THE DETERMINATION OF SODIUM IN SMALL VOLUMES OF FLUID BY FLAME PHOTOMETRY

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(With Two Text-figures)

INTRODUCTION

A method of measuring the freezing-point of small quantities (10^{-3} cu.mm.) of fluid has recently been described (Ramsay, 1949). This method opens up certain lines of investigation hitherto impracticable owing to the small quantities of fluid available. But measurements of total osmotic concentration as given by freezing-point are of limited value by themselves, and it is obviously useful to develop analytical methods which will give the concentrations of the various substances present in the fluid. The prime requirement is that such methods shall be applicable to samples of this same order of size; even fairly rough approximations might give valuable information in the present state of knowledge.

There are difficulties in reducing the scale of operation of ordinary chemical methods. These difficulties are first apparent in gravimetric work, then in volumetric and finally in colorimetric work, when quantities eventually become too small to give a measurable depth of colour. It is natural, therefore, that attention should turn to spectrometric methods which are less subject to these limitations, and more particularily so because the alkali metals sodium and potasssium, which are rather difficult to determine chemically, give intense and easily excitable emission spectra.

A general account of the use of spectrographic methods in biological problems has been given by Mitchell (1947, 1948). It is a common feature of such investigations that the element to be studied is present in very low concentration in a material which is available in more than adequate quantity. In the present problem we have a different situation, the element to be studied being a major constituent of the medium but only very small quantities of the medium being available.

In the first situation it is generally necessary to employ high-energy methods of excitation—arc or spark—and a spectrometer of high quality is required to separate the lines of the trace element from the lines produced by the other elements in the material. Conventionally, the spectrogram is recorded on a photographic plate and the intensities of the various lines are measured with a densitometer; even if the lines are weak the abundance of material makes it possible to give a sufficiently long exposure. The development of high-amplification photo-multiplier tubes has now made direct reading methods practicable. With stable conditions of excitation steady levels of intensity can be determined (Dieke & Crosswhite, 1945); alternatively,

integrating devices can be used over a fixed period of exposure. Elaborate integrating instruments have been developed by Saunderson, Caldecourt & Peterson (1945) and by Hasler, Lindhurst & Kemp (1948) for use in metallurgical processes.

In the second situation, where the amount of material may be so small that measurable intensity is sustained for only a fraction of a second, the value of integrative methods is obvious. The photographic method is, of course, integrative but infinitely more laborious than the photo-electric method. Another feature of the second situation is that the emission spectra of the alkali metals can be excited at the relatively low temperatures of the air-coalgas flame which are insufficient to excite most other elements, so that, even when emission of the alkaline earths calcium and magnesium have to be reckoned with, the flame spectrogram of a biological fluid remains relatively uncomplicated. It is therefore possible to separate the components of the spectrogram with a crude spectrometer or even with a system of filters. These considerations are, in fact, basic to the design of the instrument known as the flame photometer (for a recent review see Leyton, 1948).

A flame photometer is described by Barnes, Richardson, Berry & Hood (1945). The solution containing the element to be determined is atomized into a flame, and the light from the flame passes through an appropriate filter to a photo-cell and galvanometer. The application of this apparatus (and of the related Perkin-Elmer Model No. 18) to the determination of sodium and potassium in biological fluids is described by Hald (1947) and by Overman & Davis (1947). Other types of flame photometer are described by Belke & Dierkesmann (1948) and by Domingo & Klyne (1949). These authors were attracted to the method because of its speed for routine purposes, and were accustomed to use 0·1–2·0 ml. of blood, urine, etc., diluted as required, so that it is not easy from their work to discover the ability of these instruments to deal with small quantities. From the original description by Barnes we learn that 2–3 ml. of diluted fluid are required to obtain a reading. From Bills, McDonald, Niedermeier & Schwartz (1949) it appears that the Perkin-Elmer has a lowest standardization range of 0–1 p.p.m., implying that 1 p.p.m. can be set to give a deflexion of 100 scale divisions. 2 ml. of a 1 p.p.m. solution contain 2×10^{-3} mg. sodium; human serum contains about 3·15 mg./ml. so that the volume of serum required to give 2 ml. of 1 p.p.m. sodium is 0·635 cm.mm.—more than 600 times greater than the volume specified as a prime requirement. Existing flame photometers have two main disadvantages. First, only a portion of the sample is effectively atomized into the flame, the rest going to waste; secondly, a steady level of emission is required for a reading, and during the time taken for the instrument to settle down some of the sample is burned away to no purpose. These two disadvantages can be avoided by the use of a method whereby the whole of the sample is passed through the flame and the total emission is integrated. In addition, the use of photo-multiplier tubes offers possibilities of greater sensitivity.

When considering the possibility of applying integrative flame photometry to this problem of dealing with small volumes I was fortunate in meeting Dr T. M. Sugden who had already made use of the principle on a larger scale (Belcher & Sugden, 1950). I am glad to have this opportunity of thanking Dr Sugden for the information

and advice he has given me on various aspects of chemical spectroscopy. I was also fortunate in that this problem aroused the interest of Mr S. W. H. W. Falloon, and in collaboration with him and with Mr K. E. Machin the spectrometer and integrating system were designed and built. This apparatus is described in detail elsewhere (Ramsay, Falloon & Machin, 1951). The purpose of the present paper is to describe the techniques which are special to the use of this apparatus with small samples of biological fluid.

PRINCIPLE

A small volume—of the order of 10^{-3} cu.mm.—of fluid containing sodium is drawn up into a silica capillary pipette to a mark. This sample is then transferred to a platinum wire and dried. The platinum wire is thrust into an air-coalgas flame by a mechanical device. The light from the flame enters a spectrometer; the spectrum falls upon a mask having a slit which allows the sodium D lines to pass through to the cathode of a photo-multiplier tube. The photo-multiplier tube is connected to an integrating circuit and the reading is presented on a meter. The instrument is calibrated with known solutions of sodium which are drawn up to the same mark in the capillary pipette.

APPARATUS

This apparatus is fully described elsewhere (Ramsay, Falloon & Machin, 1951), and only a bare outline will be given here.

The platinum wire is carried into the flame by a rotating arm, actuated by a spring and set in motion by a cable release.

The burner is of the Meker type, using coalgas and compressed air; it is surrounded by an outer tube or sleeve which carries filtered air so that dust-laden air from the room is not carried into the flame. Owing to pressure variations in the air and gas mains it is necessary to use air and gas reservoirs of the gasometer type which can act as regulators or can be used as the sole source of supply just when the observation is to be made.

The spectrometer has a slit 1 in. in height and 0·015 in. in width. The lens system is of 2·9 in. aperture and a large carbon disulphide prism is used. The photo-multiplier, type 931A, has a stabilized power supply. The output of the photo-multiplier is taken via a cathode follower to a Miller integrator. The meter effectively records the change in anode voltage of the integrator. It can be adjusted to zero before each reading. The meter scale is 0–250 and can be read to the nearest unit.

In order to cut down the amount of light when more concentrated solutions are being tested an iris diaphragm is mounted just in front of the slit.

MANIPULATION OF THE SAMPLE

The use of fine silica capillaries for manipulating small volumes of water under liquid paraffin is described under the freezing-point method (Ramsay, 1949), and the technique used in the present work follows the same lines. The pipette into which the sample is drawn resembles a 'freezing-pipette' in that it is pulled from 1 mm.

diameter thin-walled 'Vitreosil' silica tubing and has a long gentle taper. The bore over the terminal 5 mm. or so should be about 20 μ and as uniform as possible. This terminal portion is bent at an angle of about 30° to the rest. It is given a thin coat of Bakelite 'Damarda' lacquer which being hydrofuge serves to prevent water creeping along the outside. The lacquer also serves to hold a short (2–3 mm.) piece of glass rod about 20 μ in diameter against the capillary, so that the end of the rod can be used as a reference mark about 2 mm. from the tip (Fig. 1 A). The pipette is fixed with sealing wax to a mercury reservoir and screw-plunger, which in its turn is mounted on an adjustable stand and arranged so that the bent terminal portion of the pipette is horizontal. Mercury is driven to the tip, and is then withdrawn under liquid paraffin which is allowed to fill the tapering portion of the pipette.

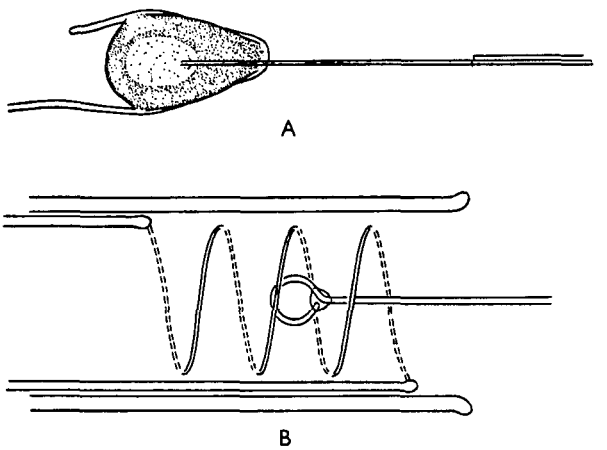

Fig. 1. A, the silica capillary pipette with tip inserted into drop of distilled water in platinum wire V; the sample about to be ejected. B, the platinum wire (which carries the sample into the flame) seen inserted into the tube containing the coil of nichrome wire. The drop from the storage tube is seen deposited upon the neck of the loop.

Subsequent operations are carried out under a medium-power binocular microscope. The droplet of the solution to be examined is kept under liquid paraffin in a watch-glass varnished internally with Bakelite lacquer. The watch-glass is placed on the stage of the microscope, the pipette is lowered into it, and by moving the microscope on the bench the droplet can be impaled upon the tip of the pipette. The solution is then drawn up into the pipette as far as the reference mark, the high viscosity of the liquid paraffin making control of this movement quite easy.

At this stage the sample might be transferred directly to the platinum wire which is to carry it into the flame, but since the process of drawing up and ejecting a measured sample takes time, and since the sensitivity of the apparatus is not constant over long periods, it is more convenient to accumulate several samples and to run them through the flame as rapidly as possible. The sample is therefore stored in another silica tube until required.

The storage tube is drawn in the same way as a 'freezing pipette', but is cut off so as to be about 100 μ in diameter at the tip and is lacquered and waxed on the

outside. It is mounted with sealing wax upon a short piece of ordinary glass tubing which pierces the cork of a small test-tube; the storage tube projects into the test-tube and is thus kept protected and in a moist atmosphere. The sample is transferred to the storage tube in the following way. A piece of thin (0·005 in.) platinum wire is mounted upon a base which slides over the stage of the microscope and carries the wire about 2 cm. above the stage. The end of the wire is bent into a V. This V is brought into the field of the microscope and a drop of distilled water, about 1 cu.mm., is deposited from a storage tube into the V. The pipette is raised, its tip is inserted into the drop and the sample is expelled—see Fig. 1 A. The drop now containing the sample is allowed to flow back into the storage tube.

The platinum wire which carries the sample into the flame is 0·015 in. in diameter, about 1·5 in. long and the free end is formed into a loop of about 0·1 in. diameter. This wire is permanently mounted on the arm which carries it into the flame. A low-power binocular microscope is arranged so as to be above the wire when it is in the 'out' position. The drop from the storage tube is blown out and deposited upon the neck of the loop. A coil of nichrome wire, formed inside a glass tube of about 0·4 in. internal diameter and heated to a dull red, is passed over the wire and held in position until the drop has evaporated (Fig. 1 B). The sample is then ready to be passed into the flame.

The meter and the various other controls are grouped at the photo-cell end of the spectometer. The air and gas valves in the 'off' position connect the mains to the gasometers, but the gasometers are not connected to the burner (except for a pilot flame). The valves are first moved to an intermediate position so that mains, gasometers and burner are interconnected and the flame is lit. The meter, which responds slightly to the lighting of the flame, is brought approximately to zero. The valves are then moved to the position where the burner is connected only to the gasometers and is isolated from the mains. The exact reading of the meter is noted and the cable release is pressed. The maximum excursion of the meter is read, corrected for the zero reading, and the net deflexion is recorded.

Given droplets of solution (uncontaminated with protein—see later) in watch-glasses under liquid paraffin, it is possible for a single operator to measure out and store twenty samples and then to run them through the flame in just under 2 hr.

ACCURACY

Before considering the accuracy of this method on the determination of sodium it is worth noting in passing certain results obtained while the apparatus was being developed—for details see Ramsay *et al.* (1951). With short flashes of light controlled by a shutter the consistency of the photocell and integrator, expressed in terms of standard deviation, was 1·8 % of the mean value. With moderately small volumes—*c.* 0·25 cu.mm.—of thallium solutions, delivered from a microburette, the standard deviation was 2 % of the mean value. *A priori*, it is unlikely that better results will be obtained with the sodium in biological fluids, of which much smaller volumes have to be accurately delivered.

In the tests with sodium the same silica pipette was used throughout. Its volume,

determined approximately from its dimensions as measured with an eyepiece micrometer, was $0{\cdot}7 \times 10^{-3}$ cu.mm. With a 0·1 % solution of NaCl, that is, with samples containing $2{\cdot}75 \times 10^{-7}$ mg. sodium, twelve consecutive readings gave an average deflexion of 110·6, with a standard deviation of 3·66, or 3·3 % of the mean. The same number of blank determinations, in which distilled water was drawn up into the silica pipette and all the other operations were the same, gave an average deflexion of 1·5 with a standard deviation of 1·44.

The relation between deflexion and concentration of NaCl was investigated over five different ranges of concentration. The results are set out in Table 1. For each

Table 1

	Concentration		Deflexions	Average deflexion
	% NaCl	Arbitrary units		
Series I. Iris aperture 1 in.; empirical factor 76·5	0·05	3·8	59, 66, 65, 65, 63	64
	0·10	7·65	120, 124, 128, 127, 125	125
	0·15	11·5	170, 181, 179, 181, 181	178
	0·20	15·3	226, 229, 236, 234, 227	230
Series II. Iris aperture $\frac{1}{4}$ in.; empirical factor 23·6	0·0	0	2, 1, 0	1
	0·10	2·35	41, 40, 41	41
	0·20	4·7	80, 81, 74	78
	0·292	6·85	116, 118, 115	116
	0·40	9·45	152, 155, 148	152
	0·50	11·8	179, 184, 171	178
Series III. Iris aperture $\frac{5}{32}$ in.; empirical factor 13·9	0·25	3·5	65, 64, 59, 61	62
	0·50	6·95	112, 113, 110, 114, 116	113
	0·73	10·15	161, 162, 165, 159	162
	1·00	13·9	220, 212, 222, 216, 211	216
Series IV. Iris aperture $\frac{3}{32}$ in.; empirical factor 8·0	0·50	4·0	68, 68, 68, 66, 67	67
	1·00	8·0	126, 133, 129, 131, 132	130
	1·50	12·0	182, 180, 188, 186, 184	184
	2·00	16·0	227, 230, 223, 236, 214	226
Series V. Iris aperture $\frac{2}{32}$ in.; empirical factor 3·7	2·00	7·4	122, 117, 131	123
	3·00	11·1	165, 159, 164	163
	4·00	14·8	211, 206, 201	206
	5 00	18·5	226, 226, 230	227
	6·00	22·2	239, 252, 256	249

series average deflexion was plotted against concentration and a curve was fitted by eye; at this stage it was obvious that the shape of the curve was very much the same in Series I–IV. Since another variable, the aperture of the iris diaphragm, was present, it was not to be expected that these curves could be superimposed as they stood; but by multiplying the concentration figures by certain factors (arrived at empirically for each series so as to give the best fit) the curves could be brought approximately into coincidence above an abscissa in which concentration was expressed in arbitrary units. The empirical factor for each series is given in Table 1. In Fig. 2 the average deflexions have been plotted against concentration in arbitrary units and for Series I–IV the relationship is reasonably represented by the curve (drawn by eye). The interpretation placed upon this relationship is that the energy falling on the cathode of the photo-cell is directly proportional to the amount of

sodium on the platinum wire and that the non-linearity arises in the electrical side of the apparatus. The results of Series V, also shown in Fig. 2, do not conform to this relationship, which is therefore not applicable to solutions more concentrated than 2 % NaCl, or, in more general terms, to quantities of sodium exceeding $5{\cdot}5 \times 10^{-6}$ mg.

From the data of Series I–IV it is possible to see how this method of determining sodium is likely to work in practice. We will assume that three measurements are to be made with a known solution in order to calibrate the apparatus for the conditions of the moment, and that it can then be used for determining sodium on unknown solutions for a limited period. An example will make this clear; the

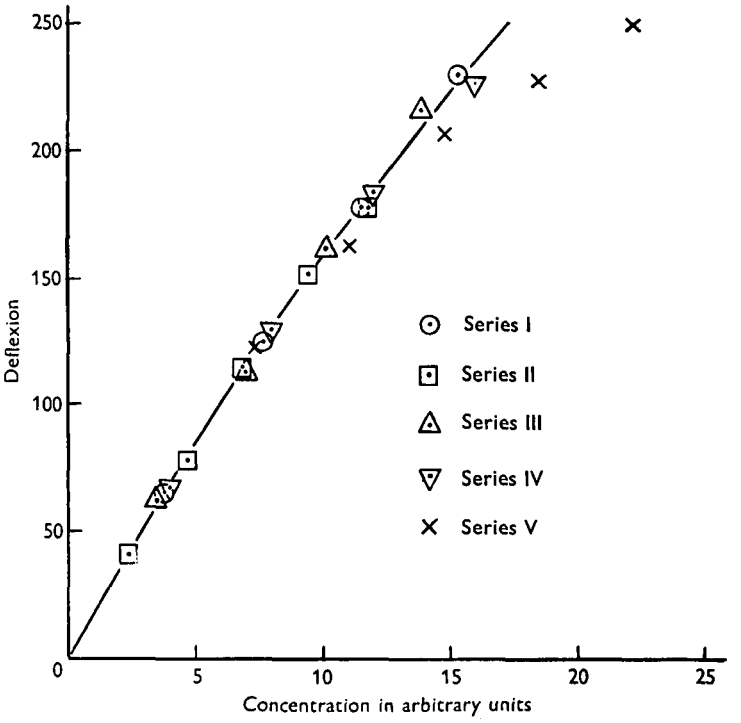

Fig. 2. For explanation see text.

treatment of Series I will be considered in detail—see Table 2. We take 0·1 % NaCl as the calibrating solution and imagine that the first three readings are our calibrating measurements. These three readings have an average value of 124. From the curve of Fig. 2 a deflexion of 124 corresponds to 7·55 arbitrary units of concentration; that is to say, for the conditions of the moment 7·55 arbitrary units can be equated to 0·1 % NaCl. We take first 0·05 % NaCl as an unknown solution. The deflexion given by each sample (column 1) is converted by means of the calibration curve into arbitrary units (column 2) and then converted into % NaCl by simple proportion from the relation established above. The error in concentration is given in column 4 and the percentage error in column 5. It is assumed that in practice the calibrating

solution will be chosen to give a deflexion of 100–150; in this particular analysis the following have been taken as the calibrating solutions:

Series I:	0·10 % NaCl	Series III:	0·73 % NaCl
Series II:	0·292 % NaCl	Series IV:	1·00 % NaCl

Table 2

(Series I: iris aperture 1 in.; calibrating solution 0·10 % NaCl; calibration readings 120, 124, 128, av. 124 ≡ 7·55 arbitrary units. 1 arbitrary unit ≡ 0·01325 % NaCl. 'Unknown', 0·05 % NaCl.)

Deflexion	Arbitrary units	% NaCl	Error	% error
59	3·45	0·0457	0·0043	−8·6
66	3·85	0·0509	0·0009	+1·8
65	3·8	0·0503	0·0003	+0·6
65	3·8	0·0503	0·0003	+0·6
63	3·75	0·0497	0·0003	−0·6

In all, the percentage errors of fifty-six samples have been calculated and they have an average value of 2·93 %. These errors are compounded of the systematic error of calibration and the random error of the individual sample and it is therefore difficult to express them in terms of standard deviation.

This treatment, whereby the accuracy of the method is assessed from data which have already been used in establishing the form of the calibration curve, is possibly open to objection on theoretical grounds. An alternative procedure, avoiding the use of a calibration curve, is to take a measurement on an unknown sample, to compare it with measurements on two known samples, one of higher and one of lower concentration, and to determine the unknown by linear interpolation. Taking Series II we can treat 0·10 % NaCl as unknown, and imagine that distilled water and 0·20 % NaCl are the calibrating solutions; similarily with 0·20, 0·292 and 0·40% , *mutatis mutandis*. This treatment provides the errors on twelve samples from Series II alone, and the average error is found to be 3·12 %. All in all, it is probably not wide of the mark to assess the average error of the method at about 3 %, for pure solutions of sodium chloride and for less than $5{\cdot}5 \times 10^{-6}$ mg. sodium. In practice, of course, we have to deal with solutions in which other substances are present and may give rise to error in other ways. These matters are discussed below.

SOURCES OF ERROR

Only a small fraction of the light energy emitted from the flame is made use of by the photo-cell; the success of any method of flame photometry depends in very great measure upon the possibility of controlling other variables so that the amount of light falling on the cell is related only to the amount of element introduced into the flame. Careful stabilization of gas and power supplies is an obvious precaution. The relative importance of other factors has not been fully investigated, but experience in the present work has shown that the thickness of the platinum wire, its height above the burner, the orientation of the loop and the disposition of the salt upon it are all highly significant. Consistency is achieved by strict attention to a rigid routine

in which the same procedure is followed in every detail. This is attended with good results when pure solutions of sodium chloride are used, but when biological fluids are used two other sources of error become apparent. The first is the effect of protein—and presumably of other surface-active substances—on the process of measuring out the sample in the silica pipette (volumetric error). The second is the effect of one element upon the emission of another (interference error).

(1) *Volumetric error.* The difference between the standard deviation of 2 % obtained with thallium delivered from a micrometer burette and the standard deviation of 3·3 % obtained with sodium delivered from a silica pipette as described may well be due to the error in measuring out the much smaller quantities of fluid. When one considers that relative to the volume of fluid the area of contact with the wall of the silica pipette is very large, it is perhaps surprising to find the error so small. No measurement of the contact angle between water, liquid paraffin and silica has been made, but inspection of the interface in the pipette shows that the water tends to displace the paraffin from the surface; yet results indicate that when the sample is ejected the amount of water remaining must be extremely small. A test was carried out in which the pipette was first washed out with 1 % NaCl and then two samples of distilled water were taken; if appreciable quantities of watery fluid adhere to the walls of the pipette the first sample of distilled water should give a higher deflexion than the second.

First sample	Second sample
6	2
2	2
3	0
2	2
Av. 3·25	Av. 1·5

The value of 1·5 for the second sample is identical with the value quoted in the previous section for twelve successive samples of distilled water; from the difference between 3·25 and 1·5 it can be calculated that all but 3 % of the sample is ejected.

Conditions are very different when the solutions used contain protein. When a solution containing protein is drawn into the pipette for the first time it appears to behave more or less normally, and if it is ejected slowly (the process taking about 30 sec.) the paraffin appears to replace it completely. But when the attempt is made to draw up a second sample the solution shows a strong tendency to creep rapidly up the walls of the pipette in an irregular manner, and generally fails to displace the paraffin completely. This trouble can be avoided by cleaning out the pipette after each protein-containing sample has been handled. It is sometimes necessary to resort to powerful cleaning agents such as dichromate-sulphuric acid, but since this attacks the lacquer covering of the pipette it has to be used with care; in most cases normal hydrochloric acid is effective, as is shown by the following test.

Pure lactoglobulin (6 mg.) were added to 39 mg. of distilled water (acidified with CO_2) in a silica tube and brought into solution. Three test solutions were prepared: (*a*) 2·0 % NaCl plus an equal volume of distilled water; (*b*) 2·0 % NaCl plus an

equal volume of the protein solution; (*c*) the protein solution alone. Four samples of each were taken, and after each sample had been ejected the pipette was washed out with normal hydrochloric acid. The deflexions were as follows:

(*a*) 155, 154, 155, 160, Av. 156
(*b*) 149, 175, 155, 152, Av. 157·75
(*c*) 1, 1, 3, 1, Av. 1·5

The presence of protein does not apparently introduce any important systematic error; it may possibly increase the random error, as in this case, but in other tests with protein-containing solutions this effect is not conspicuous.

(2) *Interference error.* Interference effects, in the broadest sense of the term, have been noted with all types of flame photometer. Some of the very large errors reported by Parks, Hilton & Lykker (1948), using the Perkin-Elmer no. 18, have been traced to the electrical circuits of the instrument itself by Bills *et al.* (1949) but even with improved design interference phenomena between different elements are recorded, in extreme cases giving errors of up to 50 %. Other cases of interference are due to imperfections of the filter system, e.g. the interference between sodium and calcium reported by Domingo & Klyne (1949). These authors also report that the interference between sodium and potassium found in the air-acetylene flame is not found in the air-butane flame. Results are also affected by the presence of organic substances such as sucrose and urea (Berry, Chappell & Barnes, 1946). Some of this interference may be indirectly the result of changes in the conditions of atomization—surface tension effects, for example; but there is also evidence that the interference is independent of the method used for introducing the material into the flame. The physical nature of interference is not fully understood.

A few tests have been carried out with the method under present discussion to discover the magnitude of the errors likely to affect the determination of sodium in the presence of some of the other constituents of biological fluids.

A sample of human serum known to contain 3·17 mg. sodium per ml.* was tested, using 1·0 % NaCl as calibrating solution; the results showed 3·34 mg. sodium per ml., an error of +5·5 %.

A sample of sea water known to contain 11·15 g. sodium per litre* was diluted with twice its volume of distilled water and the sodium was determined using 1·0 % NaCl as calibrating solution. The amount of sodium was found to be 10·29 g. per litre, an error of −7·7 %.

The effect of the sulphate anion was tested by comparing 1·0 % NaCl with 1·216 % Na_2SO_4 which has the same concentration of sodium. The apparent concentration of sodium in the Na_2SO_4 solution was lower by 6·75 %.

The separate effects of potassium, calcium and magnesium were studied in rather more detail in a series of tests, the results of which are given in Table 3.

An examination of this table and of the results of the other tests shows: (*a*) that the interference effects do not bear any simple relationship to the concentration of the interfering substance; and (*b*) that no error greater than 7·7 % has been recorded.

* I am indebted to Mr W. T. W. Potts for these analyses.

Table 3

(Concentration of NaCl 0·1 M throughout.)

Interfering Substance	Percentage error
0·01 M-KCl	+1·6
0·10 M-KCl	+6·9
0·90 M-KCl	+5·9
0·005 M-$CaCl_2$	−1·75
0·01 M-$CaCl_2$	−6·55
0·02 M-$CaCl_2$	−3·47
0·05 M-$CaCl_2$	0·0
0·10 M-$CaCl_2$	+1·64
0·90 M-$CaCl_2$	+4·37
0·005 M-$MgCl_2$	−1·1
0·01 M-$MgCl_2$	−1·68
0·05 M-$MgCl_2$	0·0

DISCUSSION

The possibilities of extending this method to deal simultaneously with other elements besides sodium have not yet been seriously considered. The potassium lines in the near infra-red are of the same order of intensity as the sodium D lines, but although photo-multiplier tubes covering the infra-red have been produced in America they are not on the market in Britain. It may be possible to use the weaker potassium lines in the violet with the 931 A which has a maximum sensitivity in this region. Calcium is rather less promising owing to the poor sensitivity of the 931 A to the red bands of calcium oxide. Flame photometry does not appear to have been applied as yet to the determination of magnesium.

As has already been mentioned, it is the saving of time in circumstances (e.g. in hospital laboratories) where a large number of routine analyses have to be carried out that has attracted attention to the possibilities of flame photometry. Unless steps are taken, as described below, to reduce the interference errors flame photometry is distinctly less accurate than orthodox chemical methods in which the nature and causes of error are more fully understood. But if it is a requirement that the method is to operate on 10^{-3} cu.mm. of fluid then no alternative to the spectrometric method appears to be available. Even the methods involving the use of radio-active isotopes have not yet been refined sufficiently to deal with these small quantities.

Evidence has been presented to show that the average error of a series of observations on the same fluid is about 3 %; for purposes of approximation we may assume that the error of any single observation is unlikely to exceed three times the average error, i.e. 9 %. The recorded systematic errors, due to interference, have not exceeded 7·7 %. This suggests that the method will give the sodium concentration in a single 10^{-3} cu.mm. sample of unknown composition to within 20 %. This is a large figure, but in view of the variation from one animal to another, especially among invertebrates even this would be useful accuracy. When something is known about the other substances present it should be possible to reduce this figure by about half.

Interference errors can be reduced in various ways. Belke & Dierkesmann (1948) first determine sodium, and then in the subsequent determination of potassium they use calibrating solutions of potassium chloride containing sodium chloride in appropriate concentration; similarly, the calibrating solutions for calcium contain sodium chloride and potassium chloride in previously determined concentrations. The error in the determination of sodium, potassium and calcium in blood, urine, etc., is thereby reduced to about 2 %, and in spite of the more complicated procedure the time saved is still substantial. This method, involving three successive measurements and therefore three samples, is not attractive when the quantity of solution is limited.

A more promising line of attack has been described by Berry *et al.* (1946). Their method depends upon the assumption (not unreasonable in view of their results) that interference effects upon related elements are similar. Thus, if a solution is to be analysed for sodium (or potassium) in the presence of interfering substances, an amount of another alkali metal, e.g. lithium, is added to bring the concentration of lithium in the sample to a known value. The lithium is then determined in the same way as the sodium and if the apparent concentration of lithium differs from the known concentration by a certain factor, that same factor is applied as a correction to the apparent concentration of sodium. This method of correction is of course inapplicable to cases of mutual interference by alkali metals (Bernstein, 1950). From the point of view of the manipulations involved the application of this principle to the method here under discussion does not appear to present any difficulty.

Of the possibility of reducing the volume of the sample below 10^{-3} cu.mm. it is not possible to say much. Naturally it will depend a great deal upon the concentration. In its present form the method has given a standard deviation of 3·3 % with samples of 0·1 % NaCl, of volume $0{\cdot}7 \times 10^{-3}$ cu.mm.; this could hardly be reproduced on a 0·01 % solution owing to the error in reading small deflexions of the meter. On the other hand, with concentrations greater than 1·0 % NaCl, requiring as they do some reduction in the aperture of the iris diaphragm, it is clearly possible to use smaller quantities of solution. The factors affecting the accuracy of measuring out the sample have received only a superficial examination so far. It may well prove possible to develop a better shape of pipette, with a smaller surface/volume ratio and with a local constriction at the reference mark. A substantial reduction in volume, say by a factor of 10, is possible but problematical.

SUMMARY

1. A method of integrative flame photometry is described, by which the amount of sodium in biological fluids can be determined, using samples of the order of 10^{-3} cu.mm.

2. With pure solutions of sodium chloride, of concentration from 0·05 to 2·0 %, the average error of the method is about 3 % of the concentration.

3. When other substances are present systematic errors of up to 7·7 % have been recorded.

4. The possibilities of reducing these systematic errors and of extending the method to deal with other elements are discussed.

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