THE APPLICATION OF FLAME PHOTOMETRY TO SODIUM AND POTASSIUM DETERMINATIONS IN BIOLOGICAL FLUIDS*

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(Received for publication, January 3, 1947)

The need for a rapid, accurate method to determine quantitatively Na and K present together in biological fluids has been met by the procedures to be described. The necessity of separating these elements prior to their determination by the various chemical methods usually employed has led to procedures which are often prohibitively tedious and time-consuming. The recent development of flame photometry has now made possible physical methods of analysis in which chemical separation of Na and K is unnecessary (1, 2).

Although we are dealing with Na and K determinations only, the principle of flame photometry originally used by Lundegardh (3–5), and modified and called the air-acetylene flame method by Cholak and Hubbard (6), can theoretically be applied to the determination of all the alkaline metals. At present the available commercial instruments employ a relatively low temperature flame which activates the ions rather than the metals themselves; the sensitivity of the photocells is such that the lines of the metals themselves would not be of sufficient intensity to measure, and suitable optical filters for other metals are not readily available.

The present report concerns the use of the Perkin-Elmer flame photometer (model 18)[1] in sodium and potassium determinations on blood, plasma, red blood cells, and urine.

Materials and Methods

The Instrument—The model 18 Perkin-Elmer flame photometer[2] was developed primarily for use in industry when rapid and accurate Na and K determinations on inorganic solutions are desired. The instrument consists

* The work described in this paper was supported by a grant from the United States Public Health Service.

[1] Manufactured by the Perkin-Elmer Corporation, Glenbrook, Connecticut.

[2] Development of flame photometry to the point of commercial manufacture of a laboratory instrument was carried out at the Stamford Research Laboratories of the American Cyanamid Company. The instrument used in these investigations was designed by the Perkin-Elmer Corporation to meet the demand of investigators who had used or heard of the instrument developed at the American Cyanamid Company.

essentially of (*a*) an aspirator and atomizer operated by compressed air, (*b*) a modified Meeker burner, (*c*) two photocells with filters, (*d*) an amplifier, and (*e*) a galvanometer. Solutions are aspirated and atomized at a steady rate, rise into the flame of the burner where the elements are heated to incandescence, and the amount of light emitted at particular wavelengths is measured by a null point galvanometer (1). The light to the Na photocell passes through an optical filter whose highest per cent transmission is 5844 A (5465 to 6500 A), while the K photocell is covered by a filter whose peak transmission is 7716 A (7000 to 9240+ A).[3]

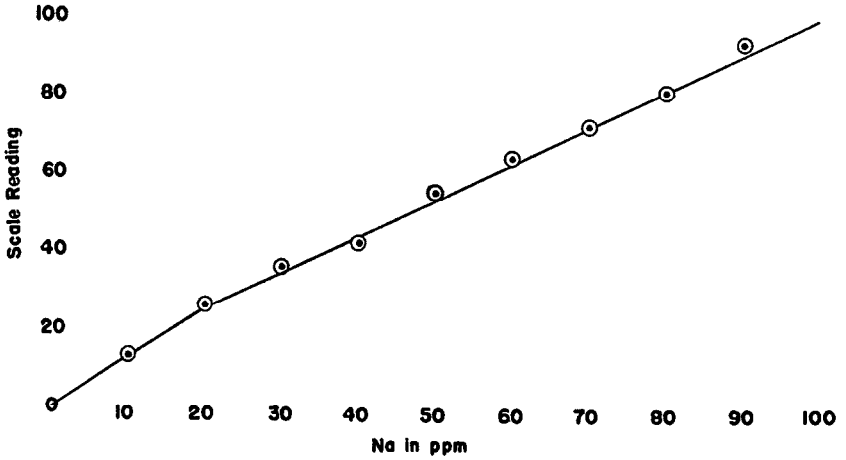

FIG. 1. Calibration chart for Na (0 to 100 p.p.m.) in the presence of 0 to 100 p.p.m. of K.

In practice the instrument is first calibrated with solutions of known ionic strength covering the range of concentrations expected in the unknown solutions. Thus the zero point of the vernier controlling the null point galvanometer may be set with distilled water and the 100 per cent point with a solution containing 100 parts per million (0.01 mg. per cent) of Na by weight. By making determinations on known solutions between these two points, calibration curves are constructed (Figs. 1 and 2) covering the range of concentrations expected in the unknown solutions to be tested.

Although with suitable calibrations a concentration of Na as high as 10,000 p.p.m. (1 mg. per cent) can be determined, actual experience has revealed that the most satisfactory results are obtained on solutions containing less than 100 p.p.m. each of Na and K (1). Consequently, dilutions of blood, plasma, red cells, and urine are necessary before determinations are made.

[3] Fraser, W. A., personal communication.

Since the presence of a high concentration of one element *may* affect the readings made on another element, it is likewise important to use solutions for calibrating the instrument which contain approximately the same *relative* amounts of Na and K as are to be expected in the unknown. In this regard, it has been shown (7) that a solution containing 1000 p.p.m. of Na will give a deflection amounting to 3 p.p.m. on the K photocell, while a solution containing 1000 p.p.m. of K will give a galvanometer deflection corresponding to 0.2 p.p.m. on the Na photocell. At the concentrations of Na and K encountered in the procedures to be described, these errors are almost negligible; nevertheless it has seemed advisable to prepare the standards with this interference in mind.

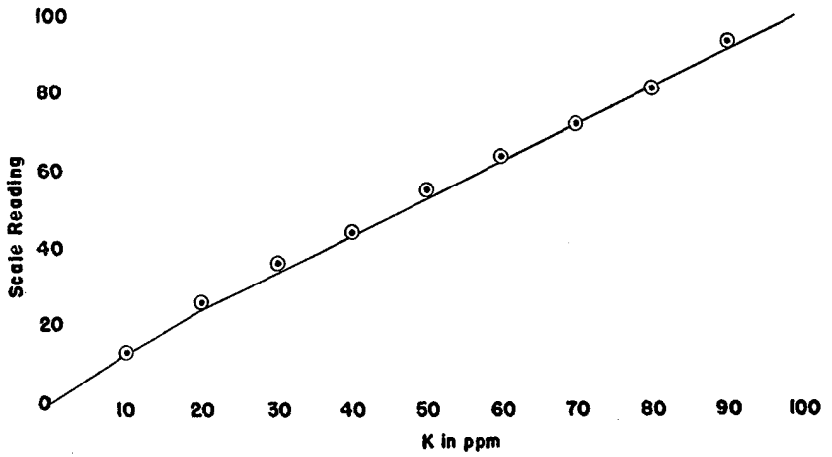

FIG. 2. Calibration chart for K (0 to 100 p.p.m.) in the presence of 0 to 100 p.p.m. of Na.

Before each unknown solution is run through the instrument, the zero point and the 100 per cent point are checked with the standard solutions. It has been found that running an "intermediate standard" (*i.e.*, some solution of known concentration between 0 and 100 per cent) is an additional check on the accuracy of the calibration.

The error encountered in flame photometric determinations on biological fluids is due to the fact that runs are made on diluted samples and consequently the small machine error (1 or 2 p.p.m) must be multiplied by a relatively large dilution factor (usually a factor of 5 or 6). However, operation of the instrument in the manner described and making multiple (three or five) readings on each unknown will produce results which have an accuracy of ± 4 per cent of the total amount of Na present and ± 3 per cent of the total amount of K present (Table I). Thus, the accuracy

of the flame photometric determinations of Na and K in blood, plasma, and urine approximates that of the microchemical methods (8). Owing to the introduction of the dilution factor in magnifying the machine error, it will be apparent that the recoveries of small amounts of added Na, for example, from relatively large amounts already present (Table I, first line), will result in sizable *recovery* errors. The solution of this problem may lie in "internal standard" flame photometry as described by Berry, Chappel, and Barnes (2).

TABLE I

Recovery of Na and K from Dog Plasma by Flame Photometry

The results, expressed in mg. per 100 ml., are the averages of fourteen determinations.

Present	Added	Total	Found	Average recovery	Per cent error
			Sodium		
367	8	375	375, 368, 368, 354, 361	365	−2.66
367	32	399	394, 396, 400, 425, 413	406	+1.75
367	98	465	451, 473, 465, 451, 413	457	−1.72
367	168	535	550, 538, 562, 535, 444	545	+1.87
367	221	588	600, 626, 575, 581, 585	593	+0.85
			Potassium		
7.7	24.3	32	31.3, 30.6, 30.6, 31.3	31.0	−3.13
17.0	81	98	91, 103, 101, 94	97	−1.02
17.0	148	165	163, 168, 172, 163	167	+1.21
17.0	205	222	231, 228, 231, 228	229	+3.15
17.0	253	270	281, 281, 281	281	+4.07
17.0	333	350	362, 362, 356	360	+2.86

The accuracy of reading the instrument, and consequently the accuracy of the determination, can be improved further by decreasing the ratio between the concentration and the scale (vernier) reading. Thus, if a solution containing 50 p.p.m. is used to set the zero point and one containing 80 p.p.m. is used to set the 100 per cent point (range setting), each of the 100 scale divisions will equal 0.3 p.p.m. instead of 1 p.p.m. as is the case when the zero is set with distilled water (0 p.p.m.) and a 100 p.p.m. solution is used to set the 100 per cent point. By thus reducing the concentration-scale division ratio, the accuracy can be increased to ± 3 per cent of the amount of Na present and ± 2 per cent of the amount of K present.

Preparation of Standard Solutions

The primary standards for flame photometric analysis are prepared by making separate solutions of sodium and potassium of 1000 p.p.m. concentration by weight. These solutions are diluted before use to the proper concentrations in the following manner.

For whole blood, 10 ml. of the sodium standard plus 10 ml. of the potassium standard are diluted to the mark in a 100 ml. volumetric flask.

For plasma, the standard for whole blood is used for the sodium determination. For the potassium determination 5 ml. of the potassium standard plus 30 ml. of the sodium standard are diluted to the mark in a 100 ml. volumetric flask.

For red blood cells, the standard for whole blood is used for potassium. For sodium, 5 ml. of the sodium standard plus 50 ml. of the potassium standard are diluted to the mark in a 100 ml. volumetric flask.

For urine, any one of the standards may be employed (depending on how dilute the urine is) with the provision that the standard must contain a higher concentration of the element being determined.

Preparation of Samples

Plasma—2 ml. of plasma are diluted with 20 ml. of distilled water and the proteins precipitated by slow addition of 3 ml. of 20 per cent trichloroacetic acid. The precipitate is removed by centrifugation and a portion of the supernatant (20 ml.) is run through the flame photometer to determine the K concentration. Since normal plasma contains between 315 and 345 mg. per cent of Na and but 16 to 22 mg. per cent of K, and since the presence of large amounts of Na affect the K readings (but not vice versa), calibration of the instrument, as noted above, *must* be based on solutions containing similar relative proportions of these ions. In practice, the calibration curve used for K in plasma is constructed from readings on solutions containing between 0 and 300 p.p.m. of Na and 0 and 50 p.p.m. of K (Fig. 3). At the dilution of plasma used, the scale readings lie between 20 and 35, which represents between 13 and 20 p.p.m. of K.

5 ml. of the supernatant are further diluted to 25 ml. with distilled water and the determination of Na is made on the resulting solution. The Na determination in plasma is based on a calibration curve obtained on solutions containing from 0 to 100 p.p.m. of Na and 0 to 100 p.p.m. of K. In the dilution used, the scale readings for plasma Na lie between 57 and 70, which corresponds to concentrations of from 52 to 62 p.p.m. of Na in the diluted unknown.

Whole Blood—2 ml. of heparinized blood are diluted with 20 ml. of distilled water with a consequent laking of the red blood cells. The

protein is precipitated by the slow addition of 5 ml. of 20 per cent trichloroacetic acid and the precipitate is removed by centrifugation. 10 ml. of the supernatant are diluted to 50 ml. with distilled water and both Na and K are determined on the resulting solution. Since whole blood contains approximately equal concentrations of Na and K, the calibration curve in this case is based on solutions containing between 0 and 100 p.p.m. of Na and 0 and 100 p.p.m. of K. In the dilution of whole blood used, scale readings for Na lie between 20 and 45, corresponding to between 15 and 40 p.p.m. of Na, while the scale readings for K lie between 30 and 40, corresponding to between 20 and 35 p.p.m. of K in the diluted unknown.

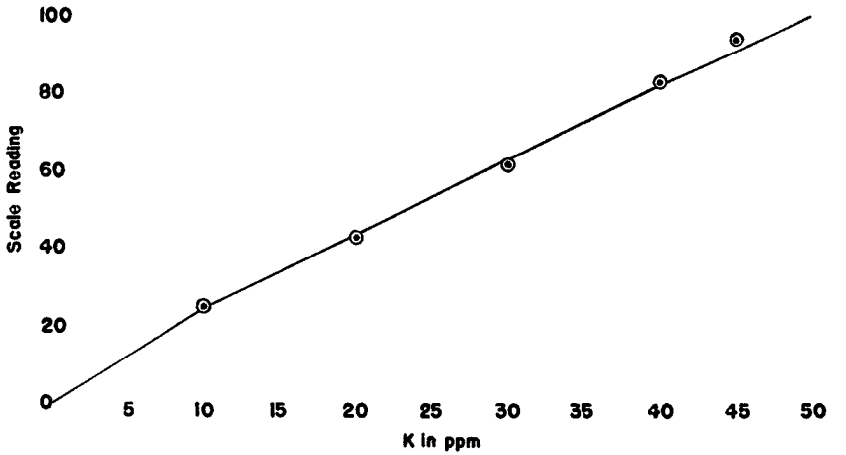

FIG. 3. Calibration chart for K (0 to 50 p.p.m.) in the presence of 0 to 300 p.p.m. of Na.

Red Blood Cells—Erythrocytes are separated from plasma by repeated centrifugation at 3000 R.P.M. for a total of 1 hour. The plasma and the top layer of red cells are removed by a suction pipette and 1 ml. of red cells is pipetted from the bottom of the centrifuge tube and is diluted and laked with 20 ml. of distilled water. The protein is precipitated by the slow addition of 3 ml. of 20 per cent trichloroacetic acid. The precipitate is removed by centrifugation and Na is determined on the resulting supernatant. By this method normal human red cells appear to contain less than 20 mg. per cent of Na.

10 ml. of the supernatant are further diluted to 50 ml. with distilled water and K is determined by means of a calibration curve based on solutions containing between 0 and 100 p.p.m. of Na and 0 and 100 p.p.m. of K. Scale readings, with such a dilution, lie between 35 and 40, corresponding to between 30 and 35 p.p.m. of K.

Urine—1 to 3 ml. of urine is diluted to 100 ml. with distilled water and the resultant solution is used directly to determine both Na and K. The calibration charts used for these determinations depend entirely upon how concentrated the urine is. Trial runs are made in order to ascertain whether the scale is to be set at 100 p.p.m. or higher.

With the methods of sample preparation described above, values for Na and K in whole blood, plasma, red blood cells, and urine have been obtained on 60 human subjects. The average values and the ranges are presented in Table II. It will be seen that the average values obtained by this method agree quite well with the usually accepted values established by the more laborious and tedious chemical methods (9–12).

TABLE II

Comparison of Average and Range Data of Flame Photometric and Chemical Methods for Na and K Determination in Human Body Fluids

		Flame photometer, average*	Flame photometer, range	Average previously reported†	Range previously reported†
Whole blood	Na	218	190–257	200	170–225
	K	222	145–257	200	150–250
Plasma	Na	352	323–366	340	315–345
	K	20	14– 28	20	16– 22
Red blood cells	Na	14	Trace to 31	0	
	K	437	425–444	420	
Urine	Na	220	80–385		
	K	197	105–465		

*Average of determinations on thirty human subjects.

†See references (9–12).

DISCUSSION

The methods described here should be valuable not only because they give results as accurate as do the usual chemical methods but also because they materially reduce the time necessary to determine both Na and K. Perhaps the greatest advantage of flame photometry lies in the fact that it is not necessary to separate Na from K in order to determine either of them by this method.

However, it must be pointed out that considerable care is required in making standards, in calibrating the instrument and rechecking the calibrations during the runs, in preparation of the unknown solutions, and in operation of the instrument in order that one may rely on the results.

Numerous other methods of preparing biological fluids for flame photometric determination of Na and K were tried unsuccessfully. Wet ashing methods with various combinations of sulfuric and nitric acids, sulfuric and perchloric acids, and sulfuric acid and hydrogen peroxide were attempted. Each of these methods depends upon the presence of some sulfuric acid which must be neutralized (with NH_4OH) before the solution can be run through the flame photometer. None of these methods has been successful, since, apparently, the presence of large amounts of salt (in this case $(NH_4)_2SO_4$) causes a depression of both the Na and K curves (2).

Dry ashing methods are likewise unsuccessful because splattering and creeping of the solutions occur, the masses of residue left after ashing small blood samples are extremely minute and consequently difficult to handle quantitatively, and, finally, in the case of whole blood or red cells, complex iron compounds are formed which possess extremely low solubilities.

In flame photometric determination it is not necessary to remove all organic material before the solution is run through the instrument. It *is* advantageous to be rid of most of the protein, which may clot or precipitate and clog the aspirator tubing. Such a degree of protein removal is readily obtained with 20 per cent trichloroacetic acid and, since the samples are so highly diluted, no serious coprecipitation of ions with the protein is encountered.

SUMMARY

1. Rapid procedures for flame photometric analysis of Na and K in blood, plasma, red blood cells, and urine are described.

2. The accuracy of these more rapid methods approximates that of the more laborious chemical methods usually employed in Na and K analysis.

3. Average and range data from 60 control determinations on human subjects are presented and compared with data previously reported.

4. Some of the precautions to be taken in the operation and calibration of the flame photometer are discussed.

The authors wish to acknowledge the cooperation and assistance of Dr. T. S. Hill, Chief of the Division of Neurology and Psychiatry, University of Tennessee College of Medicine, throughout the course of this study.

BIBLIOGRAPHY

1. Barnes, R. B., Richardson, D., Berry, J. W., and Hood, R. L., *Ind. and Eng. Chem., Anal. Ed.*, **17**, 605 (1945).

2. Berry, J. W., Chappel, D. G., and Barnes, R. B., *Ind. and Eng. Chem., Anal. Ed.*, **18,** 19 (1946).
3. Lundegardh, H., Die quantitative Spektralanalyse der Elemente, Jena, pt. 1 (1929); pt. 2 (1934).
4. Lundegardh, H., *Lantbruks-Högskol. Ann.*, **3,** 49 (1936).
5. Lundegardh, H., and Phillipson, T., *Lantbruks-Högskol. Ann.*, **5,** 249 (1938).
6. Cholak, J., and Hubbard, D. M., *Ind. and Eng. Chem., Anal. Ed.*, **16,** 728 (1944).
7. Instruction manual for operation of model 18 flame photometer, Perkin-Elmer Corporation, Glenbrook (1945).
8. Peters, J. P., and Van Slyke, D. D., Quantitative clinical chemistry, Methods, Baltimore, 741 (1932).
9. Andes, J., and Eaton, A. G., Synopsis of pathological chemistry, St. Louis (1941).
10. Levinson, S. A., and MacFate, R. P., Clinical laboratory diagnosis, Philadelphia, 2nd edition (1943).
11. Kracke, R. R., and Parker, F. P., Textbook of clinical pathology, Baltimore, 2nd edition (1940).
12. Todd, J. C., and Sanford, A. H., Clinical diagnosis by laboratory methods, Philadelphia, 10th edition (1943).