Supplementary Material (ESI) for Chemical Communications

Supporting Information

„Noncovalent insertion of ferrocenes into the protein shell of apo-ferritin“

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Experimental section

Materials

All chemicals were purchased from *Wako*, *Aldrich* or *Nacalai Tesque* and used without further purification. Racemic 1-Dimethylaminoethylferrocene was prepared according to a reported procedure.[1] Recombinant L-chain apo-Fr from horse liver was prepared in NovaBlue competent cells (Novagen) transformed with the expression vector pMK2 kindly supplied by Prof. Ichiro Yamashita. The culture and purification of the protein was performed according to a previous literature.[2] The concentration was determined by UV-spectroscopy using the reported extinction coefficient at 280 nm.[3]

Physical Measurement

NMR-spectra were recorded on a *JEOL A-400* spectrometer. UV-Vis spectra were recorded on a Shimadzu UV-2400 PC Recording spectrometer. The concentrations of Fe-atoms in the composites were determined using ICP Optical Emission Spectroscopy (Varian) after digestion of the proteine by 0.5 mol/L HNO_3, using a standard solution of iron (WAKO, 1000 mg/L) for calibration and a standard solution of $Y(NO_3)_3$ (WAKO, 1000 mg/L) as internal standard. Proteine concentrations were determined by the bicinchoninic acid (BCA) method.[4]

Preparation of the Apo-ferritin – ferrocene composites (general procedure)

To 45 ml of a 1.11 μmol/L solution of apo-ferritin (50 nmol) in a buffer of 50 mmol/L Tris/HCl, 0.15 mol/L sodium chloride (pH=8) were added 5 ml of a 100 mmol/L solution of the corresponding ferrocene derivative (5 mmol, 10000 equivalents) in acetonitrile.

The resulting mixture was stirred for another 10 hours at room temperature and then dialyzed against 3 L of 0.15 mol/L sodium chloride at 4 °C overnight. The sample was concentrated to 10 ml by membrane filtration, passed through a syringe filter (0.22 μm) and subsequently purified by G200 size-exclusion chromatography using an aqueous buffer of 20 mmol/L Tris, 0.15 mol/L sodium chloride (pH=8) as the eluent.

1 · apo-ferritin: Yield (based on apo-ferritin): 72%, Fe-atoms/apo-ferritin (based on ICP/ BCA): 45.7 ± 6.5, **UV/Vis** (20 mmol/L Tris, 0.15 mol/L NaCl)**:** λ_{max} (ε) = 450 (10200), 325 (99500), 280 (547000) nm.

2 · apo-ferritin: Yield (based on apo-ferritin): 61%, Fe-atoms/apo-ferritin (based on ICP/ BCA): 57.3 ± 3.5, **UV/Vis** (20 mmol/L Tris, 0.15 mol/L NaCl)**:** λ_{max} (ε) = 450 (30900), 325 (187000), 280 (665000) nm.

3 · apo-ferritin: Yield (based on apo-ferritin): 67%, Fe-atoms/apo-ferritin (based on ICP/ BCA): 18.3 ± 2.4, **UV/Vis** (20 mmol/L Tris, 0.15 mol/L NaCl)**:** λ_{max} (ε) = 450 (15200), 325 (61300), 280 (576000) nm.

Crystallization, X-ray data collection and crystallographic refinement

Proteine crystallization was performed using the hanging drop vapor diffusion method at 20 °C. Reservoir solutions of 1 ml were used, containing $(NH_4)_2SO_4$ (0.4 – 0.9 M) and $CdSO_4$ (10 mM – 30 mM). The crystallization drop was created by mixing 3 μl of the reservoir solution with 3 μl of the proteine solution (containg about 10 mg proteine / ml). The slightly yellow crystals appeared within one to three days

X-ray diffraction data for **1 · apo-ferritin** and **3 · apo-ferritin** were collected at 100 K using Rigaku FR-E X-ray generator (wavelength: 1.5418 Å, Cu-Kα) and R-AXIS VII detector at the High-Intensity X-ray Diffraction Laboratory, Nagoya University.

The crystals of **2 · apo-ferritin** were analyzed using synchrotron radiation. The synchrotron radiation experiments were performed at the BL44B2 in SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI). The crystal parameters and data collection statistics are summarized in Table 1.

Refinement:

The structures of protein moieties of the apo-ferritin-ferrocenes composites were determined by molecular replacement with *MOLREP* using an apo-Fr structure (pdb ID: 1DAT) as the initial model. Although refinement of the protein structures were performed using *REFMAC5* in the *CCP4* suite and *COOT*, the structures of ferrocene derivatives were not determined because electron densities corresponding to ferrocene complexes were not clear. The anomalous fourier difference maps around two-fold symmetry axis of **2 · apo-ferritin** are shown in Figure 1.

Table1: Summary of X-ray data for **1/2/3 · apo-ferritin**

	1•apo-ferritin	**2•apo-ferritin**	**3•apo-ferritin**
Data collection statics			
X-ray wavelength, Å	1.5418	1.0000	1.5418
Space group	*F*432	*F*432	*F*432
Unit cell, Å	180.579	181.951	181.832
Resolution range	50-1.95	100-1.60	100-2.00
(outer shell) (Å)	2.02-1.95	1.66-1.60	2.07-2.00
Total observations	657,959	1,011,468	716,754
Unique reflections	18,989	34,606	17,951
Completeness (%)[a]	99.9 (100)	99.9 (99.8)	99.8(100)
R_{merge} (%)[a,b]	5.9 (31.0)	5.8(22.7)	7.7(25.4)
I/σ (I)[a]	66.5 (19.2)	52.4 (12.1)	79.1(36.4)

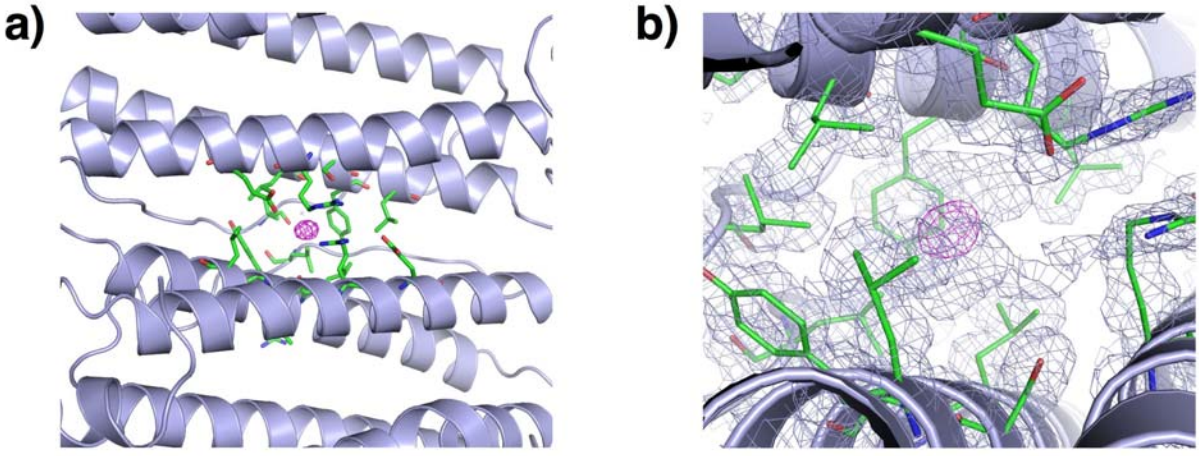


Figure 1. Crystal structure of **2•apo-ferritin**. (a) two-fold symmetry axis of apo-ferritin molecule. (b) close up view of the two-fold axis. The anomalous difference Fourier maps at 3.0 σ are shown in magenta. The selected 2*F*o-*F*c electron density maps at 1.0 σ are shown in light blue.

Electrochemical measurements

Cyclovoltammetric and differential pulse voltammetry experiments were performed under Argon using a Au working-electrode, a Pt counterelectrode and an SCE reference electrode (saturated KCl). Sample volumes were 180 µl each, the electrolyte solution was an aqueous solution of 1 mol/L NaCl at the desired pH, using 20 mM Tris/HCl (for pH = 8 and 9), Mops (for pH = 6 and 7) or HOAc (for pH = 4 and 5) buffer systems. All measurements involving proteins were performed with a protein concentration of 20 µmol/L. The ferrocene reference spectra were conducted at ferrocene concentrations of 1.2 mmol/L (dimethylaminomethylferrocene), 0.8 mmol/L (ferrocene carboxylic acid) or 0.4 mmol/L (1-dimethylaminoethylferrocene), respectively.
Cyclovoltammetry was conducted in a range from 0 - 0.5 volts at a scan rate of 20 mV/sec. Differential pulse voltammetry was conducted in a range of 0 - 0.5 volts with an increasing width of 0.002 V. The data analysis was performed using an Ocean Optics Inc. OOIBase 32^{tm} program.

Figure 2: UV-spectra of a) apo-ferritin, b) **1**·apo-ferritin, c) **2**·apo-ferritin and d) **3**·apo-ferritin.

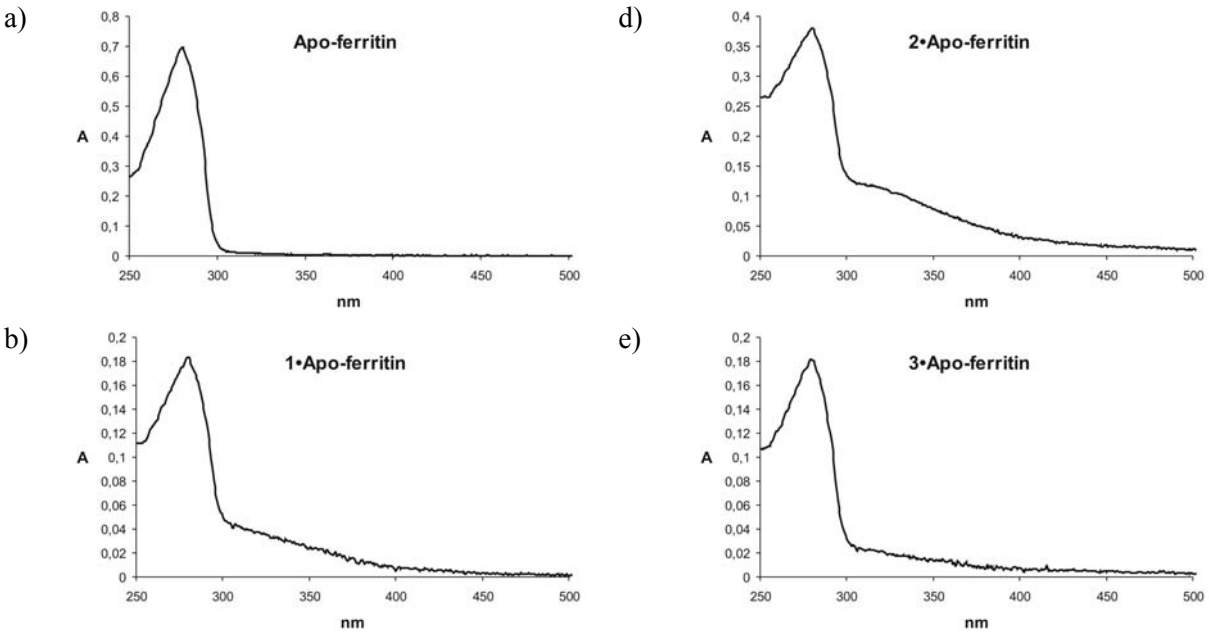


Figure 3: Cyclovoltammetry of apo-ferritin (20 µmol/L) at pH = 8

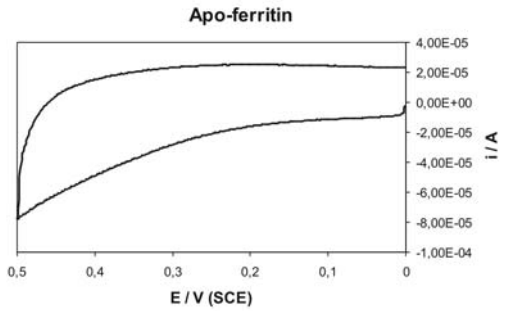
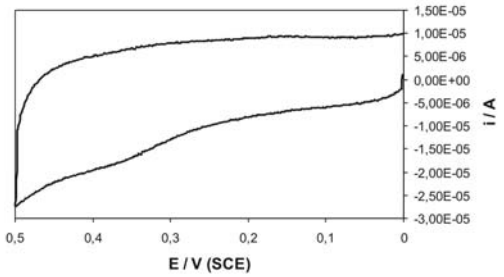


Figure 4: Cyclovoltammetry of **1**·apo-ferritin (20 μmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9

a)



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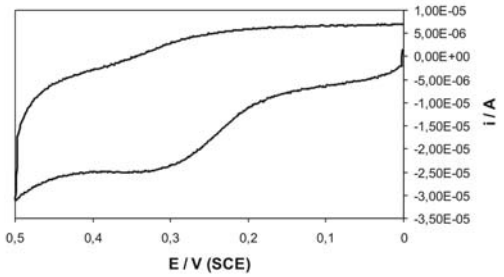

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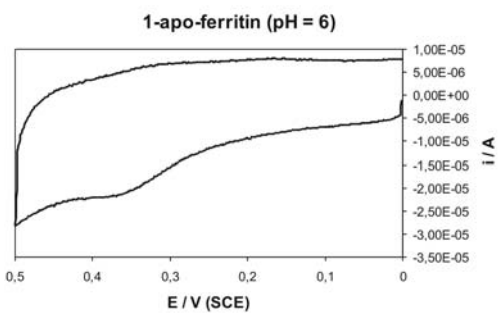

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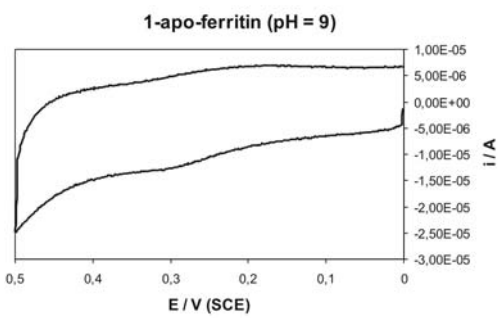

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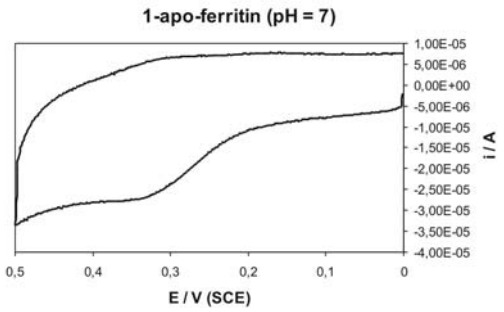
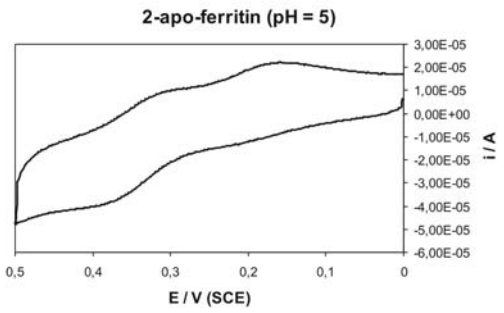


Figure 5: Cyclovoltammetry of **2**·apo-ferritin (20 μmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9

a)



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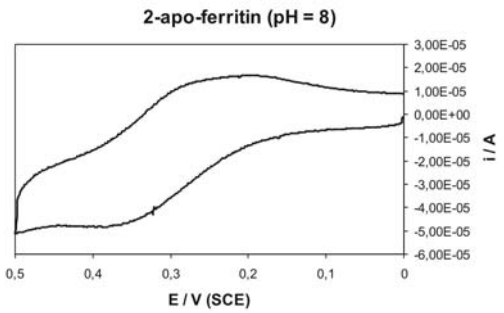

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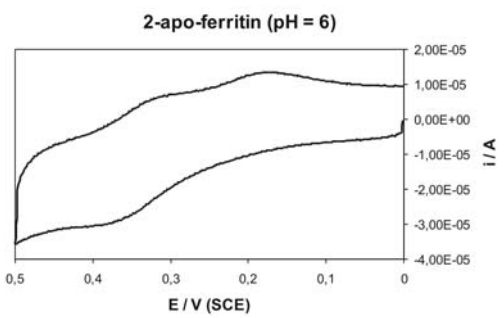

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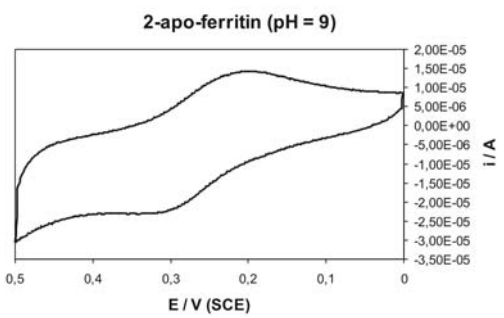

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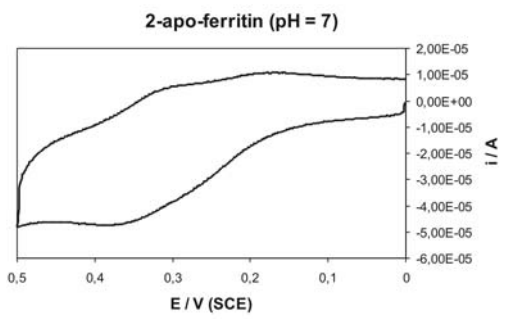
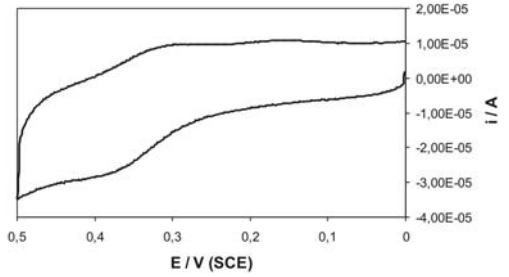


Figure 6: Cyclovoltammetry of **3**·apo-ferritin (20 μmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9

a)



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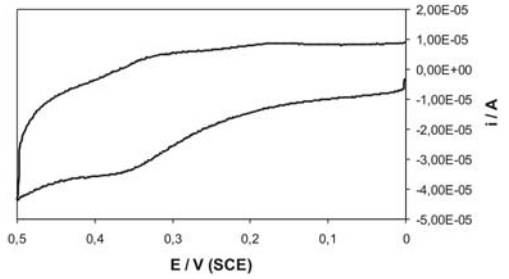

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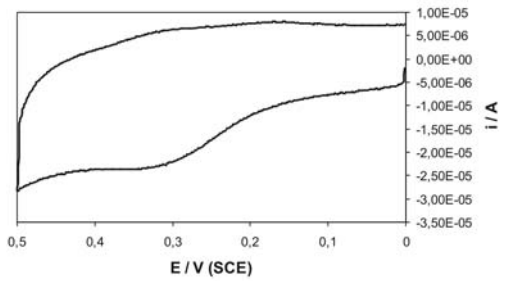

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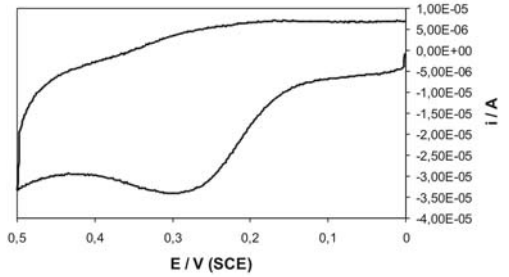

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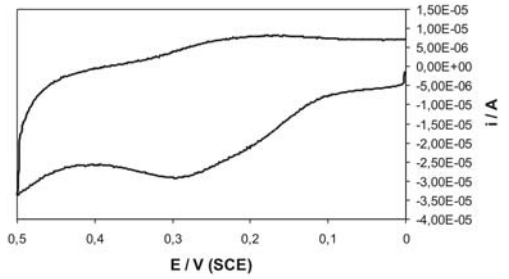


Figure 7: Cyclovoltammetry of ferrocene carboxylic acid (0.8 mmol/L) at a) pH = 4; b) pH =5, c) pH = 6, d) pH = 7, e) pH = 8 and f) pH = 9

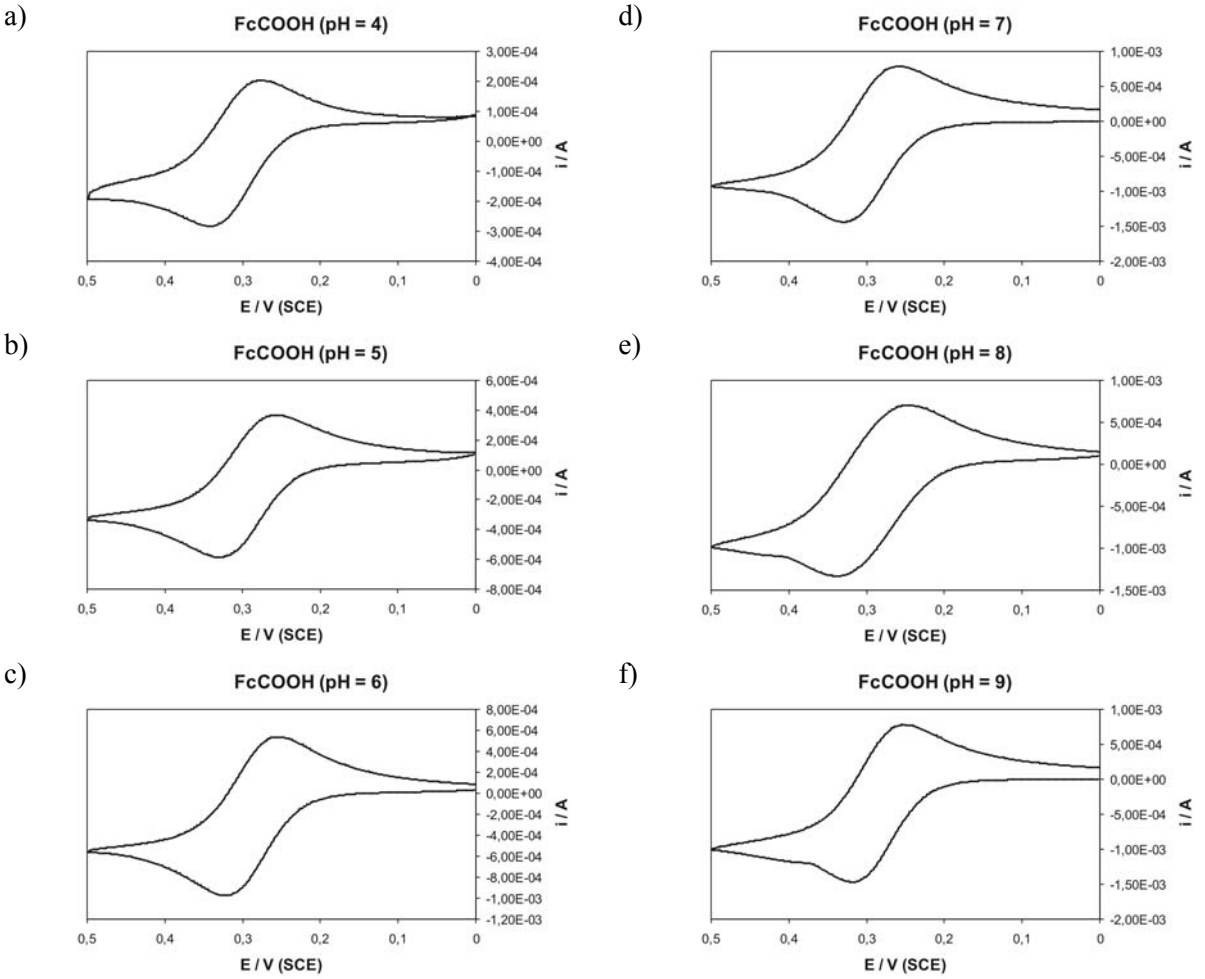
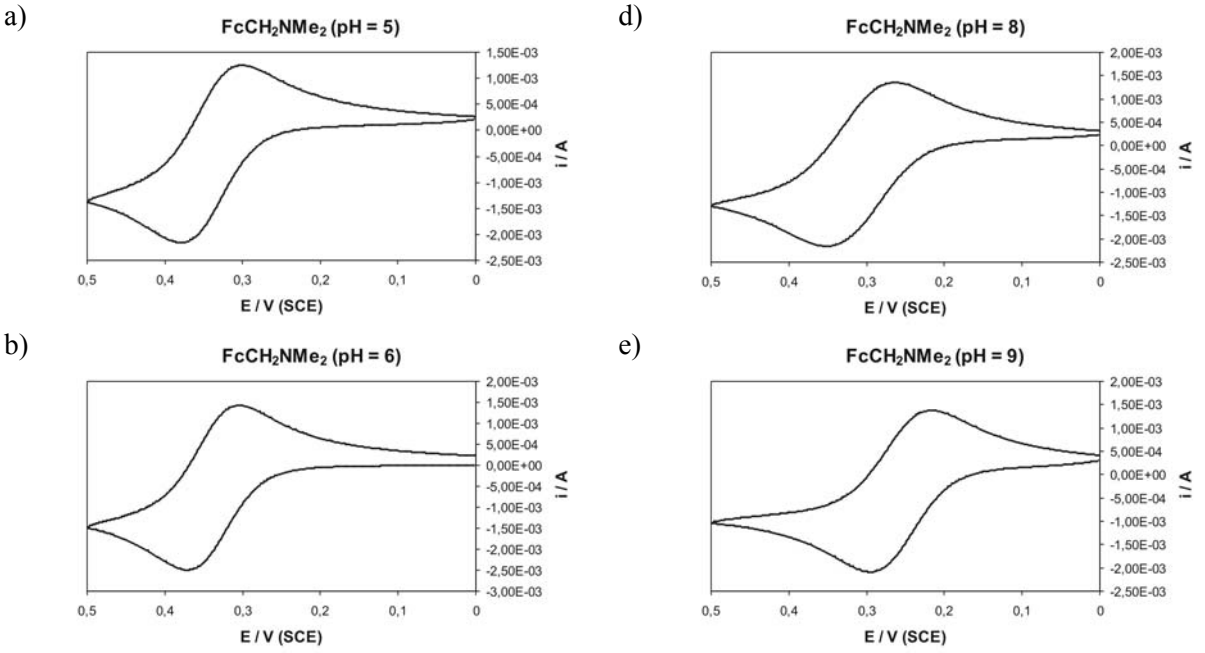


Figure 8: Cyclovoltammetry of dimethylaminomethylferrocene (1.2 mmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9



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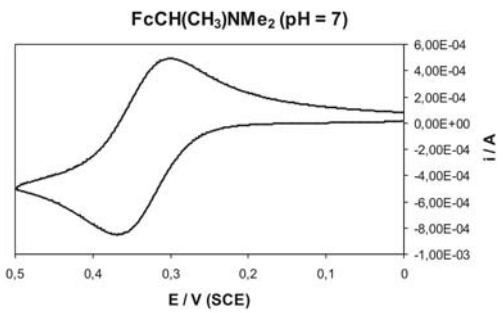
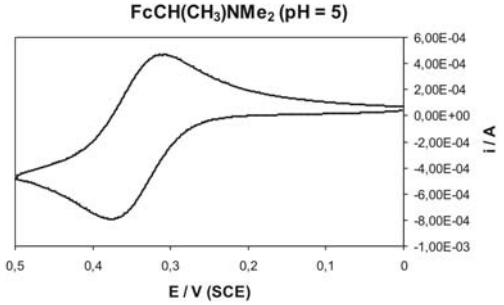


Figure 9: Cyclovoltammetry of 1-dimethylaminoethylferrocene (1.2 mmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9

a)



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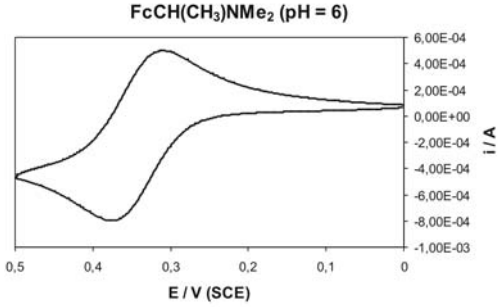

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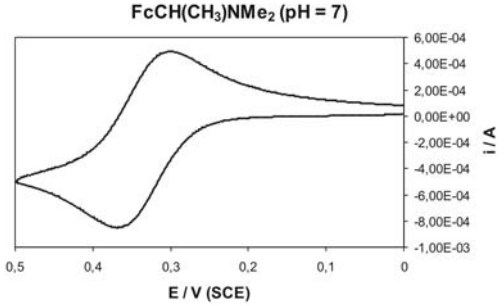

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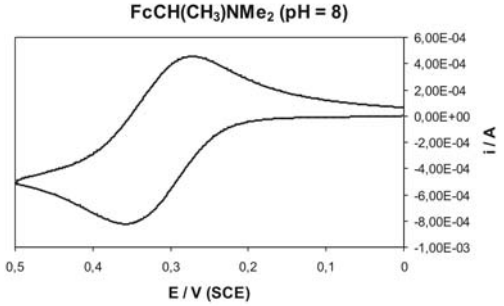

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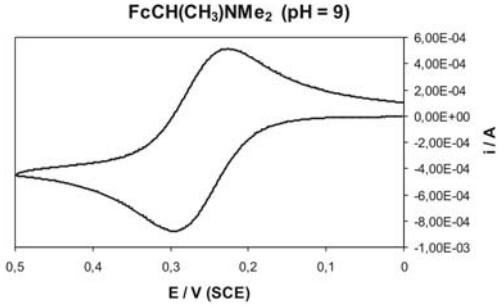


Figure 10: Differential pulse voltammetry of apo-ferritin (20 μmol/L) at pH = 8

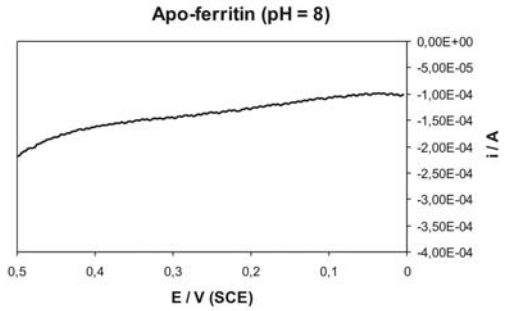
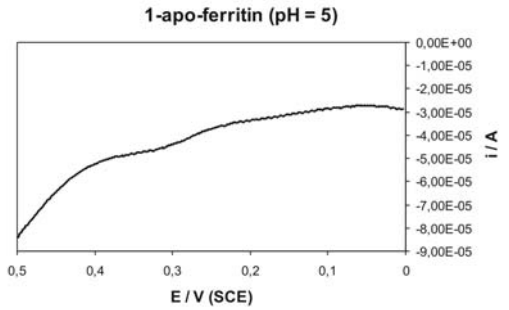


Figure 11: Differential pulse voltammetry of **1**· apo-ferritin (20 μmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9

a)



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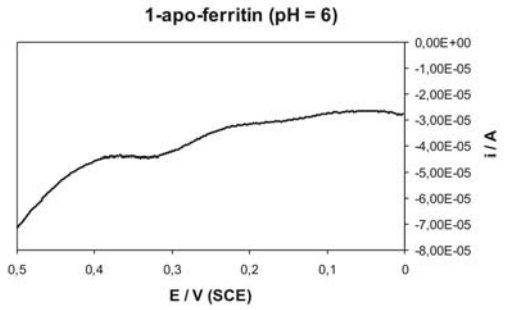

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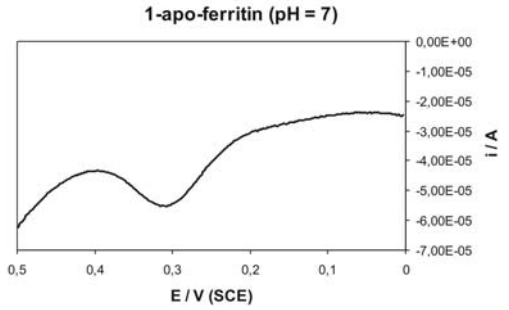

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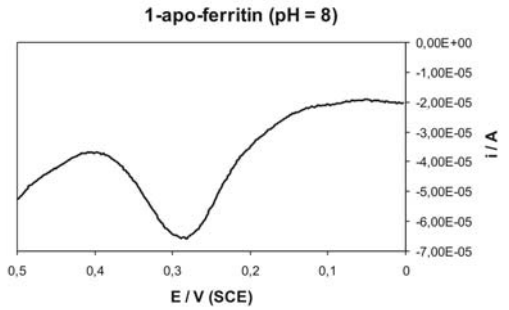

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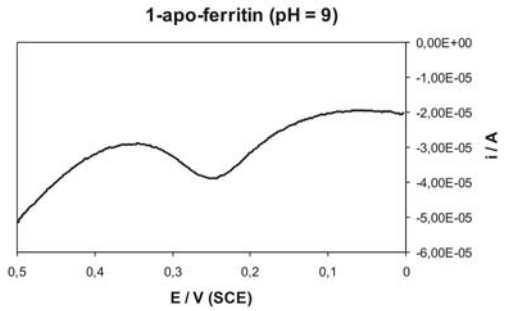


Figure 12: Differential pulse voltammetry of **2**· apo-ferritin (20 μmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9

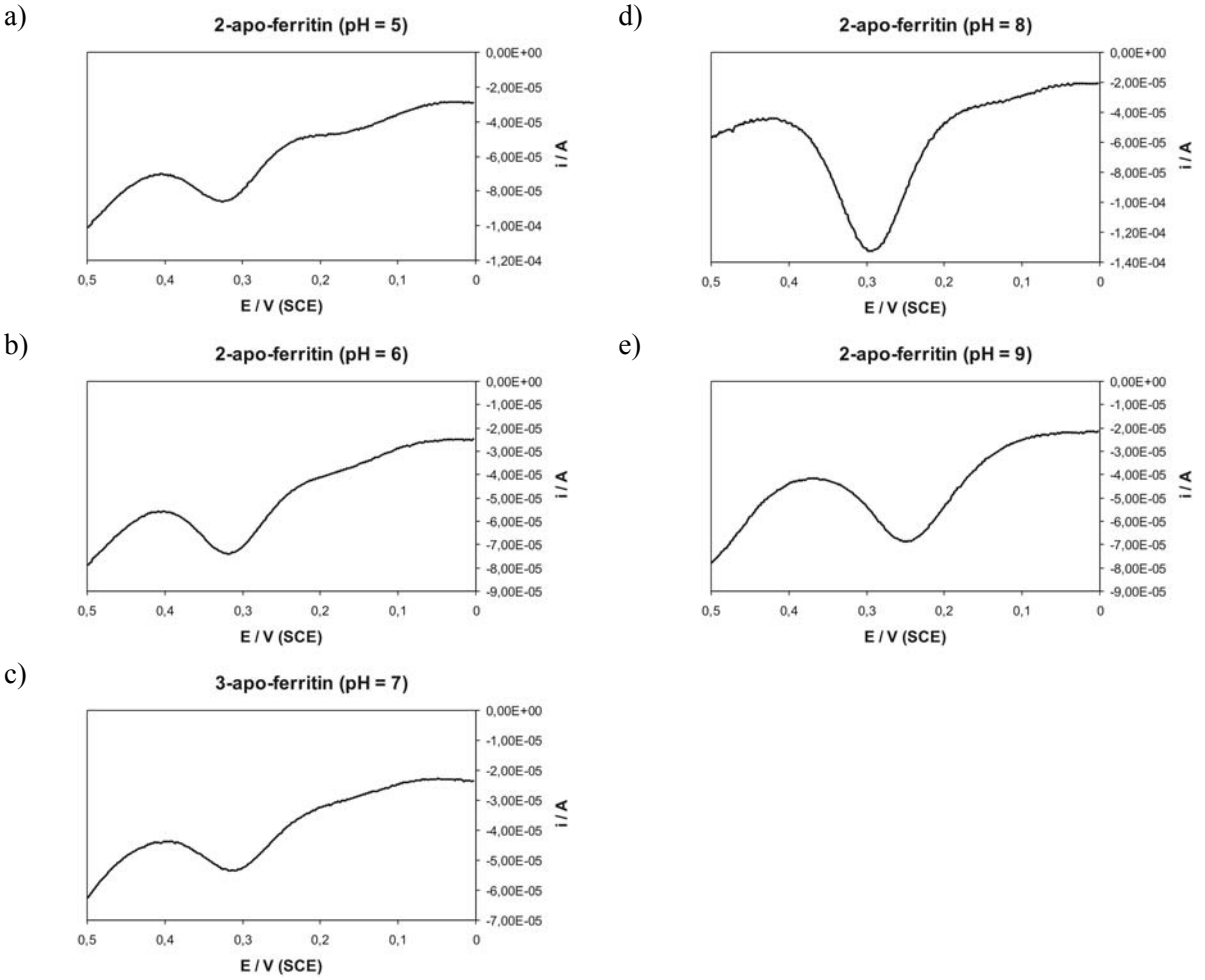
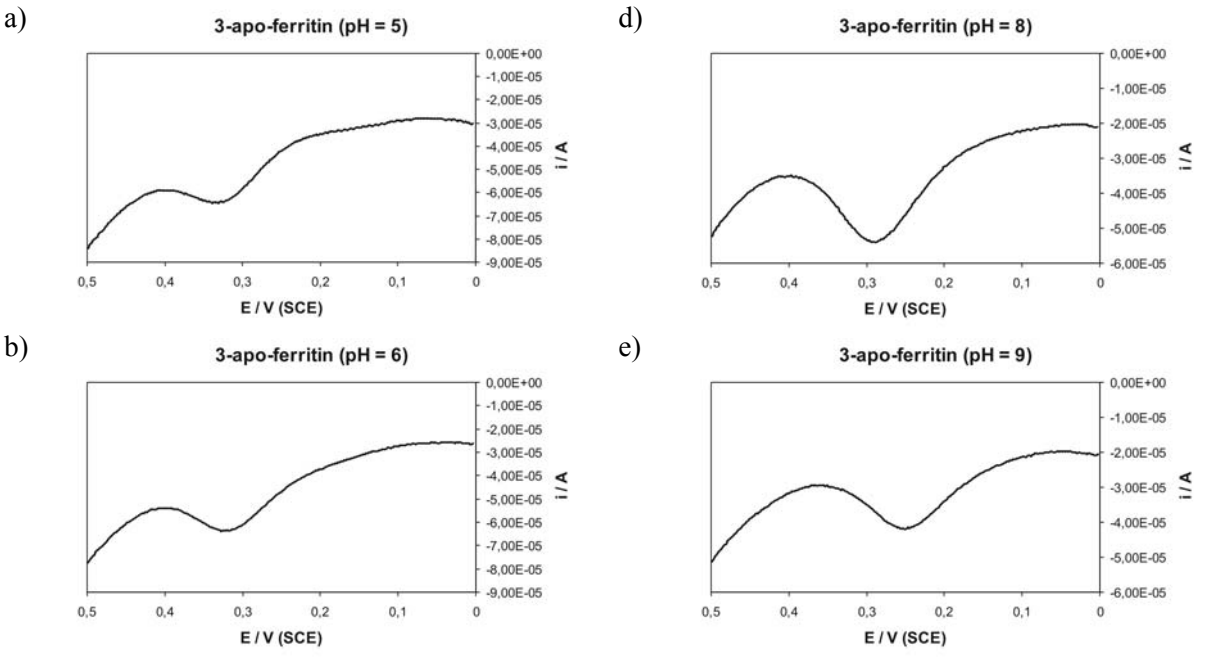


Figure 13: Differential pulse voltammetry of **3**· apo-ferritin (20 μmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9



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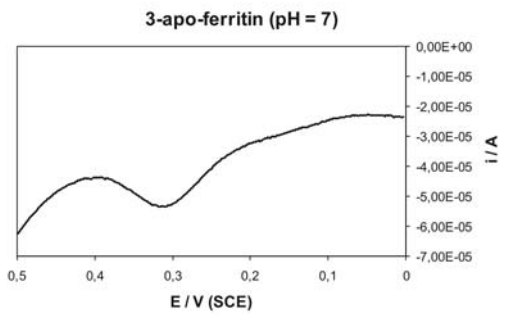
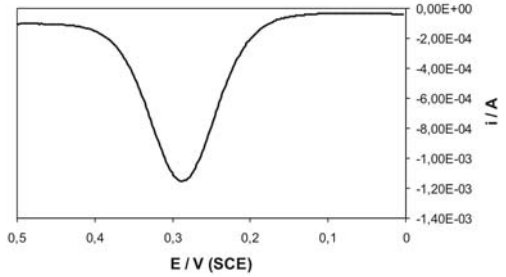


Figure 14: Differential pulse voltammetry of ferrocene carboxylic acid (0.8 mmol/L) at a) pH = 4; b) pH =5, c) pH = 6, d) pH = 7, e) pH = 8 and f) pH = 9

a)



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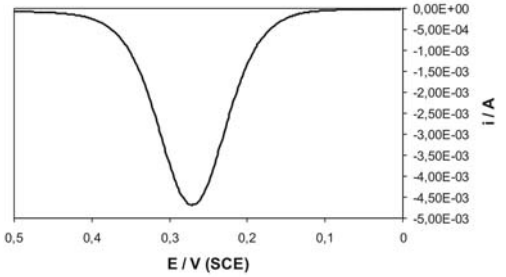

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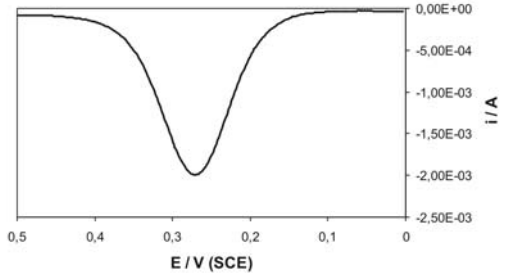

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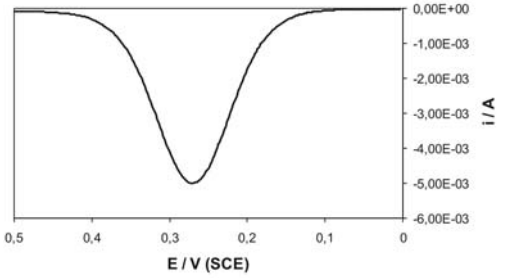

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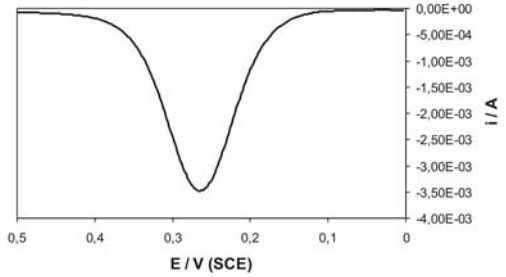


f)

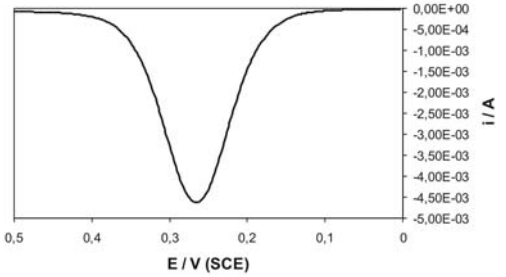
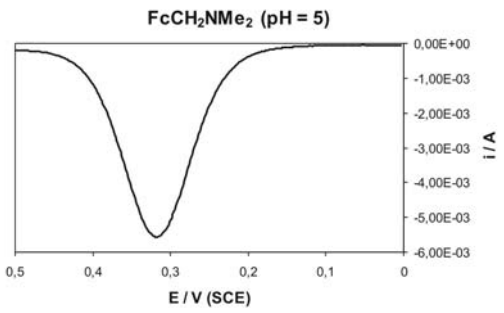
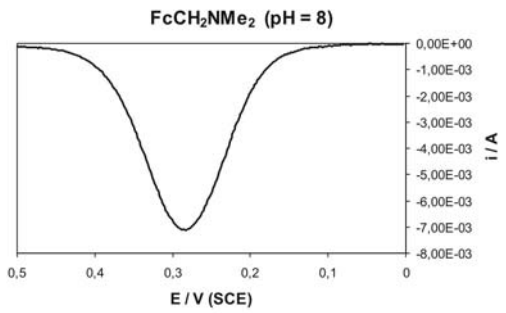


Figure 15: Differential pulse voltammetry of dimethylaminomethylferrocene (1.2 mmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9

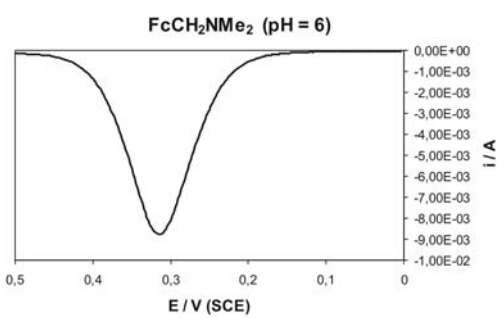
a)



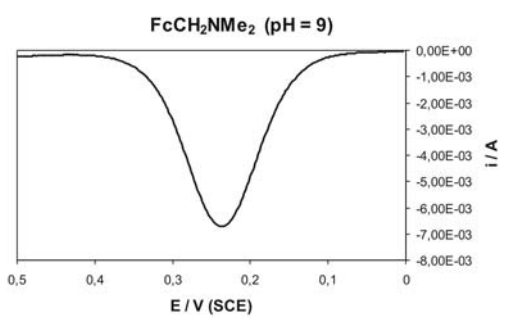
d)



b)



e)



c)

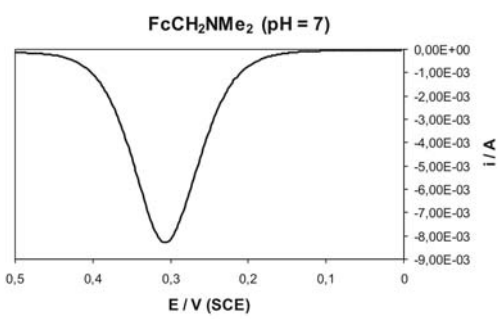
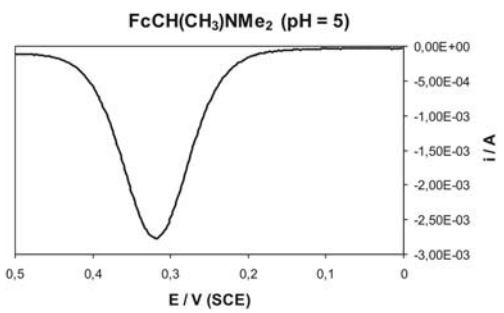
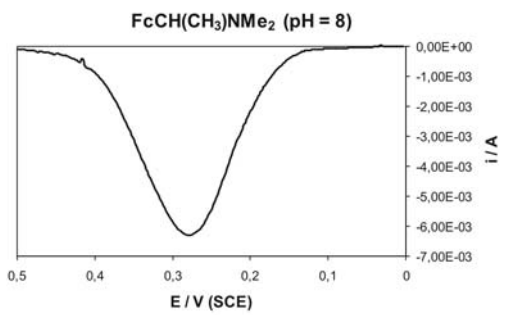


Figure 16: Differential pulse voltammetry of 1-dimethylaminoethylferrocene (1.2 mmol/L) at a) pH =5, b) pH = 6, c) pH = 7, d) pH = 8 and e) pH = 9

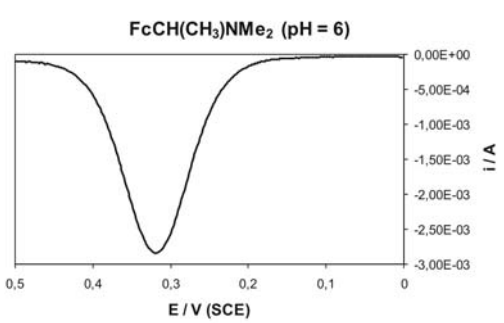
a)



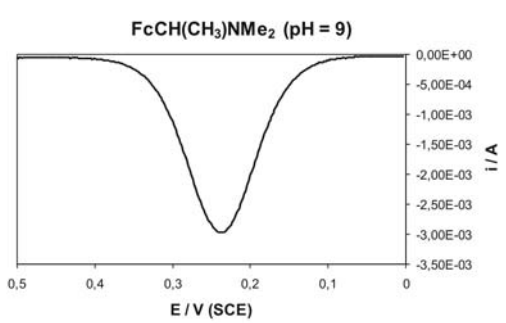
d)



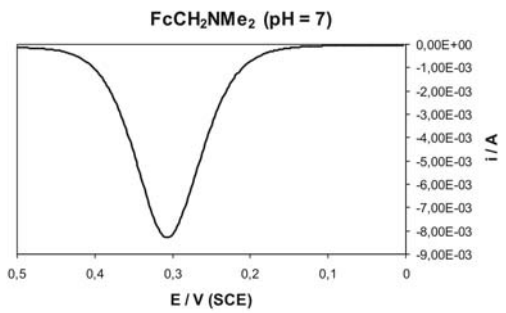
b)



e)



c)



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