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Sterility Testing by Capillary Electrophoresis: A Comparison of On-line Preconcentration Approaches in Capillaries with Greater Internal Diameters

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Abstract

Detection of microbial contamination is of critical importance in the medical and the food industry. Rapid tests for the absence or presence of viable microorganisms are in urgent demand. Capillary electrophoresis is a modern analytical technique that can be adapted for rapid screening of microbial contamination. However, the small dimensions of capillaries allow introduction of only a small fraction of the sample, which can be problematic when examining large samples. In this article, we examine the possibilities of introducing larger sample volumes using capillaries with greater internal diameters (i.d.) together with different stacking techniques. The use of 0.32 mm i.d. capillary and the injection of 60 % of the capillary volume led to approximately 120-fold improvement of the injected sample volume over the classical injection 5% of a 0.10 mm i.d. capillary. The setup we described opens new possibilities in sterility testing using capillary electrophoresis.

Keywords

Capillary electrophoresis; sterility test; *Salmonella subterranea*; on-line preconcentration; capillary diameter

1 Introduction

The detection and identification of microorganisms in samples, especially pathogenic microorganisms, is a crucial and necessary procedure to ensure the safety and quality in the food/beverage [1, 2], pharmaceutical [3] and medical industries [4, 5]. Given the large numbers of diagnostic tests required, there is a strong demand for fast and accurate methods to assess sterility of products/samples. Traditionally, the direct inoculation method and its

modifications involve time consuming cultivation in a sterile growth medium with an aliquot of sample, which usually take days to weeks and works only for a defined group of microorganisms [2, 6]. Existing fast approaches such as hybridization, immunoassay and nucleic acid amplification (PCR) are more complex to carry out, require professional personnel training, and are organism specific rather than general all-encompassing techniques [2, 5, 7, 8].

Recently, capillary electrophoresis (CE) has been established as an approach for the fast separation and identification of microorganisms [5, 9-12]. Hjertén et al. [13] first showed that microbes and viruses have an ability to migrate in the electric field together with the electroosmotic flow. The actual effect of the orientation of a virus on its electrophoretic mobility was examined by Grossman and Soane [14]. In 1993, Ebersole and McCormick first successfully employed CE in the separation and identification of a series of four bacteria [15]. Under similar conditions, the electrophoretic mobilities of 3 different bacteria populations were determined and their separations were achieved by Pfetsch and Welch. [16]. However, these initial works required 250 cm long capillaries, showed large peak widths, long migration times and small differences in the migration times as compared to the CE of molecules. A method for fast separation of microorganisms with sharp peaks were established by Armstrong and coworkers [17-22] by introducing poly(ethyleneoxide), PEO, to the running buffer. The mechanism was explored [18, 22-25]. Two models of CE behavior were introduced: (i) interaction between the PEO molecules and microbes decreased the zeta potential of the microorganisms and induced aggregation to sharp zones, (ii) non-uniform velocities of non-spherical microorganisms caused collisions and similarly to the previous model the therefore aggregation. This technique was successfully applied in the determination of cell viability, identification of the causative pathogens of urine tract infections and food contamination [19-21]. Covalent coating of capillary wall was also used to minimize the microorganism-wall interaction and thus obtain good peak efficiencies [24, 26-28]. A “three injection method” for quick sterility test (which is to give a binary answer regarding the presence/absence of a wide variety of microorganisms) using a “blocking agent”, where all the microorganisms are concentrated and “swept” to one single peak was developed [9, 11, 12].

Despite the fact that small sample solution injection volume is an advantage for the CE analysis of small molecules, it can become an intrinsic disadvantage when it comes to analysis of microorganisms. On a per-particle basis, microbial solutions are generally more dilute than solutions of molecules. Typically the injection volume does not exceed a few tens of nanoliters, which raises the problem in real world analysis: Is the sample solution injected representative of the real sample? Supposing the sample concentration is 10^3 cfu/ml, which means it contains 1 microorganism in every μ L solution and the injection volume is 25 nL, then the probability of injecting a microbe is only 2.5%. This would lead to false-negative results and inaccurate quantification. One solution is on-line analyte concentration, which is performed by injecting large volumes of sample solutions and then focusing analytes to a narrow zone before analysis (see e.g. reviews [29 - 33]). Relatively few reports are focused on online concentration methods for the analysis of microbes [24, 34].

These facts (*vide supra*) give rise to an important challenge, which is how to introduce larger sample volumes while maintaining all of the other positive features of CE. The injected volume can be enlarged by extending the length of the capillary, like the large volume sample stacking method presented by Yu and Li [34], or by increasing the capillary diameter. Previous reports on CE analysis of bacteria used different capillary diameters: the group of Armstrong (e.g. [9, 11, 12, 17, 35]) employed 0.100 mm i.d. capillaries as well as Hjertén et al.[13], Yu et al. [34] and Ebersole et al.[15]; the group of Buszewski (e.g. [5, 28]) as well as Petr et al. [36] used 0.075 mm i.d. capillaries; and Pfetsch et al. [16] believed that the 0.250 mm i.d. capillary was better for determination of the electrophoretic mobilities of bacteria.

The influence of a capillary diameter on the CE performance has been studied extensively for molecular compounds [37]. Table 1 lists the physical/geometrical characteristics of different i.d. capillaries. Some of the conclusions from these studies mainly for the practical point of view are summarized below: (i) Poiseuille's equation relates the injection volume (V_c) to the applied pressure (P), inner diameter (d), injection time (t), solution viscosity (η) and capillary length:

$$V_c = \frac{\Delta P \pi d^4 t}{128 \eta L}. \quad (1)$$

The biquadrate of the capillary i.d. in equation (1) has a huge effect. The volume injected in the 0.32 mm i.d. capillary is more than 100-times higher than that in the 0.10 mm i.d. capillary while the total capillary volume increased only approximately 10-times. The pressure and injection time should be adjusted for percentage of injection volume over the total capillary volume when the i.d. of capillary is varied. In our study, injection pressure and duration was calculated based on Poiseuille's equation to meet these needs (see Table 1). The viscosity could be determined from equation (1) with apparatus by using pressurized drive of the marker (e.g. N,N-dimethylformamide) in the solution of interest [38].

(ii) Ohm's law describes the relationship between voltage (U), the current (I), the length of capillary (L), the inner diameter (d) and the buffer conductivity (κ):

$$U = IR = I \frac{L}{S \kappa} = I \frac{4L}{\pi d^2 \kappa}. \quad (2)$$

If the voltage 30 kV results a current of 20 μ A in the 0.10 mm i.d. capillary, the same voltage in the 0.32 mm i.d. capillary will result in a current of 200 μ A according to equation (2), which is unacceptable for CE analysis. In order to maintain the current (for reproducible results and to control the joule heating), the applied voltage needs to be lowered when large i.d. capillary is used, which greatly increase the analysis time.

These facts indicate that capillaries with larger i.d.s are possible to use, but they introduce additional mitigating factors that must be accounted for. The aim of this work was to compare different on-line preconcentration approaches with a possibility of using large volume injection in capillaries with higher i.d.s, mainly for the task of fast sterility testing.

2 Materials and methods

Tris(hydroxymethyl)-aminomethane (Tris), cetyltrimethylammonium bromide (CTAB), sodium hydroxide, hydrochloric acid and luria broth were obtained from Sigma Aldrich (Milwaukee, WI). Citric acid was purchased from Fisher Scientific (Fair Lawn, NJ). Nutrient and brain heart infusion broths were products of Difco Laboratories (Franklin Lakes, NJ). Dimethyl sulfoxide (DMSO) was purchased from EM Science (Gibbstown, NJ) and used as EOF marker. *Escherichia coli* (ATCC no. 10798), *Bacillus subtilis* (ATCC no. 12695), *Candida albicans* (ATCC no. 10231), *Rhodotorula* (ATCC no. 20254), and *Salmonella subterranea* (ATCC no. BAA-836) were purchased from American Type Culture Collection (Manassas, VA). Uncoated fused silica capillaries with i.d.s of 0.100 mm were purchased from Polymicro Technologies (Phoenix, AZ) and bare silica capillaries with i.d. 0.100 mm, 0.250 mm, 0.320 mm (Supelco brand) were purchased from Sigma Aldrich (St. Louis, MO).

All experiments were performed on a Beckman Coulter P/ACE MDQ capillary electrophoresis system equipped with photodiode array using capillaries with total length of 30 cm (20 cm to the detector). New capillaries were rinsed with 0.5 M NaOH, deionized water, 0.5 M HCl, and running buffer each for 10 min for conditioning before use. Between runs, the capillaries were washed with 0.5 M NaOH, deionized water, and running buffer for 5 min each. Working citrate/Tris buffers were prepared by dissolving an appropriate amount of citric acid in deionized water and then adjusted by titration with Tris to desired pH. CTAB was added to the final buffer with concentration of 1 mg/mL. All bacteria and fungi were cultured according to the instructions from the supplier. The microorganisms were initially grown in the appropriate liquid broth, and then plated on agar growth media and stored under refrigeration. All broths and agar were autoclaved (Primus autoclave, Omaha, NE) for 1 h prior to inoculation. For experiments, fresh liquid broth was inoculated with a single microbe colony that was taken from the agar plate. These cells were grown at 37 °C under gentle agitation for approximately 24 h, producing a cellular concentration of 10⁸ colony forming units (CFU)/mL. Cell concentrations were approximated by serial dilutions and plate-count methods when necessary. The microorganisms were centrifuged down, and the excess broth was removed. These cells were then washed with working citrate/Tris buffer or water, re-centrifuged, and finally re-suspended in the fresh buffer or water (same volume as the culture broth to maintain the microbe concentration) for analysis. All samples were vortexed for 30 s and sonicated briefly prior to analysis to disperse cellular aggregates. All run buffers, solutions, and vials used in the CE analysis were autoclaved prior to the run, too.

3 Results and discussion

3.1 Electroosmotic flow

The electroosmotic flow (EOF) has a large effect on the migration of bacteria. Therefore the effect of capillary i.d. on the EOF mobility was first evaluated. Capillaries from Supelco with three different i.d.s (0.10 mm, 0.25 mm and 0.32 mm) and capillaries from Polymicro with an i.d. of 0.10 mm were compared in Figure 1. The EO mobility was measured in 10 mM citrate/Tris buffers with pHs that varied from 3.0 to 8.0. In cases when the EOF was

weak, the method published by Williams and Vigh [39] was employed. Significant differences in the EO mobilities in all the capillaries were observed. However, all the curves presented a analogous EOF profiles [40] as shown in Figure 1. The EO mobility also differed in the capillaries with same i.d. (0.10 mm) from different manufacturers. Similar results were reported previously by Kohr et al [41].

3.2 Normal stacking mode

The normal stacking mode is the simplest sample concentration method [31]. It is based on an injection of a long plug of sample in a low conductivity matrix followed by applying high voltage for analysis. According to Ohm's law, the field strength of the sample zone will be higher than that of the rest of the capillary. As a result, the sample will stack near the interface [42]. The preconcentration effect could be enhanced by using a large volume injection; sometimes a sample is injected to more than 60 % of a capillary volume [29, 43]. This technique was explored primarily by using 10 mM citrate/Tris buffer at pH 7.0 (similar results were obtained for buffer with pH 8.0) in our study using an injection of 5 % of capillary volume. The preconcentration effect was first studied with the microbes suspended in deionized water using 0.10 mm i.d. capillaries from Polymicro. The preconcentration effectiveness was evaluated as the ratio of the peak height of microbes when suspended in deionized water versus when suspended in the running buffer. Following results were obtained: 2.2 for *Escherichia coli*, 1.8 for *Bacillus subtilis*, 2.2 for *Candida albicans*, 2.3 for *Rhodotorula*, and 2.5 for *Salmonella subterranea*. Since the purpose of this work was to optimize a method for sterility testing where separation of individual types of microorganisms was not needed, only *Salmonella subterranea* was further studied as a model microorganism.

Next we compared the analysis of the model microbe *Salmonella subterranea* in capillaries from Supelco with different i.d.s, as shown by the electrophoregrams in Figure 2. The percentages of injection volumes were kept the same (here, 72 - 75% of the total capillary volume). The peak height significantly increased with higher capillary i.d.s. This is because: 1) a larger sample volume was injected when using the larger i.d. capillaries (i.e. 10 times when the 0.32 mm i.d. capillary was used compared to that when the 0.10 mm i.d. capillary was used); 2) The optical pathlength was longer for larger i.d. capillaries. As a result, the peak height was 20 times higher when the 0.32 mm i.d. capillary was used compared to that obtained with the 0.10 mm i.d. capillary. As capillary i.d. was increased, the applied voltage was also adjusted in order to maintain the current around 60-80 μA . Therefore the migration time of the microbe sample was longer in the larger i.d. capillaries. The apparent mobilities of the microbes were determined to be the same ($22 \text{ m}^2\text{V}^{-1}\text{s}^{-1}$), but the relative standard deviations increased with higher capillary i.d.s (2.2 % for 0.10 mm i.d. capillary, 6.5 % for the 0.25 mm i.d. capillary, and 9.6 % for the 0.32 mm i.d. capillary).

Figure 3 depicts the peak height as a function of injection volume percentage (over the total capillary volume). Assuming that the absorbance is directly proportional to the length of the absorbing media, the peak height was normalized in regard to optical path length using an i.d. increment factor (1.0 for 0.10 mm i.d., 2.5 for the 0.25 mm i.d. capillary and 3.2 for the 0.32 mm i.d. capillary), where i.d. increment factor is the ratio of capillary i.d. to 0.10 mm

(which is the smallest capillary i.d. used). The preconcentration effect was evaluated as the corrected peak height, which was calculated as peak height divided by the i.d. increment factor. As shown in Figure 3, the corrected peak heights all increased as the injection volume percentage increased for all three capillaries with different i.d.s, while the effect is more significant in larger i.d. capillary. The increase between 0.10 mm i.d. capillary and 0.25 mm i.d. capillary is not as high as was expected. Two explanations are possible. First, the mechanism of aggregation depends on the free movement of bacterial cells and deviations from the flat EOF profile in the larger capillaries could have an additional effect on the aggregation. Second, the aggregation is affected by the electric field strength as described by Zheng and Yeung [23]. In the larger capillaries, current requirements cause a decrease in the electric field strength and therefore it could affect the aggregation.

3.3 Stacking in the reverse EOF mode

Cetyltrimethylammonium bromide (CTAB) has been used to reverse the EOF in CE [11, 12, 44, 45]. Generally, the method with reversed EOF represents a typical option for analysis of anionic species [45, 46]. As with methods that use the normal direction of the EOF (from the anode to the cathode), stacking based on-line preconcentration could be used in the reversed EOF mode as well [44]. In our case, a BGE containing 10 mM citrate/Tris pH 7.0 with 1 mg/mL CTAB was used. This CTAB concentration was found to be sufficient to form the anodic EOF [45]. The EO mobility was measured in all the three Supelco capillaries using dimethylsulfoxide as the EOF marker. The EO mobility was measured: -42 (SD for 5 runs was 3) $\times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ for the 0.10 mm i.d. capillary, -48 (7) $\times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ for the 0.25 mm i.d. capillary, and -53 (10) $\times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ for the 0.32 mm i.d. capillary. This indicates that EO mobilities and deviations increase with capillary i.d.s. Similarly to stacking with normal EOF conditions, the preconcentration effectiveness in terms of peak height in all the three capillaries was studied as a function of the injection volume percentage (Figure 3). The migration time of *Salmonella subterranea* increased from approximately 6.9 min when using a 0.10 mm i.d. capillary to approximately 15 min for the 0.32 mm i.d. capillary, which means there was a 2.5 times prolongation of the analysis time with a 10-fold increase of the injection volume. However, the peak height in 0.32 mm capillary was 20 times greater than that in 0.10 mm capillary, which is the result of a combination of the larger injection volume, longer optical path length and the stacking effect. Moreover the use of CTAB had additional advantages. The formation of random spikes in CTAB based electrolytes was fully suppressed, probably due to the dynamic coating of a capillary wall by CTAB molecules and the overall equilibrium in the capillary.

3.4 Stacking induced by pH

The next method examined for the on-line preconcentration of microorganisms was the use of a junction between electrolytes with different pHs (pH induced stacking) [31, 47, 48]. Generally, two possible setups could be used: 1) the microbes are suspended in acidic buffer solution while the running buffer has basic pH, or 2) the microbes are diluted in the basic buffer solution and the running buffer has an acidic pH. The stacking mechanism is based on the assumption that the mobility of microbes will be different in the acidic BGE than that in the basic BGE. However a side effect of the use of a low pH electrolyte was described earlier. The microbes have an increased tendency to form clusters [11], not only composed

from single species but also hybrid clusters from more than one species [49]. Nevertheless in the case of sterility testing, there is no need to separate the microbe clusters.

We studied the potential of pH induced stacking with 10 mM citrate/Tris pH 3.0 and 10 mM citrate/Tris pH 8.0 in all three capillary dimensions (of the Supelco brand). Since the difference in EO mobility in those BGEs is approximately 10-fold (Figure 1), the analysis time increased in the BGE at pH 3. *Salmonella subterranea* suspended in water gave a peak at approximately 14 min in 10 mM citrate/Tris pH 3.0 while it gave a peak at approximately 3 min in 10 mM citrate/Tris pH 8.0, both in the 0.10 mm i.d. capillary. When the 0.32 mm i.d. capillary was used, the current was not stable and the analysis time was more than two hours, which was not acceptable for a fast and efficient analysis. Figure 4a shows an example of the *Salmonella subterranea* analysis where the microbes were re-suspended in the BGE at pH 8.0 and the separation was performed in the BGE with a pH of 3.0. Increasing the injection volume from 5 % to 20 % of the total capillary volume did not adversely affect the preconcentration. The opposite system, where *Salmonella subterranea* was suspended in the BGE at pH 3.0 while the separation in the BGE at pH 8.0, was tested, too (Figure 4b). The Again, the increase in the injection volume did not show any effect on inhibit the preconcentration. Generally, the difference of the EO mobility or more precisely the mobility of the pH boundary had an important role here. However, in the same manner, decreasing the electric field strength in 0.25 mm i.d. capillaries could affect the formation of aggregates and the separation.

3.5 Electrokinetic injection

The last tested on-line preconcentration technique was electrokinetic injection. Electrokinetic injections can achieve from 100 to over 100,000-fold sample preconcentrations [47, 50 - 52] when combined with different stacking modes, such as field amplified sample stacking (FASS, sometimes called field enhanced sample injection, FESI) and sweeping combined with cation (or anion) selective exhaustive injection. The following equation can be used to estimate the amount of analyte injected:

$$n_i = S c_i l_i = S c_i \mu_{app} E t_{inj} \quad (3)$$

Where S is the cross-sectional area, c_i is the concentration of the ion species i , μ_{app} is the apparent mobility of the ion species i , E is the electric field and t_{inj} is the injection time.

According to equation (3), the beneficial preconcentration effect from electrokinetic injection using larger capillary i.d.s would be negated by the fact that injection voltage has to be lowered to maintain the current below 80 μ A. However, the sensitivity can still benefit from the increased optical path length and the greater injection volume.

These supposed effects were then confirmed experimentally. The FASS technique was tested for the *Salmonella subterranea* standard sample. In this technique, a short water plug was hydrodynamically introduced prior to the electrokinetic injection of the sample solution. 10 mM citrate/Tris (pH 8.0) with 1 mg/mL CTAB was used as the background electrolyte and *Salmonella subterranea* was suspended in buffer solution that was diluted ten times

from BGE or in plain water. However, analysis using capillaries with 0.25 mm and 0.32 mm i.d. were not successful due to the long analysis times and unstable currents. A successful analysis was performed only in the 0.10 mm i.d. capillary using an injection voltage of 10 kV in the reverse polarity mode (Figure 5). The influence of injection time was studied in the range of 10 – 90 s. However, generally longer injections resulted in unstable currents and irreproducible results. Analysis obtained with microorganisms re-suspended in diluted BGE was more reproducible than in water. Figure 5 compares the electropherograms obtained with electrokinetic injection and hydrodynamic injection.

4 Conclusions

To improve the sensitivity of CE analysis of microorganisms and the reliability of sterility tests of dilute microorganism solutions, several preconcentration techniques combined with injection volume increases using capillaries with different i.d.s were explored. Possible theoretical benefits were examined experimentally. A comparison of all the studied approaches was made (Table 2). The use of large volume sample stacking in the 0.32 mm i.d. capillary with a 60% injection volume gave a 120-fold increase in the injection volume compared to the use of a 0.10 mm i.d. capillary with 5% injection volume. Thus the probability of a positive match between injected sample and real sample can be greatly improved when using very dilute samples. Another advantage of using large i.d. capillaries is the increase in the optical path length, which in turn leads to increased sensitivity and an improvement in the detection limits. Interestingly, the preconcentration effect was also improved when larger i.d. was used. It was shown that a 16-fold increase was observed for the corrected peak height when large volume sample stacking was used with 60% injection volume in the 0.32 mm i.d. capillary compared to regular CZE with 5% injection in a 0.10 mm i.d. capillary.

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Figure 1. A dependence of the EO mobility on the electrolyte pH

(a) 0.25 mm i.d. capillary (Supelco), (b) 0.32 mm i.d. capillary (Supelco), (c) 0.10 mm i.d. capillary (Polymicro), (d) 0.10 mm i.d. capillary (Supelco); BGE: 10 mM citrate/Tris buffer. See section 2 for details.

Figure 2. Analysis of *Salmonella subterranea* upon stacking conditions in capillaries with different inner diameters

Conditions: 10 mM citrate/Tris pH 7.0; 0.10 mm i.d. capillary: 0.5 psi for 60 s injection (75 % of total volume), 30 kV; 0.25 mm i.d., 0.1 psi for 50 s (72%), 5 kV; 0.32 mm i.d., 0.1 psi for 30s (74%), 2.5 kV. See section 2 for details.

Figure 3. The effect of injected volume percentage on corrected peak heights in capillaries with different i.d.s

A: Normal stacking conditions; BGE: 10 mM citrate/Tris pH 7.0, **B:** Stacking conditions in the reverse polarity mode using CTAB; BGE: 10 mM citrate/Tris pH 7.0 with 1 mg/mL CTAB; Other conditions are the same for both modes: *Salmonella subterranea* was suspended in water, injection pressure and duration was calculated with Poiseuille's equation (a) 0.32 mm i.d. capillary, (b) 0.25 mm i.d. capillary, (c) 0.10 mm i.d. capillary (all the capillaries are from Supelco). See section 2 for details.

Figure 4. Analysis of *Salmonella subterranea* upon pH stacking conditions in capillaries with different inner diameters

Conditions: A: 10 mM citrate/Tris pH 8.0 as BGE, sample was suspended in 10 mM citrate/Tris pH 3.0; B: 10 mM citrate/Tris pH 3.0 as BGE, sample was suspended in 10 mM citrate/Tris pH 8.0; voltage: 10 kV for 0.10 mm i.d. capillary, 5 kV for 0.25 mm i.d. capillary. See Section 2 for details.

Figure 5. A comparison of injection types in the analysis of *Salmonella subterranea*
Conditions: 10 mM citrate/Tris pH 8.0 with 1 mg/mL CTAB as BGE, -10 kV (reverse polarity). Electrokinetic injection: first inject water plug by pressure at 0.5 psi for 2s; then inject *Salmonella subterranea* in 1 mM citrate/Tris pH 8.0 by voltage (-10 kV) for 90s; hydrodynamic injection: inject *Salmonella subterranea* in water with pressure 0.5 psi for 5s (6% of the capillary volume). See section 2 for details.

Table 1

Capillary characteristics

capillary i.d.	0.10 mm	0.25 mm	0.32 mm
capillary volume (length 30 cm)	2.36 μL	14.7 μL	24.1 μL
capillary volume increase	1.0 times	6.3 times	10.2 times
probability of a positive match with 60 % volume injection from 1cfu/50 μL sample	2.8 %	17.7 %	29.0 %
injected volume (5 s by 0.5 psi) *	0.1 μL	4.2 μL	11.4 μL
% of capillary injected *	4.6 %	28.8 %	47.2 %

* calculated for injection time 5 s, pressure 0.5 psi, capillary length 30 cm and sample viscosity 1.3 mPas

Table 2

A Comparison of different on-line preconcentration approaches in terms of relative corrected peak heights^{*} for analysis of *Salmonella subterranea* in capillaries with different i.d.

Online preconcentration approaches	0.10 mm	0.25 mm	0.32 mm
Normal CZE mode (injection from BGE, 5 % of the cap. vol.)	1.0	1.3	1.7
Normal stacking mode (injection from water, 5 % of the capillary volume)	2.5	3.1	3.6
Large volume sample stacking mode (60 % of the capillary volume)	5.4	6.0	12.5
Large volume sample stacking in the CTAB mode (60 % of the capillary volume)	5.7	8.1	16.0
pH stacking mode with BGE pH 8.0 (injection from pH 3.0)	3.0	6.3	-
pH stacking mode with BGE pH 3.0 (injection from pH 8.0)	3.2	4.1	-
electrokinetic injection (90 s, -10 kV)	8.6	-	-

* The relative corrected peak heights were the ratios of the corresponding corrected peak heights over the corrected peak heights obtained in the normal CZE mode in 0.10 mm i.d. capillary