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Synthesis and Cyclic Voltammetry of A2E

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Introduction and purpose

As aging occurs, great exposure to light leads to the build-up of fluorescent materials called lipofuscin in the retinal pigment epithelial cells (Parish, Hashimoto, Nikanishi, Dillon, & Sparrow, 1998). This accumulation of lipofuscin is correlated to the progression of age related macular degeneration and other retinal dystrophies (Dorey, Wu, Ebenstein, Garsd, & Weiter, 1989). No cure for macular degeneration has been found, so lipofuscin is of great interest. The lipofuscin has been associated with an increase in radiation intake and a loss of photoreceptors in the retina (Dorey et al., 1989; Taylor et al., 1990). The compound A2E is being studied in this project because it is frequently found in the lipofuscin (Zhou, Jang, Kim, & Sparrow, 2006). According to Sakai et al. (1996) and Parish et al. (1998), A2E is made up of two molecules of all-*trans*-retinal (vitamin A aldehyde) and a molecule of ethanolamine, which are both found in the visual cycle. The goal of this project is to further characterize A2E in the hope of better understanding retinal diseases. In this study, A2E was synthesized and cyclic voltammetry was used in an attempt to determine the reduction potential. This information may be helpful in clarifying the chemical properties of A2E.

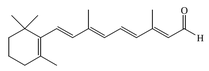

Figure 1. All-*trans*-retinal

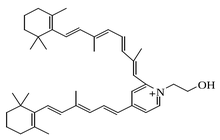

Figure 2. A2E
Sakai et al., 1996

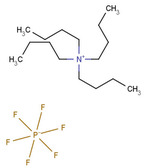

Figure 3. Tetrabutylammonium hexafluorophosphate

For further information

Please contact *wharper@olivet.edu*.

Materials and methods

- A2E was synthesized with a 2:1 molar ratio for all-*trans*-retinal:ethanolamine in the presence of acetic acid, with methanol as a solvent. The mixture was stirred in the dark for two days.
- A2E was purified through gravity chromatography on a silica gel column. The mixture was eluted with 5:95 CH_3OH:CH_2Cl_2 and 8:92:0.002 CH_3OH:CH_2Cl_2:trifluoroacetic acid (TFA).
- The sample taken from the gravity column was analyzed with a Hitachi HPLC system (Elite LaChrom, L-2130) to determine the presence of A2E. The mixture was eluted with a Nova-PAK C18 3.9X150mm column, using 75% methanol: 25% water: 0.1% TFA for 10 minutes; a gradient increased the solvent to 100% methanol between 10 and 25 minutes; 100% methanol was held until 35 minutes; and the original conditions were restored from 35 to 40 minutes. The sample was monitored at two wavelengths: 330nm and 430nm.
- The A2E sample was analyzed with a cyclic voltammeter (Epsilon from Bioanalytical Systems) for determination of the reduction potential of A2E. The sample was tested in methanol with 0.1 M tetrabutylammonium hexafluorophosphate (TBAHFP) used as a supporting electrolyte.

Current results

Figure 4 shows the results for the cyclic voltammetry analysis on an A2E sample in methanol. Both the blank and the A2E contained the TBAHFP in the test solution. A2E1, A2E2, and A2E3 are three different runs that were separated in time. Figure 5 displays an analysis with only the supporting electrolyte in methanol. This test was performed to investigate the behavior of the TBAHFP alone. This solution was analyzed twelve times with five minutes between each run. The graph contains six of these runs, each recorded ten minutes after the previous. When one looks at the two graphs, it appears as though there is a small reduction peak. There also seem to be two oxidation peaks in each graph. The second peak has a negative current and is much more clearly defined than the first. Both the A2E/TBAHFP and the TBAHFP solutions show this pattern in the cyclic voltammograms. Since this is such a unique shape, it appears that the TBAHFP may be responsible for the results. When A2E was run in methanol without the supporting electrolyte, no peaks were detected. This circumstance indicates that the A2E may not be responsible for the shape of the voltammogram. As the solutions equilibrated, the peaks became much larger and more defined. The longer the solutions stayed in the vial before testing, the more the results changed. This may mean that the TBAHFP reacts during the process, possibly with oxygen in the air. Therefore, perhaps only the first run is accurate.

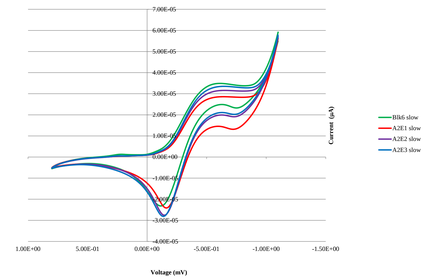

Figure 4. Cyclic voltammogram with blank (TBAHFP and methanol) and A2E (A2E, TBAHFP and methanol)

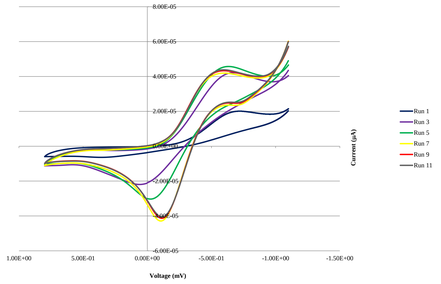

Figure 5. Cylcic voltammogram of supporting electrolyte in methanol. The six runs shown were separated by ten minutes.

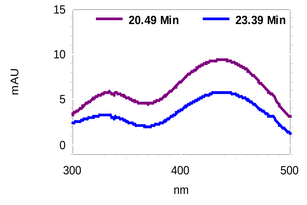

Figure 6. Spectrum of A2E in HPLC at times 20.49 and 23.39 minutes.

Future studies

- The questionable results obtained with tetrabutylammonium hexafluorophosphate indicate the need for further experiments. These should be carried out with another supporting electrolyte.
- The voltammograms changed over time. More experiments should be performed on the A2E in the presence of TBAHFP and TBAHFP alone. The analyses should be designed so that the solutions can be analyzed immediately upon solvation.

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Acknowledgments

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