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In-house packing and testing of capillaries for capillary electrochromatography using simple equipment

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Abstract

The feasibility of producing packed capillaries for capillary electrochromatography (CEC) is evaluated. Emphasis is put on the fact that only material was used that is already available in any LC/CE orientated laboratory. An experimental set-up is developed for filling the capillaries by use of an ordinary LC pump, and frits are sintered with a glowing resistance wire, fed by a d.c. power supply. Electrochromatography was carried out in an in-house built capillary electrophoresis apparatus, without pressurizing the vials. Under these conditions, the capillaries performed well, producing up to 190 000 plates per meter. Bubble formation did not appear, on condition that the mobile phase was thoroughly helium degassed. Even at ambient temperature, electrophoresis obeyed Ohm's law up to a voltage of 30 kV, proving that Joule heating was not a major concern.

Keywords: Electrochromatography; Capillary electrochromatography; Capillary packing; Capillary testing

1. Introduction

Capillary electrochromatography (CEC) has in the last few years, been receiving increasing attention as a high efficiency separation technique [1,2]. Depending on one's scientific background, the principle of CEC can be interpreted differently. For analysts who have been trained in liquid chromatography (LC), CEC can be considered as a process where electro-driven flow instead of high pressure driven flow propels the mobile phase through a narrow liquid chromatography tube. From a capillary electrophoresis (CE) point of view, electrophoretic separation is taking place in a fused silica capillary, with a superimposed chromatographic process. Various modes, all named CEC, have been proposed: open capillary tubes with chemically modified walls (open tubular CEC) [3,4], capillaries with in-situ polymerized continuous beds [5,6] or molecularly imprinted polymers [7], and capillaries packed with silica particles [8–12]. The last mentioned, packed column CEC, is the most widely known and studied form. Electro-osmotic flow (EOF), arising

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from ionization of silanols on the silica wall, and residual silanols on the silica particles, moves the mobile phase through the capillary with a nearly flat flow profile, thus decreasing band broadening and increasing efficiency. Moreover, EOF does not change with the particle diameter, allowing the use of particles as small as 0.5 μm, giving rise to very high efficiencies because of reduced eddy diffusion and improved mass transfer [13].

In the first days of CEC, capillaries were slurry-packed in the lab, whether electrokinetically or under high pressure. More recently, a packing procedure has been described where supercritical CO_2 is used as the slurrying solvent to drive the stationary phase particles in the capillary [14]. The electrokinetic packing of capillary columns has been patented [15,16], and capillaries produced in this way are commercially available. Other manufacturers offer high pressure slurry packed capillaries, ranging from 25 to 320 μm I.D. and from 15 to 75 cm in length, and packed with a variety of C6, C8, C18 or other stationary phases, mostly with 3 μm particles. These fused silica capillaries have a packed section, two frits to retain the particles, and a detection window in the unpacked part of the capillary, close to the outlet frit. Most problems associated with these capillaries can be nailed down to the two following issues: (1) the capillaries are extremely brittle and can easily be broken at the detection window or at the frits, where the coating has been removed, and (2) the generation of air bubbles during electrochromatography can necessitate external vial pressurization [17]. Capillary breakage usually makes the capillary useless, unless it's the inlet frit which can in some cases be remade. If a part of the capillary dries out, i.e. so-called bubble formation, only time-consuming flushing under pressure will drive the bubble out.

In order to deal with these problems, we tried to pack fused silica columns with silica particles ourselves, only making use of material that's normally available in any laboratory where LC and CE is performed. Furthermore, it was tried out whether it is possible to use capillaries produced in this way in CE equipment at ambient pressure and temperature.

2. Materials

2.1. Experimental set-up

2.1.1. Capillary rinsing device

A 10 ml glass reservoir (SGE CWR-10, Alltech, Laarne, Belgium), coated on the outside with a protective plastic film, is connected through a stainless steel T-piece with the fused silica capillary that pierces a septum for airtight connection. On the other end of the T, a 1/16 in. O.D. line is led to a helium gas cylinder. If the glass reservoir is filled with solvent and pressurized, the capillary will be flushed, on condition the capillary is dipped in the solvent. This device allows for capillary washing up to 5 bar.

2.1.2. High-pressure packing apparatus

A scheme of the packing apparatus is represented in Fig. 1. An L-6200 Intelligent Pump from Merck-Hitachi (Darmstadt, Germany) was connected through 1/16 in. O.D. stainless steel (ss) tubing to a T-piece (Valco) that acted as a flow splitter. A piece of ss tubing, 10 cm in length and 1 mm I.D. acts as the slurry-chamber and is mounted between two female-female unions

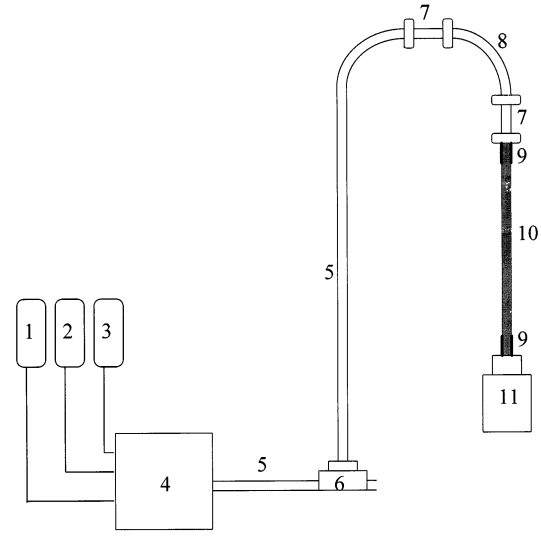

Fig. 1. Scheme of the high pressure packing device: 1, acetone; 2, water; 3, mobile phase; 4, LC pump; 5, SS tubing; 6, T-piece; 7, female–female union; 8, slurry chamber; 9, PEEK sleeve; 10, silica capillary; 11, in-line filter.

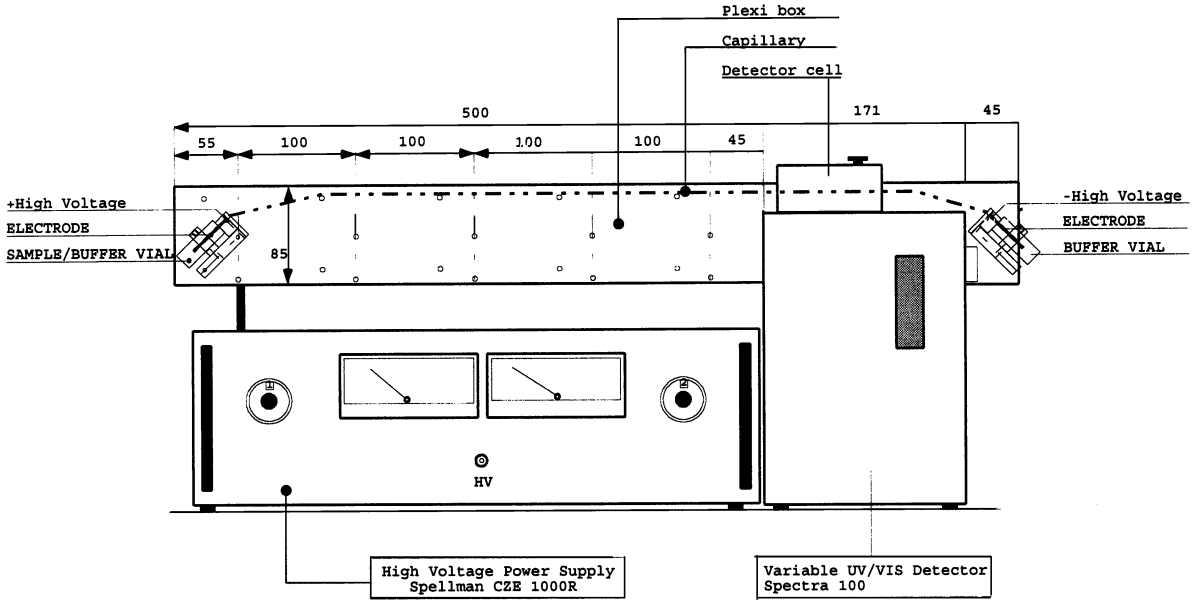

Fig. 2. Schematic presentation of CE instrumentation.

(Valco), downstream of the T-piece. A fused silica capillary, 375 μm O.D. and 50 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) is fitted immediately after the slurry chamber. For this purpose, a PEEK sleeve of 0.015 in. I.D. and 1/16 in. O.D. (Upchurch Scientific, Oak Harbor, WA, USA) is slid around the capillary inlet and fitted in the 1/16 in. I.D. female–female connection. On the other end of the capillary, an identical sleeve is used to fit the capillary into an in-line filter. In our set-up, the capillary was mounted on top of an empty 4.6 mm LC column, with the metal frit acting as the filter. The diameter of the pores of this filter was 2 μm.

2.1.3. Frit fusing device

A 0.2 mm thick and 6 cm long resistance wire was bent halfway to make a small loop. Whenever frits were made, the capillary was threaded through this 500 μm diameter loop, and voltage was applied over the filament. The d.c. power supply (DF1730, D.C. Electronics) was operated at its maximum of 3 amps (± 3.5 V).

2.1.4. CE equipment

The CE instrumentation (see Fig. 2) was assembled in-house from commercially available components. Electric fields were applied with a Spellman CZE 1000 R high voltage power supply (Plainview, NY, USA). Detection was achieved with a Spectra 100 variable UV-Vis detector, equipped with an on-capillary detection cell, both purchased from Thermo Separation Products (Fremont, CA, USA). Electrochromatograms were recorded on a HP 3396 Series II integrator (Hewlett-Packard, Avondale, PA, USA) and stored on a personal computer running HP Peak 96 software. A plexi glass box was constructed on top of the UV detector and contained the capillary, electrodes, cathodic and anodic buffer vials, and detection cell. A removable cover protects the analyst from exposure to high voltage. All experiments were carried out at ambient pressure and temperature.

2.2. Chemicals

As stationary phase, Hypersil ODS 3 μm reversed phase particles were used (Shandon Scientific, Runcorn, UK). Acetone and acetonitrile

were of analytical grade and obtained from BDH (Poole, UK) and Acros Organics (Geel, Belgium) respectively. Caffeine was purchased from Knoll AG (Ludwigshafen am Rhein, Germany), phenol from UCB (Drogenbos, Belgium), thiourea from Merck (Darmstadt, Germany), and benzylalcohol and sodium tetraborate from Acros, all analytical grade. The steroids that were used in this study were bulk samples from Roussel Uclaf (Romainville, France) or Diosynth (Oss, The Netherlands). Throughout the study, Milli-Q water (Millipore, Milford, MA, USA) was used, and solutions were filtered through 0.2 μm nylon filters (Euroscientific, Lint, Belgium) before use.

3. Procedures

3.1. Packing procedure

Fused silica capillary of 50 μm I.D. is cut to a length of 55 cm and mounted in the capillary rinsing device. The capillary is flushed consecutively with water, 0.1 M NaOH, water, and acetone, each time for about 5 min at a pressure of 3 bar. After this preconditioning step, the capillary is fixed in the high pressure packing setup, making sure the capillary is led through the loop of the heating filament. All solutions are thoroughly helium degassed immediately before use. The capillary is then acetone-washed for an extra minute at a flow rate of 25 μl min^{-1} with one end of the T-piece (Figs. 1–6) shut off.

Meanwhile, 100 mg of stationary phase is slurried in 5 ml acetone, and ultra-sonicated for at least 10 min in order to break up all the aggregates. At this point, the slurry chamber is completely free of the set-up and fixed with the two openings facing up. A syringe is filled with 0.5 ml extensively vortexed slurry, and locked on the slurry chamber. The slurry is injected and the compartment is promptly put back into position in the setup. The pump is switched on again at a flow rate of 0.1 ml min^{-1} with acetone as the displacing solvent. As soon as the pressure reaches 100 bar, the flow rate is reduced to 0.05 ml min^{-1} (up to 200 bar), and further to 30 μl min^{-1} (up to 300 bar), and finally to 10 μl min^{-1}

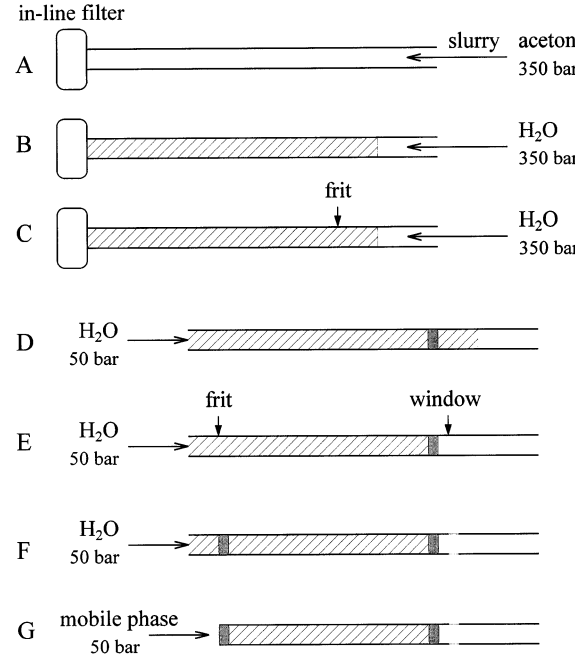

Fig. 3. Schematic view of the packing process.

(pressure around 350 bar). During this process, the slurry compartment is continuously tapped and vibrated with a small vortex mixer. As soon as the packing level does not rise any further, or when the capillary is completely filled with the stationary phase, the pump is switched off, and the pressure is allowed to slowly drop to atmospheric pressure. A schematic view of this step

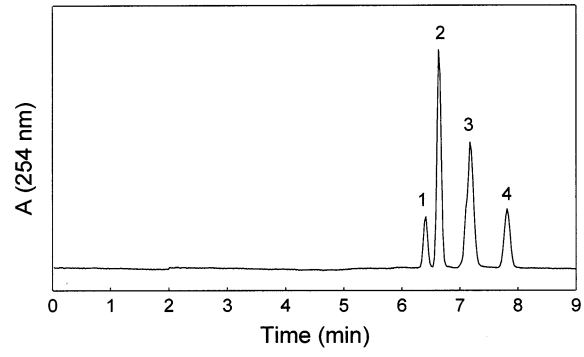

Fig. 4. Electrochromatogram of test mixture 1. Applied voltage is 10 kV. For other conditions, see Section 3.2. Peak identification: 1, thiourea; 2, caffeine; 3, phenol; 4, benzylalcohol.

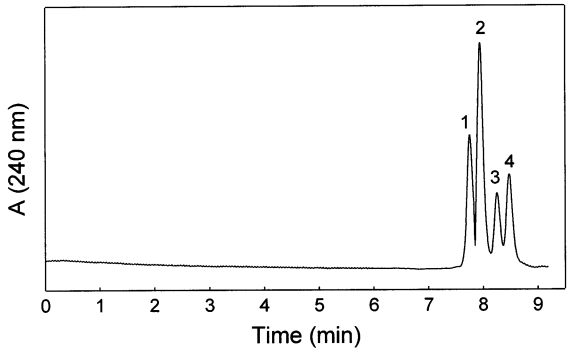

Fig. 5. Electrochromatogram of test mixture 2. Applied voltage is 10 kV. For other conditions, see Section 3.2. Peak identification: 1, dexamethasone; 2, hydrocortisone; 3, prednisolone acetate; 4, cortisone acetate.

and any further steps of the packing process is shown in Fig. 3.

The coupling between capillary and slurry chamber is disconnected, and all lines are primed with water. The capillary is mounted again and the capillary is washed with water at a flow rate of 5 μl min^{-1} for at least 30 min (300–350 bar) (Fig. 3B). The resistance wire loop is held at the position where a frit is wanted, and the voltage is switched on for 60 s (Fig. 3C) as to sinter a frit. The pump is switched off, and pressure is allowed to slowly dissipate. The in-line filter is removed from the end, and the capillary is fixed with that side first in the solvent stream. Water is pumped through the capillary at a maximum pressure of 50 bar (Fig. 3D) and particles after the frit are washed away. It might be necessary to put the capillary end in an ultrasonication bath to drive out the compressed particles. In the same way as the first frit, a second frit and a detection window next the original frit are made on the capillary (Fig. 3E–F) while washing at 50 bar. Finally, the packed section before the second frit is cut off, lines are primed with the mobile phase, and the capillary is conditioned at a maximum pressure of 50 bar for at least 1 h (Fig. 3G).

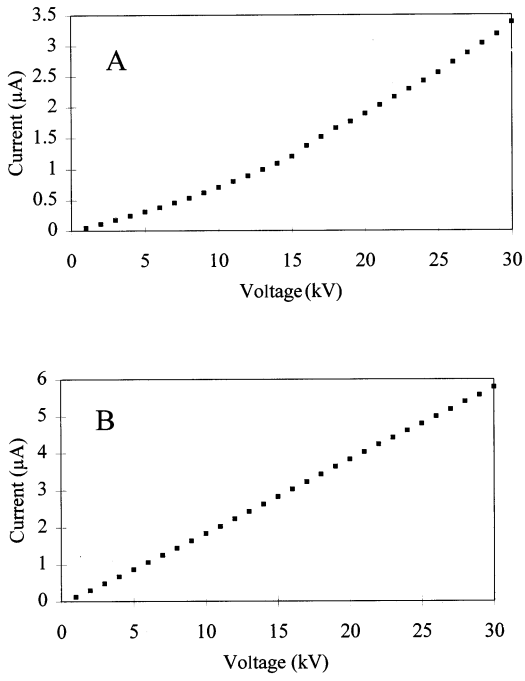

Fig. 6. Ohm's plot. (A) Without degassing or stabilizing, (B) after helium degassing and capillary stabilizing.

3.2. Capillary testing

A helium degassed mixture of acetonitrile and 5 mM sodium tetraborate pH 9.0 (80/20) was used as the mobile phase, and capillary as well as anodic and cathodic buffer vials were filled with mobile phase. The capillary (final dimensions: 37 cm total length, 19 cm packed length) was rapidly taken from the packing apparatus and fixed in the CE equipment. Test mixture 1 contained caffeine (35 μg ml^{-1}), phenol (350 μg ml^{-1}), thiourea (12 μg ml^{-1}), and benzylalcohol (600 μg ml^{-1}), all dissolved in mobile phase. Test mixture 2 consisted of hydrocortisone, prednisolone acetate, cortisone acetate, and dexamethasone, all at a concentration of 5 mg ml^{-1} in mobile phase. Injections were performed electrokinetically for 3 s at 5 kV, and detection was achieved at 254 nm for mixture 1 and at 240 nm for mixture 2. Electrochromatograms were recorded after application of the specified voltage.

4. Results and discussion

4.1. Packing procedure

Sodium hydroxide and water preconditioning

cleans and activates the capillary wall prior to the packing operation. When mounting the capillary in the high pressure packing device, care should be taken to make leak-free connections. For instance, 0.015 in. PEEK sleeves are only 6 μm wider than the width of the capillary, and the use of appropriate ferrules allows for high pressure proof transition from 1 mm I.D. ss tubing to 50 μm I.D. fused silica capillary. Ideally, the capillary should protrude the sleeve for about 2 mm. The high pressure pump used is a ternary gradient LC pump, but an isocratic pump could serve just as well, as long as the pump can be operated up to 400 bar, and the flow rate can be adjusted down to 1 μl min^{-1}. If the latter is possible, the T-piece (Figs. 1–6) acting as a flow splitter, becomes obsolete.

When the slurry is injected into the slurry chamber, and this compartment is put back into the solvent flow, manipulations should be sufficiently fast to prevent the particles from sedimenting. Tapping and vibrating the tubing has the same function, and is especially important during the first moments after the pump is switched on. Packing of the capillary takes place starting on the down end upwards, and at the same time the pressure increases. In order to prohibit the pressure from exceeding 350 bar, the flow rate is continuously monitored and adjusted if necessary. The packing progress was observed through the capillary wall with the aid of a magnifying glass.

Each time the pump is switched off, the pressure gradually decreases. It is of utmost importance that this process is given time (at least 30–60 min) before the solvent lines are somewhere interrupted. If not, stationary phase particles will shoot out of the packed capillary. Before frits are made, acetone is replaced with water as the solvent in the capillary. There are different ways of sintering polysilicate frits [18], yet in-situ sintering of the packing material is the most elegant solution. However, not all packing materials can be sintered into frits, and then other ways need to be sought [12,19]. The Hypersil ODS 3 μm particles are easily sintered into a semi-permeable frit if the capillary is locally heated up with the red-glowing resistance wire. During this process, the capillary remains water pressurized to prevent drying out of the frit zone [20]. The strength of the frit is a crucial factor in the capillary packing procedure. A frit that is too strong becomes impermeable for the mobile phase, while a too weak a frit is easily flushed out at a pressure under 50 bar. An ideal frit keeps the stationary phase particles in place, while the particles after the frit can be washed away.

4.2. Capillary performance

Electrochromatograms of test mixtures 1 and 2 are shown in Figs. 4 and 5. In Fig. 4, the unretained and neutral molecule thiourea moved together with the EOF and was detected first. The three other compounds were resolved in less than 9 mins, producing between 70 000 and 190 000 plates m^{-1}). Efficiencies for the steroid mixture ranged between 100 000 and 150 000 plates m^{-1}. These plate numbers are well in agreement with what other researchers have reported for neutral compounds [2].

One way to assess the performance of a CEC system is to examine in what voltage range Ohm's law is obeyed. According to Ohm's law, the applied voltage should be directly proportional to the observed current if the resistance is constant. In Fig. 6, the current is depicted as a function of the applied voltage. As can be seen in Fig. 6B, voltage is proportional to current up to 30 kV, if the mobile phase is extensively degassed and the capillary is given time to stabilize (60 min at 10 kV). If this is not the case (Fig. 6A), the graph deviates from linearity above 10 kV, showing changing resistance and possible bubble formation. Even at 30 kV, the voltage did not breakdown, mainly because each voltage stage was only kept for 2 min for the construction of this figure. Currents attained in Fig. 6B (5.8 μA at 30 kV) were considerably higher than in Fig. 6A (3.4 μA at 30 kV), most probably due to acetonitrile evaporation after degassing and stabilizing. It has long been thought that bubbles were generated as a result of Joule heating. More recently, it has been postulated that a change in EOF from the packed to the unpacked section of the capillary is the actual cause of local drying out of the capillary [21].

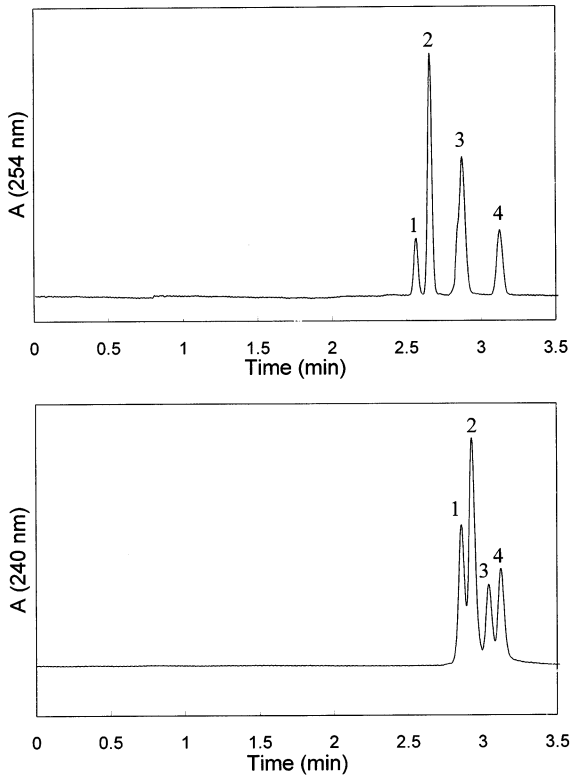

Fig. 7. High speed separation of test mixtures 1 and 2. Applied voltage is 30 kV. Other conditions as in Figs. 3 and 4.

The efficiency of the packed capillaries was further evaluated by attempting some high speed separation of the test mixtures. At 30 kV, separation of the test mixtures was achieved under 3.5 min. As can be seen from Fig. 7, there was no apparent loss in efficiency compared to the electrochromatography at 10 kV. Based on this finding, a plot was constructed of plate height versus linear velocity. Fig. 8 shows such a typical H versus u plot for a mixture containing thiourea and caffeine. Reduced plate heights decrease with increasing linear velocity and then stay more or less constant (minimal reduced plate height (only use equal sign for character and value. In this case, the word 'is' is more appropriate) = 2.2). Both compounds, retained and unretained, show the same behavior, as others have described [20]. A pronounced plate height minimum, as the Van Deemter equation predicts for LC [1], is not present in CEC.

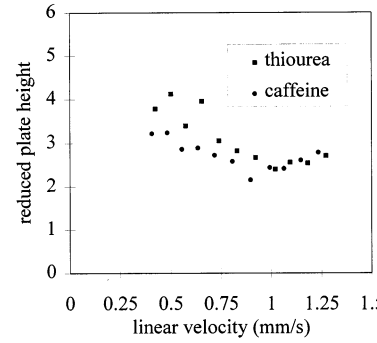

Fig. 8. H–u curves for thiourea and caffeine. Experimental conditions as in Section 3.2.

5. Conclusions

It has been demonstrated that it is feasible to produce and test packed capillary columns for CEC with very simple and straightforward equipment, in contrast to what other groups have described [16,22]. Numerous tips given in this paper and elsewhere should help in some way to circumvent a few of the pitfalls that are associated with the technique. Whether CEC will improve any particular separations, or make the unattainable separation possible, strongly depends on the application. Up to now, most work in this field has been done with uncharged molecules, and it stays questionable whether the same degree of performance will be reached for highly basic or acidic compounds. Nevertheless, we have shown that simple CEC can be performed at ambient temperature and pressure. This should allow any separation scientist to try out separations with this fascinating chromatographic technique.

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