Evanescent-Wave Cavity Enhanced Spectroscopy as a Tool in Label-Free Biosensing

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Abstract: A variety of evanescent-wave cavity-enhanced techniques is used in studying interfacial kinetics as well as the performance of anti-biofouling coatings, demonstrating the potential of these techniques in label-free biosensing.

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1. Introduction

Because of its simplicity and universality, UV absorbance detection is a generic detection method in liquid-phase analytical chemistry. However, the sensitivity of direct optical absorption detection is inferior to that of zero-background techniques such as fluorescence detection. The sensitivity of absorption detection in the visible range can be enhanced dramatically when the sample is placed in an optically stable cavity; the increased sensitivity stems from the increased optical path-length.

Conventionally, Cavity ring-down spectroscopy is based upon the abrupt termination of the optical excitation of a stable optical cavity followed by the measurement of the exponential decay of the light circulating in the cavity [1]. The exponential decay time decreases when an absorber is introduced into cavity. The sensitivity of CRDS stems from the increased optical path-length through the sample and the insensitivity to light source instabilities.

Incorporation of a fused silica substrate in the cavity allows for evanescent-wave (EW) sensing: only the highly localized evanescent wave of a total internal reflection event at the fused silica interface is used for measurements. By functionalizing the substrate surface, EW-CRDS has the potential to be used as a platform for label-free biosensing, providing an alternative for surface plasmon resonance detection.

2. Applications of cavity-enhanced techniques

Protein adsorption to silica surfaces is a notorious problem in analytical separations, and much effort has been and still is being dedicated to the development and characterization of anti-biofouling coatings [2]. Evanescent-wave cavity ring-down spectroscopy (EW-CRDS) and capillary electrophoresis (CE) were employed to investigate the capability of charged non-covalent coatings to minimize the adsorption of basic proteins. Conventionally, the performance of such coatings is quantified using separation parameters such as plate number, peak asymmetry and repeatability.

With EW-CRDS, the decrease of adsorption of cytochrome c to silica upon coating with a single and triple (sandwich) layers of polybrene and dextran sulfate could be directly quantified for the first time [3].
CE analyses of cyt c were performed with and without the respective coatings applied to the fused-silica capillary wall. Monitoring of the electroosmotic flow and protein peak areas indicated a strong reduction of irreversible protein adsorption by the positively charged coatings. Determination of the electrophoretic mobility and peak width of cyt c revealed reversible protein adsorption to the PB coating. The combination of results from EW-CRDS and CE provides highly useful information on the adsorptive characteristics of bare and coated silica surfaces toward basic proteins.

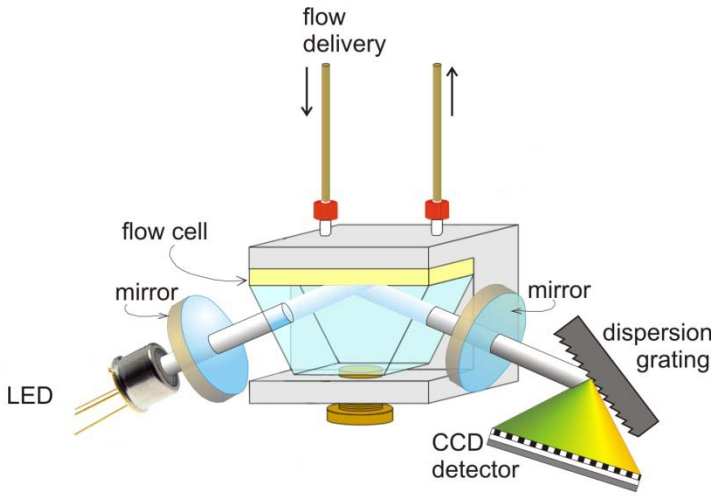

Fig. 1. Basic lay-out of an EW-CEAS system utilizing a LED as excitation source.

A novel broad-band mode of cavity-enhanced techniques is CEAS. A conventional absorbance spectrometer can be made into a CEAS setup simply by encompassing the sample in an optically stable cavity. A white light-emitting diode (LED) with emission between 420 and 700 nm and a supercontinuum (SC) source with emission between 450 and 2500 nm have been compared for use in evanescent wave broadband cavity-enhanced absorption spectroscopy (EW-BB-CEAS) [4]. The method is calibrated using a dye with known absorbance. While the LED is more economic as an excitation source, the SC source is superior both in terms of baseline noise (noise equivalent absorbances lower than 10^{-5} compared to 10^{-4} absorbance units) and accuracy of the measurement; these baseline noise levels are comparable to evanescent wave cavity ringdown spectroscopy (EW-CRDS) studies while the accessible spectral region of EW-BB-CEAS is much larger (420-750 nm in this study, compared to several tens of nanometres for EW-CRDS). The improvements afforded by the use of an SC source in combination with a high sensitivity detector are demonstrated in the broadband detection of electrogenerated Ir(IV) complexes in a thin-layer electrochemical cell arrangement. Excellent signal to noise is achieved with 10 ms signal accumulation times at a repetition rate of 600 Hz, easily fast enough to follow, in real time, solution kinetics and interfacial processes.

3. Conclusion and future perspectives

The applicability of EW-CRDS in studying surface adsorption processes has been demonstrated by directly quantifying for the first time the amount of reversibly and irreversibly adsorbed protein on anti-biofouling coatings frequently used in CE.

Since CEAS is compatible with broad-band lightsources such as LEDs, CEAS is a promising route to cost-effective and compact detectors of biomarkers. The performance of EW-CEAS using a super-continuum source and a LED as excitation source shows that the system utilizing an SC source gives superior detection performance but compromises the size and cost of the total system.

4. References

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