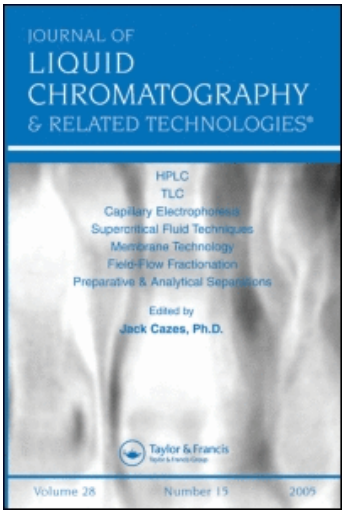

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Improved Detection System for Whole Column Detection in Capillary Isoelectric Focusing

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IMPROVED DETECTION SYSTEM FOR WHOLE COLUMN DETECTION IN CAPILLARY ISOELECTRIC FOCUSING

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ABSTRACT

An improved whole capillary scanning device was constructed which permitted the scanning of capillaries. This device allowed examination of electropherograms during the course of development, as well as eliminated the need to handle the capillaries after separation. The scanning device consisted of a detector constructed around the capillary, which was laterally driven by a DC motor via a rubber belt along polished steel rail. This design produced noise levels better than the earlier prototype on a separation of test proteins. The S/N ratio is close to that of static detection, provided a bare fused silica capillary is scanned. UV transparent capillaries still produced unacceptable noise levels, probably due to optical inhomogeneities in the outer coating which exaggerated minor positional changes due to vibration.

INTRODUCTION

The success of capillary electrophoretic techniques have spawned interest in capillary isoelectric focusing (IEF) (1-5). In IEF typical capillary dimensions are 25 - 200 μm i.d. and 12 - 20 cm long with on-column absorbance detection. Capillary IEF bears the general advantages of capillary electrophoresis, such as high efficiency, high speed and small amount of sample. In most reported capillary IEF techniques it is necessary to mobilize solutes after focusing in order to pass them through the detection region of the capillary. Mobilization can be accomplished by replacement of the basic catholyte with acid, or the acidic anolyte with base, by addition of salt to the catholyte or anolyte, or by hydrodynamic elution.

Since a focused IEF separation represents an equilibrium situation, whole-column detection without mobilization offers potential advantages both in time and in maintaining separation integrity. Early work by Brumbaugh and Ackers (6) attempted to scan a gel-chromatographic column 1 cm in diameter, with limited success. Rowlen *et al.* (7) recently were able to perform whole column detection on an HPLC column. More recently, Wu and Pawliszyn (8) constructed a concentration gradient imaging detector in which a segment of capillary was illuminated by a laser beam, using a photodiode mounted on a scanner to measure the transmitted light, or diode array. While attractive in many regards, a disadvantage of this approach is that only a small segment (2cm) can be scanned, mechanical noise is still present in the scanning diode, and detection is limited to fixed available laser wavelengths, neccessitating derivatization in many cases. This research group (9) has recently demonstrated that a conventional light source can be used to scan the entire capillary by pulling the capillary past a fixed monochromator and diode, with results similar to that achieved using conventional (static) detection. In this paper a new whole column scanning device is demonstrated in which the S/N ratio was improved compared with earlier prototype. The advantages of this design are that examination of electropherograms during the course of development is possible and the need to handle the capillary after separation is eliminated.

EXPERIMENTAL

Figure 1 is a schematic diagram of the capillary scanning apparatus. The detector was a Linear model 204 UV/Vis dual wavelength HPLC detector (Linear, Reno, NV) with output recorded on an integrator (Chromajet, Spectra-Physics, San Jose, CA). An optical fiber bundle (Superguide G-ES 20, Fiberguide, Stirling, NJ) was used to deliver light from the lightsource housed with the HPLC detection system. This radiation was re-focused to a spot of dimension ~ 0.5 mm onto the capillary using a bi-convex fused silica lens (model SBx010, Newport, Fountain Valley, CA) placed at the terminal end of the optical fiber. The photodiode was mounted on the oposite side of the light source as shown in Figure 1. The position of the optical fiber and photodiode was adjustable using a micro-positioner (model 450A, Newport). A precision moving stage was constructed and was driven via a rubber belt by a synchronous DC motor (model DA-1, 1 r.p.m., Hurst, Princetone, IN). The scan speed was variable by replacing the drive gear. Contact with buffer solution and electrical leads was achieved through the use of specially designed buffer wells. The capillary was held in the detection apparatus by two holes (360 μm x 10 mm)

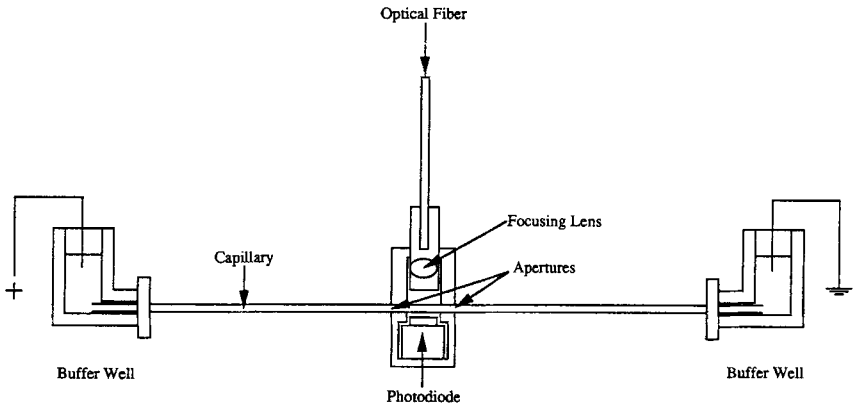

Figure 1. Cross-sectional view of the whole column detection system

drilled on the side wall of the cell. The scan cycles were controlled using a latching relay and two momentary switches at the maximum travel of the stage. The entire apparatus was enclosed in a black acrylic light-tight enclosure. The equipment for capillary electrophoresis was described in reference (9).

MATERIALS AND METHODS

Bovine albumin, ovalbumin, β-lactoglobulin B and carbonic anhydrase were obtained from Sigma (St. Louis, MO), pH 3-10 ampholytes from Fischer (Fair Lawn, NJ) and all other chemical were of analytical grade from Aldrich (Milwaukee, WI). Fused silica capillaries (various diameters) were purchased from Polymicro Technologies (Phoenix, AZ). The details of procedures for IEF and the pre-treatment of capillary were as described in our earlier report (9).

RESULTS AND DISCUSSION

Whole column detection is limited by the noise levels which can be achieved, at least in comparison with static, conventional detection. With chromophores exhibiting appreciable molar absorptivities in the 10^4 range, the detection limits for conventional, commercial instrumentation is typically in the μg/ml range. For *IEF*, original analyte concentrations can be even lower, since they are focused, and hence, concentrated

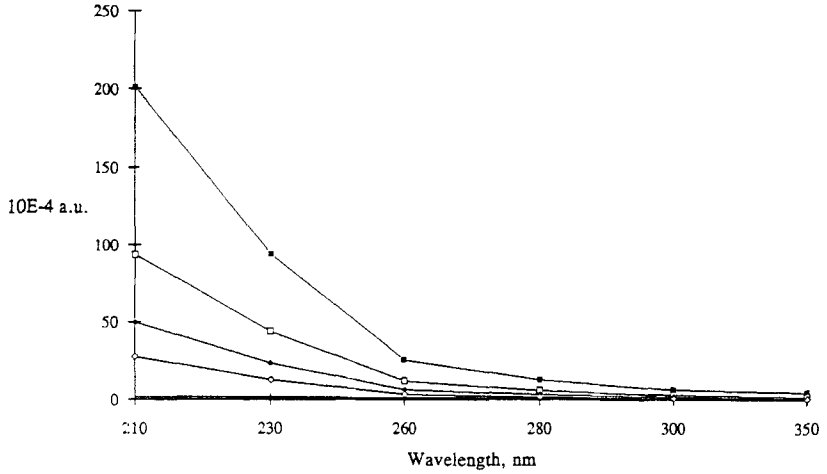

Figure 2. Comparision of noise levels of different capillaries and inner diameters: (■) UV-transparent coated capillary (75μm i.d.); (□) stripped silica capillary (75μm i.d.) measured by pulling mechanism; (◆) stripped silica capillary (75μm i.d.) and (◇) stripped silica capillary (254μm i.d.) measured by capillary scanning mode; (−) stripped silica capillary (75μm i.d.) measured by static. All capillaries were ~355 μm o.d. and filled with water, scanning or pulling speed was 0.25 mm/s.

in the solution. Preliminary data indicate that optical noise in moving column detection arises from mechnical vibrations and from optical imperfections of the capillary, as evidenced by the reduced noise when the outer capillary coating was stripped as shown in Figure 2. The improvement in the S/N ratio is due to the decreased in dynamic noise using a steady moving stage instead of the pulling operation in which vibration and friction were the major sources of dynamic noise. The mechnical vibration was also minimized using a rubber drive belt to move the scanning stage while insulating it from motor vibration. Because the capillary on the moving stage was not directly in contact with the drive motor, mechnical noise was substantially reduced compared to the pulling mechanism (9). However, it is still difficult to fabricate a precision moving stage which minimizes variation along direction perpendicular to the capillary movment. This positional variation was ~25μm in a 10 cm distance for this device which prevents the use of smaller i.d. capillaries. This positional variation is significantly decreased as larger capillaries are used. In Figure 2, the relationship between noise and capillary i.d. is presented.

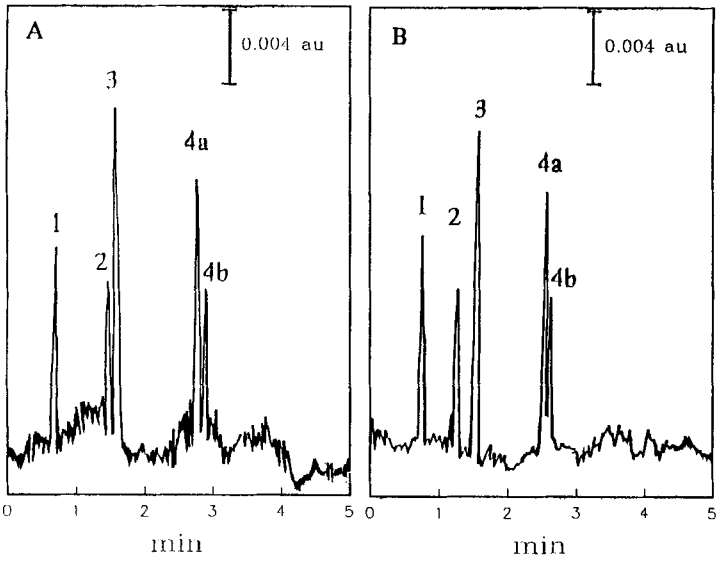

Figure 3. Isoelectropherogram measured using the capillary scanning device. (A) separated on a PEG 8M-10 treated capillary of 75μm i.d x 18 cm in 2% ampholyte (pH 3-10) and (B) separated on a bare capillary of 75μm i.d. x 18 cm in 1% Triton 100 R - 2% ampholyte (pH 3-10). Development voltage 8 kv; 6 min focusing time and scanning speed of 0.25 mm/s detection at 280 nm. Proteins: (1) ovalbumin; (2) bovine albumin; (3) β-lactoglobulin B; (4a) and (4b) carbonic anhydrase, 0.05mg/ml each.

In order to reduce the positional variation during capillary movement, small apertures (~360μm) which held the outer diameter (~355μm) of the capillary were made on the detection cell to align the position of capillary in the detection region. This enhancement in positional precision was accompanied by increased mechanical friction. In addition, we have found that the use of dual wavelength detection in order to further reduce noise due to capillary imperfections, light scattering, and vibration yields further improvements in S/N ratio. In order to maximize the effect of dual-wavelength detection the two wavelengths should be as far apart as possible. Limitations of the detector prevented experiments with wavelength variations larger than 10 nm due to the longer wavelength switching times required.

Finally, the scanning electropherogram of several proteins in Figure 3 also shows an improvement in S/N ratio compared to the previous design (9). In conclusion, this whole column detection method has

significantly improved upon previous designs by further minimizing sources of dynamic noise. The significance of the further development of such a device would be widespread. In the area of IEF, this method provides an improvement over standard IEF separations, in which a mobilization step is required for detection. Parabolic flow or the need to change the inlet buffer limit the efficieny and utility of capillary IEF in its present form. Whole capillary detection is a possible solution to these problems and provides new challenges in capillary detection technology.

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