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Short communication

# Simple method for preparing cross-linked polyacrylamide gel-filled capillaries

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**Abstract**

In this paper, a simple, step-wise polymerization method for preparing a cross-linked polyacrylamide for capillary gel electrophoresis column is presented. With this method, cross-linked polyacrylamide capillary gel columns can be prepared easily.

*Keywords:* Capillary columns; Gel-filled capillaries; Polyacrylamide gel columns

## 1. Introduction

Since the introduction of cross-linked polyacrylamide capillary gel electrophoresis (CGE) by Hjertén [1], followed by the works of many other researchers [2–9], the technique has shown an extraordinary separation power and great potential for the separation of biological macromolecules because of the dual effects of sieving and differential migration of analytes in an electric field. However, the preparation of a bubble-free CGE column still seems to be a problem. The use of CGE is still limited by both the instability of its cross-linked gel in the capillary [10] and the difficulty for common researchers to access CGE columns easily in their own laboratories according to those published methods. To avoid these difficulties, many researchers turn to non-gel sieving. However, non-gel sieving cannot always take the place of cross-linked gels because of its inferior column efficiency [11,12]. Besides, methoxylated agrose gel has been developed to achieve a similar separation ability as CGE and permits automated runs [13] at the same time.

In this paper, we present a simple method for preparing cross-linked polyacrylamide gel columns. The principle of the method is based on step-wise polymerization, and the procedure is much simpler than those in previous works. Since the viscosity of gel increases exponentially with the $T$ value even when $C=0\%$ [14], the electroosmotic force is not large enough to extrude the cross-linked gel out of the capillary, and it is unnecessary to chemically bond the gel to the internal wall of the capillary, making the pretreament of the capillary easier. In addition, this method of preparation shows a very

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high success rate and can be easily mastered even by a novice.

## 2. Experimental

### 2.1. Apparatus and materials

The CE system contains an Isco $CV^4$ UV detector, a d.c. high-voltage power supply (Glassman Instruments, USA), and a high-voltage supply with current display (laboratory-made). The recorder is a HP 3394 integrator. Fused-silica capillaries (50, 75, 100 $\mu$m I.D., 375 $\mu$m O.D.) are from the Hebei Yongnian Optical Fiber Factory (Hebei Province, China) and rinsed with NaOH (0.1%), water, $CH_3OH$ and $H_2O$ successively for 30 min each. The on-column detector window is created by removing a short segment of the polyimide coating from the capillary with a blade after the gel has formed in the capillary.

Acrylamide and N,N′-methylenebisacrylamide are dissolved in the TBU buffer (0.1 mol/l Tris+0.25 mol/l boric acid+7 mol/l urea, pH 8.3) to form T=8%, C=5% stock solution A. All the reagents above are from Fluka (Switzerland) N,N,N′,N″-Tetramethylethylenediamine (TEMED, Fluka) (10%, w/v) and 10% (w/v) ammonium persulfate (APS, Beijing Chemical Factory, China) are added into solution A successively to initiate the polymerization. The standard test sample $Pd(A)_{40-60}$ (1 unit/100 $\mu$l) is from Pharmacia Biotech (Sweden) and dissolved in pH 7 phosphate buffer. Water was distilled thrice before use.

### 2.2. Preparation of the column

Add 2 $\mu$l APS and 4 $\mu$l TEMED solutions to 1 ml degassed solution A in a small test tube. Then introduce the prepolymer solution into the capillary by vacuum or with a device described in Ref. [4]. Seal both ends of the capillary immediately by inserting both ends into two small pieces of silicone rubber, respectively, and immerse the capillary into the ice water in an open, rectangular glass chamber (length=30 cm, width=10 cm, height=20 cm, formerly used for TLC), with the central one third of the capillary above the water surface. Let the center of the capillary at least 5 cm above the cold bath to create a temperature gradient region as well as to accelerate the polymerization process in this part. The shrinkage of the polymerized gel in this part can be compensated for by the inflow of the prepolymer solution that is of low viscosity and very mobile at the bath temperature(0~4°C) under the water surface. After about 50 min, when the first step of polymerization of the prepolymer solution in the temperature region above the water surface has been completed, start the second step of polymerization by lifting one half of the two remaining parts of the capillary immersed in the water out of the ice water to hasten the polymerization reaction. Finally, let the capillary stand overnight with its rest parts in the ice water to complete the reaction.

## 3. Results and discussion

### 3.1. Performance of the prepared CGE columns

The performance of the prepared columns, including their reproducibility, life time and column efficiencies, was investigated with a standard sample $Pd(A)_{40-60}$. The electropherograms of $Pd(A)_{40-60}$ are shown in Fig. 1. Several kinds of columns with different internal diameters were compared. The results are shown in Table 1.

For calculating R.S.D.s of the migration times, corrections were made for the gradual diminishing of the effective length of the capillary after the injection end was trimmed due to serious bubble formation as evidenced by the broadening of peaks. Nevertheless, the electric field in the capillary was kept constant at 250 V/cm throughout the evaluation process. The R.S.D.s of migration times are generally less than 3%.

The life time of a column is defined as the cumulative run time before trimming of the tip is needed. Since the gel column can be as long as 70 cm, we can continue to use it even after multiple trimmings. In fact, the actual serviceable time of a column can be at least several times more than the life time. For instance, a CGE column with T=8%, C=5%, I.D.=75 $\mu$m, L=65 cm, was once used with multiple trimmings for about 30 h in running $Pd(A)_{40-60}$ at an electric field of 250 V/cm.

The column efficiencies are the average plate

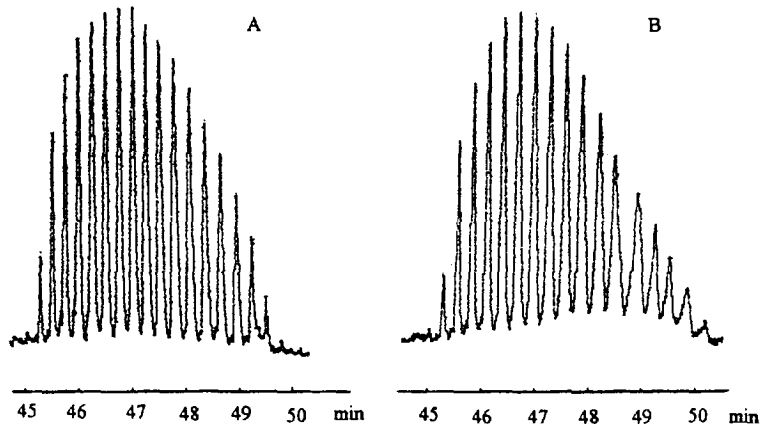


Fig. 1. Electropherograms of $Pd(A)_{40-60}$ obtained from the sixth injection (A) and sixteenth injection (B). The peaks began to broaden after 15 runs. Electric field, 250 V/cm; current, 1.6 μA; capillary, 54 cm (effective length 35 cm)×50 μm I.D.; boric acid+7 mol/l urea, pH 8.3.

numbers per meter for three peaks (5th, 10th and 15th peaks shown in Fig. 1) and one third of the total runs for three columns randomly chosen. The 50 μm I.D. column showed the highest efficiency, above $10^7$ plates/m, as expected by theory.

### 3.2. Success rate of the method

The success rates of this method varied with the T value. When T≤12% and C=5%, the success rate can be as high as 90%, based on the statistics of 50 times of preparation. This range of T value can meet the need of most CGE uses. With this method, we have prepared columns with T up to 16% and C= 5%. However, in this case the success rate fell to around 50%, based on the result of 15 experiments.

### 3.3. Some remarks on the preparation method

During the whole preparation process, attention should be paid not to touch the capillary filled with prepolymer solution directly with warm fingers to avoid initiating the polymerization at the touched places. Instead of with fingers, we used two pairs of tweezers with their tips wrapped with segments of rubber tubing for handling.

To maintain a proper reaction rate in the first step of polymerization is essential for the success of the method. The time needed for the completion of the polymerization can be indicated by the gel formation in a small test tube filled with the initiated prepolymer solution placed near the capillary and above the ice water. The reaction rate can be adjusted by either increasing or decreasing the amount of APS and TEMED added to keep the reaction time within 30 to 60 min. All the columns were prepared under room temperature ranging from 15 to 25°C.

Finally, it is desirable to let the prepared capillary stand for about one week before use. For a freshly prepared gel column, there are two void spaces of about 1 cm long at both ends and these voids will extend to about 3 cm long after standing for one

Table 1
Performance of CGE columns with different I.D. (T=8%, C=5%, $n$=3)

| Column internal diameter (μm) | R.S.D (%)[a] | | Life time (h) | Column efficiency (plates/m) |
|---|---|---|---|---|
| | Run-to-run | Day-to-day | | |
| 100 | 1.2 | 1.0 | 9.0 | $1.1 \cdot 10^6$ |
| 75 | 0.3 | 1.3 | 12.5 | $7.9 \cdot 10^6$ |
| 50 | 2.4 | 2.7 | 15.5 | $1.6 \cdot 10^7$ |

[a] Migration time reproducibility of the 10th peak shown in Fig. 1.

week. Prior to sample introduction, trimming of the tips and prerun for about one hour are needed.

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