MICROELECTRODIALYSIS IN VOLUMES OF 10^{-6}-10^{-7} LITER IN CAPILLARY TUBES

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A rapid and highly effective method of separating substances of low and high molecular weights by electrodialysis of 10^{-6}-10^{-7} liter of fluid in capillary tubes filled with 48% polyacrylamide gel as the separating phase is suggested.

KEY WORDS: dialysis of microvolumes; microelectrodialysis; capillary microgel filtration; polyacrylamide gel.

Little attention has been paid to the development of techniques for the dialysis of microvolumes of biological samples, despite the rapid development of microchemical methods of analysis [2, 4]. So far in the literature methods of microdialysis of volumes of the order of 15-120 μl through semipermeable polyhydroxymethylene films have been described [3, 4]. These methods have important disadvantages. First, they cannot be used to dialyze smaller volumes of samples (of the order of 0.1-1 μl) such as are being increasingly encountered in microchemical investigations. Second, the proposed methods of equilibrium dialysis cannot be used when the time for dialysis is severely restricted by the experimental conditions (determination of enzyme activity, analysis of unstable compounds, and so on). Microgel filtration through Sephadex columns also still has limited use because of the considerable dilution of the substances to be separated [2]. Nevertheless, in microchemical experiments carried out with single cells there is a need for rapid dialysis or change of buffered medium in microdrops.

The object of this investigation was to develop a technique of electrodialysis of microvolumes, using 48% polyacrylamide gel (PAA gel), into which high-molecular-weight compounds do not penetrate during electrofiltration, as the separating medium.

EXPERIMENTAL METHOD

Clean glass capillary tubes, rendered water-repellent, 30 mm in length and 600-700 μ in internal diameter, were filled for two thirds of their length with 48% solution of PAA gel. The gel was polymerized actually in the capillary tubes [1, 2]. After polymerization, the prepared capillary tubes were placed in a solution of 0.38 M tris-HCl, pH 9.2. By means of a micropipet held in a micromanipulator 0.1-1 μl of the fluid for dialysis was applied to the surface of the gel. Next, an "electrode bridge," consisting of a glass capillary tube with an internal diameter of 40-50 μ, filled with 48% PAA-gel, was carefully lowered into the capillary tube containing the sample from the top by means of the micromanipulator (Fig. 1). After formation of the "electrode bridge" the top end of the capillary tube containing gel was sealed with plasticine and the capillary tube was held by means of a special holder so that its bottom end was in the anode chamber and its top end in the cathode chamber. The electrode buffer in the chambers was 0.005 M tris–0.077 M glycine, pH 8.3. The Teflon cell and apparatus for capillary microdisk electrophoresis devised by Sandakhchiev and co-workers [2] was adapted for electrodialysis. Electrodialysis was carried out with a voltage of 120-

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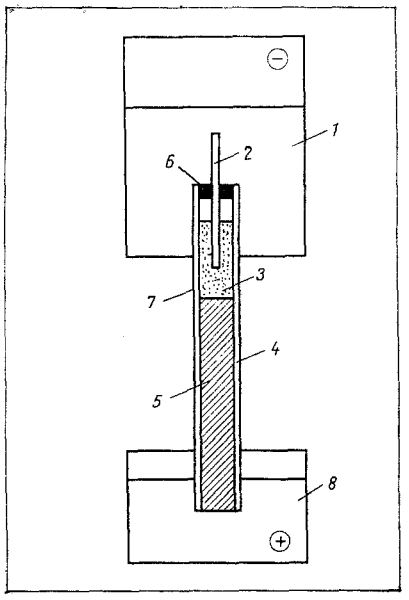

Fig. 1. Scheme showing principles of microdialysis apparatus: 1) top electrode chamber (cathode); 2) capillary tube 40-50 μ in diameter (electrode bridge); 3) sample for dialysis; 4) capillary tube with 48% PAA-gel; 5) column of 48% PAA-gel; 6) plasticine seal; 7) surface of PAA-gel; 8) bottom electrode chamber (anode).

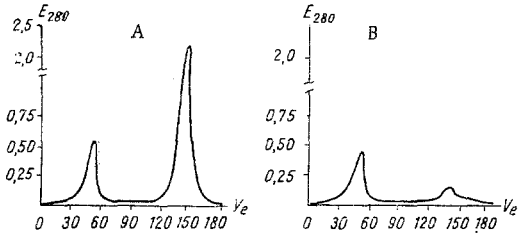

Fig. 2

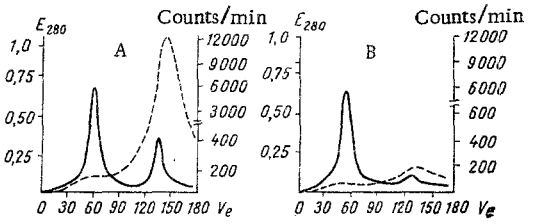

Fig. 3

Fig. 2. Microgel chromatography of a mixture of AMP (1 mg/ml) and rabbit γ-globulin-FITC (5 mg/ml) on a Sephadex G-75 column: A) before, B) after electrodialysis. Abscissa, elution volume; ordinate, optical density (E) at 280 nm.

Fig. 3. Microgel chromatography of a mixture of γ-globulin-FITC, valine-H^3, phenylalanine-H^3, and leucine-H^3, with a total radioactivity of 2.5×10^4 counts/min, on a Sephadex G-75 column: A) before, B) after electrodialysis. Abscissa, elution volume; ordinate (right), optical density (E) of eluate at 280 nm, (left) radioactivity of fractions (in counts/min) each 20 μl in volume.

150 V and a current of 2-3 μA. Migration of low-molecular-weight substances into the gel was assessed from the movement of a band of Bromphenol Blue previously added to the sample for dialysis. The band of dye advanced into the gel for a distance of 15-20 mm, the current was switched off, and the capillary tube with the gel was broken so that not more than 5-8 mm remained from the beginning of the gel on the side of the sample for dialysis to the end. The end of the capillary tube was then reimmersed in the bottom electrode chamber, the polarity of the electrode chambers was changed, and electrophoresis was carried out under the previous conditions for 1-2 min to elute the proteins which had settled on the gel surface. The current was then switched off, the capillary bridge was removed, the top end of the capillary tube filled with plasticine was cut off, and the contents of the sample above the gel were aspirated with a micropipet. The effectiveness of dialysis was monitored by microgel filtration on a Sephadex G-75 (superfine) column

measuring 1 × 160 mm (V_0 = 55 μl, V_t = 135 μl). After 1 μl of the sample had been applied to the gel surface chromatography was carried out at the rate of 2.5 μl/min. The column was connected by a thin polyethylene capillary tube to a microsyringe. The optical density (E) of the eluate was recorded continuously by means of an MSFP-1 two-wavelength microspectrophotometer with a working cell volume of 1 mm^3 (width 1 mm). The concentration of the substances in the original sample and after dialysis was calculated from the value of E of the fraction having the same V_e value as the corresponding compound. The whole procedure of chromatography took 1.5-2 h. Radioactivity of the samples was measured with a Nuclear Chicago scintillation counter. To 5 ml of the toluene scintillator 20 μl of sample was added. In the first experiment, a mixture of AMP and rabbit γ-globulin, labeled with fluorescein isothiocyanate (FITC), was separated by electrodialysis (Fig. 2). The original substances were dissolved in 0.06 M tris-HCl, pH 6.7. These experiments showed that 90-95% of the AMP could be removed from the sample by dialysis for 60-90 min. In the second experiment of mixture of γ-globulin-FITC and three tritiated amino acids was separated (Fig. 3). In this case 98% of label was removed from the sample by electrodialysis if the separation continued for 60-90 min.

By means of the suggested method of microelectrodialysis, compounds with high and low molecular weights in microvolumes of solvent can thus be quickly and effectively separated.

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