ELSEVIER

Journal of Chromatography A, 700 (1995) 21–26

JOURNAL OF CHROMATOGRAPHY A

Influence of capillary dimensions on the performance of a coaxial capillary electrophoresis–electrospray mass spectrometry interface

L.W. Tetler[a,*], P.A. Cooper[a], B. Powell[b]

[a]*Michael Barber Centre for Mass Spectrometry, UMIST, PO Box 88, Manchester, M60 1QD, UK*
[b]*Zeneca Specialties, PO Box 42, Hexagon House, Blackley, Manchester, M9 3DA, UK*

Abstract

The dimensions of the capillaries used to construct a typical coaxial capillary electrophoresis–mass spectrometry (CE–MS) interface, i.e. the inner diameter, the outer diameter and the wall thickness, have been shown to affect the performance of the CE–MS system. The influence of these parameters has been investigated in both MS and MS–MS modes. The initial results indicate that by reducing all the sheath capillaries' dimensions and the CE capillary outer diameter, better operation and increased sensitivity can be achieved. The capillary arrangement which gives optimum sensitivity and stable operation has been suggested.

1. Introduction

Capillary electrophoresis–electrospray mass spectrometry (CE–MS), since its introduction in 1987 [1], has become an established technique in many research laboratories, mainly because of improvements made in interface design, stability and operation. Pioneering research in the late 1980's resulted in the emergence of two distinct types of CE–MS interface, the coaxial and the liquid junction. The liquid junction design was introduced by Minard et al. [2], using continuous flow fast atom bombardment, and by Henion and co-workers [3], using electrospray. A coaxial design was initially reported by Smith et al. [4] in 1988. Both interface designs have been shown to produce good data for a wide range of compounds, but subsequent research by Pleasance et al. [5] and our own investigations have tended to indicate that the coaxial arrangement is the more suitable of the two methods for interfacing with electrospray MS, offering robust and reproducible operation along with maintenance of both the CE and MS performance.

A typical coaxial interface (Fig. 1) comprises three concentric capillaries. The innermost capil-

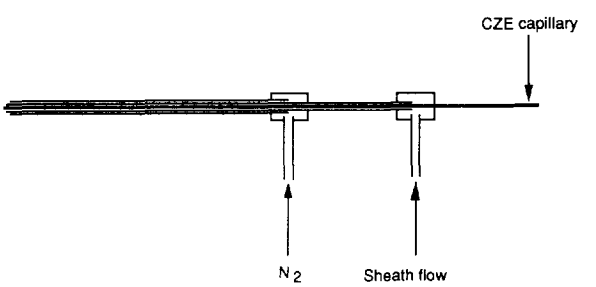


Fig. 1. A typical coaxial CE–MS interface.

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)01171-0

lary (generally fused-silica) is used for electrophoresis with the middle capillary (usually stainless steel) providing a sheath flow of liquid, which is required for a number of reasons. First, the extra liquid compensates for the low flow emerging from the electrophoresis capillary. Secondly, it provides unbroken electrical contact between the electrospray needle and the CE capillary, thus defining the potential difference across the CE capillary. Thirdly, it allows the incorporation of solvents and/or reagents which can be used to stabilise the electrospray process or to perform post-separation sample or buffer modification. The outer capillary, also stainless steel, supplies a flow of nebulising gas which aids droplet formation during the electrospray process and also provides a certain amount of cooling for the CE capillary.

The dimensions of these capillaries, i.e. their inner diameter, outer diameter and hence wall thickness, are usually determined by availability and the current instrument requirements, but they will have an influence on the spray characteristics of the electrospray, the degree of mixing at the probe tip, the siphoning effect of the nebulising gas and the electrical environment that the cathode end of the electrophoresis capillary experiences. All these parameters will affect the sensitivity and performance of the system.

The durability of the electrophoresis capillary is an important factor that should be considered. Thinner walled fused-silica capillaries tend to become brittle and suffer from 'electrodrilling' processes when subjected to the high voltages used in capillary electrophoresis. Our experience is that a thicker walled capillary (75 μm I.D., 375 μm O.D.) can last up to 4–5 times longer than a thin walled capillary (75 μm I.D., 150 μm O.D.) before any evidence of arcing or breakage is noted.

We therefore decided to investigate various combinations of capillaries in order to find whether changing from thin to thick walled capillaries has an effect on performance and also to find the optimum capillary arrangement in terms of sensitivity, ease of optimisation and robustness. The initial results from our on-going research are reported here.

2. Experimental

The CE instrument was constructed in-house (Zeneca Specialties, Blackley, Manchester, UK) and utilised a 30 kV Brandenburg Power supply (Astec, Stourbridge, UK). The injection end of the CE capillary was housed within a perspex case which could be pressurised to enable pressure injections to be made or the capillary to be flushed. The sample and buffer reservoirs were situated on a rotating table, the height of which was adjustable, thus facilitating hydrostatic injections. The separation capillaries were all untreated fused-silica (Polymicro Technologies, Phoenix, AZ, USA), 1 m in length. The diameters of the capillaries are included in Fig. 2.

The sample used was an aqueous peptide mixture containing bradykinin (M_r = 1064), angiotensin I (M_r = 1296), angiotensin II (M_r = 1040) and substance P (M_r = 1348) at a concentration of 200 pmol/μl per component. Injections were performed hydrostatically at a height of 10 cm for 5 to 10 s (depending on the interface arrangement used). This corresponds to injection volumes of 6–12 nl.

The electrophoresis buffer was a mixture of formic acid–acetonitrile–water (5:50:45). The applied CE voltage was 30 kV, which resulted in a potential drop of 25 kV (250 V/cm) across the capillary (as the cathode end of the capillary was at the same voltage as the ionspray needle, i.e. 5 kV).

MS was accomplished using a PE Sciex API III instrument (Sciex, Toronto, Canada) operated in the ionspray configuration. The ionspray voltage and the orifice potential were maintained at 5 kV and 70 V, respectively. Nitrogen was used as both drying gas (1 l/min) and nebulising gas [0.8–1.8 l/min, 25–60 p.s.i. (172–414 kPa) depending upon the interface arrangement] and the source temperature was 60°C throughout.

MS spectra were acquired by scanning Q1 from 400 to 700 amu at a rate of 1.6 s/scan. MS–MS spectra were acquired using the RAD software, which allows an optimised set of MS–MS parameters to be used for individual components. Q1 was switched manually between precursor ions as the peaks were eluted and Q3 was scanned from 50 to 1350 amu. The collision

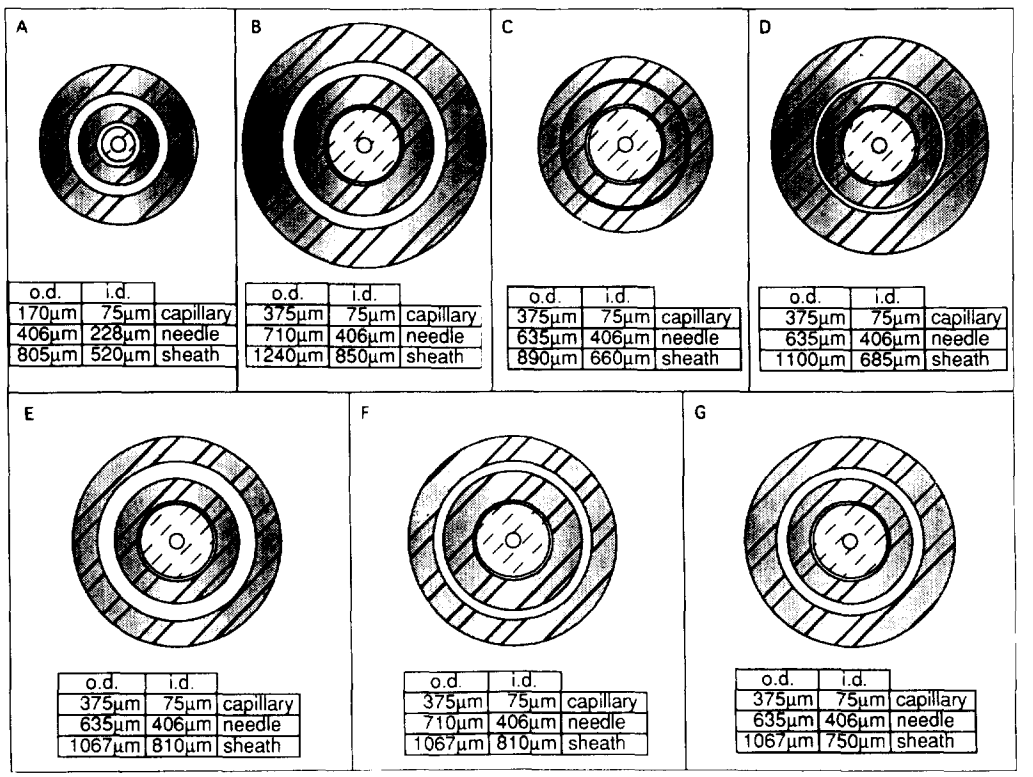


Fig. 2. Capillary combinations investigated.

gas employed was argon, at a collision gas thickness of 300×10^{13} atoms cm^{-2}.

The coaxial interface was operated with a sheath liquid comprising of a mixture of methanol–water (1:1) to which 0.1% v/v formic acid was added. The sheath liquid was delivered at a flow-rate of 8 µl/min by an Isco Model 1000 DM syringe pump (Isco, Lincoln, NE, USA).

The various capillary combinations considered in this study are shown drawn to scale in Fig. 2.

2.1. Chemicals

The peptides were purchased from Sigma (Poole, UK) and used without any further treatment. HPLC grade water, methanol and acetonitrile was obtained from Rathburn Chemicals (Walkerburn, UK). The Formic acid was supplied by Aldrich (Gillingham, UK).

3. Discussion

Our initial experiments were conducted using the original Sciex capillary dimensions which are reproduced in Fig. 2A. As expected, this arrangement resulted in a stable spray with good sensitivity, as reflected in the total ion chromatogram (TIC) of the peptide mixture (Fig. 3). However, this arrangement uses thin walled fused-silica CE capillaries which, as stated earlier, become brittle and break easily after prolonged application of high voltage. We therefore looked for capillary combinations which would allow the use of larger O.D. CE capillaries. The subsequent capillary combinations were chosen (depending upon availability) so that the ratios of the cross sectional areas of the three outlets (CE eluent, sheath flow and nebulising gas) formed a range around those of the original arrangement (Table 1). For these early experi-

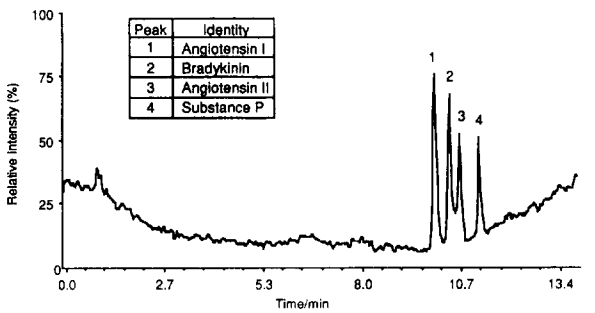


Fig. 3. CZE–MS TIC trace of the peptide mix using the original Sciex capillaries (arrangement A).

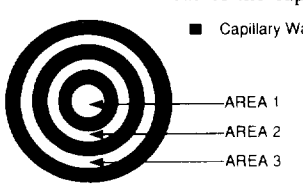
ments, the I.D. of the CE capillary was fixed at 75 μm in order to maintain sample loadings and separation parameters. Area 2 was chosen to be as small as possible. The only suitable size of capillary that was readily available had an I.D. of 406 μm. The next size up has an inner diameter of 510 μm which may have been usable, but the O.D. of this capillary meant that a larger outer capillary would have to be used and the O.D. of that capillary was too large to use with the standard 1/16 in. fittings.

The combinations shown in Figs. 2C and 2D were found to be unsuitable because of the tight fit of the middle capillary within the outer capillary. In the case of Fig. 2C, the capillaries were physically too tight to manoeuvre into position. The interface shown in Fig. 2D could be constructed without difficulty, but the closeness of the capillaries led to a restriction in the flow of nebulising gas. This meant working with very high pressures of nebulising gas, which made it very difficult to achieve stable electrospray conditions for any length of time, thus leading to fluctuation in the ion current and poor results.

The arrangements reproduced in Figs. 2B, 2E and 2F eventually all gave stable conditions but with varying degrees of difficulty. Table 2 is a comparison of the signals obtained with each of these arrangements and the Sciex original. The normalised values (relative to the largest signal for the particular peptide) are shown in parentheses and the overall relative sensitivity is shown in the final column. The ion currents have been calculated per mole of peptide. This compensates for differences in the composition between batches of the peptide mix. These discrepancies are reflected by the differences in relative peak heights in the TIC traces in Figs. 3, 4a and 5a.

It can be seen from Table 2 that capillary combination F gives the greatest sensitivity in terms of peptide ion current. However, this arrangement was more difficult to optimise and a stable spray could not always be achieved. This

Table 1
Cross sectional areas of the capillary combinations investigated



Capillary Arrangement (See Fig. 2)	Area (μm^2)	Area 2 (μm^2)	Area 3 (μm^2)	$\frac{\text{Area 3}}{\text{Area 2}}$	$\frac{\text{Area 2}}{\text{Area 1}}$
A	4416	18 121	82 868	4.1	4.6
B	4416	19 006	171 444	4.3	9.0
C	4416	19 006	25 414	4.3	1.3
D	4416	19 006	51 810	4.3	2.7
E	4416	19 006	198 507	4.3	10.4
F	4416	19 006	119 320	4.3	6.3
G	4416	19 006	125 031	4.3	6.6.

Table 2
Sensitivities of the capillary combinations investigated

Capillary arrangement (see Fig. 2)	Angiotensin I	Bradykinin	Angiotensin II	Substance P	Rel. sensitivity
A	326 000 (0.68)	214 000 (0.91)	184 000 (1.00)	75 000 (0.49)	0.8
B	300 000 (0.63)	188 000 (0.80)	135 000 (0.73)	48 000 (0.31)	0.7
E	256 000 (0.54)	226 000 (0.97)	113 000 (0.61)	145 000 (0.95)	0.7
F	476 000 (1.00)	234 000 (1.00)	131 000 (0.71)	153 000 (1.00)	1.0

Figures are ion current per mole of peptide.
Normalised value shown in brackets.

led to instability in the electrical contact at the probe tip, which caused the separation to be degraded and the baseline to fluctuate.

Arrangements B and E were less sensitive to changes in operating parameters and therefore optimisation was simpler and prolonged operation was attainable. The separation integrity was maintained when either of these arrangements was used.

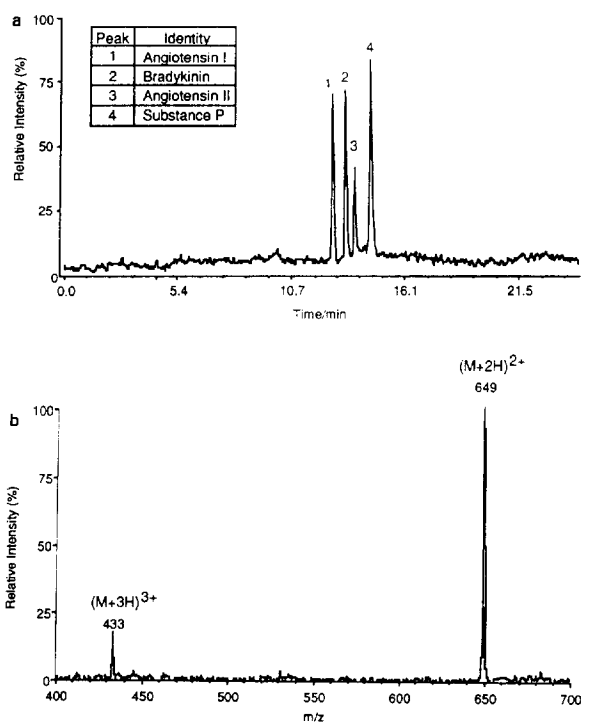


Fig. 4. (a) CE–MS TIC trace of the peptide mix using arrangement E. (b) ESI spectrum of angiotensin I, taken from peak 1 of Fig. 4a.

Fig. 4a is the TIC trace obtained from a CE–MS analysis using arrangement E. The spectrum of angiotensin I taken from the apex of the first peak is shown in Fig. 4b. To illustrate the performance of the system, CE–MS–MS experiments were performed and the TIC trace arising from interface B is reproduced in Fig. 5a. Fig. 5b is the product ion spectrum of angiotensin I taken from the first peak of the TIC.

Taking into account both sensitivity and the ease of optimisation and operation of the interfaces, the optimised capillary combination is suggested to have a surface area ratio which lies between those of arrangements F and B, and one such possibility is shown in Fig. 2G. As yet this combination has not been attempted because of the difficulty in obtaining the non-standard sized capillaries.

4. Conclusions

The dimensions of the capillaries in a coaxial capillary electrophoresis–electrospray mass spectrometry interface have been shown to affect the performance of the system in terms of both sensitivity and stability. These initial results suggest that a relationship exists between the ratio of the cross sectional areas of the outlet orifices between capillaries and the performance of the system, although further confirmation using different samples and buffer systems is

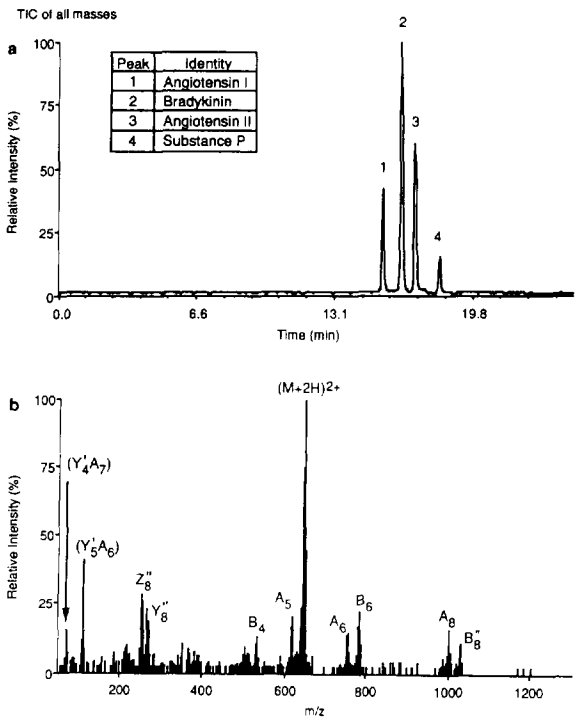


Fig. 5. (a) CE–MS–MS TIC trace from the peptide mix using arrangement B. (b) Product ion spectrum of angiotensin I, taken from peak 1 of Fig. 5a.

required. This relationship has led to the suggestion of an 'optimum' capillary arrangement. Currently we are unable to construct this interface as the stainless-steel capillary sizes required are non-standard and thus not readily available.

It has also been shown that it is possible to convert from thin to thick walled capillaries without any loss (indeed in some cases with a gain) in the performance of the system.

Acknowledgements

P.C. acknowledges the EPSRC and Zeneca Specialties (Blackley, Manchester, UK) for provision of funding for the project.

References

[1] J.A. Olivares, N.T. Nguyen, C.R. Yonker and R.D. Smith, *Anal. Chem.*, 59 (1987) 1230.

[2] R.D. Minard, D. Chinn-Fatt, P. Curry Jr. and A.G. Ewing, proceedings of the *36th ASMS conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 1988*, p. 950.

[3] E.D. Lee, W. Muck, J.D. Henion and T.R. Covey, proceedings of the *36th ASMS conference on Mass Spectrometry and Allied Topics, San Francisco, CA, 1988*, p. 1085.

[4] R.D. Smith, C.J. Baringa and H.R. Udseth, *Anal. Chem.*, 60 (1988) 1948.

[5] S. Pleasance, P. Thibault and J. Kelly, *J. Chromatogr.*, 591 (1992) 325.