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Automated microgradient system for capillary electrochromatography

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Abstract

A microprocessor controlled gradient elution system suitable for capillary electrochromatography has been developed and tested. It is based on a liquid handling device described previously which is capable of liquid transport with both low and high fluid dispersion. The low dispersion region formed by stainless steel needle 250 μm I.D. serves for sample injection, while the high dispersion region, created by steep extension of tube diameter, is used for continuous mobile phase gradient generation. A homologous series of seven alkylphenones was electrochromatographycally separated on a monolithic polyacrylic column under gradient conditions. An S-shaped acetonitrile gradient (30–70%) was applied. A high reproducibility of retention times (RSD about 0.1%) was obtained, indicating accuracy of automated gradient operations.

Keywords: Electrochromatography; Instrumentation; Automation; Gradient elution; Monolithic columns; Alkylphenones

1. Introduction

Capillary electrochromatography (CEC) is soon to become a standard analytical method. Especially the progress achieved in the field of column technology [1–3] and new applications [4–6] facilitate wider utilisation of this technique. The main obstacle for the routine use of CEC nowadays is the lack of dedicated and dependable instrumentation. The ideal CEC apparatus should be fully automated to be capable of unattended operations, and a high reproducibility of the gradient elution should be obtained.

Several methods of gradient elution CEC with different degree of automation were suggested, as reviewed in literature [7–9]. These methods could be roughly divided into three groups. The simplest are step gradients [10,11]. They can be easily generated by commercial CE instrumentation. But continuous gradients are much more powerful. The second method is the application of two computer-controlled high voltage supplies [12]. Principally, this is very near to CEC because two electroosmotically driven streams of weak and strong mobile phases merge in the CEC column. Problems with prediction of gradient shape and reproducibility are to be expected in this case because of the dependence of the electroosmotic flow magnitude on the fluid composition and capillary inner surface. This variant could be advantageously used in microchip format [13]. The most straightforward and frequently used is the third method, employing an external HPLC gradient pump equipped with an injector and joined to the CEC

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column via a splitter interface [14–22]. In addition, pressurised CEC can be carried out with this method but the disadvantage is a relatively bulky and expensive instrumentation.

Recently, we have described a simple gradient preparation method [23], based on turbulent mixing of weak and strong mobile phase at the spot of a steep change of tubing diameter. In spite of manual operations, the reproducibility of emerging gradients measured as reproducibility of retention times was relatively good (RSD ~0.5–3.3%). In this work, a liquid handling device [24] was used which was originally implemented as microcolumn liquid chromatograph and later used for handling of isotachophoretic focusing [25–27]. Our main goals were to run CEC columns in this apparatus and to achieve an unattended gradient preparation, high flexibility of mobile phase manipulations and high retention time reproducibility.

2. Experimental

2.1. Reagents and materials

Seven homologous alkylphenones (acetophenone–octanophenone) were obtained from Aldrich (Milwaukee, WI, USA). Test mixture was prepared by dissolution of alkylphenones (2 m*M* each) in acetonitrile (ACN)–water (30:70, v/v). Vinylsulfonic acid (25% solution in water), poly(ethylene glycol) with an average molecular mass of ≈10 000, tris(hydroxymethyl)aminomethane, lauryl acrylate, *N*-methylformamide, 3-(trimethoxysilyl)-propyl methacrylate were purchased from Aldrich. *N*,*N*′-methylenebisacrylamide, ammonium persulfate, acetone, *N*,*N*,*N*′,*N*′-tetramethylethylene-diamine (TEMED) and boric acid were obtained from Merck (Darmstadt, Germany). Acetonitrile G Chromasolv was from Riedel–de Haën (Seelze, Germany). Fused silica capillaries (75 μm I.D.×360 μm O.D.) and optical fibers (300 μm core, 360 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Column preparation

The monolithic CEC column [*net*-poly(acrylamide-co-*N*,*N*′-methylenebisacrylamide-co-laurylacrylate-co-vinylsulfonic acid] was synthesised following the procedure described by Palm and Novotny [28]. Pre-treatment of the inner wall of the capillaries was performed at room temperature by a statical etching with 1 *M* sodium hydroxide twice for 15 min, followed by a rinsing with 0.1 *M* hydrochloric acid for 30 min and a subsequent flushing with deionized water for 30 min. Thereafter a 50% (v/v) solution of 3-(trimethoxysilyl)propyl methacrylate in acetone was introduced and left inside the capillary twice for 30 min. Finally the capillary was rinsed with water and acetone.

Acrylamide (30.2 mg), *N*,*N*′-methylenebisacrylamide (60 mg), vinylsulfonic acid (25% solution in water, 24.8 μl), polyethylene glycol (60 mg) and lauryl acrylate (24.5 μl) were dissolved in 1.85 ml of *N*-methylformamide containing 100 μl of 200 m*M* Tris–300 m*M* boric acid buffer. A freshly prepared solution was filtrated and deoxygenated with helium for 30 min. To start the polymerisation, 2 μl of 100% TEMED and 5 μl ammonium persufate were admixed to 250 μl of the monomer solution described above. The solution was quickly introduced into the freshly activated capillary which was than placed in water bath with a temperature of 15 °C. The polymerisation started in about 5 min which was evident as a white turbidity in the rest of polymerization mixture and proceeded overnight at the same temperature. Next day, the column was cut to appropriate length and about 2 mm of the polyimide coating was removed by sharp blade to create optical window. The gel spans from one end of the column to the other which means that ''through gel'' detection was used.

2.3. Instrument

The automated gradient system is schematically represented in Fig. 1. It consists of three main parts: liquid handler, splitter, and CEC separation unit. The liquid handling device/gradient generator (0–8) includes a liquid distribution block (5) with five inlets (0–4) for sample and gradient transport, sample injection, weak and strong mobile phase feed, and waste outflow, respectively, and a 75 μl syringe (7) with a needle (6). The needle has a side opening (0.15 mm in diameter), which can be connected to

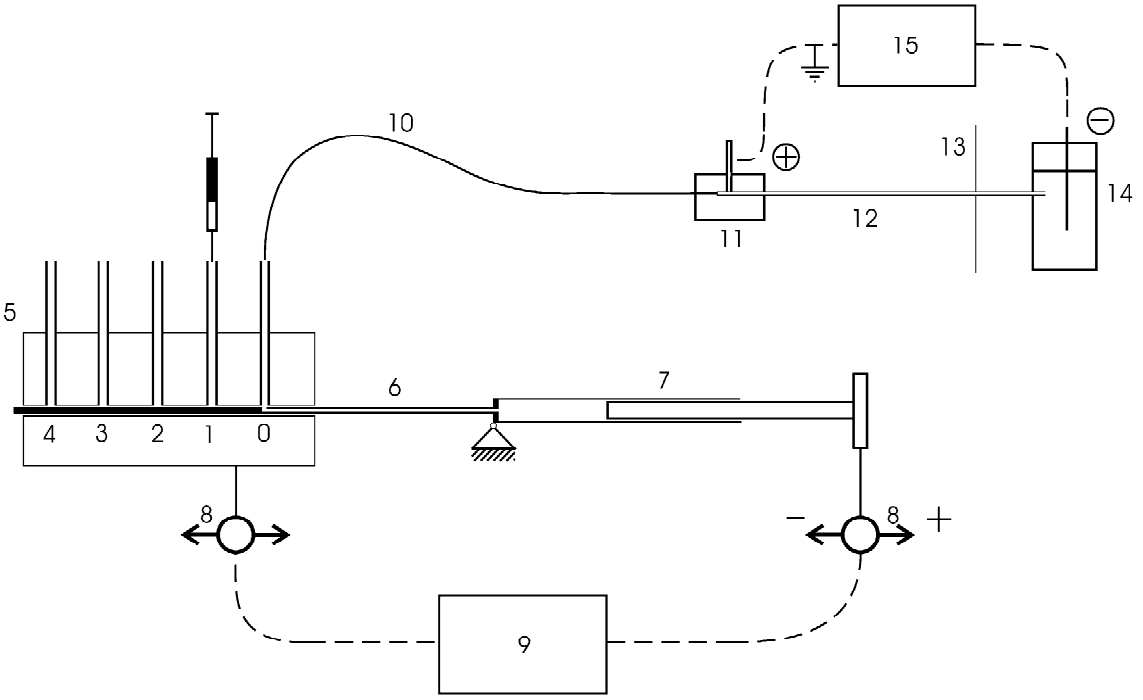

Fig. 1. Instrument design. (0) Sample and mobile phase outlet, (1–3) inlets for the sample and week and strong mobile phases, respectively, (4) waste, (5) liquid distribution block, (6) injection needle (0.25 mm I.D.×0.5 mm O.D.) with side orifice (0.15 mm), (7) 75 μl glass syringe, (8) stepping motors, (9) microprocessor control, (10) connecting capillary (50 μm I.D.×360 μm O.D.×250 mm length), (11) splitter, (12) CEC column [105 mm (effective length 80 mm)×75 μm I.D.×360 μm O.D.], (13) optical fibers for UV detection, (14) electrolyte chamber, (15) high voltage power supply.

one of the five inlets (0–4). All operations are executed by means of two computer controlled (9) stepping motors (8) according to an appropriate algorithm.

The liquid handler is joined by the connecting capillary (10) to the splitter (11), which is depicted in detail in Fig. 2. The splitter is part of the CEC separation unit and serves as grounded anode. The grounding is essential to avoid electrical interference with the liquid handler microprocessor. The CEC column (12) is inserted in the splitter and in another electrolyte chamber (14). A high voltage power supply (HVS) (15) from Spellman (Plainview, NY, USA) was used to generate electric fields. Optical fibers (300 μm core, 360 μm O.D.) are attached to the CEC column for UV detection as already described [25,29]. The optical fibers were joined to a SpectraFocus UV detector (Spectra-Physics, San Jose, CA, USA). DataApex (Czech Republic, Prague) integration software was used for data collection.

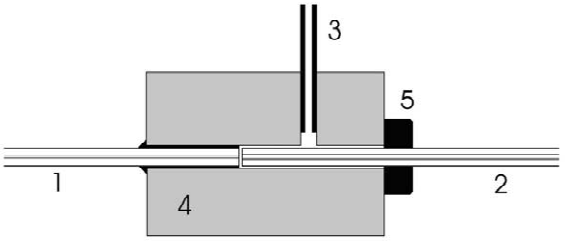

Fig. 2. Splitter. (1) Connecting capillary (50 μm I.D.×360 μm O.D., 250 mm length) fixed by epoxy glue, (2) CEC column (75 μm I.D.×360 μm O.D.), (3) stainless steel tubing waste capillary and grounded anode, (4) splitter glass body (380 μm I.D.×7 mm O.D.), (5) silicon rubber sealing.

Table 1
Example of a control algorithm used for the separations shown in Fig. 3

	Step	Position[a]	Piston[b]	Volume (μl)	Flow (μl s^{-1})	Pause[c] (s)	Voltage (7 kV)	Duration (s)
Gradient generation	0	3	+	15	3.3	2	Off	11.6
Sample injection	1	2	+	10	3.3	2	Off	
	2	1	+	0.5	0.2	1	Off	25.8
	3	0	−	0.75	0.2	6	On (1 s)	
	4	0	−	3.75	0.3	0	Off	
Analysis	5	0	−	21	0.07	0	On	300
Column regeneration	6	2	+	12	3.3	1	On	137.1
	7	0	−	1.5	0.3	0	On	
	8	0	−	2.5	0.02	0	On	
	9	4	−	8	3.3	0	Off	
Syringe flushing[d]	10	3	+	5	3.3	1	Off	8.1
	11	4	−	5	3.3	0	Off	
	12	3	+	5	3.3	1	Off	
	13	4	−	5	3.3	0	Off	

[a] Position of the liquid distribution block, see Fig. 1.
[b] + = sucking, − = displacement.
[c] Pause for negative pressure release after quick sucking.
[d] Exchange of the weak for the strong mobile phase in the syringe needle.

2.4. Instrument function

The electrochromatograph operates according to an algorithm, shown exemplarily in Table 1. The dedicated program enables one to set (i) a position (0–4) (Fig. 1) of the liquid distribution block (5), (ii) the direction of piston move, (iii) the volume size, (iv) the volumetric flow and (v) a pause. In the actual case, it consists of 13 single steps in five sets (gradient generation, sample injection, analysis, column regeneration and syringe flushing). At the beginning, the syringe is quickly filled first by the strong and then by the weak mobile phase (step 0 and 1). At the sucking of the week mobile phase, the gradient is formed by a turbulent mixing in the place of the steep extension of the tube diameter at the mouth of the needle (6) into the syringe (7) (Fig. 1). After that, 0.5 μl sample is slowly sucked (step 2) into the needle. The sample plug is then transported slowly into the splitter (step 3) with minimal dispersion, so as not to decrease the sample concentration. There is a pause in step 3 (6 s) to allow one to electrokinetically inject the sample (here 1 s) into the CEC column. A flushing of the splitter follows in step 4, removing all rests of sample and bringing the weak mobile phase (outset of gradient) into the splitter. Now, the analysis can begin (step 5) by switching the voltage on. The flow, which is forced by the liquid handler into the splitter, must be higher than the flow through the CEC column. The overflow of mobile phase leaks into a waste capillary. After analysis is completed, the CEC column must be regenerated (steps 6–9). Therefore, a volume of 12 μl of weak mobile phase is sucked into the syringe (step 6) and a portion of 1.5 μl is quickly displaced into the splitter (step 7) to flush out any rest of the strong mobile phase. In this and the following step 8 voltage is switched on so as the regeneration could start immediately and continue during step 8. Step 9 serves for displacement of the rest of the mixture of week and strong mobile phase in order not to enter the CEC column. The syringe flushing steps 10–13 are inserted for replacing rests of week mobile phase remaining in the needle by strong mobile phase so that the device is ready for the next analysis.

3. Results and discussion

3.1. Creation of low and high fluid dispersion

In the liquid handler used [24], the fluid passes through conduits with low and high mixing capabilities. The part of the needle (I.D. 0.25 mm) from the side orifice (Fig. 1, position 6) to the mouth into the glass barrel of the syringe (Fig. 1, position 7) represents the low dispersion region, serving for sample sucking and displacement. The high dispersion region is created by a steep extension of the tube diameter in the mouth of the needle (Fig. 1, position 6) into the 75-μl syringe cylinder (I.D. 1.14 mm). The 4.56 times increase in diameter and 20.8 times increase in cross-section means that the linear velocity is 20.8 times slower in the syringe cylinder. The resulting deceleration causes turbulent mixing. In addition to the geometrical preconditions for low/high dispersion, we have used the computer controlled movement of the syringe piston and resulting changes in volumetric flow as an additional mean to influence the mixing in a desirable way. As shown formerly [24], both the slow sucking and slow displacement leads to minimum peak dispersion which ensures a constant sample concentration. Consequently, a flow of 0.2 $\mu l\ s^{-1}$ (Table 1, step 2 and 3) was set for sample sucking from the inlet and the transport into the splitter. On the other hand, a much higher flow of 3.3 $\mu l\ s^{-1}$ (Table 1, step 0 and 1) was used to intensify mixing of the gradient.

3.2. Isocratic vs. gradient elution

To test the gradient capabilities of the device for monolithic CEC columns we used a mixture of seven homologous alkylphenones (acetophenone–octanophenone) as analytes. First, isocratic runs with a weak elution strength (ACN–water 30:70, v/v), and a high elution strength (ACN–water 70:30, v/v) of the mobile phase were carried out. In the first case the retention times of the homologues increased progressively (3.0, 3.4, 4.1, 5.2, 7.4, 11.6, and 18.6 min), as expected from the reversed-phase mechanism of retention. In the second case (70% ACN) only one peak containing all phenones appeared with a retention time of 2.6 min. Then we used an S-shaped gradient from 30 to 70% ACN for elution of the same test mixture. The preparation of the gradient follows the algorithm listed in Table 1. A simple optimisation was done which consisted in an alteration of the sucked volumes in step 0 and 1, and the flow in step 5 (see Section 3.3) such that all peaks of the homologous series were affected by the gradient. The result is presented in Fig. 3. In comparison to the weak mobile phase isocratic elution, a compression of the retention times occurred, indicating that the gradient mechanism was at work. In addition, the decreasing peak widths with growing retention times indicate the focusing ability of the gradient elution. To visualise the gradient, we added 0.5% of acetone into the strong mobile phase. The result can be seen in Fig. 3 (track g). Besides, the ACN gradient caused a systematic drift of the baseline between 2 and 4 min, induced by a refractive index change. The slightly higher than usual noise (about 0.1 mAU) can be attributed to the ''through gel'' detection. After the gradient run, column regeneration was carried out, filling the CEC column by week mobile phase. The column is than ready for a next analysis. In Fig. 3, the end of regeneration is noticeable as a sudden increase of the baseline at 7.4 min.

3.3. Flexibility of liquid manipulations

Synergy of both low/high fluid dispersion capabilities and simple software control of liquid sucking and displacement brings high degree of liquid manipulation flexibility. Although not studied in detail, some examples are obvious from Table 1. The extent of initial weak and terminating strong mobile phase segments, bracketing the S shaped gradient, could be controlled by changing the sucked volumes (Table 1, step 0 and 1, column 5). The steepness of the gradient could be controlled by simply changing of the flow (Table 1, step 5, column 6). Even more complicated schemes for linear gradient generation could be accomplished by software-controlled mixing of weak and strong mobile phase segments.

3.4. Reproducibility of retention times

To test the uniformity of repeated gradients, the

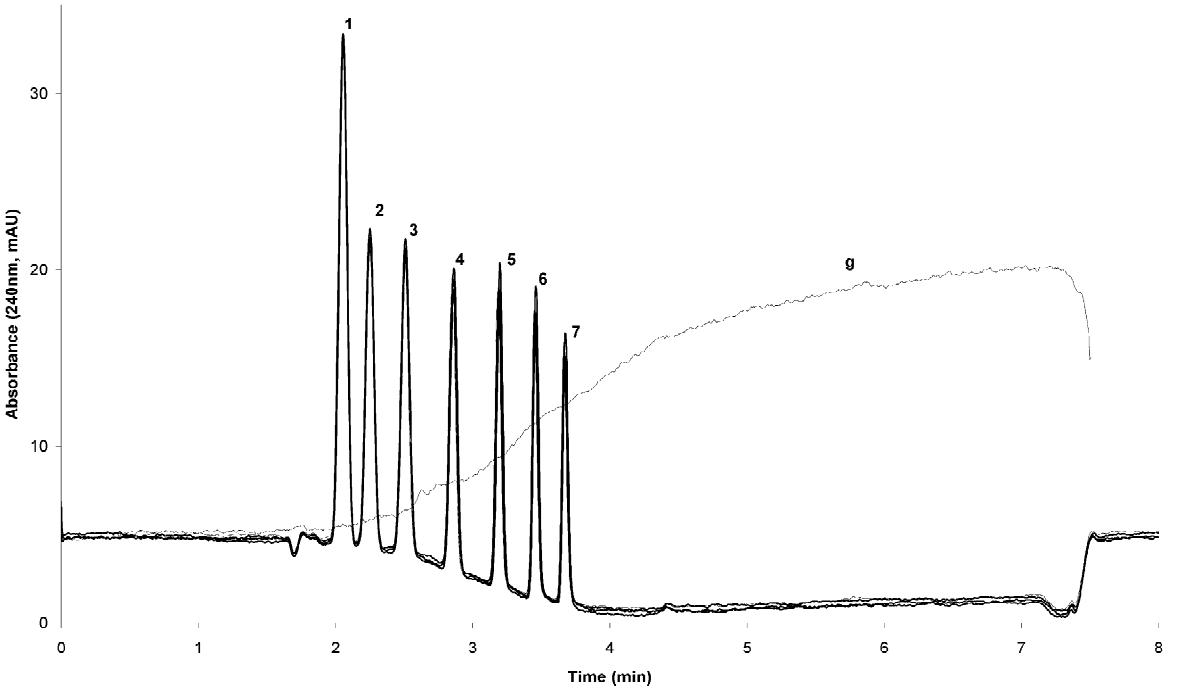

Fig. 3. Four successive gradient separations of alkylphenones. Peak description: 1 = acetophenone, 2 = propiophenone, 3 = butyrophenone, 4 = valerophenone, 5 = hexanophenone, 6 = heptanophenone, 7 = octanophenone; g = gradient track. Column: fused-silica capillary [105 mm (effective length 80 mm) × 75 μm I.D. × 360 μm O.D.] length) filled by a synthetic monolith; S-shaped mobile phase gradient: 30–70% ACN–5–7.5 m*M* Tris–borate; electric field strength: 670 V cm^{-1}; current 2: 2.5 μA.

reproducibility of retention times was measured. The statistically evaluated data from four successive runs are presented in Table 2. A much higher reproducibility in comparison to manually generated gradients [23] was achieved (RSD ~0.09–0.15%), in spite of the fact, that the CEC column was not thermostated and high voltage power supply was switched manually for electrokinetic sample injection and start of analysis. The piled up tracks of four consecutive runs are depicted in Fig. 3. The high reproducibility of retention times is clearly visible here too.

Table 2
Reproducibility of retention times in gradient elution

Solute	Retention time (min)	RSD (%, $n = 4$)
Acetophenone	2.054	0.105
Propiophenone	2.250	0.104
Butyrophenone	2.508	0.091
Valerophenone	2.860	0.087
Hexanophenone	3.192	0.105
Heptanophenone	3.456	0.146
Octanophenone	3.671	0.120

4. Conclusions

A new possibility of automated mobile phase gradient generation in capillary electrochromatography was described. It was shown that the suggested principle is applicable as a basis for a design of CEC instrumentation and can bring the following advantages:

(i) An automated gradient preparation is facilitated through a simple and straightforward computer controlled liquid handler which is driven by two stepping motors. (ii) A high degree of sample and mobile phase manipulation flexibility can be achieved by a combination of high/low dispersion capabilities of the device and a simple computer control of liquid sucking and displacement. (iii) High reproducibility of retention times (RSD ~0.1%) can

be realized, documenting the high uniformity of gradient operations.

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