Photoluminescence Following Electron Transfer for Electrochemical Detection

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We have been applying electrochemical detection to bioanalytical problems for a considerable time. The strengths are in the selectivity. The weaknesses are generally in the area of sensitivity and reproducibility of the sensitivity. Any surface-based detection technique is susceptible to the problems incurred by the alterations in the surface that occur as the detection technique is used. We have attempted to find a method that will have the selectivity of electrochemical detection, but which will not suffer the disadvantages of a surface-based technique. Photoluminescence following electron transfer (PFET) is such a method.

In PFET, we use homogeneous oxidation of analytes, typically emanating from an HPLC column, with a reagent that is not fluorescent, but which becomes fluorescent upon reduction by analyte. There is a postcolumn reactor in which complexes like $M(bpy)_3^{2+}$, M=Ru, Os, are oxidized in a packed bed of PbO_2 to the nonluminescing +3 state. This stream is mixed with HPLC effluent. Oxidizable analytes produce the fluorescent $M(bpy)_3^{2+}$ which can be quantitated with laser induced fluorescence.

We have determined the proclivity of various biomolecules to react with $M(bpy))_3^{3+}$. This guides us in determining the appropriate metal to use for a particular application. Several catecholamines react with $Os(bpy))_3^{3+}$ as shown below. Our preliminary data with M=Ru indicate that the homogeneous reactions leading to fluorescence go to completion. This implies that the technique is like a fluorescent coulometry. Coulometry has the advantage of being an absolute measurement technique, requiring a minimum of calibration.

Although the Os complex is not as fluorescent, it is more selective. For example, the reaction of the Ru(III) complex with water and hydroxide ion limits its use to low pH. The Os complex on the other hand, is useful up to pH 9.

We have also optimized the mass transport using a novel approach so that the signal-producing reaction occurs in the minimal volume

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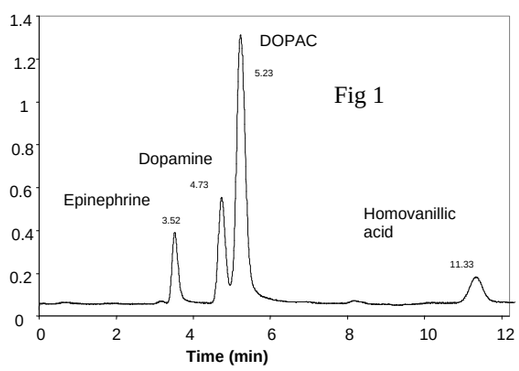

Fig 1