

# Evaluating Synergisms and Antagonisms of Ascorbic Acid (AA) Redox Mechanism in Oxidative Potential (OP) Measurements

E. J. dos S. Souza, K. W. Fomba, M. van Pinxteren, N. Deabji and H. Herrmann.

**TROPOS**

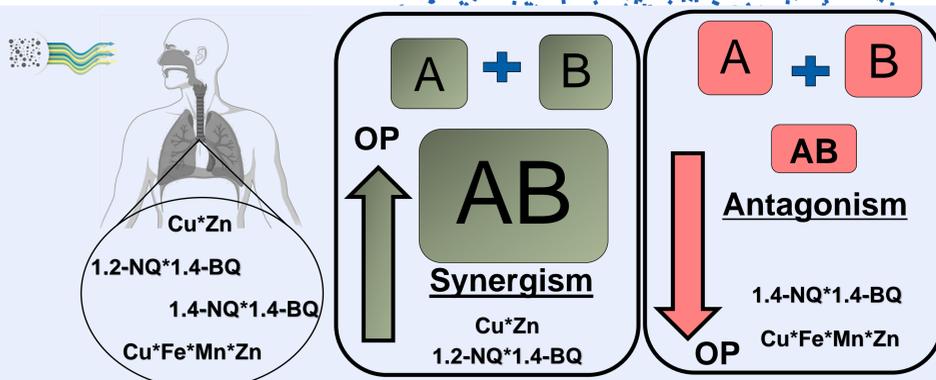
Leibniz Institute for Tropospheric Research

Leibniz Association

Atmospheric Chemistry Department (ACD), Leibniz Institute for Tropospheric Research, Leipzig, Germany

## Introduction

**Synergistic** and **antagonistic effects** have the interesting ability to explain over or underestimated values of specific outcomes that is not fully understood by applying a simple sum of individual values. The oxidation of **ascorbic acid (AA)** - one of the most abundant antioxidants present in lung fluids - can be investigated through the dehydroascorbic acid formation when redox-active species in PM are reduced (Visentin, 2016). This study presents a critical evaluation of chemical interactions of two **oxidative potential (OP)** assays of the AA mechanism: simple chemical assay (**OP<sup>AA</sup>**) and the related redox potential (RP) in a simulated epithelial lining fluid (**SELF** and hence **OP<sup>AA-SELF</sup>**). A statistical model evaluation was carried out using **factorial design (FD)** - 2<sup>4</sup> (three central points) with 19 experiments - and **interaction factor (IF)** analysis to understand divergences between available OP assays since chemical interactions have shown to present different redox mechanisms which may influence the toxicity prediction of aerosol samples.



## Methods

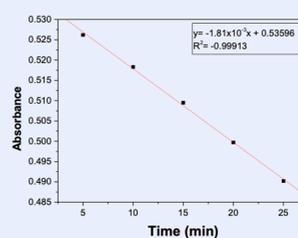
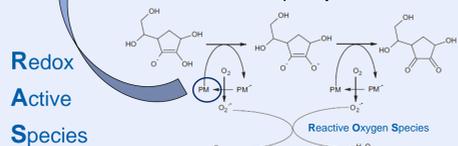
### Oxidative Potential Assay

- OP<sup>AA</sup> and OP<sup>AA-SELF</sup> assays were investigated under 25 min of incubation.
- 30 μL of 10 mM AA - which contains 300 nmol of AA - into PM extract solutions and standard solutions were investigated.
- The AA depletion was investigated directly in the spectrophotometric cuvette due to the AA ion formation at 265 nm under 37 °C (ε=14500M<sup>-1</sup>cm<sup>-1</sup> at pH 7.4).
- The SELF in this work was made of phosphate-buffer saline solution, which was composed of a mixture of glutathione (GSH), citric acid (CA), and uric acid (UA) (200 μM each).

### Factorial Design Investigation

Chemical species	Low (-) (nM)	Central point (0) (nM)	High (+) (nM)
1,2-naphthoquinone (1.2-NQ)	0	40	80
1,4-naphthoquinone (1.4-NQ)	0	40	80
9,10-phenanthroquinone (9.10-PQ)	0	40	80
1,4-benzoquinone (1.4-BQ)	0	40	80
Cu	0	50	100
Fe	0	500	1000
Mn	0	500	1000
Zn	0	500	1000

AA oxidation by redox active species in PM with subsequently ROS formation.



$$\sigma_{AA} = \sigma_{Abs} * (N / Abs)$$

Abs slope  
Abs- intercept  
N- Initial mol of AA  
V<sub>a</sub> Volume incubated with AA  
V<sub>e</sub> Extraction volume  
V Total air volume sampled  
A Area taken for AA assay  
A<sub>Total</sub> Total area of the sample  
M PM mass concentration (μg m<sup>-3</sup>)

### OP Calculation

$$\frac{\sigma_{Sample} - \sigma_{Blank}}{\frac{V_a}{V_e} \times V \times \frac{A}{A_{Total}}} \quad \frac{AA_V}{M}$$

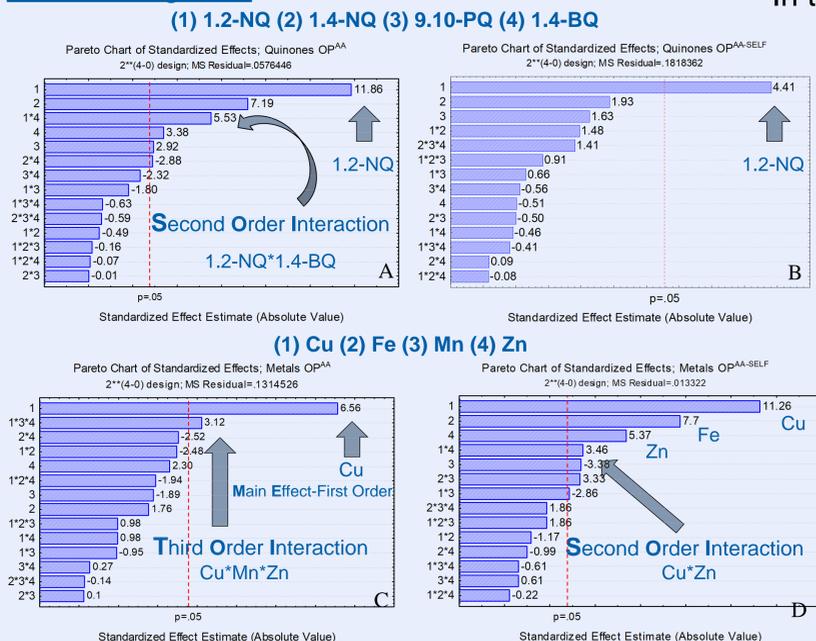
### IF Calculation

$$IF_{A,B,...,k} = \frac{OP_{AB...k}}{OP_A + OP_B + \dots + OP_k}$$

IF < 1 Antagonism  
IF > 1 Synergism  
IF = 1 ± 0.2 Additive

## Results

### Pareto Diagrams



In total, 55 different mixtures were investigated

38 Antagonisms

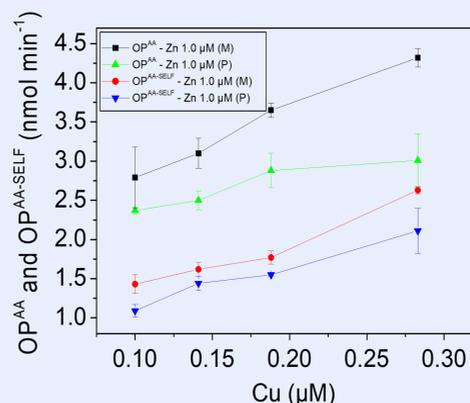
15 Synergisms

2 Additives

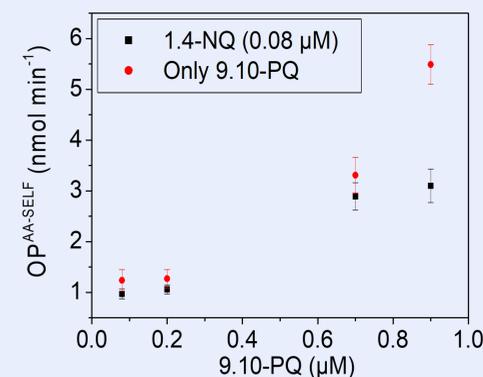
### IF for binary, ternary and quaternary mixtures

Chemical Interaction	Interaction Factor	
	OP <sup>AA</sup>	OP <sup>AA-SELF</sup>
Cu*Zn	1,18	1,31
Cu*Fe*Zn	0,46	0,81
1.2-NQ*1.4-BQ	2.64	1.33
1.4-NQ*9.10-PQ*1.4-BQ	0.59	0.33
Cu*9.10-PQ*Zn*1.4-NQ	-	0.36

- 1.2-NQ is a significant parameter for both assays (Fig. 1 A&B), but second-order interactions involving 1.2-NQ is only representative for OP<sup>AA</sup>
- 1.4-NQ, 9.10-PQ and 1.4-BQ are also significant parameters to modify OP<sup>AA</sup> but in a smaller order of magnitude
- Cu is a statistically significant factor in both assays achieving higher effects in OP<sup>AA-SELF</sup>, which showed a higher estimated factor compared to Fe.



Evaluating the OP<sup>AA-SELF</sup> assay under variation on 9.10-PQ concentration in the presence of 0.08 μM of 1.4-NQ



Measured and predicted OP values for the binary mixture of Cu and Zn in OP<sup>AA</sup> and OP<sup>AA-SELF</sup> assays.

## Conclusion

- AA assays present more antagonistic effects compared with synergistic.
- The redox system involving the depletion of AA by redox active species poorly depends on additive effects.
- Cu and Zn have demonstrated strong synergism for both OP assays.
- 1.4-NQ combined with 9.10-PQ cause antagonisms for binary, ternary and quaternary mixtures.

## References

Visentin et al. Env Pol 2020  
Pietrogrande et al. Atm Env 2019  
Bates et al. Env Sci Tec 2019