

MASTER'S THESIS

Investigation of the effects of the 1) UV absorbance of halide ions and 2) wall adsorption of marker ions for indirect detection in capillary electrophoresis

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Date of Award:
2001

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**Investigation of the Effects of the 1) UV Absorbance of
Halide Ions and 2) Wall Adsorption of Marker Ions for
Indirect Detection in Capillary Electrophoresis**

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**A thesis submitted in partial fulfillment of the requirements
for the degree of
Master of Philosophy**

**September 2001
Hong Kong Baptist University**

Abstract

The use of halide salts in combination with sulfonated calixarenes has been recently demonstrated as a novel and effective approach for the indirect detection of a number of significant analytes, including amino-acids, biogenic amines, inorganic cations and anions in capillary zone electrophoresis (CZE). In this method, a sulfonated calixarene, such as p-sulfonic calix[6]arene (SCX6), was employed as the selectivity modifier as well as the UV-absorbing background electrolyte, for the indirect detection of the sample ions, while the halide salt was supposed to serve as the 'ionic strength adjuster' in the run buffer. However, although halide ions are not commonly employed as UV-active chromophores in CZE for the indirect detection of various analytes, certain halide ions, especially Br⁻ and I⁻, are capable of absorbing appreciable amounts of UV photons near 200 nm. Therefore, in a run buffer system containing a calixarene compound and a halide salt, the overall background absorbance can arise from both of these background electrolytes.

In the present work, new insights into the influence of halide ions on the detector response patterns (both peak intensity and direction) were provided by examining the effects of the presence of p-sulphonic calix[6]arene (SCX6) and/or halide ions in the run buffer on the indirect detection of various model analytes. The results indicated that halide ions played a significant role in governing detection sensitivity, while the cations of the halide salts were

also found to influence the peak intensity, as well as the peak direction for the various analytes studied. These response patterns were briefly discussed in terms of mathematical treatments based on the Kohlrausch regulation function.

In the second part of this thesis, the effects of the wall adsorption of the visualization agent on baseline noise characteristics in indirect UV detection in capillary electrophoresis (CE) were investigated by employing long chain cationic surfactants (compared to bromide ion) as probe molecules. New insights into the possible role of adsorption/desorption processes occurring at the capillary surfaces on baseline fluctuations (a fluctuation of the visualization agent concentration within the capillary) were provided by monitoring the baseline characteristics, i.e., frequency and intensity, as a function of experimental parameters such as pH, applied voltage, ionic strength, acetonitrile, β -cyclodextrin and jacket inserted along the capillary. In contrast to characteristics predicted for non-detector noise that are thermal in origins, i.e., fluctuations arose from Joule heat production and non-uniform thermostating along the capillary, the magnitude of baseline noise levels were found to decrease (rather than increase as previously reported for thermally-induced noise), as the applied voltage (current) or conductivity (ionic strength) of the background electrolyte (BGE) was increased when using a long chain cationic surfactant (C_{16} : alkylbenzyltrimethyl ammonium halide with sixteen carbon atoms in the hydrophobic alkyl

chain) as the visualization agent.

Other interesting effects observed in the present study were: 1) when the pH of the BGE was increased from 3 to 8, the magnitude of baseline fluctuations appeared to go through a maximum near pH 5, and 2) when organic additives such as acetonitrile and β -cyclodextrin, were added into the BGE or when a jacket was inserted along a portion of the capillary, baseline disturbances were found to change considerably. All the above observations suggest that when using certain UV active chromophores such as C_{16} as the visualization agent, which has a strong tendency of adsorbing onto the capillary surfaces due to both hydrophobic as well as electrostatic interactions, it is likely that baseline noise characteristics are not solely governed by mechanism that is thermal in origin, but rather, the interplay of both thermal as well as adsorption events occurring within the capillary is likely responsible for the fluctuation of visualization agent concentration at the detector.

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