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Change in conductivity in non-cross-linked polyacrylamide capillary electrophoresis
Effects of aging polyacrylamide and buffer composition

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Abstract

Upon application of an electric field to a polymer-filled capillary, there is a rapid expulsion of excess ions associated with the polymerization mixture. This expulsion is complete in about 10–15 min. A much slower transport of ions occurs at one end of the capillary; the transport arises because of the difference in transference number between the buffer reservoir and the polymer filled capillary. This ionic transport leads to a change in the conductivity of the polymer at one end of the capillary. We have monitored the conductivity profile in 5% T non-cross-linked polyacrylamide capillaries at 300 V/cm for different ages of polyacrylamide. The profile was fit with a double-exponential, corresponding to the rapid expulsion of reaction ions and the slow transport of ions due to differences in transference number. The fit indicates that there are more ions in polyacrylamide as it is aging, and that the depletion of ionic concentrations is less important in aged polyacrylamide than in fresh polyacrylamide. The effect of the buffer composition was studied. By changing from 1×TBE buffer reservoirs to a mixture of 30 m*M* KCl and 1×TB buffer, the depletion of ionic concentration at the injection end disappears and is replaced by an increase of ionic concentration at the ground end. By changing 1×TBE buffer reservoirs to 1×TBE, 5% T non-cross-linked polyacrylamide and seven molar urea buffer reservoirs the depletion of ionic concentration at the injection end disappears.

Keywords: Buffer composition; Polyacrylamide aging

1. Introduction

Capillary electrophoresis is a powerful method for the separation of biological materials. A promising application of capillary electrophoresis is the separation of DNA sequencing samples [1–15]. In early applications of capillary electrophoresis for DNA sequencing, cross-linked polyacrylamide was very popular as the separation medium [1–11]. However, these gels are quite rigid; as a result, the entire capillary must be replaced when the separation medium fails. Non-cross-linked polyacrylamide was then proposed as a separation medium because the low viscosity material can be pumped from the capillary and replaced with fresh matrix as necessary.

The reuse of the separation medium for several DNA sequencing runs is attractive. Capillary replacement and optical realignment are avoided for cross-linked polyacrylamide. Replacement of the matrix with fresh material is avoided for non-cross-linked polymers. In our experience, the migration time of DNA systematically decreases with subsequent runs [16–18]. This change in mobility is due to the difference in transference numbers for ions in the buffer reservoirs and ions in the polymer-filled

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capillary [16]. This depleted area forms at one capillary-buffer interface and extends further into the capillary with time [16]. There is a corresponding asymmetry in the electric field throughout the capillary: the field is higher in the first few centimetres of the capillary and lower in the rest of the capillary. This increasing asymmetry of the electric field distribution with time causes the migration time of DNA to increase for subsequent DNA sequencing runs.

We found that the reproducibility of replicate sequencing runs on the same gel increases by use of older polyacrylamide [17] or reversing the polarity for a short period between runs [18]. Older polyacrylamide was shown to be less affected by ionic depletion. Reversing the polarity between runs pumps ions into the depleted area. A better way of obtaining reproducible migration time and stable resolution is to pressure-refilled low viscosity non-cross-linked polyacrylamide in the capillary in between each runs [10]. Refilling the capillary with fresh polyacrylamide between each run replaces the depleted area with fresh polymer. All those methods to stabilize the migration time are correlated with a stabilization of the ionic depletion at the injection end [16].

In this report, we continue our study of the effect of changes in transference number at the reservoir–capillary interface. In particular, we show the effect of buffer additives on the change in transference number at the reservoir–capillary interfaces.

2. Experimental

The experimental procedure has been described elsewhere [16]. Polyacrylamide solutions (5% T) were prepared by mixing a 625 μl aliquot of a 40% acrylamide (Bio-Rad, CA, USA) stock solution with 1 ml of 5×TBE buffer (2.7 g Tris (ICN Biomedicals, Cleveland, OH, USA), 1.37 g boric acid (BDH, Toronto, Ontario) and 1.0 ml 0.5 *M* disodium EDTA diluted to 50 ml in deionized filtered water) and 2.1 g of urea (Gibco BRL, Gaithersburg, MD, USA) and making the solution up to 5.0 ml in deionized and filtered water. Oxygen was flushed from the solution by bubbling argon gas for 20 min. The polymerization reaction was initiated and catalyzed by adding 20 μl of 10% ammonium persulfate (Boehringer Mannheim, Indianapolis, IN, USA) and 2 μl N,N,N′,N′-tetramethylethylenediamine (TEMED) (Gibco, BRL). Immediately following the addition of initiator, the solution was injected into a fused-silica capillary (Polymicro, Phoenix, AZ, USA), typically 30 cm long, 32 μm I.D. and 143 μm O.D. The inner wall of the capillary had been pretreated with γ-methacryloxypropyl trimethoxysilane (Sigma, St. Louis, MO, USA) for 1 h [2]. All chemicals are electrophoretic grade. The day of preparation is day zero, capillaries older than that were left to age on the bench. The dried ends of the capillary are trimmed by several centimetres before use.

The capillary electrophoresis system used for these experiments is an in-house design from a Spellman CZE 1000 (Plainview, NY, USA) power supply and a locally-constructed Plexiglas safety-interlocked box. In these experiments, current was estimated from the voltage drop across a 1.1 MΩ resistor, added in series between the injection and detection buffer reservoirs.

3. Results

The effect of transference number discontinuities at the reservoir–capillary interface depends on the age of the polyacrylamide, the buffer composition, the electric field, and the total electrophoresis run time. For some buffer systems, a depletion of ions forms at the injection end of the capillary. For other buffer systems an increase in the ionic concentration forms at the ground end. As explained by Spencer [19–21] the effect observed at a reservoir–capillary interface depends on the ions in the buffer. If there is a discontinuity in the transference number of the ionic species at the interface, then there will be changes in the ionic composition near the interface. At the cathode (injection end of the capillary in DNA sequencing), anions move into the polymer from the buffer reservoir and encounter more resistance due to the relatively dense polymer matrix. Cations move from the polymer to the other buffer reservoir and meet less resistance. Depending on the sizes of the ions (i.e. if one of them encounters more resistance in the polymer than the other) a change in transference number at the interface induces a

change in ionic concentration at the interface. We will address the effect that age of the polyacrylamide and the buffer composition have on the changes of transference number at the reservoir–capillary interfaces.

3.1. The age of the polyacrylamide with 1×TBE buffer

In a previous report, we showed that the effect of transference is less important in aged polyacrylamide than in fresh polyacrylamide [16]. We have also shown that the migration time of DNA fragments is more stable in aged polyacrylamide [17]. Here, we present a more detailed study of the effect of polyacrylamide age on the depletion of ions at the injection end of capillaries. The buffer used in this study is 1×TBE. With this buffer, the ionic concentration is depleted at the injection end of the capillary whereas no change is observed at the ground end [16]. We prepared several 5% T non-cross-linked polyacrylamide capillaries from completely different starting solutions, and let them age by storage at room temperature on a laboratory bench. After the dried-out portion near the capillary tip was trimmed, each capillary was run at −300 V/cm for at least 6 h with 1×TBE as the running buffer. The current was monitored as a function of time and used to compute the time-dependent conductivity. Each capillary was only used once and discarded. A double exponential decay was fit to the conductivity versus time profile by use of a non-linear regression algorithm:

$$\kappa(\Omega^{-1}\mathrm{m}^{-1}) = W_0 + W_1 \exp\left(\frac{-t}{W_2}\right) + W_3 \exp\left(\frac{-t}{W_4}\right) \quad (1)$$

The equation consists of three parts. The first term, W_0, equals the steady state conductivity of the capillary. The second part, $W_1 \exp(-t/W_2)$, corresponds to an exponential decrease in conductivity, with magnitude W_1 and time constant W_2; this decrease is associated with the expulsion of excess polymerization and hydrolysis ions from the capillary over a period of a few minutes. The last part of the equation, $W_3 \exp(-t/W_4)$, corresponds to another exponential loss of ions, this time due to the effect of transference numbers on the ionic composition in the polymer. The latter process occurs slowly and requires several hours to reach steady state.

Our results suggest that the magnitude of conductivity decrease due to the rapid expulsion of ions, W_1, increases slightly with the age of the polymer, Fig. 1. The difference in ionic concentration between the polymer and the running buffer increases slightly with the age of the polyacrylamide. This increase could be caused by the hydrolysis of some amino groups of polyacrylamide to form acrylic acid. However, the time constant for the expulsion of small ions, W_2, is independent of the age of the polymer and has a mean value of 8±4 min. The polymer structure does not seem to change sufficiently with age to affect the mobility of small ions, because the time constant is independent of the age of the polymer.

The last term in the equation is related to the depletion of ions caused by differences in transference numbers in the buffer reservoir and in the capillary [16]. The magnitude of the loss in ions associated with transference number effects, W_3, decreases with the age of polyacrylamide, Fig. 2. Fresh polymers undergo much greater change in conductivity than do older polymers. The change in conductivity due to transference number differences decreases roughly exponentially with the age of the polymer (smooth curve in the figure):

$$W_3\,(\Omega^{-1}\mathrm{m}^{-1}) = 1.2\pm0.4\ \Omega^{-1}\mathrm{m}^{-1} + 3.2\pm0.4\ \Omega^{-1}\mathrm{m}^{-1} \exp\left(\frac{-\ \mathrm{day}}{3.5\pm1.2\ \mathrm{day}}\right) \quad (2)$$

This result is consistent with the observation that the depletion of ions is smaller for older polymers than for younger polymers. It also indicates that there still is a small depletion of ions (1.2±0.4 $\mathrm{h}\Omega^{-1}\ \mathrm{m}^{-1}$) in older polyacrylamide. W_4 is constant at 108±50 min.

The constant W_0 is the steady-state or limiting conductivity of the polymer, Fig. 3, and increases exponentially with the age of the polymers.

$$W_0\,(\Omega^{-1}\mathrm{m}^{-1}) = 6.4\pm0.4\ \Omega^{-1}\mathrm{m}^{-1} - 4.8\pm0.4\ \Omega^{-1}\mathrm{m}^{-1} \exp\left(\frac{-\ \mathrm{day}}{5\pm1\ \mathrm{day}}\right) \quad (3)$$

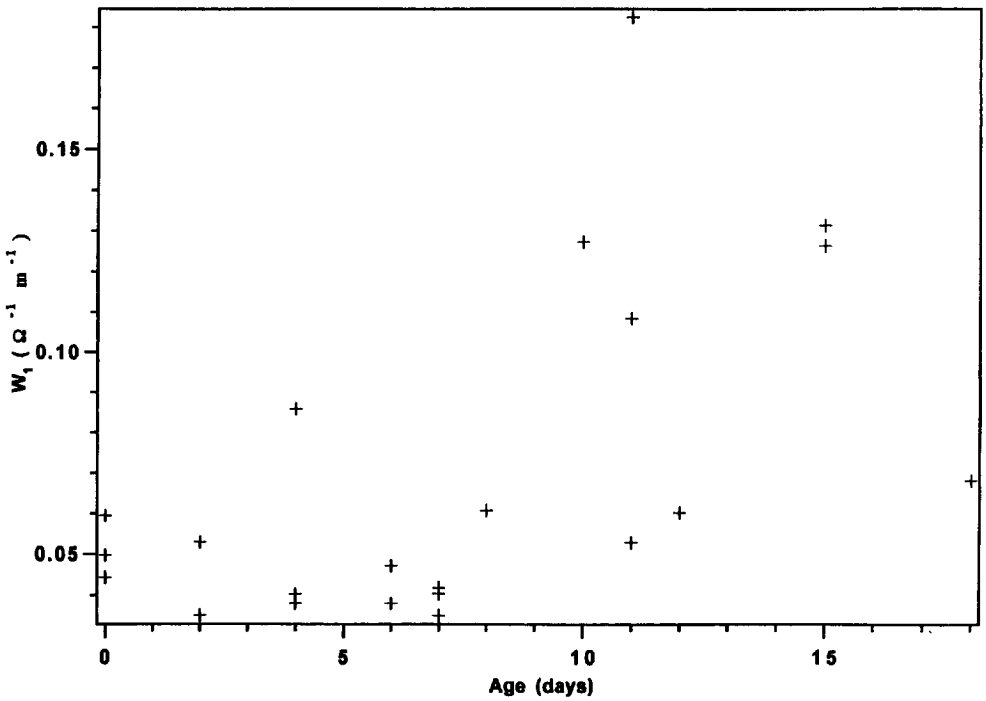

Fig. 1. W_1 versus the age of polyacrylamide.

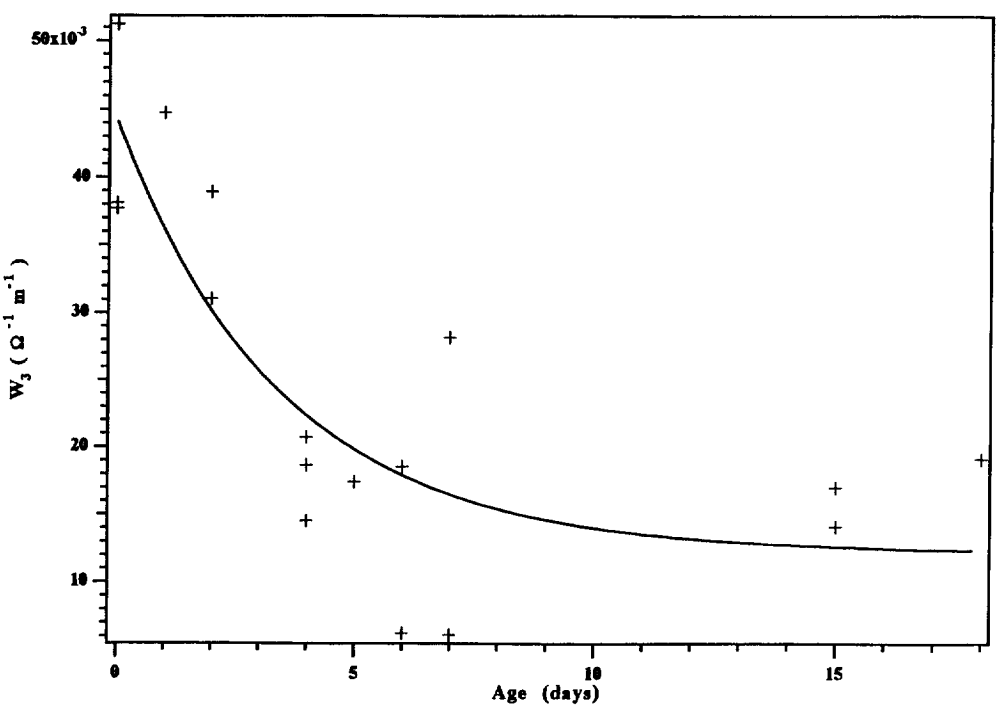

Fig. 2. W_3 versus the age of polyacrylamide.

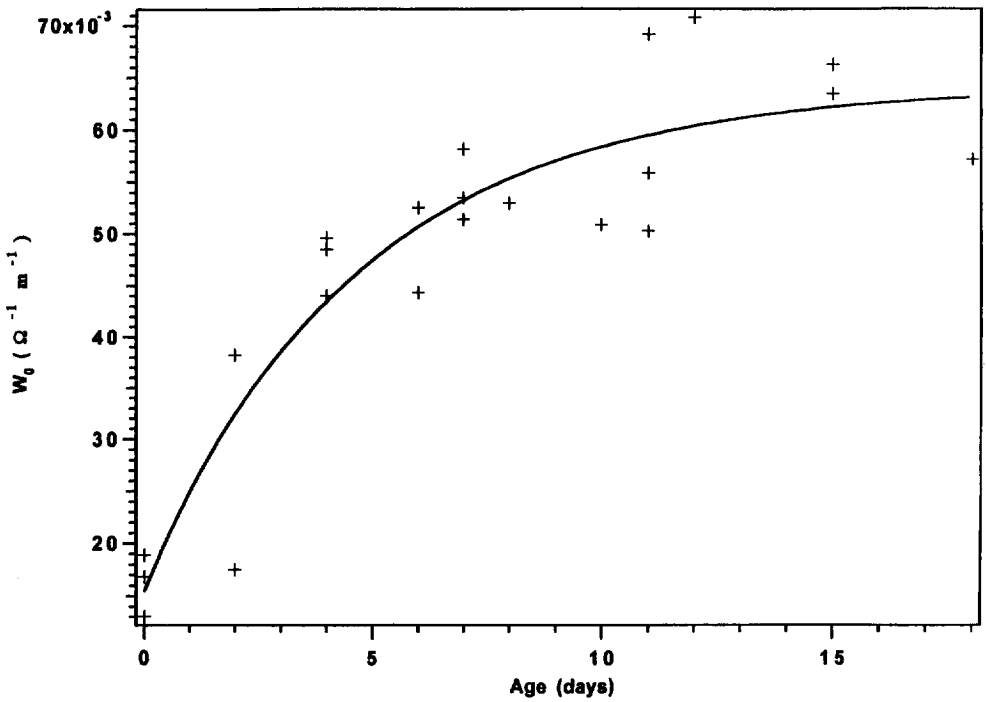

Fig. 3. W_0 versus the age of polyacrylamide.

The maximum depletion of ions due to transference effects is 4.8±0.4 $k\Omega^{-1}$ m^{-1}. It occurs for fresh capillaries and is 71% of the steady-state conductivity. This huge loss of ions is localized at the injection tip of the capillary and will strongly affect the migration time of DNA sequencing fragments through the polymer. Older capillaries develop a smaller loss in conductivity, and the loss decays with a time constant of 5±1 day. That is, a five-day-old capillary will generate a relative conductivity drop of $e^{-1} \times 71\% = 26\%$ of the steady-state value. After 15 days, the depletion is only 10% of the depletion observed for fresh capillaries. These results clearly indicate that older polyacrylamide generates higher conductivity than does fresh polyacrylamide.

3.2. Buffer system

In this section we demonstrate the effect of the buffer system on the change in transference number at the reservoir–capillary interface. Each capillary was run at −300 V cm^{-1} for at least 3 h. To obtain the conductivity versus the length of the capillary, pieces of the capillary were trimmed from one end of the capillary. The electric field was applied for 20 s after each cut in order to measure the current through the capillary. The electric field was kept constant by readjusting the potential after each cut. The conductivity was calculated from the values of the current and the electric field.

3.3. Effect of adding KCl

The buffer reservoirs for the capillary were filled with 30 m*M* KCl plus 1×TB. Only a small amount of KCl, compared to the buffer concentration, was required to make KCl the main charge carrier. The steady-state conductivity for this new buffer system is 24 $h\Omega^{-1}$ m^{-1}, which is four times higher than in the case of 1×TBE alone. In all cases, the conductivity decreased when pieces of the capillaries were trimmed from the ground end. This result indicates that an increase of ionic concentration occurred at the ground end. No significant change in conductivity was noticed at the injection end. Thus, in the case of 30 m*M* KCl+1×TB, the ions at the

injection end were expelled out of the capillary into the buffer reservoir, and the area of increased concentration at the ground end moved into the capillary. These results are the opposite from that observed with 1×TBE. The depleted area and the area of increased concentration move in or out of the capillary, depending on the change of the transference number across the boundary [19–21]. This change in conductivity depends on the type of ions involved in conduction. For some buffers, like 1× TBE, the depleted area moves into the capillary and the area of increased ionic concentration moves out of the capillary; those buffers are called normal buffers. For some other buffers, like 30 m*M* KCl+ 1×TB, the depleted area moves out of the capillary, and the area of increased ionic concentration moves in the capillary; those buffers are called abnormal buffers.

3.4. Effect of 5% T non-cross-linked polyacrylamide-filled reservoir

Swerdlow noticed that by filling the buffer reservoirs with a mixture of polymer and buffer, the depletion of ionic concentration is reduced [22]. In this section, we filled both reservoirs with 5% T non-cross-linked polyacrylamide containing 1×TBE and 7 *M* urea. The steady-state conductivity, $12.0\pm0.8\ \Omega^{-1}\ m^{-1}$ is higher than the one obtained with the 1×TBE buffer reservoirs. This result occurs because ammonium persulfate and TEMED are also present in the non-cross-linked polyacrylamide-filled reservoirs. After the initial expulsion of polymerization ions, the conductivity drops slightly. This conductivity decrease was traced to a 10% decrease in conductivity at the injection tip, extending a few millimetres into the capillary. This decrease in conductivity was nearly a factor of ten smaller than that observed with 1×TBE in the buffers.

4. Discussion

The conductivity temporal profile was modeled with a double exponential decay. The parameters obtained suggest that the depletion of ionic concentration decreases as the age of the polyacrylamide increases. Clearly, the various behaviours of different ages of capillaries are related to a change in the properties of polyacrylamide. Change in the properties of cross-linked polyacrylamide as it ages were previously reported [23–27]. Our results also indicate that ionic depletion is less important for older polyacrylamide. This result could be explained by the presence of charges on the polymer, perhaps due to the hydrolysis of some amino groups of polyacrylamide to carboxylate groups. The presence of charges on the stationary polymer would ensure a minimum conductivity and would disturb the effect of the changes in transference number. Alternatively, there may be a change in the pore structure of the polymer, which also changes the relative transport of small ions. However, the independence of the ionic depletion rate on the age of the polymer argues against this model.

The addition of KCl transformed the buffer system from a normal buffer system to an abnormal buffer system. In this abnormal buffer system, an area of increased ionic concentration moves into the capillary at the ground end, and a depleted area at the injection end moves out of the capillary and disappears in the buffer reservoir. This abnormal behaviour was also described by Spencer [19–21].

The 1×TBE buffer reservoirs were replaced by 5%T non-cross-linked polyacrylamide in 1×TBE. The depletion of ionic concentration at the injection end was not observed. These results are expected because the transference number is the same in the polyacrylamide reservoirs and the capillary. Thus, no change in ionic concentration can occur at either end of the capillary. One would expect a change in the ionic concentration near the electrodes; however, due to the size of the reservoirs compared to the capillary, the change in ionic concentration at the electrodes has a negligible effect on the conductivity.

In the case of DNA sequencing there are advantages to the use of a non-cross-linked polymer with greater chemical stability than polyacrylamide. Any polymer with similar pore size and viscosity as polyacrylamide, and inert toward the DNA sample, is a good candidate as a matrix for DNA sequencing by CGE. The recent work by Fung and Yeung demonstrates that polyethylene oxide is an excellent polymer for separation of sequencing fragments [28].

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