Focusing Review

Development of Capillary Liquid Chromatography

Toyohide Takeuchi

Department of Chemistry, Faculty of Engineering, Gifu University, 1−1 Yanagido, Gifu 501−1193, Japan

Received for review November 22, 2004. Accepted January 18, 2005.

Keywords: liquid chromatography, capillary columns, instrumentation

1. Introduction

Ishii's group at Nagoya University started microcolumn liquid chromatography (μLC) more than 30 years ago [1]. His ideas have been realized in user−friendly μLC systems which are now commercially available from a number of manufacturers. In the meantime, injection valves with low dead volume, pumping systems working at low flow rates, high−resolution columns, and novel detection systems have been developed by manufacturers and research institutes. The present review will focus on methods and ideas for improving μLC systems proposed in the author's laboratory.

2. Development of related techniques

Table 1 shows development history of capillary−based separation methods and their related techniques [1−9]. In the early stage of the development of μLC, the researchers competed for achieving higher resolution. However, the pressure drop across the separation columns was a restring parameter as long as densely packed columns were employed. Although open−tubular capillary columns [2] and packed microcapillary columns [3] were then appeared and expected to provide higher resolution than densely packed columns owing to their higher permeability, these separation methods still have not been popularized, yet. One of the plausible reasons lies in the development of electrically−driven separation methods such as capillary zone electrophoresis [5], electrokinetic chromatography [6] and capillary electrochromatography, which have a potential to produce much higher theoretical plates than pressure−driven separation methods. Monolithic silica capillary columns, which have intermediate permeability between open−tubular columns and densely packed columns, are expected to produce higher resolution in the LC mode [9]. It should be noted that all these capillary−based separation methods have expanded owing to the development of fused silica capillaries [4].

Table 1. Development history of capillary−based separation methods and their related techniques

Year	Developed capillary separation methods and their related technique	References
1974	μLC	1
1978	Open−tubular capillary LC	2
1978	Packed microcapillary LC	3
1979	Fused−silica capillary	4
1981	Capillary zone electrophoresis	5
1985	Electrokinetic chromatography	6
1987	Capillary electrochromatography	7,8
1998	Monolithic silica capillary columns	9

3. Features of capillary LC

LC can be classified based on the column diameter. Columns with the diameter smaller than 0.075 mm are used in nano−LC and its flow rate is less than 1 μL/min, whereas the column diameter in μLC is 0.2−0.8 mm and its flow rate is 1−20 μL/min. In this article capillary LC is defined as the one involving nano−LC and μLC.

The features of capillary LC are attributed to the use of

Table 2. Classification of LC

Purpose	Classification	ID mm
Analytical	Nano−LC	～0.075
	μLC	0.2~0.8
	Semi−μLC	1.0~2.1
	Conventional LC	4.0~6.0
Preparative	Preparative LC	10~

Correspondence:
E−mail: takeuchi@apchem.gifu−u.ac.jp

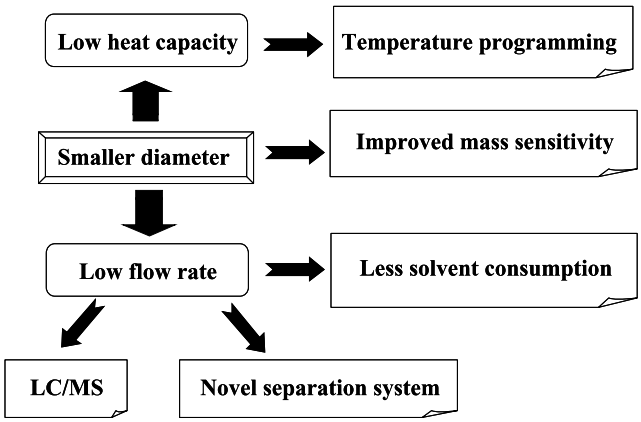

Fig. 1. Features of capillary LC

smaller–diameter columns and lower eluent flow rate, as shown in Fig. 1 [10]. The lower flow rate saves solvents, reagents and packing materials, compared with conventional LC using 4–6 mm ID columns. This situation is environmentally friendly. Improved mass sensitivity can be expected owing to small–volume detection in capillary LC. This is especially of benefit when the sample amounts available are restricted as in the case of biological samples. Low flow rate allows us to use exotic mobile phases or mobile phase additives, leading to achievement of novel separation selectivity or detection systems. Low heat capacity of capillary columns facilitates the control of column temperature. This feature allows us to carry out temperature programming more easily not only in capillary LC but also in supercritical fluid chromatography and gas chromatography (GC). The most significant feature of capillary columns lies in direct coupling of LC and mass spectrometry (MS), which is an ultimate detector for chromatography.

4. Novel detection systems based on capillary columns

On–column fluorimetric detection

On–column detection is sometimes indispensable for narrow–bore columns in order to avoid additional extra–column band broadening. This is not convenient to handle, and it leads to deterioration of the sensitivity. However, in some cases the sensitivity can be improved by detecting analytes in the presence of the stationary phase. It can be expected that the signal intensity does not depend on the retention time in on–column detection because both the analytes existing in the stationary and mobile phase are subjected to the detection in on–column detection. Fig. 2 compares the chromatograms obtained in the absence or presence of the stationary phase.

As for fluorimetric detection of dansyl amino acids, the signal was increased with increasing retention time, owing to the fact that the fluorescence was enhanced by the hydrophobic stationary phase [11]. The on–column fluorimetric detection can improve the detection limits of the analyte eluting late.

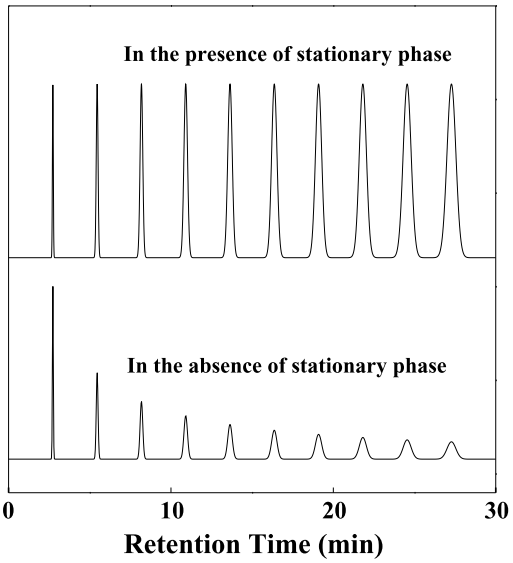

Fig. 2. Comparison of chromatograms obtained in the absence or presence of the stationary phase.

Indirect photometric detection

When appropriate detection methods are not available, indirect detection can be a candidate in ion chromatography, reversed–phase LC and ion–pair chromatography. Indirect detection is classified according to the type of interaction between the analytes and the visualization agents. Analytes are usually visualized in indirect detection via ion exchange or an interaction with a mobile–phase additive in the column. The following equation was derived for the indirect detection of non–electrolytes in reversed–phase LC [12]. The peak area (S_p) of the analyte can be correlated with operating conditions and the parameters.

$$S_p \propto C_{adM} W_{an0} f k_{ad} (k_{an} + 1)/(k_{an} - k_{ad}) \quad (1)$$

where C_{adM} is the concentration of the additive in the eluent, W_{an0} is the amounts of the analyte injected, *f is the coefficient accounting for the signal direction and intensity,* k_{ad} and k_{an} are the retention factor of the additive and the analyte, respectively. Eq. (1) indicates that the analyte signal is higher when the analyte elutes close to the additive system peak and that the signal direction changes between before and after the system peak.

Direct coupling with MS

Low flow rate is advantageous for direct coupling LC with MS. We have developed an interface for capillary LC and fast atom bombardment (FAB) MS [13]. The interface was composed of capillary tubing, the top of which was attached with a metal frit. The eluent containing a matrix was eluted on the surface of the frit, where xenon beam was irradiated and ionized. The stainless steel frit (1/16") employed for this purpose is illustrated in Fig. 3. The Frit FAB LC–MS interface is now commercially available.

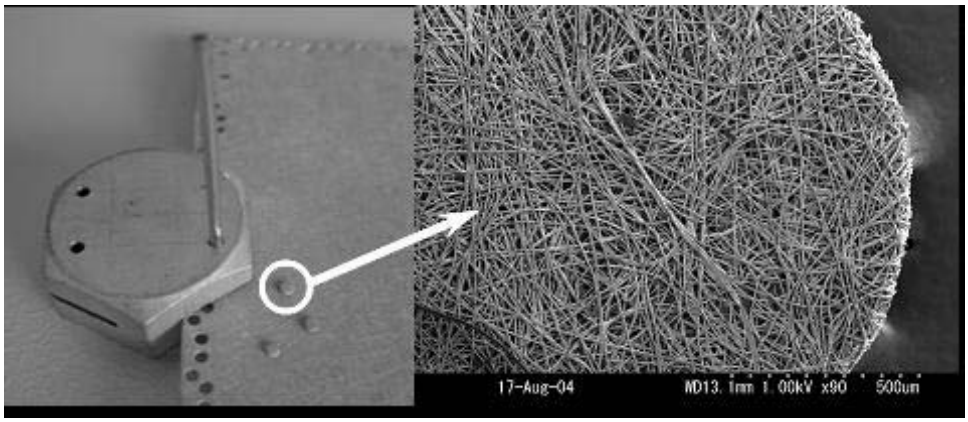

Fig. 3. Stainless steel frit for Frit FAB LC–MS

5. Use of accessories for capillary columns

Unions, 3–way connectors and 6–way switching valves for capillary tubing or columns are now commercially available. These accessories possess low dead volumes as low as a few tens of nL, and they therefore allow us to conduct pre–column enrichment [14], recycle separation [15], split and bypass flow system [16] and post–column reaction [17] with tolerable band broadening.

An on–line sample enrichment system was designed using monolithic pre–columns in capillary LC [14]. The monolithic ODS capillary columns were prepared *via in–situ* sol–gel processes. The limits of detection were improved by more than 2000–fold and the system was applied to the determination of phthalates contained in laboratory distilled water and tap water samples.

The pre–column enrichment system was applied to the determination of ethanol in breath [18]. Fig. 4 demonstrates the detection of ethanol in breath of a volunteer who drank 350 mL beer. The breath sample was taken right after the drinking. The concentration of ethanol in the breath was determined to be 0.29 mg L^{-1}. The time course of the breath sample could be monitored. As a result, the concentrations in the breath of the person in 5, 10, 30 and 60 min after drinking 350 mL beer were 0.044, 0.021, 0.015 and 0.007 mg L^{-1}, respectively.

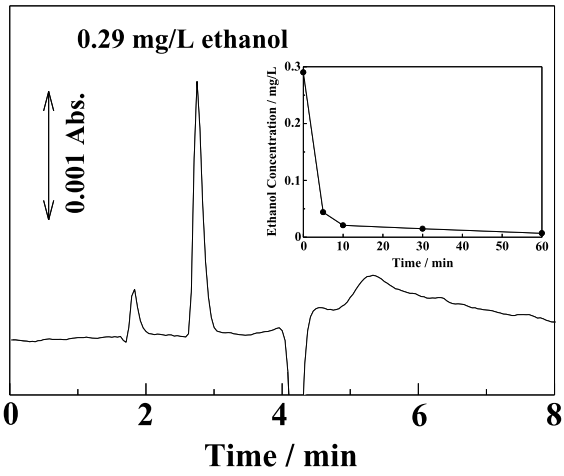

Fig. 4. Determination of ethanol in breath.
Column: Develosil C 30–UG–5, 100 × 0.32 mm I.D. Eluent: aqueous solution of 0.5% propionic acid. Flow rate: 4.2 μL min^{-1}. Enrichment column: quartz wool, 1.7 × 0.25 mm ID. Sample: Sample: 2 mL of breath of a person who drank 350 mL beer. Wavelength of UV detection: 210 nm.

An alternate–pumping recycle system was developed for capillary LC with an aid of a commercially available low dead–volume 6–way switching valve [15]. The recycle system utilized two separation monolithic columns, and the dead volume of the recycling lines was kept to a minimum by avoiding passing the sample through the pump chamber and sample injector. It was observed that the theoretical plate number (N) increased linearly with increasing number of cycle, and the N per unit time increased with increasing inlet pressure.

Split flow and bypass flow systems were assembled using Nano Y Connectors with low dead volume commercially available for capillary LC [16]. The split ratio could be controlled by changing the dimension of restriction tubing and applied back pressure to the restriction tubing. The bypass flow system uses two Nano Y Connectors, where the eluent split at the first Nano Y Connector, which is located in the inlet of the separation column, is merged again into the effluent from the column at the second Nano Y Connector. The bypass flow system could avoid on–column detection and allowed us to use flow cells, leading to improved sensitivity.

6. Future prospects of capillary LC

Development of capillary columns with much higher efficiency than conventional LC columns will shift users from conventional to capillary LC, as seen in the development of capillary GC [10]. Columns with higher column efficiency must have higher permeability in comparison with common densely packed columns. Open tubular capillary columns and monolithic columns are expected to achieve higher column efficiency because of their higher permeability. At this moment, monolithic silica columns can be one of the most hopeful candidates for such a highly permeable

separation system. On the other hand, when the pressure limit can be increased up to much higher levels, densely packed columns will come back to the candidate.

Ultra–high–speed separation, ultra–micro pumping systems, mixing and reaction system with an aid of micromachining technique, downsizing of a whole capillary LC system, *etc.* are to be considered in the near future.

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