# In-situ Monitoring of Lipid Oxidation using Electrochemistry-Mass Spectrometry

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# 1. Objective

Using Electrochemistry/MS to mimic and to predict the oxidation of phospholipids

- Electrochemistry as biomimetic tool
- Mimicking ROS (Reactive Oxygen Species) reactions
- Mimicking enzymatic oxidation (lipoxygenase)

# 2. Introduction

Phosphatidylethanolamines (PEs) are a major class of phospholipids present in cellular membranes and lipoproteins of mammalian organisms. Oxidation of PEs fatty acyl chains generates several oxidized products, exerting a vast number of biological functions, not totally unveiled yet. *In vitro* biomimetic models have been used to identify oxidized PEs and to develop analytical strategies for their targeted *in vivo* detection. Most of the models are based on reactive oxygen species (ROS), but the oxidative metabolism of PE also relies on controlled reactions catalyzed by enzymes as lipoxygenase (LOX), which can be mimicked by electrochemical (EC) oxidation. In this study, three PE standards (POPE, PLPE, and PAPE) were oxidized by EC oxidation, using an EC flowthrough cell system and identified by ESI/MS and MS/MS.

## 3. Instrumentation



Figure 1: (**A**) Schematics infusion Electrochemistry/MS system: Infusion pump, ROXY Potentiostat (Antec), equipped with (**B**) μ-PrepCell2.0 and Boron Doped Diamond electrode (Magic Diamond) operated at 37°C

## 4. Experimental

#### Reagents/Chemicals

Phospholipid standards POPE, PLPE, PAPE were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA) and used without further purification.

#### Electrochemical Oxidation

The EC reactions were conducted in the ROXY EC system (Antec Scientific, Zoeterwoude, NL) consisting of the ROXY potentiostat a flow-through µ-PrepCell 2.0 equipped with a Magic Diamond<sup>™</sup> working electrode (BDD: Boron Doped Diamond) and a Pd/H<sub>2</sub> reference electrode. Phospholipid standards were diluted to 25 µM in 1:1 MeOH: 20 mM ammonium acetate (pH 7.4) and infused through the EC cell at rate of 20 µL/min. During the reaction, the temperature of the ROXY EC system was constantly maintained at 37°C. The ROXY EC system was directly coupled to the ESI-HCT Ion Trap mass spectrometer. A voltage of 3.00 V was selected for POPE and 2.50 V was selected for PLPE and PAPE.

#### Mass Spectrometry

Negative mode and positive mode ESI-MS and MS/MS experiments were done, using a spherical high capacity (HCT plus) ion trap mass spectrometer (Bruker, Millerica, MA, USA). For tandem MS (MS/MS) analysis collision-induced dissociation (CID) was applied.

## 5. Results

The analysis of the MS data in negative and positive mode, for all the electrochemically oxidized phosphatidylethanolamines (POPE, PLPE, PAPE) showed an oxidation pattern characterized by three major oxidative modifications: **Long chain** oxidation products, **short chain** oxidation products and **PE adducts** (only observed for POPE and PLPE).

### 5.1. Spectra of Electrochemically Oxidized Phospholipids



Figure 1: ESI-MS spectrum in negative ion mode showing the [M-H]<sup>-</sup> ions of POPE (**A**), PLPE (**B**) and PAPE (**C**) after the electrochemical oxidation

#### 5.2. Examples of Polyoxygenated PAPE Derivatives



Figure 2: Polyoxygenated PAPE derivatives generated in the ROXY EC system and analyzed by ESI-MS/MS: (**A**) hydroxyhydroperoxy-PAPE; (**B**) dihydroperoxy-PAPE; (**C**) ketohydroxy-hydroperoxy-PAPE



## 5.3 Long Chain Oxidation Products

Mass Increment	m/z [M+H] <sup>+</sup>					
	POPE	Relative abundance	PLPE	Relative abundance	PAPE	Relative abundance
	718.6	100.00	716.5	64.93	740.5	100.00
14 (+O-2 Da)	732.5	20.44	730.5	32.81	754.5	11.21
16 (+0)	734.6	59.44	732.5	36.20	756.5	13.11
28 (+2O-4 Da)	746.5	15.26	744.4	8.67	768.5	9.16
30 (+2O-2 Da)	748.5	51.12	746.5	36.39	770.5	12.77
32 (+20)	750.6	47.52	748.5	100.00	772.5	19.70
44 (+30-4 Da)	762.5	27.50				
46 (+3O-2 Da)	764.5	53.07	762.5	<mark>64.1</mark> 8	786.5	32.64
48 (+30)	766.6	49.01	764.5	73.97	788.5	39.35
60 (+40-4 Da)	778.5	18.93	776.5	9.88	800.5	12.05
62 (+40-2 Da)	780.6	28.14	778.5	38.65	802.5	40.95
64 (+40)	782.5	13.30	780.5	46.60	804.5	50.93
70 (+50-10 Da)	788.5	12.48				
72 (+50-8 Da)	790.6	9.91				
76 (+50-4 Da)	794.6	20.15	792.5	19.61	816.5	21.68
78 (+50-2 Da)	796.6	15.28	794.5	68.13	818.6	45.78
80 (+50)			796.5	<u>11.82</u>	820.5	35.64
92 (+6O-4 Da)					832.6	31.62
94 (+6O-2 Da)			810.5	10.72	834.6	47.04
96 (+60)					836.6	29.20
108 (+70-4 Da)			<mark>824.5</mark>	7.85	848.6	34.33
110 (+70-2 Da)					850.6	32.63
112 (+70)					852.6	17.39
122 (+8O-6 Da)					862.6	13.55
124 (+80-4 Da)					864.6	28.27
126 (+8O-2 Da)					866.6	22.48
128 (+80)					868.6	9.80
138 (+90-6 Da)					878.5	13.94
140 (+90-4 Da)					880.6	18.19
142 (+90-2 Da)					882.6	10.92
156 (+100-4 Da)					<mark>896.6</mark>	11.11

Table 1: Long chain oxidation products observed in the positive ESI-MS spectra from each oxidized PE (POPE, PLPE and PAPE) with the identification and the indication of the m/z values of the [M+H]<sup>+</sup> ions.

# Conclusions

The on-line coupling of Electrochemistry/MS allows for direct monitoring and identification of lipid oxidation products. In case of the 3 studied Phospholipids this approach gave information on the PE metabolism on two levels:

- Non-enzymatic, radical-driven PE oxidation
- Enzymatic, Lipoxygenase (LOX) catalyzed oxidation

## Reference

M. Rosario Domingues, paper submitted to JASMAS