THE MICRO-ESTIMATION OF SODIUM, POTASSIUM, CALCIUM, MAGNESIUM, CHLORIDE, AND SULPHATE IN SEA WATER AND THE BODY FLUIDS OF MARINE ANIMALS

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INTRODUCTION

Much progress has been made in the last ten or fifteen years, chiefly by medical biochemists working on problems of clinical interest, in improving the accuracy and sensitivity of the methods employed for the estimation of the principal inorganic constituents of blood and other mammalian body fluids. If, however, attempts are made to apply directly these methods to the micro-analysis of sea water, or of the blood and body fluids of marine invertebrates, they are often unsuccessful. This is only to be expected if one considers that the much higher concentration of salts in these fluids not only brings about a possibility of direct chemical interference, particularly in the form of co-precipitation, but also by decreasing the activity coefficients of their constituent ions may cause a significant increase in the solubility of "insoluble" salts. Furthermore, the higher concentration of all the ions in question means that methods are sometimes available for the analysis of the body fluids of marine animals which are not sufficiently sensitive to be used for mammalian material. Since in many physiological experiments on marine animals, especially those relating to excretion or permeability of the integument, it is necessary to be able to perform accurate analyses both of the body fluids and of the surrounding sea water, the former being often available only in small quantities, we have attempted to devise suitable methods. The aim we set ourselves was to find methods for the estimation of sodium, potassium, calcium, magnesium, chloride and sulphate, which could be performed on 1 ml. samples of sea water or of a body fluid of approximately similar composition, and whose maximum error did not exceed 2%. In this aim we may claim to have largely succeeded; only in the methods for potassium and sulphate is the accuracy somewhat less than might be wished, but even for these two radicals the number of analyses in which the error exceeds 2% is small, and may be practically eliminated by performing duplicate determinations

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The need for an improvement in the accuracy of analytical methods for these purposes is seen when one examines the results obtained by previous workers. In Table I are shown the ionic ratios for sea water as calculated from the analyses published by different authors who were investigating the mineral balance of marine invertebrates or the mineral composition of their blood and tissues. The important point to observe is that these figures were obtained by the same analytical methods as were the data of physiological interest furnished by the same authors. In the table are set out for comparison the most likely figures for the true values of the ratios; also the mean result of four or more determinations for each ratio made by us at Millport by the micro-methods described in this paper. It is easily seen that of the previous authors cited only Macallum used satisfactory methods, and in his case they were all performed on a large scale by gravimetric processes that are mostly long and tedious.

Table I

Author	Na/Cl	K/Cl	Ca/Cl	Mg/Cl	SO_4/Cl
Macallum (1903)	0·5550	0·02025	0·02123*	0·06683	0·1370
Page (1927)	0·4796	0·02245	0·02302*	0·07087	0·1425
Bethe & Berger (1931) (Dec.)	0·6005	0·02858	0·02252	—	—
,, (Feb.)	0·5398	0·01740	0·02201	0·05922	—
Bialaszewicz (1932)	—	0·02361	0·02286	0·07524	0·1419
,, (1933)	—	0·02173	0·02304	0·06758	0·1351
Robertson & Webb (this paper)	0·5532	0·02011	0·02115	0·06692	0·1401
True value	0·5514	0·01997	0·02122*	0·06694	0·1396
Authority	Dittmar (1884)	Dittmar (1884)	Thompson & Wright (1930)	Thompson & Wright (1930)	Thompson, Johnston & Wirth (1931)

* Gravimetric results; the strontium error has therefore been scaled down to render them comparable with the volumetric figures.

In view of the fact that analyses of this kind must often be carried out at a marine station care has been taken to render the methods independent of elaborate pieces of apparatus, such as the micro-balance, photo-electric colorimeter, or muffle furnace, which are unlikely to be available. Only a centrifuge and ordinary glass-ware are required. It is perhaps unnecessary to add that for accurate results it is essential that all volumetric apparatus, even of high grade, should be standardized.

In order to determine the accuracy of the method for the estimation of calcium it was necessary to have recourse to spectrographic analysis of precipitates, filtrates, and standard solutions. These analyses were carried out in accordance with the principles detailed by Webb (1937). Copper electrodes were used, and the standard mixtures for comparison were prepared from Hilger's "Specpure" salts.

All the methods were tested out on a synthetic sea water made up according to the following formula,[1] which is calculated from the most probable figures for the various ratios as given by Thompson & Robinson (1932).

[1] This formula is preferable to that given by Allen (1914) in that sodium sulphate may be weighed out with greater accuracy than magnesium sulphate.

	g. per litre
NaCl	23·4651
KCl	0·7246
$CaCl_2.6H_2O$	2·2040
$MgCl_2.6H_2O$	10·6364
Na_2SO_4 (anhydrous)	3·9282

Expressed in terms of the individual ions the composition of this solution is as follows:

	g. per litre
Na	10·5044
K	0·3800
Ca	0·4032
Mg	1·2722
Cl	19·0000
SO_4	2·6562

Kahlbaum or B.D.H. "Analar" salts were used, and the solution was stored in a wax-lined bottle. The sodium chloride, potassium chloride and sodium sulphate were weighed out directly, after drying at 100° C.; the calcium and magnesium chlorides were made up into arbitrary solutions whose strength was then estimated by titrating the chloride. Since, however, it appears that even in the purest samples of these salts there are liable to be present small quantities of excess acid or base, other methods were used to determine exactly the concentration of calcium and magnesium in the synthetic sea water.[1] These methods are described later in the sections on the two metals in question. In most cases the final testing of the method was performed on quantities of synthetic sea water measured out by one of us and unknown to the other who performed the analysis.

As an expression of the accuracy of each method we have given the probable error, calculated according to the ordinary root-mean-square formula from about a dozen analyses. It is not an entirely satisfactory measure, since the formula is valid only if the analytical results fall along a normal frequency curve with the theoretical value as mean, and this is probably seldom the case. Nor is the number of analyses in any series large enough to warrant any kind of statistical treatment. It has, nevertheless, at least as much meaning as the customary "maximum error", which is bound to increase with the number of analyses performed. In general it may be said that differences amounting to over three times the probable error are unlikely to be due entirely to experimental error.

PREPARATION OF BIOLOGICAL SAMPLES

The methods as described below are applicable directly to sea water or to any similar solution free from organic solutes. Most animal body fluids, however, contain quantities of protein which are sufficient to interfere with these methods and cause serious errors. Except in the case of the chloride method, where it does not give rise to any error, this protein must be removed. This may be accomplished either by

[1] In any case the traces of calcium found in even the purest sodium chloride and sulphate are sufficient to cause a significant error.

ashing (wet or dry) or by precipitation with one of the recognized protein precipitants. The latter procedure is rapid, and for this reason nearly always employed in routine clinical work, but its use is open to certain objections. Either the precipitated protein must be washed repeatedly on the filter or in the centrifuge tube, thus bringing the total volume of fluid to an inconveniently large figure; or else, if an aliquot of the supernatant fluid is taken for analysis, it must be assumed that the volumetric distribution of solutes is the same throughout the precipitated protein clot as in the supernatant fluid. It has, however, been shown repeatedly (e.g. by Van Slyke *et al.* (1927)) that this is not always the case, even when the volume of the protein itself is negligible, and that errors of the order of 4% may easily be introduced by this procedure.

In the estimation of sulphate, however, precipitation of the proteins is the only course possible, since on ashing much of the organic sulphur is bound to be converted to sulphuric acid, of which some at least will remain behind in the ash. Among the various deproteinizing agents available it seemed to us that a neutral or alkaline reagent would be preferable on *a priori* grounds, in order to avoid adsorption of anions on the coagulum, which is likely to occur if precipitation is carried out at or on the acid side of the iso-electric point. Trichloracetic acid was therefore ruled out. Tungstic acid precipitates barium as an insoluble tungstate and is therefore impossible. Somogyi's (1930) alkaline zinc mixture was tried, but the zinc ions were found to react with the indicator to form a stable orange complex which rendered the end point almost impossible to observe. Eventually mercuric chloride was tried and found to be satisfactory.

For the metals there remained the choice between wet and dry ashing. With the former there is certain to be no loss of base, but the large quantities of acid that must be employed are undesirable, since it is usually necessary to neutralize them before proceeding further with the analysis, and this involves not only a great increase in the total salt content of the solution, but also the risk of introducing small quantities of the metal that is being estimated, in the form of impurities, either in the acid or in the base that is used to neutralize it. With dry ashing on the other hand there is a danger of loss of sodium and potassium by volatilization of their chlorides. This source of error may be completely eliminated by careful control of the temperature, but such control is difficult to achieve without the use of a muffle furnace. If, however, the sample is heated with an excess of sulphuric acid before ignition, all the chlorides are converted into sulphates, and the salts may be safely heated to a bright red heat (800° C.) without loss of base by volatilization.

The methods adopted for the preliminary treatment of samples which contain protein are accordingly as follows.

For the estimation of Na, K, Ca, Mg, *and also of* Cu (*vide section on magnesium*). The 1 ml. sample is measured out into a small silica or porcelain crucible,[1] about

[1] Glazed silica crucibles are best, but good quality porcelain ones with the glaze intact may also be used, except for potassium. For potassium estimations only silica crucibles are safe, since some qualities of porcelain liberate appreciable amounts of potassium when ignited in the presence of sulphuric acid. As soon as any part of the inner surface of a crucible becomes in the slightest degree rough to the touch it should be rejected.

0·2 ml. (three to four drops) of 25 % sulphuric acid is added, and it is evaporated down on a steam bath. The excess acid is then driven off by very cautious heating over a flame until no more fumes are given off. Finally, the crucible is ignited till all the carbon has been burnt away. If a considerable quantity of organic matter is present it is necessary, during the process of ignition, to allow the crucible to cool and to add once more one or two drops of sulphuric acid. This assists in the oxidation of the carbon, and prevents the formation of carbonates and oxides which are liable to attack the surface of the crucible.

For the estimation of SO_4. To the 1 ml. sample are added 4 ml. of distilled water followed by 2 ml. of a 5 % solution of mercuric chloride. The mixture is gently warmed, with stirring, to coagulate the protein precipitate; it is then filtered and the filter washed with 5 ml. portions of distilled water until it runs through chloride-free. The combined filtrate and washings are evaporated down to a volume of 10 ml.

For the estimation of Cl. No previous treatment is necessary. The sample is measured directly into the reaction flask.

SODIUM

The standard zinc uranyl acetate method gives excellent results with sea water, and hardly any modifications are required. Since the weight of the precipitate is 67 times that of the sodium it contains, and since sea water contains about 10 mg. of sodium per ml., it is clear that even with as little as 0·1 ml. gravimetric methods will yield results of the degree of accuracy required. We have not, therefore, investigated any of the volumetric methods proposed for the estimation of the precipitate. The method, due originally to Barber & Kolthoff (1928), was applied to biological material by Butler & Tuthill (1931), from whom we differ in one point only—the length of time that the precipitate must be left in the desiccator before weighing. Barber & Kolthoff considered 10 min. sufficient; Butler & Tuthill extended this to ½ hr.; but we find that 3–4 hr. are necessary before constant weight is attained. Possibly this is due to the greater bulk of precipitate in our analyses.

Barber & Kolthoff showed that potassium does not interfere unless its concentration exceeds 25 g. per litre in the original solution; we have confirmed this for the much smaller concentrations in which it is likely to occur in body fluids of any kind. Butler & Tuthill recommend that phosphates be removed by precipitation with calcium hydroxide, but in the material for which our methods are devised this ought never to be necessary. Assuming that all phosphate is precipitated as uranyl phosphate it may be shown by calculation (and we have confirmed it by experiment) that even with 100 mg. of P per litre present as phosphate the error will be less than 0·25 %. All recorded figures for phosphate in the blood of invertebrates are much smaller than this. It is possible that higher concentrations might be found in excretory fluids, but one of us has estimated the phosphate in the antennary gland secretion of *Cancer pagurus* and found only negligible quantities, so that it appears unlikely that serious errors can arise from this source.

Procedure. The precipitating reagent, which consists of a solution of zinc and uranyl acetates, is made up as follows. 80 g. uranyl acetate are dissolved, with heating, in 425 ml. of water to which are added 14 ml. glacial acetic acid. 220 g. zinc acetate are dissolved separately in 275 ml. water with 7 ml. glacial acetic acid, and the two solutions are mixed while hot. A trace of sodium acetate may be added to ensure that the solution is saturated with respect to the triple salt (sodium zinc uranyl acetate), but unless the salts used are of analytical grade sufficient sodium will already be present as an impurity. The mixture should be allowed to stand for 24 hr. before being filtered and used. Since the temperature coefficient for the solubility of the triple salt is relatively high the solution should be stored, preferably at a fairly constant temperature, in contact with excess of the salt, and well shaken up and filtered immediately before use. This applies also to the washing fluid, which consists of 95 % alcohol saturated with the triple salt.

A sintered glass filter crucible of medium porosity and 30 ml. capacity is washed through with ether, sucked dry by means of a filter pump, and weighed after standing for an hour or more in a desiccator. Its lower opening is then fitted with a rubber bung, which prevents the passage of any fluid through the filter. Into the upper part are put 20 ml. of the freshly filtered zinc uranyl acetate solution, and to this is added the sample. Sea-water samples may be delivered directly into the reagent from a pipette; with ashed samples of blood, etc., it is best to extract with 1 ml. of water which is poured into the reagent, the crucible being washed out first with two $\frac{1}{2}$ ml. portions of water and finally with three 1 ml. portions of the reagent. The important point to observe is that the volume of the reagent must be at least ten times that of the water added in the sample and washings.

The contents of the filter crucible are stirred for 2 min. with a glass rod,[1] from which the precipitate is then washed off with a further ml. of the reagent, and the crucible is covered and allowed to stand for 1 hr. at a temperature within a few degrees of that at which the reagent was filtered. The bung is then removed and the crucible fitted to an aspiration flask. After drawing off all the fluid the precipitate is washed with five 2 ml. portions of the freshly filtered alcoholic washing fluid, and then with two 5 ml. portions of pure ether. After it has been sucked as dry as possible it is left in a desiccator for 4 hr. before being reweighed. The weight of the precipitate multiplied by 0·01495 gives the weight of sodium in the sample.

The analytical results are shown in Table II.

POTASSIUM

Of the insoluble salts of potassium those that have been most used for the estimation of the metal are the cobaltinitrite and the chloroplatinate. The difficulty of obtaining reliable results with the former is notorious; with the platinum method the composition of the precipitate is more constant, but the method is less easily adapted to small quantities of potassium, and its expense is a disadvantage for its

[1] Unless the rod is perfectly clean there is a tendency for the precipitate to adhere to it. It may of course be weighed along with the crucible throughout.

Table II

No.	Nature of sample	Na present mg.	K present mg.	P present as phosphate mg.	Na found mg.	Error %
1	NaCl solution	10·44	—	—	10·47	+0·3
2	,,	10·44	—	—	10·47	+0·3
3	Synthetic sea water	10·70	0·39	—	10·71	+0·1
4	,,	10·70	0·39	—	10·69	−0·1
5	,,	10·70	0·39	—	10·67	−0·3
6	,,	10·70	0·39	—	10·66	−0·4
7	,,	10·44	0·38	—	10·41	−0·3
8	,,	10·44	0·38	—	10·42	−0·2
9	,,	10·44	0·44	0·05	10·48	+0·4
10	,,	10·44	0·44	0·05	10·41	−0·3
11	,,	10·44	0·51	0·1	10·45	+0·1
12	,,	10·44	0·51	0·1	10·43	−0·1
13	,,	10·44	0·63	0·2	10·43	−0·1
14	,,	10·44	1·38	—	10·39	−0·5

Deviation of mean −0·08 %. Probable error 0·2 %.

use in routine work. It has been known for some years, however, that the double cobaltinitrite of silver and potassium is much more insoluble than the simple salt, and Breh & Gaebler (1930), who were the first to use it for quantitative work, obtained encouraging results. It has since been employed by Robinson & Putnam (1936) and by Ismail & Harwood (1937). In our experience this method, although somewhat capricious, can with care be made to give results whose error seldom exceeds 2 %.

The precipitate, once obtained, may be estimated in several ways, titrimetric or colorimetric. We have adopted the estimation of the nitrite radical by titration with ceric sulphate as the most convenient. Most authors, when performing ceric sulphate titrations of this type, have added excess of ceric sulphate to the nitrite, then added excess of ferrous ammonium sulphate, and titrated this against more ceric sulphate added from a burette. We have followed Lindner & Kirk (1937) in cutting out one stage of this procedure by adding excess of ceric sulphate and titrating this directly with ferrous ammonium sulphate from the burette.

If the ferrous ammonium sulphate is made up in decinormal acid as directed below it keeps almost indefinitely without change of titre. It should, however, for safety be standardized against the ceric sulphate solution once a fortnight.

A large number of redox indicators are available for ceric sulphate titrations. We have tried only two, ferrous orthophenanthroline, as supplied in solution by B.D.H., and Lissamine green (also known as Erio green). The former gives a somewhat sharper colour change, but gives rise to a much larger blank. This may, however, be made to cancel out by observing the same proportion in all titrations between the volumes of indicator and of ceric sulphate solution. The choice between the two indicators is largely a matter of personal preference.

Unfortunately, it seems that even when the silver salt is used it is difficult to obtain a constant factor, whether theoretical or empirical, to express the relation

between the titre of the precipitate and its potassium content. Temperature and length of precipitation time both seem to be of importance in determining the composition of the precipitate, and if these were rigidly standardized it might be possible to obtain constant results. But it is better, both for accuracy and convenience, to run standards simultaneously with the unknowns, so that fluctuations in the composition of the precipitate, arising from chance variations in technique, may be compensated. With each batch of analyses two standards of known potassium content should also be analysed. When this is done it is of course unnecessary to know the strength in absolute units of either the ceric sulphate or of the ferrous ammonium sulphate solution.

Owing to the presence of silver ions in this method it is necessary to eliminate all chlorides from the solution before carrying out the precipitation. In the case of blood samples treated as described above this has already been done; sea-water samples must be similarly evaporated down with sulphuric acid and the excess acid cautiously driven off by heat.

Procedure. To the sample, which is in the form of solid mixed sulphates, are added 2 ml. of water and a drop of bromocresol green (0·04 % solution). If the indicator shows that any free acid is present the extract should be evaporated down again and heated more strongly; or it may be neutralized by adding drop by drop 1 % sodium hydroxide or carbonate, provided that it is free from all traces of potassium. (We have found Merck's sodium carbonate to be quite satisfactory.) The liquid is then transferred to a centrifuge tube, the crucible being washed out with three 1 ml. portions of water. 1 ml. of pure acetone is added and the contents of the tube well stirred. It is then immersed in a water bath at 65° C. for 2 min. Meanwhile, a 25 % solution of sodium cobaltinitrite has been made up; this is mixed with an equal volume of 1 % silver nitrate solution, and of the mixture (which should be filtered if any precipitate appears) 2 ml. are added with stirring to the contents of the tube, the rod being rinsed off with the last few drops. The tubes are then allowed to cool and are left overnight (i.e. for 12–18 hr.) at room temperature. Next morning they are centrifuged, the fluid siphoned off, and the precipitate washed three times with 3 ml. portions of the washing fluid, which consists of its saturated aqueous solution. A suitable siphon is made by joining with rubber tubing two glass capillaries, one of which has an upturned end. The flow is regulated by a spring clip. During the last wash the precipitate should be well agitated and suspended in the liquid by tapping the end of the tube. After the final centrifugation and siphoning 5 ml. of approximately 0·02 *N* ceric sulphate solution (12 g. "purified" ceric sulphate made up to a litre with 25–30 ml. concentrated sulphuric acid) are added to the precipitate, and the tube is immersed in a water bath at 70° C. and stirred until all the precipitate has been dissolved and oxidized. This should only take 2 or 3 min. The tube is then emptied into a 100 ml. beaker, rinsed out well with water, and the excess ceric sulphate titrated with approximately 0·02 *N* ferrous ammonium sulphate, made up by dissolving 8 g. $FeSO_4.(NH_4)_2SO_4.6H_2O$ and making up with 3 ml. of concentrated sulphuric acid to a litre. For this, as for all the titrations described in this paper, we use a pinchcock burette of 5 ml.

capacity, graduated in 0·05 ml. divisions, and delivering about 50 drops per ml. Such a burette is cheap, convenient, easy to standardize accurately, and capable of giving results whose reading error need never exceed 0·005 ml.

It is best to defer adding the indicator until the yellow colour of the ceric ions has almost disappeared. If the ferrous *o*-phenanthroline indicator is used the solution as supplied by the manufacturers should be diluted four or even eight times, and one drop added for each titration in which 5 ml. of ceric sulphate solution are used. If Lissamine green is used one drop of 0·1 % aqueous solution is required.

If x represents the titration figure given by the sample, y that given by 1 ml. of a standard potassium sulphate solution, containing 0·8914 g. K_2SO_4 (= 0·400 g. K) per litre, and z the volume of ferrous ammonium sulphate solution that is equivalent to 5 ml. of the ceric sulphate solution, then the potassium content of the sample is

$$\frac{0{\cdot}4\,(z-x)}{z-y} \text{ mg.}$$

The analytical results are given in Table III. All the samples analysed were synthetic sea water.

Table III

No.	K present mg.	K found mg.	Error %
1	0·3847	0·3795	−1·4
2	0·3847	0·3871	+0·6
3	0·3847	0·3813	−0·9
4	0·3847	0·3854	+0·2
5	0·3847	0·3786	−1·6
6	0·3847	0·3746	−2·6
7	0·3847	0·3848	0·0
8	0·3847	0·3883	+0·9
9	0·4373	0·4451	+1·8
10	0·3233	0·3242	+0·3
11	0·4944	0·4994	+1·0
12	0·6180	0·6133	−0·8

Deviation of mean −0·2 %. Probable error 0·85 %.

CALCIUM

In spite of certain disadvantages that appear to be almost inseparable from it, precipitation as oxalate remains the principle on which nearly all micro-methods for the determination of calcium are based. Bolliger (1935) has recently proposed the use of picrolonic acid as a precipitating agent, but his results seem to indicate that the difficulties likely to be encountered in applying this method to sea water would be even greater than those inherent in the oxalate method. We have not therefore investigated this reagent.

The chief obstacle to the accurate determination of calcium in solutions of the type of sea water is the ease with which magnesium is co-precipitated with the calcium. This may be overcome by dissolving the precipitate and reprecipitating it, twice if necessary. This adds, however, both to the tediousness of the method and

to the chance of losing part of the calcium, and since certain authors (Fiske & Logan, 1931; Gripenberg, 1937) have claimed to be able to effect a complete separation of calcium from large quantities of magnesium with only one precipitation, we decided to investigate these claims. After performing nearly two hundred analyses we have been led to the conclusion that a complete separation of the calcium from the magnesium in sea water cannot be brought about under any conditions by a single precipitation, and that correct results by such methods are achieved only by virtue of a compensation of errors.

Since Gripenberg's method was specifically designed for the analysis of sea water we examined it in detail. A sample of synthetic sea water was treated exactly in accordance with her description, and the precipitate and filtrate were examined spectrographically. As we expected calcium was present in the filtrate and magnesium and sodium in the precipitate. The positive and negative errors, on whose equivalence the accuracy of the method relies, are of the order of 2–3%. The method may therefore be adequate for the routine analysis of sea-water samples whose composition shows only a small range of variation, but would clearly be unsafe if used for body fluids in which much wider fluctuations in composition might be expected.

In the light of these results the method of Fiske & Logan, which was designed for the analysis of urine, was not examined, since it appears probable that it relies on a similar compensation of errors. Indeed, the high dilution at which the precipitation is carried out would seem to render it almost inevitable that significant quantities of calcium should remain behind in the filtrate.

We have therefore adopted a two-precipitation method which is essentially an adaptation to a micro-scale of the method of Kirk & Moberg (1933) with certain modifications in detail. Thompson & Wright (1930) after a careful investigation came to the conclusion that three precipitations are necessary, but as Kirk & Moberg point out this is probably because they precipitated in alkaline solution.

Occlusion of magnesium in the precipitate is by no means the only source of error to which the oxalate method is open. If the calcium oxalate is precipitated from a solution containing any considerable concentration of sodium, even as little as 2 g. per litre, a significant quantity of sodium oxalate is co-precipitated with the calcium. This important fact was noticed by Dittmar (1884), but has been ignored by later authors. A similar occlusion of sodium has been recorded when calcium is precipitated as sulphate (Plimmer & Page, 1913). A recalculation of Dittmar's analyses shows that if his crude first precipitate of calcium oxalate had been estimated volumetrically the error due to sodium would have been 2·9% and that due to magnesium 3·5%. The quantity of sodium occluded will presumably be even greater when sodium salts such as sodium acetate are used as buffers. Our spectrographic analyses show that even in Gripenberg's method, in which the sea water is diluted to five or six times its original volume before precipitation takes place, the amount of sodium in the precipitate is far from negligible.

A third possibility of error arises from incomplete precipitation of the calcium. This is most likely to take place under just those conditions which minimize errors

due to co-precipitation of sodium and magnesium, i.e. low *p*H and considerable dilution. On theoretical grounds one might expect that it would also be favoured by an increase in the total quantity of soluble salts present, and for this reason we do not use any buffer other than the ammonium oxalate that serves as a precipitating agent.

Finally, there remains to be considered the influence of the presence of strontium on the results of calcium estimations. This has already been discussed elsewhere (Webb, 1938), and it is sufficient to say that if the convention suggested there is adopted (the interpretation of "calcium" in sea-water analysis so as to mean "calcium after the strontium and barium have been replaced by calcium") no correction is necessary for results obtained by volumetric methods. If the true calcium figure is required the results must be decreased by an appropriate correction, which, in the case of normal sea water or any other solution in which the atomic ratio Ca/Sr approximates to 65, is equal to 1·5%.

It is not claimed that the method described below eliminates all these errors completely. Spectrographic examination of precipitates and filtrates shows that, even after two precipitations, some magnesium may be detected in the precipitate (although sodium is virtually absent), and that, even with an enormous excess of oxalate ions present, some calcium remains behind in solution after the first and to a less extent after the second precipitation. These errors are opposite in sign and more or less equal in magnitude, but it must be emphasized that each is only of the order of 0·5%, so that even if they failed to compensate one another the accuracy of the method within the limits aimed at would be unimpaired.

As an oxidizing agent for the volumetric estimation of the precipitated calcium oxalate potassium permanganate is generally used, but for this purpose, as for many others, it may with advantage be replaced by ceric sulphate, which is more stable, gives a sharper end-point with dilute solutions, and does not require careful control of the temperature during titration. These advantages are more than enough to compensate for the necessity of preparing an extra standard solution (ferrous ammonium sulphate).

As has already been mentioned, it was found impossible to make up the synthetic sea water in such a way that its calcium content would be accurately known without further standardization. It was estimated, therefore, by analysing separately the solutions of the various constituent salts. The calcium of the calcium chloride solution was determined gravimetrically by precipitating as oxalate and weighing as carbonate after ignition at 500° C., as described by Willard & Boldyreff (1930). The calcium in the other four salts was estimated spectrographically. It was found to be about 1% of the calcium in the calcium chloride solution.

Procedure. The sample is transferred to a centrifuge tube and made up to 4 ml. with water. To it are added two drops of bromo-cresol green, two drops of approximately twice normal hydrochloric acid, and 1 ml. of a filtered saturated solution of ammonium oxalate. It is then heated in a water bath to about 90° C. and 2% ammonia (i.e. 1 part of 0·880 ammonia diluted with 49 of water) added drop by drop till the colour has just passed from green to blue. After 10 min. in the water

bath it is left at room temperature overnight.[1] Next morning it is centrifuged, the liquid siphoned off, and the precipitate washed once with 2 % ammonia and redissolved by adding three drops of twice normal hydrochloric acid and 4 ml. of water and heating in a water bath. As soon as the precipitate is dissolved it is reprecipitated by adding as before indicator, ammonium oxalate and ammonia. After 10 min. in the water bath it is left for 4 hr. at room temperature, centrifuged, and the precipitate washed three times with 2 % ammonia. After the final washing 5 ml. of approximately 0·006*N* ceric sulphate solution (3·5 g. "purified" ceric sulphate made up with 25–30 ml. concentrated sulphuric acid to a litre) are added to the tube, the precipitate stirred up, and the tube warmed on a water bath until the precipitate is dissolved. The excess ceric sulphate is then titrated with approximately 0·006*N* ferrous ammonium sulphate (2·5 g. $FeSO_4.(NH_4)_2SO_4.6H_2O$ made up with 3 ml. concentrated sulphuric acid to a litre) as has already been described in the section on potassium. The relative strengths of the ceric sulphate and ferrous ammonium sulphate solutions are found by titrating 5 ml. of the former against the latter, and their strengths in absolute units by adding to 1 ml. of standard decinormal sodium oxalate (6·6995 g. $Na_2C_2O_4$ per litre) 20 ml. of the ceric sulphate solution, warming for a few minutes until the reaction is complete, and titrating the excess ceric sulphate, using four drops of indicator.

If the volume of ferrous ammonium sulphate solution required for the three titrations, in the order in which they are described above, are x, y and z ml. respectively, then the quantity of calcium in the sample is

$$\frac{2{\cdot}004\,(y-x)}{4y-z}\ \text{mg.}$$

Table IV

No.	Ca present mg.	Ca found mg.	Error %
1	0·4162	0·4207	+1·1
2	0·4162	0·4159	−0·1
3	0·4162	0·4171	+0·2
4	0·4162	0·4146	−0·4
5	0·4162	0·4159	−0·1
6	0·4162	0·4153	−0·2
7	0·4162	0·4183	+0·5
8	0·4162	0·4195	+0·8
9	0·4162	0·4222	+1·4
10	0·4162	0·4213	+1·2
11	0·5058	0·5056	−0·04
12	0·5058	0·5056	−0·04
13*	0·5058	0·5056	−0·04
14*	0·5058	0·5056	−0·04

Deviation of mean† +0·4 %. Probable error† 0·5 %.

* Only one precipitation. † Calculated on results for synthetic sea water only.

The analytical results are shown in Table IV. The first ten were performed on synthetic sea water, the last four on a pure calcium chloride solution made by

[1] 4 hr. is sufficient, but the time schedule given here will usually be found the most convenient.

weighing out pure calcium carbonate and dissolving it in hydrochloric acid. These latter results show that the method may be accurately standardized by analysing a standard calcium chloride solution, only one precipitation being necessary.

MAGNESIUM

The most accurate method for the estimation of magnesium is still the classical one of precipitation as magnesium ammonium phosphate. But since it is hard to find a satisfactory volumetric means of determining the precipitate the method is not very suitable for work on a micro-scale; besides, the necessity, demonstrated by Thompson & Wright (1930), of performing three precipitations for sea water, apart from the preliminary precipitations of calcium, make it extremely tedious. Another method has been available since Berg (1927, 1935) introduced the use of 8-hydroxyquinoline into analytical chemistry. This reagent possesses the property of forming insoluble compounds with many metals, including magnesium, and the precipitate may be conveniently estimated by bromination, followed by iodometric titration (Greenberg & Mackey, 1932; Greenberg *et al.* 1935). Matthews & Ellis (1928) used this reagent for the estimation of magnesium in sea water, but with indifferent results, and Thompson & Wright (1930), when comparing the phosphate and hydroxyquinoline methods, were unable to reduce the error of the latter, using the technique of Matthews & Ellis, below a minimum of 1·5%. In these early attempts, however, the calcium was first removed as oxalate before the precipitation of the magnesium, in order to prevent the co-precipitation of calcium hydroxyquinolinate. A better procedure, and one that we find gives excellent results, is to purify the magnesium hydroxyquinolate by means of a second precipitation; this serves to eliminate all the calcium from the precipitate without the risk of losing any magnesium as oxalate.

Particular attention may be drawn to three points in the procedure described below:

(1) The small amount of acid used to dissolve the first precipitate. Nicolaysen (1932) has shown that ammonium salts, if present in a concentration greater than $N/30$, have an inhibitory effect on the precipitation of magnesium. Since the acid must be neutralized by ammonia before the second precipitation, its quantity should clearly be limited to the minimum necessary to dissolve the precipitate.

(2) The great dilution at which precipitation is carried out. If the solution is too concentrated there is a risk of magnesium hydroxide being precipitated before the hydroxyquinoline salt has come down. Previous authors have overcome this by adding ammonium chloride, but this is, for the reasons just stated, undesirable. Furthermore, the greater the dilution the less calcium is precipitated.

(3) The small excess of hydroxyquinoline present at the second precipitation. If this is exceeded the results are high, owing either to adsorption of the reagent or to co-precipitation of calcium a second time.

A large number of other metals besides magnesium form insoluble salts with hydroxyquinoline, and among them are copper and zinc. Both are found in traces in sea water, but in quantities far too small to cause any appreciable error in

magnesium determinations. But in the blood of certain marine invertebrates the concentration of copper may be as high as 200 mg. per litre. If all this were precipitated with the magnesium it would give rise to an error of about 6%; and although the copper is present in organic combination as haemocyanin the ashing of the blood, which is a necessary preliminary to the estimation of magnesium, reduces the copper to inorganic form.

The investigations of Fleck & Ward (1933) and of Moyer & Remington (1938) on the effect of hydrogen-ion concentration on the precipitation of various metals by hydroxyquinoline seemed to indicate that a preliminary precipitation at *p*H 5 should remove all the copper and zinc, and that the magnesium could then be precipitated in alkaline solution after filtration. This was tried out with synthetic sea water to which had been added known amounts of copper, but the results were not very encouraging, negative errors up to 4% being found. Since Moyer & Remington present no data for copper, and Fleck & Ward confined their investigations to the precipitation of single metals from pure solutions, it is possible that co-precipitation of copper with magnesium might begin at a *p*H too low to bring about the precipitation of magnesium from pure solutions. An alternative method, by which it appears to be easier to obtain accurate results, is to estimate the mixed hydroxyquinolates precipitated from alkaline solution, which may be presumed to contain all the magnesium and copper, and then estimate the copper separately and make allowance for it. According to Berg (1935) the bromination technique is inapplicable to the determination of the copper salt of hydroxyquinoline. We have found, however, that the presence of 0·1–0·2 mg. of copper in a 1 ml. sample causes an apparent increase in the magnesium content by the theoretical amount, so that the estimation by bromination of the mixed hydroxyquinolates may be considered to be a justifiable procedure when the amount of copper present is of this order of magnitude.

This procedure assumes that copper is the only metal, other than magnesium, that is liable to be precipitated in significant quantities. The assumption is probably quite justified for the type of biological material for which these methods are intended; for although zinc may be present in tissues in considerable quantity there is no evidence that it is ever found in the blood or body fluids. Mendel & Bradley described a zinc-containing protein "haemosycotypin" from the blood of *Busycon*, but a re-examination of the blood by Gatterer & Philippi (1933) showed by spectroscopic analysis that this was erroneous, and that zinc was absent from the blood of this, as of certain other species that contain haemocyanin.

For the estimation of copper we have adopted a colorimetric method with sodium diethyldithiocarbamate, following on the whole the procedure of Hoar (1937), though the absence of iron in significant quantity from our material renders the addition of citric acid unnecessary. There is no necessity for any very refined form of colorimeter, since the degree of accuracy required for the estimation of copper is much less than that aimed at in the estimation of magnesium. In a typical haemocyanin blood, such as that of *Carcinus*, the ratio of copper to magnesium is of the order of 1 : 15; from this it may be calculated that an error of 10% in the

estimation of the copper will produce an error of only about 0·25 % in the value for magnesium.

As in the case of calcium, it was impossible to make up the synthetic sea water so as to contain a known concentration of magnesium. The standard value against which the method was tested was obtained therefore by three precipitations as magnesium ammonium phosphate, using 50 ml. samples, according to the method of Thompson & Wright (1930).

Procedure for the estimation of magnesium. The sample is transferred to a 100 ml. beaker and diluted to 50 ml. with water. It is then heated to about 85° C. on a water bath and 5 ml. of a 1 % solution of 8-hydroxyquinoline in 70 % alcohol are added, followed by five drops of 0·880 ammonia diluted with an equal volume of water. After 20 min. on the water bath it is cooled and filtered through no. 40 Whatman paper or through a small sintered glass Buchner funnel. Both beaker and filter are washed with 2 % ammonia. The precipitate is dissolved with successive portions of *N*/20 hydrochloric acid, added 1 or 2 ml. at a time, and not exceeding 20 ml. in all. The liquid is collected in the original beaker, made up to 50 ml. with water, and reprecipitated by heating on the water bath as before and adding 0·4 ml. of hydroxyquinoline solution followed by eight drops of 50 % ammonia. It is once more left on the water bath for 20 min. and then cooled and filtered. The beaker and filter are well washed with 2 % ammonia, and the precipitate is then dissolved in 10 ml. of hot normal hydrochloric acid, the liquid being again collected in the original beaker. The filter is washed out with water into the beaker, and the contents of the latter transferred to a conical flask of about 250 ml. capacity, fitted with a ground-glass stopper. A further 40 ml. of normal hydrochloric acid are added, and sufficient water to bring the volume to about 100 ml. 10 ml. of a standard decinormal bromate-bromide solution are added from a pipette, and the flask immediately stoppered, gently shaken, and allowed to stand for at least 2 min. This solution contains 2·7836 g. $KBrO_3$ and about 11 g. KBr per litre. In acid solution the bromate and bromide react to liberate bromine, some of which combines with the hydroxyquinoline. 2 ml. of 20 % potassium iodide solution, or 0·5 g. of the solid salt, are added, and the flask stoppered again and well shaken. The iodine liberated from the potassium iodide by such of the free bromine as had not combined with the hydroxyquinoline is titrated with approximately decinormal sodium thiosulphate, using starch as indicator. The thiosulphate solution is standardized by titrating against 10 ml. of the bromate-bromide solution, to which have been added 50 ml. of normal hydrochloric acid, followed after 2 min. by excess of potassium iodide. The flask is kept stoppered, as described above, until all the iodine has been liberated, in order to prevent the escape of bromine vapour.

1 ml. of decinormal bromate solution is in this method equivalent to 0·304 mg. magnesium. If, therefore, x is the titration figure for a sample, and y the titration figure for 10 ml. of bromate-bromide solution alone, then the apparent magnesium content of the sample is

$$\frac{3{\cdot}04\,(y-x)}{y} \text{ mg.}$$

But this requires correction if copper is present in significant quantities. If the copper content of the sample is z mg. the correction amounts to

$$\frac{24{\cdot}32}{63{\cdot}57}\,z,$$

and hence the true magnesium content is

$$\frac{3{\cdot}04\,(y-x)}{y} - 0{\cdot}38\,z \text{ mg.}$$

Procedure for the estimation of copper. The sample is ashed, as for the estimation of the other metals, and the ash extracted with water and made up to a volume of about 20 ml. To it are added in the following order 5 ml. of a 1 % solution of gum arabic or gum tragacanth, 7 ml. of 50 % ammonia, 5 ml. of a 0·2 % solution of sodium diethyldithiocarbamate, and sufficient water to bring the volume to 100 ml. The intensity of the brownish yellow colour is compared with that of a suitable standard made up from a copper sulphate solution containing 0·3928 mg. $CuSO_4.5H_2O$ (= 100 mg. Cu) per litre. Beer's law is well obeyed over a considerable range of dilution, but it is best for the strength of the standard to be such that its copper content is between 50 and 200 % of that of the sample.

The analytical results of the application of the method to synthetic sea water are shown in Table V. It will be seen that there is a general tendency for the results to be high, and possibly a more accurate result might be obtained if a routine correction of −0·5 % was applied.

Table V

No.	Mg present mg.	Cu present mg.	Mg found* mg.	Error %
1	1·305	—	1·315	+0·8
2	1·305	—	1·312	+0·5
3	1·305	—	1·321	+1·2
4	1·305	—	1·312	+0·5
5	1·305	—	1·321	+1·2
6	1·311	—	1·320	+0·7
7	1·311	—	1·317	+0·5
8	1·311	—	1·301	−0·8
9	1·311	—	1·323	+0·9
10	1·311	—	1·314	+0·2
11	1·311	—	1·322	+0·8
12	1·311	0·1	1·309	−0·2
13	1·311	0·1	1·309	−0·2
14	1·311	0·2	1·332	+1·6
15	1·311	0·2	1·333	+1·7

Deviation of mean +0·65 %. Probable error 0·6 % (0·45 % if all results are scaled down by 0·5 %).

* Corrected for Cu where necessary.

CHLORIDE

For the determination of chlorides in sea water Mohr's method of direct titration with silver nitrate, using potassium chromate as indicator, has long been classical, and does not need to be improved upon. It may easily be performed on 1 ml. samples with an error not exceeding 0·5 %.

This method cannot, however, be used directly for solutions that contain even a small amount of protein. Since dry ashing of organic matter cannot be accomplished without some loss of chloride, no matter how carefully the temperature is controlled (Baerts & Vandewijer, 1937), it has been customary either to precipitate the proteins or else to destroy them by digestion with nitric acid in the presence of excess silver nitrate, the excess silver being estimated by a Volhard titration. Recently Sendroy (1937) has introduced a method which is both quicker and more sensitive than either of these procedures, and which gave in his hands very accurate results. We have tried out this method on sea water and on the body fluids of *Echinus* and *Carcinus*, and have obtained satisfactory results. We have therefore adopted it, with a few minor modifications, as our standard method for the analysis of body fluids.

It consists essentially in the addition of solid silver iodate to the solution to be analysed; this salt, though almost insoluble, is more soluble than silver chloride, so that silver chloride is precipitated and iodate ions pass into solution. When the reaction is complete the solution is filtered and the iodate estimated iodometrically in an aliquot of the filtrate. Since silver is never present in solution except in minute concentrations deproteinization is unnecessary. The reaction is represented by the equation

$$AgIO_3 + NaCl = AgCl + NaIO_3.$$

This implies that for every chloride ion originally present one iodate ion is released. Owing to the slight solubility of both silver iodate and silver chloride this condition is seldom exactly attained, and it is necessary to correct the results both for dissolved silver iodate and for incomplete precipitation of silver chloride. These corrections, both of which vary with the initial concentration of chloride, tend in opposite directions, so that the resultant correction may be either positive or negative, but in samples containing over 7·5 mg. of chloride it is always less than 0·5 %. The formula for calculating the corrected value is given below; for a discussion of the theoretical aspects of the equilibrium, from which the formula is derived, reference may be made to Sendroy's paper.

In volumetric determinations on human plasma Sendroy found that his results were unaccountably low by an error of slightly over 1 %; this he assumed to be due to the presence of some substance which reduced the iodate, and he compensated for it by an empirical correction. With the blood of *Carcinus* this phenomenon does not seem to occur; we have found no tendency for results obtained by Sendroy's method to be constantly lower than those obtained by open Carius digestion and Volhard titration, nor did a sample of blood to which potassium iodate had been added liberate iodine or iodides on standing for several hours. These analyses of *Carcinus* blood are given in Table VI.

Procedure. The silver iodate is prepared by adding to a solution of silver nitrate a very slight excess of potassium iodate, filtering through a Buchner funnel, and washing the precipitated silver iodate ten or more times with distilled water. The washings are collected at intervals and tested by the addition of hydrochloric acid

and potassium iodide. The yellow colour of the liberated iodine should diminish in intensity in the successive washings until it settles down to a constant intensity which is determined by the solubility of silver iodate. When this occurs washing is complete and the silver iodate is dried at air temperature and stored in the dark.

Table VI

Sample no.	Method	Chloride found mg.	Deviation from mean of other methods
1	1	17·22	—
1	1	17·20	—
1	2	17·26	—
1	2	17·24	—
1	3	17·30	+0·4%
1	4	17·36	+0·8%
2	1	15·59	—
2	3	15·49	−0·6%

Key to numbering of methods: 1, Open Carius digestion and Volhard titration. 2, Mohr titration after deproteinizing by Somogyi's (1930) method, the protein being washed free of chlorides. 3, Sendroy's method. 4, Sendroy's method performed on the filtrate after Somogyi deproteinization.

The sample is measured directly into a 100 ml. standard flask, and diluted with about 60 ml. of water. 5 ml. of a solution of phosphoric acid consisting of 230 ml. of the syrupy acid diluted to a litre (about 3·4 *M*) are added, followed by about 15–20 mg. of silver iodate for each mg. of chloride expected in the sample. The flask is corked and shaken vigorously for 3 min. At the end of this time the solid at the bottom is inspected to see that a fair excess of silver iodate is still present; it may be easily distinguished from the chloride by its greater speed in settling to the bottom and by its failure to turn mauve on exposure to light. If none is visible some more must be added and the shaking repeated. At least 1 g. may be added before the error due to the volume it occupies becomes at all significant. If protein is present the foam is removed by the addition of a minute drop of caprylic alcohol. The flask is then made up to the mark with water and well mixed, and the liquid filtered through no. 1 Whatman paper into a clean dry vessel. If protein is present in considerable quantity the silver salts do not flocculate so well and it may be necessary to use a double thickness of paper, or a more retentive grade such as no. 3, but ashless papers, which nearly always contain some chloride, are best avoided.

20 ml. aliquots of the filtrate are taken for titration. To each is added sufficient potassium iodide to dissolve the iodine that it liberates, and the iodine is titrated with approximately decinormal sodium thiosulphate. If protein is present it is precipitated by the iodine; this renders the approach of the end-point less easy to predict, but otherwise does not seem to interfere.

The sodium thiosulphate may be standardized by titration against 5 ml. of a standard decinormal potassium iodate solution, containing 3·5670 g. KIO_3 per litre, after adding 1 ml. of the phosphoric acid solution and excess of potassium iodide. In our experience, however, the bromate-bromide solution described in the section

on the estimation of magnesium forms a more permanent and reliable standard for iodometry.

Each iodate ion liberates six equivalents of iodine, according to the equation

$$HIO_3 + 5HI = 3I_2 + 3H_2O.$$

If therefore x be the volume in ml. of thiosulphate solution used in the titration and n its normality, the quantity of iodate in the flask must have been $\frac{1}{6}xn$. This would also represent the original amount of chloride present in the sample were it not for the corrections made necessary by the solubility of silver chloride and iodate. Using the data furnished by Sendroy the following formula is obtained to express the chloride content of the sample in mg.:

$$Cl = 35{\cdot}457\left(0{\cdot}8369\,nx - \frac{0{\cdot}000033}{nx}\right).$$

For all values of x above 6 (assuming $n = 0{\cdot}1$) the second term inside the bracket becomes so small as to be negligible. Under these conditions, which cover the analysis of 1 ml. samples with a chlorinity of 18‰ and over, the formula may be taken as

$$Cl = 29{\cdot}67\,nx \text{ mg.}$$

The method was tested on natural sea water whose chlorinity had been determined by direct titration with silver nitrate. The results are shown in Table VII.

Table VII

Cl present mg.	Cl found mg.	Error %
21·63	21·50	−0·6
21·63	21·76	+0·6
21·63	21·77	+0·6
21·63	21·70	+0·3
18·68	18·65	−0·2
18·68	18·81	+0·7
18·57	18·68	+0·6
18·57	18·66	+0·5
18·57	18·65	+0·4

Deviation of mean +0·3 %. Probable error 0·35 %.

SULPHATE

A satisfactory method for the micro-determination of sulphates still awaits discovery. Unless a micro-balance is used gravimetric determination as barium sulphate can scarcely be applied to quantities of less than 25 mg. of sulphate without the risk of an appreciable error arising from weighing operations alone; to provide this quantity about 10 ml. of sea water would be required. In clinical work precipitation as benzidine sulphate is largely employed, but the difficulty of obtaining accurate results in the presence of high chloride concentrations is notorious. Various methods have been proposed by which chromate ions are brought into solution by the decomposition of barium chromate to form barium

sulphate, and are then estimated iodometrically, but Manov & Kirk (1937) have found that even the best of these methods depend for their success on the compensation of relatively large errors, which can be eliminated only by taking rather elaborate precautions such as using ammonia specially purified to free it from carbon dioxide, and performing the titration in an atmosphere of nitrogen, etc. Van Slyke *et al.* (1927) proposed a barium iodate method analogous to Sendroy's silver iodate method for chlorides, but an hour's vigorous shaking is required to bring the reaction to completion, and even then the results are not stoichiometric but require correction by means of an empirically constructed correction curve.

The method here proposed is of an order of accuracy somewhat inferior to the methods for the other radicals, and a little practice is required for judging the end-point, but it has the merit of simplicity and speed, and the errors are probably no greater than would be given by any other existing method if applied to the same material.

It has been known for some years that rhodizonic acid reacts with barium ions to form a brilliant red compound, barium rhodizonate, and that this colour is discharged when sufficient sulphate is added to precipitate the barium as sulphate. Several attempts have been made to use this as the basis of a direct titration method for barium or sulphate, but at first they met with little success except in relatively concentrated solutions. Recently, however, it has been shown by Mutschin & Pollak (1937) that much more satisfactory results may be obtained by adding excess of barium chloride and back-titrating with sodium sulphate. They also showed that a high chloride concentration hastens the precipitation of barium sulphate. Abrahamczik & Blümel (1937) have found that by the use of a buffered solution it is possible to bring the *p*H to a value at which the red barium rhodizonate is stable while the yellow colour of the free indicator is destroyed almost as soon as it is liberated, thus rendering the end-point easier to observe. The method as here presented incorporates features from both these papers as well as certain original ones.

We have found that glassware cleaned with concentrated sulphuric acid needs to be washed with much more than ordinary care if it is to be rendered completely free of sulphate. It should be washed with soap and warm water and then repeatedly rinsed with distilled water.

Procedure. The indicator, sodium rhodizonate, is ground up in a mortar with about 250 times its weight of pure (sulphate free) sodium chloride and stored in this form, since it is stable for only a few hours in solution. The sodium chloride assists in the precipitation of barium sulphate, and by conferring bulk on the indicator makes it easier to handle in minute quantities.

Blood samples are deproteinized by the addition of mercuric chloride as already described in the introductory section. The volume of the sample is brought by evaporation or dilution to about 10 ml., and it is transferred to a Pyrex boiling tube of 50 ml. capacity, greased at the rim on the outside. In a similar tube 0·5 g. of the indicator-salt mixture (enough to produce a deep orange colour) is dissolved in 5 ml. of water, and when it has completely dissolved 5 ml. of alcohol are added.

Previous authors have used acetone or methyl alcohol; we find ethyl alcohol quite satisfactory, and certainly preferable to acetone. Next there is run in from a burette a measured quantity of *M*/100 barium chloride solution (2·4431 g. $BaCl_2.2H_2O$ per litre) such that it exceeds by 5–35 % (preferably by about 10 %) the equivalent of the sulphate expected in the sample. For 1 ml. of normal sea water this quantity will be about 3 ml. A deep cherry pink colour with a slight yellow fluorescence is developed, which often becomes more intense on allowing the solution to stand for 5 min. If at the end of this period the colour is at all inclined towards orange or brick red either the reagents have been added in the wrong order, or else there is not enough alcohol present. In any case it is useless to attempt the titration until the proper colour has been attained.

To the same tube there are next added 2·5–3 ml. of the buffer solution. This is made by adding 5 ml. of concentrated hydrochloric acid to a litre of water and saturating this solution with potassium hydrogen phthalate. Its *p*H is about 2·4, and when added in the quantity stated brings the *p*H of the solution during titration to about 3. This figure was established by Abrahamczik & Blümel as the optimum, but their own buffer solution (citrate), whose initial *p*H is 3, was diluted four times in making up with barium chloride into the final solution, and then further in the course of titration, so that the final *p*H must have been well above 3. At any rate we found that their directions, when applied to the quantities of barium chloride used by us, did not produce a sufficiently rapid fading of the yellow colour of the rhodizonate ions.

The barium solution, after the buffer has been added, is poured into the tube containing the sample, and the solutions are well mixed, now and throughout the titration, by pouring from one tube to another. Since there is an excess of barium over sulphate the solution remains pink. The titration is carried out by running in from a burette *M*/100 sodium sulphate solution (1·4205 g. anhydrous Na_2SO_4 per litre) until all traces of pink have disappeared and the solution remains pale yellow or dirty white. Near the end-point a minute should be allowed to elapse between the last addition of sodium sulphate and the final adjudication of the colour. The use of two boiling tubes for the titration sounds extremely clumsy, but it enables the approach of the end-point to be predicted, and its value determined, more easily than would otherwise be possible, since the solution may be divided into two sub-equal portions, one in each tube, and the colours compared after adding one or more drops of the sodium sulphate solution to one of them. With a little practice the end-point error can always be kept below 1 %.

If x ml. of barium chloride solution have been added, and y ml. of sodium sulphate solution are required in the titration, then the amount of sulphate in the sample is

$$0{\cdot}9606\,(x-y)\text{ mg.}$$

The analytical results are shown in Table VIII. Nos. 1–6 were performed on natural sea water whose sulphate content was calculated from the chlorinity, assuming $SO_4/Cl = 0{\cdot}1396$. Nos. 7–10 were performed on synthetic sea water.

Table VIII

No.	SO_4 present mg.	$BaCl_2$ added ml.	Excess of $BaCl_2$ over SO_4 (%)	SO_4 found mg.	Error %
1	3·012	3·49	11	3·014	+0·1
2	3·012	3·50	11	3·034	+0·7
3	3·012	3·71	18	3·027	+0·5
4	3·012	3·89	24	2·958	−1·8
5	3·012	3·25	4	3·003	−0·3
6	3·012	3·52	12	2·986	−0·9
7	3·044	3·35	8	2·976	−2·2
8	2·305	3·175	33	2·354	+2·1
9	2·679	3·13	11	2·712	+1·2
10	2·503	3·205	24	2·486	−0·7

Deviation of mean −0·15 %. Probable error 0·9 %.

SUMMARY

Methods are presented for the estimation of sodium, potassium, calcium, magnesium, chloride and sulphate in sea water and in other solutions, such as the blood and body fluids of marine animals, whose inorganic composition is similar to that of sea water.

The estimations may be performed on 1 ml. samples, and the limit of error is about 2 %.

Sodium is precipitated and weighed as sodium zinc uranyl acetate; potassium is precipitated as potassium silver cobaltinitrite which is titrated with ceric sulphate; calcium is titrated with ceric sulphate after two precipitations as oxalate; magnesium is precipitated with hydroxyquinoline and the precipitate brominated and estimated iodometrically; chloride is treated with silver iodate and the released iodate estimated iodometrically; sulphate is titrated with barium chloride using sodium rhodizonate as indicator.

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