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Rapid and Precise Analysis for Calcium in Blood Serum

A simple, precise, and accurate method for determining the level of calcium in blood serum was required that needed only microquantities of the samples. Volumetric titrations, the standard methods for such determinations, require large samples and a fair amount of manipulation; more-recent methods by atomic-absorption spectrophotometry, although rapid and accurate, require large samples, and the serum protein clogs the jets.

A differential-absorption spectrophotometric technique has been developed that, using murexide, gives a highly precise analysis of calcium in volumes of serum as small as 0.01 ml. The method of additions and proper timing allows compensation to be made for fading, variation in type of serum or plasma, and aging of the specimen. The method is very rapid, especially for large numbers of samples; precision and accuracy are about 1.5%.

The levels of calcium are measured on a double-beam recording spectrophotometer with 1.0-cm silica cells. The indicator solution contains murexide at 0.1 mg/ml in a mixture of 2% water in propylene glycol. The optical density is used as a check on the concentration of the dye; 1.5 ml of indicator in 2.5 ml of 0.044M KOH, compared to a reference cell containing H₂O, should read approximately 1.4 absorbance units (A), at 545 m, 5 minutes after addition to the KOH solution. The working calcium standard is the stock solution diluted 1:10 with water to give a calcium concentration of 0.1 g/liter. The calcium concentration of the diagnostic reagent is 10.5 mg%.

In the analysis, 2.50 ml of 0.044M KOH is pipetted into two 7-ml self-capping polyethylene vials. A 10:1 to 15:1 sample is pipetted into one of the vials,

and the pipette is rinsed with the contained solution. The murexide indicator (1.50 ml) is added to both vials in rapid succession with a commercially available Repipet dispensing unit; then the solutions are mixed by repeated inversions slow enough to avoid foaming.

The solutions are then placed in clean, dry cells before the absorption peak of the sample solution is read against the reagent blank at 497 m, 5 minutes after addition of the dye. The ratio of absorption by the sample to absorption by an equal volume of standard calcium solution (0.1 g/liter), multiplied by 10, equals the concentration of calcium in the sample (in milligrams percent). The rapidity of operations allows three samples and a reagent blank to be dispensed and run at 1-minute intervals without loss of accuracy.

Reference:

1. F. H. Ilcewicz and R. B. Holtzman, *ANL-7360* (Argonne National Laboratory, Argonne, Ill., June 1967), p. 89.

Notes:

1. Medical laboratories, hospitals, and blood centers may be interested in this information.
2. Inquiries may be directed to:

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Reference: B69-10160

Source: F. H. Ilcewicz and R. B. Holtzman
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(continued overleaf)

Patent status:

Inquiries concerning rights for commercial use of this innovation may be made to:

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