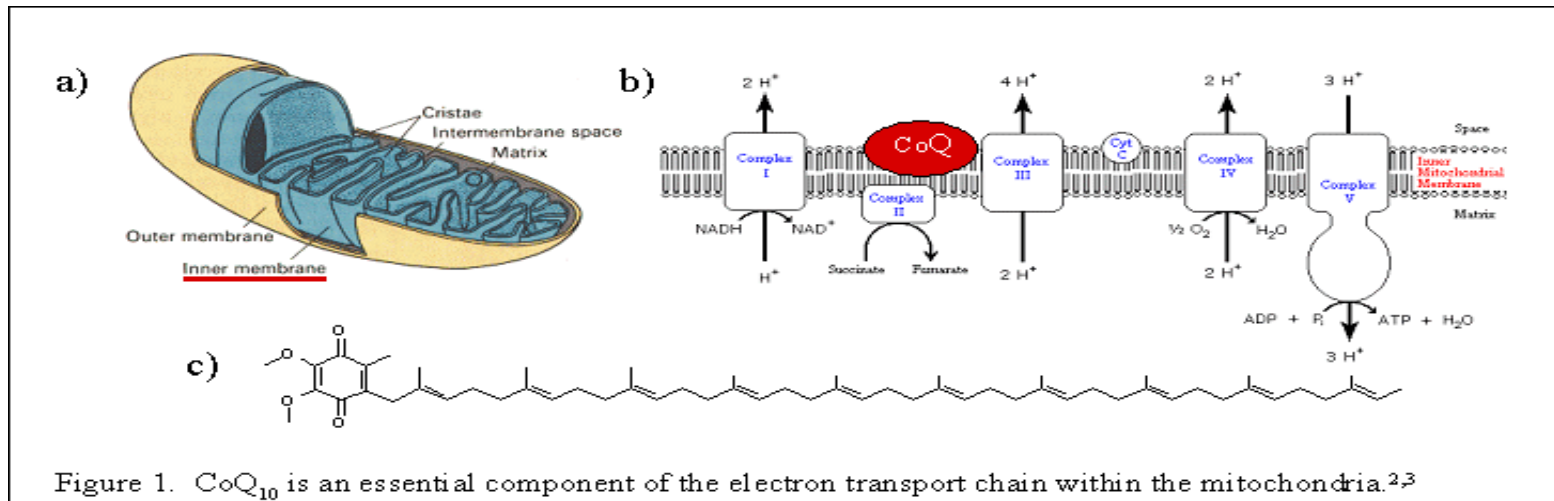
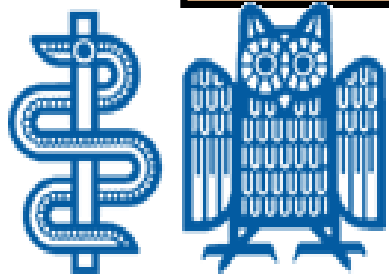


INSIGHTS INTO A NEW FUNCTION of COENZYME Q-10 (CoQ10)

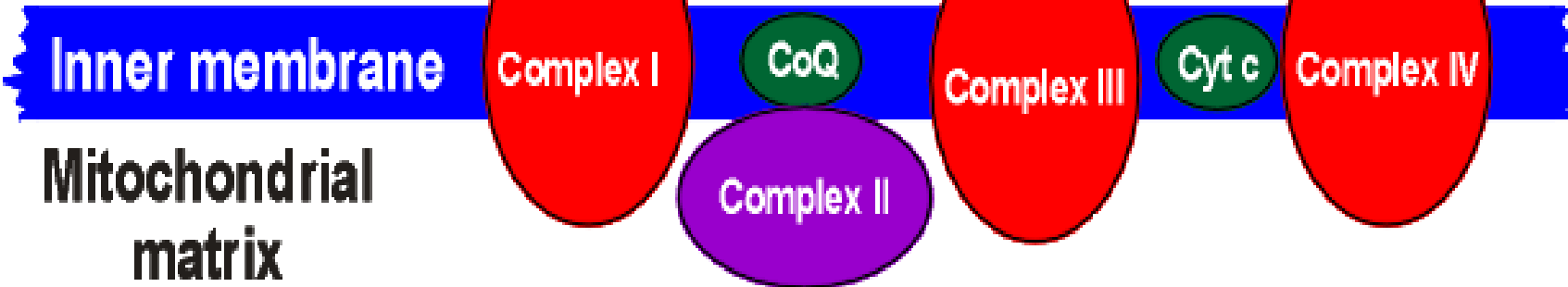
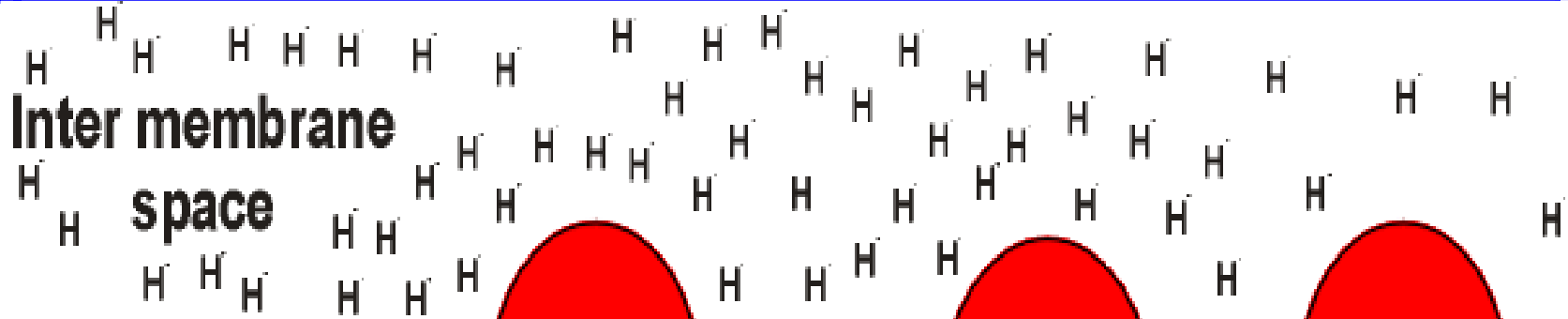
PART 1. *Alkaline Synthesis and Properties of New Types Methoxy-Benzoquinones-*

*Rubin Gulaboski, Ivan Bogeski, Reinhard Kappl,
Richard Kohler, Markus Hoth*



Cytoplasm

Outer membrane

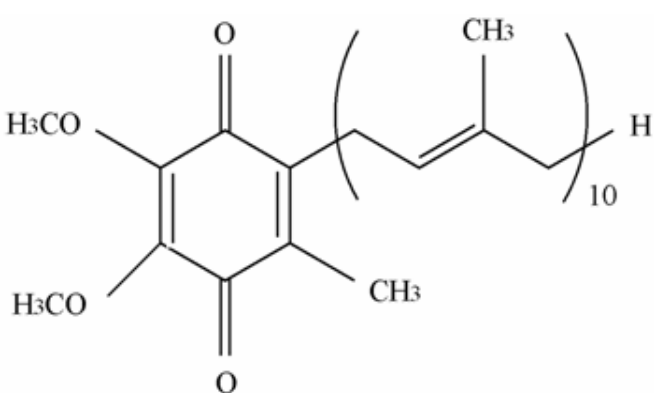


CoQ10 mediates the electron transfer between Complexes I, II and III

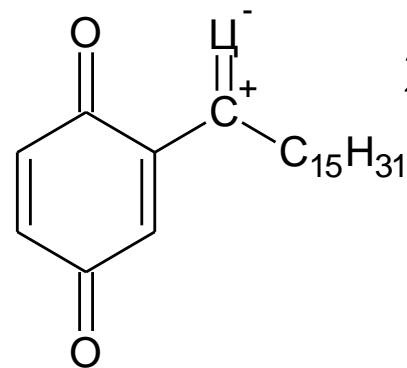
in the mitochondrial electron transfer chain, **and...**

...IT HAS THOUSANDS MORE FUNCTIONS

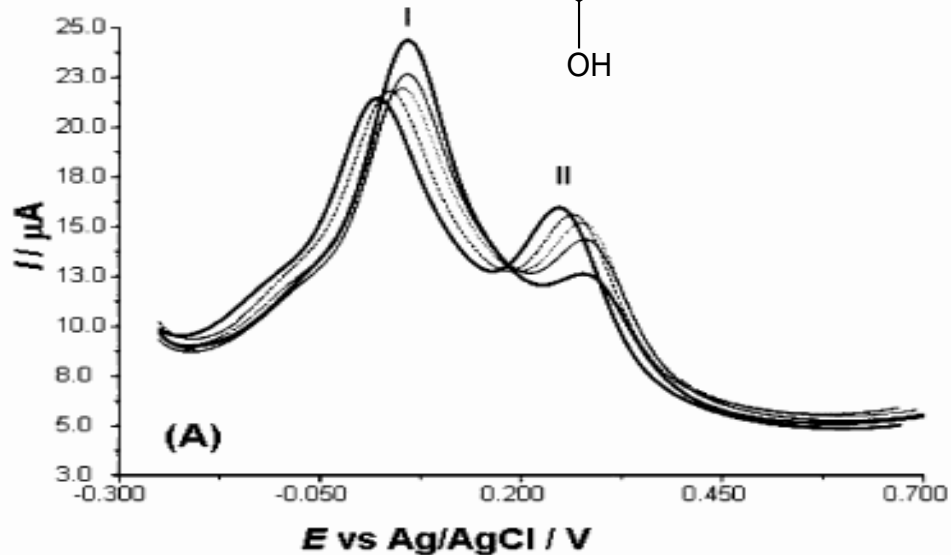
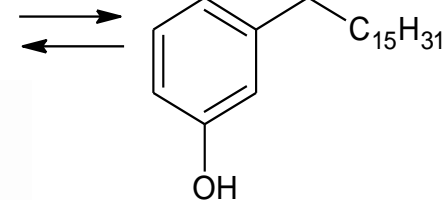
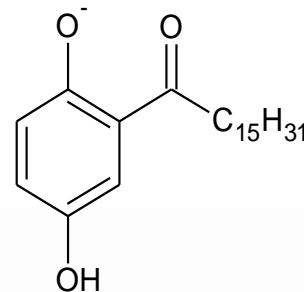
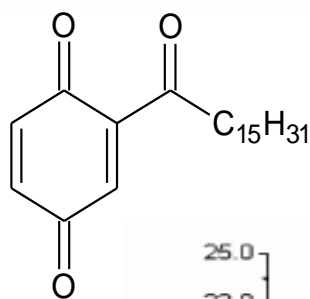
Our Initial Idea-to probe whether CoQ10 can bind and transfer Ca^{2+} ?

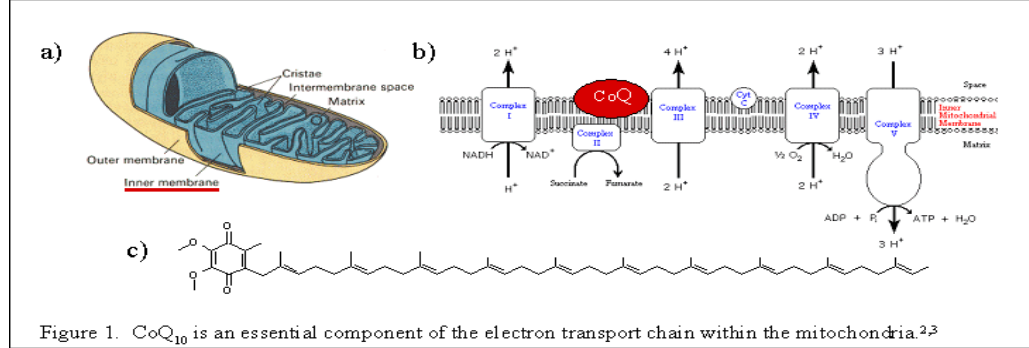
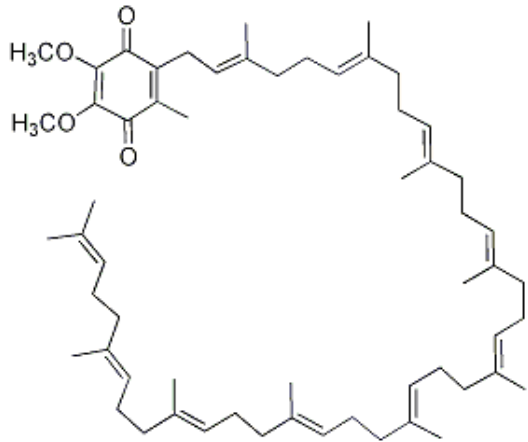


CoQ10



2-palmitoyl quinone

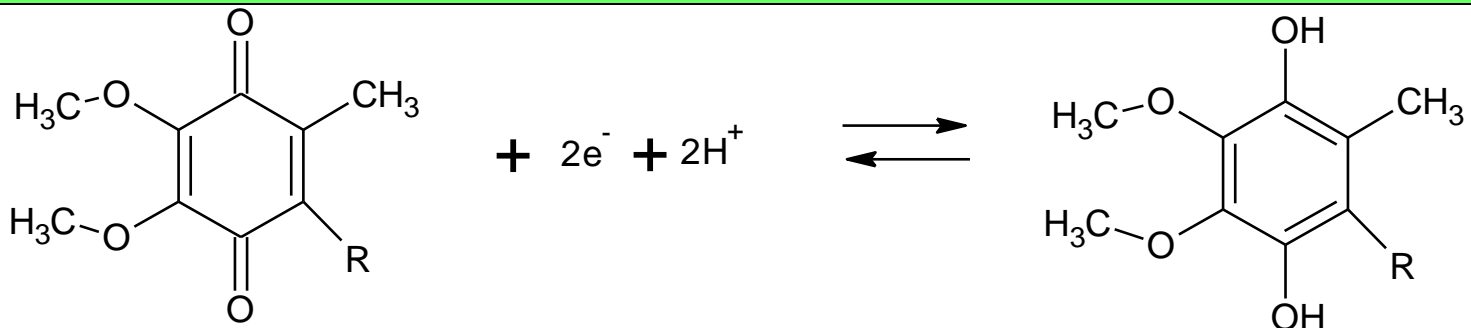


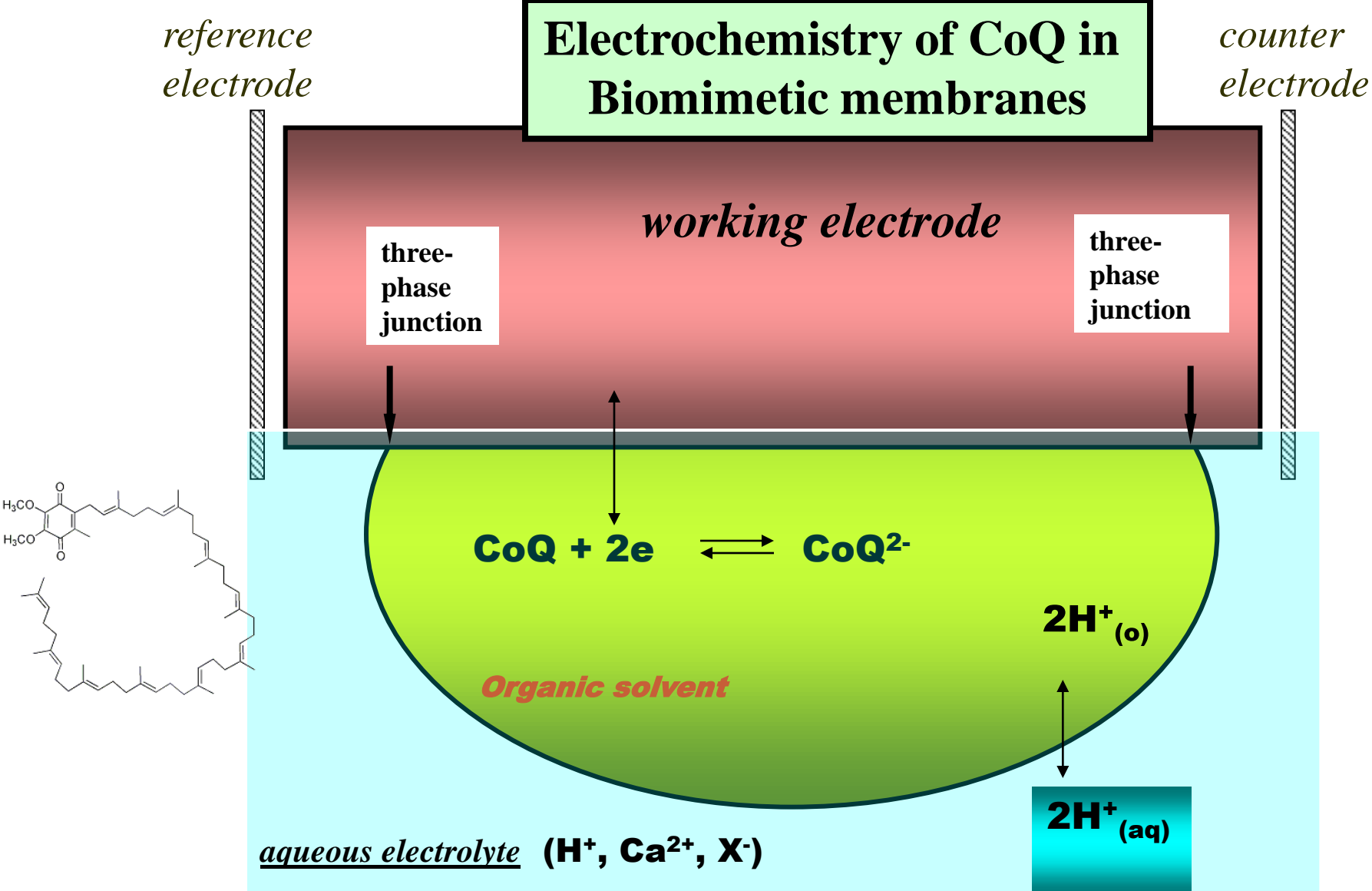


CoQ10 has multifacated functions

- crucial *cofactor* for at least three mitochondrial enzymes- Compl. I, II, III
- the reduced form of CoQ10 is a very *effective antioxidant-radical scavenger*
- inhibits lipid and protein peroxidation*
- CoQ10 can stabilize myocardial calcium-dependent ion channels, **decreases blood viscosity ...**

***In 99.99 % of the cases, the biological functions of CoQ10 are linked to the redox reaction at the *2 carbonyl C=O* groups placed in para position**



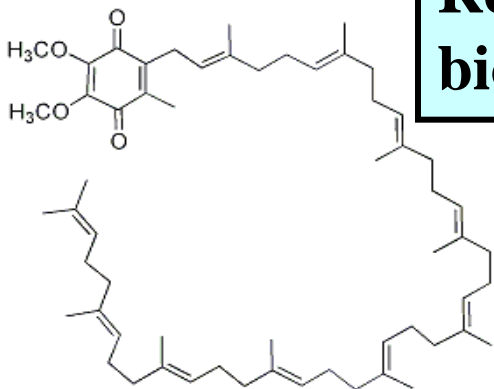


In the organic layer: $\text{CoQ} + 2e^- \rightleftharpoons \text{CoQ}^{2-}$

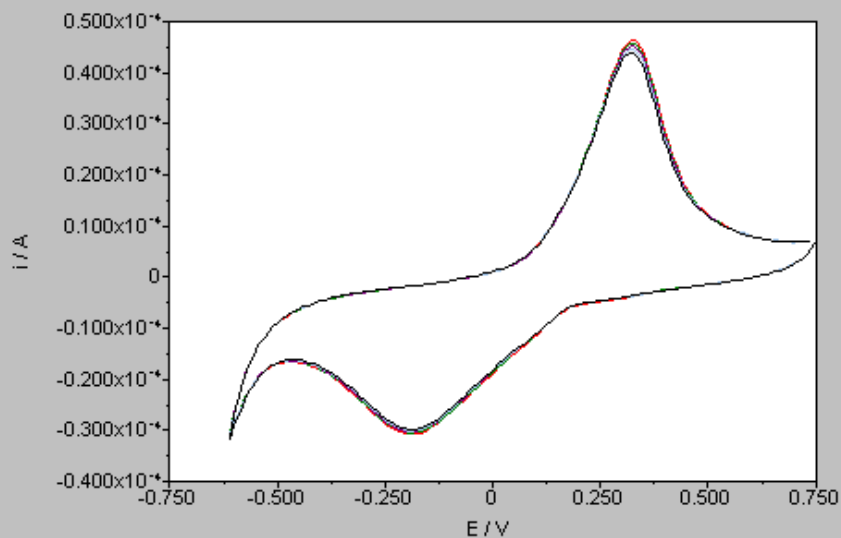
**Charge transfer across liquid-liquid interface:
 $2\text{H}^+(\text{water}) \rightleftharpoons 2\text{H}^+(\text{organic medium})$**

Results of Voltammetry of CoQ10 in biomimetic membranes

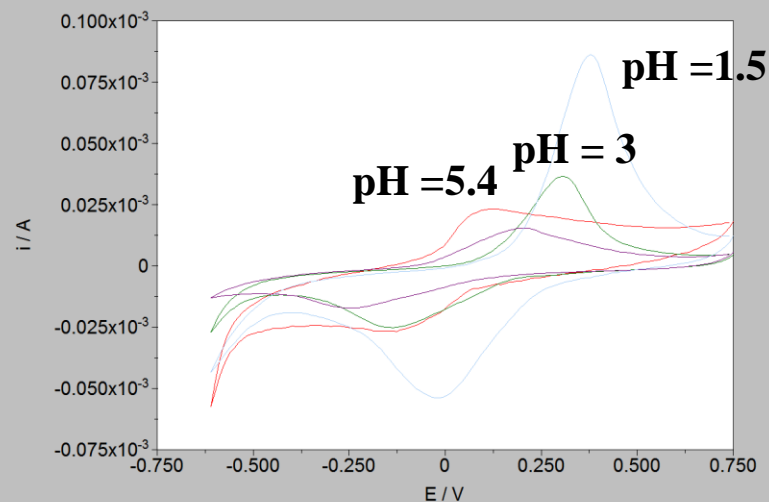
Sensitive to H⁺



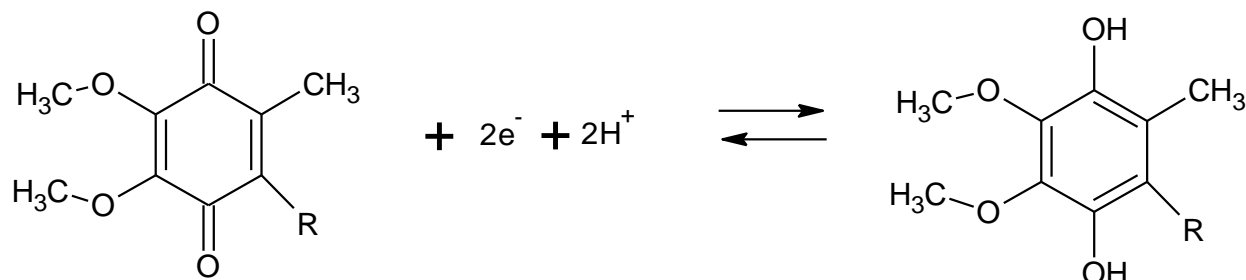
CoQ in NPOE + 0, 10, 25, 50 and 100 mM Ca²⁺



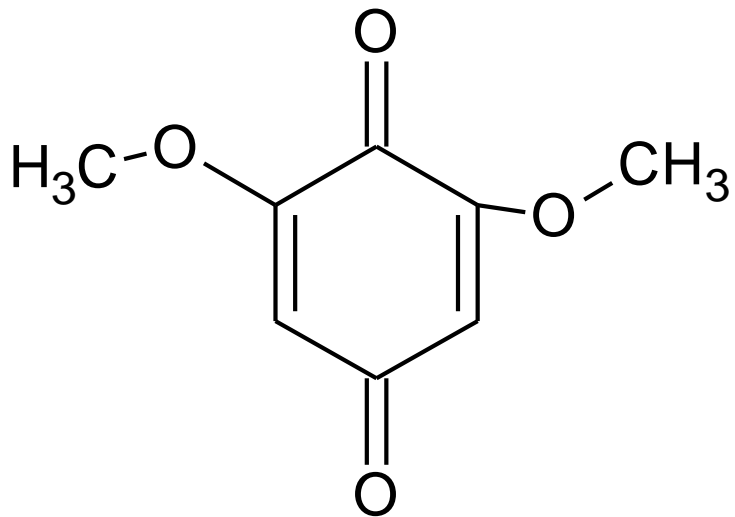
CoQ in NPOE + 0 Ca²⁺ - Effect of pH 1.5, 3, 4 and 5.4



But, ... **INSENSITIVE** to Ca²⁺!!!



Problem No#1: Find whether *the aromatic part* of the structure can *BIND* Ca^{2+} ?



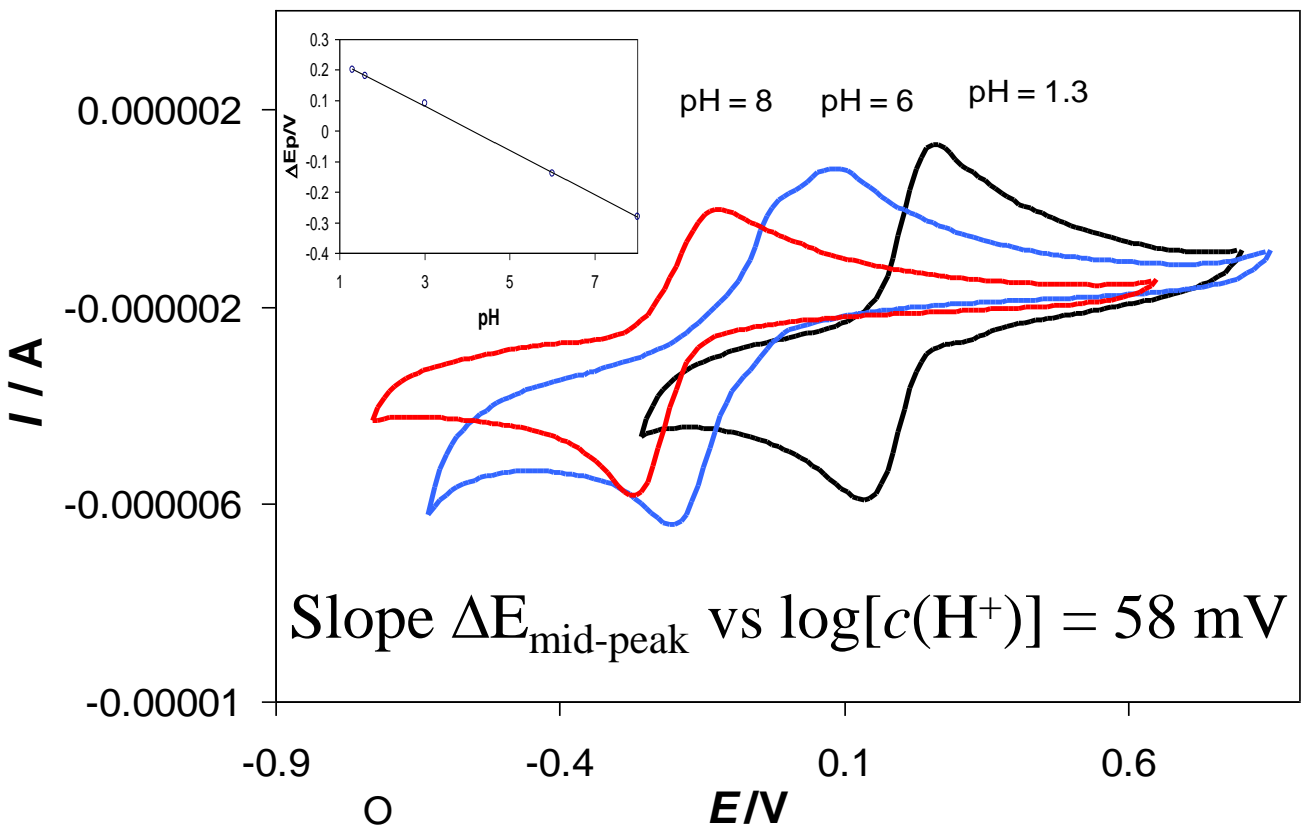
2,6-dimethoxy 1,4-benzoquinone

2,6-dimethoxy benzoquinone

Structurally similar to the *aromatic part* of CoQ10.

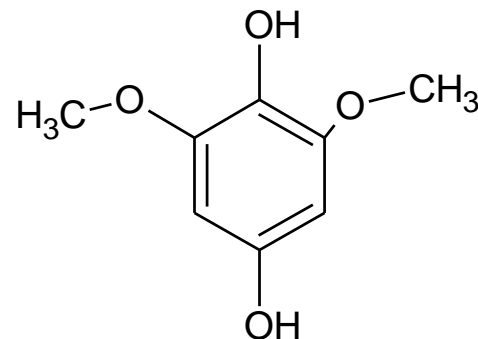
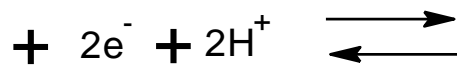
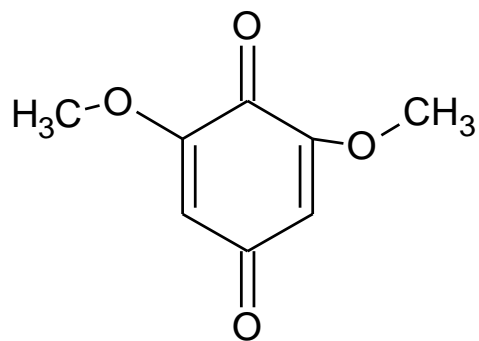


**Dissolved in
*Neutral media***



Cyclic voltammograms of 2,6-dimethoxy BzQ as a function of pH.

Stock solution of BzQ in pH of 7.00



$$E = E^{\circ} + (2.303RT/2F) \log \frac{c(\text{BzQ})_{\text{ox}}}{c(\text{H}_2\text{BzQ})} + (2.303RT/2F) \log c(\text{H}^+)^2$$

B

$$2 \times 2,303 \times RT / (2F) = 59 \text{ mV}$$

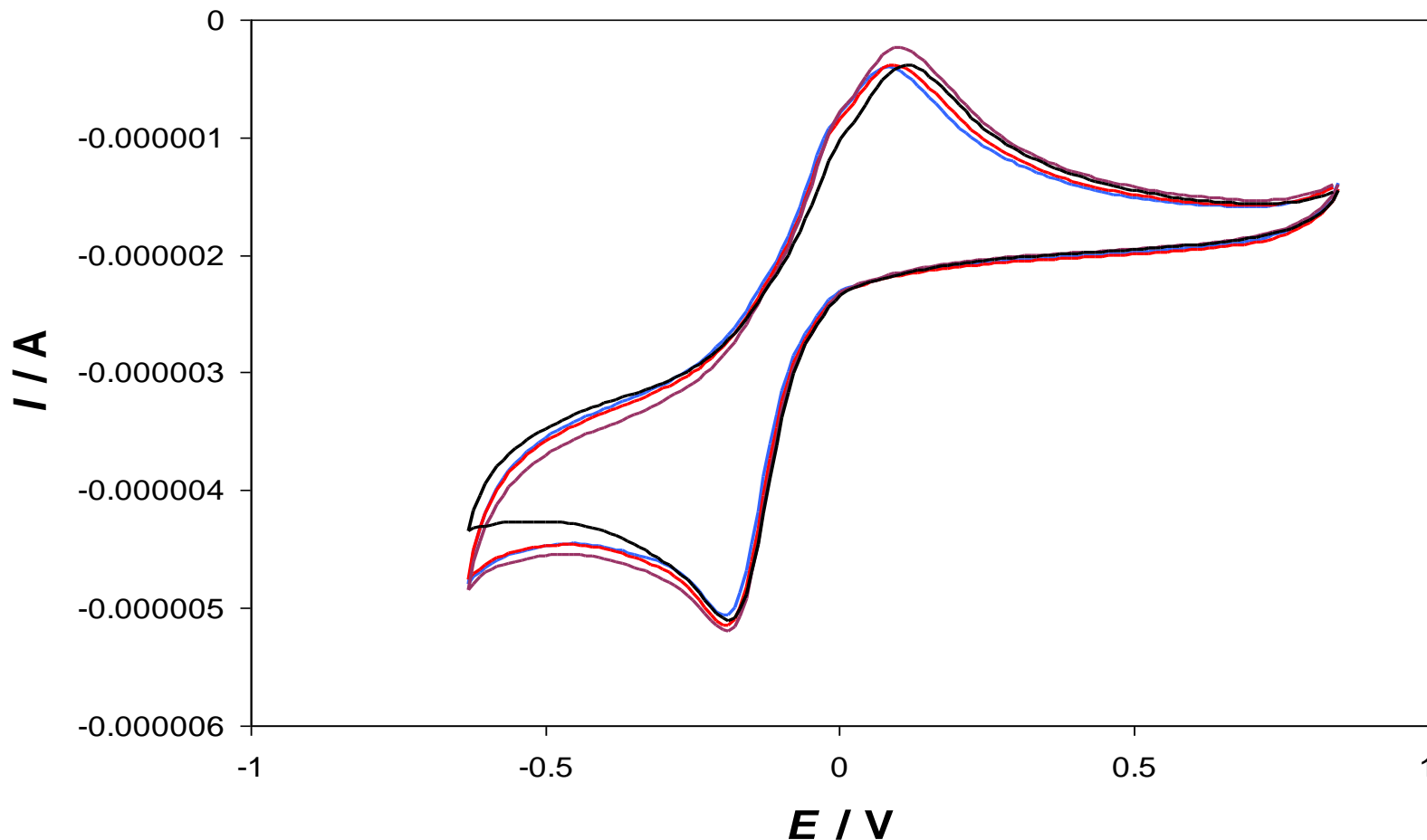
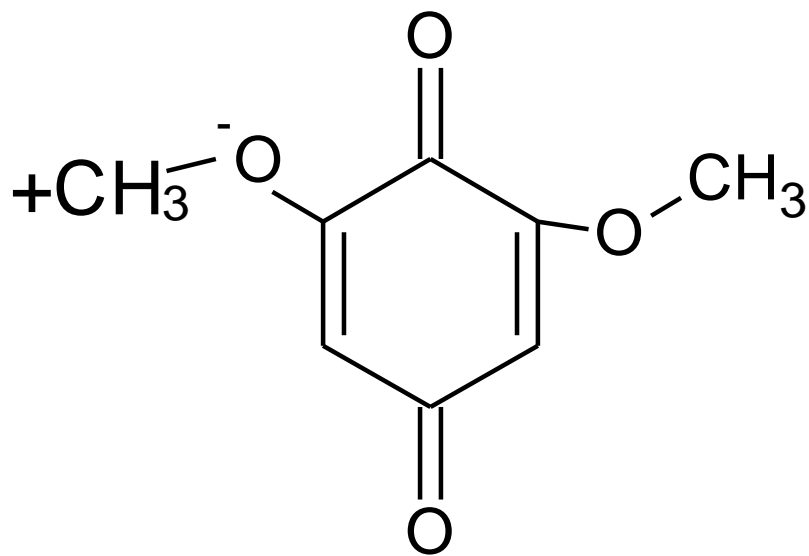


Figure 1. Cyclic voltammograms of 2,6-dimethoxy BzQ as a function of *calcium concentration* in pH = 7.00.

Scan rate of 25 mVs, $c(2,6\text{-Dm-BzQ}) = 1\text{mM}$.

$c(\text{Ca}^{2+}) = 0, 5, 10 \text{ and } 50 \text{ mM}$

CONCLUSION: THE NATIVE FORM OF METHOXY BEZOQUINONES CAN NOT BIND Ca^{2+}



This would mean-

END OF THE STORY!!?

Or may be Not?





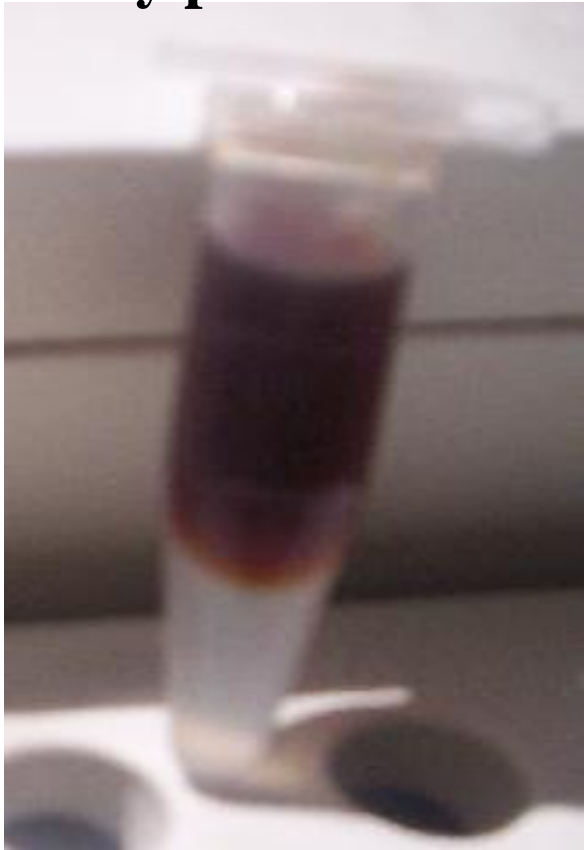
BzQ Dissolved in
Strong alkaline media

B

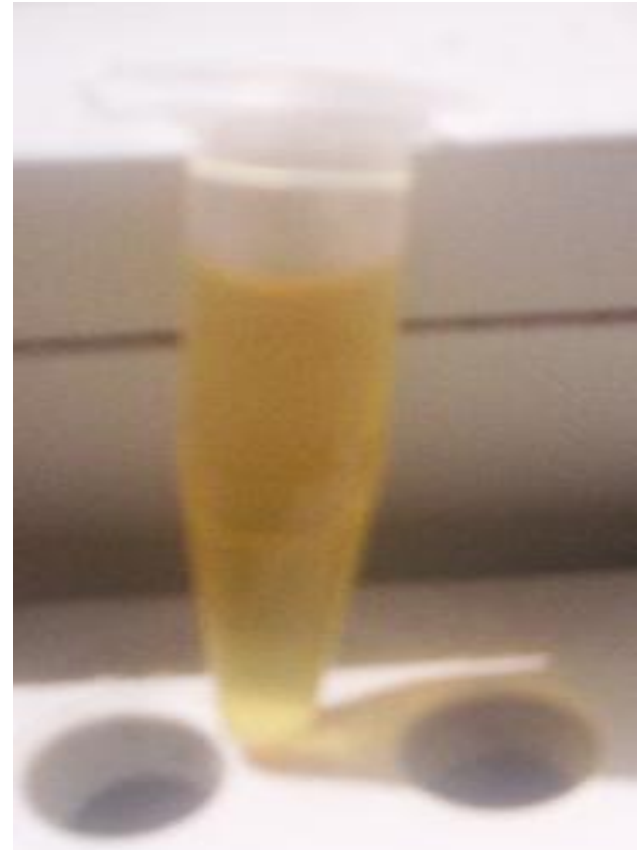


BzQ Dissolved in
Neutral media

**BzQ initially dissolved in NaOH-
HIGHLY HYDROPHILIC-it means
-it is very polar!!!**



Neutral BzQ-AMPHIPHILIC



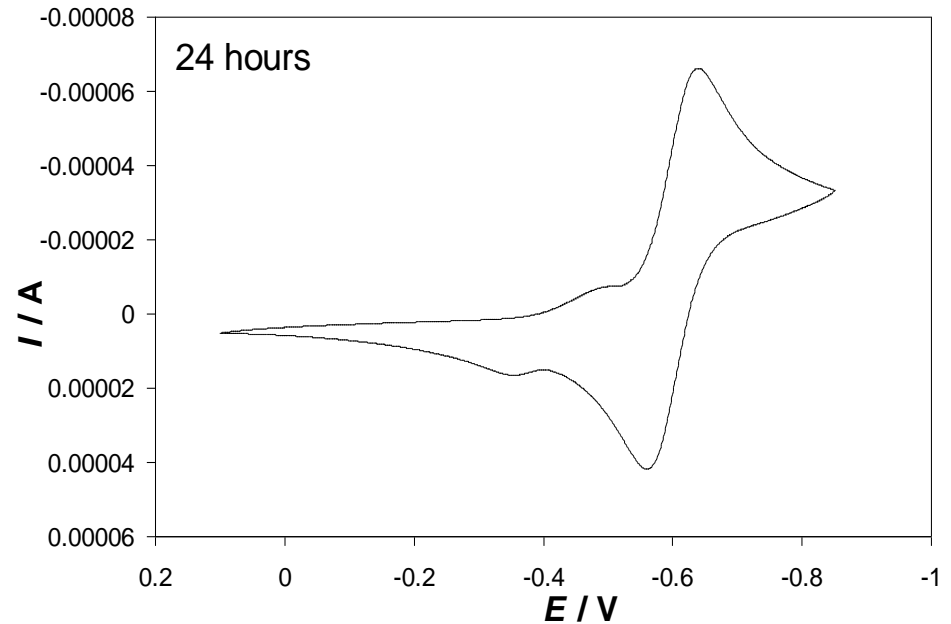
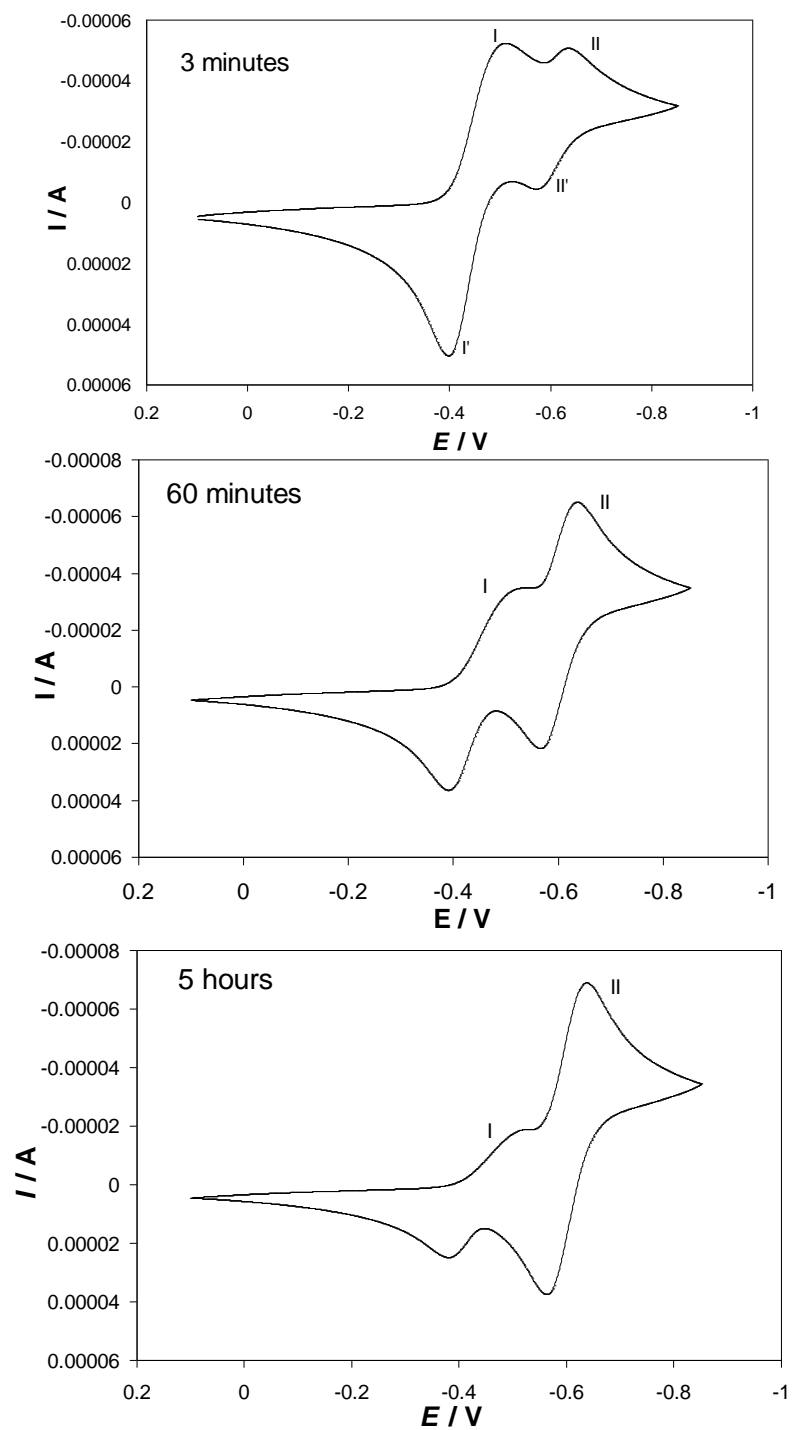
**BzQ firstly dissolved
In 1 M NaOH**

**NEUTRAL form
of BzQ**

**Partition experiments between
water and 1,2-dichloroethane**

B

**Chemical reaction takes place
between 2,6-BzQ and NaOH**

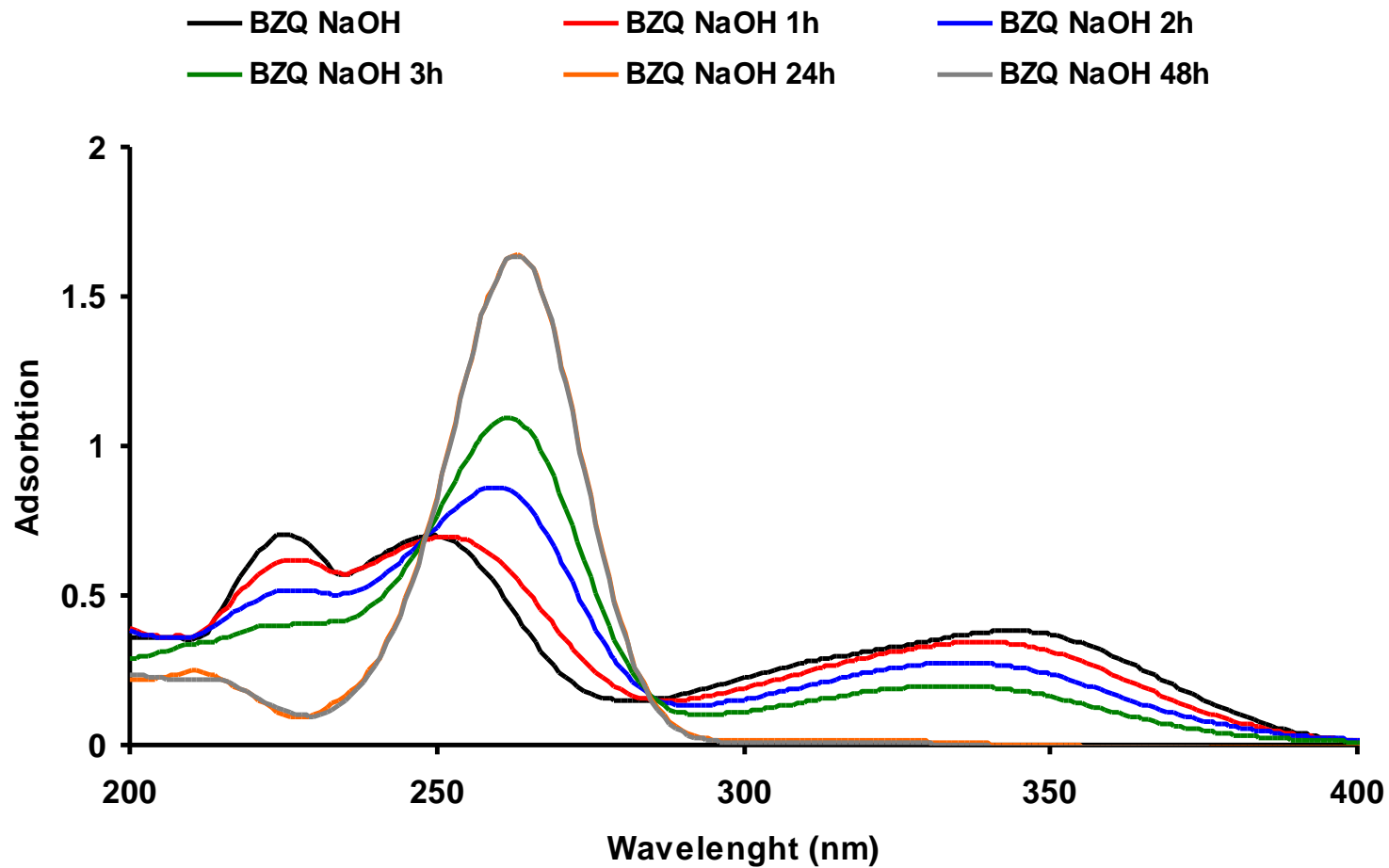




**BzQ dissolved directly
in *Neutral media* $pH = 7$**



**BzQ dissolved initially
dissolved in 1M NaOH 45 min
and *pH adjusted to 7***

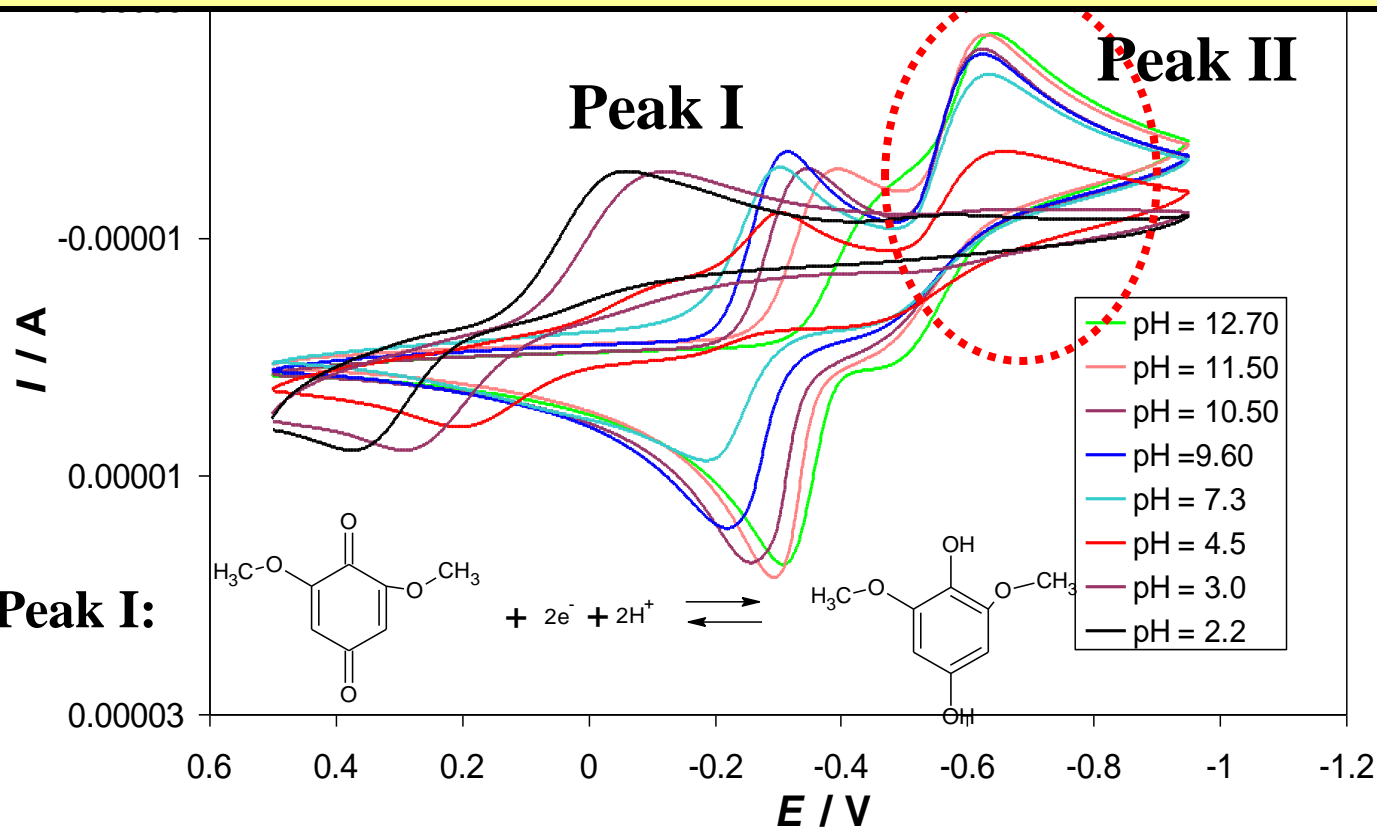


UV-VIS of 2,6-diMethoxy BzQ in 1 M NaOH

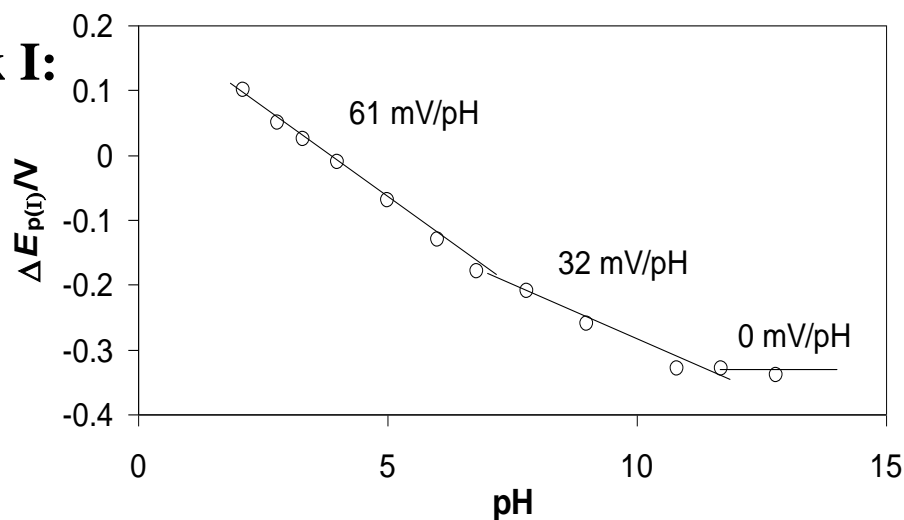
Testing the electrochemical properties of the formed product

CV-2,6-di-Metoxy-
BzQ-
pH dependence
2,6-diMetoxy-BzQ
firstly dissolved in
1M NaOH for
45min.
 $c(\text{BzQ})=1\text{mM}$

for Peak I:

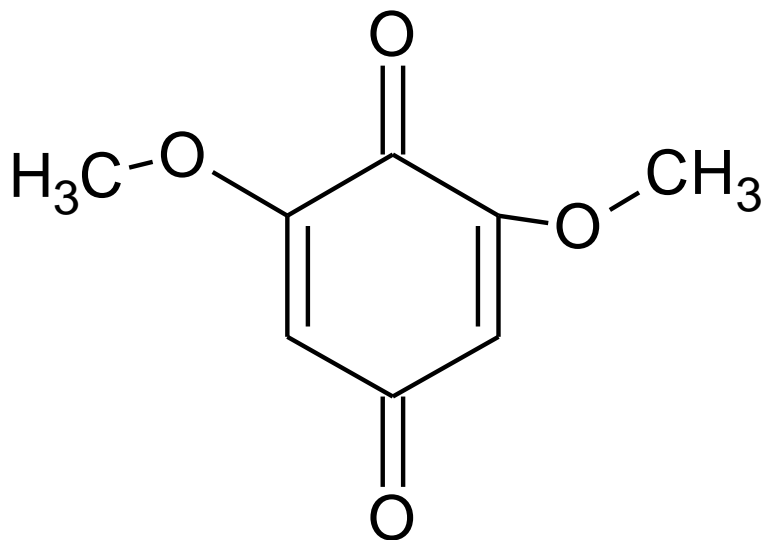


for Peak I:

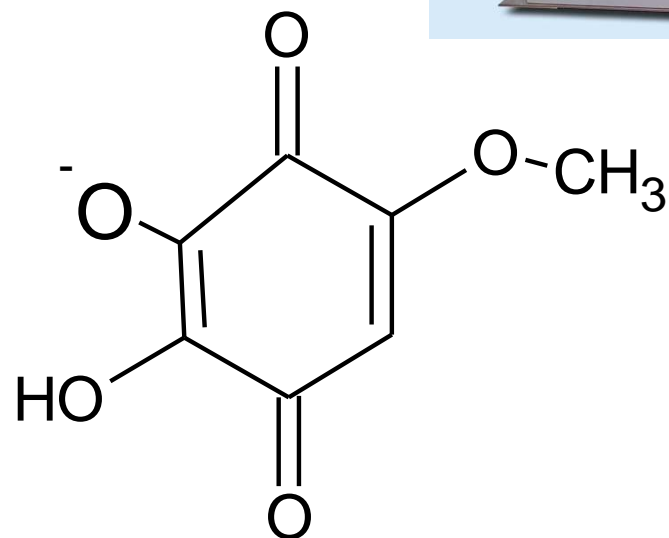


Question: what is the structure of the formed product in alkaline media, and how it is stabilized?

From *Electron Spin Resonance (ESR)* Experiments, we found that the structure of the product of the reaction of 2,6-dimethoxy Benzoquinone in Alkaline media is:



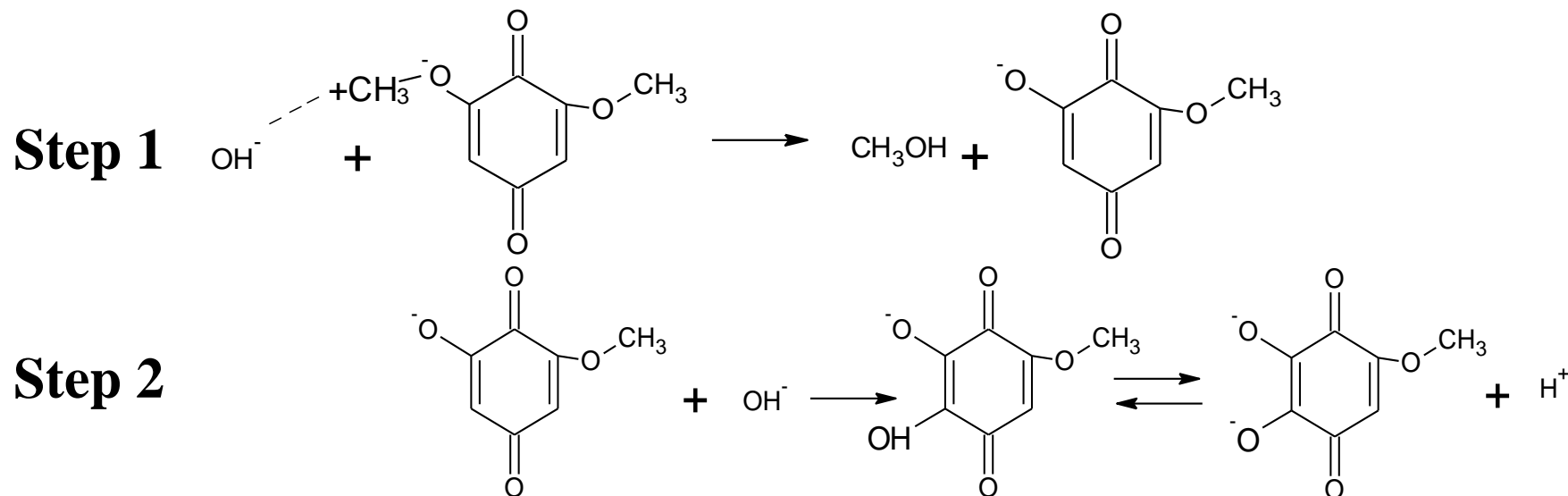
Original structure of native 2,6-diMetoxy Benzoquinone



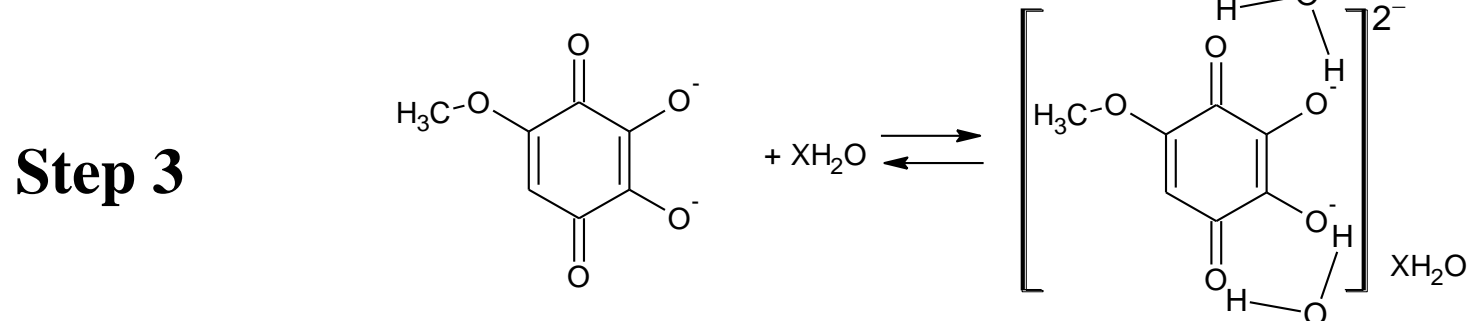
Structure of Product obtained in Strongly alkaline media

What is happening with metoxy BzQ in strongly alkaline media?

A. Scission of the methoxy etheric-bond and formation of a Di-anion in reaction with NaOH



B. Stabilization of the di-anion via the hydrogen bonds with the water molecules

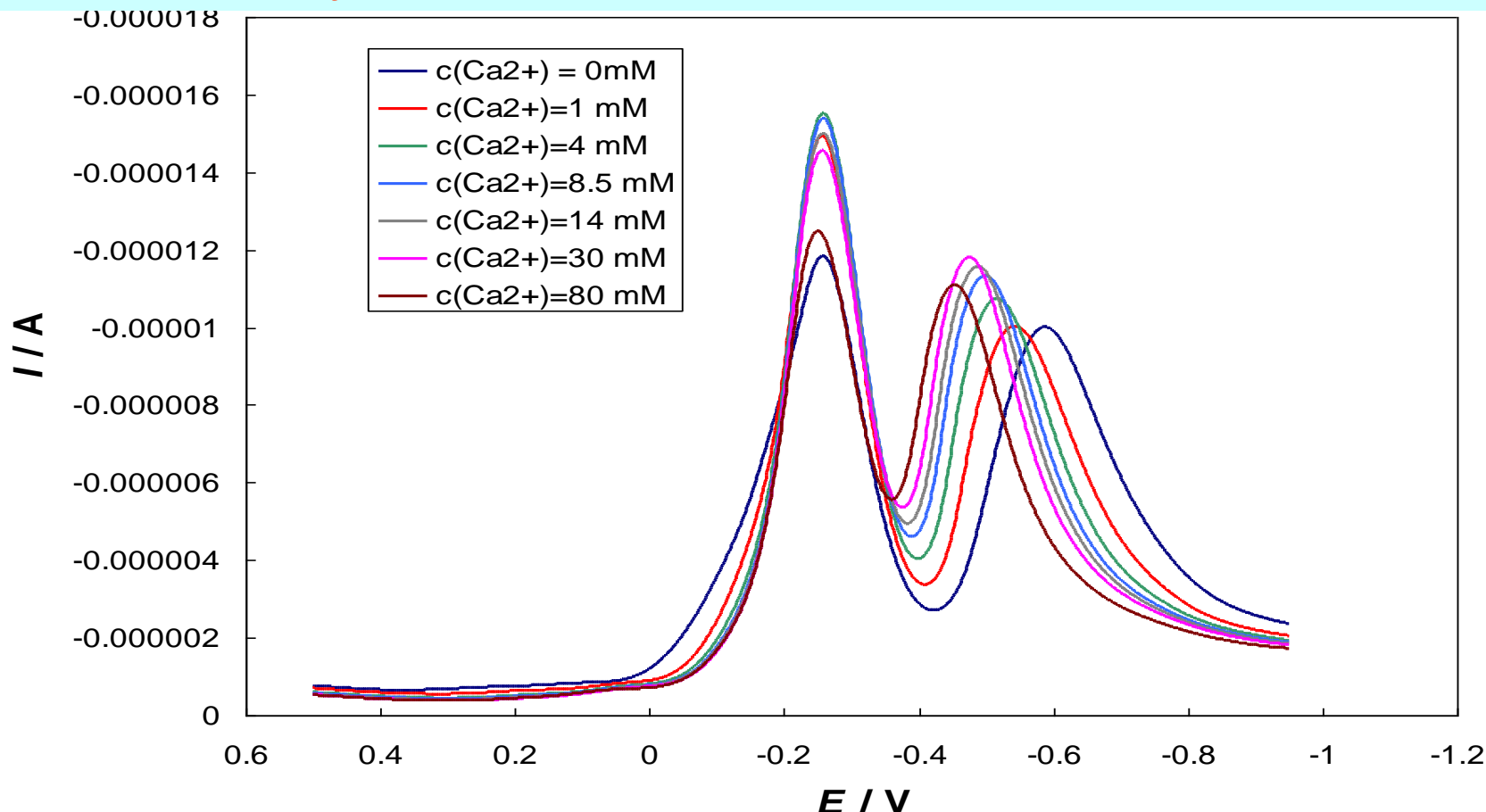


Manojkumar et al. *J. Chem Phys* 118 (2003) 8681

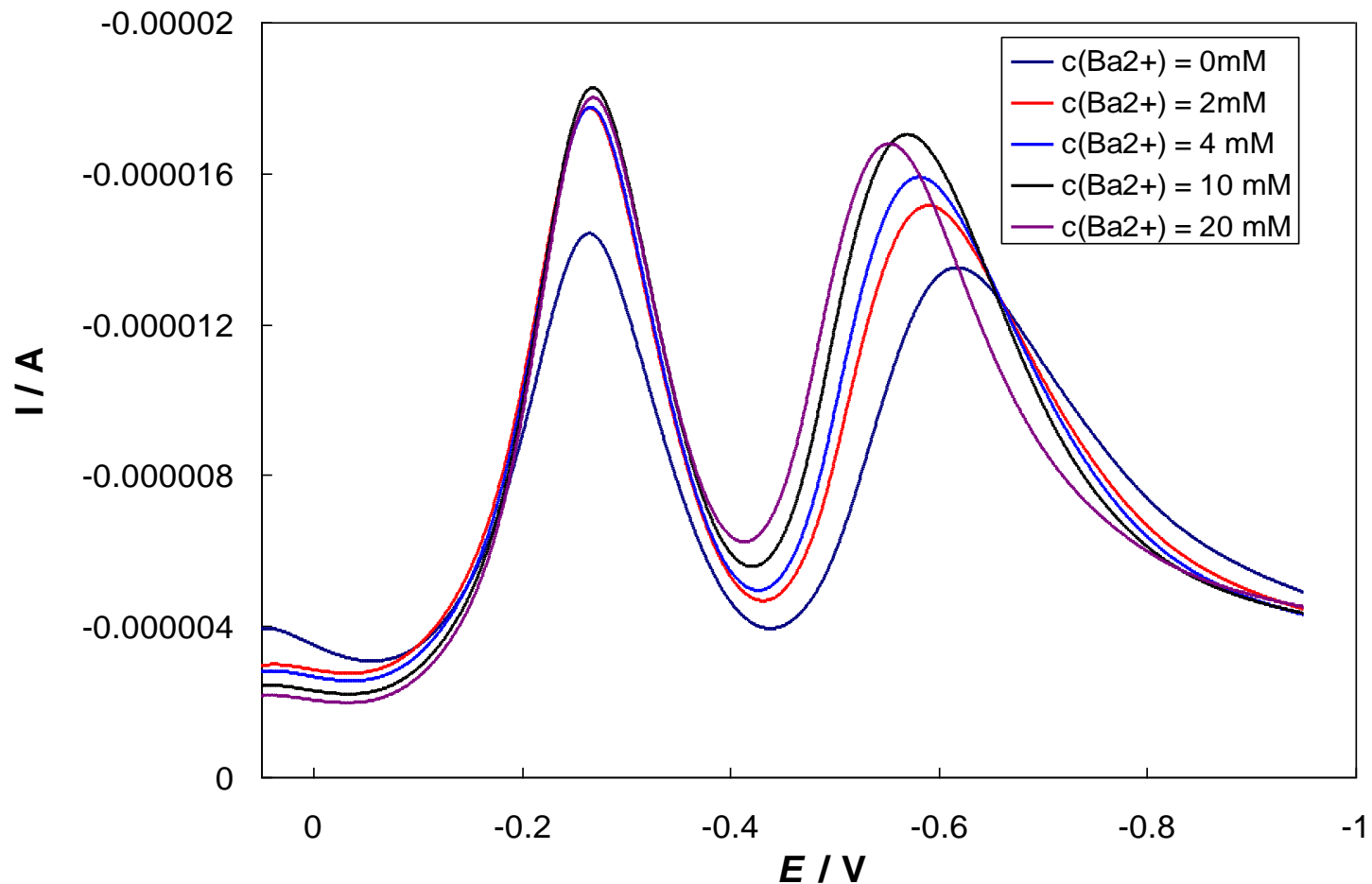
M. Quan et al. *J Amer Chem Soc.* 129 (2007) 12847

Ability of the new product to complexate Alkaline and Earth-alkaline ion

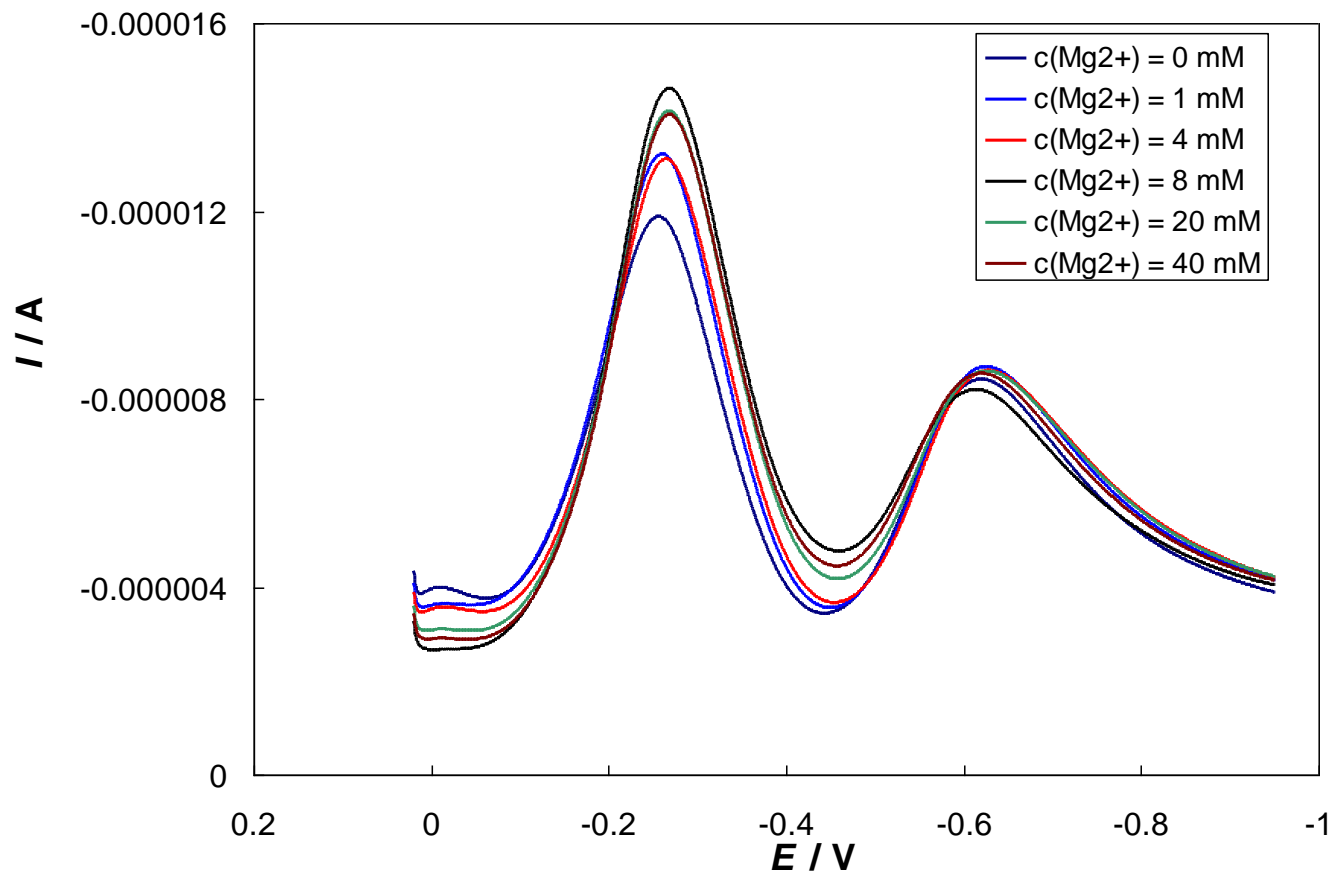
NO-sensitivity to H^+ and alkaline metal ions- Na^+ , K^+ , Cs^+ , Rb^+



SWV-2,6-dimethoxy-BzQ-*EFFECT* of Ca^{2+} . pH = 7.20
2-Methoxy-BzQ firstly dissolved in 1M NaOH for 30 min.
 $c(2,6-DiM-BzQ) = 1mM$, scan rate of 30 mV/s



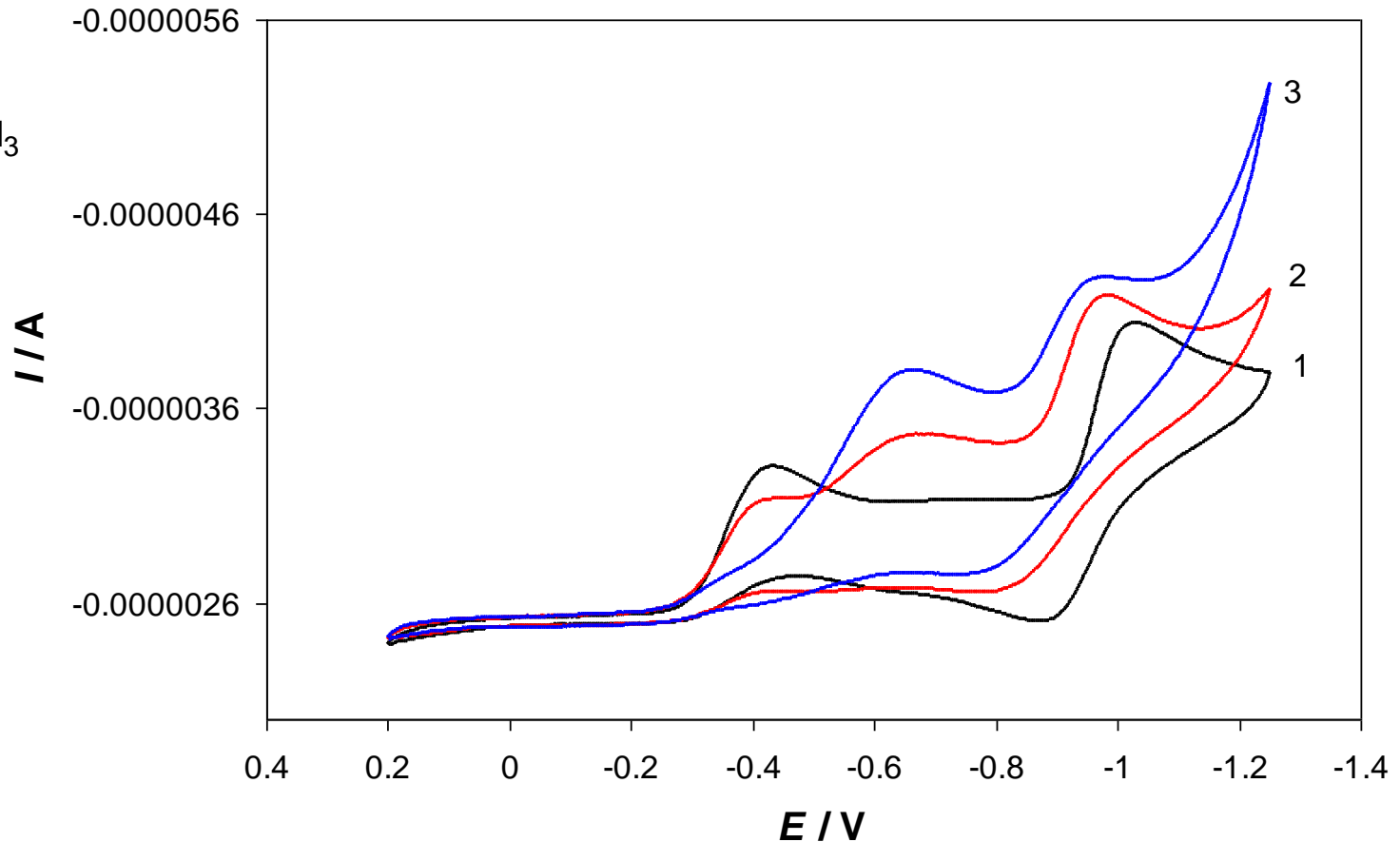
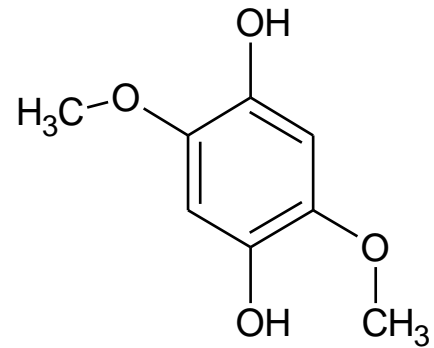
CV-2-Metoxy-BzQ-*EFFECT* of Ba^{2+} . pH = 7.20
 2,6-diMetoxy-BzQ firstly dissolved in 1M NaOH for 45 min.
 $c(2,6\text{-DiM-BzQ})=1\text{ mM}$, scan rate of 30 mV/s



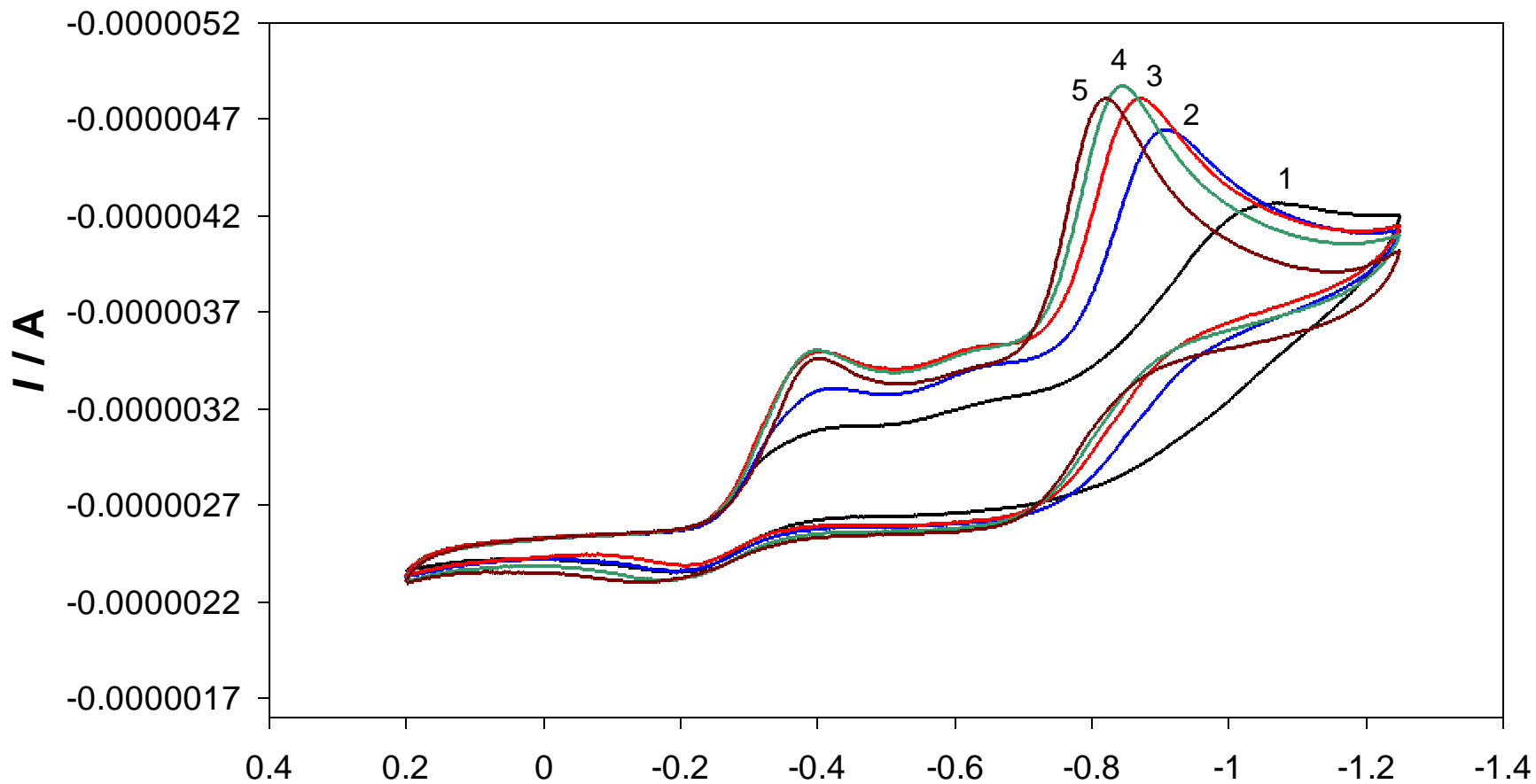
CV-2,6-diMethoxy-BzQ-*EFFECT* of Mg^{2+} . pH = 7.20
2,6-Methoxy-BzQ firstly dissolved in 1M NaOH for 30 min.
 $c(2,6\text{-DiM-BzQ})=1\text{mM}$, scan rate of 30 mV/s

C

2,5-diMetoxy-BzQ behaves almost identically as 2,6- diMetoxy-BzQ



CV-2,5-diMetoxy-BzQ-pH dependence from pH 12.7 to pH 8.0.
2,5-diMetoxy-BzQ firstly dissolved in 1M
NaOH for 1h. $c(2,6\text{-DiM-BzQ})=1\text{mM}$, scan rate of 30 mV/s



CV-2,5-diMethoxy-BzQ-*EFFECT* of Ca^{2+} .

pH = 7.20

2-Methoxy-BzQ firstly dissolved in 1M NaOH for 45 min.

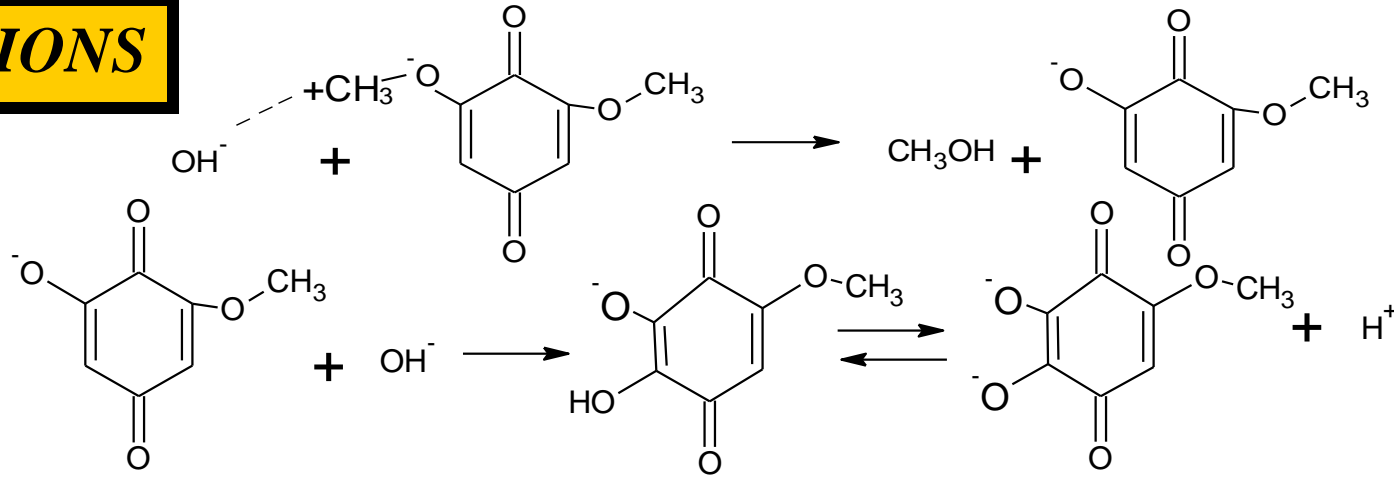
$c(2,5\text{-diMethoxy-BzQ})=1\text{mM}$, scan rate of 10 mV/s

$c(Ca^{2+})/\text{mM} = 0(1), 1(2) 3(3) 6(4) \text{ and } 10(5)$

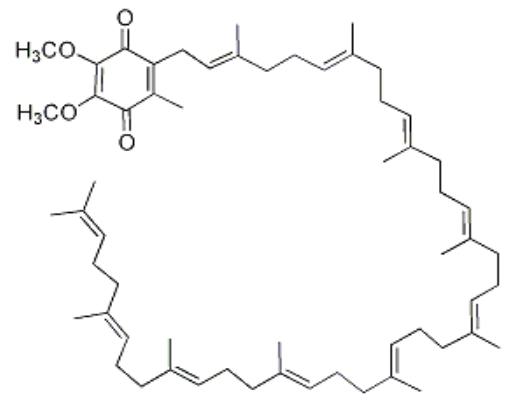
B

MAIN CONCLUSIONS

What we learned from this study?

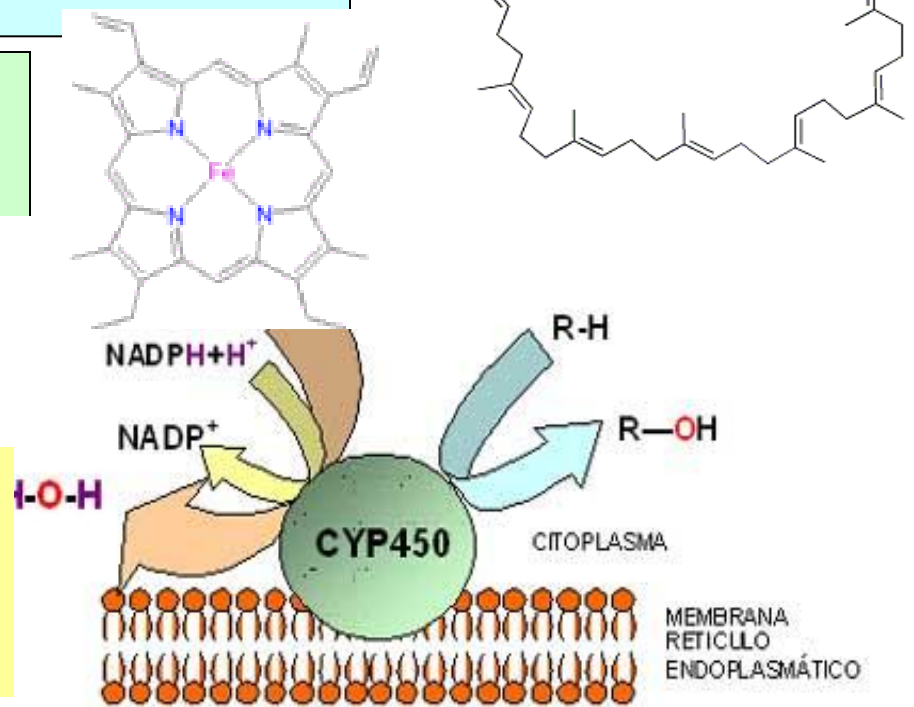


How do we find connection between the behaviour of metoxy-benzoquinones in alkaline media and CoQ10?



The same effect that NaOH can produce in the structure of Metoxy-BzQ, can be reproduced by the *P-450 enzymes*!!!

It is well-known that P-450 can contribute in scission of various etheric bonds



**The effect of P-450 and NaOH to CoQ1 and CoQ10,
and the features of the obtained methoxy dealkylated products...**

-
-
-

***Herr Bogeski* beim nexten Laborseminar**

**May be the Major conclusion of this lecture is:
DON'T EVER GIVE UP!!!**

Thanks to: Markus, Ivan, Reinhard, Richi, Martin Jung.



**The speaker's attendance
at this conference was sponsored
by the
Alexander von Humboldt Foundation.**

▶ <http://www.humboldt-foundation.de>