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Polyimide Stripping Device for Producing Detection Windows on Fused-Silica Tubing Used in Capillary Electrophoresis

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INTRODUCTION

Separations in capillary electrophoresis (CE) are usually performed in a length of small inner diameter fused-silica capillary which is clad with a layer of polyimide to protect the fragile silica tubing underneath from breakage. Although the polyimide coating imparts substantial flexibility and durability to the capillary, it also masks the superior optical properties of the underlying silica. Thus, a small region (5–10 mm) on the exterior of the capillary must be stripped of the polyimide before that section can be used as the detection cell when installed in the optical path of an absorbance (*1*) or a fluorescence (*2*) detector.

Removal of the polyimide to expose the underlying silica tubing is commonly accomplished by using a small flame (*3*) or a heating element (*4*, *5*), and a device is commercially available (Polyimide Stripper, Polymicro Technologies, Phoenix, AZ) which employs a resistive heating coil energized with a rechargeable battery power supply to burn away the polyimide. Although these methods produce optically clear windows by removing the polyimide coating, they both have limitations. Use of a flame requires caution, as it can sometimes soften and consequently distort the silica capillary. The commercially available polyimide stripper, which has a maximum operating temperature below the softening point of silica, obviates this problem, though the device is rather expensive. However, since these approaches remove the polyimide by thermally decomposing the polymer, the capillary is subjected to considerable heat. Neither method can be used to remove polyimide from chemically derivatized (*6*) or gel-filled (*7*) capillaries, as the intense heat would pyrolyze the bonded phase or disrupt the integrity of the polyacrylamide or agarose matrices, respectively, in such capillaries.

For preparation of derivatized or gel-filled capillaries, the window must be formed before the capillary is chemically derivatized or is filled with gel medium. However, removal of the polyimide weakens the mechanical strength of the capillary in the denuded region, and hence the fragile capillaries must be handled gingerly during the derivatization or gel-filling procedure to prevent breakage. Removal of small sections (e.g., <1-mm widths) of the polyimide has been reported not to decrease substantially the flexibility of capillaries (*4*). Some protection can also be afforded to the fragile window by enclosing the denuded region or the entire capillary in a sheath of protective tubing. However, these precautions are time-consuming and inconvenient to implement, and loss due to breakage is still high.

This report describes a simple device for removing polyimide from a section of fused-silica capillary without subjecting it to extreme heat. The approach affords the researcher the ability to prepare extended lengths of chemically derivatized or gel-filled capillaries, from which sections of the desired dimension can be cut and fabricated into working lengths for separations. Use of protective sheaths is obviated, and loss of capillaries due to breakage in the detection window region is substantially reduced.

RESULTS AND DISCUSSION

The method of polyimide removal described here is based on mechanical stripping of the polymer using the apparatus shown in Figure 1. This device is a lathelike instrument that supports and protects the capillary so that a section of polyimide can be scraped away with a chisel-pointed knife. A jig formed from two concentric sections of 12- and 15-gauge hypodermic tubing (Small Parts, Inc., Miami, FL) with two cutouts milled near the ends constitutes the heart of the device. A capillary on which the window is to be formed is inserted through these two sections of tubing and one end is attached to a variable-speed motor (Dremel Moto-Tool, Racine, WI) by a force-fit chuck attachment on the motor. The other end of the capillary is supported in a section of 15-gauge hypodermic tubing; this section of tubing, as well as the previously mentioned jig, are mounted (by using epoxy) on sliding supports (fabricated from Lucite) which can be positioned relative to the motor to vary the placement of the window along the length of the capillary, as well as to ac-

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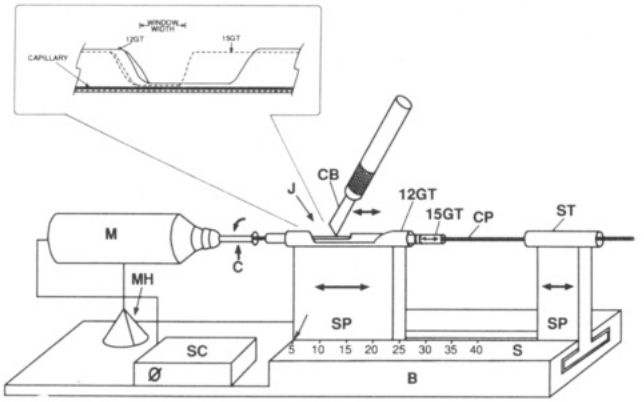

Figure 1. Capillary stripper apparatus for polyimide removal from silica tubing. The capillary is held in a jig (J) formed from two concentric sections of hypodermic tubing mounted on a sliding support standard (SP). One end of the capillary (CP) is attached to a motor (M) powered by a speed controller (SC) using a force-fit chuck (C) attached to the motor. Milled-out regions in the two concentric sections of the jig assembly define the region where the window is to be made. The cutout on the 23 cm long section of the 12-gauge tubing (12GT) is 20 mm long and 1.5 mm deep. The milled-out region on the 30 cm long section of the 15-gauge tubing (15GT) is 10 mm long and 1.2 mm deep. The length of the support tubing (ST) is 4 cm. The motor is attached to a Lucite base (B) using a Moto-Tool Holder (MH), which is positioned so that the axes of the motor and the sections of hypodermic tubing are aligned. The location of the window on the capillary is determined by positioning the jig an appropriate distance from the motor using the scale (S) on the base. An X-Acto chisel blade (CB) removes the polyimide from the capillary in a gentle, back-and-forth motion with the point of the blade resting on the bottom of the cutout in the 15-gauge tubing. (A second support tube may be positioned between the motor and the jig to provide additional stability when windows are being formed near the center of long (75–125 cm) capillaries.)

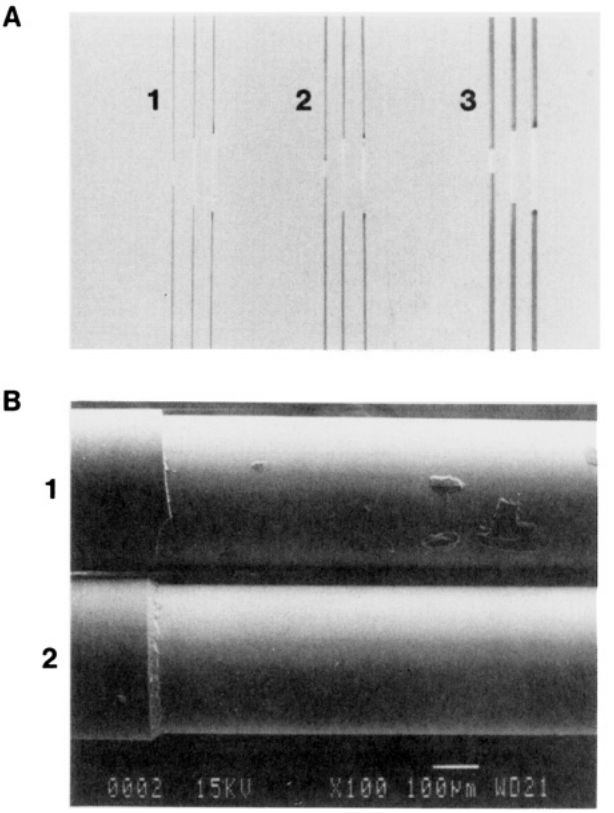

Figure 2. (A) Photograph of sections of capillary of various sizes with windows produced by the conventional (flame) method and the method described here (mechanical stripper). Capillary dimensions are (1) 50 µm i.d./200 o.d., (2) 250 µm i.d./350 µm o.d., and (3) 530 µm i.d./700 µm o.d. Window dimensions in each group are 2 mm (stripped), 10 mm (stripped), and ~10 mm (flame). (B) Electron photomicrographs of the silica capillary surface comparing surface quality of denuded capillaries. The presence of charred flakes of polyimide is evident on the window (capillary 1) produced by using flame. The surface of the mechanically stripped capillary (capillary 2) is clear and smooth, with no evidence of scoring from the chisel blade.

commodate capillaries of various lengths. The force-fit chuck is simply an Eppendorf Gel-Loader 200 pipet tip (Brinkmann Instruments, Westbury, NY) inserted into the chuck of the Moto-Tool; direct mechanical coupling of the capillary to the motor using only the collet usually crushes the end of the capillary. To maintain the motor and the hypodermic tubing sections in alignment along a common axis, the sliding support standards, as well as the motor, are positioned on a Lucite base. As designed, the apparatus (Figure 1) occupies about 1 m of bench space and can accept capillaries up to 1.25 m in length, though the device could easily be modified to accommodate capillary sections of longer dimensions.

To remove the polyimide and form the detection window, the capillary is spun at a moderate speed (~5000–8000 rpm) and the polymer cladding is gently scraped away with a back-and-forth motion using a No. 19 or No. 24 X-Acto chisel blade (X-Acto, Long Island City, NY) held at an ~45° angle from vertical in the opening formed by the milled-out sections of the hypodermic tubing (Figure 1). Usually, fewer than a dozen passes of the chisel blade are sufficient to remove all of the polymer cladding and expose the silica to form the window. Only gentle pressure is applied to the chisel blade, allowing the spinning motion of the capillary to do most of the work of removing the polyimide. (It is also possible to remove polyimide by laying the capillary on a flat surface and scrapping away the polymer with gentle pressure while manually turning the capillary; however, extreme care (*4*) is required to prevent breakage of the capillary by excessive pressure from the chisel blade or by inadvertently applying torque to the partially denuded section while turning.)

The location of the window is selected by positioning the jig at the desired distance from the motor using the centimeter scale attached to the base of the apparatus; in the design shown, a window can be placed as close as 5 cm from the end of the capillary. By variation of the size of the gap in the jig formed by the lengths of the milled-out sections of the hypodermic tubing, the width of the window can be adjusted. Polyimide-free windows on capillaries of varying diameters (50–530 µm i.d., 200–700 µm o.d.) have been produced by using this apparatus (Figure 2A). The device is capable of making windows on small outer-diameter capillaries (e.g., 50 µm i.d., 200 µm o.d., Figure 2A-1) as well as on fragile, thin-walled ones (e.g., 250 µm i.d., 350 µm o.d., Figure 2A-2). Windows ranging from 1–10 mm in length can be prepared by using the design shown in Figure 1, though longer (or shorter) windows might be produced with modification to the instrument.

Removal of polyimide using this technique does not distort or destroy the optical clarity of the silica tubing (Figure 2B), which is important for maintaining high sensitivity in the detection system. In Figure 2B, no scratches are evident on the stripped section of capillary; such scratches are to be avoided as they could increase radiation scattering in the optical system of the detector as well as further weaken the denuded section of capillary. Capillaries with windows prepared by this method have been used at high sensitivity in both absorbance (*6*) and fluorescence (*8*) detection systems. Finally, unlike the flame method for producing windows, this technique does not produce charred flakes of burned polyimide (Figure 2B) which can sometimes become dislodged from the capillary when placed in the optical path of the detector and subsequently introduce noise into the detection system.

This method of removing the polyimide also does not alter

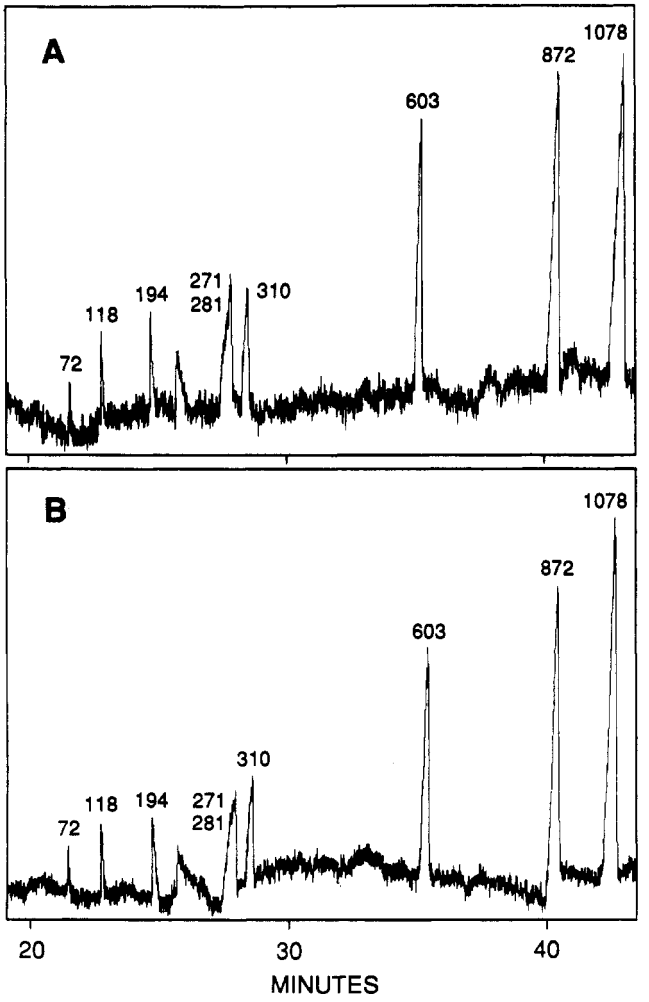

Figure 3. Comparison of gel electrophoretic separations in (A) a capillary with the window produced by the conventional (flame) method *before* the capillary was filled with gel and (B) a capillary with window produced by the mechanical stripper described here *after* the capillary was filled with gel. The bands in the separation correspond to the 72–1078 base-pair fragments of phiX174 RF DNA digested with *Hae* III restriction enzyme. The separations were run at 5-kV applied potential in 60-cm lengths (25 cm from cathode to detector) of 100 μm i.d./350 μm o.d. fused-silica capillaries derivatized with ((methacryloxy)propyl)trimethoxysilane (*10*) and filled with 1.934% acrylamide, 0.066% bis(acrylamide) gel mix (2% T, 3.3% C). The reservoir and gel buffers were 90 mM Tris, 90 mM boric acid, and 2 mM EDTA (pH 8.2), and detection was by UV absorbance at 260 nm.

the interior of the capillary, which may be chemically modified or filled with an electrophoretic gel matrix (in principle, the device may also be useful for removing polyimide from capillaries packed with porous microspheres and used in miniaturized HPLC (*9*)). Figure 3 compares the separation achieved in a 2.0% polyacrylamide gel-filled capillary on which the window was prepared by using the device described here (Figure 3B) with a separation in an identical capillary on which the window was prepared by the conventional (flame) method *before* filling the capillary with gel (Figure 3A). The 2.0% polyacrylamide gel is not very mechanically stable and is easily damaged; however, the spinning motion to which the capillary (and gel) was subjected in the polyimide stripping procedure was sufficiently gentle so as not to damage the gel matrix. As shown in Figure 3, retention and resolution are comparable in the separations on the two capillaries prepared by the different methods.

CONCLUSIONS

The device described here has several advantages over the conventional method of removing polyimide for producing detection windows on capillary tubing to be used for CE separations. Sharp, well-defined windows can be formed on chemically derivatized or gel-filled capillaries without altering or destroying the chemical functionalities or gel matrices at the window region inside the capillaries. Intercapillary reproducibility of separations might thus be improved since an extended length of tubing can be chemically derivatized or filled with gel and then sections of this bulk capillary fabricated into numerous identical working capillaries. Window placement and width are variable and small sections of the bulk capillary that are damaged (or that contain bubbles or voids in the case of gel-filled capillaries) can be discarded while the remainder of the spool is still utilized. Finally, the apparatus can be fabricated from readily available parts costing under $200.

Registry No. SiO_2, 60676-86-0.

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