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TRENDS

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Microscale liquid phase separations

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Abbreviations *CEC* Capillary electrochromatography · *CE* capillary electrophoresis · *micro-LC* microscale liquid chromatography · *MEKC* micellar electrokinetic chromatography

Miniaturization and microfabrication of analytical instrumentation for chemical and biological analyses has been proceeding at a rapid pace for a few decades, and has gained momentum in the past decade with the introduction and application of microfabricated instruments. Motivating factors for performing microscale separations and analyses are reduced reagent, solvent and sample consumption, reduced cost, improved analysis speed, sensitivity and/or separation efficiency, and the ability to perform multiple, often parallel, analyses on a large number of samples. Examples of areas where this is of importance are genomics, proteomics, screening of combinatorial libraries, and remote sampling and analysis.

The recent trend toward miniaturization is perhaps most evident in the area of liquid phase analytical separations. Rapid development in the areas of capillary electrophoresis (CE) and microscale liquid chromatography (micro-LC) has led to their acceptance and routine use in the analytical laboratory over the past 15 years. Research and development continue with the introduction of new separation modes and materials, sensitive and selective detection methods, new applications, and further miniaturization and application of these techniques in microfabricated devices.

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Microfabricated instruments

The demonstration of CE separations in etched microchannels in a glass substrate in 1992 [1] has led to a very large number of studies concerning the development of microfabricated instruments (instruments and instrument components on microchips) employing electrokinetically-driven liquid-based separations. Microchips allow chemical and biological assays to be performed very quickly with reduced sample consumption and cost. A variety of separation modes have been demonstrated with these instruments, including free zone and gel electrophoresis, electrochromatography, and micellar electrokinetic chromatography (MEKC). Recent reports have demonstrated complete CE separations in milliseconds [2]. Two dimensional separations combining either MEKC [3] or electrochromatography [4] with free zone electrophoresis have also been reported.

There has been a great deal of interest of late in fabrication of microchips from plastics or polymeric materials [5]. These materials allow easier, more cost-effective, and more varied fabrication techniques to be utilized.

Detection technologies include off-chip UV or mass spectrometric (MS) detection and on-chip fluorescence and electrochemical detection. MS detection is an extremely important capability for microscale separation devices because of its sensitivity, selectivity and qualitative capabilities.

Sorbents and surface modification

The open channels produced in microchip devices may be used without further modification, or with surface modification to control electroosmotic flow in CE and MEKC. Surface modifications can also be made to facilitate sorption of analytes on the channel walls, such that open tubular capillary electrochromatography (CEC) separations can be achieved. The use of packed beds [6] or monoliths [7, 8] in microchannels or capillaries increases sample capac-

ity. These sorbents can be produced by a variety of methods, and can support a variety of different chromatographic modes of separation. The porous nature of both conventional and monolithic sorbents has led to the development of models with reasonable ability to describe and predict electroosmotic transport of eluents through pores [9, 10].

Novel materials have also been introduced and investigated for application as pseudo-stationary phases in MEKC. Ionic amphophilic polymers have generated considerable interest due to applicability in a variety of buffer matrices, unique chemical selectivity, and applicability with MS detection [11].

On-column preconcentration

Several on-column preconcentration or stacking methods have been introduced in the past decade to increase the detection sensitivity of CE 10–1000 fold [12]. Electrophoretic stacking mechanisms can efficiently stack ionic or ionizable analytes. Analytes can also be preconcentrated on a short section of chromatographic packing material at the head of the capillary column or by using membrane concentrators. Ionic and neutral analytes can be preconcentrated using a relatively new method in which the sample is swept or focused through interaction with a pseudostationary phase as it migrates through the sample zone. Concentration enhancement up to 5000-fold has been observed for non-ionic hydrophobic solutes [13], and enhancement of nearly a million-fold has been achieved through combination of this method and field amplified sample stacking [14]. Several hundred-fold concentration enhancement has also been reported using high conductivity sample buffers [15].

Enrichment methods have also been employed in CEC and capillary LC. El Rassi has used sequential frontal and elution steps in electrochromatography to provide for trace analysis [16]. Solid phase micro extraction has also been employed on-line with microscale separation methods. The higher loading capacity of capillary LC and CEC has in general allowed those techniques to avoid some of the detection sensitivity pitfalls addressed by preconcentration methods in CE. That said, the challenge of dealing with small sample volumes in a quantitatively reproducible manner still remains.

Applications

Microscale liquid phase separations methods continue to see moderate growth in applications. Not surprisingly, these methods are applied predominantly in cases where the improved separation efficiency, selectivity and speed are of particular importance, or where they can provide complementary information to more established techniques. Developments in CEC, capillary LC, CE and MEKC provide a range of selectivities and separation mechanisms that extend the reach of microscale separations to encompass a large pool of prospective analytes and matrices. However, there remains concern about whether techniques that rely on electroosmotic transport can generally meet the reproducibility requirements of regulatory agencies. The complementary nature of these techniques – along with their similar requirements of scale, geometry, and detection – will likely result in their use in various combinations on microchips and in more conventional formats to solve increasingly complex separations problems.

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