DUAL ELECTRODE DETECTION IN LC-EC ANALYSIS OF SANGER DERIVATIZED AMINO ACIDS

Dong Wang, Paulina Guerrero, John Becker, Haoran Sun, and Miles Koppang*
Department of Chemistry
University of South Dakota
Vermillion, SD 57069
*Corresponding author email: mkoppang@usd.edu

ABSTRACT

In 1958, Frederick Sanger won the Nobel Prize in Chemistry for determining the structure of insulin using 2,4-dinitrofluorobenzene (DNFB) [Erk, A., J. Mottishaw, J. Kramer, H. Sun, and M. Koppang. 2015. Using ESP Maps to visualize chemical reactivity in Sanger’s reagent. Journal of Chemical Education 92(11):1846-1852. http://pubs.acs.org/doi/10.1021/ed5006344. Sanger, F. 1945. The free amino groups of insulin. Biochem Journal 39(5):507–515.] DNFB derivatized the N-terminus of peptides by adding a chromophoric “tag” which allowed for spectroscopic detection in liquid chromatography (LC) analysis of non-chromophoric peptides. We investigated the reductive electrochemistry of nitro-substituted aromatics to assess the feasibility of electrochemical detection in LC analysis of DNFB tagged amines, amino acids and peptides. Cyclic voltammetry (CV, glassy carbon working electrode and Ag/AgCl reference) of nitrobenzene (1) produced an irreversible reduction wave for a variety of aqueous buffers. Following reduction, a new reversible redox couple appears. We concluded that 1 was reduced to the n-phenylhydroxylamine (2) in a four electron, four proton process. The product 2 can be reversibly oxidized to nitrosobenzene (3) in a two electron, two proton process. We also ran CV on 2,4-dinitroaniline (4) in the different buffers and observed a similar irreversible reduction followed by formation of a new, reversible redox couple, similar to nitrobenzene reduction. Instead of one reduction peak, we observe two reduction peaks corresponding to reduction of each nitro group and formation of two adjacent new, reversible redox couples. We have begun investigations of dual electrode (in series) detection in LC-EC analysis of DNFB tagged analytes. Reductive detection at an upstream electrode is followed by oxidative detection at a downstream electrode. Reductive detection requires removal of oxygen from the mobile phase.