APPLICATIONS OF FLAME PHOTOMETRY TO THE ANALYSIS OF ALKALIS AND CALCIUM IN SALINE SOLUTIONS FOR INFUSION

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THE practical use of flame photometry for the determination of the alkali and the alkaline earth metals in different materials has widely increased during the last few years. Even though a great number of other elements can now be determined with the flame photometer, the application of flame photometry to the alkali analysis was greeted with special enthusiasm. The analytical chemist has always been faced with long, tedious "wet" methods for sodium and potassium, often involving time-consuming separations. Rapid determination of sodium and potassium in plasma, urine, etc., has become of increasing importance not only in research, but also in diagnosis and treatment. By the technique of flame photometry the clinician can obtain data on sodium and potassium concentrations in these materials in a few minutes. The superior rapidity of the flame photometric methods has made them very advantageous for routine analyses. The great number of papers dealing with the practical aspects of flame photometry published during the last few years in various scientific journals indicate that flame photometry has assumed an important position among the practical sciences. In a previous paper the author has given a review of flame photometry, involving the developments in instruments and techniques and the limitations and advantages, which are inherent in the method[1].

The theoretical connection between the concentration of the element in the flame and the intensity of its emission is not yet elucidated. Therefore quantitative flame photometric analysis must be based upon an empirical comparison of the test solution with standard solutions. Besides the instrumental errors, which are exclusively dependent on the instrument used, the interferences, caused by the effect of dissolved substances on the emission of the test element, are the most troublesome aspects of flame photometry. These interferences are as yet so little known that no generalisations can be made. The observations on interferences made by different authors have often been divergent, probably due to different instrumental and other measuring conditions. The investigations described in this paper were carried out in order to serve as a basis for the application of flame photometry to the routine control of saline solutions in this laboratory.

APPARATUS

A Beckman DU spectrophotometer with the flame photometer attachment model 9200 was used. The instrument is exhaustively described in the manufacturers instruction manual[2], and therefore no detailed description of it will be given here.

This new Beckman flame photometry attachment incorporates an all-metal combination burner-atomiser in which the sample is sucked into a vertical palladium capillary and is sprayed directly into the flame. A concentric channel provides oxygen to operate the atomiser and the flame. An additional concentric channel provides acetylene or hydrogen for the flame. In this atomiser the spray is introduced directly into the flame and no condensing chamber is used. Since no pre-mixing of gases is necessary, backfiring is eliminated. The burner consumes 1·5 to 2 ml. of liquid per minute. Figure 1 shows the construction of the burner-atomiser.
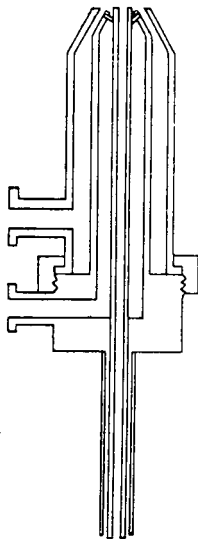
The flame photometer is equipped with pressure regulators on a control panel.

A base assembly unifies the DU spectrophotometer and the attachment into a single instrument. The burner housing is fixed with hinge pins in sockets on the base assembly. When not in use the housing can be easily swung out of the way to permit the use of a lamp. A resistance box assembly on the phototube housing permits the quick interchange of resistor for emission and absorption work. For flame photometry the model DU spectrophotometer is provided with a 10,000-megohm phototube load resistor to increase the sensitivity 5 times above that normally available with the standard 2000-megohm resistor. According to Gilbert[3] this is the practical limit of the increase of sensitivity.

By the use of the spectrophotometer in the flame photometer the combination of adjustable slit, sensitivity control, and wavelength drum enables the operator to select the most favourable ratio between background and analytical line radiation and more effectively to isolate the radiation due to the element analysed.

FIG. 1. Integral atomiser–burner unit.

EXAMINATION OF INSTRUMENTAL CONDITIONS

The instrumental conditions for reproducibility and stability were considered first. The instrument, used in this work, had an acetylene burner. The burner was supplied with tank acetylene and oxygen. At an oxygen pressure of 20 lb./sq.in. the burner consumed 1·5 ml. of liquid per minute. The manufacturer recommends an atomising rate of 1·5 to 2 ml. of liquid per minute. The suction pressure in the capillary will then be about 100 cm. of water. With the oxygen pressure at 20 lb./sq.in. an acetylene pressure of 4 lb./sq.in. gave a flame of desired height and appearance. These pressure settings, i.e., oxygen 20 lb./sq.in. and acetylene 4 lb./sq.in. pressure, were used for all measurements of sodium potassium and calcium described in this paper.

The instrumental conditions at these pressure settings were examined in respect to flame background, stability and sensitivity, and furthermore the reproducibility of the settings was tested.

Flame background. The flame background consists of the spectrum of the flame itself. This was established by setting the instrument to zero with the shutter closed, and then rebalancing the galvanometer circuit with the shutter open by rotating the transmission knob. In Figure 2 these readings are plotted against the wavelength. All measurements were made with the sensitivity control at the midpoint.
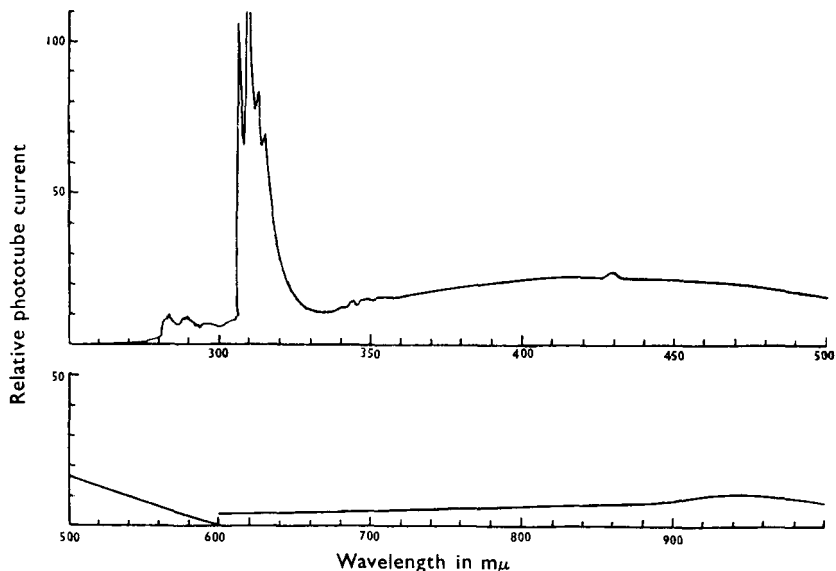

FIG. 2. Flame background.

Between 250 and 600 mμ the blue sensitive phototube is used. Slit width 0·2 mm.
Between 600 and 1000 mμ the red sensitive phototube is used. Slit width 1 mm.

The acetylene-oxygen burner had strong emission bands within the wavelength range 260 to 350 mμ belonging to the OH spectrum. The background radiation decreased then continuously towards the infra-red. A weak maximum could, however, be noticed in the near infra-red after increasing the width of the slit.

The background radiation as a function of the width of the slit is shown in Figure 3 at 554 mμ (calcium), 589 mμ (sodium) and 768 mμ (potassium). Here, too, the sensitivity was held constant with the sensitivity control at the midpoint. At this setting 1 galvanometer scale division is generally equivalent to 1 transmission scale
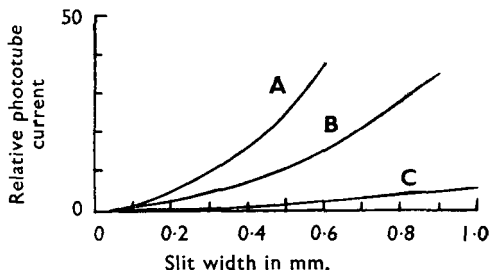

FIG. 3. Flame background as a function of slit width. Wavelengths shown as parameters.
A. 554 mμ.
B. 589 mμ.
C. 768 mμ.

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division with selector switch at 0·1. The measurements were made with distilled water spraying, which decreased the background radiation somewhat.

Flame conditions. A number of measurements were carried out on solutions of sodium and calcium in order to give a view of how the radiation intensity varied with the gas pressure. The results are graphically shown in Figures 4 and 5. Figure 4 shows relative photocurrents for the sodium lines at 589 mμ and the calcium band at 554 mμ as a function of acetylene pressure at the oxygen pressure of 20 lb./sq.in.
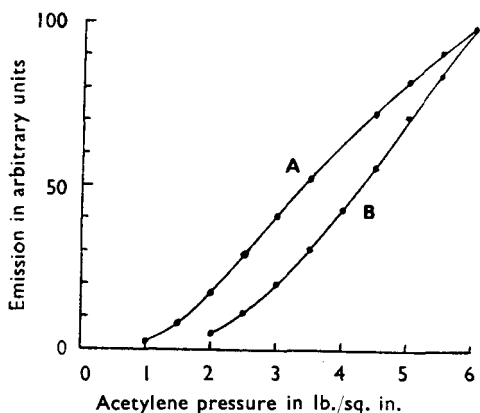

FIG. 4. Curve A. Sodium emission at 589 mμ. Oxygen pressure 20 lb./sq.in. Slit width 0·05 mm. Concentration of sodium 100 p.p.m. Curve B. Calcium emission at 554 mμ. Oxygen pressure 20 lb./sq.in. Slit width 0·1 mm. Concentration of calcium 500 p.p.m.

Figure 5 shows readings at the same wavelengths as a function of oxygen pressure at the acetylene pressure of 4 lb./sq. in. Net readings (with background subtracted) are plotted against pressure settings.

The luminosity showed no maximum or plateau with respect to acetylene and oxygen pressure. For a given oxygen pressure the luminosity increased steadily with the acetylene pressure.

Flame stability. The Beckman flame spectrophotometer is designed for standardisation with external standards. This direct intensity procedure assumes that the flame is a constant, steady source of light and that the rate of atomisation is constant. The stability of the flame, obtained with above stated standard settings of gas pressure, was tested by taking series of readings on a solution containing 100 p.p.m. of sodium. Operating conditions for the flame spectrophotometer were: wavelength at 589 mμ, transmission at 100, slit at 0·05 mm., and selector switch at 0·1. Between each reading the test solution was lowered from the capillary
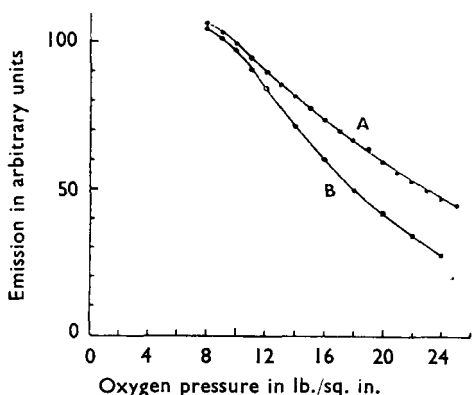

FIG. 5. Curve A. Sodium emission at 589 mμ. Acetylene pressure 4 lb./sq.in. Slit width 0·05 mm. Concentration of sodium 100 p.p.m. Curve B. Calcium emission at 554 mμ. Acetylene pressure 4 lb./sq.in. Slit width 0·1 mm. Concentration of calcium 500 p.p.m.

and the photocell shutter was closed. In each series 10 readings were taken. 3 series of readings gave mean deviations from the average readings of 0·3, 0·2 and 0·4 per cent. The results showed that the stability of the flame was satisfactory. In practice, in routine analyses, the stability of the flame is further checked by repeated standardisations during the measuring of a series of test solutions.

The reproducibility of gas pressure settings. In order to check the performance of the gauges the following measurements were carried out. 30 readings were serially taken on a solution containing 100 p.p.m. of sodium. The instrumental conditions were the same as described above under flame stability. Between each reading the test solution was lowered from the capillary after closing the photocell shutter, the oxygen and acetylene pressures were changed and then restored exactly to their original values. No other adjustments were made. These readings showed a mean deviation from the average reading of 0·5 per cent. The reproducibility of the pressure settings was consequently very good.

Sensitivity. The investigation of the sensitivity for sodium, potassium, and calcium described below, was not intended to establish the absolute efficiency of the instrument in this respect. It may only be considered as an examination of the possibilities in practice under the instrumental conditions used here. With further adjustments of pressure settings, etc., in measuring the different elements the sensitivity can probably be increased.

All measurements were carried out with the sensitivity knob at the midpoint and the selector switch at 0·1. With 1 p.p.m. of sodium at 589 mμ and with a slit width of 1 mm., a net reading (background subtracted) of 70 transmission scale divisions was obtained. With 10 p.p.m. of potassium at 768 mμ and slit at 1·5 mm., the net reading was 85 scale divisions. 10 p.p.m. of calcium at 554 mμ and with a slit width of 0·8 mm. gave a net reading of 31 scale divisions.

INVESTIGATION OF INTERFERENCES

The term "interference" covers various effects of dissolved substances on the light produced by the test substance. Many authors have studied these interference effects. Published results are apparently greatly dependent upon instrumental and other measuring conditions and cannot directly be applied. For that reason experiments were carried out to elucidate the importance of interferences in the determination of sodium, potassium and calcium in saline solution for infusion. These solutions contain sodium, potassium and calcium as salts of various acids, and the metals are often present together in various ratios.

Compounds used in interference tests

The metal salts used in the experiments were for the most part of reagent purity. Before use they were dried at 105° C. to constant weight. In some cases chemicals of Swedish Pharmacopœia purity were used. Solutions of lactates and acetates of sodium and potassium were prepared from the acids and the metal hydroxides. In all calcium salts the content

of calcium was determined by titration with the disodium salt of ethylene-diaminetetra-acetic acid[1].

Sodium and potassium

Anion interference. A study was made on the interference effects of a number of anions, several of which are often met with in the saline solutions. This study was carried out as a comparison of sodium chloride with the sodium salts mentioned in Table I and of potassium chloride with the potassium salts in Table II. The relation of concentration of test element to the anion interference was also studied by determining the interference effect at two different concentrations of the metals. The solutions containing 100 p.p.m. of the test element were prepared from the solutions containing 1000 p.p.m. by dilution with distilled water.

TABLE I

INTERFERENCE BY ANION ASSOCIATED WITH SODIUM

Compound	Change of emission in per cent. at 589 mμ compared with sodium chloride	
	1000 p.p.m. Na	100 p.p.m. Na
Sodium lactate	0	0
Sodium citrate	0	0
Sodium bicarbonate ..	0	0
Sodium acetate	−5	0
Sodium nitrate	−4	0
Sodium iodide	−3	0
Sodium sulphate	−8	0
Disodium phosphate ..	−6	0

It was observed that only at the higher concentration of sodium the light output in some cases was affected by the difference in the anions associated with the metal. The lactate, citrate and bicarbonate gave also at the higher concentration the same readings as the chloride. In all cases the measurements of the solutions containing 100 p.p.m. of sodium were interference free. Solutions containing 10 p.p.m. of sodium were also assayed. No interference was noticed.

Similar results (recorded in Table II) were obtained on repeating the investigations with potassium instead of sodium.

From these findings it is clear that flame photometric determinations of the sodium and potassium salts of the saline solutions can be made with the chlorides as standards.

TABLE II

INTERFERENCE BY ANION ASSOCIATED WITH POTASSIUM

Compound	Change of emission in per cent. at 768 mμ compared with potassium chloride	
	1000 p.p.m. K	100 p.p.m. K
Potassium lactate	0	0
Potassium acetate	0	0
Potassium bicarbonate ..	0	0
Potassium iodide	−2	0
Potassium nitrate	−3	0
Potassium sulphate	−4	0
Monopotassium phosphate ..	0	0

Interference effect of some acids. An excess of free acid is not often found in the saline solutions. For that reason less attention was given to the effect of free acids on the determination of sodium and potassium. The observations made on the interferences engendered by smaller amounts of some acids are recorded in Table III.

TABLE III

INTERFERENCE BY ACIDS WITH SODIUM AND POTASSIUM

		Change of emission in per cent.	
Acid	Concentration of acid	Sodium at 589 mμ 100 p.p.m.	Potassium at 768 mμ 500 p.p.m.
Hydrochloric acid	0·1N	0	0
Sulphuric acid ..	0·01N	0	0
	0·1N	0	−4
Nitric acid	0·1N	0	0
Phosphoric acid	0·01N	0	0
Citric acid	0·1N	0	0
Lactic acid	0·01N	0	0

A number of sodium chloride and potassium chloride solutions were prepared containing the metals and acids in amounts stated in Table III. These solutions were then analysed flame photometrically for sodium and potassium, using pure sodium and potassium chloride solutions of the same metal content as comparison standards. It is observed that the presence of 0·7 per cent. (0·1N) of citric acid did not affect the determination of sodium or potassium.

Interference of calcium with sodium and potassium and interference of sodium and potassium with each other. Several authors have shown that the alkali metals enhance each other, and that the enhancement is not proportional to their concentration. In order to investigate the magnitude of these interferences under the instrumental conditions used in this laboratory, quantitative measurements were made. The results of these

TABLE IV

MUTUAL INTERFERENCE OF SODIUM AND POTASSIUM AND INTERFERENCE OF CALCIUM WITH SODIUM AND POTASSIUM

Interfering element	Weight ratio of interfering element to test element	Change of emission in per cent.			
		Sodium at 589 mμ		Potassium at 768 mμ	
		100 p.p.m.	10 p.p.m.	200 p.p.m.	50 p.p.m.
Sodium ..	1:10	—	—	0	0
	1:2	—	—	0	0
	1:1	—	—	+4	0
	5:1	—	—	+12	+7
	10:1	—	—	+16	+15
Potassium	1:10	0	0	—	—
	1:2	0	0	—	—
	1:1	+1	0	—	—
	5:1	+5	+2	—	—
	10:1	+8	+3	—	—
Calcium	1:10	0	0	0	0
	1:2	0	0	0	0
	1:1	0	0	0	0
	5:1	+4	0	+6	+3
	10:1	+6	+1	+8	+5
Slit width		0·05 mm.	0·15 mm.	0·2 mm.	0·4 mm.

measurements are recorded in Table IV. With transmission scale at 100 (or at 90 when the enhancement was greater than 10 per cent.) and selector switch at 0·1, the flame photometer was standardised with a solution containing the test element only. Then readings were taken on solutions containing the same quantity of the test element but with various amounts of interfering element added. The effect of dilution on the interference was studied. At first the stronger solutions were assayed, then they were diluted and assayed at the lower concentration range. The chlorides of the metals were used.

It is evident from the findings that the presence of potassium in the same (or larger) concentration as sodium gave a positive interference in the determination of sodium at the 100 p.p.m. concentration. The effect of calcium on sodium was smaller. The interference of sodium with potassium was stronger than *vice versa*, while calcium increased the emission of potassium in about the same degree as the emission of sodium. In all cases dilution decreased the interference, even though the ratio of interfering element to test element remained constant.
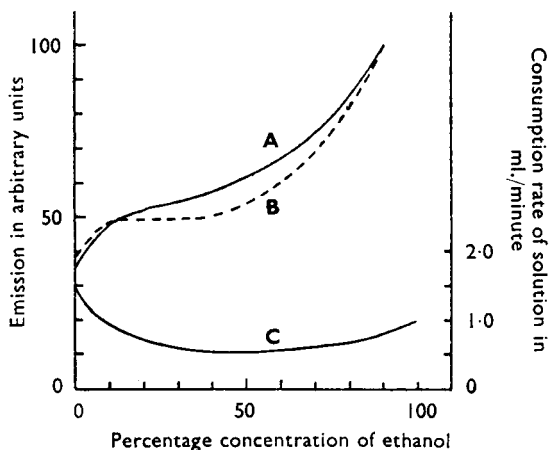

Since the saline solutions generally contain less potassium and calcium than sodium, most determinations of sodium in these solutions can be made using a standard solution containing only sodium chloride.

In connection with the measurements of interference described above, experiments were carried out to ascertain if the increased readings included also a continuum interference, i.e., an increased background radiation due to continuous spectral emission by the interferant. For that reason solutions containing only the interfering metal, in the same quantities as the solutions assayed above, were measured. No significant increase of the background was observed in any case.

Fig. 6. Effect of ethanol on the emission of sodium and potassium.
Curve A. Sodium emission at 589 mμ. Slit width 0·05 mm. Concentration of sodium 100 p.p.m.
Curve B. Potassium emission at 768 mμ. Slit width 0·1 mm. Concentration of potassium 500 p.p.m.
Curve C. Consumption rate of the sodium solution as a function of ethanol concentration.

Effect of ethanol and dextrose on the determination of sodium and potassium. It has been reported[5,6,7] that light emission is actually increased by the addition of ethanol. The cause of this interference is not known. This observation was verified by experiments. The results are given in Figure 6, which shows the readings of sodium and

potassium at different concentrations of ethanol. The solutions of sodium and potassium used in these experiments contained the metals as iodides.

Osborn and Johns[8] consider that this interference is a physical effect resulting from an increased rate of atomisation owing to the lowered surface tension of the test solution. In order to check this hypothesis the rate of atomisation was measured at different concentrations of ethanol. The results of these measurements are recorded in Figure 6 in the form of a curve showing the rate of atomisation in ml./minute as a function of the concentration of ethanol in the test solution. From the curves in Figure 6 it is evident that the interference of ethanol cannot be attributed to an increased rate of atomisation. The highest rate of consumption was obtained with the pure water solution.

Dextrose is reported[9,10] to cause negative errors in the determination of sodium and potassium with an instrument containing a condensing chamber in the atomiser. On the other hand, Weichselbaum and Varney[11] found that a concentration of 4 per cent. of sugar did not effect the readings. In the Weichselbaum atomiser the spray was introduced directly into the flame and no condensing chamber was used. The presence of dextrose or sugar changes the viscosity of the solution. From these findings it might be concluded that the atomiser with condensing chamber is more readily affected by changes in viscosity resulting in changed spray rate.
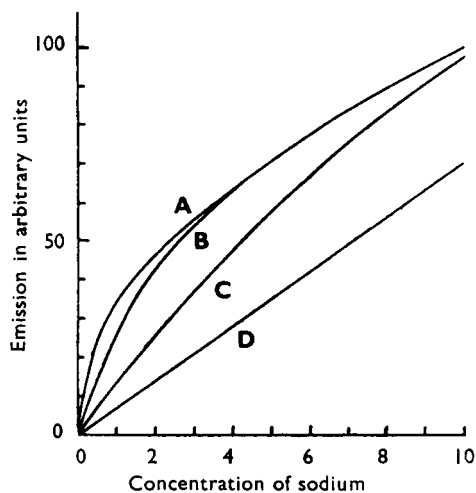
As mentioned before the

FIG. 7. Calibration curves for sodium at 589 mμ. Linear co-ordinates.
A. 0 to 1000 p.p.m.
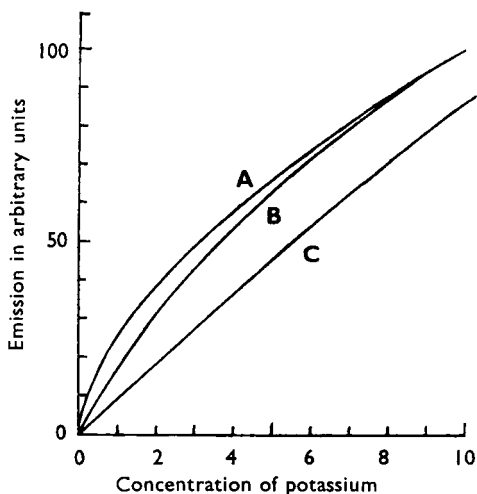B. 0 to 100 p.p.m.
C. 0 to 10 p.p.m.
D. 0 to 1 p.p.m.

FIG. 8. Calibration curves for potassium at 768 mμ. Linear co-ordinates.
A. 0 to 1000 p.p.m.
B. 0 to 100 p.p.m.
C. 0 to 10 p.p.m.

atomiser in the Beckman instrument sprays the solution directly into the flame. Measurements of the rate of consumption by atomising solutions of sodium chloride containing 0, 1, 2 and 3 per cent. of dextrose showed that these quantities of dextrose did not change the spray rate.

Calibration curves. Figures 7 and 8 show the relative photocurrents for sodium and potassium in different ranges of concentration. These curves are actual working curves. The net readings are plotted as ordinates.

The sodium lines at 589 mμ show, at higher concentration of the metal, a pronounced upward convexity due to self absorption. At the 0 to 1 p.p.m. range the curve is linear.

With potassium at 768 mμ and 0 to 10 p.p.m. range, the curve obtained was nearly linear.

Bills *et al.*[7] showed that, in the determination of sodium and potassium, reduction in interference, both mutual and from foreign solutes, was effected by working at low concentration ranges. The results of Tables I, II and IV also confirm this finding. There is, too, another advantage of standardising for a low concentration range. At, for example, the 0 to 1 p.p.m. range, the relation between concentration and dial reading is linear. Hence no calibration curve is required. But certain disadvantages are also associated with the measurements of solutions containing very small quantities of the metals. The light output is of course lower, and therefore an increase in the slit width is necessary. This increase in slit width has certain undesirable effects in that the spectral band width is increased, resulting in an increased background and possible inclusion of lines of other elements in the sample. The reproducibility of the readings, too, becomes less satisfactory. Furthermore, it is very important to handle and preserve very diluted solutions of alkali with care to avoid the possibility of accidental contamination with the metal to be determined. The accidental introduction of sodium or potassium, as from a particle of dust, tobacco smoke, or fingerprint, can seriously upset the analysis. Glass containers too may contribute important quantities of sodium or potassium. These sources of practical error make the work with very diluted solutions in routine analyses less reliable.

For reasons discussed above, and with the guidance of the results obtained by the investigations of interference, the concentration ranges 0 to 100 p.p.m. for sodium and 0 to 200 p.p.m. for potassium are generally used in the analyses of the saline solutions in this laboratory. Because the saline solutions contain comparatively large amounts of the alkalis, the use of these relatively high concentration ranges also reduces the work of preparing the solutions to be atomised. The amounts of interfering substances in the solutions are known, and therefore radiation interferences are eliminated by including the proper amounts of the interfering substances in the standards, which is the most effective remedy for radiation interference.

For sodium the wavelength setting at 589 mμ was used measuring the radiation of the sodium lines at 589·0 and 589·6 mμ. The determination of potassium was based on the radiation of the potassium

lines at 766·5 and 769·9 mμ. The calibration curves were found to be reproducible.

Calcium

The examination of the measuring conditions for the determination of calcium by flame photometry was, for various reasons, given little room in this investigation. In the last few years rapid and sensitive chemical methods for the determination of calcium have been described in the literature[1]. Furthermore, calcium is met with in only a small number of saline solutions for infusion. The experiments described below might, however, show that several causes of interference complicate the flame photometric determination of calcium. Above all, the high concentration of sodium to calcium in saline solutions renders this particular determination most difficult.

All determinations of calcium have been carried out by measuring the radiation of its spectral emission band at 554 mμ. At this wavelength the flame background (Fig. 3) is higher than at the wavelength settings used in the determination of sodium and potassium.

Previous studies[12] showed that the band spectra of calcium and the other alkaline earths are affected by the nature of the anion present in the solution. This is to be expected since the band system originates from the thermal excitation of calcium-containing molecules (probably the oxide or chloride). Measurements of this anion interference were carried out by comparing solutions of 4 calcium salts containing the same content of calcium. The solution of calcium chloride was used as standard. The results are recorded in Table V.

TABLE V

INTERFERENCE OF ANION ASSOCIATED WITH CALCIUM

Compound	Change of emission in per cent. at 554 mμ compared with calcium chloride			
	1000 p.p.m. Ca	500 p.p.m. Ca	100 p.p.m. Ca	50 p.p.m. Ca
Calcium bromide	−32	−16	0	0
Calcium gluconate	−28	−21	−7	−3
Calcium lactate	−17	−17	−5	−4

When the concentration was decreased the interference effect decreased rapidly. From these results it is clear that the standards used in the flame photometric determination of calcium must be prepared from the same compound or compounds in the same ratio as that contained in the sample being analysed.

The presence of an excess of hydrochloric acid changed the luminous output from calcium (as chloride). Thus, solutions of calcium chloride containing 500 p.p.m. of calcium, which were 0·01N and 0·1N in respect to hydrochloric acid, gave 3 to 9 per cent. higher readings than a solution of only calcium chloride with the same content of the metal. Since the solutions were diluted 10 times (to 50 p.p.m. of calcium), no interference was observed.

Sodium and potassium increased strongly the emission radiation of

calcium. In order to investigate the effect of these cations, series of readings were taken to compare a pure solution of calcium chloride with solutions containing the same amount of calcium chloride, but with various quantities of sodium or potassium as chlorides added. The results are recorded in Table VI.

TABLE VI

INTERFERENCE OF SODIUM AND POTASSIUM WITH CALCIUM AT 554 Mμ

Interfering element	Weight ratio of interfering element to test element	Background radiation in scale divisions	200 p.p.m. Calcium	
			Change of reading in per cent.	Change of emission of calcium in per cent.
Sodium	0	5·1	—	—
	1:10	5·5	0	0
	1:2	5·8	−5	−5
	1:1	6·0	−9	−10
	5:1	8·2	−18	−20
	10:1	11·0	−14	−20
Potassium	0	5·1	—	—
	1:10	5·2	+1	+1
	1:2	5·4	0	0
	1:1	5·9	−3	−4
	5:1	7·3	−15	−17
	10:1	9·1	−22	−25
Slit width		0·2 mm.		

At a concentration of 200 p.p.m. of calcium, 200 p.p.m. (or more) sodium depressed the light output of calcium. Similar results were obtained with potassium in a concentration of 200 p.p.m. or more. Table VI also shows the results of background readings obtained by measuring the light output, when solutions containing only the interfering metal were atomised. A pronounced increase of the background radiation was observed.

Calcium gave for the concentration range 0 to 1000 p.p.m. a slightly S-shaped calibration curve. At the 0 to 100 p.p.m. range a linear curve was obtained. Figure 9 (p. 524) shows these curves. The net readings are plotted against the concentrations.

EXAMPLES OF DATA FOR VARIOUS TYPES OF DETERMINATIONS

The procedures for the analyses of various types of saline solutions, which were arranged with the guidance of the results obtained by the investigations described above, were applied to about 100 solutions of varying composition. These solutions, prepared in Swedish pharmacies, might be a representative material of saline solutions for infusion used in this country. The solutions contained sodium as chloride, bicarbonate, phosphate, lactate and citrate, potassium as chloride, lactate and phosphate, and calcium as chloride. Solutions of the alkalis containing dextrose were also analysed. Determinations of the sodium and potassium salts based on their anions were made, and for the sake of comparison the results of these determinations are also recorded in the tables below. Sodium and potassium were not assayed with chemical methods. Tables VII to XIII show some of the results.

TABLE VII

SOLUTIO NATRII CHLORIDI PHYSIOLOGICA PHARM. SVECICA ED. XI

This solution contains 9·0 mg./ml.

Sample No.	Sodium chloride mg./ml. determined	
	Argentimetrically as chloride	Flame photometrically as sodium
1	8·9	9·0
2	8·9	9·0
3	8·9	9·0
4	8·9	9·0
5	8·1	8·1
6	8·9	9·0
7	9·1	9·2
8	9·3	9·4
9	8·7	8·8
10	9·0	9·0
11	9·1	9·1
12	9·1	9·1

TABLE VIII

INJECTABILE NATRII BICARBONATIS 1·3 PER CENT.

Sample No.	Sodium bicarbonate mg./ml. determined	
	Acidimetrically as bicarbonate	Flame photometrically as sodium
1	12·9	12·9
2	13·2	13·0
3	12·9	12·9
4	12·9	13·0
5	12·8	13·0
6	13·0	13·2
7	12·9	12·9
8	12·9	12·9
9	13·0	13·0
10	13·3	13·2
11	13·0	13·0

TABLE IX

SOLUTIONS OF SODIUM CHLORIDE WITH GLUCOSE

Sample No.	Glucose mg./ml.	Sodium chloride mg./ml.		
			Determined	
		Stated	Argentimetrically as chloride	Flame photometrically as sodium
1	16·3	6·0	6·0	6·0
2	18·0	6·0	6·1	5·9
3	23·4	4·5	4·4	4·5
4	24·5	4·5	4·5	4·6

TABLE X

SOLUTIONS OF SODIUM CITRATE

Sample No.	Sodium citrate mg./ml.		
		Determined	
	Stated	As citric acid	Flame photometrically as sodium
1	30·0	29·7	30·2
2	30·0	31·0	30·8
3	38·0	39·3	39·2
4	38·0	37·5	37·8

TABLE XI

SOLUTIONS OF SODIUM CITRATE WITH CITRIC ACID AND GLUCOSE

Sample No.	Glucose mg./ml.	Citric acid mg./ml.	Sodium citrate mg./ml.		
				Determined	
			Stated	As citric acid	Flame photometrically as sodium
1	23	7·5	21·0	21·6	22·2
2	22	6·3	21·0	20·0	20·6
3	23	8·0	22·0	22·2	21·7
4	24	8·3	22·0	22·3	22·0
5	16	5·0	13·2	12·9	13·4

Solutions containing different salts of sodium. The determinations of sodium in these solutions were all made in the following manner. The saline solution was diluted with distilled water to a sodium content of about 100 p.p.m. With wavelength scale at 589 mμ, transmission scale at 100, selector switch at 0·1 and slit at 0·05 mm., the flame spectrophotometer was standardised with a solution of sodium chloride containing 100 p.p.m. of sodium, and then the intensity of the unknown was determined. The standard solution was freshly prepared every day from a stock solution. A calibration curve for the 0 to 120 p.p.m. range was used to convert the ratio of readings to the ratio of concentrations.

Solutions containing both sodium and potassium. These solutions (Table XII) had the following compositions:—

FIG. 9. Calibration curves for calcium at 554 mμ. Linear co-ordinates.
A. 0 to 1000 p.p.m.
B. 0 to 100 p.p.m.

Darrow's solution:—Potassium chloride 2·0 g., sodium chloride 3·0 g., 1 molar solution of sodium lactate 40 ml., sterile water 710 ml.

Butler's solution:—Sodium lactate 2·2 g., sodium chloride 0·6 g., potassium chloride 1·0 g., dipotassium phosphate 0·5 g., sterile water to 50 ml.

Inject. natrii et kalii lactat.:—Potassium chloride 3·8 g., 20 per cent. stock solution of sodium lactate 51·5 g., monosodium phosphate 0·3 g., disodium phosphate 1·2 g., sterile water 950 g.

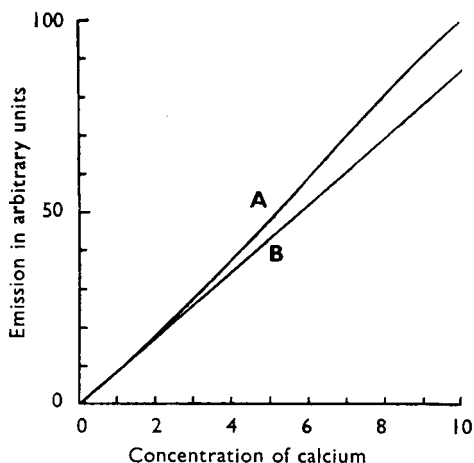With the exception of Inject. natrii et kalii lactat. the sodium content of these solutions was determined as above described, using sodium chloride as standard. In the analysis of Inject. natrii et kalii lactat. the proper amount of potassium was included in the sodium standard

in order to compensate for the interference of potassium with sodium. Potassium was added as potassium chloride.

All determinations of potassium were carried out with self-compensating standards containing potassium and sodium in the same ratio as the test solutions. The standard solutions were prepared from sodium chloride

TABLE XII

Sample	Potassium mg./ml.		Sodium mg./ml.	
	Stated	Found	Stated	Found
Darrow's solution	1·40	1·37	2·80	2·86
,, ...	1·40	1·38	2·80	2·86
Butler's solution	15·0	14·7	13·7	13·7
,, ...	15·0	14·8	13·7	14·0
Inject. natrii et kalii lactat. ..	1·99	2·00	2·47	2·50

and potassium chloride. The saline solutions were diluted to a potassium content of 200 p.p.m. With transmission scale at 100 the flame spectro-photometer was standardised with the standard. The standard solutions were freshly prepared every day from stock solutions. The measurements were made at 768 mμ with a slit width of 0·2 mm. A calibration curve for the 0 to 220 p.p.m. range was used to convert the ratio of readings to the ratio of concentrations.

Solutions containing sodium, potassium and calcium

Ringer's solution:—This saline solution contained, in each l., 9·0 g. of sodium chloride, 0·42 g. of potassium chloride and 0·24 g. of calcium chloride ($CaCl_2,6H_2O$).

Determination of sodium. The determination of sodium was made as described above, using sodium chloride as standard.

Determination of potassium. Sodium and potassium were included in Ringer's solution in the ratio of 16 to 1. The comparatively large content of sodium affected strongly the light output of potassium, and therefore a standard solution of potassium containing the proper amount of sodium had to be used for the standardisation of the instrument, and furthermore, the determination was carried out at a lower concentration range than usually. The saline solution was diluted 4 times. The solution, to be assayed, then contained 55 p.p.m. of potassium. The small amount of calcium did not affect the potassium light. The calibration curve was obtained in the following way. With the transmission scale at 100 and slit at 0·5 mm., the instrument was standardised with the standard solution. Then a number of solutions containing the same amount of sodium as the standard but with different quantities of potassium added were measured. No increase of the background radiation was observed at 768 mμ, when a solution containing only sodium in the same concen-tration as the standard (885 p.p.m.) was assayed.

The effect of small variations of the concentration of sodium on the determination of potassium in Ringer's solution was also tested. When the concentration of sodium was changed within ±10 per cent. no change of the readings of potassium was noticed, but the light output decreased

about 1 per cent. when the concentration of sodium was decreased 20 per cent. and increased about 1 per cent. when the content of sodium was increased 20 per cent. This result demonstrates the principle underlying the use of radiation buffers to reduce interference[13]. At higher concentrations of interfering materials small variations in the amount of the interferant have relatively little effect on the analysis.

Determination of calcium. Ringer's solution contained calcium, potassium and sodium in the ratio of 1 to 5 to 80. The possibilities of determining calcium flame photomet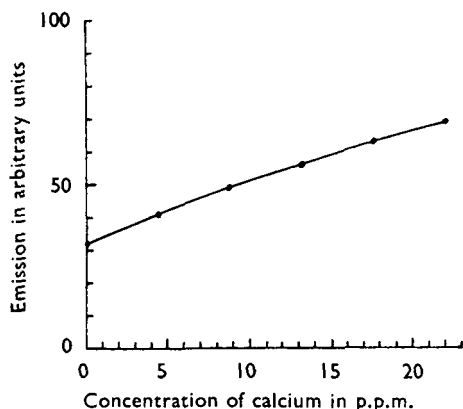rically in the presence of the relatively large amounts of sodium and potassium were investigated. The saline solution was diluted with an equal volume of water ($=22$ p.p.m. of calcium) and the measurements were carried out at 554 mμ with slit at 0·4 mm. The calibration curve (Fig. 10) was obtained in the same way as the potassium curve above. With transmission scale at 100 and slit at 0·4 mm., the instrument was standardised with a standard solution of the same composition as the test solution.

FIG. 10. Calibration curve for calcium in Ringer's saline solution.

Then a number of solutions containing the same amount of sodium and potassium as the standard but added different quantities of calcium were measured. The concentration is plotted against the net reading (the radiation of the flame fed with pure water is subtracted).

As Figure 10 shows, the relatively high concentration of the alkali metals caused a pronounced increase of the background radiation. Therefore the ratio between background and band radiation of calcium was unfavourably low. The reproducibility of the readings was less satisfactory. The accuracy of these measurements could be estimated to about ±5 per cent. Further, it was found that a little change in the content of alkali metals affected the reading. Thus an increase or a decrease of the concentration of the alkali metals of 10 per cent. increased or decreased the reading about 5 per cent., giving an error of ±10 per cent. in the determination of calcium. This effect was due to a change of the background radiation. The background radiation made up about 50 per cent. of the reading obtained (Fig. 10). This error can of course be reduced. At first, flame analyses are made of sodium and potassium, and then the proper amounts of the metals shown in these analyses are added to the standard for the determination of calcium.

In Table XIII results of some analyses of Ringer's solutions are recorded. Calcium was also determined by chemical means with ethylenediamine-tetra-acetic acid[4].

With the application of flame photometry the assay of saline solutions for infusion could be organised on a rational basis in this laboratory. The cost of this analytical control could be reduced considerably by using these rapid and labour-saving methods. It is evident, too, that

TABLE XIII

SOLUTIO RINGERI PHARM. SVECICA ED. XI

Sample No.	Sodium as NaCl mg./ml.	Potassium as KCl mg./ml.	Calcium as $CaCl_2,6H_2O$ mg./ml. determined	
			Volumetrically	Flame photometrically
1	9·0	0·42	0·243	0·25
2	9·0	0·42	0·236	0·25
3	9·0	0·42	0·243	0·25
4	9·0	0·42	0·246	0·24

flame photometry can successfully be applied to many other materials in pharmaceutical analytical work. The Swedish Pharmacopœia Commission has recently proposed flame photometry for the assay of saline solutions for infusion.

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