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Journal of Chromatography B, 770 (2002) 19–23

JOURNAL OF CHROMATOGRAPHY B

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Versatile tool for the manipulation of electrophoresis chips

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Abstract

This communication describes a versatile tool allowing standard operations (i.e. washing, pre-conditioning, separation, inner surface modification of the chip channel) with capillary electrophoresis chips. Through currently designed for a chip of maximal dimensions 30×60 mm, other formats of the chip require only a minimum adjustment of the equipment, namely setting of the chip sliding rails and adequate arrangement of the exchangeable heads. The application of the tool is demonstrated by the separation of the standard set of inorganic cations.

Keywords: Electrophoresis chips

1. Introduction

Capillary microchip electrophoresis opens new dimensions in miniaturised separation technology. While numerous attempts of microchannel fabrication can be traced in the literature (all of them are based on the photolithography process similar to the semiconductor technology) [1,2], no adequate instrumentation opening the possibility of exploiting at least a certain category of microchips in the capillary electrophoretic routine has been published so far [3]. While microfabricated devices have been applied for the separation of fluorescently labeled amino acids [4], DNA restriction fragments [5–7], PCR products and oligonucleotides [8], applications to other categories of compounds are rare at the moment [9,10]. Current trends in capillary electrophoresis chips are directed mainly to multiple channel analysis [11] and towards on-chip pre- or post-column derivatisation reactions [12].

In order to be able to exploit fully the advantages of capillary chip electrophoresis, such as short running times and further miniaturisation of the samples analysed, it is necessary to have a simple, versatile device allowing fast chip channel positioning, channel washing (and/or fast and reproducible chip replacement), fast electrolyte filling and reproducible sample injection. While in standard capillary electrophoresis these preliminary operations (except sample injection) take units to tens of minutes and are generally shorter than the time needed for the analysis, in capillary chip electrophoresis these operations should be reduced to tens of seconds which requires adequate instrumental background.

It was the aim of the present work to construct a simple versatile device that can be used for routine chip analysis. Because we did not find in the literature even an attempt for standardisation of the chip dimensions we have tried to develop a device which can be (with minimum modifications) used for different chip formats. The device was constructed for maximal outline dimensions of the chip 30×60 mm, and firstly matched for the chip experimentally

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1570-0232/02/$ – see front matter
PII: S0378-4347(01)00377-2

manufactured by Krejčí Engineering (Tišnov, Czech Republic).

2. Description of the tool

Fig. 1 shows the overall view of the equipment. The outer dimensions of the box are 350×110×135 mm. A small compressor and vacuum pump (ASF Thomas 7010 and 5002; ASF Thomas Industries, Puchheim, Germany) are placed in the left half of the inside space in the box. There are five operating sections on the upper desk. The chip can be shifted in a slide way in the front part of the upper desk to the individual sections (positions). The first three positions are intended for ''wet'' manipulations: washing, flushing and filling of the channels in the chip. The fourth position is prepared for other possible functions (e.g. surface treating of the channel or additional washing operations). The actual electrophoretic analysis takes place in the last position.

All the described positions ensure an appropriate contact with the chip. Each position is provided with an arm fastened to the rear part of the upper desk by a close-running fit. This fit is provided by a groove in the arm and a flat spring fastened to the bottom side of the arm which presses the arm to a horizontal bar. Each of the arms can be lifted by means of a cam to enable slanting of the arm from the chip. The pin in the middle of the arm (Fig. 2) serves for lifting the arm while the lower part of this pin is in contact with a cam inside of the box. A screw with a spring in the arm serves for setting the contact pressure between the seal and the chip. The arms carry special heads matched for the different chip operations.

Fig. 2 shows one of the first three arms (position a) with the heads (position b) in the lowered position, i.e. with the contact with the chip (position d). The close running fit of the arm (position c) is seen on the left-hand-side while on the right-hand-side of the arm we can see the head. In front of the head there is a stainless tube for liquid introduction by pressure (for details, see Fig. 3). The seal to the chip is secured by a piece of silicon rubber hose around the central stainless steal tube which feeds the chip with the liquid. The filling tube is connected to a washing-bottle containing the desired liquid by means of a silicon rubber hose (Fig. 2, position f) visible on the left side of the head. The necessary pressure is applied by compressed gas, the inlet of which to the washing-bottle is controlled by an electromagnetic valve. The holders of the washing-bottles (Fig. 1, position f) are visible on the left of the back side. The electromagnetic valves (Fig. 1, position g) are placed also on the right of the back side. The tubes on the right-hand-side of the heads are connected to

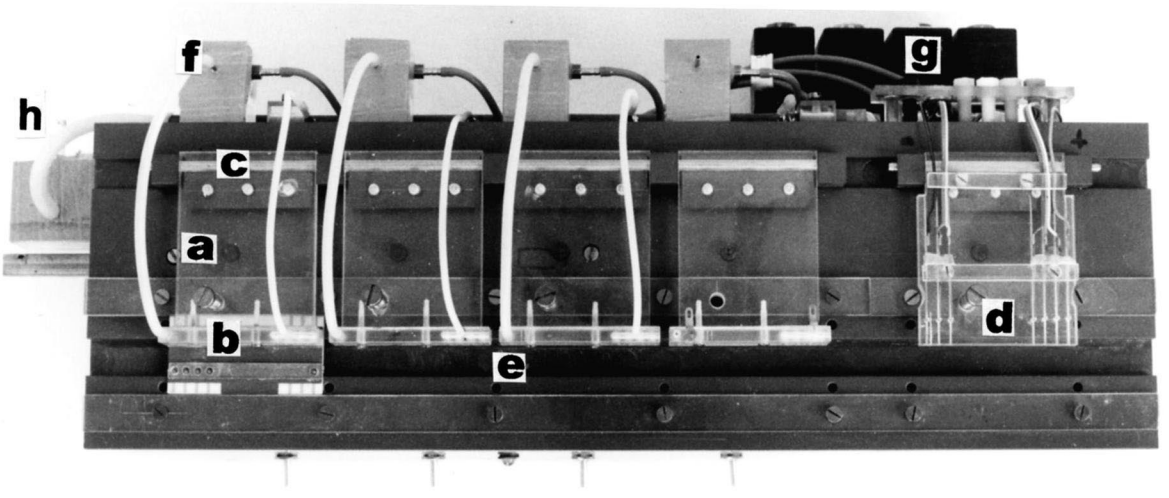

Fig. 1. Overall view on the tool. (a) ''Wet'' arm, (b) head of the arm, (c) close running fit, (d) arm with electrical contacts, (e) slide-way, (f) holder for washing bottle, (g) electromagnetic valve, (h) liquid separation bottle.

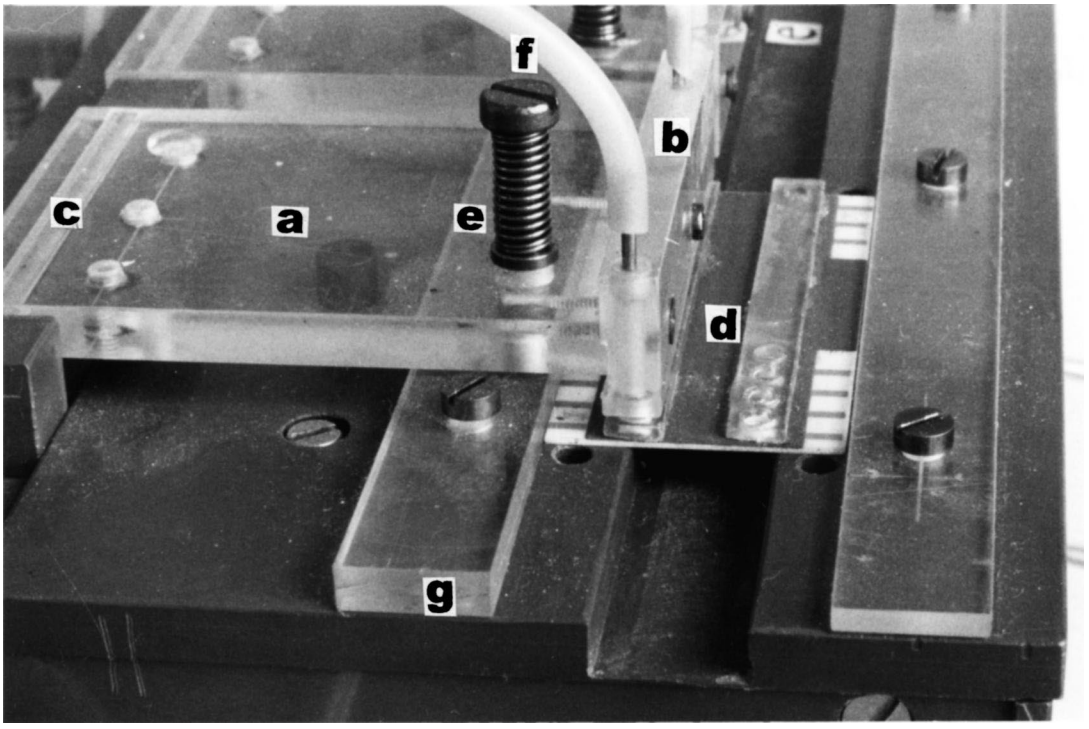

Fig. 2. Detail view on the arm with the head and the chip in the first position. (a) Arm, (b) head, (c) close running fit, (d) chip, (e) screw for setting the contact pressure, (f) silicon rubber hose, (g) rails of the slide-way.

a vacuum pump across a liquid separation bottle (Fig. 1, position h) which is placed on the left side of the box. This prevents the liquid from overflow the inlet jars (Fig. 3, position i).

Fig. 4 presents a scheme of the experimental chip used. The chip contains two combinations of the separation channels of different length. The geometry of the jars and the contacts are symmetrical; by

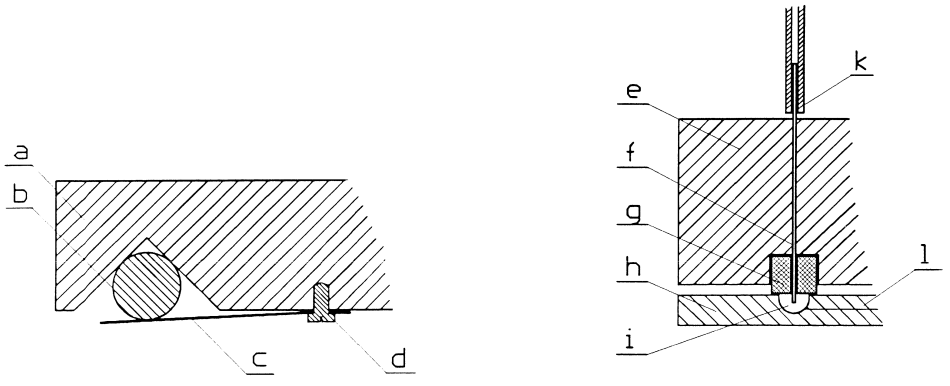

Fig. 3. Cross-section details. Left: close running fit: (a) arm, (b) horizontal bar fastened to the upper desk of the tool, (c) flat spring, (d) fixing screw for the spring. Right: part of the head with the filling nozzle: (e) head, (f) stainless tube, (g) silicon rubber seal, (h) chip, (i) inlet jar, (k) inlet silicon rubber hose, (l) capillary channel in the chip.

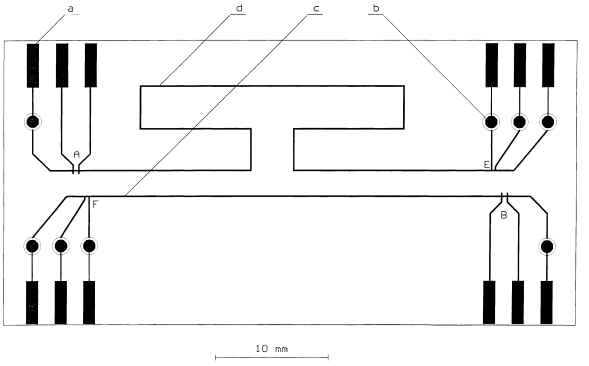

Fig. 4. Schematic view of the experimental chip. (a) Electrical contact, (b) jar, (c) channel 1, (d) channel 2. (A,B) Contacts for conductivity detection, (E,F) channels for sample introduction.

turning the chip 180°, the position of the jars and electrical contacts do not change.

In the position for filling the chip with the electrolyte, a small amount of the liquid remains in the jar because the sucking pipe is shorter than the depth of the jar (The depth of the jar in the experimental chip Fig. 4 is about 1 mm and the sucking pipe is immersed to the half of this depth). The suction is very important because when high potential is applied for electrophoresis, any humidity layer on the chip would cause a surface leakage current disturbing the analysis. In the fourth position, there is an arm available for possible future application. In the last position, the arm is prolonged in order to cover the chip and to, thereby, prevent the evaporation of the liquid from the jars. There are small grooves in the arm which guide stainless steel wire springs. The parts of the springs over the chip are equipped with rectangular hooks for electrical contacting of the chip. One couple of the electrodes serves for the application of high voltage during the analysis, the other couple is used for electromigration sample introduction, while the third couple contacts the electrodes of the chip to the detector (if conductometric detection is used). Both the arms and the heads are manufactured of Perspex.

In the present stage of development, the tool is set for conductivity detection only. For UV and/or fluorescence detection, the light from an appropriate light source (monochromator, deuterium lamp, xenon lamp) enters the tool from the right hand side. A 45° surface mirror is attached inside the box under the last position which deflects the light to the chip. The position of this mirror can be finely adjusted as necessary. The arm for the UV detection bears contact springs for electrical connection and a solar blind phototube (Hamamatsu R 1826, Hamamatsu Photonik, Japan). Our experience has shown that only a simple shield against direct light is sufficient [13]. The situation with the fluorescence detection is quite different. The intensity of the emitted light is minimal and occurs frequently in the visible range of the spectrum. Therefore, perfect light shielding is necessary. For this purpose grooves in the upper desk should, with a matched arm, form a light-trap. The light is directed by a light guide to an external photomultiplier. The exact positioning of the chip under the arms is secured by four guiding pins, the upper parts of which are conical and shifted out after the chip is placed in either position. Thus the position of the chip in the direction of the slide-way is better than ± 0.1 mm. In the perpendicular direction, the clearance of the guiding of the chip is smaller than ± 0.05 mm. Due to the close-running fit of the arms there is nearly no clearance perpendicular to the longitudinal direction of the tool. The clearance in the longitudinal direction is smaller than ± 0.05 mm. Test with the chip used (Fig. 4) have shown that the positioning of the chip is quite sufficient for tightening of pressures up to 200 kPa and the electrical contacts with the chip are reliable with a high isolation between them.

During the development, care was taken to ensure versatility. For other chips geometry, the matching is quite simple. The arms are easy to exchange by manipulation of just four screws. The heads are fastened to the arms with only two screws. For another width of chip, the slide-way can be easily accommodated by the reposition of the guiding rails.

3. Apparatus testing

Practical applicability of the system described was demonstrated by separating a three cation mixture (consisting of Na, K, Li as chlorides purchased from Lachema, Brno, Czech Republic; p.a. quality) using the chip described in Fig. 4 (length of the separation channel: 40 mm; capillary dimension: 50×50 μm). Samples was applied electrokinetically 5 s at 200 V

in a concentration of 20 m*M* of each cation separated. Background electrolyte: 20 mmol MES–20 mmol His (Sigma Chemical, St. Louis, MO, USA); run voltage: 600 V. The channel was pre-conditioned by 100 m*M* SDS, 50 m*M* methanol, water and run buffer (15 min each wash). The test was similar to those used by Guijt et al. [14]. The result is shown in Fig. 5.

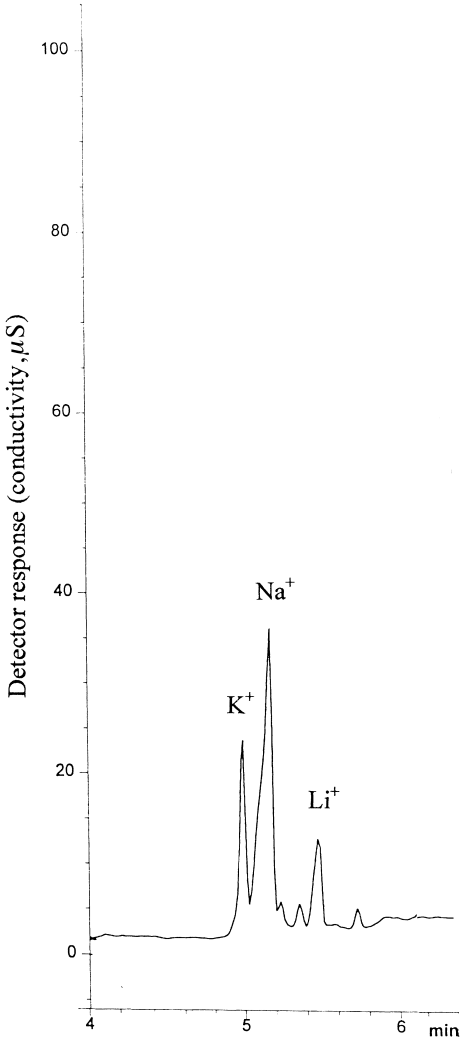

Fig. 5. Separation of Na, K and Li (as chlorides) in 20 m*M* MES–20 mmol His, pH 5.5; electrokinetic injection: 200 V/5 s; separation voltage: 600 V. Cathode on the right-hand-side.

Acknowledgements

The work was supported by the Grant Agency of the Czech Republic, Grant No. 203/01/0288.

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