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Optimization of the loading limit for capillary zone electrophoresis of synthetic opioid and tachykinin peptides: a study of the interactions among the amount of peptide, resolution, saturation, injection volume and capillary diameter

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Abstract

The upper loading limit in the capillary zone electrophoresis (CZE) of a mixture of fourteen synthetic opioid peptides and one tachykinin peptide was determined for capillaries of 50-, 75-, 100-, 130-, 150-, and 200-μm internal diameter (I.D.) to optimize the separation of these neuropeptides. The loading limit is an important parameter for preparative post-CZE studies. Electrophoretic resolution is illustrated for each capillary I.D. as the injection volume and amount of injected peptide increase. Loading limit is evaluated, based on the considerations of resolution, saturation, and current-stability. Among the capillaries studied, the capillary with 100 μm I.D. gives the optimal loading limit (39–78 pmol of each peptide).

1. Introduction

Since the work of Jorgenson and Lukacs [1,2], capillary zone electrophoresis (CZE) has become an important technique in separating peptides based on each peptide's charge and mass parameters [3–14]. Together, the high electrophoretic resolution that can be achieved using a high voltage (10–30 kV), and the selectivity that is different from conventional reversed-phase high-performance liquid chromatography (RP-HPLC), make CZE an excellent method for peptide separation and analysis. Other advantages of CZE include a short analysis time, high detection sensitivity, on-line detection, interfacing to a mass spectrometer (MS), long column life, low sample/reagent consumption, and automation.

The biomedical applications of CZE have been reviewed recently [15–17]. For many years, the aim of this laboratory has been to analyze neuropeptides extracted from various biological sources, including human tissues and fluids, using multi-dimensional RP-HPLC for sample preparation [18], and using radioimmunoassay

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(RIA) [19], MS [20], and tandem MS (MS–MS) [21] for qualitative and quantitative analyses [22]. CZE may also be used to prepare biological samples prior to the RIA, MS, and MS–MS detection of neuropeptides.

In order to fully utilize CZE in a preparative application, one must perform the separation with a maximal amount of sample without "overloading" the capillary. In this manuscript, the term "loading amount" is used to describe that experimental feature. This objective can be achieved by using a capillary of larger cross-sectional dimension (e.g. >50-μm I.D.). Others [23–25] have reported the use of capillaries as large as 100-, 150-, and 200-μm I.D. for fraction collection prior to peptide sequencing analyses. Theoretically, the increase in the amount of sample loading with capillaries with an increasing I.D. is a direct function of the capillary volume, or of the square of the capillary radius. However, increasing the capillary I.D. will also commensurately increase the current and thus Joule heating. Consequently, the resolution and current-stability will decrease; however, use of a cooling system in an instrument may minimize these deteriorations in system performance. The magnitude of the current and Joule heating will also depend on the ionic strength of the CZE buffer used for the separation. Therefore, in practice, it is not likely that one could accurately predict the increase in sample loading with larger I.D. capillaries without some basic evaluations obtained under a well-defined set of experimental conditions.

The present experiments were designed to study the inverse relationship between the increase in sample loading capacity and the decrease in resolution and current-stability by separating fourteen synthetic opioid peptides and one tachykinin peptide with CZE using capillaries with increasing I.D. (50, 75, 100, 130, 150, and 200 μm). The increase in sample loading is qualitatively and quantitatively determined by comparing the loading limits (defined here as the maximal amount loaded without significant deterioration of the separation and defined to be a function of the injection volume, amount of peptide injected, resolution, capillary volume, and current-stability) of these capillaries. Electrophoretic resolution of this mixture of 15 synthetic peptides is demonstrated for these capillaries as the injection volume, and thus the amount of peptides, increases. This present investigation aims to determine empirically the capillary I.D. that optimizes the fraction collection of selected neuropeptides such as substance P and methionine enkephalin from biological sources.

2. Experimental

2.1. Reagents and materials

All synthetic peptides were purchased from Sigma (St. Louis, MO, USA), and used without further purification. The synthetic peptides were methionine enkephalin (ME), ME-K, ME[O], ME-NH_2, ME-RGL, ME-RF, leucine enkephalin (LE), LE-R, LE-K, LE-NH_2, dynorphin (Dyn)A_{1-17}, DynB, preproenkephalin (PreproE), substance P (SP), and DynA_{1-13}, where K, [O], R, G, L, F, and NH_2 are lysine, sulfoxide, arginine, glycine, leucine, phenylalanine, and the uncharged carboxamide group, respectively. A detailed description of the amino acid sequence, molecular mass, and charge characteristics of these synthetic neuropeptides are given elsewhere [14]. The volatile CZE buffer was 20 m*M* ammonium formate (J.T. Baker, Phillipsburg, NJ, USA), titrated to pH 2.5 with trifluoroacetic acid (Pierce Chemical Co., Rockford, IL, USA). Fused-silica capillaries with 50, 75, 100, 130, 150, and 200 μm I.D. and 360 μm O.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Instrumentation

An ISCO Model 3140 Electropherograph (ISCO, Lincoln, NE, USA) outfitted with an IBM Personal System/2 Model 30 286 computer (IBM, Armonk, NY, USA) was used. The instrument has four vacuum levels: two for sample injections and two for column washing. A detailed description of these vacuum levels is given

elsewhere [26]. Operation of the CZE instrument and data collection were controlled by the ISCO ICE 3.1.0 level software.

2.3. Capillary zone electrophoresis

All fused-silica capillaries used (50–200 μm I.D.) were 98 ± 0.2 cm long, with a 68 ± 0.2 cm length from the capillary inlet to the middle of the detector window. Table 1 summarizes several of the pertinent parameters (measured, calculated, and experimental) for these capillaries. Injection volumes, denoted low and high, are used. Low refers to < 0.5% and high to 2% of the capillary volume. Prior to daily use, each capillary was preconditioned with the following sequence of solvents by applying high vacuum for ca. 2–13 (depending on the capillary I.D.) capillary volumes for each solvent: water, 1 *M* NaOH, water, 0.1 *M* HCl, water, and finally the ammonium formate buffer for the experiment. A volatile buffer system similar to that described by Johansson et al. [27] was chosen so that the collected sample could be processed further for MS detection. A low CZE buffer concentration (20 m*M*) was used throughout this study to minimize the current and Joule heating when larger I.D. capillaries were used, while still providing a sufficient electrophoretic focusing effect [28]. The peptide mixture contained 0.1 μg (47 to 180 pmol) of each peptide per μl of water. The injection volumes were determined as described [26]. The applied voltage was 27, 20, or 12.5 (± 1%) kV, and the temperature was regulated to 30 ± 0.5°C by the electropherograph's built-in air-circulating system.

3. Results and discussion

3.1. 50-μm I.D. capillary

Fig. 1A shows a typical analytical CZE separation of the 15 synthetic peptides using a 50-μm I.D. capillary, with a low injection volume (4 nl) and an amount (233–902 fmol) of each peptide at the subpicomole level. The capillary volume is 1.9 μl (Table 1). Thus, the 4-nl injection volume represents only a small portion (0.2%) of the capillary volume. The calculated surface area (μm^2)-to-volume (μm^3) ratio for a 50-μm I.D. capillary is 0.080 (Table 1). Because this value is high, the Joule heating generated in the capillary [1,2] is efficiently dissipated. Furthermore, Table 1 shows that the power (0.5 W) generated in the 50-μm I.D. capillary is the lowest among the capillaries studied because of its low current (ca. 20 μA).

In general, combination of these experimental factors (minimum amount of peptide injected, minimum injection volume, minimum analyte/sample-buffer concentrations, and a relatively high area-to-volume value for the capillary) is required for high CZE resolution [1,2,29,30]. Unfortunately, the high level of analytical performance as exemplified in Fig. 1A is of little practical value in preparative CZE, because often an insufficient amount of peptide can be collected for further manipulations, such as confirmation of its identity via sequencing analysis.

Fig. 1B–D shows the electropherograms when larger amounts [4.8, 9.8, and 24.3 pmol (average of the sum of the amount of each peptide)] of each peptide were injected (31, 63, and 157 nl,

Table 1
Summary of the measured, calculated, and experimental parameters of the capillaries

Capillary I.D. (μm)	Capillary volume (μl)	Area-to-volume ratio	Applied voltage (kV)	Monitored current (μA)	Power (W)
50	1.9	0.080	27	20	0.5
75	4.3	0.053	27	40	1.1
100	7.7	0.040	27	85	2.3
130	13.0	0.031	20	130	2.6
150	17.3	0.027	20	160	3.2
200	30.8	0.020	12.5	175	2.2

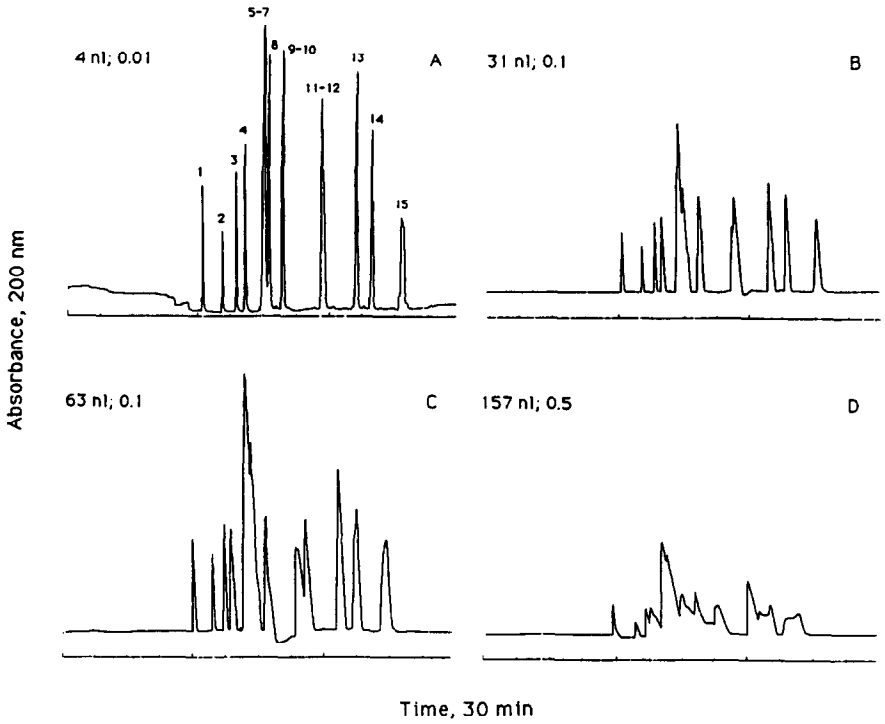

Fig. 1. Electropherograms of the peptide mixture on a 50-μm I.D. capillary at four different injection volumes. At 4-nl injection (A), peptides are: $DynA_{1-13}$ (No. 1; 312 fmol), $DynA_{1-17}$ (No. 2; 233 fmol), SP (No. 3; 371 fmol), DynB (No. 4; 318 fmol), PreproE (No. 5; 364 fmol), LE-K (No. 6; 731 fmol), ME-K (No. 7; 712 fmol), LE-R (No. 8; 702 fmol), ME-RF (No. 9; 571 fmol) ME-RGL (No. 10; 555 fmol), LE-NH_2 (No. 11; 902 fmol), ME-NH_2 (No. 12; 873 fmol), LE (No. 13; 899 fmol), ME (No. 14; 871 fmol), and ME[sulfoxide] (No. 15; 848 fmol). Injection volumes are 4, 31, 63, and 157 nl, and the second number represents the appropriate absorbance unit full scale (AUFS), respectively. Thus, the peptide amount is in the range of 0.2–0.9 (average = 0.6 pmol of each peptide), 1.8–7.1 (4.8 pmol), 3.7–14.2 (9.8 pmol), and 9.1–35.4 (24.3 pmol) pmol for (A–D), respectively. The monitored current was ca. 20 μA; the applied voltage was 27 kV.

respectively; representing 8×, 9×, and 40× more sample injected). These injection volumes represent 1.6, 3.3, and 8.3% of the capillary volume, respectively. Peak-broadening is apparent in Fig. 1B,C, but separation is largely maintained. Fig. 1D shows the decrease of the separation when the capillary is "overloaded" with peptide (24.3 pmol of each peptide) in an injection volume of 157 nl (8.3% of the capillary volume). This severe loss of resolution may be ascribed to saturation (i.e. the condition where the capillary gives an electrophoretic separation that is severely affected by excessive sample loading) of the capillary column. These data suggest that an injection volume >3% but ≪ 8% of the capillary volume may be used to increase the amount of sample loaded on the column for preparative purposes under the experimental conditions used; however, as a rule of thumb, the injection volume in CZE is recommended to not exceed 2% of the capillary volume [29]. If the maximum loading limit for these experiments is between the values shown in Fig. 1C and D, then an amount of peptide in the range 10–24 pmol is the loading limit that could be achieved with a 50-μm I.D. capillary under these experimental conditions. A loading amount close to the upper limit should be

avoided to prevent the loss of the electrophoretic resolution and peak-height as observed in Fig. 1D.

3.2. 75-μm I.D. capillary

Fig. 2 shows the CZE separation of the peptide mixture using a 75-μm I.D. capillary and six different injection volumes and amounts of peptide injected. The capillary volume is 4.3 μl, and the area-to-volume ratio is 0.053 (Table 1). The applied voltage is also 27 kV, but the monitored current increases nearly two-fold to ca. 40 μA. This two-fold increase in the monitored current also commensurately doubles the power (1.1 W) generated inside this capillary. Table 1 clearly demonstrates the interaction of these parameters; for example, as the capillary I.D. increases, the area-to-volume ratio decreases linearly, whereas the power increases linearly at the same applied voltage. Therefore, it is essential to know empirically when the power (Joule heating) becomes too large and the area-to-volume ratio too small to allow an efficient rate of Joule heat dissipation.

At the low injection volume of 10 nl (0.2% of

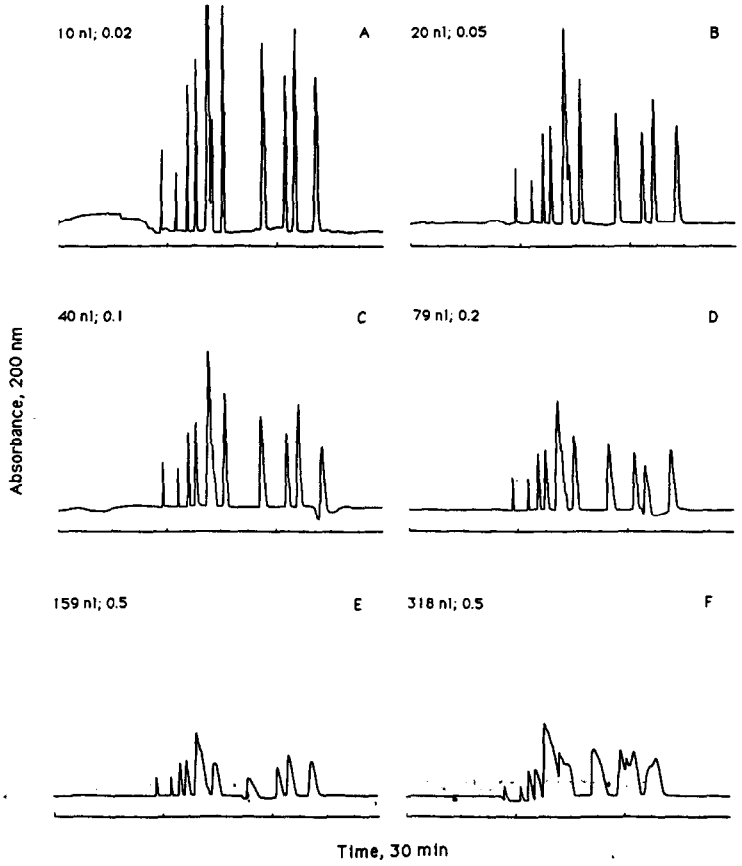

Fig. 2. Electropherograms of the peptide mixture on a 75-μm I.D. capillary at six different injection volumes. Injection volumes and AUFS are shown. Peptide amount is in the range of 0.5–2.3 (average = 1.6 pmol of each peptide), 1–4.5 (3.1 pmol), 2–9 (6.2 pmol), 4–17.8 (12.2 pmol), 8–35.8 (24.6 pmol), and 15.9–71.6 (49.3 pmol) pmol for (A–F), respectively. The monitored current was ca. 40 μA; the applied voltage was 27 kV.

the capillary volume), corresponding to 1.6 pmol of each peptide, the resolution in Fig. 2A is comparable to the resolution observed in Fig. 1A. The resolution deteriorates slightly as the injection volume increases to 20, 40, and 79 nl (representing 3.1, 6.2, and 12.2 pmol of each peptide in Fig. 2B–D, respectively). These injection volumes represent 0.5, 0.9, and 1.8% of the capillary volume, respectively. The observation that the resolution is relatively constant in Fig. 2B–D agrees with the fact that these electropherograms all have an injection volume $<2\%$ of the capillary volume. When the injection volume is increased to 159 nl (3.7% of capillary volume), which is an injection volume comparable to that of Fig. 1D, separation is still maintained, whereas the 50-μm I.D. capillary was obviously saturated at that point. The separation deteriorates when 318 nl (7.4% of the capillary volume) and 49.3 pmol of each peptide were injected (Fig. 2F); however, the loss of resolution and peak-height is less compared to the electropherogram shown in Fig. 1D, confirming that a 75-μm I.D. capillary may tolerate a larger injection volume, and thus a corresponding larger amount of peptide. These observations again indicate that an injection volume $>2\%$ of the corresponding capillary volume and 24.6 pmol of peptide may be used to maximize the loading in CZE used for preparative purposes.

If we consider that the electrophoretic separation deteriorates somewhere between the amounts of peptide used in Fig. 2E and 2F, then the maximal loading limit is in the range 25–49 pmol of each peptide. This range represents a ca. twofold improvement over that of the 50-μm I.D. capillary, which is in good agreement with the fact that the capillary volume of the 75-μm I.D. is ca. two times larger than the volume of the 50-μm I.D. capillary. Dissipation of Joule heating must be sufficient in the 75-μm I.D. capillary for this relationship to be observed.

3.3. 100-μm I.D. capillary

Fig. 3A shows the electropherogram of the 100-μm I.D. capillary with an injection volume of 16 nl (0.2% of the capillary volume) and an amount of 2.5 pmol of each peptide. Compared to Figs. 1A and 2A, where the injection volumes also are 0.2% of the corresponding capillary volume, Fig. 3A shows an increase in peak-width. The capillary volume is 7.7 μl, and the area-to-volume ratio is 0.040 (Table 1). A voltage of 27 kV can still be applied, because the monitored current (ca. 85 μA) remains below the limit of 100 μA and thus excessive Joule heating is avoided. However, both the increase of the current and the power and the decrease in the area-to-volume ratio may account for the loss of resolution seen in Fig. 3A, probably caused by the increased Joule heating. The loss in resolution for this mixture is relatively minor; furthermore, analytical electrophoretic separation is not the objective of this study.

When the injection volume is increased to 31, 63, and 126 nl (representing 0.4, 0.8, and 1.6% of the capillary volume, and 4.8, 9.8, and 19.5 pmol of each peptide, respectively) in Fig. 3B–D, respectively, the resolution remains relatively constant. This observation, which is similar to the discussion of Fig. 2B–D, indicates that the larger 100-μm I.D. capillary tolerates a correspondingly greater injection volume and also a correspondingly larger amount of peptides than the 75-μm I.D. capillary. No relatively greater loss of resolution is observed until the injection volume reached 3.3% (251 nl, Fig. 3E) of the capillary volume. Increasing the injection volume to 502 nl (6.5% of the capillary volume) gives a separation (Fig. 3F) that is similar to that shown in Fig. 2F, although the injection volume in Fig. 3F is much larger ($1.6\times$) than the injection volume in Fig. 2F. The electropherogram in Fig. 3F also shows a slightly better resolution than that shown in Fig. 2F. Again, Fig. 3E,F suggests that an injection volume $>2\%$ of the capillary volume may be used to maximize sample loading per separation.

Fig. 3E,F represent an amount of peptide of 38.9 and 77.8 pmol of each peptide, respectively, indicating that the loading limit of this peptide mixture was reached between these two values. This range represents a ca. four-fold improvement over that of the 50-μm I.D. capillary, in

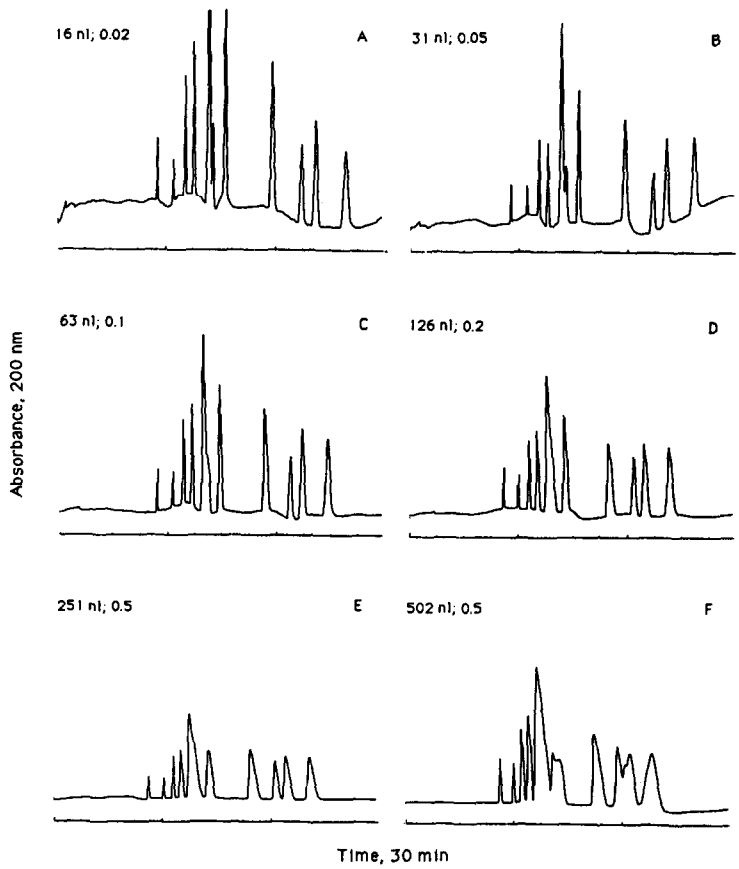

Fig. 3. Electropherograms of the peptide mixture on a 100-μm I.D. capillary at six different injection volumes. Injection volumes and AUFS are shown. Peptide amount is in the range of 0.8–3.6 (average = 2.5 pmol of each peptide), 1.6–7.0 (4.8 pmol), 3.2–14.2 (9.8 pmol), 6.3–28.4 (19.5 pmol), 12.6–56.5 (38.9 pmol), and 25.1–113.0 (77.8 pmol) pmol for (A–F), respectively. The monitored current was ca. 85 μA; the applied voltage was 27 kV.

good agreement with the four-fold increase in the capillary volume calculated for the 100-μm I.D. capillary. Again, the dissipation of the Joule heating must be sufficient for this relationship to be observed. The separation obtained with the 100-μm I.D. capillary gives a good example of the favorable trade-off between the slight loss of analytical resolution for that mixture and the multi-fold increase in the amount of peptide injected.

3.4. 130-μm I.D. capillary

Fig. 4A–D show the electropherograms for CZE performed on a 130-μm I.D. capillary at four different injection volumes and amounts of peptide injected. The capillary volume is 13 μl, and the area-to-volume ratio decreases further to 0.031 (Table 1). The applied voltage was reduced to 20 kV to reduce the monitored current to ca. 130 μA. The data in Table 1 clearly indicate that the power (2.6 W) generated inside of this capillary remains relatively high, while the area-to-volume ratio for this capillary decreases further to 0.031.

At a low injection volume (90 nl, 0.7% of the capillary volume), Fig. 4A shows that peptides No. 13 and 14 are now barely separated at baseline. These two peptides are well-separated at an injection volume of ca. 0.7% of the respective capillary volume for the 50-, 75-, and 100-μm I.D. capillaries (e.g. Fig. 2B,C,

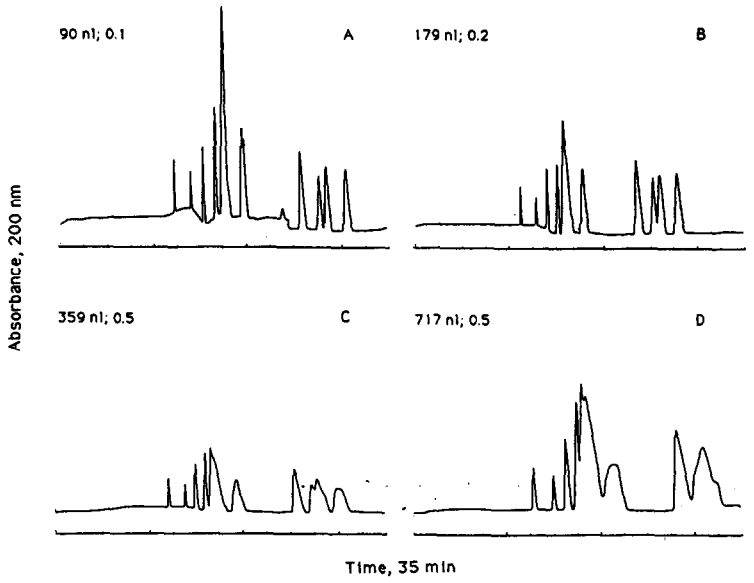

Fig. 4. Electropherograms of the peptide mixture on a 130-μm I.D. capillary at four different injection volumes. Injection volumes and AUFS are shown. Peptide amount is in the range of 4.5–20.3 (average = 14.0 pmol of each peptide), 9.0–40.3 (27.7 pmol), 18.0–80.8 (55.6 pmol), and 35.9–161.3 (111.1 pmol) pmol for (A–D), respectively. The monitored current was ca. 130 μA; the applied voltage was 20 kV.

and Fig. 3C, respectively). No baseline separation of these two peptides occurs when the injection volume was doubled (Fig. 4B), compared to the good separation of these two peptides at a comparable ratio of injection volume-to-capillary volume on the 50-, 75-, and 100-μm I.D. capillaries (Fig. 1B, 2D, and 3D, respectively). At an injection volume of 359 nl, representing only 2.8% of the capillary volume, Fig. 4C exhibits a far inferior separation compared to that shown in Fig. 1C, 2E, and 3E, which all have an injection volume-to-capillary volume in the range 3–4%. Also, comparison of Fig. 4C with Fig. 3E,F suggests that the 100-μm I.D. capillary is capable of providing better resolution than the 130-μm I.D. capillary at the same injection volume used in Fig. 4C. Finally, the separation deteriorates in Fig. 4D (717 nl, 5.5% of the capillary volume). These observations and comparisons suggest that the 130-μm I.D. capillary did not improve the loading limit because of the rapidly deteriorating resolution, especially compared to the 100-μm I.D. capillary.

Furthermore, in the process of testing this 130-μm I.D. capillary, the monitored current became unstable more quickly as the number of experiments increased, although the applied voltage was reduced. This instability is most likely due to the fact that the dissipation of heat is less efficient and because the temperature increase is faster inside this particular capillary. One way to circumvent this current instability is to replenish the capillary more often with cooler buffer. This process obviously consumes more time, and these enhanced experimental costs do not make it worthwhile to replace the 100-μm I.D. capillary in order to scale-up the separation.

3.5. 150-μm I.D. capillary

Fig. 5 shows the electropherograms obtained at two different injection volumes using a 150-μm I.D. capillary. The capillary volume is 17.3

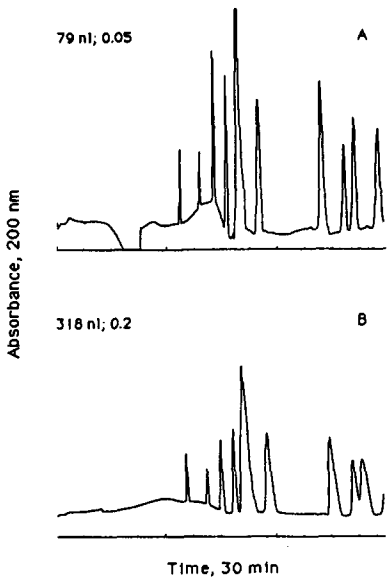

Fig. 5. Electropherograms of the peptide mixture on a 150-μm I.D. capillary at two different injection volumes. Injection volumes and AUFS are indicated. Peptide amount is in the range of 4.0–17.8 (average = 12.2 pmol of each peptide) and 15.9–71.6 (49.3 pmol) pmol for (A) and (B), respectively. In (B), the last peak that migrated after 30 min was present, although the computer failed to record it because of the pre-set processing time. The monitored current was ca. 160 μA; the applied voltage was 20 kV.

μl, and the area-to-volume ratio is 0.027 (Table 1). A constant voltage of 20 kV was applied, and the current increases further to ca. 160 μA. Although the problem of Joule heating and current is more severe than for the 130-μm I.D. capillary, this 150-μm I.D. provides a superior scale-up performance, compared to the 130-μm I.D. capillary.

Fig. 5A demonstrates a well-behaved separation at an injection volume of 79 nl (0.5% of the capillary volume), and exhibits a slightly better resolution than that shown in Fig. 2D, which also employed an injection volume of 79 nl. When the injection volume was increased ca. four-fold (318 nl), Fig. 5B shows a separation that is comparable to that shown in Fig. 3E, but represents a higher peptide amount of 49 pmol for each peptide.

Fig. 5B can also be compared to Fig. 2F, where the same injection volume was used. Such a comparison shows that the data in Fig. 5B represent a far better resolution than those in Fig. 2F. With an injection volume that is only 1.8% of the capillary volume, Fig. 5B also suggests that the injection volume and thus loading may be increased further, while still maintaining the separation without loss of resolution caused by saturation. However, as mentioned above for the 130-μm I.D. capillary, one should be prepared to deal with the problems associated with the increases in Joule heating and current.

Compared to the 130-μm I.D. capillary, Table 1 indicates that the power generated (3.2 W) in the 150-μm I.D. capillary increases by 23% at the same applied voltage, that the area-to-volume ratio decreases by 13%, and that the capillary volume increases by 33%. The fact that the 150-μm I.D. capillary performs better than the 130-μm I.D. capillary suggests that the decrease in area-to-volume ratio and the increase in Joule heating are relatively minor, compared to the increase in the capillary volume.

3.6. 200-μm I.D. capillary

Fig. 6 shows the electropherograms obtained at two different injection volumes using a 200-μm I.D. capillary. The capillary volume is 30.8 μl, and the area-to-volume ratio is 0.020 (Table 1). The applied voltage had to be further reduced to 12.5 kV to maintain the current (175 μA) below 200 μA. As previously reported [24], a capillary as large as 200-μm I.D. is capable of maintaining a well-behaved resolution as confirmed by Fig. 6A (126 nl, 0.4% of the capillary volume), although the separation time (e.g. 60 min) is longer due to the lower operating voltage permissible because of the relatively high current and Joule heating that result from operating at a higher voltage (e.g. 27 kV). Fig. 6B shows the separation when the injection volume was increased four-fold (502 nl; 1.6% of the capillary volume). Compared to the other capillaries at a similar injection volume-to-capillary volume ratio (Fig. 1B, 2D, 3D, 4B, and 5B), the data in Fig. 6B exhibit the worst resolution, in agree-

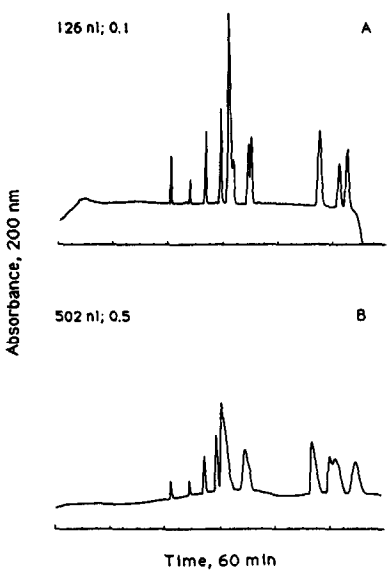

Fig. 6. Electropherograms of the peptide mixture on a 200-μm I.D. capillary at two different injection volumes. Injection volumes and AUFS are indicated. Peptide amount is in the range of 6.3–28.4 (average = 19.5 pmol of each peptide) and 25.1–113 (77.8 pmol) pmol for (A) and (B), respectively. In (A), the last peak was present, although the peak is not seen due to the negative dip. The monitored current was ca. 175 μA; the applied voltage was 12.5 kV.

ment with the fact that the increased Joule heating in this relatively large I.D. capillary deteriorates the resolution.

This figure can also be compared with Fig. 3F, where the same injection volume was applied. The differences between these two electropherograms are not significant enough to make one to choose 200-μm I.D. capillary over the 100-μm I.D. capillary at this injection volume. However, the 200-μm I.D. capillary will probably tolerate a higher injection volume before the separation degrades. For example, the data in Fig. 6B and Fig. 4C both exhibit very similar resolution, but the injection volume in Fig. 6B is much larger than that for Fig. 4C. In spite of this factor, one should, in general, be prepared to expect a relatively longer separation time (because of the reduced high voltage) and to face the problems associated with Joule heating and current like those encountered when using 130- and 150-μm I.D. capillaries.

4. Conclusions

The loading capacity for the CZE separation of fourteen synthetic opioid peptides and one tachykinin peptide is demonstrated using 50-, 75-, 100-, 150-, and 200-μm I.D. capillaries. The objective of this study is to experimentally determine an optimal I.D. capillary for scaling-up the CZE separation of these types of peptides for preparatory post-CZE purposes. This scaling-up process is in fact a trade-off between the benefit of using larger I.D. capillaries with a greater capillary volume and the two disadvantages of the decrease in capillary area-to-volume ratio that makes heat dissipation less efficient and of the increases in current and in current-instability. From examining and comparing the total of 24 electropherograms obtained from testing these 50–200-μm I.D. capillaries, the 100-μm I.D. capillary seems to be the best choice, also suggesting that Joule heating at 2.3 W can be tolerated by a capillary with an area-to-volume ratio of 0.040. The 24 electropherograms and the parameters in Table 1 will be useful in aiding one to predict the relationships among the amount of peptide, resolution, saturation, injection volume, and capillary diameter and to achieve optimal sample loading with the required resolution.

Acknowledgement

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