Determination of Calcium in Official Calcium Phosphates

By JUNG SOOK KIM and MURRAY M. TUCKERMAN

The assay of official calcium phosphates for calcium can be brought into the general method for calcium determination by titration with EDTA using hydroxynaphthol blue indicator by dissolving the sample in a strictly limited amount of hydrochloric acid, passing an aliquot of the solution through a column of anion-exchange resin in the chloride form, washing out the calcium ion with water, and determining calcium in the eluate. The method is comparable in precision with the official method for calcium in dibasic calcium phosphate, but requires much less time.

In 1965 Butler, Maurina, and Morecombe (1) showed the utility of hydroxynaphthol blue as an indicator in the direct titration of calcium with disodium ethylenediaminetetraacetate (EDTA). All of the pharmaceutical substances official in U.S.P. XVII and N.F. XII which are assayed for calcium are assayed by direct titration with standard EDTA and this indicator with the exception of dibasic calcium phosphate U.S.P., which is assayed by the titrimetric determination of oxalate in a precipitated calcium oxalate with standard permanganate. This duality in methods arises from the present impossibility of a direct complexometric titration of calcium in the presence of large amounts of phosphate.

Calcium has been determined complexometrically in the presence of phosphate by addition of excess complexing agent and determination of the excess (2–9) and also after separation of calcium from phosphate by ion exchange (10–20). From a review of the literature it appeared that a simple, rapid, elegant, direct titrimetric determination of calcium in calcium phosphates was possible.

EXPERIMENTAL

Reagents—All reagents employed were reagent grade chemicals. All standard solutions employed were prepared and standardized according to the official compendia.

Methods—*A*—N.F. XI method (21).

B—Proposed Method—Charge a glass column about 37 cm. long and 2.5 cm. in diameter, fitted with a stopcock and delivery tip, with about 40 Gm. of Amberlite IRA-400 (Cl) resin previously hydrated for about 1 hr. Place about 500 mg. of dibasic calcium phosphate, previously ignited at 800° to constant weight and accurately weighed, in a 100-ml. beaker and dissolve it in 2.5 ml. of 1:2 hydrochloric acid by heating. Transfer the solution to a 100-ml. volumetric flask, dilute to volume, and mix well. Pass a 20.00-ml. aliquot of the sample solution through the ion-exchange column at a flow rate of about 0.5 ml. per minute, collecting the effluent in a 500-ml. conical flask. Wash the column with 175 ml. of water, passing the first 25 ml. at a rate of 0.5 to 1 ml. per minute, the next 50 ml. at about 4 ml. per minute, the next 50 ml. at about 8 ml. per minute, and the last 50 ml. at free flow, collecting the effluents in the same conical flask as the sample aliquot. Add 15 ml. of sodium hydroxide T.S. and about 300 mg. of hydroxy naphthol blue, A.R. indicator, and mix well. The pH of the solution should be between 12.5 and 13.0. Titrate the solution with standard 0.05 *M* disodium ethylenediaminetetraacetate to a change of color from purple to deep blue.

After passage of three samples the ion-exchange column is regenerated by passing through it about 200 ml. of 1 *M* sodium chloride solution at about 5 ml. per minute and washing with 3 to 4 L. of water until there is little free chloride present as shown by testing the effluent with silver nitrate.

RESULTS

Five determinations of a single sample by method *A* gave an average result of 98.9 ± 0.1%. Five determinations of the same sample by method *B* gave an average result of 99.1 ± 0.8%. The standard deviations were calculated from the range of values obtained by the method of Dean and Dixon (22).

Method *B* was also applied to tribasic calcium phosphate N.F. using an ignited sample of about 200 mg. Five determinations gave an average value for calcium of 37.5 ± 0.3%.

DISCUSSION

This assay is of interest from the philosophical standpoint of development of standard methods of assay for specific ions and also from the physical chemistry of the ion-exchange process. For reasons of convenience and economy, it was decided to attempt to use the anion-exchange resin in the commercially available chloride form rather than in the hydroxyl form which is expected to be more efficient. The resin has less affinity for the dihydrogen phosphate anion than for chloride (23).

Since the reactions of ion exchange are stoichiometric and reversible, one may write the equilibrium $aM_1/bR + bN_1/aX \rightleftarrows aN_1/aR + bM_1/bX$, in which M is the anion attached to the univalent resinate cation R and N is the anion added with the univalent

Received April 14, 1967, from the School of Pharmacy, Temple University, Philadelphia, PA 19122

Accepted for publication June 22, 1967.

Presented to the Drug Standards, Analysis and Control Section, A.PH.A. Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.

Abstracted in part from a thesis submitted by Jung Sook Kim to the Graduate School, Temple University, Philadelphia, Pa., in partial fulfillment of Master of Science degree requirements.

cation X. If these letter symbols are allowed to stand for concentration, and the subscripts r and w are used to indicate the resin and the outside solution, respectively, then one can write the classical law of mass action $N_r{}^a M_w{}^b / M_r{}^a N_w{}^b \simeq K$. If K is approximately constant for a given exchange, the numerical value is a measure of preference of the resin for the ion. Note, however, that K is dimensionless only if a and b are equal, so that comparison of ion affinities can be made only for exchanges of ions of the same type, that is, univalent-univalent, univalent-bivalent, *etc.* Despite the limitations of the theory, there is general knowledge that for similar ions affinity increases with increasing ionic charge.

On the basis of the foregoing discussion it is seen that the concentration of hydrochloric acid is critical. There must be enough to dissolve the sample in a reasonable time but not so much chloride ion present as to prevent complete exchange of phosphate. Additionally, a low hydrogen-ion concentration favors the multivalent anion in the equilibrium $H_2PO_4^- \rightleftarrows H^+ + HPO_4^{-2}$.

The importance to be attached to the difference in standard deviations between methods A and B is difficult to evaluate. The standard deviation for the assay of dibasic calcium phosphate is expected to be about 0.4 for either method. A collaborative study with sufficient replicates is needed to establish any real difference in the reproducibility of the two methods. The two methods yield the same result within the expected standard deviation.

No comparisons can be made in the assay of tribasic calcium phosphate as the official method is the determination of phosphate.

CONCLUSIONS

The proposed method is much more rapid than the official method of assay for dibasic calcium phosphate, has acceptable reproducibility, and yields the same result as the official method.

REFERENCES

(1) Butler, A. Q., Maurina, F. A., and Morecombe, F. A., *J. Pharm. Sci.*, **54**, 134(1965).
(2) Banks, J., *Analyst*, **77**, 484(1952).
(3) Betz, J. D., and Noll, C. A., *J. Am. Water Works Assoc.*, **42**, 49(1950).
(4) Biedermann, W., and Schwarzenbach, G., *Chimia*, **2**, 1(1948).
(5) Botha, G. R., and Webb, M. M., *J. Inst. Water Engrs.*, **6**, 459(1952).
(6) Brooke, M., and Holbrook, M., *Chem. Analyst*, **41**, 80(1952).
(7) Brunisholz, G., Genton, M., and Plattner, E., *Helv. Chim. Acta*, **36**, 782(1953).
(8) Conners, J. J., *J. Am. Water Works Assoc.*, **42**, 33 (1950).
(9) Marcy, V. M., *Power*, **94**, 105(1950).
(10) Mustafin, I. S., and Kruchkova, E. S., *Zh. Anal. Khim.*, **15**, 20(1960).
(11) Sobel, A. E., and Hanok, A., *Proc. Soc. Exptl. Biol. Med.*, **77**, 737(1951).
(12) Banewicz, J. J., and Kermer, C. T., *Anal. Chem.*, **24**, 1186(1952).
(13) Cheng, K. L., Kurtz, T., and Bray, R. H., *ibid.*, **24**, 1640(1952).
(14) Cheng, K. L., and Bray, R. J., *Soil Sci.*, **72**, 449(1951).
(15) Budo, Y., and Takagi, H., *Japan Analyst*, **9**, 998 (1960).
(16) Graue, G., and Zahler, A., *Angew. Chem.*, **65**, 532 (1953).
(17) Schafer, H. N. S., *Anal. Chem.*, **35**, 53(1963).
(18) Kruchkova, E. S., and Mustafin, I. S., *Zabod. Lab.*, **27**, 668(1961).
(19) Cluley, H. L. J., *Analyst*, **79**, 561(1954).
(20) Nielsch, W., *Glas-Emsil-Keramo-Tech.*, **6**, 73(1955).
(21) "The National Formulary," 11th ed., J. B. Lippincott Co., Philadelphia, Pa., 1960, p. 69.
(22) Dean, R. B., and Dixon, W. J., *Anal. Chem.*, **23**, 636(1951).
(23) Peterson, S., *Ann. N. Y. Acad. Sci.*, **57**, 144(1953).

Technical Articles

Pharmaceuticals Stored in Plastic Containers

By HAROLD M. BEAL, ROBERT J. DICENZO, PAUL J. JANNKE, HENRY A. PALMER, JULES PINSKY*, MORRIS SALAME*, and TULLY J. SPEAKER

IN THE DEVELOPMENT of light weight highly mobile combat support hospitals and medical treatment facilities, the U. S. Army Medical Research and Development Command recognizes the need for the standardization of pharmaceutical containers in addition to the reduction of their weight, cube, and breakage. The following excerpt from the USAMRDC bulletin, "Development Requirements," describes the general characteristics for nonglass containers which are sought for drugs, biologicals, and reagents:

"Purpose: The purpose of this requirement is to develop a family of immediate containers for drugs, biologicals and reagents that will materially reduce the packaged bulk of such items. These containers are to be compatible with contents and be suitable for use in any field environment and under all tactical conditions. They will be of standard size and shape, light weight, small cube and chemical-gas-temperature-breakage resistant."

Quite logically, the question arose as to the stability of drugs packaged in containers other than glass. Since certain plastics possess, at least in part, the characteristics sought, a project

Received May 4, 1967, from the School of Pharmacy, University of Connecticut, Storrs, CT 06268
Accepted for publication July 14, 1967.
* Monsanto Co., Bloomfield, CT 06002
This investigation was supported by the Medical Research and Development Command, U.S. Army Surgeon General's Office, under contract DA-49-193-MD-2568, Washington, D.C.