carried out in duplo or triplo.

UV-Vis titrations

A 10 mM aqueous dispersion of the surfactant (prepared as described above) was sonicated in a bath-type sonicator for 60 min at 50°C. An 0.1 ml to 0.9 ml aliquot of this solution was added to 0.1 ml of a 10 mM aqueous copper(II) chloride solution in a quartz cuvette. The total volume was adjusted to 1.0 ml by addition of water and the spectrum was recorded after 30 s. The reference cuvet contained an aqueous solution with the same concentration of surfactant but without copper(II) chloride. The temperature during the titrations was kept at 20°C.

DSC measurements

To 0.075 ml of an 0.1 M aqueous dispersion of the surfactant (prepared as described above) in a DSC cup was added 0.075 ml of water or an aqueous 0.2 M solution of copper(II) chloride. The cup was closed and the sample was incubated for 1 h at 50°C. After cooling to room temperature the cups were placed in the DSC apparatus and heating scans were recorded with a scan rate of 2°C/min.

Electron microscopy

Samples for freeze-fracture electron microscopy were prepared as described for the DSC measurements.

EPR measurements

To 0.25 ml of an aqueous 0.1 M dispersion of the surfactant (prepared as described above) in an Eppendorf cup was added 0.005 ml of an aqueous 0.1 M solution of copper(II) chloride. An 0.05 ml aliquot of this solution was transferred to a capillary tube (diameter ~1 mm) which was closed on one side. The other side was sealed with parafilm and the capillary tube was placed in an EPR tube filled with paraffin oil. The latter tube was placed in the cavity of the spectrometer. The temperature in the cavity was controlled and set with the help of a flow of nitrogen. Prior to each experiment the temperature in the cavity was measured via a thermocouple placed in a EPR tube which was filled with paraffin oil.

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References and notes

1a *F.M. Menger, L.H. Gan, E. Johnson, D.H. Durst*, J. Am. Chem. Soc. **109**, 2800 (1987);
b *R. Fornasier, P. Scrimin, U. Tonellato, N. Zanta*, J. Chem. Soc. Chem. Commun. 716 (1988);
c *R. Fornasier, P. Scrimin, P. Tecilla, U. Tonellato*, J. Am. Chem. Soc. **111**, 224 (1989);
d *T. Fujita, K. Ogino, W. Tagaki*, Chem. Lett. 981 (1988).
2 *J. Simon, J. Le Moigne, D. Markovitsky, J. Dayantis*, J. Am. Chem. Soc. **102**, 7247 (1980).
3 *T. Kunitake, Y. Ishikawa, M. Shimomura*, J. Am. Chem. Soc. **108**, 327 (1986).
4 *M. Shimomura, T. Kunitake*, J. Am. Chem. Soc. **104**, 1757 (1982).
5 *J. Le Moigne, J. Simon*, J. Phys. Chem. **84**, 170 (1980)
6 *T. Shimuzu, M. Mori, H. Minamikawa, M. Hato*, J. Chem. Soc. Chem. Commun. 183 (1990).
7 *J. van Esch, A.L.H. Stols, R.J.M. Nolte*, J. Chem. Soc. Chem. Commun. 1658 (1990).
8 *D.R. Christ, N.J. Leonard*, Angew. Chem. **81**, 953 (1969).
9 *J.P. Guthrie, D.C. Pike*, Can. J. Chem. **65**, 1951 (1987).
10 *M.S. Fernandez, P. Fromherz*, J. Phys. Chem. **81**, 1755 (1977).
11 *C.J. Drummond, F. Grieser, W. Healy*, J. Chem. Soc., Far. Trans. I **85**, 521 (1989).
12 *J. Kibblewhite, C.J. Drummond, F. Grieser, P.J. Thistlethwaite*, J. Phys. Chem. **93**, 7464 (1989).
13 *J. Marra*, J. Phys. Chem. **90**, 2145 (1986).
14 *J. Reedijk*, Recl. Trav. Chim. Pays-Bas. **88**, 1451 (1969).
15 *E. Bernarducci, W.F. Schwindinger, J.L. Hughey, K. Krogh-Jespersen, H.J. Schugar*, J. Am. Chem. Soc. **103**, 1686 (1981).
16 Perchlorate was used as the counter-ion instead of chloride since in organic solvents like ethanol and chloroform, chloride ion is a strong ligand for the Cu(II) ion. Titration of $CuCl_2$ with **1**·HCl in ethanol/chloroform (1/3, v/v) reveals binding of only one molecule of **1** to the copper ion, the other coordination sites being occupied by the chloride ions. In aqueous solutions the chloride ion is strongly hydrated and is a much weaker ligand.
17 *J.D.R. Hille, G.M. Donné-Op den Kelder, P. Sauve, G.H. de Haas, M.R. Egmond*, Biochemistry. **20**, 4068 (1981).
18 *J. Reedijk*, J. Inorg. Nucl. Chem. **33**, 179 (1971).
19 *G. Palmer*, Biochem. Soc. Trans. **13**, 548 (1985).
20 *A. Pezeshk, M. Pasenkiewicz-Gierula, W.K. Subczynski, W.E. Antholine*, J. Phys. Chem. **94**, 451 (1990).
21 *J.N. Israelachvili, S. Marcelja, R.G. Horn*, Quart. Rev. Biophys. **13**, 2 (1980).
22 *J.N. Israelachvili, D.J. Mitchell, B.W. Ninham*, J. Chem. Soc. Far. Trans. 2, **72**, 1525 (1976).
23 *J.F. Nagle, D.A. Wilkinson*, Biophys. J. **23**, 159 (1978).
24 *R.G. Laughlin, R.L. Munyon, Y.-C. Fu, A.J. Fehl*, J. Phys. Chem. **94**, 2546 (1990).
25 *D.M. Small*, J. Lipid. Res. **8**, 551 (1967).
26 Comprehensive Inorganic Chemistry; ed. *A.F. Trotman-Dickinson*, Pergamon Press, Oxford, 1973, vol. 3, 1.
27 *G. Franson, B.K.S. Lundberg*, Acta. Chem. Scand. **26**, 3969 (1972).
28 *D.D. Perrin, B. Dempsey*, Buffers for pH and metal ion control, Wiley and Sons, London, 1974.
29 CRC Handbook of Chem. Phys., ed. *C.R. Weast*, CRC Press INC., 1980, 61st edition, Boca Raton, Florida.