

LITERATURE CITED

- (1) Amberg, S., Loevenhart, A. S., *J. Biol. Chem.* **4**, 149 (1908).
- (2) Belcher, R., Leonard, M. A., West, T. S., *J. Chem. Soc.* **1959**, 3577.
- (3) Belcher, R., West, T. S., *Talanta* **8**, 863 (1961).
- (4) Beveridge, J. M. R., Johnson, S. E., *Canadian J. Res., Sec. E* **27**, 159 (1949).
- (5) Fine, L., Wynne, E. A., *Microchem. J.* **3**, 515 (1959).
- (6) Linde, H. W., *ANAL. CHEM.* **31**, 2092 (1959).
- (7) Loevenhart, A. S., Peirce, G., *J. Biol. Chem.* **2**, 397 (1907).
- (8) Megregian, S., *ANAL. CHEM.* **26**, 1161 (1954).
- (9) Nielson, H. M., *Ibid.*, **30**, 1009 (1958).
- (10) Reiner, J. M., "Behavior of Enzyme Systems," p. 153, Burgess, Minneapolis, 1959.
- (11) Reiner, J. M., Tsuboi, K. K., Hudson, P. B., *Arch. Biochem. Biophys.* **56**, 165 (1955).
- (12) Singer, L., Armstrong, W. D., *ANAL. CHEM.* **31**, 105 (1959).
- (13) Stetter, H., *Chem. Ber.* **81**, 532 (1948).
- (14) Wharton, H. W., *ANAL. CHEM.* **34**, 1296 (1962).

RECEIVED for review June 8, 1964.
Accepted August 26, 1964.

SCIENTIFIC COMMUNICATIONS

Submicroanalysis by Radiochromatography

SIR: Published radiometric precipitation techniques have been used to determine as little as 0.01 $\mu\text{g.}$ of calcium as C^{14} -labeled calcium oxalate and F^{18} -labeled calcium fluoride (1, 2). Employing S^{35} -labeled sulfates, P^{32} -labeled phosphates, and improved radiometric techniques, sensitivities as low as 0.0001 $\mu\text{g.}$ for Ca, Sr, Ba, Pb, Be, and Zr have been achieved in this laboratory. When paper chromatographic separations were performed prior to radiometric precipitation, mixtures of the above elements have been analyzed simultaneously.

Two small aliquots of the original sample in HCl are spotted adjacent to each other on a Whatman No. 42 filter strip (2 \times 30 cm.); tracer activities of the elements to be analyzed are added to one of the aliquots and the chromatogram is developed. For Ca, Sr, and Ba, a mixture of 2 parts ethanol, 2 parts methanol, and 1 part 2N HCl is used. For Pb, a mixture of 5 parts ethanol, 3 parts methanol, and 2 parts 1.6N nitric acid is used. For Zr, a mixture of 30 parts acetone, 17 parts isobutanol, and 1 part 12N HCl is used and for Be, a mixture of 1 part tetrahydrofuran and 1 part dilute HCl is used.

The location of the unknown element is then determined by scanning the added tracer activities. If several tracers are used the areas are identified by beta absorption techniques.

The labeled sulfates of Ca, Sr, Ba, and Pb are precipitated on the paper by adding S^{35} -labeled sulfuric acid 2 cm.

above the area where the unknown was originally spotted and migrating the reagent with a 50% ethanol- H_2O mixture through the elements. The migration is continued at least 10 cm. beyond the unknown area to remove excess acid. For Be and Zr, the labeled phosphates are precipitated by adding P^{32} -labeled phosphoric acid 2 cm. above the first origin and migrating with a mixture of 14 parts isopropanol, 1 part trichloroacetic acid, and 6 parts water.

The paper is then dried and the area cut out for radiometric analysis. The labeled sulfate precipitates are assayed with a liquid scintillation counter and the phosphates with a low background beta scintillation counting system.

Preliminary results have shown that complete chromatographic separations are achieved with the reagents used. In addition, the precipitation on the paper is complete, and the precipitate remains in the areas indicated by the tracers.

For each of the elements a linear relationship was observed between counting rate and weight of the element in the precipitate.

Table I lists detection limits observed for Ca, Sr, Ba, and Pb using 0.3N sulfuric acid with 6.3×10^6 d.p.m. of S^{35} per meq., and for Be and Zr using 0.1N phosphoric acid with 6.3×10^7 d.p.m. of P^{32} per meq. These detection limits represent sample counting rates which are twice instrumental background and are inversely proportional to the specific activity of the precipitant.

Table I. Detection Limits

Element	Observed detection limit, $\mu\text{g.}$
Ca	0.002
Sr	0.005
Ba	0.008
Pb	0.01
Be	0.0001
Zr	0.0002

The radiochromatographic technique described is applicable to many other precipitants such as Cr^{51} -labeled chromate, Ti^{44} -labeled titanate, Zr^{95} -labeled fluorozirconate, C^{14} -labeled carbonate, S^{35} -labeled sulfide, and P^{32} -labeled phosphomolybdate. Therefore, all elements that can be isolated chromatographically can be determined at these low sensitivity levels.

LITERATURE CITED

- (1) Chiriboga, J., *ANAL. CHEM.* **34**, 1843 (1962).
- (2) Kudahl, J. H., *Nature* **197**, 1199 (1963).

G. A. WELFORD
E. L. CHIOTIS
R. S. MORSE

Health and Safety Laboratory
U. S. Atomic Energy Commission
New York, N. Y. 10014

RECEIVED for review July 21, 1964.
Accepted August 19, 1964.