

# Redox Activity and Diffusion of Hydrophilic, Hydrophobic, and Amphiphilic Redox Active Molecules in a Bicontinuous Cubic Phase

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The objective was to examine how a bicontinuous cubic phase influences the diffusion and electrochemical activity of dissolved molecules. The cubic phase is a structure with three-dimensional continuous channels of water separated by an apolar membrane. A redox active molecule can dissolve in three different environments. A hydrophobic molecule will prefer the interior of the membrane, a hydrophilic molecule will prefer the water channels, and an amphiphilic molecule will be situated with its headgroup at the surface of the membrane and its tail in the interior. The electrochemical activity was measured with cyclic voltammetry and the transport behavior with chronocoulometry. All the molecules were redox active in the cubic phase; that is, all the molecules could reach the surface of the electrode and react. The cubic phase made the kinetics of the charge transfer slower, showing a quasi-reversible behavior. The reason may be that a layer of the membrane adheres to the hydrophobic electrode surface. The diffusion experiment showed that the diffusion was slower than in solution. The molecules that were restricted to diffuse within the membrane gave particularly low mass transport rates.

## Introduction

Polar lipids in water form a variety of self-assembled aggregates. The bicontinuous cubic phases are particularly interesting with their two continuous water domains separated by the lipid membrane.<sup>1</sup> The bicontinuous cubic phase is crystalline in three dimensions. The structure is built from a twisted bilayer separating two independent systems of water channels that permeate the crystal but have no contact with each other.<sup>1</sup> Both the bilayer and the two water spaces are continuous. The surface defined by the ends of the hydrocarbon tails can be described by a so-called minimal surface that is equally convex and concave in every point.<sup>2</sup> The cubic phase has recently become of interest for a number of potential applications. The phase can be used to crystallize proteins<sup>3,4</sup> as well as used as a drug delivery vehicle.<sup>5</sup> If redox active enzymes are added, the system can be used as a biosensor.<sup>6</sup> A number of studies have shown that redox enzymes and other proteins can be incorporated in the cubic phase, with increased stability and retained catalytic activity.<sup>6–10</sup> The cubic phase is in itself very interesting, with its continuous channels and the ability to incorporate hydrophilic, hydrophobic, and amphiphilic molecules.

An electrode in contact with such a phase would ideally be in contact with the membrane and at least one of the aqueous subspaces. These features enable measurement of diffusion of redox active molecules moving both in the water channels and in the apolar membrane. A few studies have previously reported both diffusion coefficients and redox activities for a number of molecules.<sup>7,8,11–13</sup> All hydrophilic molecules studied in the cubic phase retained the redox activity, but the decrease in diffusion rate varied widely; the redox protein cytochrome *c*<sup>7</sup> had a diffusion coefficient 70 times lower in the cubic phase than in an aqueous buffer, whereas the decrease for the smaller molecules glucose<sup>8</sup> and  $\text{Ru}(\text{NH}_3)_6^{3+}$ <sup>12</sup> was only four times. Also,

redox active hydrophobic molecules have been studied in the cubic phase, such as ferrocene (and derivatives thereof) and ubiquinone-10.<sup>11,14</sup> Both substances had substantially reduced diffusion rates in the cubic phase.

The electrochemical response depends on the rate of diffusion. For both polar and nonpolar compounds restrictions and obstructions in the cubic phase decrease the diffusion rate. Also, the crystal properties will influence the diffusion, e.g., if the sample is a single crystal or if it comprises several microdomains, shifting the diffusion characteristics from a semiinfinite pattern to a finite pattern.<sup>15,16</sup> The interaction between the investigated phase and the electrode surface is also important. In this study a gold surface was used, which is hydrophobic, and may have a monolayer adsorbed onto the surface.

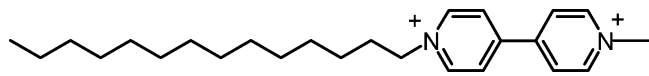
This contribution reports the results of electrochemical studies of probes in a bicontinuous cubic phase situated on an electrode surface. We have investigated several hydrophilic and hydrophobic molecules and also an amphiphilic viologen using the same experimental conditions so that properties such as redox activities and diffusion coefficients can be directly compared.

## Experimental Section

**Materials.** Monoolein (glycerol-1-mono(9-octadecenoate), MO) (Danisco Ingredients, Brabrand, Denmark) was used as received. The fatty acid chain composition of monoolein was 92% C18:1, 6% C18:2, and 2% saturated acids. *N*-Tetradecyl-*N'*-methylviologen (TMV, see Figure 1) (Aldrich), ruthenium(II)hexaammine chloride (Aldrich), ferrocene (Aldrich), potassium hexacyanoferrate(III) (Merck), NaBr (Aldrich), and NaCl (Ridel-de Haën) were of highest purity and used as received. The water was purified with a Millipore water purification system.

**Equipment.** Electrochemical measurements were performed with a 660A potentiostat from CH-Instruments. Small-angle X-ray scattering (SAXS) diffractograms were recorded with a

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**Figure 1.** Molecular structure of *N*-tetradecyl-*N'*-methylviologen (TMV).

Kratky camera (Hecus M. Brown, Graz). Cu K $\alpha$  radiation with a wavelength of 1.542 Å was used. The exposure time was 2 h. The data processing and evaluation were made with the software 3D-view (v. 4.2). The cubic phase samples were placed between two mica sheets in a sample holder.

**Procedure.** The bicontinuous cubic phase was made from monoolein and water, a well-characterized system.<sup>1,17</sup> The phase diagram displays a large cubic area with two different types of cubic phase, the D and G structures. The one-phase region of the D structure is connected to pure water by a large two-phase region. The samples were made in the two-phase region to avoid swelling of the cubic phase during the electrochemical measurements. The cubic samples were prepared in vials by melting the monoolein (at 38 °C) and mixing it with the electrolyte (200 mM KCl in water). The hydrophobic ferrocene and the surfactant TMV were mixed with the melted monoolein before the electrolyte was added. The hydrophilic redox active molecules were mixed with the electrolyte solution before it was added to the melted monoolein. After the samples were prepared, the vials were kept at 38 °C for an hour to help mixing. The samples were centrifuged at 3600g for an hour and then stored at 25 °C. The samples were centrifuged a couple of times to help mixing during the equilibration period, which was continued until no further visual change could be observed. The equilibration time was usually 1 week.

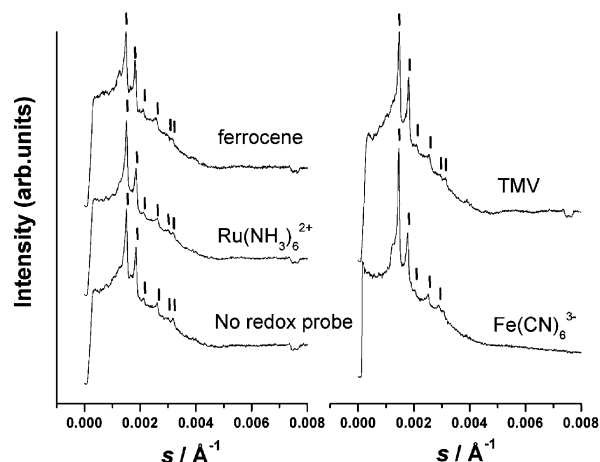
A gold electrode (CH-Instruments, Austin, TX), ca. 2 mm in diameter, was polished on a polishing cloth (Buehler) with the use of a homemade rotor, in a water–aluminum oxide slurry (Al<sub>2</sub>O<sub>3</sub> (Buehler), particle size 0.3 μm) for 3 min. The electrode was rinsed with water and sonicated in water for 10 min. The measurement cell was purged with nitrogen for at least 40 min prior to each experiment and then kept under a blanket of nitrogen during the experiment. A three-electrode setup was used with a Ag|AgCl|KCl(3.0 M) ( $E^\circ = 0.2059$  V vs NHE) (Metrohm) as reference electrode and as counter electrode a coiled platinum wire put into a separate electrolyte vessel. All measurements were carried out at 25.0 ± 0.1 °C. All reported potentials refer to the Ag|AgCl reference electrode.

To ensure that the electrochemical signal only came from the cubic phase, the electrode was covered by a close-fitting cylindrical Teflon cap containing a 1 mm deep small circular hole (with the same size as the electrode surface) wherein the cubic phase was deposited, completely filling the hole. The electrolyte outside the sample contained the same concentration of the electroactive substance as the sample (except for the hydrophobic ferrocene) to avoid leakage during the measurement.

The redox activity was measured with cyclic voltammetry (scan rates from 10 to 1000 mV/s), and the diffusion coefficients were estimated from chronocoulometry, using the Cottrell equation to describe the transient assuming a semiinfinite diffusion

$$Q = \frac{2nFAD^{1/2}c^*t^{1/2}}{\pi^{1/2}} \quad (1)$$

where  $Q$  is the accumulated charge,  $n = 1$  is the number of electrons in the reaction,  $F$  is the Faraday constant,  $A$  is the electrode area,  $D$  is the diffusion coefficient,  $c^*$  is the



**Figure 2.** Diffractiongram for the MO–electrolyte system (200 mM KCl) with different redox probes added.  $s$  is the reciprocal space ( $s = 2 \sin \theta / \lambda$ , where  $2\theta$  is the scattering angle and  $\lambda$  is the wavelength). The markers point out the position for peaks associated with the  $Pn3m$  space group, the D-structured bicontinuous cubic phase. The compositions for the samples are reported in Table 1.

concentration, and  $t$  is the time. The chronocoulometry measuring time was 5 s. A precondition potential was first applied for 120 s at a potential where the species were thermodynamically stable, thereby building up a large diffusion layer that should not be depleted during the 5 s measurement. Each experiment was repeated three times with satisfactory reproducibility.

In the application of the Cottrell equation for the determination of the diffusion coefficients, the cubic phase at the electrode was regarded as a homogeneous medium in contact with all of the electrode area  $A$  and containing a concentration of the redox probe that was the average concentration over the whole volume of the phase. In this way the value of diffusion coefficient will depend not only on the viscosity of the medium but also on effects from the microstructure of the cubic phase, i.e., the inhomogeneous distribution of the probe, according to its polarity, the reduced accessible area of the electrode, and constrictions and other hindrances for the diffusive movement within the phase.

The average concentrations used in the determination of the diffusion coefficients were calculated differently for the different molecules depending on their solubility properties. Ferrocene and TMV were assumed to be completely incorporated in the membrane. From the phase diagram<sup>1</sup> the fraction of electrolyte and cubic phase could be calculated under the assumption that the diagram had not changed from the addition of salt. Assuming that the cubic phase had a density of 1 g/cm<sup>3</sup>, the volume of the cubic phase was calculated and from that the average concentration of the molecules in the entire cubic phase. For the hydrophilic molecules it was assumed that the concentration was the same in the water channels of the cubic phase as in the electrolyte. Knowing the volume fraction of cubic phase, the average concentration in the entire cubic phase could be calculated.

## Results and Discussion

**SAXS.** The effect of the incorporated molecules on the cubic structure was assessed by SAXS measurements. The diffractiongrams all corresponded to the  $Pn3m$  space group, which is the D structure (see Figure 2). The lattice parameter varied slightly for the different redox molecules (see complete data for the diffractiongrams in Table 1). The lattice parameter for MO–electrolyte was 94.2 Å. The system with the ruthenium complex

**TABLE 1: X-ray Diffraction Data of the Bicontinuous Cubic Phase of Monoolein in Water and with Added Redox Probes<sup>a</sup>**

sample	$d/\text{\AA}$	$hkl$	$a_0/\text{\AA}^c$	space group
MO-W <sup>b</sup>	66.5 (vs)	110	94.0	<i>Pn3m</i>
54.1:45.9 wt %	54.5 (s)	111	94.5	
	47.1 (m)	200	94.2	
	38.4 (m)	211	94.1	
	33.4 (m)	220	94.4	
			<b>94.2</b>	
MO-Ru(NH <sub>3</sub> ) <sub>3</sub> <sup>2+</sup> -W	66.5 (vs)	110	94.0	<i>Pn3m</i>
56.2:0.06:43.7 wt %	54.2 (s)	111	93.8	
	47.1 (w)	200	94.2	
	38.4 (m)	211	94.1	
	33.4 (m)	220	94.4	
			<b>94.1</b>	
MO-Fe(CN) <sub>6</sub> <sup>3-</sup> -W	68.8 (vs)	110	97.2	<i>Pn3m</i>
55.0:0.08:44.9 wt %	56.1 (s)	111	97.2	
	48.8 (w)	200	97.6	
	39.8 (m)	211	97.4	
	34.4 (m)	220	97.3	
			<b>97.3</b>	
MO-TMV-W	67.6 (vs)	110	95.6	<i>Pn3m</i>
55.1:0.31:44.6 wt %	55.3 (s)	111	95.8	
	47.6 (w)	200	95.3	
	39.1 (m)	211	95.8	
	33.8 (w)	220	95.6	
			<b>95.6</b>	
MO-ferrocene-W	67.59 (vs)	110	95.6	<i>Pn3m</i>
55.0:0.05:45.0 wt %	55.3 (s)	111	95.8	
	47.6 (w)	200	95.3	
	39.2 (m)	211	95.9	
	33.8 (m)	220	95.5	
			<b>95.6</b>	

<sup>a</sup> Data are given for the first five peaks corresponding to the *Pn3m* space group. <sup>b</sup> In all samples, W is a 200 mM KCl water solution. <sup>c</sup> The values in bold letter are mean values.

had a similar value (94.1 Å). The ferrocene and the ferricyanide gave slightly larger spacing, 95.6 and 97.3 Å, respectively. The TMV system (at the concentration used in the electrochemical measurements) had also a larger spacing 95.6 Å. A sample with a lower concentration was also measured, however, in which case the lattice parameter (92.5 Å) was smaller than in the MO-electrolyte system. It seems that a small amount of the charged surfactant viologen can be accommodated in the phase without swelling but that TMV somehow change the packing of the membrane.

**Electrode Area Determination.** The electrode area was measured electrochemically by using chronocoulometry with a long measuring times (5 s) in an Fe(CN)<sub>6</sub><sup>3-</sup> solution (in 0.2 M KCl). Using the diffusion coefficient ( $7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ ) from von Stackelberg et al.,<sup>18</sup> the area was calculated to 0.036 cm<sup>2</sup> from the Cottrell equation (see eq 1). This area is close to the geometric area, which is consistent with the long measuring time. A long measuring time gives a diffusion layer that is not affected by the surface roughness.

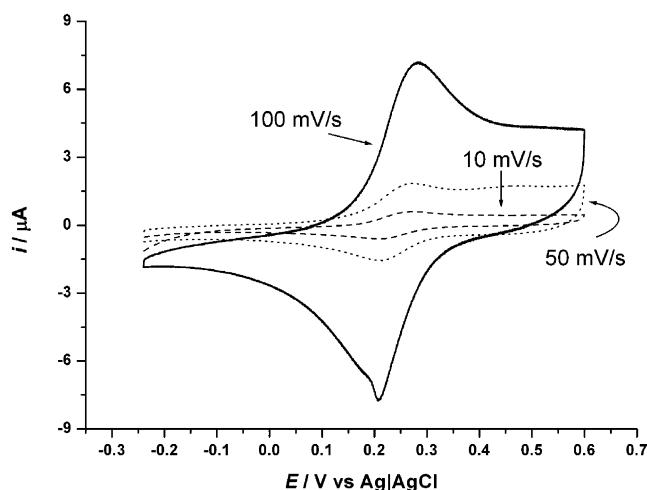
**Redox Potentials and Reversibility.** The electrochemistry of three hydrophilic (methylviologen, rutheniumhexaammine, and hexacyanoferrate), one hydrophobic (ferrocene), and one amphiphilic (TMV) substance, incorporated in the cubic phase, was investigated. All the redox active substances made contact with the electrode. Cyclic voltammetry resulted in voltammograms (see Figures 3–6) with peak splits larger than 59/*n* mV, suggesting that the molecules are not surface adsorbed but free to diffuse toward the electrode surface.

In Table 2 the values of the formal potentials in the cubic phase and in pure electrolyte (200 mM KCl) are given. A comparison between the formal potentials in water solutions

**TABLE 2: Redox Potentials and Concentration of the Redox Molecules in Electrolyte Solution and in the Cubic Phase<sup>a</sup>**

redox molecule	bare electrode		cubic phase	
	$c/\text{mM}$	$E^0$ <sup>b</sup>	$c/\text{mM}$	$E^0$
Fe(CN) <sub>6</sub> <sup>4-/3-</sup>	1.94	$0.230 \pm 0.001$ <sup>c</sup>	0.779	$0.244 \pm 0.002$
ferrocene <sup>0/+</sup>			2.49	$0.387 \pm 0.001$
methylviologen <sup>2+/+</sup>	2.43	$-0.633 \pm 0.002$	0.97	$-0.628 \pm 0.002$
TMV <sup>2+/+</sup>	1.72	$-0.462 \pm 0.005$	7.11	$-0.511 \pm 0.001$
Ru(NH <sub>3</sub> ) <sub>6</sub> <sup>2+/3+</sup>	1.06	$-0.132 \pm 0.001$	0.416	$-0.140 \pm 0.001$

<sup>a</sup> The supporting electrolyte solution was a 200 mM KCl water solution. <sup>b</sup> Vs Ag|AgCl. <sup>c</sup> The uncertainty value is the 95% confidence values.



**Figure 3.** Cyclic voltammogram of 1.94 mM Fe(CN)<sub>6</sub><sup>3-/4-</sup> in the bicontinuous cubic phase (scan rate was 100 mV/s). The sharpness of the reduction peak suggests some sort of surface adsorption; this can also be seen at the bare electrode. The voltammograms for the slower scan rates does not show this feature. The amount of current originating from the surface adsorbed species decreases when the scan rate is decreased because there is more time for molecules to diffuse to the surface and react. The supporting electrolyte solution was a 200 mM KCl water solution.

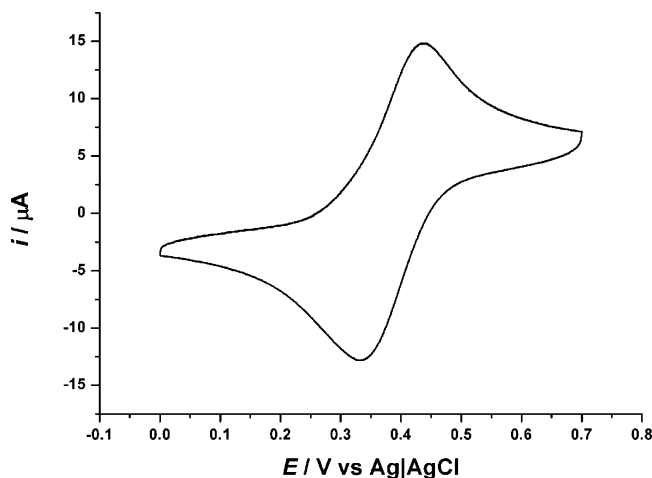
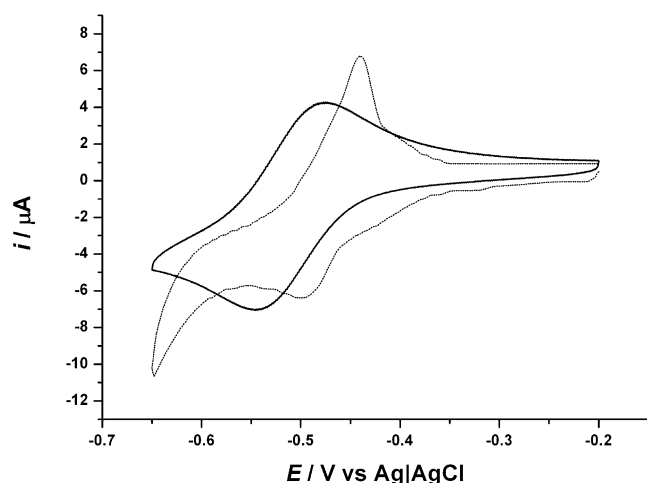
and in the cubic phase can be made for the all substances, except for ferrocene. The incorporation in the cubic phase facilitated reduction of Fe(CN)<sub>6</sub><sup>3-/4-</sup> and oxidation of Ru(NH<sub>3</sub>)<sub>3</sub><sup>2+/3+</sup>; i.e., the form with the highest charge was stabilized by the cubic phase. This result is unexpected since the surrounding is more hydrophobic in the cubic phase.

The redox potential for methylviologen was the same, within the experimental errors, as in water (see Table 2). For TMV, however, the redox potential was more negative in the cubic phase. The shape of the voltammograms in solution is different from the expected for a redox probe diffusing in the solution toward the electrode surface (see Figure 5). It has been shown earlier that alkyl viologens precipitate in the reduced form at the surface from the solution, especially if the alkyl chain is longer than eight carbons.<sup>19</sup> As the molecules are adsorbed on the surface, the local charge density is increased and will destabilize the 2<sup>+</sup> state more than the 1<sup>+</sup> state, causing the redox potential to be more positive.<sup>20</sup> The surfactant viologen in the cubic phase, however, will spread out more, since it is energetically favorable to spread out into the entire apolar membrane of the cubic phase.

All substances showed quasi-reversible behavior: the peak splits were larger than 59 mV, and the split grew with increasing scan rate (Table 3). As judged from the peak split, Fe(CN)<sub>6</sub><sup>3-/4-</sup> showed quasi-reversible behavior both at the bare electrode and

**TABLE 3: Peak Split Values from Cyclic Voltammetry Measurements for the Different Redox Probes in Solution as Well as in the Cubic Phase (All Values in mV)**

scan rate, mV/s	Fe(CN) <sub>6</sub> <sup>4-/3-</sup>		ferrocene		MV <sup>2+/+</sup>		TMV <sup>2+/+</sup>		Ru(NH <sub>3</sub> ) <sub>6</sub> <sup>2+/3+</sup>	
	bare	cubic	bare	cubic	bare	cubic	bare	cubic	bare	cubic
10	67	57	—	78	58	59	58	64	72	65
50	67	67	—	96	60	62	67	70	73	67
100	68	73	—	101	58	63	72	69	69	69
200	69	84	—	117	57	62	69	76	70	70
500	82	113	—	145	68	78	64	91	70	87
1000	85	131	—	187	67	96	65	110	71	99

**Figure 4.** Cyclic voltammogram of the hydrophobic molecule ferrocene (2.49 mM) in the bicontinuous cubic phase. The scan rate was 100 mV/s. The supporting electrolyte solution was a 200 mM KCl water solution.**Figure 5.** Cyclic voltammogram of the amphiphile TMV<sup>2+/+</sup> in the bicontinuous cubic phase (solid line, 7.11 mM) and in the solution (dotted line, 1.72 mM). Note that the anodic peak in the solution is sharper than the other peaks. This can be explained by precipitation of the TMV<sup>+</sup> radical ion on the surface. The scan rate was 100 mV/s. The supporting electrolyte solution was a 200 mM KCl water solution.

in the cubic phase. All the other investigated molecules were reversible in the water solution, but when incorporated in the cubic phase showed quasi-reversible electrochemical behavior. If there were an insulating layer (monolayer or bilayer) on the electrode surface, the heterogeneous charge-transfer rate would be slowed down since the hydrophilic molecules would have to react through a tunneling mechanism. However, it is unlikely that such a layer would be free from defects. If there are small pores in it, an increased peak split could result.

**Diffusion Measurements.** Figures 7–9 show the chrono-coulometry response for the redox active molecules in the cubic

**TABLE 4: Diffusion Coefficients for the Redox Active Molecules in the Solution and in the Cubic Phase<sup>a</sup>**

redox molecule	$D_{app} \times 10^6 \text{ s/cm}^2$		potential step/ $E/V \text{ vs Ag AgCl}$
	bare electrode <sup>b</sup>	cubic phase	
Fe(II)CN <sub>6</sub> <sup>4-</sup>	$9.2 \pm 0.1$	$2.5 \pm 0.07$	−0.24 to 0.6
Fe(III)CN <sub>6</sub> <sup>3-</sup>	$7.6 \pm 0.4$	$4.2 \pm 1.5$	0.6 to −0.24
ferrocene		$0.027 \pm 0.005$	0 to 0.7
ferrocinium		$0.11 \pm 0.02$	0.7 to 0
methylviologen <sup>2+</sup>	$7.7 \pm 0.6$	<sup>c</sup>	−0.4 to −0.8
TMV <sup>2+</sup>	$9.8 \pm 1.4$	$0.20 \pm 0.04$	−0.2 to −0.7
Ru(II)(NH <sub>3</sub> ) <sub>6</sub> <sup>2+</sup>	$5.6 \pm 0.4$	$1.2 \pm 0.08$	−0.4 to 0.2
Ru(III)(NH <sub>3</sub> ) <sub>6</sub> <sup>3+</sup>	$9.7 \pm 0.6$	$2.3 \pm 0.1$	0.2 to −0.4

<sup>a</sup> The concentrations are given in Table 2. The supporting electrolyte solution was a 200 mM KCl water solution. <sup>b</sup> The uncertainty value is the 95% confidence values. <sup>c</sup> It was not possible to calculate a value since there was not a linear response.

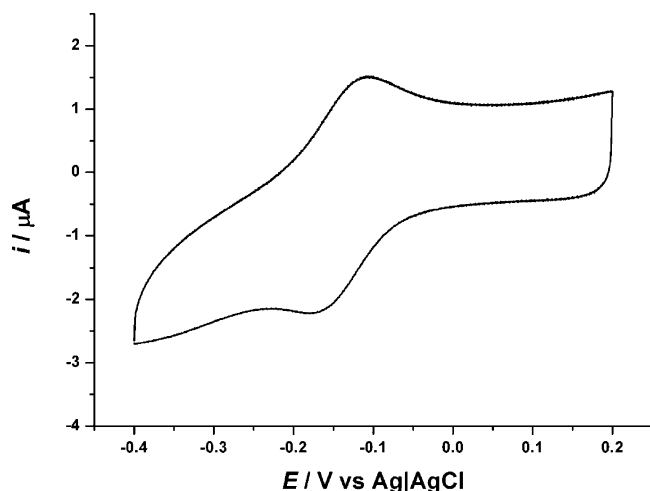
phase. The potential step was large enough to ensure that the diffusion was limiting the current response. The measurement time was 5 s.

Methylviologen, Ru(NH<sub>3</sub>)<sub>6</sub><sup>2+/3+</sup>, and Fe(CN)<sub>6</sub><sup>3-/4-</sup>, all hydrophilic, can be expected to diffuse through the water channels. TMV, being a surfactant, should diffuse laterally along the membrane. Ferrocene being highly hydrophobic is expected to diffuse inside the membrane, whereas the ferrocinium ion is more hydrophilic and probably prefers the aqueous domains.<sup>21</sup>

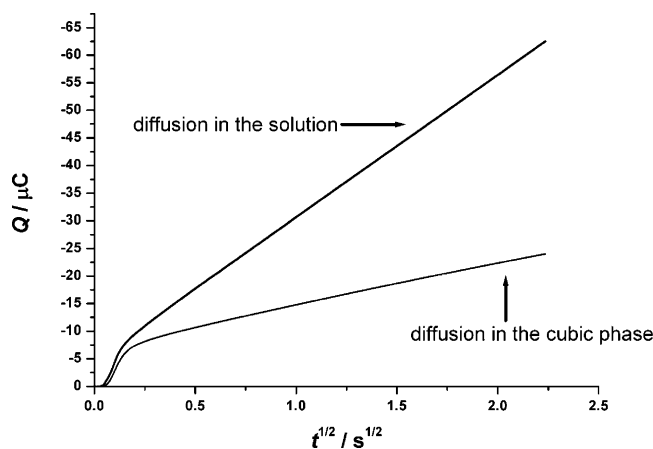
In the beginning and end of the measurement, the chrono-coulometry experiments showed different slopes. This was also observed in the experiments with a bare electrode and can probably be attributed to charging of the electrode. The effect appears larger in the cubic phase due to the smaller diffusion currents. The slope from the late part of the measurement was used to calculate apparent diffusion coefficients using the Cottrell equation (see eq 1). The values of the diffusion coefficients are collected in Table 4.

The Fe(CN)<sub>6</sub><sup>4-</sup> diffusion rate decreased by almost a factor of 4 compared to the value on the bare electrode; the corresponding decrease for the oxidized form was a factor of only 2. The diffusion rates of the two redox forms of the ruthenium complex decreased by a factor of 4 to 3. The value of  $2.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  for Ru(NH<sub>3</sub>)<sub>6</sub><sup>3+</sup> correlates very well with the results of Rowiński et al.<sup>12</sup> For methylviologen, on the other hand, the Cottrell plot does not give a straight line (see Figure 9), in contrast to the expected behavior and the behavior as shown by the other hydrophilic molecules. The slope increases with time, which cannot be explained by finite diffusion lengths since then the slope should decrease with time. One explanation is that close to the electrode there is a layer blocking the methylviologen or giving it a very slow diffusion rate, but outside this layer the diffusion is unhindered. Then the diffusion layer will not be depleted, as it normally would be in potential step techniques when the sample is homogeneous outside the electrode surface. Since the curve is not a straight line, a unique slope cannot be obtained.





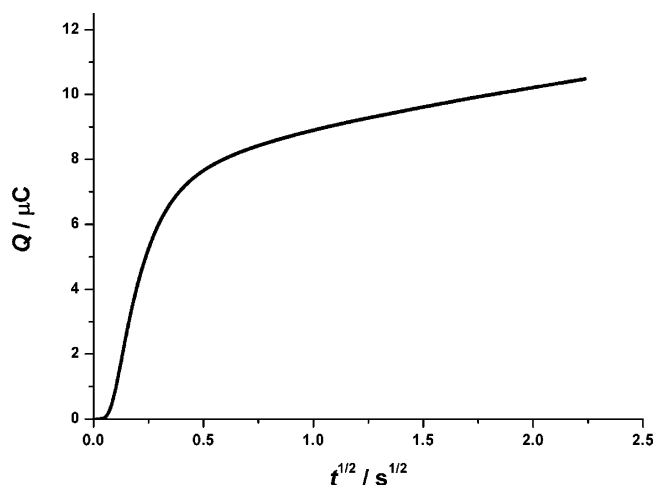
**Figure 6.** Cyclic voltammogram of  $\text{Ru}(\text{NH}_3)_6^{2+/3+}$  (0.416 mM) incorporated in the bicontinuous cubic phase. The supporting electrolyte solution was a 200 mM KCl water solution. The scan rate was 100 mV/s.



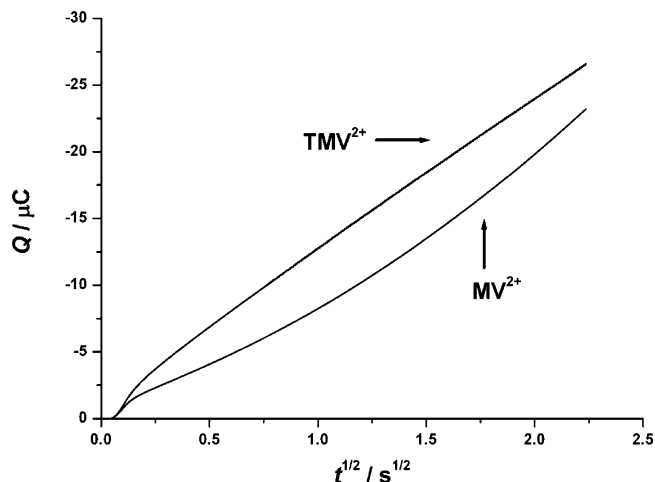
**Figure 7.** Chronocoulometry of  $\text{Fe}(\text{CN})_6^{3-}$  in the bicontinuous cubic phase and in solution. Note the similar shape of the two curves in the beginning of the measurement. The concentration in the solution was 1.94 mM and in the cubic phase 0.779 mM. The supporting electrolyte solution was a 200 mM KCl water solution.

Ferrocene has a very low diffusion rate in the cubic phase, almost 100 times slower than the hydrophilic probes. The amphiphilic  $\text{TMV}^{2+}$  was also severely hindered in the cubic phase. The diffusion coefficient was 50 times lower than at the bare electrode.

One important point to address is that according to the diffusion coefficients measured (Table 4) the root-mean-square displacement,  $(2Dt)^{1/2}$ , is 5.2  $\mu\text{m}$  to 64.8  $\mu\text{m}$  for the slowest and fastest redox probe, respectively, during the measuring time at 5 s.<sup>22</sup> There is no risk that the 1 mm cubic phase would be depleted. The gold surface is hydrophobic, but it difficult to say how that affects the cubic phase close to the surface. Possibly there is a layer adsorbed at the surface. The increase in the peak split suggests that this is the case. But the layer must be thin since the hydrophilic compounds made contact with the electrode, giving a current almost as high as in the experiment with the bare electrode, which would not be possible if the layer thickness was comparable to the diffuse layer. On the other hand, if there is thin layer with small pores, the measurement of the diffusion coefficient will not be affected since the diffusion layer, in a 5 s long measurement, is much thicker than the thickness of a surface adsorbed layer. It would be completely different if the measurement time were only a



**Figure 8.** Chronocoulometry of ferrocene (2.49 mM) in the bicontinuous cubic phase. The charging process of the electrode dominates over the diffusion current at the beginning of the experiment. The supporting electrolyte solution was a 200 mM KCl water solution.



**Figure 9.** Chronocoulometry of  $\text{MV}^{2+}$  and  $\text{TMV}^{2+}$  in the bicontinuous cubic phase. Note that the curve for  $\text{MV}^{2+}$  is not straight, which suggests that the condition for an unrestricted semiinfinite diffusion does not apply. The supporting electrolyte solution was a 200 mM KCl water solution.

few milliseconds. Then the surface properties would then affect the result severely.

A decrease of the diffusion coefficients for the hydrophilic molecules is expected because they have to diffuse longer distances inside the cubic matrix. The large decrease for  $\text{TMV}^{2+}$  and the very slow diffusion of ferrocene are more difficult to understand. An explanation could be that the cubic phase is not continuous throughout the entire sample but comprises microdomains with grain boundaries in between. Another possibility is that the morphology of the cubic phase is changed close to the electrode by interactions with the surface, forming a layer that can inhibit the diffusion from the outer part of the phase. This would result in charge transients with finite diffusion characteristics.<sup>16</sup> However, this was not seen in our experiments. Notice that the value for ferrocene ( $2.7 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ ) is lower than what Barauskas et al. reported ( $11 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ ).<sup>11</sup>

## Conclusions

We have shown that molecules with different properties (hydrophilic, hydrophobic, and amphiphilic) are still redox active while incorporated in the cubic phase. The kinetics is slowed

down, and all molecules showed a quasi-reversible behavior; the peak splits are larger than 59/n mV and increase with increasing scan rates. A possible explanation could be that there is a hydrophobic layer adsorbed at the electrode surface.

The bicontinuous cubic phase slowed down the mass transport; i.e., the diffusion coefficients decreased. Some molecules were more hindered than others. The diffusion coefficient of the amphiphilic viologen decreased by a factor of 50. Also, ferrocene had a much lower diffusion coefficient compared to the value in acetonitrile according to a study by Barauskas et al.<sup>11</sup> Since it is restricted to the membrane, the rate of movement is dependent on the integrity of the structure. If the membrane is not continuous throughout, it will slow the movement severely. The diffusion coefficients for the hydrophilic probes decreased not as much, only by a factor of 2–4. The charge transient for methylviologen, with a continuous increase in the slope, deviated from the linear result expected of a molecule with an unhindered movement. It is difficult to explain why the methylviologen behaved differently than the other hydrophilic molecules.

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