Microcolumn separation techniques developing in Japan

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Chromatography was initiated and named by a Russian botanist, M.S. Tswett in the beginning of this century [1,2]. Since then a number of significant innovations and inventions have contributed to the advancement of chromatography, and it is still in progress now.

Miniaturization of separation columns has been one of the most epoch-making topics in chromatography. The term “miniaturization” in science and technology generally imagines high efficiency, high speed, and integration as in the case of semi-conductors. Miniaturization of separation columns in chromatography also leads to high efficiency and high speed.

There have been several remarkable inventions to be noted in the history of microcolumn separation. Golay columns in gas chromatography (GC) [3,4], fused-silica capillary tubing [5], capillary electrophoresis (CE) [6] and electrokinetic chromatography (EKC) [7] are outstanding breakthroughs in microcolumn separation.

In August, 1982 Japan-U.S. joint seminar was held in Honolulu, where thirty-two researchers from Japan and U.S. together with four participants from other countries joined and discussed on microcolumn separations and ancillary techniques [8]. The seminar was organized by Ishii, Nagoya University, Nagoya, Japan and Novotny, Indiana University, Indiana, U.S. The main topic of this seminar was the miniaturization of chromatographic techniques.

In May of this year the 20th International Symposium on Capillary Chromatography and Capillary Electrophoresis was held in Riva del Garda, Italy. This symposium originally started as the International Symposium on Capillary Gas Chromatography in 1975 at Hindelang, Germany. After Ishii, Nagoya University, Japan, presented the work on capillary liquid chromatography (LC), the symposium name was changed to International Symposium on Capillary Chromatography since the fourth symposium. At the recent symposia, topics on CE and its related techniques are also included. In the meantime, this series of symposium was held twice in Japan, at Gifu in 1986 and at Kobe in 1990. In November, 1999, the 22nd International Symposium on Capillary Chromatography will be held at Gifu, organized by Jinno, Toyohashi University of Technology, Toyohashi, Japan.

As seen from the above stories, it is understandable that Japan is one of the leading countries in the field of micro-column separation. In this dossier recent topics on capillary GC, supercritical fluid chromatography (SFC), micro-column LC, CE, EKC, capillary electrochromatography (CEC) and optical chromatography are collected. Twelve articles are contributed by Japanese outstanding well-known analytical scientists.

Fused-silica is now the most frequently used as the material for open-tubular capillary columns in GC owing to its inertness and flexibility. Watanabe et al., Frontier Laboratories, have developed metallic capillary columns having slanted multi-layers, which improved temperature durability, build-ups and sample loading capacity, compared to fused silica capillary columns.

High-density gas such as supercritical carbon dioxide is used as the mobile phase in SFC. Since supercritical fluids possess solubility properties like liquid and faster diffusivity than liquid, SFC has a potential to analyze non-volatile compounds that cannot be subjected to GC separation in a shorter time than LC. Hishimoto and Hirata, Toyohashi University of Technology, have developed a flow switching interface for two-dimensional SFC, which allowed the separation of complex mixtures of ethoxylate oligomers and fatty acid cholesteryl esters.

Miniaturization of separation columns in LC generates several advantages over conventional LC. Low consumption of mobile and stationary phases allows the use of expensive, exotic phases; decreased flow rate facilitates the direct coupling to a mass spectrometer as a detector; improved mass sensitivity is of benefit to the analysis of biological samples; and decreased heat capacity of the separation column facilitates the temperature control. The concentration sensitivity of microcolumn LC can also be improved by on-line enrichment of analytes onto a pre-column or a separation column as well as by solid-phase extraction.

Jinno et al., Toyohashi University of Technology, describe microcolumn LC coupled with solid phase micro extraction for the analysis of benzodiazepines in human urine. The hyphenated technique is realized for the toxicological and forensic drug analysis.

Enami and Nagae, Nomura Chemical Co., describe UV absorption detection using a packed flow cell in microcolumn LC and find that the sensitivity of analytes eluting late are improved by the detection in the presence of the stationary phase.

Shirota et al., Shiseido Research Center, apply semi-microcolumn LC to the forensic analysis and find that pre-concentration using a switching valve provides a high concentration sensitivity which seems to be the most suitable for the quantitation of low-concentration analytes.
CE has rapidly been advancing since Jorgenson’s work triggered it in 1981 [6] because of its high resolution and high speed of analysis. The high efficiency of CE attributes to the fact that electrically-driven flows, e.g., electrophoresis and electroosmosis flows, are not laminar as in chromatography, but are flat. In addition to the fact that CE achieves a better separation efficiency, CE can be applied to the study of interaction between chemical species as well as to the kinetic study.

Honda et al., Kinki University, study on carbohydrate-protein interaction by CE and find that the association constant of a protein to an acidic carbohydrate can be conveniently estimated from the migration times.

Shibukawa et al. couple CE with frontal analysis (FA) for the study of drug-protein binding. CE/FA allows a simple and quantitative assay of enantioselectivity of the binding using a chiral selector.

Odake et al., The University of Tokyo, describe ultrasensitive on-column detection using a laser-induced capillary vibration method for CE and apply their techniques to the biological materials such as DNA fragments.

Basically, CE cannot separate neutral components. Terabe et al. [7] used a buffer solution containing micelles in the electrophoretic solution. The micelles work as the stationary phase and neutral components can also be separated depending on the difference in the distribution coefficients. The method is not electrophoresis, but chromatography, called as EKC. EKC can achieve the separation of ionic compounds as well as neutral components. When the pseudostationary phase is micelle, the method is called as micellar EKC (MEKC).

Otsuka and Terabe describe about recent developments in MEKC-MS. They investigate how to couple MEKC with MS from the viewpoint of the micelle type and the ionization mode of MS.

In CEC electroosmosis can be used as the driving force of the mobile phase instead of the pump as in LC. It is considered that the flow pattern driven by electroosmosis is still flat even if packed columns are used. This means that the contribution of the mass transfer resistance in the mobile phase to the plate height can be minimized in CEC, leading to the achievement of higher efficiency than LC.

Tsuda describes his recent topics on capillary electrochromatography, electrically enhanced concentration, instrumentation of a chromatographic system for space station and dynamic observation of human perspiration.

Fujimoto, Hamamatsu University School of Medicine, has developed fritless packed columns for use in CEC and shows several approaches which enable more reliable production of CEC columns.

Besides chromatography and electrophoresis, there has been reported a novel separation method using capillary tubing. Imasaka et al. have developed optical chromatography for the separation of particles [9]. The particle is trapped at the position where the scattering force is identical to the force induced by the medium flow. Optical chromatography is not really chromatography because there is no stationary phase.

Microcolumn separation methods have a number of advantages over conventional separation methods as viewed in this dossier. Micropacked separation, arrayed capillary separation and separation on a microtip will be next generation of microscale separation methods. Finally, it should be remarked that owing to the limited pages the issue could not include other interesting researches on capillary separation progressing in Japan.

References