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Tapers and restrictors for capillary electrochromatography and capillary electrochromatography–mass spectrometry

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Abstract

Tapered and narrow restrictor capillaries have been investigated for use in capillary electrochromatography (CEC) and have been shown to offer viable, more reproducible, alternatives to silica frits and may aid in suppression of bubble formation, a recognised problem in CEC. In addition, these capillaries have been coupled to mass spectrometry, especially for low-flow and nano-flow electrospray ionisation, where results have shown better maintenance of chromatographic efficiency and higher sensitivity, compared with more conventional interfaces. The use of tapered CEC column inlets is suggested as being useful in sampling from micro-environments, such as cell contents.

Keywords: Electrochromatography; Tapers; Restrictors; Electrospray ionisation mass spectrometry; Steroids

1. Introduction

Capillary electrochromatography (CEC) is now widely recognised as a technique of growing importance, especially in terms of efficiency and the ability to cope with small sample volumes. Mass spectrometry (MS), with its high sensitivity and unrivalled specificity has become established as an extremely powerful adjunct to liquid chromatographic techniques. Initially it was necessary to split the flow from typical analytical HPLC columns to allow ca. 5 μl/min into the mass spectrometer, or reduce the amount of solvent by evaporation. The advent of capillary separation techniques, including CE and CEC, offered more compatible flow-rates, but still not ideal, conversely being too low and requiring a "make-up" flow of solvent. The first coupling of CEC with MS utilised fast atom bombardment (FAB) ionisation and incorporation of "make-up" flow at a tee-piece [1], but subsequent reports have used electrospray ionisation (ESI), usually via co-axial liquid–sheath interfaces for incorporation of "make-up" flow [2–5]. ESI is inherently most suitable for introduction of solutions into the mass spectrometer and this is likely to remain the case for the foreseeable future. However, the CEC eluent is diluted in liquid–sheath interfaces, and ionisable species in the sheath solvent compete with the analyte for charge, both effects presumably causing a reduction in sensitivity. Sheathless interfaces have been used for CE [6–8] and a recent report described the use of 100 μm I.D. CEC columns for sheathless ESI, where the flow-rate is sufficiently high at 1–2 μl/min for ESI, however, no comparison with sheath-flow was made [3]. More typical CEC columns utilise capillaries of smaller internal diameter than 100 μm with correspondingly lower flow-rates, which are highly compatible with the flow-rates in

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the recently reported technique of "nano-ESI" [9–11]. This technique requires tapered capillaries with metallised tips, for electrical contact and which have inner diameters in the low μm range at the MS probe tip, enhancing the electrical field, allowing the electrospray phenomenon to occur at ca. 20–30 nl/min flow-rates. This results in improved ionisation efficiency and hence improved sensitivity [7,8,10,12].

We have investigated the use of capillaries for CEC in which the end is drawn out to a fine taper or coupled to a short length of smaller I.D. capillary, obviating the requirement for an end frit and being suitable for low flow ESI–MS. Tapered capillaries would also provide opportunity for sampling from micro-environments such as the interior of a cell, when a taper is at the inlet end of the capillary. Similar tapers and flow restrictors have been described for use in open tubular supercritical fluid chromatography to maintain supercritical conditions throughout the column [13–17]. We have found one previous report on the use of tapered capillaries for LC by Alborn and Stenhagen [18], who used 0.22 mm I.D. capillaries with an outlet taper in which 30–50 μm silica was used to retain 3/5/10 μm packing. These authors also used the tapered configuration especially for MS coupling, but used an electron impact source rather than ESI as in our case.

In CEC where a length of unpacked capillary is present, such as for UV detection via a "window" in the capillary, bubble formation in the unpacked section has been frequently observed. Joule heating and consequent solvent de-gassing seems an unlikely explanation, since current carried by typical CEC systems is too low for this effect. The bubbles appear to form at the packed–unpacked interface, i.e., at the frit, where there is a pressure differential, the frit probably acting as a nucleation site. We have investigated a second use of capillary tapers, as a replacement for outlet frits immediately prior to a detection "window". Smith and Evans [19] successfully developed an idea of Knox and Grant [20], on the use of a pressurised CEC system to suppress bubble formation in the capillary and many workers now use this technique. However, pressurised systems are not applicable to MS coupling. In addition we have investigated the use of "restrictor" capillaries post detection "window" to provide flow resistance in the unpacked section of capillary, with a view to suppressing bubble formation.

2. Experimental

2.1. Materials

Fused-silica capillary tubing (Polymicro Technologies), was obtained from Composite Metal Services, Worcester, UK. Chromatographic packing materials, from Phase Separations, Deeside Industrial Park, Deeside, UK. Epoxy adhesives and circuit board repair pen from RS Components, Corby, UK. All solvents used were of HPLC grade obtained from Rathburn, Walkerburn, UK, and were filtered before use through a 0.2 μm PTFE filter (Alltech Associates Applied Science, Carnforth, UK). Steroids were obtained from Sigma, Poole, UK and used as received. All other reagents from BDH, Poole, UK.

2.2. Preparation of capillary tapers/restrictors and packing of columns

Tapered capillaries 50/75/100 μm I.D.×375 μm O.D., were produced using an "in-house" capillary puller (Fig. 1), in which fused-silica capillaries were clamped with "Swagelock" fittings and tensioned, before heating with a micro-burner. Tapers were produced either at the end of the capillary or mid-way and are depicted in Figs. 2a and 3a, respectively. The former were produced for use at either the outlet end, for ESI–MS, or inlet end, being especially suitable for sampling from micro-environments, particularly in the case of Fig. 2a. The mid-way

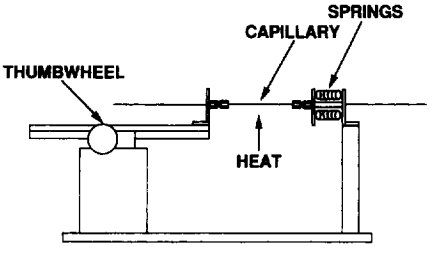

Fig. 1. Apparatus for production of tapered capillaries.

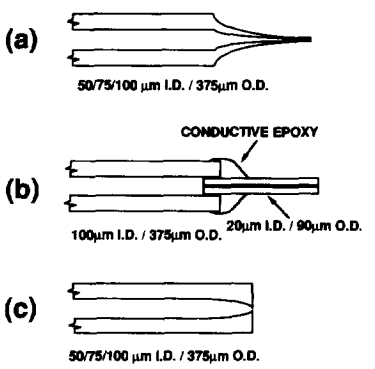

Fig. 2. Cross sections of capillary inlet/outlet profiles. (a) Single external taper for column outlet/inlet. (b) Restrictor capillary for column outlet/inlet. (c) Single internal taper for column outlet.

tapers in Fig. 3a were produced to retain column packing prior to the detection "window" and were reinforced by fixing an outer sheath of capillary as shown in the diagram. Experiments showed that it was only necessary to taper the capillary to ca. 10 μm I.D. for a "particulate keystone" effect to retain 3 μm silica.

Flow of liquid through the taper was at least as good compared to a more usual sintered silica frit, allowing easy packing of columns with 3 μm Spherisorb ODS-1, as described in more detail elsewhere [1]. For ESI–MS, capillaries were completely packed and an inlet frit was formed by heating the actual packing material and the extraneous capillary removed. Similar frits were formed prior to a "window" in capillaries without mid-way tapered constrictions, where detection was by UV absorbance.

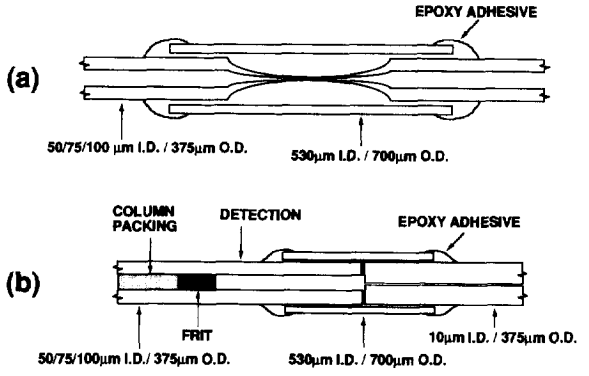

Fig. 3. Cross sections of capillary profiles for through-wall detection. (a) Double internal taper (constriction). (b) Post detection "window" restrictor for flow resistance and bubble suppression.

The capillary restrictor as shown in Fig. 2b, was produced by fixing a 20 μm I.D.×90 μm O.D. capillary into a 100 μm I.D.×375 μm O.D. capillary, using conductive epoxy adhesive in this case for electrical contact. This type of capillary also packed easily, retaining 3 μm silica at the joint between the two capillaries. A mid-way restrictor was produced, as shown in Fig. 3b, post detection "window", to provide flow resistance as an alternative to using a pressurised system, to inhibit bubble formation. This may be used in combination with the internal taper shown in Fig. 3a. An elegant method of joining capillaries as recently described by Hows and Perrett [21], may find application for the production of these restrictors.

Finally, a further method of forming outlet tapers was investigated. The profile shown in Fig. 2c was produced by heating the tip of a capillary, thereby forming a sealed internal taper and then grinding to produce an opening. These capillaries retained a thick wall at the tip and were consequently more robust than those with an external taper, but were not used for ESI–MS.

Tapered outlet sections of capillaries shown in Figs. 2a and 2b were coated externally with silver using a circuit board repair pen (RS Components), for electrospray electrical contact and forming a CEC cathode.

2.3. Chromatography

CEC separations were performed using a Waters Quanta 4000 electropherograph (Milford, MA, USA). A mixture of steroids were analysed (Fig. 4), cardenolides having a 5-membered lactone ring, bufadienolides with a 6-membered lactone (pyrone) ring. Our interest in these compounds relates to the identification of a human endogenous bufenolide, which we believe is involved in regulation of blood pressure [22]. The compounds were dissolved in acetonitrile, 10 nmol/μl each component and were

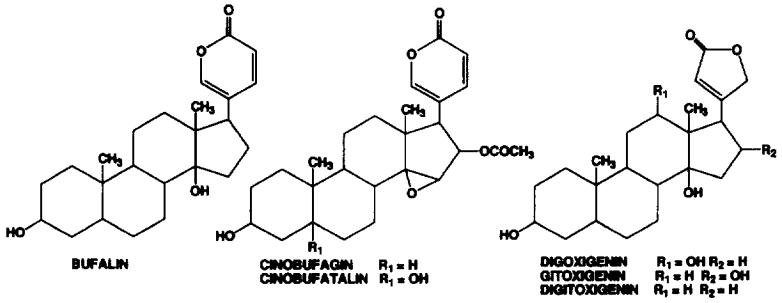

Fig. 4. Bufadienolide and cardenolide structures.

applied to the column electrodynamically. Detection was by either UV absorbance (214 nm) through a "window" in the capillary wall 26 cm from the inlet (MS off-line), or by MS on-line. For the latter case the anode was removed from the electropherograph cabinet (hazard!) and placed close to the ESI–MS co-axial probe (details in Section 2.4), to minimise capillary length to 45 cm. High voltage was applied to the anode buffer solution [acetonitrile–4 m*M* aqueous sodium tetraborate (70:30, v/v), adjusted to pH 8 with dilute sodium hydroxide solution], into which the inlet end of the capillary was immersed. The outlet end of the capillary was linked to the mass spectrometer as described in Section 2.4 below.

2.4. Mass spectrometry

MS was carried out on a Quattro MK.I quadrupole mass spectrometer fitted with an ESI source (Micromass, formerly VG Organic, Altrincham, UK), upgraded to Quattro MK.II specifications. The CEC capillaries were linked via a co-axial interface-probe as described previously [2] and sheath flow, when used consisted of acetonitrile–water (50:50, v/v) with 0.1% formic acid. A voltage of 3.5 kV was applied to the stainless steel capillary tip and a cone voltage of 30 V was used. MS acquisitions in positive mode were made scanning the range *m/z* 370–470 and were initiated approximately 10 min after application of sample onto the column and commencement of elution.

Bufalin alone was introduced by infusion into an unpacked capillary (50 μm I.D.×375 μm O.D.), at a flow-rate of 5 μl/min, using a syringe driver (Harvard Apparatus, S. Natick, MA, USA).

3. Results and discussion

Fig. 5 shows a UV chromatogram (MS off-line), for the separation of a mixture of bufadienolides and cardenolides, for comparison with MS on-line chromatograms. The peaks are well resolved, but with slight tailing apparent, particularly for peak 3 (cinobufatalin).

MS on-line experiments were compared using tapered capillaries as in Figs. 2a and 2b, necessitating the use of 100 μm I.D. capillaries and were found to produce seemingly identical results, in terms of chromatographic efficiency and MS sensitivity (data not shown). Fig. 6 shows selected ion chromatograms for the protonated molecule ions of a similar separation in a 100 μm I.D. CEC column with external tapered outlet (profile as in Fig. 2a). This compares sheathless, low-flow ESI in Fig. 6a with more conventional co-axial make-up sheath flow at 6 μl/min in Fig. 6b. It is apparent that the former situation displays better efficiency e.g., digoxigenin peak 19 s wide at half height in Fig. 6a compared to 27 s wide in Fig. 6b. Both mass chromatograms reflect the UV chromatogram in the previous figure, again showing peak tailing, especially with cinobufatalin but also with bufalin, which was not so apparent in the UV chromatogram. Some loss of chromatographic resolution is apparent at the mass spectrometer, compared with the UV chromato-

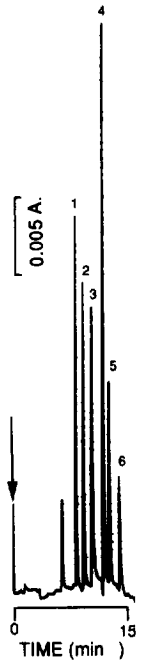

Fig. 5. UV chromatogram of steroid separation. Column: 50 cm×50 μm I.D., packed to 25 cm with 3 μm Spherisorb ODS-1. Injection: 10 s, 5 kV. Applied voltage: 15 kV. Detection at 214 nm. 0.05 A F S. Other conditions as described in Section 2.3. 1= Digoxigenin, 2=gitoxigenin, 3=cinobufatalin, 4=digitoxigenin, 5=cinobufagin, 6=bufalin.

gram, even when the differences in I.D. are taken into account. We have commented upon this previously [1,2], when we attributed band broadening chiefly to the length of unpacked capillary post packed section which was used in those cases (Lane and coworkers have reported the use of fully packed capillaries in light of this [4,5]). However, in this case the capillary was fully packed with no unpacked section, terminating only at the MS ionisation point. Therefore it seems likely that band broadening must be more attributable to effects in the ionisation source of the mass spectrometer. We postulate that this arises from dispersion of the eluting narrow band of analyte in solution to the gas state, into the relatively large volume of the ionisation source and depends on speed of removal of ions from source to analyser. However, the specificity of MS obviates this problem by detection of the unique mass of each compound, and only fails when dealing with closely eluting isobaric compounds, such as digoxigenin and gitoxigenin. Clearly the ideal situation is where the chromatographic performance apparent by UV detection is reflected by the mass spectrometer.

A drawback with the removal of make-up sheath flow is the lack of opportunity to alter the pH of the eluent, which can be advantageous to improve ESI–MS sensitivity. Fig. 7 shows comparative spectra for bufalin (500 pmol/μl in CEC buffer–acetonitrile–4 m*M* aqueous sodium tetraborate, 70:30, v/v), when infused into the mass spectrometer after 100× dilution in either CEC buffer, Fig. 7a, or acetonitrile–water (70:30, v/v) with 0.1% formic acid, Fig. 7b. The spectrum in Fig. 7a shows a prominent $[M+Na]^+$ ion at *m/z* 409, together with ions at *m/z* 425 $[M+K]^+$ and *m/z* 450 $[M+Na+acetonitrile]^+$, whilst the protonated molecule ion $[M+H]^+$ at *m/z* 387 is of very low intensity. The spectrum in Fig. 7b shows a dominant $[M+H]^+$ ion with the other ions of much less significance. In Fig. 6a and 6b, protonated molecule ions were chosen for the selected ion chromatograms and show similar sensitivity. However, if $[M+Na]^+$ was selected in Fig. 6a, there should be an approximate twenty-fold increase in sensitivity over 6b, in accordance with the expected gains in sensitivity of low-flow ESI. Severs et al. [23], recently described a novel means of acidifying CE analytes at the CE–ESI–MS interface, without increasing the flow-rate, via micro-dialysis tubing. This technique may also prove useful for post separation solution changes in CEC.

The mid-way tapers and restrictors post detection "window" appear to function well and subjectively seem to suppress bubble formation, but a longer, more detailed study is required.

4. Conclusions

Tapered and narrow restrictor capillaries offer a viable alternative to silica frits in CEC, are more reproducible and may be used in a variety of situations. When used as capillary outlets we have shown that they can provide better maintenance of chromatographic performance and higher sensitivity in low-flow, sheathless ESI–MS compared with ESI using a liquid sheath. In addition, tapers at the column inlet are applicable to small volume sam-

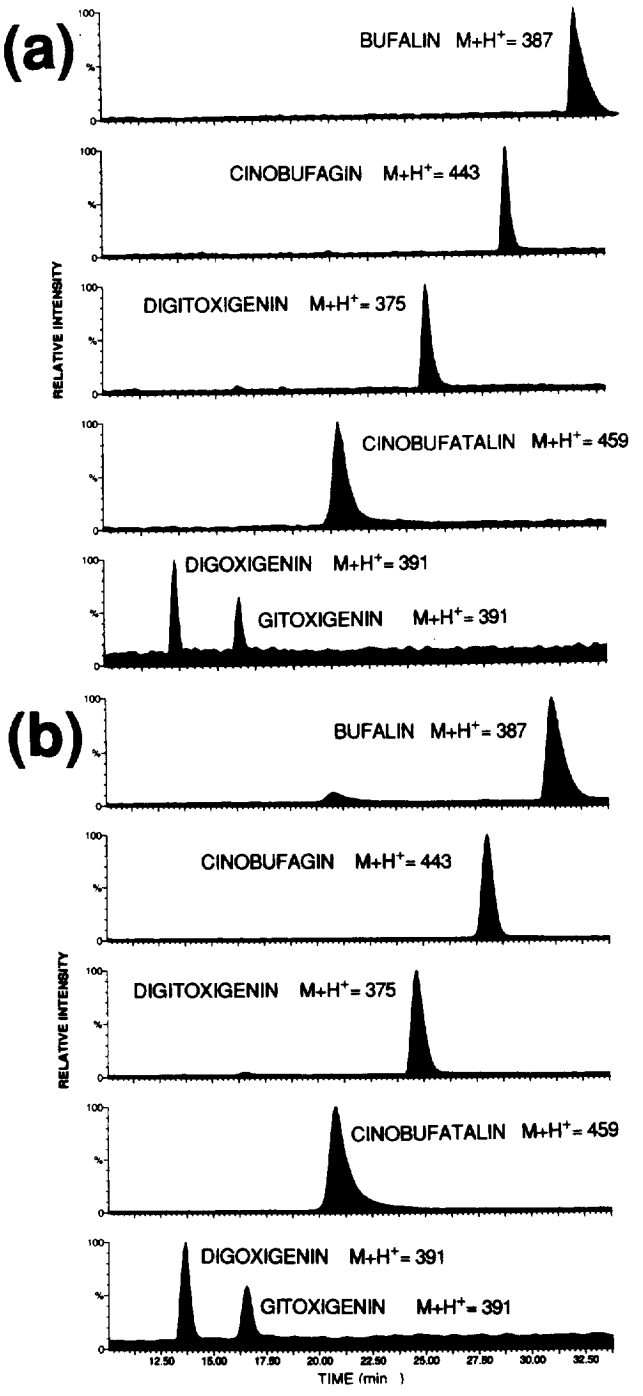

Fig. 6. On-line CEC–MS separation of steroids; selected ion chromatograms of protonated molecule ions $(M+H)^+$ from full scan data (m/z 370–470). Column: 45 cm×100 μm I.D., fully packed with 3 μm Spherisorb ODS-1. Injection: 10 s, 5 kV. Effective applied voltage: 21.5 kV. Other conditions as described in Section 2.3. (a) Low-flow "sheathless"+ESI; no co-axial make-up flow. (b) Conventional+ESI with 6 μl/min make-up flow [acetonitrile–water (50:50, v/v) with 0.1% formic acid].

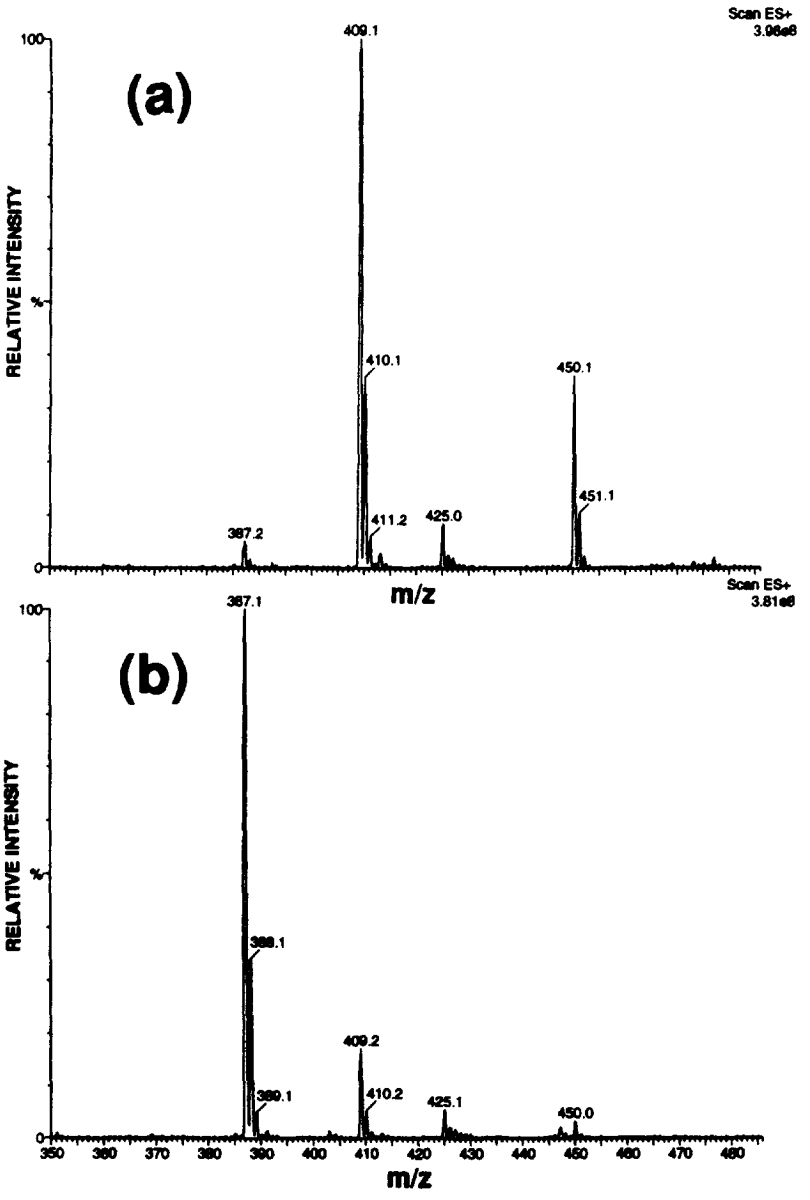

Fig. 7. +ESI spectrum of bufalin. (a) Using CEC buffer (acetonitrile–aqueous 4 m*M* sodium tetraborate, 70:30, v/v). (b) Using acetonitrile–water, (50:50 v/v) with 0.1% formic acid. Other conditions as described in Section 2.4.

pling, offering potential use for sampling cell contents. Tapers in place of frits prior to detection "windows" in CEC show potential in suppressing bubble formation, as do narrower restrictor capillaries coupled to packed capillaries.

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