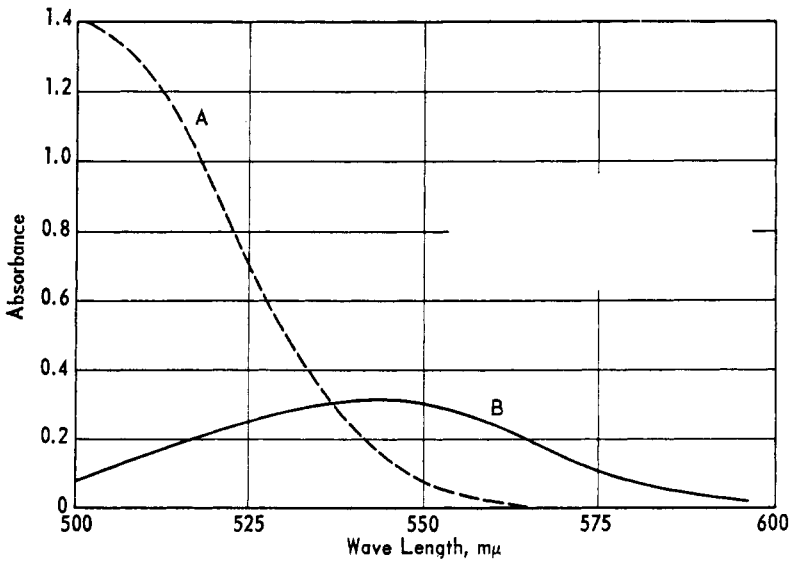

Figure 1. Absorbance of thorin and neptunium-thorin complex

A. Reagents, distilled water reference
B. Neptunium-thorin complex, (5.78 μg./ml. Np), reagent reference
1-cm. cells

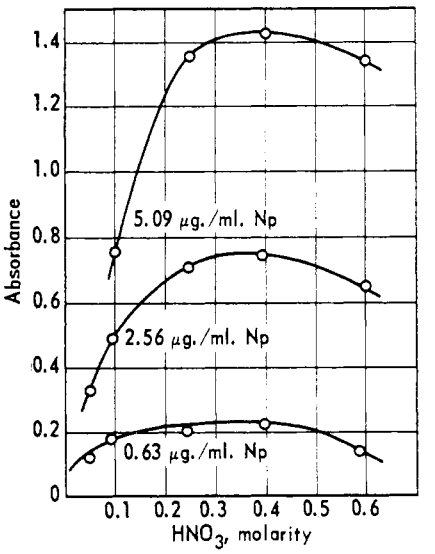

Figure 2. Absorbance of neptunium-thorin complex as a function of nitric acid concentrations at various neptunium concentrations

Procedure. Known amounts of neptunium covering the range 15.8 to 127 μg. were added to 25-ml. volumetric flasks that contained 0.5 ml. of 2*M* ferrous sulfamate and sufficient nitric acid to yield a final acid concentration of 0.4*M* after dilution. The contents were mixed and allowed to stand 15 minutes. Finally 2.5 ml. of 0.1% aqueous solution of thorin was added and the contents were diluted to volume and allowed to stand 30 minutes (Table II). The blank was treated in the same manner as the standards.

Figure 1 shows the absorbance spectra of the neptunium-thorin complex and thorin. Figure 2 shows the effect of the concentration of nitric acid on the absorbance of solutions containing 0.63, 2.56, and 5.09 μg. of neptunium per ml.

The data in Table II show that the complex was stable enough to make the readings after 1 hour. Beer's law was obeyed over the entire range.

A discussion of the interferences in the plutonium-thorin procedure and methods of removing them, given by Healy and Brown (*2*), applies equally well to the neptunium-thorin procedure.

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AIDS FOR THE ANALYST

Quartz Flow Cells for Continuous Spectrophotometric Analysis of Column Effluents

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QUANTITATIVE spectrophotometric analysis of liquid chromatographic column effluents containing ultraviolet-absorbing materials (proteins, peptides, nucleotides, etc.) has generally been done manually on collected fractions. With controlled flow rates, however, the absorption may be recorded continuously with increased resolution, accuracy, and convenience. The major difficulty has been lack of a flow cell of good optical quality that contains a minimum volume of fluid, has no liquid-retaining corners, and connects to small-bore plastic tubing. This paper describes suitable quartz cells and a two-cell holder for the Beckman DU spectrophotometer. Two cells of different optical path length (1.00- and 0.20-cm.) may be used in series to record a greater range of densities. Another method is to connect two identical cells in parallel, with the input to the chromatographic column flowing through one cell and the column effluent through the other. The blank cell may then be moved into position at intervals to check the zero absorbance settings. To match the light transmittance through the two cells, movable masks are provided, which may be adjusted when the cell holder is in the spectrophotometer. With minor changes this system may be used for double-beam recording.

A drawing of the apparatus is shown in Figure 1. The cell holder, 1, is constructed from a standard Beckman DU aluminum cuvette holder (Beckman Catalog No. 5010). The 1.00-cm. path quartz cell, 2, and the 0.20-cm. path cell, 15, (Oak Ridge quartz flow cells, Quaracell Products Co., New York 13, N. Y.) are both 22 mm. long, 12 mm. deep, and 13 mm. wide. Round connecting holes 4 mm. in diameter and 3 mm. deep are provided at both top and bottom. The 0.20-cm. path cell contains 0.064 ml. in a chamber 0.2

[1] Operated by Union Carbide Corp. for the U. S. Atomic Energy Commission.

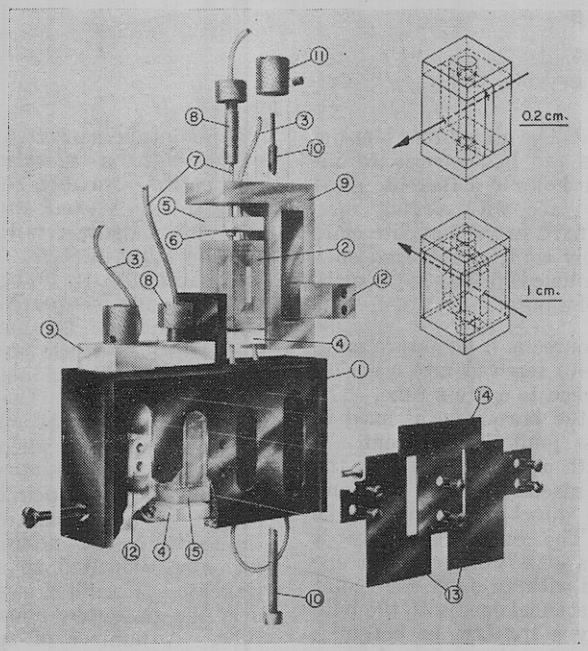

Figure 1. Quartz flow cells and holder

1. Modified Beckman DU cell holder
2. 1.00-cm. path quartz flow cell
3. Inlet tubing
4. Lower Teflon pressure plate
5. Upper Teflon pressure plate
6. O-ring
7. Outlet tubing
8. Upper pressure plate screw
9. Pressure plate screw and mask holder
10. Mask adjusting screw
11. Mask adjusting screw knob
12. Mask attaching piece
13. Adjustable mask
14. Fixed upper mask
15. 0.20-cm. path quartz flow cell

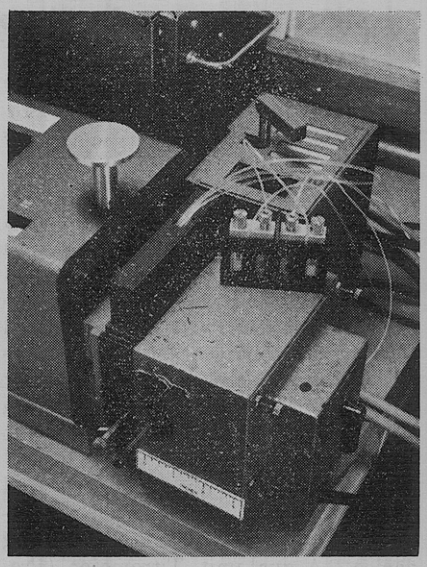

Figure 2. Flow cell and light-tight top enclosure for connecting tubing to cells in spectrophotometer

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cm. square while the 1.00-cm. path cell holds 0.284 ml. in a chamber 0.2 cm. wide, with four triangular quartz corner inserts to minimize dead space in the cell. Flow through the cells is always from bottom to top to sweep out air bubbles.

The inlet tubing, 3, connects with the lower Teflon pressure plate, 4, which presses against the lower surface of the flow cell. Both the lower and upper Teflon pressure plate, 5, have cylindrical projections which fit snugly into the flow cells and are undercut to accept a $^1/_8$-inch I.D. silicone rubber O-ring, 6 ($^1/_{16}$ inch wide, $^5/_{32}$-inch I.D., $^9/_{32}$-inch O.D., AN-6227-2 56022, Leinarts, Inc., Knoxville, Tenn.). The outflow tubing, 7, passes through the upper pressure plate screw, 8, which in turn is threaded into the pressure plate screw and mask holder, 9. The movable mask, 13, is fixed on the mask-attaching piece, 12, which is moved vertically by the mask-adjusting screw, 10, and knob 11. The fixed upper mask, 14, masks the top 3 mm. of both cells to minimize the effects of small bubbles and to match the 0.2-cm. path cell to the 1-cm. path cell with its corner inserts. All masks are adjusted laterally to prevent light from passing through the cells on either side of the cell fluid chamber.

PE 190 polyethylene tubing (1.19-mm. I.D., 1.70-mm. O.D., Clay Adams Co., Inc., New York 10, N. Y.) is drawn out manually to a diameter to pass through the No. 54 (0.055-inch) drill holes in the Teflon pressure plates. The tubing is pulled through the pressure plate until an unconstricted segment is being pulled through. The tubing is then cut off about 0.5 mm. from the inside surface of the Teflon and flared with a small flame. Because pressure seals these connections, no leakage is experienced up to 70 p.s.i. (the highest pressure tested). Connections between segments of tubing outside the cell are made with 12/1 ball socket joints cut off $^3/_4$ inch from the joint. PE 190 polyethylene tubing is drawn through the glass capillary as described for the Teflon pressure plates. If the ball part of the connection is constructed from a glass O-ring sealing ball (made from size 12/5 No. 7775 Orseal ball connectors, California Scientific Glass Co., Pasadena, Calif.), no lubrication is required.

For low pressure work connections between sections of PE 190 tubing are made easily by pushing the square ends into a half-inch piece of PE 205 polyethylene tubing (1.57-mm. I.D., 2.08-mm. O.D., Clay Adams Co., Inc., New York 10, N. Y.). When smaller diameter tubing is required for connection between widely spaced devices, AWG-22 Teflon tubing (nominal 0.69-mm. I.D., 1.29-mm. O.D., Thermatic Inc., Elm City, N. C.) is used and fits snugly into PE 190 polyethylene tubing.

The tubing from the flow cells is led out through the black-anodized aluminum light trap shown in Figures 2 and 3. The trap is constructed in two parts and does not require that the tubing be threaded through it. The

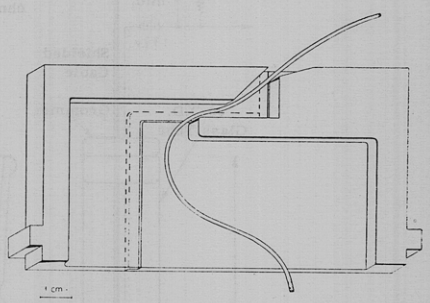

Figure 3. Light-tight top closure for Beckman DU spectrophotometer (section)

absorbance of the stream flowing through the cell may be recorded on a conventional strip chart recorder using a spectral energy adapter (Beckman Catalog No. 5800).

The flow cells have been in continuous use for more than six months, monitoring effluents from nucleotide ion exchange columns and modified cellulose columns for fractionating protein mixtures.