Detecting a transition-metal ammine at tailored surfaces

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The fabrication of surfaces by forming Langmuir films, which incorporate amphiphiles containing hydrophilic 18-crown-6 (18C6) derivatives, at a gas/water interface is described. These Langmuir films can be transferred to a hydrophobised quartz crystal microbalance (QCM), using the Langmuir–Blodgett technique. The QCM response has been measured in aqueous solution as a function of the concentration of the transition metal complex $[Co(NH_3)_6]Cl_3$ which was injected into a vial in which the film-coated QCM had been immersed. By comparing various surfaces covered with hydrophilic polyether and hydroxy functions and hydrophobic methyl groups, and by varying the composition of the films so as to increase the separation between the 18C6 macrocycles, it has been demonstrated that surfaces can be tailored that will enhance the binding of the $[Co(NH_3)_6]^{3+}$ trications.

In 1959, Sauerbrey[1] showed that, when a quartz crystal comes into contact with a gas, the change in frequency DF of the quartz crystal, sandwiched between two excitation electrodes, of natural resonant frequency F_0 (s^{-1}), density r (2.648 g cm^{-3}), and shear modulus m (2.947×10^{11} dyn cm^{-2} for AT-cut quartz) is related to the adsorbed mass Dm by the relationship:

$$\mathrm{D}F = -\frac{2F_o^2}{A(\mathrm{rm})}\frac{\mathrm{D}m}{1/2} \quad (1)$$

where A is the exposed surface area (m^2) of the quartz. This pioneering activity led to the development of the quartz crystal microbalance (QCM). The QCM has proved to be a highly versatile instrument for the determination of the amount of material deposited from the gas phase[2] on to a solid surface. Applications include thickness monitors in metal evaporation and deposition processes, and the detection of gas-phase analytes, such as hydrocarbons, water vapour and other volatile compounds. A more demanding, but potentially more important, area in which the QCM is being employed currently, is in liquid media,[3] where it has been used to measure interfacial processes at electrode surfaces.[4] It has also been employed as an immunosensor[5] at the nanogram level to monitor antibodies,[6] bacterial growth,[7] cells,[8] proteins,[9] and microbes,[10] as well as to detect surfactants,[11] anaesthetics,[12] antibiotics,[13] bitter and odorous substances,[14] DNA hybridisation,[15] pH changes,[16] enzyme reactions,[17] liposomes,[18] chiral recognition,[19] intercalation,[20] and even cell growth.[21] Many of these experiments involve the molecular recognition of a substrate from the subphase to a biological receptor which has been deposited on the QCM surface. There are, however, very few examples in the literature where the QCM has been utilised to detect recognition events involving totally synthetic systems.[22]

The research reported in this paper relates to detecting molecular recognition events within a wholly synthetic system. It is known from many crystal structures[23] that transition-metal (ammine) complexes (TMCs) hydrogen bond[24] *via* the hydrogen atoms of their ammine ligands to the oxygen atoms of crown ethers, *e.g.* 18-crown-6 (Fig. 1). This type of molecular recognition is an example of second-sphere coordination,[25] a concept first discussed by Alfred Werner[26] in 1912 when he noted that the optical properties of chiral transition-metal ammine complexes depend significantly on the nature of the solvent molecules. In the times of Werner, these non-covalent bonding interactions were not well understood at a molecular level. However today, with the advent of various spectroscopic techniques and X-ray crystallography, intermolecular interactions are becoming more fully understood. Indeed, new areas of science are emerging from the study of molecular interactions: they include crystal engineering,[27] host–guest[28] and supramolecular chemistry.[29] They are all disciplines which rely upon the natural concepts of self-assembly[30] and self-organis-

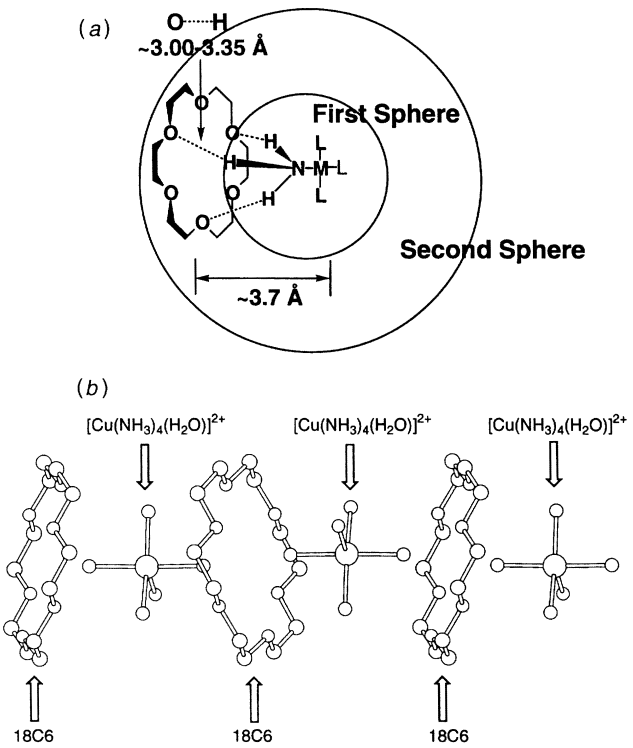


Fig. 1 Second-sphere coordination in the solid state: (*a*) illustrating the first- and second-sphere ligands, and (*b*) illustrating the supramolecular 1:1 polymer formed between $[Cu(NH_3)_4(H_2O)]^{2+}$ and 18C6 (hydrogen atoms are omitted for clarity)

ation[31] to construct arrays of molecules held together by non-covalent bonding interactions.[32]

Here, we report the chemical modification of the 18-crown-6 (18C6) macrocycle in order to facilitate its incorporation[33] into Langmuir films which can be deposited on to a QCM by the vertical dipping method, such that the hydrophilic head groups are exposed to the aqueous environment. An aqueous solution of $[Co(NH_3)_6]Cl_3$ can then be injected into the aqueous environment in which the LB film-coated QCM is immersed, and the frequency response can be measured as a function of the concentration of $[Co(NH_3)_6]Cl_3$ to yield information about the kinetics and thermodynamics of the recognition process.[34]

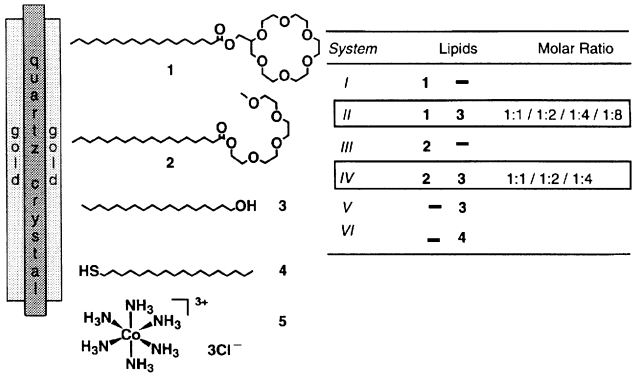
Results and Discussion

General remarks

The compounds, which were used in the present research, are listed in Fig. 2. Compound **1** is a chemically modified 18C6 derivative in which one of the methylene hydrogen atoms has been replaced by an oxymethylene octadecanoate chain to make it amphiphilic in nature. Compound **2** is a linear polyether analogue of **1** in which the polyether is bonded covalently to the aliphatic chain by an ester linkage. Compounds **3** and **4** are simply the commercially available octadecanol and thiooctadecanol, respectively. Compound **5** is the kinetically inert transition-metal ammine complex $[Co(NH_3)_6]Cl_3$. Compounds **1**–**4** were chosen for several reasons. Firstly, **1** and **2** were utilised to establish if a macrocyclic effect was operating in addition to purely non-specific binding. The hydroxy compound **3** was employed to establish if the transition-metal ammine complex had any affinity for a hydrophilic surface. Conversely, the thiol **4** was utilised to establish if the $[Co(NH_3)_6]Cl_3$ **5** had an affinity for a hydrophobic surface. Additionally, the hydroxy amphiphile **3** was employed to increase the separation of the polyether headgroups in cospread monolayers incorporating **1** and **2**. This incorporation of **3** into monolayers formed from ether containing compounds **1** and **2** allows control over the intermolecular separation between the ether moieties in these monolayers, something which is crucial for the transition-metal ammine complex to become inserted into the monolayer. Thus, various systems were investigated in which the molar ratio of the octadecanol to the ether amphiphiles was increased systematically. These systems are illustrated in Fig. 2.

Monolayer formation

A few points are noteworthy about the Langmuir film forming ability of the single and mixed component systems. Firstly, consider the single component systems, systems I, III and V.



System	Lipids		Molar Ratio
I	1	–	
II	1	3	1:1 / 1:2 / 1:4 / 1:8
III	2	–	
IV	2	3	1:1 / 1:2 / 1:4
V	–	3	
VI	–	4	

Fig. 2 Lipid compounds and transition-metal ammine complexes used in this research, showing the orientation of the molecules when adsorbed on to the QCM. The tabulated information describes the constitutions of systems I–VI.

Systems I and III form poorly expanded monolayers with extremely low collapse pressures of approximately 32 and 28 $mN\,m^{-1}$, respectively (Fig. 3). Presumably, the poor stability of these films is a result of (i) the larger area requirement of the polyether moieties relative to the alkyl chains (especially in the case of the crown ether lipid), (ii) the electronic repulsion between electron lone pairs on the oxygen atoms, and (iii) the high solvation of the polyether moieties by the water molecules. Conversely, the isotherm of the octadecanol **3** monolayer is very stable, collapsing at just below 60 $mN\,m^{-1}$, forming a solid analogous phase. Thus, a compromise is required between the poorly expanded films of **1** and **2** and the well condensed film of **3**. This compromise was achieved by cospreading the two ether compounds **1** and **2** with octadecanol **3**. Fig. 3 shows the isotherms of the monolayers formed from systems II (**1** with **3**) and IV (**2** with **3**) in which one molar equivalent of octadecanol **3** is cospread with the ether amphipiles **1** and **2**, respectively. The isotherms of systems II and IV are less expanded, relative to systems I and III, and these two component films are stable until around 45 $mN\,m^{-1}$. It should be noted that, at pressures between 30 and 45 $mN\,m^{-1}$, the isotherms have a short phase change from a liquid analogous phase to a close-packed phase. Isotherms of cospread mixtures with molar ratios of the ether compounds to octadecanol **3** of 1:2 and 1:4 (and 1:8 in the case **1**) were recorded and showed the general trend that, as the amount of octadecanol was increased, the film became less expanded. This point can be appreciated from inspection of data recorded in Table 1 where the area per molecule at 25 $mN\,m^{-1}$ (the pressure at which the films were transferred to the QCM for all systems) decreases as the proportion of octadecanol **3** increases.

QCM studies

The monolayers at the gas/water interface transferred with good transfer ratios (0.85–0.95) on to hydrophobised quartz supports by the vertical dipping mode into the aqueous subphase, thus achieving X-type deposition.

The QCM was covered with a monolayer of the various systems I–V by the same vertical dipping technique, after the QCM surface was hydrophobised with a polymer solution, leading to an X-type deposition which is illustrated in Fig. 4. The QCM is held on to a Teflon case by a vacuum and remains immersed in a vial which contains 3 ml of water and a stirrer bar. Several 50 µl aliquots of a 0.1 mM aqueous $[Co(NH_3)_6]Cl_3$ were injected into the vial and the change in frequency of the QCM was monitored as each injection was performed.

The QCM responses for the 1:2 ratios for systems II and IV, together with system V, the one component octadecanol **3** system, are shown as a function of time in Fig. 5. It can be observed that initially the QCM frequency drops rapidly after each injection and then reaches a plateau at equilibrium between the $[Co(NH_3)_6]^{3+}$ trications being adsorbed and not

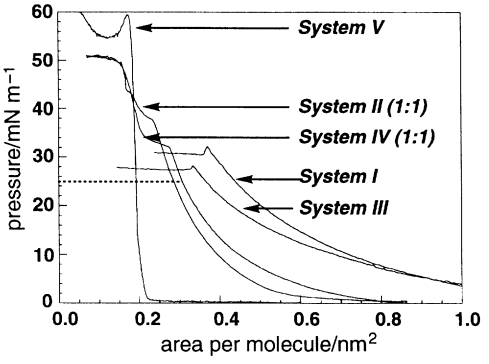


Fig. 3 Isotherms of systems I–VI

Table 1 Thermodynamic data for systems II, IV, V, VI adsorbed on to the QCM upon injections of 50 ml of 0.1 mM aqueous $[Co(NH_3)_6]Cl_3$ solution into 3 ml of water in which the QCM was immersed

system (ratio)	A^a/nm^2	$n \times 10^{-14b}$	DF^c/Hz	relative binding index^d	K_a/dm^3 mol^{-1} ^{e}	K_a/dm^3 mol^{-1} ^{f}
II (1:1)A	0.318	1.62	1234	20	373	297
II (1:1)B	0.318	1.62	1308	22	354	363
II (1:2)A	0.289	1.78	4399	66	121	131
II (1:2)B	0.289	1.78	4452	67	128	141
II (1:4)A	0.253	2.04	3508	46	150	163
II (1:4)B	0.253	2.04	3796	49	131	143
II (1:8)A	0.201	2.56	1765	18	395	448
II (1:8)B	0.201	2.56	1900	20	319	339
IV (1:1)A	0.299	1.71	2422	22	262	283
IV (1:1)B	0.299	1.71	2259	35	253	278
IV (1:2)A	0.281	1.82	1568	23	343	383
IV (1:2)B	0.281	1.82	1960	19	250	282
IV (1:4)A	0.254	2.02	1778	26	361	328
IV (1:4)B	0.254	2.02	2063	27	272	289
VA	0.189	2.73	1735	17	339	375
VB	0.189	2.73	2213	22	224	244
VI	—^{g}	2.73	102	1	87	97

^{a}Area per molecule when film is transferred to the QCM. ^{b}Number of amphiphiles transferred to the QCM; calculated from the area per molecule at 25 mN m^{-1} for the Langmuir films of systems II and VI and 50 mN m^{-1} for system V multiplied by the area of the gold electrode on to which the films were transferred (area = 0.513 cm^2). ^{c}Change in frequency at infinite $[Co(NH_3)_6]Cl_3$; calculated from the Lineweaver Burke plot of the reciprocal of concentration of TMC against the reciprocal of the change in frequency. ^{d}Calculated from normalising the change in frequency at infinite $[Co(NH_3)_6]Cl_3$ concentration. Normalisation achieved by accounting for the fact that different numbers of amphiphiles were transferred to the QCM for the various systems. ^{e}Association constant (k_1/k_{-1}). ^{f}Association constant calculated from the quotient of the gradient and the value of the intercept on the y-axis of the straight line obtained from the Lineweaver Burke plots (LWB). ^{g}Chemisorbed from solution.

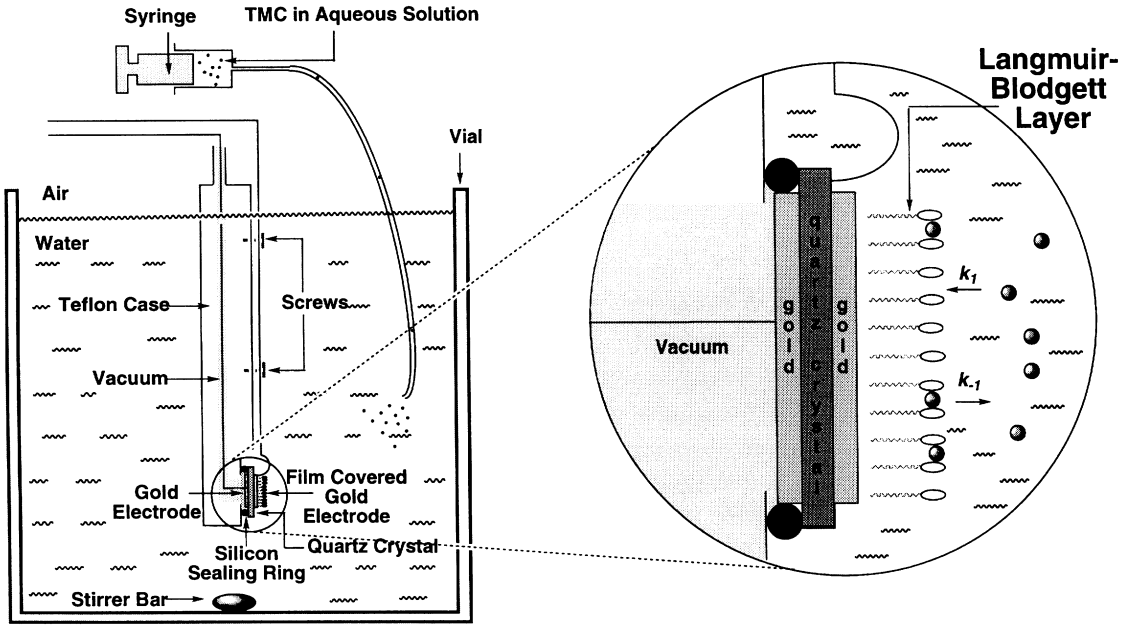


Fig. 4 Diagrammatic representation of the QCM experimental set-up with a magnification of a cartoon representation of the recognition event on the QCM to detect complexation on a receptor-derivatised QCM surface in contact with a solution containing complementary guest molecules

adsorbed on to the surface. The three curves demonstrate that, after each successive injection of the $[Co(NH_3)_6]Cl_3$ aqueous solution, (i) the change in frequency is less, (ii) the crown ether containing film, system II, has a considerably greater response than its linear counterpart, namely system IV, and (iii) the one-component octadecanol **3** system, which presents a purely hydrophilic surface to the aqueous environment, has a similar response to system IV.

This data is more clearly presented in a concentration dependent titration curve for the QCM response. Fig. 6 shows the QCM response for system IV, the octadecanol **3** and the amphiphilic polyether **2**, in the molar ratios 1:1, 1:2 and 1:4, together with system V, pure octadecanol **3**, and system VI, the self-assembled monolayer of thioocatedecanol **4**. The first point to note is that system V, which presents a purely hydrophobic surface to the aqueous environment, has a very small response to the TMC as subsequent injections are made. Indeed, the same response was recorded when pure water was injected. Thus, the small changes in frequency are not a result of the TMC having an affinity for this hydrophobic surface, but merely a result of the physical changes experienced by the QCM as the water level rises which, in turn, exerts a slightly greater pressure on the QCM.[35] Contrast this result with the hydrophilic surfaces of systems IV and V. In these cases, the QCM response is much larger than for system VI, or when pure water is injected into the vial containing the QCM with

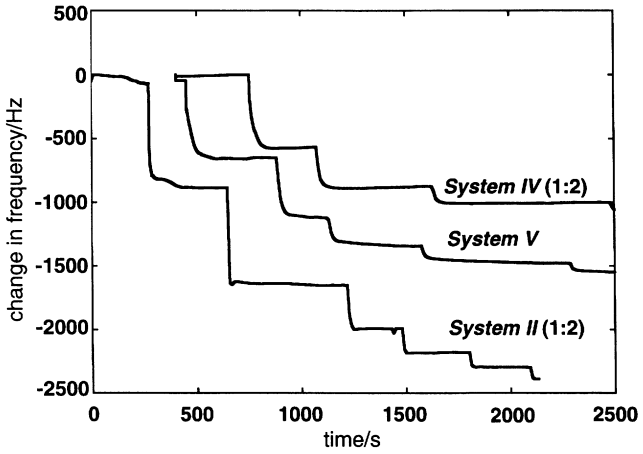


Fig. 5 Comparison of the time-dependent titration curves for system II (1 : 2) and system IV (1 : 2) as 50 ml aliquots of a 0.1 mM $[Co(NH_3)_6]Cl_3$ are injected into the vial containing the QCM

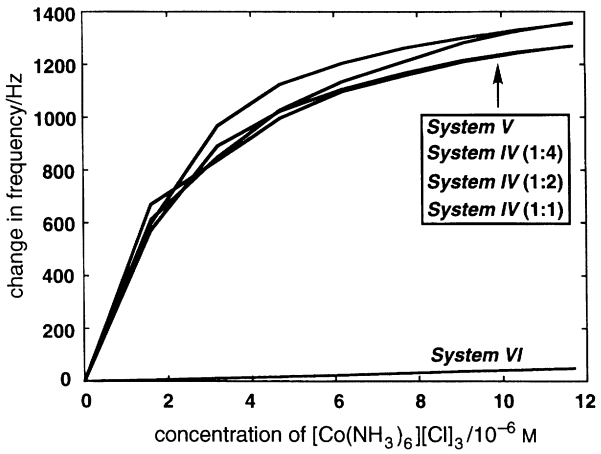


Fig. 6 QCM concentration-dependent titration curves for the hydrophilic systems IV and V, and the hydrophobic system V, upon injection of 50 ml aliquots of 0.1 mM aqueous $[Co(NH_3)_6]Cl_3$, illustrating the non-specificity of all the hydrophilic surfaces

these hydrophilic surfaces. However, for all of these hydrophilic surfaces, the responses are very similar. This result indicates that the complexation event on the surfaces formed from system IV and V is not a result of a complementary molecular recognition event, *i.e.*, it is a result of non-specific non-covalent bonding interactions.

The non-discriminatory nature of the molecular recognition event between the $[Co(NH_3)_6]^{3+}$ trications and systems IV and V, illustrated in Fig. 6, contrasts extremely well with the amphiphilic crown ether containing films of system II (Fig. 7). Initially, when only one equivalent of octadecanol **3** is present in the film with the amphiphilic crown ether, an intermediate QCM response is obtained between that of the purely hydrophobic surface of system VI and the non-specific hydrophilic surfaces of system IV and V. However, when two molar equivalents of octadecanol **3** are introduced into the film, the response of the QCM is much greater than those of the non-specific hydrophilic surfaces of systems IV and V. When four equivalents of octadecanol **3** are introduced into the films of system II, the response of the QCM is then intermediate between the non-specific hydrophilic surfaces and the film formed from 1.0 molar equivalent of **1** and 2.0 molar equivalents of **3**. Finally, when 8.0 molar equivalents of octadecanol **3** are incorporated into the film with the crown ether amphiphile, the QCM response is very similar to the non-specific hydrophilic surfaces of systems IV and V.

The kinetic data for these systems is shown in Table 2, where

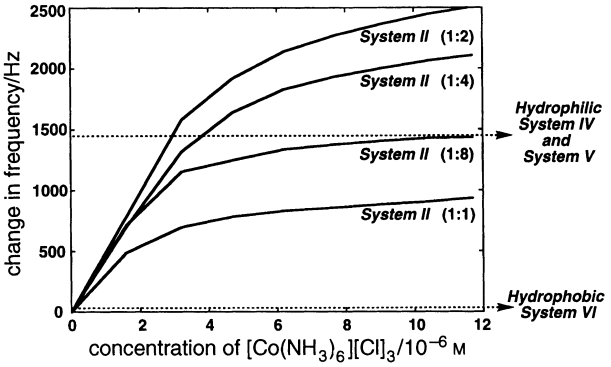


Fig. 7 QCM concentration-dependent titration curves for the hydrophilic system II upon injection of 50 ml aliquots of 0.1 mM aqueous $[Co(NH_3)_6]Cl_3$, illustrating the significantly different responses for the various ratios of **1** : **3** and the significantly greater response for the 1 : 2 ratio system II, relative to the hydrophilic surfaces of system IV and V

DF_t, DF_{eq}, and DF_{max} are the changes in frequency after 10 s from the first injection, at equilibrium for the first injection and at infinite concentration of the TMC, *i.e.* when all the surface recognition sites are filled, obtained from the *y*-intercept of the Lineweaver Burke plot of the reciprocal of concentration against the reciprocal of change in frequency.

The results from Table 2 are depicted graphically in Fig. 8. The ratio of the molar equivalents of octadecanol **3** against the ether amphiphiles **1** and **2** is plotted on the *x*-axis and the rate of complexation (rate on, k_1) plotted on the left hand side *y*-axis and the rate of decomplexation (rate off, k_{-1}) plotted on the right hand side *y*-axis.

First of all, consider the rates of complexation and decomplexation for the linear polyether lipid in system IV. Here, it is evident that there is very little variation of these two parameters, illustrating once again the very non-specific nature of the complexation event involving the surfaces containing the linear polyether and octadecanol **3**. However, when one considers the crown ether lipid containing films of system II, it is evident that, at the 1 : 2 to 1 : 4 ratio of crown ether lipid to octadecanol **3**, maxima result for both the rate on (k_1) and rate off (k_{-1}). However, it is slightly surprising that, on closer inspection of Fig. 8, the difference between the complexation and decomplexation rate is at its smallest at the 1 : 2 ratio of system II. This result means that the binding of the TMC is apparently weakest for this ratio, even although it has been established that the QCM response is largest for system II (1 : 2). This apparently anomalous behaviour will be discussed later in the paper.

The thermodynamic data[37] for the binding events of these systems are shown in Table 1. This data is summarised graphically in Fig. 9 and 10. Fig. 9(*a*) illustrates the maximum[38] QCM responses obtained from the Lineweaver Burke plots, derived from the graphs illustrated in Fig. 6 and 7. It is clearly evident that, for the linear polyether lipid systems (system IV), the response is essentially independent of the composition of the film. This result is in contrast with system II, the crown ether lipid containing films, where the largest response by the QCM is with the 1 : 2 molar ratio film. Fig. 9(*b*) represents a normalisation of the data in Fig. 9(*a*), to compensate for the slightly greater number of amphiphilic molecules which are transferred to the QCM at 25 mN m^{-1} as the molar proportion of the octadecanol increases in the film. Thus, one could expect greater QCM responses for films with more molecules per unit area. However, upon inspection, this expected increase in response is clearly not observed. System IV surfaces still have no distinct feature, and the maximum of system II is still at the 1 : 2 ratio. Thus, the 1 : 2 ratio film of system II complexes to more $[Co(NH_3)_6]^{3+}$ trications than any of the other films

Table 2 Kinetic data[a] for systems II, IV and V adsorbed on to the QCM upon injections of 50 ml of 0.1 mM aqueous $[Co(NH_3)_6]Cl_3$ solution into 3 ml of water in which the QCM was immersed

system	$DF_{t=10s}$[b]/s^{-1}	DF_{eq}[c]/s^{-1}	DF_{max}[d]/s^{-1}	k_1	k_{-1}
II(1:1)A	222	474	1234	14.51	0.039
II(1:1)B	200	487	1308	13.17	0.037
II(1:2)A	679	738	4399	25.50	0.211
II(1:2)B	682	785	4452	21.43	0.167
II(1:4)A	626	702	3508	26.76	0.178
II(1:4)B	598	684	3796	22.36	0.169
II(1:8)A	319	700	1765	14.53	0.037
II(1:8)B	403	659	1900	19.57	0.061
IV(1:1)A	315	672	2259	11.22	0.044
IV(1:1)B	457	733	2422	17.58	0.067
IV(1:2)A	296	572	1568	15.94	0.046
IV(1:2)B	287	577	1960	12.17	0.049
IV(1:4)A	370	670	1778	18.09	0.050
IV(1:4)B	290	636	2063	11.05	0.040
VA	277	632	1735	12.58	0.037
VB	284	603	2213	10.44	0.046

[a]Y. Ebara and Y. Okahata, *J. Am. Chem. Soc.*, 1994, **116**, 1209. [b]Change in frequency 10 s after the first injection of the TMC. [c]Equilibrium change in frequency after first injection of TMC. [d]Maximum change in frequency at infinite concentration of TMC extrapolated from the Lineweaver Burke plot of reciprocal of change in frequency against reciprocal of concentration of TMC.

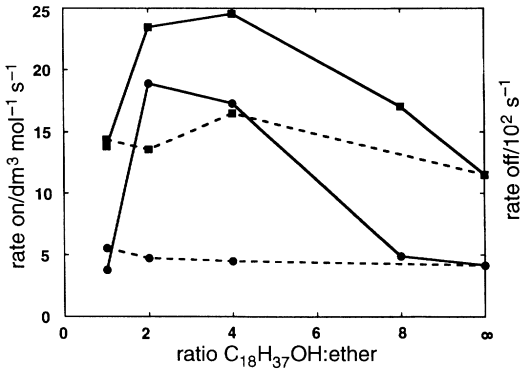


Fig. 8 Plot of the rate of complexation (rate on, &) and rate of decomplexation (rate off, $) as the amount of octadecanol increases in systems II (—) and IV (– – –)

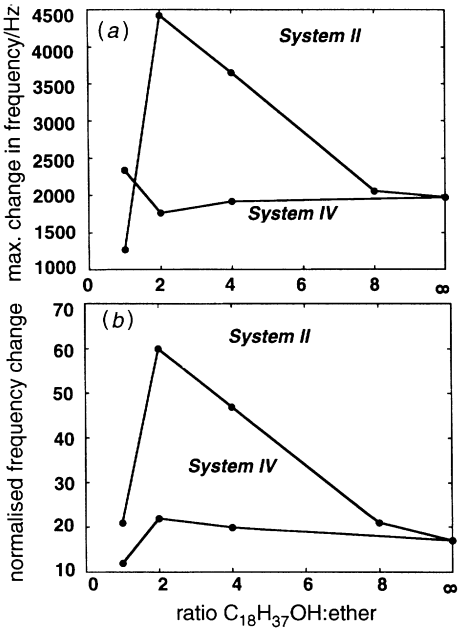


Fig. 9 Plots of (*a*) the maximum change in frequency at infinite concentration of $[Co(NH_3)_6]Cl_3$ (obtained from Lineweaver Burke plot), and (*b*) the normalised change in frequency on taking into account the different number of amphiphiles transferred to the QCM

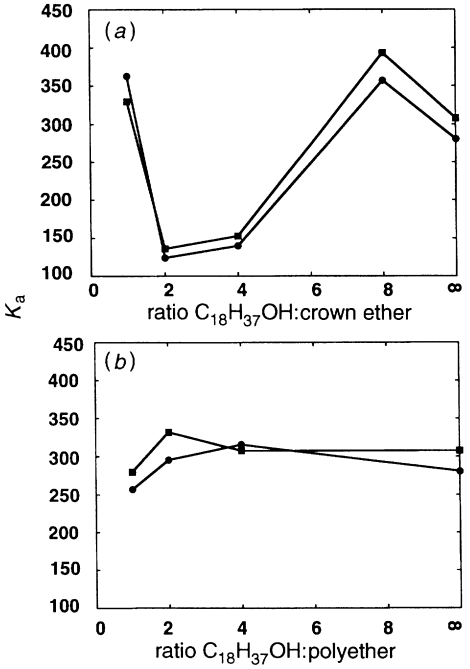


Fig. 10 Plot of the binding constants obtained both kinetically ($) and thermodynamically (&) for (*a*) system II and (*b*) system V as a function of the amount of octadecanol in the monolayer

studied in this paper, while all system IV (and system V) surfaces complex only approximately one-third of the number of trications complexed by system II (1:2 ratio) and are independent of surface composition.

Fig. 10 depicts the variation of the binding constants, calculated both kinetically (k_1/k_{-1}) and thermodynamically (Lineweaver Burke plots of data in Fig. 6 and 7) of the films towards the TMC as a function of the ratio of the ether lipids **1** and **2** and octadecanol **3**. These graphs illustrate the extremely good agreement between the kinetically established binding constants and the thermodynamically established ones. Again, it is evident that system IV and V surfaces, containing the linear polyether lipid, complex to the $[Co(NH_3)_6]^{3+}$ trications independent of the surface composition, such that they all have a K_a of approximately 300 dm^3 mol^{-1}. In contrast, it is evident once again that system II, containing the amphiphilic crown ether **1** and octadecanol **3**, has a minimum at the 1:2 ratio. However, this minimum is actually illustrating that this surface

has the lowest binding constant towards the $[Co(NH_3)_6]^{3+}$ with a value for K_a of approximately 125 $dm^3\ mol^{-1}$. This behaviour is apparently anomalous, since it has already been established that this molar ratio of system II equates with the largest QCM response, *i.e.* it complexes to the largest amount of the $[Co(NH_3)_6]Cl_3$, as illustrated graphically in Fig. 7 and 9. In order to explain this anomalous behaviour, the following two models are proposed. Firstly, consider system IV, the linear polyether **2** and the octadecanol **3** containing films. These systems have a relatively large binding constant relative to system II (1:2) but bind substantially less $[Co(NH_3)_6]Cl_3$. This behaviour can be explained by inspection of the model in Fig. 11 where we consider that each $[Co(NH_3)_6]^{3+}$ trication, when it reaches the hydrophilic surfaces is 'captured' by several polyether arms. The net effect is that, in order to break free from the surface, several polyether arms have to unravel simultaneously from the $[Co(NH_3)_6]^{3+}$ trications. Thus, the rate of decomplexation is slow relative to the rate of complexation, resulting in a relatively large binding constant, K_a.

Now consider system II (the 1:2 ratio variation). Here, the largest QCM response is observed (Fig. 9) compared with all the systems studied, yet this system has the lowest binding constant (Fig. 10). Consider the model in Fig. 12. Here, the crown ether moiety is spaced out at just the correct distance to allow the $[Co(NH_3)_6]^{3+}$ trication to slip easily into the surface cavity created by two neighbouring crown ethers. Additionally, the spacing is such that each crown ether binds to two $[Co(NH_3)_6]^{3+}$ trications such that the overall stoichiometry of the film is 1:1 with respect to the trication and 18C6 head groups. This model then establishes why this film

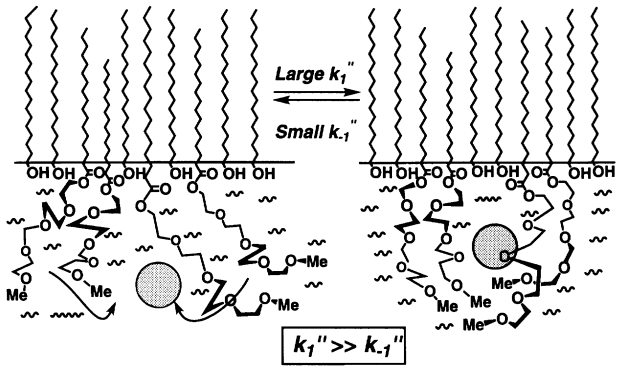


Fig. 11 Model of the complexation event between the system IV surfaces and the $[Co(NH_3)_6]^{3+}$ trications, illustrating that several polyether arms complex with the $[Co(NH_3)_6]^{3+}$, leading to a small decomplexation rate, relative to the system II (1:2) model (depicted in Fig. 12)

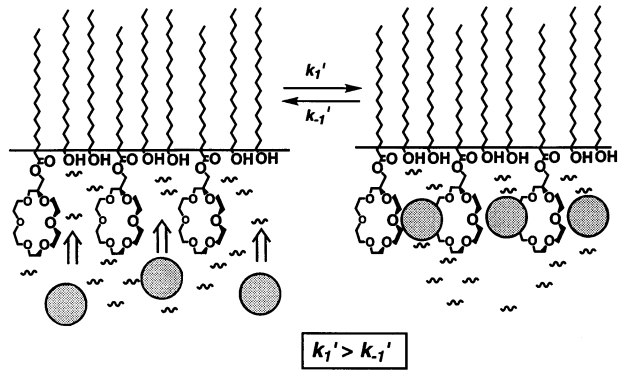


Fig. 12 Model of the complexation event between the system II surfaces and the $[Co(NH_3)_6]^{3+}$ trications, illustrating the 1:1 molar ratio between the crown ether head groups and the $[Co(NH_3)_6]^{3+}$, resulting in a high $[Co(NH_3)_6]^{3+}$ uptake coupled with the small binding constant, relative to system IV (Fig. 11)

has the greatest QCM response, as a result of the 1:1 (trication:crown ether) stoichiometry, rather than the 1:several (trication:polyether) stoichiometry for the system IV films. Furthermore, the low K_a value for system II (1:2) is a result of the fine balance between the correct steric fit of the TMC in the surface cavities and the weak second-sphere hydrogen-bonding interactions enabling the $[Co(NH_3)_6]^{3+}$ trications to slip in and out of the surface cavities easily, such that the rate off is not so different, relative, to the rate on, leading to a low K_a value for system II.

Conclusions

The observation in the solid state of the so-called second-sphere coordination between transition-metal ammine complexes and 18C6 ligands, has prompted the chemical modification of 18C6 to make it amphiphilic in nature, such that it could be incorporated into Langmuir–Blodgett films on solid supports. By utilising a QCM as the solid support, it has proved possible to detect this second-sphere coordination upon introduction of the transition-metal ammine complex $[Co(NH_3)_6]Cl_3$ into a solution in which the film-coated QCM was immersed, and has enabled the kinetic and thermodynamic characterisation of these very weak molecular recognition events, something which was not possible by other techniques. It turned out that the complexation was critically dependent on the composition of the film. The evaluation of the kinetic and thermodynamic data has enabled a model of the surface recognition event to be formulated. This model highlights that, although these very weak N—H , O hydrogen-bonding interactions are competing with the competitive aqueous environment, the recognition still occurs. By tailoring the film, the 18C6 moieties are preorganised[36] in the film such that they create many surface recognition sites which are stereoelectronically compatible with the $[Co(NH_3)_6]^{3+}$. However, the binding of these trications is weak, relative to the linear polyether containing surfaces which bind many fewer trications but in a significantly stronger manner.

The recognition in natural systems at interfaces by hydrogen-bonding interactions is of utmost importance, since it is responsible for immune responses,[37] amongst other biologically important signals. It follows that the study of simpler synthetic systems[38] is of considerable value in shedding light on the more complex biological recognition events as well as for the development of new sensors.[4]

Additionally, we have demonstrated that the QCM offers yet another valuable tool to the research worker who is studying unnatural supramolecular systems, where the recognition event is between relatively small molecules (M_r # 300 u), in contrast with the majority of studies carried out to date which have been concerned with the detection of naturally occurring macromolecules[5–21] ($M_r > 30000$ u).

Experimental

Solvents were dried using literature methods where necessary or used directly as obtained from the suppliers. Thin layer chromatography (TLC) was performed on aluminium sheets coated with Merck 5554 Kieselgel 60F. Developed plates were scrutinized in an iodine chamber. Column chromatography was performed using Kieselgel 60 (0.040–0.063 mm mesh, Merck 9385). Melting points were determined with an Electrothermal 9200 melting point apparatus and are uncorrected. Microanalyses were performed by the University of Birmingham and the University of Sheffield Microanalytical Services. Low-resolution mass spectra were obtained from a Kratos Profile mass spectrometer, operating at 4 kV and using 70 eV for electron impact mass spectrometry (EIMS). Proton nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC 300 (300 MHz) spectrometer, using the deuteriated

solvents as the lock. ^{13}C NMR spectra were recorded on the Bruker AC 300 (75 MHz) spectrometer.

The isotherm measurements were all recorded on a self-made trough with a Wilhelmy pressure pick up system. The spreading solutions consisted of $CHCl_3$ and the lipids **1**–**3**, and mixtures thereof, in the concentration range 0.5–1.0 mg ml^{-1}, of which between 25 and 50 ml were spread from a syringe on to an aqueous subphase or an aqueous subphase with the $[Co(NH_3)_6]Cl_3$ dissolved in it. Each isotherm was carried out over a 20 min period. The $[Co(NH_3)_6]Cl_3$ was of analytical quality as defined by the commercial supplier and was used without further purification. $[Co(NH_3)_6]Cl_3$ subphases were prepared freshly each day from Milli Q water (resistivity *ca.* 18 V cm^{-1}). The quartz crystal microbalance consisted of a quartz crystal (9 MHz, AT-cut, $d=8$ mm) covered with gold electrodes (area $=0.53$ cm^2) obtained from Quartzkeramik GmbH. The quartz crystal was mounted on a Teflon dipstick (Fig. 4). In order to ensure no short circuits between the two gold electrodes, a silicon sealing ring was placed between the Teflon holder and the crystal. The QCM was held in place by a vacuum on the non-covered face. The QCM was hydrophobised with a 'silicon solution' obtained from Serva. The quartz crystal was driven by an in-house oscillator (15 V, 100 mA), the oscillation shape controlled by a Hameg (HM604) oscilloscope. The frequency change was recorded with an Iwatsu universal counter (SC7201). Transfer of the Langmuir films of systems I–IV to the QCM was achieved on a computer-controlled trough from KSV Instruments (KSV5000) utilising a vertical dipping method at 20 °C and a film pressure of 25 mN m^{-1} for systems I–IV and 50 mN m^{-1} for system V, with a dipping speed of 2 mm min^{-1}. The films were held at 25 or 50 mN m^{-1} for 20 min before deposition to ensure they were stable and were compressed with a barrier rate of 5 mm min^{-1}. System VI was chemisorbed on to the QCM clean gold surface from a solution (0.1 mM) of thiooctadecanol **4** in $CHCl_3$. The gold surface was cleaned with MeOH and $CHCl_3$.

Synthesis

2-Oxymethyl-18-crown-6-octadecanoate 1. A solution of 2-hydroxymethyl-18-crown-6 **5** (0.50 g, 1.70 mmol) and NEt_3 (0.260 g, 2.60 mmol) was dissolved in dry toluene (25 ml) and it was added dropwise to a stirred solution of octadecanoyl chloride (0.64 g, 2.10 mmol) in dry toluene (10 ml) whilst maintaining the temperature at 10 °C. The reaction mixture was then allowed to warm to room temperature and H_2O (20 ml) was added. The solution was then concentrated *in vacuo* to give a yellow oil, which was dissolved in CH_2Cl_2 (20 ml) and washed with H_2O (20 ml × 2). The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and purified by silica gel column chromatography (eluent: EtOAc–Me_2CO, 3 : 2) to yield the amphiphilic crown ether **1** as a colourless oil. Yield 0.30 g (32%); ^1H NMR (300 MHz, $CDCl_3$) d 4.28–4.10 (2H, m, CO_2CH), 3.90–3.80 [1H, m, $OCH(CH_2O)_2$], 3.78–3.60 (4H, m, CH_2OCH_2), 2.40–2.30 (2H, t, CH_2CO), 1.65–1.58 (2H, m, CH_2CH_2CO), 1.30 [28H, s, $-(CH_2)_{14}CH_3$], 0.98–0.89 (3H, t, CH_3). EIMS $C_{30}H_{60}O_8$ requires m/z 560 $[M]^+$. Found: m/z 561 $[M+H]^+$. Anal. Calc.: C, 66.58; H, 10.72. Found: C, 66.67; H, 10.81%.

Monomethoxytetraethyleneglycol 7. A solution of tetraethyleneglycol **6** (2 g, 0.11 mol) in dry THF was added dropwise to a suspension of NaH (60% dispersed in mineral oil) (20 mg, 5.4 mmol) in dry THF (50 ml) under nitrogen at room temperature. The reaction mixture was then heated under reflux and stirred for a further 30 min. A solution of MeI (0.76 g, 5.4 mmol) in THF (20 ml) was then added over a period of 20 min. The reaction mixture was heated under reflux for a further 2 h. The reaction mixture was then allowed to cool to room temperature and MeOH (10 ml) was added. The solvents were removed *in vacuo* and the residue was taken up in CH_2Cl_2 (50 ml) and washed with aqueous Na_2CO_3 (50 ml) and H_2O (2 × 50 ml). The organic layer was then dried ($MgSO_4$) and the solvents were removed to afford a clear liquid which was purified by silica gel column chromatography (eluent: CH_2Cl_2–MeOH) to afford **7** as a clear oil. Yield 9.6 g (42%); ^1H NMR (300 MHz, $CDCl_3$) d 3.70–3.50 (14H, m, OCH_2CH_2O), 3.45 (3H, s, OCH_3), 2.81–2.78 (2H, t, CH_2OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) d 72.5, 71.8, 70.5, 70.5, 70.5, 70.2, 70.2, 61.6, 58.9. EIMS: $C_9H_{20}O_5$ requires m/z 208 $[M^+]$. Found: m/z 209 $[M+H]^+$.

Lipid ether 2. A solution of monomethoxytetraethyleneglycol **7** (2.0 g, 9.6 mmol) and NEt_3 (1.2 g, 11.5 mmol) dissolved in dry toluene (25 ml) was added dropwise to a stirred solution of octadecanoyl chloride (3.5 g, 11.5 mmol) in dry toluene (10 ml) maintaining the temperature below 10 °C. The reaction mixture was then allowed to warm to room temperature and H_2O (20 ml) was added. The solution was then concentrated *in vacuo* to give a waxy off-white solid which was dissolved CH_2Cl_2 (50 ml) and washed with H_2O (50 ml × 2). The organic layer was dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* and purified by silica gel column chromatography (eluent: EtOAc–Me_2CO, 3 : 2) to yield the acyclic polyether **2** as a white waxy solid. Yield 4.4 g (96%); ^1H NMR (300 MHz, $CDCl_3$) d 4.24–4.19 (2H, m, CH_2CO_2), 3.73–3.52 (14H, m, OCH_2CH_2O), 3.46 (3H, s, OCH_3), 2.32–2.28 (2H, t, CH_2OCH_3), 1.65–1.55 (2H, m, $CH_2CH_2CO_2$), 1.33–1.20 (28, s, CH_2CH_2), 0.89–0.84 (3H, s, CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) d 173.9, 72.6, 71.9, 70.6, 70.3, 69.2, 63.4, 61.7, 59.0, 34.2, 31.9, 29.5, 29.4, 29.1, 24.9, 22.7, 14.1. EIMS: $C_{27}H_{54}O_6$ requires m/z 474 $[M^+]$. Found: m/z 475 $[M+H]^+$. Anal. Calc.: C, 68.31; H, 11.46. Found: C, 68.38; H, 11.61%.

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