

Signal amplification using deuterated water-based buffers in capillary electrophoresis coupled to laser induced fluorescence detection

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Many fluorescent molecules can be quenched by OH oscillators present in water. The increase of fluorescence intensity or lifetime can be obtained by replacing the light water for the deuterium oxide. We can take advantage of this phenomena in some analytical techniques coupled to the fluorescence detection (e.g. capillary electrophoresis, high performance liquid chromatography, flow cytometry, fluorescence microscopy, etc.).

The capillary electrophoresis coupled with laser-induced fluorescence detection can be used for the determination of anthracyclines and related compounds which are widely used drugs, especially in cancer treatment. The steady-state and time-resolved fluorescence measurements of chosen anthracyclines in light and heavy water were carried out on the spectrofluorometer. Those experiments have proved that the emission signal and lifetimes of anthracyclines can be increased up to 4.5 times using deuterium water as a solvent. Therefore the deuterated water-based buffers can be used for the detection of anthracyclines by the capillary electrophoresis with LIF.

The CE-LIF separation of chosen molecules was successfully done in borate buffers prepared in light and heavy water. The fluorescence signal in D₂O based buffer increased up to 4.4 times for rhein. Around 2 times higher fluorescence emission signal was recorded also for other studied anthracyclines (doxorubicin, daunorubicin, epirubicin). The calculated limit of detection for rhein was $5.1 \cdot 10^{-8}$ mol.l⁻¹ and $5.0 \cdot 10^{-9}$ mol.l⁻¹ in light and heavy water buffer respectively. Over one order better limit of detection is not caused only by reducing of the dynamic quenching but also lower noise level achieved in the analysis in D₂O buffer. The main advantage of capillary electrophoresis is the high sensitivity combined with the lower consumption of sample and background electrolyte (about hundreds of microliters) which means that only small volume of heavy water is needed for the analysis.