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Preparation of highly condensed polyacrylamide gel-filled capillaries with low detection background

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Abstract

A method was established for the preparation of capillaries filled partially with highly condensed polyacrylamide gels (including step gradient gels) used for separation and partially with a buffer used for detection. These novel capillaries combine the high resolution of gel-filled capillaries and the low background of buffer-filled capillaries. They can be used for more than 1 week or for more than 50 injections, as demonstrated by the separation of poly-(α,β)-D,L-aspartate. The detection limit tested with diaspartate is about two orders of magnitude lower than that with the totally gel-filled capillaries. Some problems such as baseline drifting arise when using these capillaries but can be overcome by pre-running the capillaries several times with highly concentrated samples.

1. Introduction

Capillary gel electrophoresis, suggested by Hjerten in 1983 [1], has been valued as a new method for high-speed DNA sequencing [2–8] and demonstrated to be a powerful tool for the size analysis of other substances such as oligosaccharides [9–11], polyamino acids [12] and proteins [13–16]. However, the usefulness of this method depends on whether the separated bands can be detected. Gel-filled capillaries have a strong UV background, which leads to low or poor detection sensitivity. Another problem in using this method is the difficulty in preparing the gel filled capillaries: voids develop in the immobilized cross-linked gels or the gels not immobilized migrate in uncoated capillaries because of electroendosmosis [4,17], leading to irreproducible separation and/or re-formation of voids.

A way to overcome these problems is to use replaceable gels or entangled polymer solutions [18–24]. These solutions have a low background and/or are UV transparent [20]. They can easily be filled into a capillary and renewed whenever necessary. Nevertheless, Liu et al. [10] and Dolnik and Novotny [12] have demonstrated that highly condensed (or high-concentration) gels are essential to separate oligosaccharides and polyamino acids well. Unfortunately, condensed gels are not replaceable. To prepare capillaries which such condensed gels, special filling or polymerization methods are needed and one or two methods have been developed [17,25]. However, the high background of the capillaries remains a problem or becomes even more serious because

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the condensed gels such as polyacrylamide have a much stronger UV absorption than the low-percentage gels. Although laser-induced fluorescence (LIF) detection is an excellent way to overcome the detection problem [9–12], the samples should be fluorescent or must be labelled with fluorescent agents, which more or less influences the structure of the samples. It might also be questionable whether or not all the samples can be labelled or can be homogeneously labelled. In addition, the construction of the detector is much more expensive that for a UV detector. UV spectrophotometry therefore preferred as an inexpensive, universal detection method but low-background gel-filled capillaries should be available.

In this paper, we discuss a method for the preparation of such low-background capillaries. These capillaries are filled partially with polyacrylamide gels used for separation and partially with buffer used for detection. To eliminate voids, the separation gels are not immobilized but blocked by two plugs of immobilized gels to overcome the gel migration problem. With this method, capillaries with more than one step of separation gels can also be prepared. The detection sensitivity, stability and performance of the resulting capillaries were tested by the separation of polyaspartate and diaspartate. Some problems in using the resulting capillaries were found and are discussed, but the peak identities are not discussed because the elution standards available are insufficient.

2. Experimental

2.1. Materials

Tricine [N-tris(hydroxymethyl)methylglycine], bicine [N,N-bis(2-hydoxyethyl)glycine], γ-methacryloxypropyltrimethoxysilane, poly(Asp) [poly-(α,β)-D,L-aspartate Na^+, M_r (average molecular mass detected by viscosity) = 6850], aspartic acid and diaspartate were purchased from Sigma (St. Louis, MO, USA). Acrylamide and Bis [*N,N'*-methylenebis(acrylamide)], electrophoretically pure, were obtained from Bio-Rad Labs. (Richmond, CA, USA). Tris [tris(hydroxymethyl)aminomethane], TEMED (N,N,N',N'-tetramethylethylenediamine), APS (ammonium peroxodisulfate), TEA (triethanolamine) and other chemicals were of analytical-reagent grade from Merck (Darmstadt, Germany). Fused-silica capillaries of O.D. 375 μm and a wide-bore capillary of 0.53 mm I.D. were obtained from Composite Metal Services (Worcestershire, UK). The water used was purified with a Millipore Super Q system.

2.2. Filling principle

There are at least three principles that can be used for filling solutions into capillaries: (1) capillary surface tension or capillary force, that is, when a capillary is dipped into a solution, the solution will automatically flow into the capillary; (2) vacuum; (3) pressure. For filling solutions with low viscosity, using the capillary force is very convenient. The filling can be speeded up by lowering the other end of the capillary. For filling viscous liquids such as gelling solutions, using the capillary force combined with slight pressure is adopted for easy manipulation.

There are several ways to pressurize a solution slightly, of which only one way is described, that is, using sealed glass vials as micro-pumps [25]. A glass vial (Beckman, part No. 358807) is sealed using a modified screw-cap (Beckman, part No. 360004) with a rubber septum and a PTFE septum (Millipore, part No. 73005) as shown in Fig. 1A. The vial is pressurized by injection of air or solution into it and can also be evacuated by drawing the air out of the vial (Fig. 1B). These sealed vials are also used for polymerizing the acrylamide under slight pressure (Fig. 1C), for degassing a solution (after evacuation) and for drying a capillary (by injection of >10 ml of air into the vial; see Fig. 1B).

2.3. Partial silanization of capillaries [3,4,25,26]

One end of a new capillary is dipped into a 0.5% (v/v) solution of γ-methacryloxypropyl-trimethoxysilane in acetic acid–methanol (1:250, v/v) until the solution inside the capillary

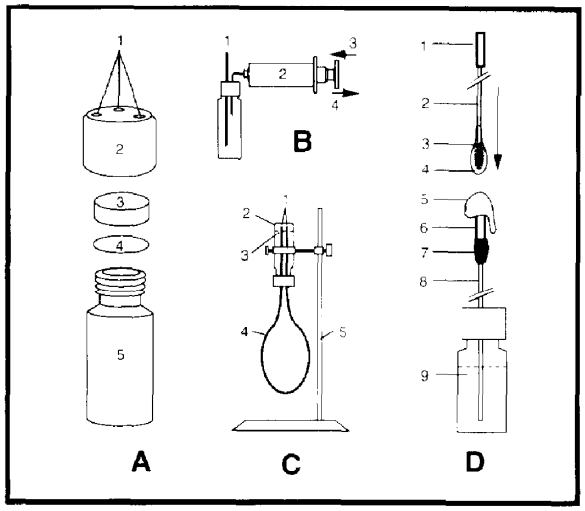

Fig. 1. (A) Structure of a sealed vial. 1 = 1.5 mm diameter holes, two used for plugging capillaries and one for syringe needle; 2 = screw-cap; 3 = rubber septum (2–3 mm thick); 4 = PTFE septum; 5 = glass vial. (B) Sealed vial with a capillary (1), pressurized (3) or evacuated (4) using a syringe (2). (C) Capillary filled with gelling solutions pressurized using a sealed vial. 1 = Capillary tips; 2 = sealed vial, pressurized by injection of 3.5–4 ml of ice-cooled water; 3 = water level; 4 = filled capillary; 5 = hanging device. (D) An important step for coupling a gel-filled capillary with a buffer-filled capillary where the arrow shows that the gel-filled capillary is being plugged into the connector against the buffer flow. 1 = Buffer-filled casing tube; 2 = gel-filled capillary; 3 = Pattex sealing membrane; 4 = a drop of buffer to prevent the polyacrylamide gel from drying out; 5 = buffer flow; 6 = connector; 7 = fixative; 8 = buffer-filled capillary; 9 = sealed vial with buffer, pressurized by injection of about 5 ml of air into the vial.

reaches the height required (measured from the dipped end). The capillary is removed from the solution and laid on a table at room temperature for 10–20 min, during which the solution filled in it will evaporate naturally. To compensate for the evaporation, the capillary end should be re-dipped into the silane solution every 2–5 min. The capillary is then washed for 5 min by drawing water into the not yet modified end and dried for 10–15 min (see Section 2.2 and Fig. 1B). It is critical to dry capillary completely, otherwise the filling speed cannot be controlled further. The other end of the capillary is modified and dried in the same way except that the water for washing is drawn in from the first modified end. All the parts filled with the silane solution are considered to be completely silanized.

2.4. Preparation of partially coated capillaries

A partially silanized capillary is completely filled with a 3.5% (w/v) solution of acrylamide in 0.1 *M* Tricine–0.05 *M* Tris containing 0.1% (v/v) TEMED + 0.1% (w/v) APS, and kept for at least 30 min. The capillary is then washed with water for 15 min and dried for 5–10 min.

2.5. Preparation of totally coated capillaries

A new capillary is washed first with 1 *M* NaOH for 1 h and then with water and methanol for 30 min each. This capillary is completely filled with the silane solution mentioned above. After keeping for 1 h, the capillary is washed again with methanol and water for 30 min each and then filled with 3.5%T gelling solution and kept for 1 h. The capillary is finally washed with 0.1 *M* Tricine–0.05 *M* Tris buffer for 30 min and dried for more than 10 min. This coated capillary can be stored at room temperature for more than 5 months.

2.6. Gelling solutions

All the gelling solutions are prepared in 0.1 *M* (final concentration) Tricine–0.05 *M* Tris from stock solutions of 40%T + *X*%C ($X \geq 0$), where %T is the grams of the total monomers in 100 ml of water solution and %C the grams Bis in 100 g of the total monomers. The solutions are placed inside evacuated glass vials and degassed for about 10 s by ultrasonic shocking [25]. Just before filling, TEMED and APS are added to a final concentration of 0.04–0.05% each. The solutions, after addition of APS and TEMED, should be filled into the capillary at 15–20°C within 8 min.

2.7. Preparation of partially or step gradient gel-filled capillaries

A general structure of the partially gel-filled capillaries, described with the positions of the

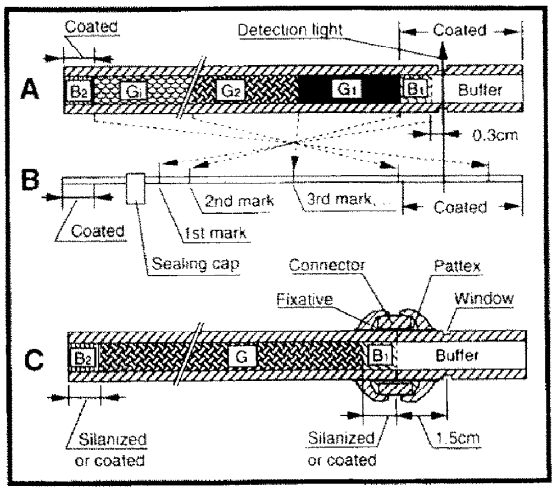

Fig. 2. Schematic configurations of (A) a partially or step gradient gel-filled capillary and (C) a coupled capillary where the boundaries between the different gels are defined by the positions of the initial solutions. (B) Capillary ready for filling gelling solutions. B_1 = immobilized cross-linked gels (<20%T); B_2 = immobilized linear gels (≥10%T) or cross-linked gels at the injection end of the capillary; G_1–G_i and G = gels used for separation.

initial gelling solutions, is shown schematically in Fig. 2A, where G_1–G_i ($i \geq 1$) are the gels used for separation and their concentrations are arranged as $G_1 > G_2 > \cdots > G_i$. B_1 and B_2 are a short plug (≥1 cm) of immobilized cross-linked gels (≤15% T + 5%C) or linear gels (≥10%T) used for preventing the separation gels from migrating. When $G_1 < 20\%T$ (+5%C) and $G_i \leq 10\%T$ (+5%C), B_1 can be replaced by a portion of immobilized G_1 and B_2 by a portion of immobilized G_i.

To prepare such a capillary, the buffer portion is generally first filled into the capillary to create a clean detection environment; it is then followed by solutions of $B_1, G_1, \ldots, G_i$. B_2 is filled into the capillary at the final step. If this filling sequence is reversed, that is, B_2 is first filled in and the buffer is filled in after all the gelling solutions, the detection background of the resulting capillaries may not clear because the buffer may be seriously contaminated by the gelling solutions remaining at the capillary wall. In this case, more than 2 h of equilibration (see below) are required to clean the background. The buffer portion can also be filled in between the gels, for instance, the capillary can be filled first with a portion of B_1 and then with a portion of the buffer followed by B_1 and other gelling solutions.

It is clear that, to fill different solutions into one capillary, the capillary should be transferred from solution to solution several times, and how the tube is transferred is critical. To prevent air bubbles from being introduced into the capillary, the transfer time should be less than 2 s, which is especially important when transferring a capillary from a buffer to other solutions because most of the buffers have low viscosity. Whenever a bubble is observed during filling, the capillary should be re-washed with water for 10 min and then dried for re-filling. A more detailed filling procedure is as follows.

(1) One end of a new capillary is coated (see above) for 3–5 cm and the other end for 10 cm (2 cm for immobilization of gel and 8 cm for buffer). The buffer part of the capillary is coated in order to reduce electroosmosis.

(2) The capillary is marked from the shorter coated end as shown in Fig. 2B and then mounted with a sealing cap, which will be used for speeding up the filling when necessary.

(3) The shorter coated end is dipped into a newly degassed buffer of 0.1 *M* Tricine–0.05 *M* Tris until the buffer level inside the capillary reaches the first mark.

(4) The capillary is vertically and quickly removed from the buffer and immediately dipped into B_1 solution until the buffer level reaches the second mark.

(5) As in (4), the capillary is transferred to the vial with G_1 solution until the buffer reaches the third mark. This step is repeated until G_i and B_2 are filled in. The buffer level should, at this point, reach the other end of the capillary. The filling, if too slow, is speeded up by sealing the vial, which is then injected with less than 2 ml of air (see also Fig. 1B).

(6) For polymerization, both ends of the filled capillary are plugged into one sealed vial and about 3.5–4 ml of ice-cooled water are then injected into the vial to build up a slight pressure of 5–7 atm and to lower the polymerization

speed at the ends of the capillary [25]. The tips of the capillary are set at a higher level than the water in the vial (Fig. 1C) so that the empty space at the ends, a result of gel shrinkage, can be measured after polymerization. The capillary is hung for about 4 h in a windless and shockless location at 15–20°C. After hanging, at least 0.5 cm of both ends of the capillary are cut off to remove the empty ends.

(7) The resulting capillary is equilibrated with 0.1 *M* Tricine–0.05 *M* Tris buffer at 200 V/cm and 25°C for at least 1 h and then stored at room temperature with both ends dipped into the buffer. The capillaries can be stored for more than 3 months.

(8) The detection window is opened just before using the capillary for the separation of samples. About 2 mm of the over-coating at the buffer part of the capillary (0.3 cm from the boundary of the buffer, Fig. 2A) are manually removed using a scalpel and further cleaned with methanol.

2.8. Preparation of capillaries totally filled with gels

Such capillaries can be prepared by cutting off the buffer part of the partially gel-filled capillaries or by the following method. A partially silanized capillary (3–5 cm of silanization from both tips) is filled with 3–5 cm of B_1 solution followed by sections of separation gelling solutions and then by 3–5 cm of B_2 solution. This capillary is pressurized for 4 h and then equilibrated with 0.1 *M* Tricine–0.05 *M* Tris buffer as described in section 2.6.

2.9. Coupling of a gel-filled capillary with a buffer-filled capillary

The structure of a coupled capillary is shown in Fig. 2C. Such capillaries are prepared as follows.

(1) One end of a totally coated capillary (ca. 10 cm) is pasted with Pattex (Henkel, Düsseldorf, Germany) and kept for 5 min. This end is then slipped into a buffer-filled connector (1 cm × 0.53 mm I.D., cut from a coated, wide-bore, fused-silica capillary) and fixed by pasting a quick-drying glue (a cyanoacrylate from UHU Vertrieb, Bühl, Baden, Germany) outside the joint. This fixative takes about 2–5 min to dry. Because a wide connector is used for each manipulation, the gap between the walls of the capillary and the connector is fairly large and should be sealed. This is achieved by pasting the capillary tip with the Pattex before coupling. The gap also retains air bubbles, which are eliminated by pre-filling the connector with a buffer. The suggested buffer is 0.1-*M* Trincine–0.05 *M* Tris. Some buffers, such as borate, dramatically reduce the adherence of the fixative.

(2) The B_1-end of a gel-filled capillary prepared in as in Section 2.7 is quickly pasted with the Pattex as in (1). To prevent the gel tips from drying out, a drop of buffer is put on the Pattex-pasted tip and the other end is slipped into a casing tube filled with buffer (Fig. 1D, upper).

(3) The capillary prepared in (1) is continuously filled a with newly degassed buffer of 0.1 *M* Tricine–0.05 *M* Tris using a sealed and pressurized vial (Fig. 1D, lower). Against the buffer flow (Fig. 1D, upper), the pasted tip of the gel-filled capillary is plugged into the connector until both ends of the capillaries meet.

(4) The new joint is held manually and the filling pressure is then released by plugging a syringe needle through the cap (do not pull the capillary out of the vial at this moment!).

(5) After the buffer outside has been cleaned with a filter paper, the new joint is fixed by pasting the quick-drying glue over it and kept for more than 2 min (the capillary can now be pulled out for further treatment).

(6) The resulting capillary is equilibrated with buffer and stored as in Section 2.6. The detection window is opened at the buffer part of the capillary, 1.5 cm from the gel tip.

2.10. Electrophoresis

Electrophoresis was performed at 25°C using a Beckman P/ACE System 5500 and 2100, controlled by an IBM SP/2 computer with System Gold software. The cartridges used were modified so that the coupled capillaries could be

mounted without breakage. For detection, the 100×200 (axial direction) μm apertures were mounted and, except where stated otherwise, the data were collected with a PMT (photomultiplier tube). The data sampling rate was 1 Hz and the rise time 1 s. When a diode-array detector was used, the detection band width was 6 nm. The running buffer (0.1 *M* Tricine–0.05 *M* Tris) was degassed just before use and renewed every five runs. The sample was introduced into the negative end of the capillary by dipping it into a sample solution for 30 s (diffusing injection [27]). To carry out the diffusing injection, the run method should be started 16 s after the capillary has been dipped into the sample solution because the response time of the software or the CE system is 14 s.

3. Results and discussion

3.1. Detection sensitivity

The most important advantage of the partially gel-filled capillaries is that they retain the high performance of gel-filled capillaries and the low detection background of buffer-filled capillaries. When using totally gel-filled capillaries to separate polyamino acids, the detection sensitivity is too low even at the optimum wavelength of 200 nm [12] and no peaks can be detected at 200 nm (Fig. 3A and B). A simple way to increase the detection sensitivity is to couple the gel-filled capillaries with a piece of buffer-filled capillary as a detection cell. Fig. 3C and E show that the peak height is doubled at 220 nm and further improved, at 200 nm, more than five-fold by using a buffer-filled detection capillary with the same inner diameter. However, the resolution is dramatically reduced. To improve the resolution, fairly condensed gels ($\geq$20%T + 5%C) are required, leading to a greatly increased separation time. In contrast, when using the partially gel-filled capillaries, a 15%T + 5%C gel can yield a good separation of poly(Asp), the resolution is the same as with the totally gel-filled capillaries (Fig. 4A and C) and the detection sensitivity is similar to that of coupled

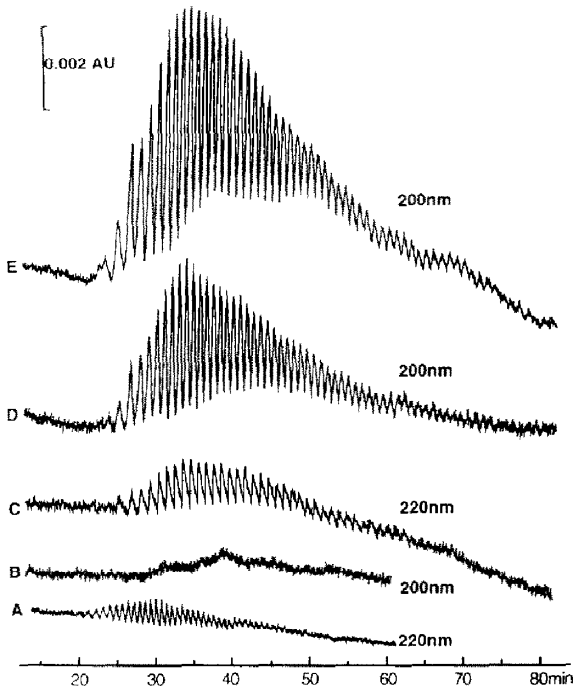

Fig. 3. Comparison of detection sensitivity between a gel-filled capillary (A and B) and a coupled capillary (C–E). Electrophoresis was performed at constant current of 3.5 μA (ca. 6.4 kV for A–B and 5.7 kV for C–E) by using the P/ACE System 2100. Capillary: A–B, 25/32 cm (separation/total lengths) $\times$ 50 μm I.D.; C–E, 25 cm $\times$ 50 μm I.D. (gel) + 8.5 cm $\times$ 50 μm I.D. (buffer). Gel: G = 20%T + 5%C; B_1 = 10%T + 5%C (2 cm solution); B_2 = 1 cm of 20%T. Sample: A–C and E, 100 mg/ml poly(Asp); D, 50 mg/ml poly(Asp).

capillaries (Fig. 4C and D). Further studies showed that the detection limit of the partially gel-filled capillaries was about two orders of magnitude lower than that of the total gel-filled capillaries. The testing was carried out with the P/ACE System 5500 as follows. Capillaries with a dimension of 32/37 cm (gel/total lengths) $\times$ 75 μm I.D. were filled totally or partially with a separation gel of 15%T + 5%C (B_2 = 1 cm of 10%T and B_1 = 2 cm of the immobilized separation gel) and run with diaspartate at an average electric field of 400 V/cm. The concentration of diaspartate was decreased until a signal-to-noise ratio of 3. The detection limit for the totally gel-filled capillary was 850 μg/ml. For a partially gel-filled capillary with the buffer not inserted between the gels, the detection limit was

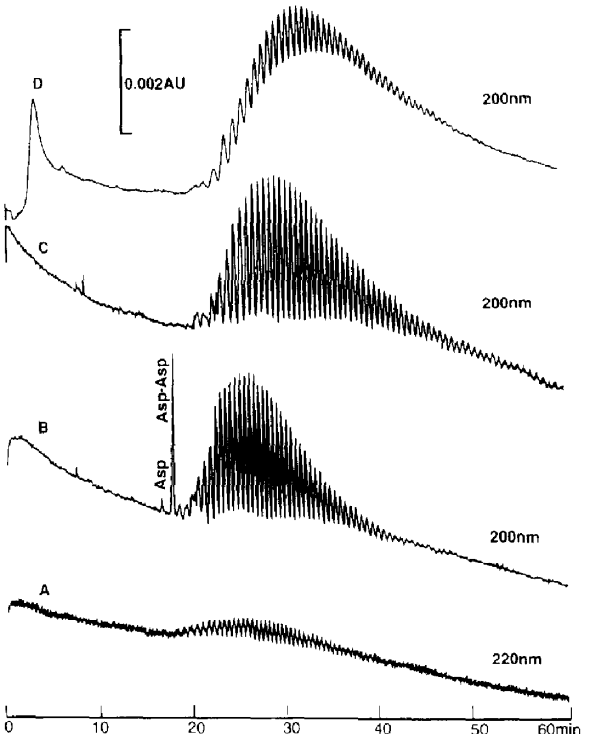

Fig. 4. Comparison of resolution between (A) a gel-filled capillary, (B and C) partially gel-filled capillaries and (D) a coupled capillary. The aspartate and diaspartate in (B) were added at concentrations of 20 and 100 μg/ml each. All the capillaries were run at 6.5 μA (ca. 7.4, 6, 7 and 7.1 kV from A to E) by using the P/ACE System 5500. Capillary: A–C, 32/39 cm × 75 μm I.D.; D, 32 cm × 75 μm I.D. (gel) + 8.5 cm × 75 μm (buffer). Gel: A, G = 15%T + 5%C, B_1 = 2 cm of the immobilized G, B_2 = 1 cm of 10%T; B, G_1 to G_5 (6 cm each) are 15%T + 5%C, 14%T + 5%C, 13%T + 5%C, 11.5%T + 5%C and 10%T + 5%C, respectively, B_1 = 2 cm of G_1, B_2 = 2 cm of 10%T; C–D, G = 15%T + 5%C, B_1 = 2 cm of G, B_2 = 2 cm of 10%T. Sample: 50 mg/ml poly (Asp).

20 μg/ml; if the buffer was inserted between the gels, the detection limit increased to 50 μg/ml, which might result from the buffer being slightly compressed in this instance. It is therefore better to use the former kind of partially gel-filled capillaries.

Similarly to the coupled capillaries, partially gel-filled capillaries also increase the separation time (compare Fig. 4A and C), but this can easily be overcome by using capillaries with step gradient separation gels (compare Fig. 4A and B). The resolution is only slightly affected. These kinds of capillaries may also be useful in the separation of complicated samples such as natural proteins or oligosaccharides, but further investigation is required.

3.2. Voids and gel migration

We found that the voids in immobilized linear gels (up to 35%T) can easily be prevented and that the voids in a short section of immobilized cross-liked gel (<20%T + 5%C) or in a long section of condensed cross-linked gel that is not immobilized can also be eliminated without too much difficulty [25]. This implies that we can establish a new method to prepare void-free and stable capillaries, that is, using the non-immobilized cross-linked gels as separation media to eliminate the voids and prevent them from migrating with two plugs of immobilized linear gels and/or cross-linked gels. It would seem difficult to introduce two plugs of immobilized gels at the ends of the separation gels, but it is actually easy to do so or at least not too difficult. The success rate of the preparation with the described method is higher than 90%. The resulting capillaries can be used continuously for more than 1 week or for more than 50 injections of poly(Asp) at about 200 V/cm (Fig. 4). This stability seems to be comparable to that of istachophoretically prepared capillaries [17].

It is essential that the gel plugs at the capillary ends (B_1 and/or B_2, Fig. 2A) should be formed at a lower speed than the separation gels, otherwise several large voids may develop in the separation gels. There are two options to control the gelatinization speed: (1) adding a larger amount of TEMED and/or APS to the separation gelling solutions than to the B_1 and B_2 solutions so that the gelatinization of the former solutions will start at least 5 min ahead of the latter; inside a capillary, it is difficult to measure the starting point of the gelatinization, and an easy way is to measure the starting point in a vial and use the measured values as a reference; (2) cooling the ends of the capillaries as described under Experimental, which is easier in practice than the first method. To eliminate voids com-

pletely, a slight pressure should be applied to the capillary during polymerization [25].

Any linear gels with concentrations higher than 4.5%T can be used as the plugs, but higher percentage linear gels such as 10%T are suggested for preparing stable capillaries. For the preparation of partially gel-filled capillaries, we suggest using a cross-liked gel as the plug in front of buffer (B_1). Linear gel plugs may sometimes cause a low detection sensitivity after several runs with the capillaries. The main reason might be that some part of the linear gels which do not bind to the capillary wall gradually dissolve into the buffer.

Interestingly, when any of the gel plugs (B_1 and B_2) is omitted, the stability of the resulting capillaries will be influenced by the direction of the applied electric field. For instance, without B_2, the capillaries may be unstable (observed by a current drop) if a positive voltage is applied to the injection end (gel end), although they are stable if a negative voltage is applied to the injection end. When B_1 is omitted, the situation is reversed.

3.3. Reproducibility and problems

The main problem in using the suggested method is that, because the flexible capillaries are generally protected by a polyimide over-coating, it is difficult to measure the correct positions of the gels after polymerization as they are different from those of the solutions, owing to the gel shrinkage during polymerization. In order to measure the gel positions photometrically, the over-coating should be removed but then the capillaries become two fragile. We therefore use the solution lengths or positions to describe the capillaries (Fig. 2A). Clearly, if the lengths of the solutions cannot be controlled correctly, irreproducible capillaries will result. To investigate the reproducibility, five capillaries with 1 cm of 10%T gel (solution) in the injection end followed by 29 cm of 10%T + 5%C separation gel (2 cm = B_1) and 7 cm of buffer were prepared and run at 400 V/cm. Each capillary was used for ten injections of diaspartate. The average elution time ± the relative standard deviation from run-to-run for each capillary were 10.65 ± 0.82, 10.78 ± 0.43, 10.96 ± 0.55, 10.77 ± 0.21 and 10.89 ± 0.37%, respectively. For capillary to capillary, the elution times of the tenth injection of each capillary were averaged, the result being 10.76 ± 1.61%. The reproducibility was not too bad. To obtain higher reproducibility, the capillaries should be run at a constant current or a constant power.

Special phenomena are observed when using the partially gel-filled capillaries and also the totally gel-filled capillaries for the separation of poly(Asp). (1) the baseline of the capillaries drifts negatively. For partially gel-filled capillaries, the drift in the first several runs may be larger than −0.01 absorbance per hour at 200 nm but is reduced to about −0.003 absorbance after about ten runs. Interestingly, the baseline drift depends on the detector and on the detection wavelength, as shown in Fig. 5. With a PMT detector, we found that different buffers or buffer components yield different drifts. Compared with Tricine–Tris, Bicine–Tris and Tricine–TEA buffers increase the drift by ca. 10%. (2) When using newly prepared capillaries, the first several separations are generally not ideal, and are accompanied by a current decrease. The current will become stable at a value lower than that without the injection of the sample. This is not due to the re-formation of voids in the gels, which can be checked under a microscope. A possible interpretation is that some pores of the gels are blocked by some large solutes because the current can be recovered by applying a reversed electric field to the capillaries (positive voltage at the injection end) for more than 1 h after separation. The separation is improved after the capillaries have been stored for 2–5 days or run more than three times with a highly concentrated sample [100 mg/ml poly(Asp)], which is a way to overcome the problems.

4. Conclusion

The described method and the resulting capillaries offer a new approach to overcoming some of the problems in capillary gel electrophoresis.

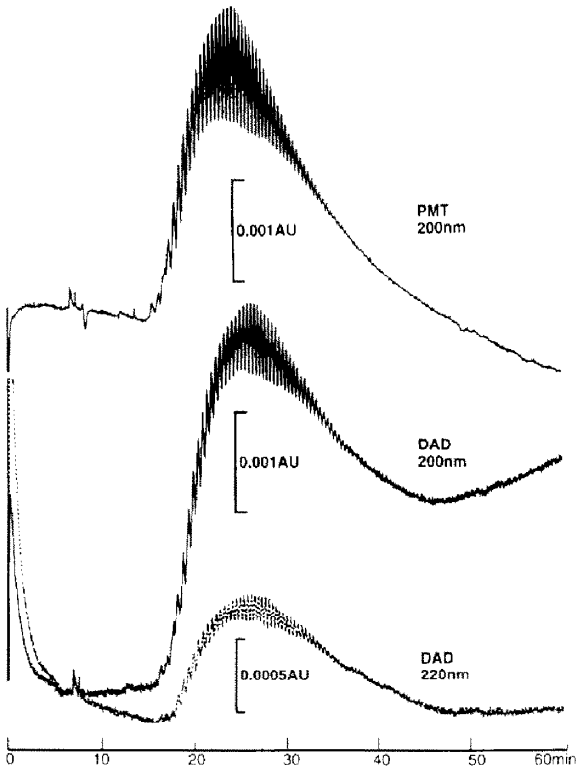

Fig. 5. Electrophoresis using a 30/37 cm × 75 μm I.D. capillary filled with 30 cm of 10%T + 5%C gel (B_1 and B_2 = 2 cm of the immobilized gel) as separation medium and with 7 cm of buffer as detection background. The capillary was run with 50 mg/ml poly(Asp) at 7.5 μA (ca. 6.7 kV) by using the P/ACE System 5500. The lower two electropherograms were generated in one run.

It is clear that the partially or gradient gel-filled capillaries will also increase the detection sensitivity of LIF or other absorption-dependent detection methods. After modification, this method could be used for the production of capillaries with different kinds of polymers, not only polyacrylamide. Polymers with stability independent of temperature, voltage and pH are highly desirable.

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