

Detection of electrochemically generated peroxide and superoxide by fluorescence microscopy

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Application Note

Introduction

Oxygen reduction and evolution reactions play a central role in numerous applications for the technical use of regenerative energy sources and many other electrochemical processes. Often the production of hydrogen peroxide and other reactive oxygen species is an unwanted parallel reaction. However, in-line monitoring of the appearance of traces of these by-products often is difficult. For this reason, specific fluorescence reporter dyes have been developed for the detection of active oxygen species.[1,2] Here we present the application of the dyes Amplex Ultra Red and 4-chloro-7-nitrobenz-2-oxa-1,3-diazole for the visualization of hydrogen peroxide and superoxide anion evolution at a polymer modified electrode.

Setup

All measurements were performed on a Leica DMIRE2 inverted microscope. Samples were excited with a tungsten lamp filtered with dichroic filter sets for 436 nm excitation/540 nm emission or 546 nm excitation/580 nm emission. Detection was achieved with a Neo DC152QC-FI sCMOS (scientific CMOS) camera from Andor Technology attached to the third optical port of the microscope. To cover the electrode diameter of 3 mm, a 5x (0.15) Leica Fluotar objective was used for all measurements. The electrochemical measurements were performed in a 10 mm diameter Teflon cell with an Ag/AgCl reference electrode and Pt wire counter electrode. The potential was applied with a PalmSens potentiostat in cyclic voltammetric mode covering a potential range of 0.4 to -1 V.

Detection of hydrogen peroxide

Generation of hydrogen peroxide was in-situ investigated by fluorescence microscopy in a time series of 100 frames using 546 nm as excitation wavelength to detect the fluorescent molecule at 590 nm. The working solution contained 0.1 mol L⁻¹ phosphate buffer (pH 7.0), 10 µM Amplex Ultra Red (dye), 10 U/mL horseradish peroxidase (1 unit U is defined as the amount of enzyme that will form 1.0 mg of purpurogalin from pyrogallol in 20 seconds). Time step is 1 s per frame.

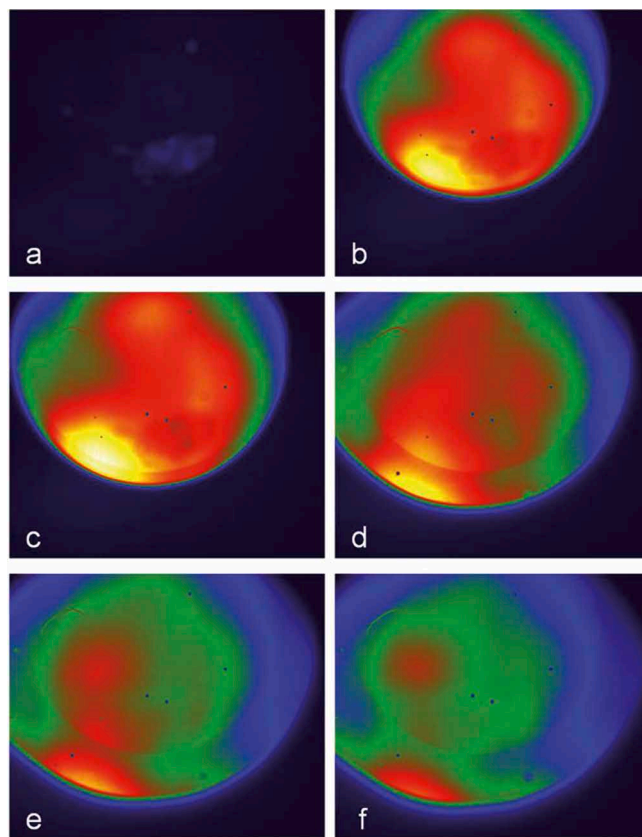


Fig 1: Time series of selected images for detection of generated hydrogen peroxide. Time step: 1 s per frame for 100 series. Sequence is from left to right and top to bottom.

Detection of superoxide anion

Generation of superoxide anions was also in-situ investigated by fluorescence microscopy in a time series using 436 nm as excitation wavelength to detect the fluorescent molecules at 550 nm. The working solution contained 0.1 mol L⁻¹ phosphate buffer (pH 7.0) and 100 µL of 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (10 mM in acetonitrile). Time step is 1 s per frame.

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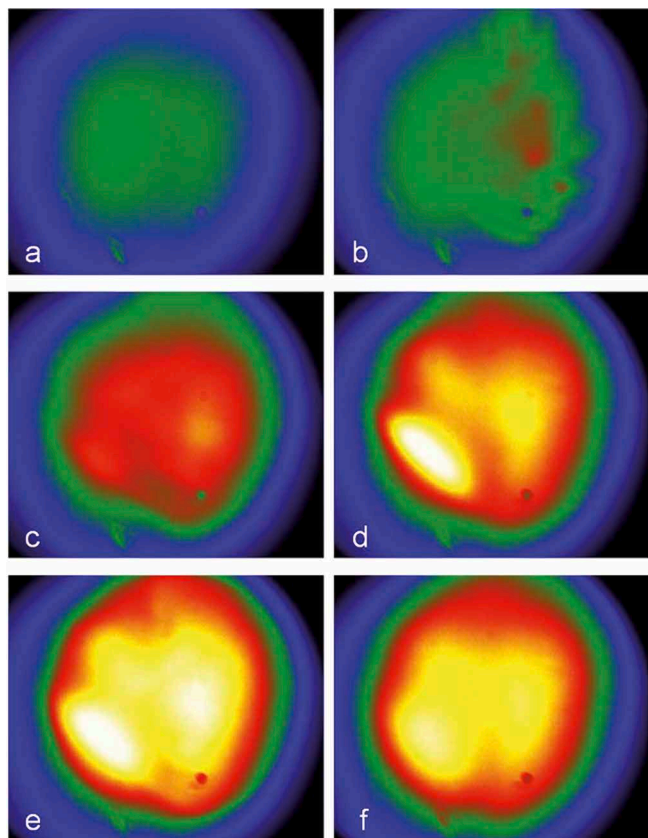


Fig 2: Time series of selected images for detection of generated superoxide anion. Time step: 1 s per frame for 100 series. Sequence is from left to right and top to bottom

Results

Figures 1 and 2 show the evolution of peroxide and superoxide generated during a CV cycle. For each series of 100 frames, representative frames for times before reaching the half wave potential, at half wave potential, at diffusion controlled state and after the return point are displayed. For hydrogen peroxide as well as for the superoxide anion, a non-homogeneous evolution of the active oxygen species is observed, showing a heterogeneous composition or thickness of the polymer layer on the electrode. Also, the formation of a diffusion layer around the electrode can be monitored in real time.

Conclusion

Fluorescent sensor dyes allow the effective and fast detection of trace compounds which are formed by unwanted parallel reactions. Especially the localization of active reaction zones on the electrode surface will provide important information on the surface reactions involved. If the detection of main compounds is in focus, the use of fluorescent reporter molecules may be obsolete as Raman microscopy will provide a sensitive and selective detection method as interesting alternative to fluorescence detection.

References

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2. R.O. Olojo, R.H.Xia, J.J. Abramson, *Anal.Biochem.* 339 (2005) 338-344.

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