A Concept for Immobilizing Catalytic Complexes on Electrodes: Cubic Phase Layers for Carbon Dioxide Sensing

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Liquid crystalline cubic phases formed with monoolein and Myrperol have been used as matrices to host a catalytic complex of nickel(II) and 1-hexadecyl-1,4,8,11-tetraazacyclotetradecane for the reduction of carbon dioxide. The structures of the cubic phases, both with and without the catalyst, were established using small-angle X-ray scattering. The catalytic reduction of carbon dioxide was performed using thin mercury film and glassy carbon electrodes modified with cubic phases containing the catalyst. The linear dependence of the catalytic reduction current on the carbon dioxide concentration allowed use of the modified electrodes as sensing devices both in solution and in the gas phase. The high reproducibility of the measurements makes this method of monitoring carbon dioxide levels attractive compared to other methods based on modified electrodes.

The measurement of carbon dioxide is important for medical applications, for atmospheric concentration control, and for indoor climate monitoring.¹–³ While direct measurements of carbon dioxide are preferred, most often the measurements are done indirectly,₄,⁵ e.g., by monitoring of pH changes. Here is reported the development of a method to measure carbon dioxide directly, using its reduction on electrodes modified with a cyclam complex. High catalytic activity of cyclam complexes in the reduction of CO₂ on mercury electrodes was reported first by Sauvage and co-workers.⁶ From the presence of carbon dioxide has been elucidated, ⁵⁻⁷,⁹ while the mechanism of reduction in the absence and presence of carbon dioxide has been elucidated,⁵⁻¹⁴ and the following scheme is now accepted:

\[
[Ni(cyclam)^{2+}]_{ads} + e^- \rightarrow [Ni(cyclam)]^{+}_{ads} \quad (1)
\]

\[
[Ni(cyclam)]^{+}_{ads} + CO_2 + H^+ \rightarrow [(Ni(cyclam)CO_2H)^{2+}]_{ads} \quad (2)
\]

\[
[(Ni(cyclam)CO_2H)^{2+}]_{ads} + e^- \rightarrow [Ni(cyclam)^{2+}]_{ads} + CO + OH^- \quad (4)
\]

Balazs and Anson⁹ reported that, in the presence of CO, the reduction of the catalyst takes place stepwise and that the Ni(cyclam)CO adduct is formed. The adduct, which interferes in the overall reduction process of CO₂ by blocking the catalytic surface, can be removed from the electrode with sufficiently positive potential pulses.¹² Immobilizing catalysts in stable and functionally active forms on solid supports is one of the main challenges in sensor technologies.³⁻⁴,¹⁵ Recently, we presented several approaches to immobilize catalysts for CO₂ reduction on electrode surfaces.¹⁶⁻¹⁸ One was based on stripping voltammetry with adsorptive accumulation of the catalyst on the mercury electrode surface. This method allows one to record a well-developed catalytic signal even when the catalyst concentration in the solution is very low—on the level of 10⁻⁸ mol/dm³.¹⁶,¹⁷ Another approach made use of

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cyclam ligands modified with long alkyl chains, to obtain amphiphilic derivatives. These molecules formed Langmuir monolayers at the air—water interface and could be transferred onto the electrode substrates. These monolayer-modified electrodes exhibited high catalytic efficiency in the reduction of CO$_2$. The cyclam complexes were also self-assembled from the solution or immobilized in Nafion layers. The use of Nafion layers was, however, limited because the diffusion of the complex within the layer was slow, and the potential window accessible was not sufficient to resolve the catalytic current from the final rise of the current. On the other hand, the stability of the Langmuir—Blodgett and self-assembled monolayers was lower, compared to the well-known stability of alkanethiol monolayers on gold. Unfortunately, the potentials needed to carry out the reduction of CO$_2$ are much more negative than the potential of thiol desorption; hence, thiol derivatives of cyclams cannot be used for CO$_2$ sensing.

The aim of this work was to prepare a catalytically active and stable layer to modify electrodes for CO$_2$ sensing. Here we propose a new method of immobilizing a cyclam complex, using liquid crystalline cubic phases. The cubic phase, first described by Luzzati et al., is a highly viscous, isotropic, and stable material, found very useful as membrane mimetic matrices for direct spectroscopic studies of immobilized enzymes. In the present work, it is shown to be also a useful matrix for holding synthetic catalyst close to the electrode surface. The lipids used for preparing the cubic phases were monoolein and Myverol. The structures of the monoolein and Myverol cubic phases had been extensively studied using spectroscopic methods, and it was shown that the liquid crystals consist of lipid bilayers folded to form channels filled with water. These systems provide a unique environment for immobilizing both water-soluble compounds and hydrophobic molecules, which remain in the lipid bilayer. The structures of the monoolein and Myverol cubic phases containing a catalytic complex of nickel(II) and 1-hexadecyl-1,4,8,11-tetraazacyclotetradecane (NiC$_{16}$) used in this study were determined using small-angle X-ray scattering (SAXS) and polarized light microscopy. The sensing systems allowed us to monitor CO$_2$ concentration in aqueous solutions and, even more importantly, in a gas phase.

**EXPERIMENTAL SECTION**

**Chemicals.** All chemicals were of analytical grade. NaClO$_4$ was obtained from Fluka, NaOH and HClO$_4$ were from POCh. M onoolein (M; 1-monooeoyl-rac-glycerol, 99%) was purchased from Sigma and Myverol 18-99 from Eastman Chemical Co. (Kingsport, TN). Myverol is an inexpensive product rich in monoglycerides. The main components of Myverol 18-99 used in this work are monoolein (60.9%), monolinolein (21.0%), monolino- nolenin (8.8%), and monopalmitin (4.1%). Water was distilled and passed through a Milli-Q water purification system (Millipore). Ni(II) cyclam derivative was a generous gift from Professor Thomas Kaden, University of Basel; the synthesis of this compound was described recently.

**Electrochemistry.** Voltammetry experiments were done using the Autolab potentiostat (Eco Chemie) in a three-electrode arrangement, with a saturated calomel electrode (SCE) as the reference and platinum foil as the counter electrode. The working electrodes were either glassy carbon electrode (GCE, BAS, 0.036 cm$^2$ area) or thin mercury film silver supported electrode (TMFE) (VME) with an area of 0.012 cm$^2$ or thin mercury film silver supported electrode (TMFE) with an area of 0.012 cm$^2$ or thin mercury film silver supported electrode (TMFE) with an area of 0.012 cm$^2$ or thin mercury film silver supported electrode (TMFE) with an area of 0.012 cm$^2$. The TMFE were polished by 0.05- or 0.25-μm alumina slurry with a cloth and then rinsed with distilled water. The experiments were performed in 0.1 M solution of NaClO$_4$, pH in the range 4.7–5.2. The pH was adjusted to the desired value by addition of HClO$_4$ or NaOH. The solution was deaerated with argon, and an argon blanket was maintained over the solution during the electrochemical experiments. In the experiments with CO$_2$, the solution was saturated with CO$_2$ by passing it for 20 min through the deaerated solution and also by maintaining the CO$_2$ blanket during experiments. The carbon dioxide concentration in the supporting electrolyte was 0.033 mol/dm$^3$, which constitutes a saturated solution. The temperature during the experiments was held constant at 20°C.

**Cubic Phases.** The cubic phase was prepared by weighing monoolein or Myverol and the cyclam and mixing in a small vial with a spatula. The closed vial was slowly heated until the complex dissolved in the melted lipid. Then water was added, the preparation was mixed again, and the vials were left for 24 h for equilibration. The ratio of components for the cubic phase was chosen on the basis of phase diagrams for the monoolein— and Myverol—water systems. The isotropy of the cubic phase within the temperature range 20–50°C was confirmed in polarized light, using a microscope with 100× magnification. The phase was yellowish due to the cyclam complex, transparent, and highly viscous, and remained unchanged for several weeks when kept in a closed vial. The stability of the system was confirmed by macroscopic observations of the sample viscosity and clarity, and if no changes were observed, the cubic phase was spread on the electrode, using a spatula. The thickness of the layer was 1–3 mm. The cubic phase remained unchanged when the sample was stored under water and during electrochemical measurements, i.e., in direct contact with the supporting electrolyte. The electrode...
modified with cubic phase was kept in the supporting electrolyte for 20 min before each experiment; this period of time was needed for equilibrating the gas concentration between the cubic phase and solution.

**SAXS.** X-ray measurements of cubic phases containing the catalyst were performed using a small-angle camera equipped with a generator providing, through a 1-mm-thick nickel filter, a Cu $K\alpha$ radiation of 1.54Å wavelength. The temperature was controlled within 0.5 °C using Peltier elements. The X-ray experiments were performed several times to ensure their reproducibility. Standard deviation of repetition distances measurements is ±0.02 nm.

**RESULTS AND DISCUSSION**

**Cubic Phases.** Cubic phases were obtained using two different monoglyceride preparations, monoolein and Myverol. The phase diagrams of monoolein in water are well documented in the literature. Myverol is a mixture of monoglycerides, with a prevailing content of monoolein. Myverol has a variety of well-established uses in food products, such as emulsification, starch-complexing, aeration, lubrication, antistalling, defoaming, and oil stabilization, and in the pharmaceutical sector for preparation of sustained-release capsules. The annual world sale of Myverol is thousands of tonnes, and unlike pure monoolein, Myverol is inexpensive. Despite Myverol's commercial importance, its phase behavior was established only recently, and it has been shown that, like pure monoolein, Myverol forms in water reverse-type structures, i.e. water in oil. With both monoolein and Myverol, the same phases are formed: reversed isotropic; and two types of liquid crystals, lamellar and cubic. Differences in the phase behavior of monoolein and Myverol are, however, observed, particularly concerning the capacity of water incorporation into the cubic phases. At 20 °C, 44% water can be incorporated into the monoolein cubic phase and only 34% into the Myverol cubic phase. This latter difference is obviously due to the presence in Myverol of different components and monoacylglycerols other than monoolein. Despite these differences, for both amphiphiles the cubic phase domain is divided in two parts, corresponding to the bicontinuous structures of $Ia_3d$ and $Pn_3m$ symmetry. While the $Ia_3d$ structure spans over a wide zone of temperature and lower water concentrations; the $Pn_3m$ structure exists only at higher concentrations of water and thus is stable in excess water. The latter property of the cubic phase is an important advantage for elaborating sensors of molecules present in water. The interconnected rods (ICR) model permits a simple description of cubic structure. The segregation of the aqueous and nonaqueous domains is obtained by formation of two interwoven, independent networks of short cylinders connected 3 by 3 in the $Ia_3d$ structure, and 4 by 4 in the $Pn_3m$ structure, but having the same diameters and length in the two cases. In our work, the sample structures were determined using SAXS. The $Ia_3d$ and $Pn_3m$ structures were differentiated, based on the ratio of the repetition distances.

corresponding to the diffraction lines of the first and the second order, and being, respectively, 1.15 and 1.23. In the structure Ia3d, the first-order reflection corresponds to the diffraction planes d [211] and d [220], while in the structure Pn3m, the first-order reflection corresponds to the diffraction planes d [110] and d [111].

The structures of the samples containing monoolein and 36 and 40% water are that of Ia3d. The addition of the cyclam reduces the cubic phase domain, which becomes comparable with such containing several types of alkyl chain Myverol—water system. The monoolein cubic phases containing 1–2% cyclam and 36 and 40% water are thus in the domain of the Pn3m structure. A study of the system based on Myverol shows that, for the concentrations of water of 29 and 34% the structure of the cubic phase is Pn3m (Scheme 1). In the latter case, the addition of cyclam does not modify the structure, and water not incorporated in the structure stays in equilibrium with the cubic phase.

**Electrochemistry.** The voltammograms recorded using mercury film electrodes modified with the monoolein cubic phase containing the Ni(II) cyclam amphiphilic derivative are shown in Figure 1A. Since an unsubstituted, hydrophilic Ni(II) cyclam was found to be easily removable from the cubic phase into the solution (results not shown), a long alkyl chain has been attached to the catalyst. The observed long-term stability of such a catalytic system can be explained by the anchoring of the cyclam alkyl chain in the lipid bilayer. The possible location of the catalyst in the cubic phase is shown in Scheme 1. The liquid crystal matrix fulfills the condition of retaining the catalyst in close proximity to the electrode surface, and it also allows fast diffusion of the analytes. The latter effect is important for using the cubic phase layers as components of sensor devices.

The shape of the voltammograms recorded using the electrode modified with the layer containing the catalyst indicates a nearly reversible electrode process of the catalyst. The peaks involved, \((a_2, c_1)\) at \(-1.37\) V in the deoxygenated solution, are shown in Figure 1A.

The diffusion coefficient of the Ni(II) cyclam amphiphilic derivative in the cubic phase was calculated based on the equations describing the reduction peak current recorded using a large glassy carbon electrode; and the plateau current of stationary currents was measured with a glassy carbon microelectrode:

\[
i_p = 2.69 \times 10^5 A C V^{1/2} n^{3/2} D^{1/2} \quad (5)
\]

\[
i_{ss} = 4 n F r CD \quad (6)
\]

In eqs 5 and 6, \(A\) (cm\(^2\)) is the electrode area, \(C\) (mol/cm\(^3\)) is concentration, \(V\) (V/s) is scan rate, \(n\) is the number of electrons, \(D\) (cm\(^2\)/s) is the diffusion coefficient, \(F\) (C/mol) is the Faraday constant, \(r\) (cm) is the microelectrode radius, \(i_p\) (A) is the cathodic peak current at the large electrode, and \(i_{ss}\) (A) is the steady-state reduction current recorded on the microelectrode.

The diffusion coefficient value was found to be \(1.90 \times 10^{-8}\) cm\(^2\)/s and the calculated concentration of the catalyst in the liquid crystal matrix (2.04 \(\times\) 10\(^{-2}\) mol/dm\(^3\)) corresponded to 0.016 mg of Ni(cyclam)\(^{2+}\)/mg of cubic phase. The calculated value is in a good agreement with the concentration based on the weighted quantities of components used for preparing the cubic phase—\(-0.01\) mg of the catalyst/mg of cubic phase.

We propose that, by analogy to aqueous medium, the voltammetric peak \(c_1\) (Figure 1A) corresponds to the reactions 2–4, while the peak \(c_2\) corresponds to the further reactions of Ni(cyclam)\(^{2+}\) in the presence of CO with the formation of [Ni(cyclam)CO]. The oxidation of the [Ni(cyclam)CO] deposit gives rise to the peak \(a_2\), close to 0 V. The symmetric shape of this peak indicates that the product is immobilized at the electrode surface. When the potential at which the scan is reversed is less negative than \(-1.6\) V, the anodic \(a_2\) signal is absent since the Ni(cyclam)CO does not accumulate at these potentials. Peaks \(c_2\) and \(a_2\) decrease at low scan rates as shown in Figure 2. This decrease indicates that the Ni(cyclam)CO\(^+\) compound may decompose given enough time, before approaching the potential of its reduction.

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For the NiLC16 complex, the catalytic reduction current peak $c_1'$ is well developed, which led us to use this catalyst in CO$_2$ sensing layers. The influence of the electrode substrate on the signal is significant, as shown in Figure 1. The catalytic effect found for the carbon electrode is smaller than for the mercury film electrode. However, the use of the carbon electrode may be advantageous for monitoring CO$_2$ levels in some biological applications where mercury electrodes are banned. When glassy carbon is used as an electrode substrate, the catalytic peak appears at less negative potentials, compared to mercury film electrode, which is advantageous. The characteristics of the electrode processes for both electrode substrates are shown in Table 1.

In the case of carbon electrodes, a poorly developed anodic signal $a_2$ corresponding to Ni(cyclam)(CO) oxidation was detected after multiple scanning. The height of this peak increases with increasing concentration of the catalyst in the cubic phase. To provide a sufficient long-term reproducibility, the 0 V potential should be applied between scans to the electrode, to remove all traces of carbonyl compounds accumulating on the electrode surface. The mean value of five measurements of $i_{c1'}$ recorded in a saturated CO$_2$ solution was 9.20 $\mu$A. Subsequently, the concentration of CO$_2$ in saturated solution is 0.033 mol/dm$^3$. Figure 3 shows the voltammograms for increasing concentrations of CO$_2$, and the catalytic peak current–concentration plot is shown in the inset. The detection limit for CO$_2$ determination was 0.001 mol/dm$^3$. The $i_{c1'}$ value is sufficiently reproducible to assume that changes of the cubic phase structure, if any, that might occur in the described system at longer working times, do not affect the measured electrochemical signal.

Interestingly, our method was found useful also in the case of monitoring CO$_2$ directly in the gas phase. In this approach, the cubic phase was placed on a microscopic slide in the electrochemical cell and all three electrodes were immersed in the liquid crystal. Good conductivity is obtained by using 0.1 mol/dm$^3$ NaClO$_4$ solution instead of water to prepare the cubic phase. Voltammograms shown in Figure 4 indicate that the current increases when the cubic phase is in contact with CO$_2$. In the absence of CO$_2$, the peaks correspond to the reduction and the oxidation of the catalyst, while signals at -1.60 and -1.65 V in the presence of CO$_2$ are due to reduction of CO$_2$ catalyzed by the Ni(II) cyclam. The first of them corresponds to the CO$_2$ reduction, while the second corresponds to the formation of CO adduct with

![Figure 2. Cyclic voltammograms recorded on TMFE modified with a monoolein cubic phase layer containing NiLC16, in 0.1 M NaClO$_4$, pH 4.8, solution saturated with CO$_2$. Scan rate: (1) 0.01, (2) 0.05, and (3) 0.1 V/s. The modifying layer contained 0.01 mg of Ni-(cyclam)$^{2+}$/mg of cubic phase.](image1)

![Figure 3. Cyclic voltammograms recorded on TMFE modified with a monoolein cubic phase layer containing NiLC16, in 0.1 M NaClO$_4$, pH 4.8, for increasing concentrations of CO$_2$: (1) 0, (2) 0.0066, (3) 0.0132, (4) 0.0198, (5) 0.025, and (6) 0.033 M. Scan rate, 0.05 V/s. Quantity of cubic phase and Ni(cyclam)$^{2+}$ is given in Figure 1A. Inset: catalytic peak current vs concentration of CO$_2$ in the solution.](image2)

<table>
<thead>
<tr>
<th>type of electrode</th>
<th>formal reduction potential of the complex in solution deaerated with Ar (V)</th>
<th>potential of the catalytic reduction of CO$_2$ (V)</th>
<th>$i_{c1'/c1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCE</td>
<td>-1.38</td>
<td>-1.45</td>
<td>1.7</td>
</tr>
<tr>
<td>TMFE</td>
<td>-1.37</td>
<td>-1.54</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of the Electrode Processes for GCE and TMFE

![Figure 4. Cyclic voltammograms recorded on TMFE modified with a monoolein cubic phase layer containing NiLC16. Electrochemical cell filled with (1) argon and (2) with CO$_2$. Scan rate, 0.05 V/s; 245.30 mg of cubic phase containing 2.45 mg of Ni(cyclam)$^{2+}$ was used.](image3)
the Ni(cyclam) complex, as described above. When the reversal potential is more negative than −1.65 V, the oxidation peak of the CO adduct, c2, is also seen. The procedure for the determination of CO2 in the gas phase is straightforward, and the results are reproducible; therefore, it can be proposed for monitoring of CO2 levels in gas samples. Elaborating an optimal, miniaturized CO2 sensing device using a cyclam/cubic phase method is underway in our laboratory.

Compared to the monoolein-based cubic phases, those based on the M yverol are somewhat less stable. Indeed, in 5–6 days after their preparation, some anisotropic spots could be observed in the otherwise isotropic phase. The voltammograms recorded using TMFE modified with a layer of M yverol containing 1% catalyst in the solutions saturated with argon, or CO2, are presented in Figure 5A. The catalytic to diffusion peak ratio (c2′/c1) is lower than for the monoolein, which may indicate that some catalytic centers are blocked. Since increasing of the catalyst content up to 5% increases the c2′ peak corresponding to the electrode blocking by the CO compounds (Figure 5B), without improving the catalytic efficiency, the content of 1% of the cyclam in the M yverol cubic phase was considered as optimal. The characteristics of the catalytic processes (given as i2′/i1 ratio) for different amounts of catalyst in the M yverol-based cubic phase are as follows: for 1% concentration of catalyst, 2.90; for 2% 2.38; and for 5% 2.35. Because M yverol is readily available in large quantities, preparation of the liquid crystal matrixes for different analytical applications is simple and inexpensive.

CONCLUSIONS

The liquid crystalline lipid phases of cubic structure were shown to have properties required for the preparation of modified electrodes. The cubic phases based on monoolein and M yverol used in this work host successfully cyclam derivatives of catalytic interest, and when spread on electrode surfaces, they remain firmly attached, allowing durable electrode modification. While hydrophilic cyclam complexes are easily removed from the liquid crystals, a long hydrocarbon chain cyclam derivative remains well anchored. The latter indicates that the catalyst is located in the lipid bilayer of the cubic phase and that, in order to achieve a stable immobilization, the catalyst has to be of an amphiphilic structure similar to that of the lipid itself.

The cubic phases containing catalytic centers were found to be useful for detecting carbon dioxide both in aqueous solutions and in gas samples, with the catalytic current response of the sensor being directly proportional to the concentration of the analyte.

Our results indicate that M yverol can be used to replace the monoolein for preparing the cubic phase matrixes. While M yverol-based cubic phases are less stable than those based on the monoolein, the low cost of their preparation is expected to be decisive in technological applications.

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Figure 5. Cyclic voltammograms recorded on TMFE modified with a Myverol cubic phase layer containing NiLC16, in 0.1 M NaClO4, pH 4.8, solution saturated with (1) argon and (2) CO2. Scan rate, 0.05 V/s. Concentration of the catalyst: (A) 1 and (B) 5 wt %. The quantity of cubic phase is given in Figure 1. The content of Ni(cyclam)2+ was 0.01 mg/mg of cubic phase in (A), and 0.05 mg of Ni(cyclam)2+/mg of cubic phase in (B).