

SIMULTANEOUS DETERMINATION OF SODIUM AND
POTASSIUM IN SMALL VOLUMES OF FLUID BY
FLAME PHOTOMETRY

BY J. A. RAMSAY, R. H. J. BROWN AND S. W. H. W. FALLOON

*The Zoological and Cavendish Laboratories,
University of Cambridge*

(Received 29 May 1952)

(With Plate 1)

INTRODUCTION

In two earlier papers (Ramsay, 1950; Ramsay, Falloon & Machin, 1951) a method was described by which it was possible to determine the concentration of sodium in samples of fluid of the order of 10^{-3} cu.mm. in volume. This method has now been extended to include the determination of potassium simultaneously upon the same sample. It is still in the experimental stage of development; we are fully conscious of its shortcomings and have in mind various possibilities for its improvement. Nevertheless, it is being used in its present form for biological work, some of which is already published (Ramsay, 1952), and it is therefore necessary that some description of it should be given.

The general principles and technique of flame photometry are becoming well known; various instruments have been described and some are on the market. In the majority of these instruments the solution to be investigated is atomized into the flame and the steady level of emission is measured. The method now under discussion differs mainly in that the whole of the sample is passed into the flame and the total emission is integrated. A small volume—of the order of 10^{-3} cu.mm.—of the fluid to be investigated 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 the flame by a mechanical device. The emission from the flame enters a spectrometer; the spectrum falls upon a mask having two slits, one passing the sodium doublet (5890–96 Å.) and the other passing the potassium doublet (7665–99 Å.), and these emissions are allowed to reach two photomultiplier tubes. The photomultipliers are connected to integrating circuits with meter presentation. The apparatus is calibrated with known solutions drawn up to the same mark in the capillary pipette.

In essence, this is the same as the method previously described, differing only in the provision of a second channel for potassium. But for operational reasons further complications become necessary. In the earlier method the meter was first brought to rest near zero and the sample was passed into the flame; the meter made a rapid excursion and then fell back slowly. The operator noted the maximum deflexion, subtracted the zero reading and recorded the net deflexion. This was

entirely practicable when only one meter had to be read, but obviously would not be practicable with two meters. The apparatus therefore had to be modified so that the meters should always start from a known zero and maintain their maximum deflexions long enough for these to be recorded. In view of the number of adjustments involved it was considered desirable to make the whole sequence of operations semi-automatic. Various other improvements were made at the same time.

MANIPULATION OF THE SAMPLE

The processes of drawing up the sample, transferring it to a storage tube, placing it upon the platinum wire and drying it are fully described in the first of the two earlier papers (Ramsay, 1950) and are still the same. Various silica capillary pipettes have been used, of volumes ranging from 0.28 to 3.8×10^{-3} cu.mm. It has been found useful to wash out the pipette with absolute alcohol after it has been out of use for some time and after cleaning fluids have been applied to it; this treatment with alcohol facilitates the displacement of the liquid paraffin by the aqueous solution at the surface of the silica.

APPARATUS

The burner is the same as that described by Ramsay *et al.* (1951), but modifications have been made in its supply system. In order to eliminate variations in pressure of the gas and air mains the burner was originally supplied from gasometers of sufficient capacity to maintain the flame for 20 sec. when isolated from the mains. In the new arrangement the flame burns continuously and the gasometers are connected to the mains through electrically controlled valves which allow them to fill and empty between certain limits. Variations in the quality of the town gas have been a source of trouble, and latterly 'Calor' gas has been used.

The device for carrying the platinum wire into the flame has been completely re-designed. The wire is now carried forwards into the flame by a piston driven by compressed air and returns under the action of a spring. The compressed air is admitted through an electrically controlled valve, and between this and the cylinder there is a second valve which can be adjusted to retard the flow of air and so to slow down the movements of the piston. This is important because violent accelerations can cause distortion of the wire while it is hot. The wire and its operating mechanism are mounted upon a carriage which can be swung away from the position under the entrance slit to a position which is convenient for loading the wire (see Pl. 1, B and C).

The spectrometer has the same lens system and prism, but ethyl cinnamate is now used in the latter instead of carbon disulphide. The entrance slit is adjustable in width and is $\frac{1}{2}$ in. in height instead of 1 in. as before. Inevitably the arrangements of the mask are very different. The two curved slits in the mask are formed by the gaps between four movable vanes, and these are under the control of a single adjustment whereby the widths of the two slits can be varied together while each slit remains centred upon its appropriate spectral line. In addition, the height of each slit can be varied independently by a second set of vanes working at right

angles to the first set. Immediately behind the mask are two mirrors set at 60° , directing the sodium and potassium emissions to their respective photomultipliers. For the details of the design and construction of the spectrometer we are indebted to Mr J. Barker of the Cambridge University Engineering Laboratory.

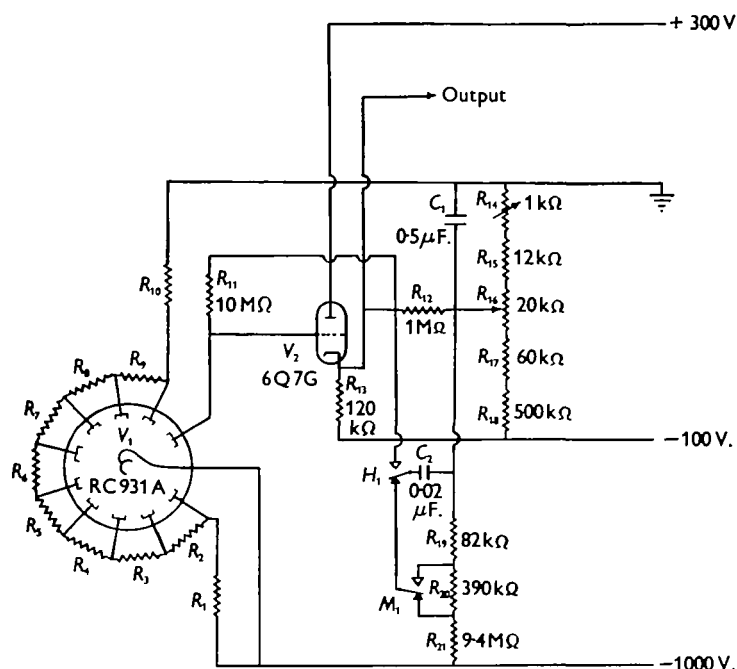
The sodium emission falls directly upon the photocathode of an RC931A photomultiplier. Unfortunately this tube is not sensitive to the red emission of potassium and there does not seem to be any photomultiplier combining red-sensitivity and high gain available on the British market. The solution adopted is the same as that adopted by Mitchell (1950), that is, to use a CV148 infra-red image converter in conjunction with the RC931A. The substage condenser of a microscope, placed as close as possible to the mirror, focuses the emission upon the photocathode of the image converter. Between the image converter and the photomultiplier are two bulls-eye lenses whereby some of the light from the image converter screen is focused upon the photocathode of the multiplier. Unfortunately, the screen of the CV148 is some distance from the back of the tube and only a small proportion of the light is captured by the lens system. Holliday & Wild (1951) have made use of the infra-red image converter ME1202. This has the advantage of having the photocathode at one end and the screen at the other, so that the photomultiplier can be brought right up to the screen and the worst losses can be avoided. Through the kindness of Dr Holliday we were able to borrow an ME1202 converter for trial. We found it better than the CV148 by a factor of 5, but in view of its high cost and in hopes of eventually obtaining a good red-sensitive photomultiplier we decided to use the CV148 in the meantime.

The connexions of the photomultiplier, V_1 , and its cathode follower, V_2 (see Text-fig. 1), present no special features. R_{14} and R_{18} are respectively the fine and coarse zero-setting controls, by which the sitting level of the cathode follower is adjusted to give zero input to the integrator, i.e. so that the meter is steady but not necessarily exactly at zero on the scale. The only complication is the test circuit. C_2 is a condenser which is charged from the resistance chain R_{19} , R_{20} , R_{21} , and can be discharged through R_{12} by operation of relay contact H_1 , thus applying a negative pulse to V_2 . Operation of relay contact M_1 allows of C_2 being charged to two different voltages which are appropriate to circumstances when the amplifier V_3 (Text-fig. 2) is or is not in circuit, according to whether or not relay M is operated. This amplifier is used in the potassium channel only.

The valves V_4 and V_6 (Text-fig. 2) and their connexions form the main integrator. The input passes via relay contact L_1 . The closure of relay contact E_1 reduces the time constant of the circuit, allowing C_3 to discharge quickly between determinations. The output of the integrator is connected via the meters either to a reference voltage provided by the resistance chain R_{45} , R_{46} , or, by the operation of relay contact F_1 , to the output of V_6 which is known as the 'follower'.

V_6 is connected as an integrator, but it is the property of maintaining a constant anode voltage with the grid disconnected (relay contact K_1) rather than the ability to integrate which is made use of. C_4 is connected to a pair of change-over contacts which are driven by a motor and operate at about 50 times per sec. C_4 is thus

charged by what is approximately the grid-anode voltage difference of the integrator and is then applied between grid and anode of the follower. In this way the grid-anode voltage difference is brought to the same value for both systems, but the actual voltages of the two anodes are not necessarily identical. To bring this difference in anode voltages under control the grid of the follower is returned

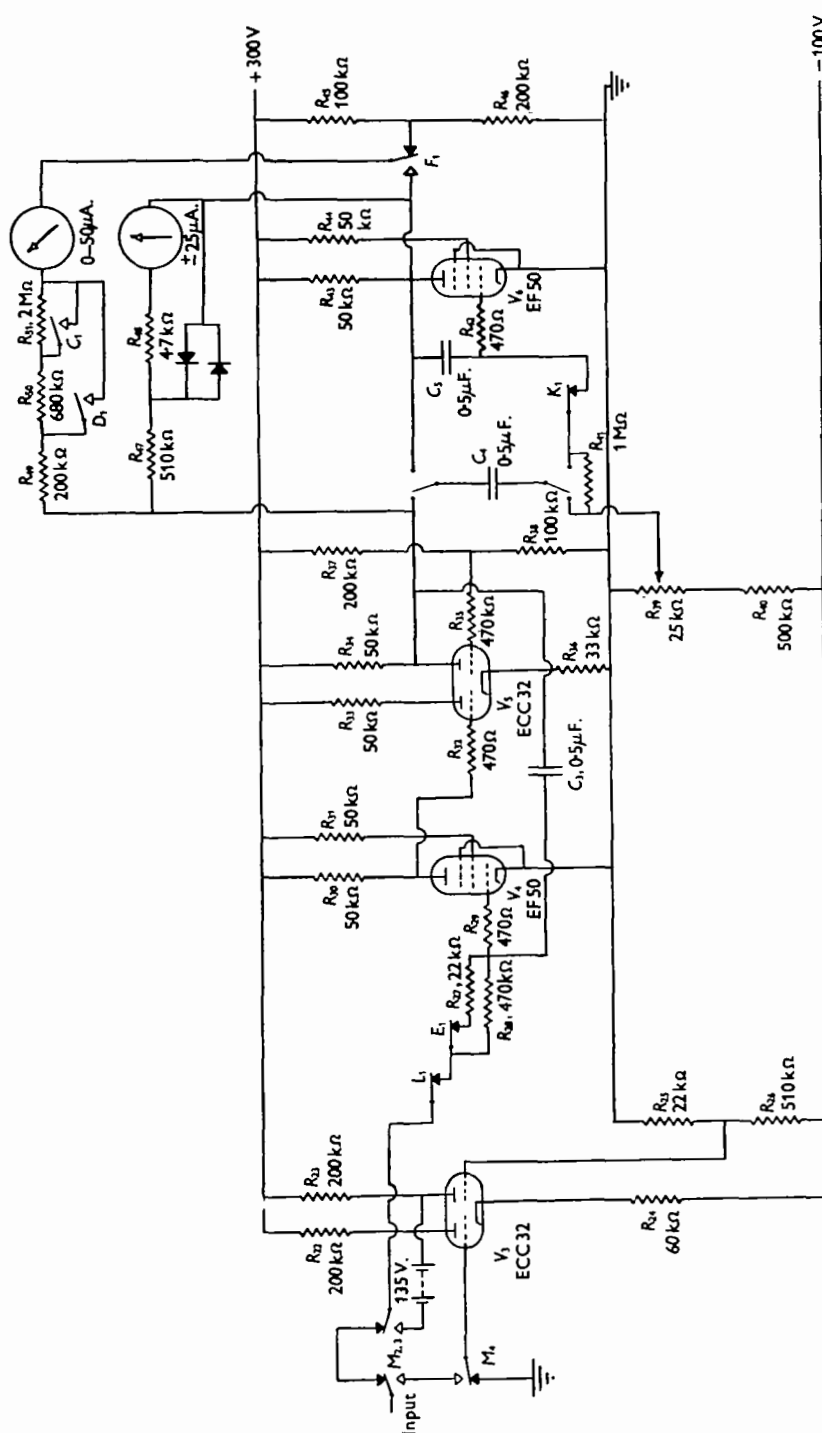


Text-fig. 1. Photomultiplier and cathode follower, zero-setting controls and test circuit.
 R_1 – R_{10} , all 120 k Ω .

through R_{41} to a potential slightly below earth on the potentiometer R_{39} ; by adjustment of R_{39} , the main meter can be set exactly to zero on the scale.

The main meter is a 0–50 μ amp. instrument with a wide scale which can be read to 0.1 μ amp. R_{49} , R_{50} , R_{51} and relay contacts C_1 and D_1 provide three sensitivity ranges. While the main meter can be connected either between integrator and reference voltage or between integrator and follower, the subsidiary meter is permanently connected between integrator and follower. It is a ± 25 μ amp. instrument, and by means of the associated resistance-rectifier network its response is made logarithmic; that is to say, it is fully sensitive to slight differences between integrator and follower voltages, and is at the same time protected from damage by large differences in voltage. The purpose of the subsidiary meter is to safeguard the main meter; it enables the operator to see that the follower is following correctly before he transfers the main meter from the reference voltage to the follower by relay contacts F_1 .

The –100V. supply is from a battery. The +4000V. (for the CV 148), the



Text-fig. 2. Amplifier, integrator and follower. Contacts as when button 1 is pressed.

+ 300 V. and the - 1000 V. supplies are provided by power packs stabilized against mains voltage fluctuations to within 0.1 %.

The 'follower' device for holding the main meter at zero was designed by Mr J. A. Popple and is published by him elsewhere (Popple, 1952). Mr Popple also made important contributions to the design of these circuits and worked out suitable values for the components.

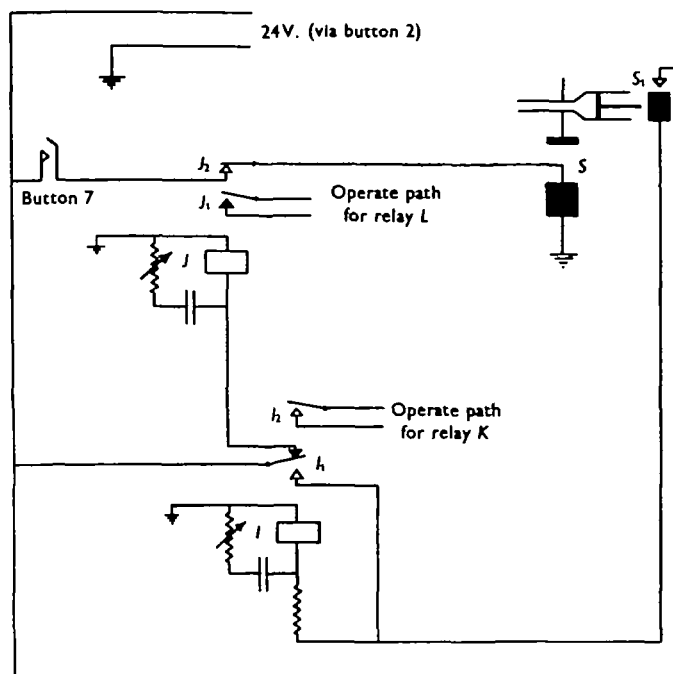
V_1 and V_2 with their connexions are mounted upon the spectrometer. The rest of the circuit is mounted upon the integrator chassis, with the two channels side by side (Pl. 1, A). Upon the front panel of the integrator chassis, under the meters, is a row of eight locking press-buttons, which are used in the operation of the various relays already mentioned. The only relay not operated by a press-button is relay M ; this is operated by a switch. A full description of the relay system and its connexions would be very long and does not seem to us to be necessary. We will simply describe the sequence of operation, going into detail at one stage only.

When button 1 is pressed the 24 V. supply to the whole system is cut and all the relays are de-energized. In this condition contacts L_1 and K_1 are closed, contact E_1 is closed (short time constant) and contacts C_1 and D_1 are open (low sensitivity); the main meter is connected to reference. The operator uses the coarse and fine zero-setting controls to bring the main meter needles to rest near zero on the scale. He then presses button 2. This supplies 24 V. to the whole system and contact E_1 opens (long time constant). Button 3 operates relay C (medium sensitivity) and button 4 operates relay D (high sensitivity). The fine zero setting controls are again used, if necessary, to bring the main meter needles to rest. When the operator is satisfied that these show no tendency to drift and that the subsidiary meters are reading zero, he presses button 6 (button 5 is idle) which operates relay F (follow), and then immediately afterwards he presses button 7 (fire), the action of which must now be described in more detail.

A simplified circuit diagram is shown in Text-fig. 3. When 24 V. is applied to the system (on pressing button 2) the master-relay \mathcal{J} is energized and its associated contacts are as shown in the figure. When button 7 is pressed the compressed air valve S is energized, compressed air is admitted to the cylinder and the platinum wire begins to move towards the flame. After it has moved a very short distance contact S_1 closes and the master-relay I is energized. Master-relay I is 'slugged' so that there is a short delay between energization and operation. As soon as it operates (1) contact I_1 locks master-relay I and de-energizes master-relay \mathcal{J} , (2) contact I_2 closes, operating relay K and disconnecting the grid of the follower. The follower now ceases to follow and provides a reference voltage; any signal entering the integrator will now be registered. Master-relay \mathcal{J} is also 'slugged' and releases after further delay. When it does so contact \mathcal{J}_1 closes, operating relay L and disconnecting the input to the integrator; no further signals can be received and the main meter now registers the voltage difference between integrator and follower. At the same time contact \mathcal{J}_2 opens, the air valve S is de-energized and the platinum wire is withdrawn from the flame.

In this way signals from the photomultiplier are registered only during the short

interval, or 'gate', between the operation of master-relay *I* and the release of master-relay *J*, and this interval can be adjusted to cover only that period during which the sample is vaporized into the flame. The reason for having master-relay *J* initially energized is that with a 'slugged' relay a longer delay can be obtained on de-energization than on energization.



Text-fig. 3. The 'gate' mechanism. Contacts as when button 2 is pressed. For further explanation see text.

Button 8 operates the test circuit. This, of course, does not come into the normal sequence of operation; when required, it replaces button 7 in the sequence. The test circuit involves the operation of the 'gate' mechanisms as before, and by means of a third 'slugged' master-relay the test signal is applied while the 'gate' is open. It does not seem to us necessary to go into the details of this circuit.

PERFORMANCE

The performance of a flame photometer is affected by a variety of factors, some of which will now be considered.

A useful measure of performance is the standard deviation of a series of observations carried out under identical conditions, using a quantity of the element near the lower limit of detection so that the instrument is operated at maximum sensitivity. The observations recorded in Table 1 were made with this in view.

In this table it will be noted that the deflexions in the sodium channel due to distilled water are considerable; this is partly due to the presence of sodium in the

distilled water—not that in the measured sample, which is of course negligibly small, but that in the droplet (about 0.5 cu.mm.) into which the sample is ejected from the pipette. These droplets when deposited directly upon the platinum wire give an average deflexion of 2.5 divisions. There must be additional contamination with sodium during the manipulation and storage of the sample, but it is not yet known how this occurs. One of the deflexions in the distilled water series appears to be anomalous, but it has been included in the calculations.

Table 1

Width of entrance slit: 0.012 in. Height of slit in mask, Na and K: 0.5 in. (full). Test solutions: 0.43 mm./l. NaCl, 0.43 mm./l. KCl, distilled water. Volume of sample: 3.8×10^{-8} cu.mm.

		Deflexions	Mean deflexion	S.D.
Sodium channel	0.43 mm./l. NaCl	9.7, 10.9, 8.7, 8.8, 8.3, 9.7, 9.1, 10.2, 10.9, 9.8	9.61	± 0.89
	Distilled water	4.3, 3.8, 4.7, 7.9, 3.3	4.8	± 1.81
Potassium channel	0.43 mm./l. KCl	1.8, 3.2, 2.6, 2.5, 2.9, 2.5, 2.6, 4.2, 2.6, 2.6, 1.0, 0.3, 0.1, 0.6, 0.3	2.75	± 0.62
	Distilled water		0.46	± 0.41

It is calculated that the sample contains 3.74×10^{-8} mg. sodium and 6.35×10^{-8} mg. potassium. To find the deflexions corresponding to these quantities we subtract the deflexions given by distilled water; the net deflexions are then 4.81 for sodium and 2.29 for potassium. Assuming that deflexion is proportional to amount of element the standard deviations are as follows:

$$\begin{aligned}\text{Sodium:} \quad & \pm 3.74 \times 10^{-8} \times \frac{0.89}{4.81} \\ & = \pm 6.9 \times 10^{-9} \text{ mg.} = \pm 3.0 \times 10^{-10} \text{ m.equiv.}\end{aligned}$$

$$\begin{aligned}\text{Potassium:} \quad & \pm 6.35 \times 10^{-8} \times \frac{0.62}{2.29} \\ & = \pm 1.72 \times 10^{-8} \text{ mg.} = \pm 4.4 \times 10^{-10} \text{ m.equiv.}\end{aligned}$$

From these figures it is possible to calculate the smallest difference which can be established at a particular level of statistical significance with a given number of determinations. For example, if we have two solutions and make four determinations on each, the smallest difference which can be established at the $P=0.01$ level of significance is 7.9×10^{-10} m.equiv. of sodium and 10.5×10^{-10} m.equiv. of potassium.

The above is only true for amounts near the lower limit of detection. When greater amounts are used it is found that, as with most analytical methods, the error is roughly proportional to the amount of the element present. Opportunities for investigating this matter have presented themselves throughout the course of biological investigations in which the method has been used. In one such investigation a solution containing 171 mm./l. NaCl and 171 mm./l. KCl was used as a

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standard of reference, and several readings were taken on this solution during each series of observations. This data is summarized in Table 2.

Table 2

Width of entrance slit: 0.10 in. Height of slit in mask: Na, 0.15 in.; K, 0.5 in. Test solution: 171 mM./l. NaCl, 171 mM./l. KCl. Volume of sample: 0.28×10^{-3} cu.mm.

Series	No. of observations	Sodium			Potassium		
		Mean deflexion	S.D.	S.D. %	Mean deflexion	S.D.	S.D. %
1	7	29.3	± 0.46	± 1.59	40.4	± 0.855	± 2.12
2	8	30.6	± 1.085	± 3.54	39.2	± 0.825	± 2.11
3	9	29.0	± 0.96	± 3.32	33.2	± 1.19	± 3.58
4	9	31.7	± 0.88	± 2.78	33.8	± 0.61	± 1.80
5	7	30.5	± 1.06	± 3.48	32.8	± 0.66	± 2.02
	Total 40	Average S.D. $\pm 2.97\%$			Average S.D. $\pm 2.36\%$		

The sample is calculated to contain 4.8×10^{-8} m.equiv. both for sodium and for potassium. Assuming that deflexion is proportional to amount, the standard deviations are as follows:

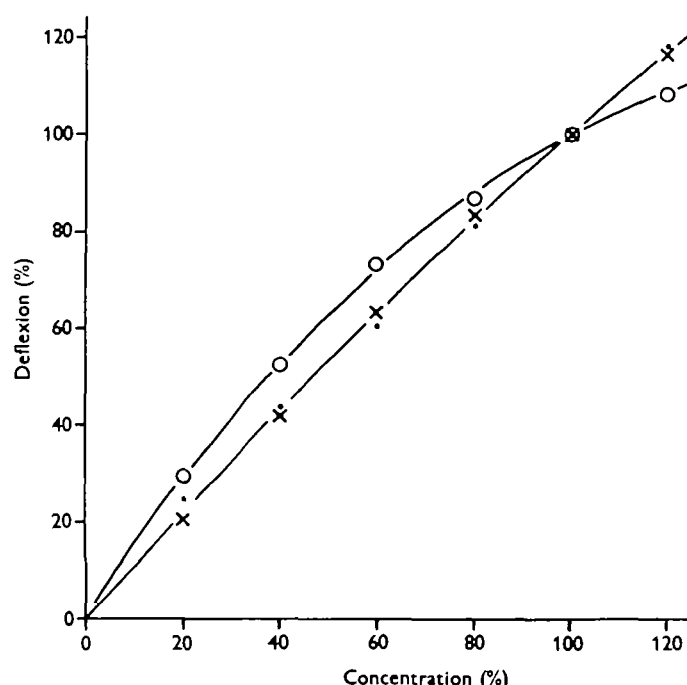
$$\text{Sodium: } \pm 4.8 \times 10^{-8} \times \frac{2.97}{100} = \pm 1.42 \times 10^{-9} \text{ m.equiv.}$$

$$\text{Potassium: } \pm 4.8 \times 10^{-8} \times \frac{2.36}{100} = \pm 1.13 \times 10^{-9} \text{ m.equiv.}$$

Consideration of this and many other series of observations carried out under a variety of conditions leads to the general conclusion that when the deflexions lie in the middle range (i.e. 20–40 divisions) of the scale, the standard deviation amounts to some 3% of the mean deflexion.

We must now examine more closely the relation between deflexion and amount of element, which has hitherto been assumed to be one of simple proportion. As will be seen from Text-fig. 4, the assumption is certainly untrue. For low concentrations there is some departure from linearity, but it is not likely to be a serious source of error. At high concentrations the departure from linearity is considerable. These results are compatible with what was found in the earlier work (Ramsay, 1950). Text-fig. 2 of that paper shows a relationship between deflexion and concentration which almost exactly fits the low-concentration curve in Text-fig. 4 of the present paper. The departure from this relationship was previously observed when the amount of sodium in the sample exceeded 5.5×10^{-6} mg. It is now observed at about 3.0×10^{-6} mg. At high concentrations the departure from linearity has the effect of reducing the accuracy of the method. For example, at a sodium concentration of 14 m.equiv./l. an error of 3% in deflexion corresponds to an error of 3.3% in concentration; at 140 m.equiv./l. it corresponds to an error of 5.7% in concentration. It is therefore desirable to work with the smallest

quantities which will give adequate deflexions. A difficulty frequently arises, however, when one element is present in much greater concentration than the other, as is generally the case with sodium relative to potassium in biological fluids. In order to get adequate deflexions on potassium it may be necessary to raise the amount of sodium in the sample beyond the point at which the percentage error begins to increase.



Text-fig. 4. Relation between deflexion and concentration. •: Na, 14 m.equiv./l.; ×: K, 40 m.equiv./l.; ○: Na, 140 m.equiv./l. Volume of pipette, 1.9×10^{-2} cu.mm. Each point is the mean of at least three determinations.

In the earlier paper the interpretation given to the relation between deflexion and concentration was that at low concentrations the emission was directly proportional to the amount of element present in the sample and that the slight non-linearity of the low-concentration curves arose in the electrical side of the apparatus. The present results are in keeping with this interpretation. The considerable departures from linearity at high concentrations are commonly experienced in other methods of flame photometry.

It is also necessary to take account of interference, i.e. the enhancement or repression of the emission of an element by the simultaneous presence of other elements or radicals. Where the concentrations of the interfering substances are known it is merely a matter of patient calibration to establish the nature and extent of the interference. But where we have to determine sodium and potassium in a solution of unknown composition the possibilities of serious error are considerable.

Fortunately in biological fluids the upper and lower limits of concentration are known with some certainty for most substances. It is therefore possible to determine by a preliminary survey the interference errors to which the method is liable for the particular fluid which is to be investigated. Purely for the purpose of illustration a short survey of interference errors has been carried out with certain substances in such concentrations as may occur in normal human urine. The substances in question and their concentrations were as follows:

Na ⁺	140 m.equiv./l.	Cl ⁻	102 m.equiv./l.	Urea	330 mm./l.
K ⁺	40 m.equiv./l.	HPO ₄ ⁻	56 m.equiv./l.		
Ca ⁺⁺	12 m.equiv./l.	SO ₄ ⁻	42 m.equiv./l.		
Mg ⁺⁺	8 m.equiv./l.				

A preliminary survey, carried out in the coal gas flame, revealed considerable mutual interference between sodium and potassium. The addition of KCl in a concentration of 40 mm./l. to a solution containing 140 mm./l. NaCl reduced the sodium deflexion by 1.5%. The addition of NaCl in a concentration of 140 mm./l. to a solution containing 40 mm./l. KCl reduced the potassium deflexion by 49%. The effect of potassium on sodium is negligible; the effect of sodium on potassium, although very great, is not of outstanding importance since these are the substances which are being measured, and adequate calibration makes full correction possible. But the effects of other substances whose concentrations are not measured by the method are more serious. These are listed in Table 3.

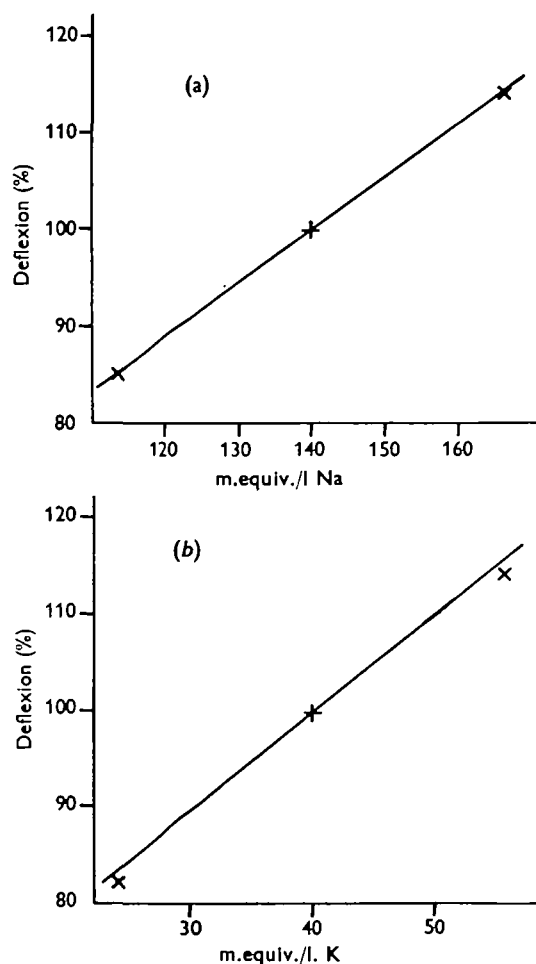
Table 3

Width of entrance slit: 0.0075 in. Height of slit in mask: Na, 0.05 in.; K, 0.5 in. Volume of sample: 1.9×10^{-3} cu.mm. The deflexions given by sodium and potassium in the solutions tested are compared with the deflexions given by sodium and potassium in a reference solution containing 140 mm./l. NaCl \times 40 mm./l. KCl. All concentrations are given in m.equiv./l. or mm./l.

Solutions tested								Interference errors (%)	
Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	HPO ₄ ⁻	SO ₄ ⁻	Urea	Na	K
140	40	12	—	192	—	—	—	- 4.0	- 2.2
140	40	—	8	188	—	—	—	- 5.0	- 4.0
140	40	12	8	200	—	—	—	- 6.2	- 4.0
140	40	—	—	124	56	—	—	+ 13.5	+ 6.2
140	40	—	—	138	—	42	—	+ 7.5	+ 7.0
140	40	—	—	82	56	42	—	+ 17.0	+ 25.0
140	40	—	—	180	—	—	330	- 2.8	- 1.9

The interference errors caused by phosphate were further tested using various concentrations of 'Specpure' ammonium phosphate instead of distilled water for the drop into which the sample was ejected from the pipette. The volume of the drop in these tests was approximately 1 cu.mm. It was found that the effect of ammonium phosphate became noticeable with solutions of 0.01 mm./l. and reached

its maximum at 0.075 mM./l., without further significant change up to 10 mM./l. It therefore seemed possible to swamp the effect of phosphate in the sample by adding excess of ammonium phosphate in the drop. The experiments summarized in Table 3 were therefore repeated using a drop of 1 mM./l. ammonium phosphate



Text-fig. 5. Calibration curves in the presence of excess ammonium phosphate. (a) For sodium, in the presence of 40 m.equiv./l. potassium; (b) for potassium, in the presence of 140 m.equiv./l. sodium.

instead of distilled water. At this stage, for other reasons, 'Calor' gas was substituted for coal gas, but it was first ascertained that phosphate interference was not substantially affected by this change.

A solution containing 140 mM./l. NaCl + 40 mM./l. KCl was used as reference and the apparatus was calibrated over the ranges $\pm 20\%$ of these concentrations. The calibrations are reproduced in Fig. 5, each point being the mean of at least three determinations. Over these limited ranges we are prepared to accept the

relation between concentration and deflexion as linear. In view of the very serious interference effect of sodium on potassium it seemed likely that further calibration curves for potassium in the presence of different concentrations of sodium would have to be prepared. Fortunately it was found that over the ranges of concentration in question the change in this effect was less than 3% and it has been neglected.

Table 4
(Conditions as described for Table 3.)

Solutions tested								Interference errors (%)				No. of observations	
								Deflexion		Concentration			
Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	HPO ₄ ⁻	SO ₄ ⁻	Urea	Na	K	Na	K	Reference	Test
140	40	12	—	192	—	—	—	-4.3	-3.0	-6.2	-3.75	8	4
140	40	—	8	188	—	—	—	+1.0	-1.0	+1.4	-1.25	5	4
140	40	12	8	200	—	—	—	0	-1.0	0	-1.25	6	5
140	40	—	—	124	56	—	—	0	-2.5	0	-3.1	4	4
140	40	—	—	138	—	42	—	-0.5	-2.5	-0.7	-3.1	4	4
140	40	—	—	82	56	42	—	0	-1.0	0	-1.25	6	5
140	40	—	—	180	—	—	330	-3.8	0	-5.3	0	8	4

The interference errors in the presence of excess ammonium phosphate are listed in Table 4, use being made of the calibration curves in converting deflexion error into concentration error. As compared with Table 3 a substantial improvement can be noted, and it is particularly satisfactory that the swamping effect of ammonium phosphate appears to make itself felt upon the sulphate error as well as upon the phosphate error.

Errors of the order of 6% may possibly be unacceptable in chemical work and in studies of mammalian physiology in which a high degree of accuracy is called for. In any case, the integrative method offers no advantage over the more usual non-integrative atomizing methods where the amount of fluid available is not a consideration. If the present method has anything to recommend it, it is that it can be used with the very small volumes which have to be accepted in physiological work upon invertebrates, where errors of 6% are by no means prohibitive. It is in this field that we believe it may find useful application.

DISCUSSION

The sensitivity of this method is greater than that of any other method hitherto described. For the Beckman flame spectrophotometer Gilbert, Hawes & Beckman (1950) state that 0.05 cc. of 0.01 p.p.m. sodium, which represents 2×10^{-8} m.equiv. of sodium, will give a deflexion 0.5% of full scale. From the data in Table 1 it can be calculated that a similar deflexion would be given by 8.5×10^{-11} m.equiv. of sodium. It would be a matter of no great difficulty further to increase the sensitivity to sodium by a factor of, say, 5. But nothing would be gained by so doing, since in practice it is almost invariably the lack of sensitivity to potassium which sets the lower limit to the volume of sample which can be used.

The consistency of the method, however, is distinctly poor by modern standards.

Our figure of $\pm 3\%$ of the total quantity is to be compared with corresponding figures of the order of $\pm 0.5\%$ reported by Weichselbaum & Varney (1949) Gilbert *et al.* (1950) and White (1952). We believe that the lack of consistency is associated with the means adopted for introducing the sample into the flame. With a platinum wire of 0.005 in. diameter the vaporization and emission are over in less than a second. Oscillograms of the pulse recorded from the cathode follower with the integrator disconnected show considerable variation in form, although taken under identical conditions.

We are not impressed with the urgency of improving the consistency of the method in view of the greater hazards to accuracy which are presented by the interference errors. This is a subject which is at the moment in some confusion. Take, for example, the mutual interference of sodium and potassium. Most observers find that the effect is one enhancement, e.g. Belke & Dierkesmann (1948), Bernstein (1950), Brealey & Ross (1951), Domingo & Klyne (1949), Knight, Mathis & Graham (1951), Riehm (1948), Smit, Alkemade & Verschure (1951), Weichselbaum & Varney (1949), West, Folse & Montgomery (1950). In the present work the effect was found to be one of repression, as was also found by Parks, Johnson & Lykken (1948). The very extensive studies made by Bills, McDonald, Niedermeier & Schwarz (1949) showed that the sign of the interference changed with dilution. Or, again, the case of interference by phosphate; in the present work phosphate has been found to enhance the emission of sodium and potassium. But Brealey & Ross (1951), Crimson (1948), Osborn & Johns (1951), Parks *et al.* (1948) found repression, while Bowman & Berliner (1949), Domingo & Klyne (1949), Riehm (1948) and Shapiro & Hoagland (1948), failed to discover any effect at all.

The only serious attempt to make a physical interpretation of interference phenomena in flame photometry is that of Smit *et al.* (1951), and their treatment is restricted to the mutual interference between sodium and potassium. It is doubtful whether our knowledge of the kinetics of reactions in flames is sufficiently advanced to promise any comprehensive theory of interference in the near future, and it seems likely that flame photometry will remain an empirical science for some time to come. We must therefore look to various empirical methods of reducing interference errors.

The device of the internal standard, introduced by Berry, Chappell & Barnes (1946), has been used with success in reducing errors due to variation in flame temperature, rate of atomization, etc. A known concentration of lithium is added to the tested solution and if the apparent concentration of lithium differs from its known concentration by a certain factor, that same factor is applied as a correction to the apparent concentrations of sodium and potassium. This method of correction is of course inapplicable to the mutual interference between sodium and potassium, as pointed out by Bernstein (1950), and it further appears that the interference effects of anions are not suffered equally by all the alkali metals (Parks *et al.* 1948; Gilbert *et al.* 1950).

Knight *et al.* (1951) make up their calibrating solutions in the form of a 'synthetic known', that is to say, the calibrating solution is made as nearly as possible identical

with the tested solution except for the element involved in the calibration. This procedure should give reliable results provided that the concentrations of the interfering substances in the tested solution can be accurately foretold. A safer method is the use of 'radiation buffers' as recommended by West *et al.* (1950), who measured the sodium, potassium and calcium concentrations in river water. For the determination of sodium they added excess of KCl, CaCl₂ and MgCl₂, thereby swamping the effects of these cations in the river water itself; similarly for potassium and calcium, *mutatis mutandis*. This line of approach has also been followed by Eggertsen, Wyld & Lykken (1951) in overcoming the interference due to acids and salts, and has been used for phosphate interference as described earlier in this paper.

The strongest impression which one gains from a survey of the literature dealing with flame photometry is that each investigation has led to a special solution. This is no doubt in part a result of the unpredictable interference effects which are encountered, but it is also a consequence of the innumerable minor variations in design which have recommended themselves to different users. If a limited number of commercial types of flame photometer come to replace the variety of 'home-made' instruments now in use, it may be that the subject will gradually be brought into some sort of order. Until some general principles can be established we remain of the opinion that, even if the same instrument is used throughout, each different kind of fluid,—blood, urine, etc.,—presents a fresh problem, requiring a fresh approach, and that attempts to argue from one set of conditions to another will be unprofitable.

Apart from its usefulness in cases where only small volumes are available, the integrative method as here described avoids the difficulties of maintaining the same rate of atomization when solutions of different physical properties are compared. It does not entail the use of special glassware for very dilute solutions. It does not seem to us that the provision of a third channel for internal standard would substantially improve its accuracy. What is felt most keenly is the lack of a suitable photomultiplier for the potassium channel. A tenfold increase in the sensitivity to potassium would avoid the loss of accuracy on sodium which occurs when more than 3×10^{-6} mg. sodium is present in the sample, by making it possible for smaller samples to be used. It is encouraging to know that experimental models of high-gain photomultipliers with suitable photocathodes have been made, and at the same time exasperating to be unable to obtain one.

SUMMARY

1. A method of flame photometry is described, by which the amount of sodium and potassium in biological fluids can be determined simultaneously, using samples of the order of 10^{-3} cu.mm.
2. When the method is tested with quantities near the lower limits of detection (of the order 4×10^{-8} mg. sodium, 6×10^{-8} mg. potassium) the reproducibility, measured as standard deviation, is $\pm 7 \times 10^{-9}$ mg. sodium and $\pm 17 \times 10^{-9}$ mg. potassium.

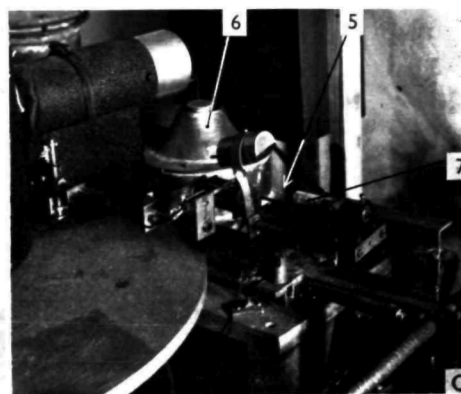
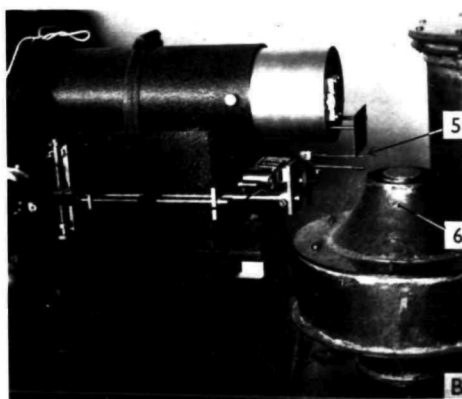
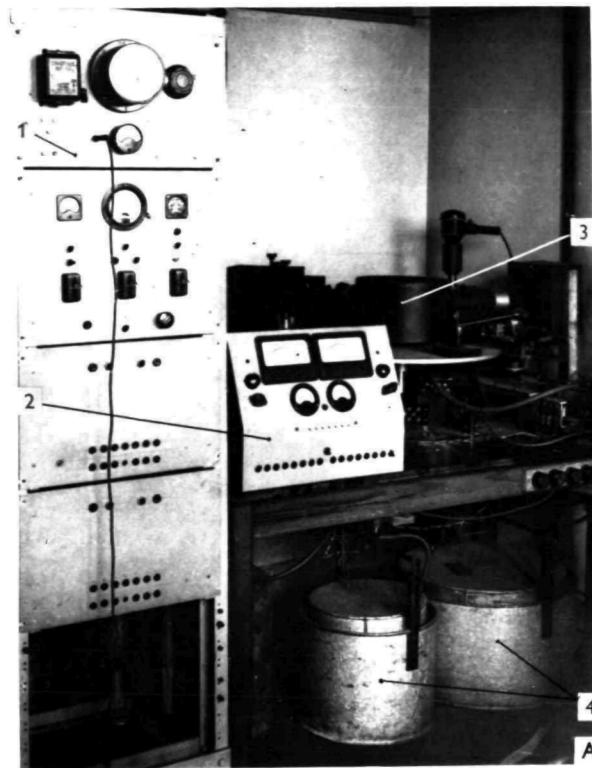
3. When larger quantities (up to certain limits) are used, the standard deviation is approximately $\pm 3\%$ of the quantity in the sample, both for sodium and for potassium.

4. As is usual in flame photometry serious errors can be caused by the presence of other substances in the sample. In the case studied for purposes of illustration it is shown that these interference errors can be reduced to the order of $\pm 6\%$ by the addition of excess ammonium phosphate.

We wish to thank Dr R. L. Mitchell and Mr K. E. Machin for their comments upon the typescript of this paper.

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EXPLANATION OF PLATE 1

- A. General view of the apparatus.
- B. Close-up, to show the platinum wire in the firing position.
- C. Close-up, to show the platinum wire in the loading position.
- 1: rack for power supplies. 2: integrator chassis and panel; main meters (square), subsidiary meters (round), coarse and fine zero-setting controls (to right and left of meters), press-buttons (white) under subsidiary meters. 3: spectrometer; entrance slit and collimator on right, box containing photomultipliers, etc., on left. 4: gasometers. 5: platinum wire. 6: burner. 7: coil of nichrome wire enclosed in a glass tube; this slides forwards on guides so as to cover the platinum wire.