Notes

DIRECT COLORIMETRIC DETERMINATION OF TRACE AMOUNTS OF CHLORIDE

(*Presented at the meeting of the Society on Wednesday, April 6th*, 1960)

RECENT work on fluorescein showed that there were solubility and spectral-absorption minima at the isoelectric point.[1] The dye or one of its derivatives might therefore have a strong affinity for some nascent precipitates, with the likelihood of a measurable change in colour. This possibility was realised with dibromofluorescein at pH 3·4 in the presence of silver chloride below the turbidimetric level.

The procedure used was—

> To 6 ml of sample solution add 0·4 ml of a 0·01 per cent. solution of dibromofluorescein in 0·001 *M* alkali, mix, add 0·4 ml of 1 per cent. v/v acetic acid, and mix again. Successively add 1, 2 and 4 drops of a 0·34 per cent. solution of silver nitrate, mixing after each addition. Measure the colour of the solution at 550 mμ or with use of a green filter at 30-second or 1-minute intervals from the addition of dibromofluorescein. Rinse glassware with dilute ammonia after every second determination.

The calibration curve does not rise above the base-line until 0·4 p.p.m. of chloride ion is present; this corresponds to the calculated solubility of silver chloride under these conditions. To provide sensitivity near the origin, 3·5 μg of chloride ion can be added to every sample. The initial sensitivity is then about the same as that for the mercuric thiocyanate method,[2] but it decreases as dye is used up at high concentrations of chloride; the range is from 0·0 to 5 p.p.m. of chloride. When an E.E.L. colorimeter (Evans Electroselenium Ltd.) was used, a series of ten rapid determinations on a solution containing 1 p.p.m. of chloride gave a value of $1 \pm 0{\cdot}05$ p.p.m. The method was developed for air samples and is applicable only to neutral unbuffered solutions containing no colloidal material or ions (other than chloride) forming insoluble silver salts at the working pH; the presence of up to 0·2 per cent. of sulphate or nitrate is tolerable. Samples from bubblers through which London air has been passed must be boiled to expel sulphur dioxide before the chloride is determined.

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DETERMINATION OF ASCORBIC ACID IN HIGHLY COLOURED SOLUTIONS WITH N-BROMOSUCCINIMIDE

THE presence of pigments, especially those absorbing light at about the same wavelength as does 2:6-dichlorophenolindophenol, may interfere with the determination of ascorbic acid when this dye is used, so that direct measurement of the decolorisation of the dye, either visually[1] or spectrophotometrically,[2] is impossible. Ascorbic acid can be determined in pigmented material by using 2:6-dichlorophenolindophenol in an electrometric procedure,[3] but specialised apparatus is required. Another modification is the use of an organic solvent to trap the excess of dye during titration and hence to indicate the end-point,[4] but, if mixing is inadequate, dye may enter the organic phase without having reacted with ascorbic acid in the aqueous phase and so give falsely low results.

Oxidation of ascorbic acid by N-bromosuccinimide is a satisfactory method for assaying ascorbic acid, and the reagent, unlike 2:6-dichlorophenolindophenol, is unaffected by reductones, reductic acid and iron salts.[5] Excess of N-bromosuccinimide is detected by the liberation of iodine from potassium iodide in acid solution and formation of the blue colour with starch, but pigments also interfere with this end-point. With pigmented material, the liberated iodine can conveniently be detected by an organic solvent when the ascorbic acid present has been preferentially oxidised. This modification was tested by using blackcurrant juice, as this material is

intensely pigmented and was found to have an absorption maximum at about 520 mμ. Measurements at this wavelength are made in the spectrophotometric method[2] and also in the phenylhydrazone method for assaying dehydroascorbic acid, the primary oxidation product of sacorbic acid.[6]

Method

Reagents—

N-*Bromosuccinimide solution*—Prepare a stock solution by dissolving 200 mg of the reagent in warm water, cooling, and diluting to 100 ml; this solution is stable for a few days at 4° C. Just before use, dilute the stock solution (1 + 9) with water; 1 ml of this solution is approximately equivalent to 0·2 mg of ascorbic acid.

Acetic acid, glacial—Analytical-reagent grade.

Potassium iodide solution, 4 per cent. w/v, aqueous—Prepare from iodate-free potassium iodide.

Diethyl ether—Peroxide-free.

Standard ascorbic acid solution, 0·2 mg per ml—Freshly prepare this solution in 1 per cent. v/v acetic acid.

Procedure—

For solutions containing red pigments, *e.g.*, blackcurrant juice, dilute the sample with 1 per cent. v/v aqueous acetic acid until it contains from 0·4 to 1·0 mg of ascorbic acid per 5 ml of solution. Transfer 5 ml of the diluted sample to a 6-inch × 1-inch test-tube, add 1 ml of glacial acetic acid, mix, add 5 ml of potassium iodide solution, and mix again. Add 3 ml of diethyl ether, and titrate the mixture with N-bromosuccinimide solution added from a 10-ml semi-micro burette. Vigorously shake the test-tube after each addition of titrant, and allow the organic layer to separate. The end-point is indicated by the first appearance of the brown colour of liberated iodine in the upper ether layer; comparison against an untitrated "dummy" mixture permits easy establishment of the end-point. Carry out a blank titration, in which 5 ml of water *plus* a volume of water equivalent to the titre replace the diluted sample, to determine the volume of N-bromosuccinimide solution necessary to impart a definite brown colour to the ether layer. Standardise the N-bromosuccinimide solution by using the same procedure, but titrating against 5-ml aliquots of standard ascorbic acid solution.

Discussion of the method

The results in Table I show that the recovery of ascorbic acid added to diluted blackcurrant juice is satisfactory within the limits of experimental error.

Table I

Recovery of ascorbic acid added to diluted blackcurrant juice

Sample	Ascorbic acid added, mg	Ascorbic acid found, mg	Recovery, %
Blackcurrant juice, commercial concentrate No. 1 ..	0·100	0·097, 0·103 (mean 0·100)	100
	0·199	0·195, 0·210 (mean 0·203)	102
	0·398	0·399, 0·399 (mean 0·399)	100
Blackcurrant juice, commercial concentrate No. 2 ..	0·102	0·101, 0·105 (mean 0·103)	101
	0·204	0·204, 0·212 (mean 0·208)	102
	0·408	0·419, 0·419 (mean 0·419)	103

Ether was used when red-coloured solutions were analysed, as the yellow-brown colour in the organic layer contrasted well with the red colour in the aqueous layer. Iodine in carbon tetrachloride has a colour similar to that of diluted blackcurrant juice, so that there is no colour contrast between the layers when this solvent is used; agreement between duplicate titrations was less good in presence of carbon tetrachloride than in presence of ether. Evaporation of ether at normal laboratory temperature was no problem, as each titration was of short duration.

The use of carbon tetrachloride or chloroform as organic phase seems to be indicated when yellow or brown solutions are analysed, as low concentrations of iodine give a pink colour in both these solvents. A few experiments with dilutions of concentrated orange juice revealed that the original method, in which starch was used as indicator,[5] was satisfactory, since the colour did not interfere when the sample was suitably diluted.

I thank Mr. M. W. Weg, who suggested to me the use of carbon tetrachloride as indicator in the N-bromosuccinimide method and so instigated this investigation.

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THE ULTRA-VIOLET SPECTROPHOTOMETRIC DETERMINATION OF 2-AMINO-2-DEOXYHEXOSES

The reaction of sulphuric acid with carbohydrates was the basis for the ultra-violet spectrophotometric determination of sugars and uronic acids described by Bath.[1] The work described here suggests that this method is also applicable to the determination of aminosugars. For these experiments, the two most extensively occurring aminosugars, 2-amino-2-deoxyglucose and 2-amino-2-deoxygalactose, were chosen as representative of this class of carbohydrates.

By carrying out Bath's procedure on 1-ml portions of the aqueous hexosamine solutions and 7-ml portions of 96 per cent. sulphuric acid, we obtained characteristic spectra (see Fig. 1) after

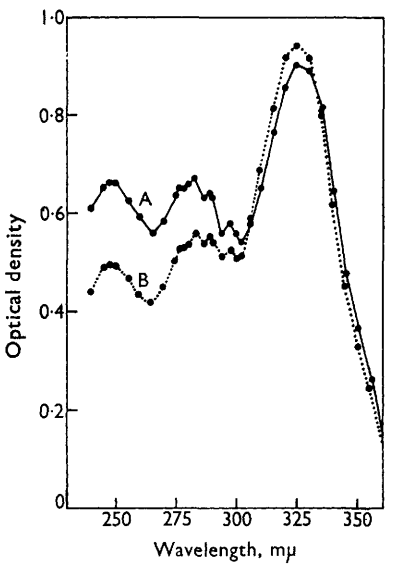

Fig. 1. Absorption spectra of reaction products formed by separately heating 200-μg amounts of each aminosugar with 7·66 ml of 89·1 per cent. sulphuric acid for 10 minutes in a boiling-water bath : curve A, 2-amino-2-deoxyglucose; curve B, 2-amino-2-deoxygalactose

heating for 10 minutes. Both aminosugars produced similar spectral patterns, which differed only in the relative intensities of individual peaks. They exhibited absorption maxima at 248 and 325 mμ, as expected,[2] and a group of closely spaced but clearly defined peaks at 283, 289 and 298 mμ. This group of peaks is not exhibited by any other class of sugars so far reported. A

discernible peak also appeared in each spectrum at approximately 278 mμ, but as it was ill-defined it was not measured. The extinction coefficients of these five peaks for both aminosugars, calculated from the equation—

$$E^{1\%}_{1\,cm} = \frac{a\lambda \times 10^4}{\text{Concentration of aminosugar, } \mu g \text{ per ml}},$$

are shown below.

Wavelength, mμ	248	283	289	298	325
Extinction coefficient for 2-amino-2-deoxyglucose	260	255	245	230	350
Extinction coefficient for 2-amino-2-deoxygalactose	190	215	210	200	360

The values of the extinction coefficients did not change throughout the observed range of concentrations (6·5 to 65 μg of aminosugar per ml of sulphuric acid solution), *i.e.* solutions of both compounds obeyed Beer's law. The coefficient of variation of the optical-density measurements at 325 and 283 mμ for a series of samples (see Table I) did not exceed 2·62 per cent.

TABLE I

COEFFICIENTS OF VARIATION OF OPTICAL-DENSITY MEASUREMENTS

Amount of aminosugar present, μg	Coefficient of variation of optical-density measurements for—			
	2-amino-2-deoxyglucose at—		2-amino-2-deoxygalactose at—	
	283 mμ, %	325 mμ, %	283 mμ, %	325 mμ, %
50	2·45	2·25	2·62	1·36
100	1·10	1·04	1·34	1·35
200	0·73	0·53	0·53	0·44

A study of the effect of time of heating on the spectra of the aminosugars revealed an interesting pattern of changes in the intensities of the individual absorption maxima. The group of peaks between 275 and 300 mμ behaved differently from those at 248 and 325 mμ (see Figs. 2 and 3). This indicates that the peaks between 275 and 300 mμ are produced by a reaction different from that producing those at 248 and 325 mμ.

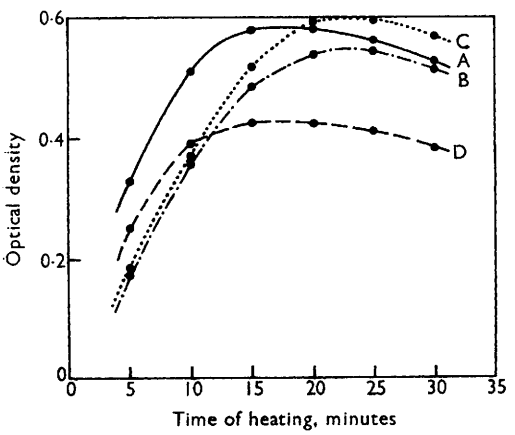

Fig. 2. Change in optical density of a solution of 2-amino-2-deoxyglucose in 89·1 per cent. sulphuric acid with time of heating at 100° C: curve A, 325 mμ; curve B, 289 mμ; curve C, 283 mμ; curve D, 247 mμ

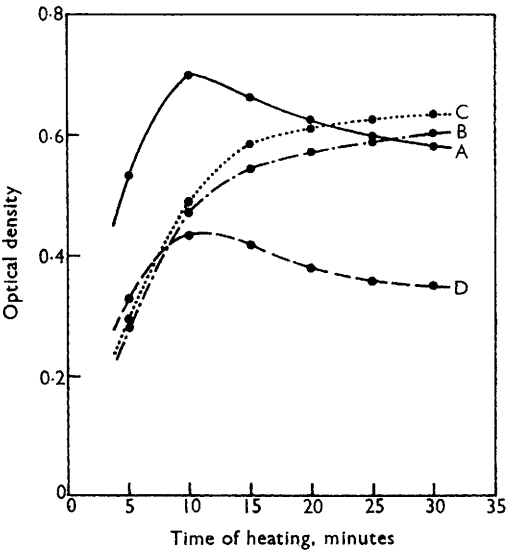

Fig. 3. Change in optical density of a solution of 2-amino-2-deoxygalactose in 89·1 per cent. sulphuric acid with time of heating at 100° C: curve A, 325 mμ; curve B, 289 mμ; curve C, 283 mμ; curve D, 247 mμ

In conclusion, it is noteworthy that, although there were no great differences between the two aminosugars in the ultra-violet region, they showed considerably different behaviour in the visible region. When a larger amount of 2-amino-2-deoxyglucose was heated with sulphuric acid, a canary-yellow colour was formed, whereas a solution of 2-amino-2-deoxygalactose in sulphuric acid showed no apparent colour, even after being heated for 1 hour in the boiling-water bath.

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THE DETERMINATION OF CALCIUM AND MAGNESIUM IN RAT LIVER

During an investigation of the variation in calcium and magnesium in certain pathological conditions of rat liver, the need became apparent for a method of determining these ions in the presence of excessively large amounts of phosphate. Griswold and Pace's method,[1] in which an ion-exchange column was used to separate the metal ions from each other and from phosphate, was satisfactory, but it was too time-consuming when many samples had to be analysed. Other recent methods involve use of ethylenediaminetetra-acetic acid (EDTA) and two indicators. One indicator is usually specific for calcium, *e.g.*, murexide or calcein; the other, *e.g.*, Eriochrome black T, is used to determine the total calcium *plus* magnesium, and the magnesium is found by difference. For systems containing large amounts of phosphate and small amounts of calcium and magnesium, such methods are suitable only when the phosphate can be satisfactorily removed.

Our procedure is based on the method described by Horner,[2] in which the phosphate was removed by precipitation with sodium tungstate and morpholine nitrate. Certain modifications were necessary, and the proposed method permits calcium and magnesium to be conveniently determined with a maximum error of approximately ± 5 per cent.

Method

Procedure—

Heat 3 to 5 g of the sample of liver in a platinum dish at 700° to 800° C overnight. When cool, add 1·0 ml of 0·5 *N* nitric acid to the ash. Set aside for some time, thoroughly wash the sides of the dish with the solution (use a fine Pasteur pipette), and transfer the solution to a 15-ml centrifuge tube with the same pipette. Repeat this procedure with a further 1·0-ml portion of 0·5 *N* nitric acid and two 1·0-ml portions of distilled water.

To the solution in the centrifuge tube add 2 ml of a 66 per cent. w/v solution of sodium tungstate ($Na_2WO_4.2H_2O$) and then 4 ml of a 28 per cent. solution of morpholine nitrate.[2] Stir the mixture with a thin glass rod, set aside for 1 hour, and spin in a centrifuge at 3000 r.p.m. and 14-cm radius for 15 minutes. (Although only analytical-reagent grade chemicals were used, significant errors due to reagents were encountered; a reagent blank solution was therefore prepared by substituting 4·0 ml of 0·25 *N* nitric acid for the sample solution.)

Titration of calcium—After centrifugation, titrate 1·0 ml of the supernatant liquid against 4 *N* sodium hydroxide, with a solution of alizarin yellow G as indicator, in order to determine the amount of 4 *N* sodium hydroxide required to bring the pH to 12. Place 1·0 ml (see Note) of the supernatant liquid in a 1-cm cuvette of a Spekker absorptiometer, and adjust the pH to 12 by adding the previously determined amount of 4 *N* sodium hydroxide. To the contents of the cuvette add 1·0 ml of a solution containing 50 μg of calcium per ml, 0·8 ml of murexide indicator solution (0·045 g per 250 ml), 0·2 ml of 4 *N* sodium hydroxide and sufficient distilled water to bring the total volume to 8·0 ml. Titrate the calcium with 0·01 *M* EDTA (previously standardised against a solution containing 100 μg of calcium per ml) added from an Agla micrometer-syringe burette as described by Dunstone,[3] and treat 1·0 ml of the supernatant liquid from the blank solution in similar fashion. Titrations were reproducible to within 0·001 ml (equivalent to 0·4 μg of calcium).

Titration of calcium plus *magnesium*—Dilute 1·0 ml of the supernatant liquid with distilled water to 10·0 ml. To a suitable aliquot of this solution add 10 ml of a solution prepared by dissolving 5 mg of Eriochrome black T in 250 ml of buffer solution of pH 10 to 10·5. (Prepare the buffer solution by mixing 57 ml of concentrated ammonium hydroxide, 6·8 g of ammonium chloride, 6·6 g of potassium cyanide and 0·2 g of hydroxylamine hydrochloride and then diluting to 1 litre with distilled water; the cyanide minimises interference from iron and copper.[1]) Titrate this solution with 0·01 *M* EDTA (previously standardised against a solution containing 100 μg of magnesium per ml) added from an Agla micrometer-syringe burette to the blue end-point. (The end-point was more easily detected when viewed in light from a tungsten lamp.) Treat 1·0 ml of the supernatant liquid from the blank solution in similar fashion. Titrations were reproducible to within 0·002 ml (equivalent to 0·3 μg of magnesium).

NOTE—The use of larger aliquots of supernatant liquid was unsatisfactory, as the higher concentration of sodium nitrate in the solution caused a decrease in the magnitude of absorption increments near the end-point and so made determination of the end-point less accurate. The addition of extra calcium was thought to be advisable, owing to the small amounts usually present in liver from normal animals.

When the amount of calcium present, as determined by a preliminary titration, is much greater than usual, suitably dilute the supernatant liquid, and treat a 1-ml portion of the diluted solution as described above. The blank solution should be similarly treated.

CORRECTION FOR VOLUME OF PHOSPHATE PRECIPITATE—

Table I shows typical results of experiments carried out to determine the calcium and magnesium contents of pure solutions. The amount of calcium or magnesium found was always about

TABLE I

EFFECT OF MORPHOLINE NITRATE AND SODIUM TUNGSTATE ON RECOVERY OF CALCIUM AND MAGNESIUM FROM PURE SOLUTIONS

Element	Amount of element found— in absence of morpholine nitrate and sodium tungstate, μM per 4 ml	Amount of element found— in presence of morpholine nitrate and sodium tungstate, μM per 4 ml	Deviation from expected result, %	Mean deviation, %
Calcium	8·1	8·3	+2·5	+3·1
	20·3	21·0	+3·4	
	40·6	41·9	+3·2	
	60·8	62·8	+3·3	
Magnesium	19	20	+5·2	+2·5
	56	56·5	+0·9	
	93	95	+2·1	
	187	190	+1·6	

3 per cent. greater than that expected, and this is attributed to the volume of the precipitate formed in the removal of phosphate. It is therefore recommended that the results of the determinations be multiplied by a factor of 0·97 to compensate for this error.

RESULTS

Table II shows typical results found when the proposed procedure was used to determine calcium and magnesium in mixtures containing known amounts of the two ions; phosphate was

TABLE II

RECOVERIES OF CALCIUM AND MAGNESIUM FROM MIXTURES CONTAINING ADDED PHOSPHATE

Mixture No.	Calcium present, μM per 4 ml	Calcium found, μM per 4 ml	Magnesium present, μM per 4 ml	Magnesium found, μM per 4 ml
1	1·00	1·00	5·02	5·35
2	2·00	1·94	8·36	8·60
3	4·00	3·85	8·36	8·36

added to these mixtures in sufficiently high concentration to be in excess of that expected to occur in a normal liver[4] (a 2-fold to 7-fold excess was used). The amounts of morpholine nitrate and

sodium tungstate solutions used have been found to remove completely 290 μM of phosphorus from 4 ml of solution.

The results of determinations carried out on fresh samples of normal rat liver were—

Sample No.	1	2	3	4	5
Calcium found, μM per g	0·73	0·96	0·67	0·97	0·96
Magnesium found, μM per g	12·3	11·7	—	10·1	—

These results agree with those reported by other workers.[4,5]

We thank Dr. C. C. Kratzing for helpful discussions during this work.

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SAND IN CANNED STRAWBERRIES

Strawberries are liable to contamination by soil, and, as a result, cans of strawberries normally contain a minute amount of sand. Although the amount involved is not usually sufficient to cause comment, the possibility of complaints of excessive amounts of sand always exists, and there are no figures available on which to base an opinion as to what constitutes a reasonable or normal amount. The ideal would be to ensure that there is no sand in preserved strawberries, but again there is no information as to how practicable is the avoidance of contamination or the removal of soil from the fruit.

We therefore determined to make a survey of as many different packs of canned strawberries as possible and to relate the sand contents to such details of canning practice and horticulture as we could discover. Experiments were also made to determine the effectiveness of various washing techniques, the maximum amount of sand likely to gain entry on the fruit, the reliability of visual inspection and the value of placing straw beneath the growing fruit.

Experimental

The amount of soil present on fresh fruit was determined by washing the fruit and collecting the soil on a tared Whatman No. 42 filter-paper, which was then dried at 100° C and weighed; several pounds of fruit were used in each assay. The sand (or, more correctly, acid-insoluble residue) in canned strawberries was assessed by using a standardised procedure consisting in washing and decantation, collection of the combined residues (also containing organic material, such as strawberry seeds, pieces of calyx, etc.) on a Whatman No. 42 filter-paper, wet oxidation of the filter-paper and contents and transfer of the insoluble residue to a tared G4 crucible. The residue was dried at 100° C and then weighed, and the amount of insoluble residue was calculated on the basis of net can content. This result for "siliceous residue" or "sand" was generally about 75 per cent. of that originally found for soil.

Discussion of results

The collection of soil washed from fresh strawberries gave the results in Table I, from which the upper limits of possible contamination without rejection of the fruit out of hand and the effects of placing straw beneath the fruit can be assessed.

The amount of soil that can attach itself to strawberries without leading to rejection of the fruit is surprisingly high (see Table I). Washing easily removed about 98 per cent. of this soil, but it must be remembered that these results are for fruit handled as experimental material; the same efficiency is not to be expected in industry. Even under the experimental conditions, 30 to 40 p.p.m. of sand still remained on the washed fruit.

The average sand content of all cans of strawberries examined (see Table II) was 105 p.p.m. Included in this average are results for cans deliberately packed with dirty strawberries; if the results for such cans and any others found to contain more than 200 p.p.m. of sand are excluded, the average is reduced to 60 p.p.m. The average sand content of all cans of unwashed strawberries was 220 p.p.m. and that of all washed strawberries 55 p.p.m.

TABLE I

RESIDUES OF SOIL AND SAND FOUND ON RAW STRAWBERRIES

Variety of fruit	Soil found on fruit, p.p.m.	Siliceous matter found on fruit, p.p.m.	Fruit washed	Fruit strawed
Talisman and Cambridge Vigour	3210	2440	No	No
Talisman and Cambridge Vigour	—	40	Yes	No
Talisman (mature)	2530	2300	No	No
Talisman (immature)	2150	1150		
Talisman	2090	1580		
Talisman	—	30	Yes	No
Cambridge Vigour	1990	1570	No	No
Cambridge Vigour	—	30	Yes	No
Talisman	1490	1090	No	Yes
Talisman	—	20	Yes	Yes
Royal Sovereign	1700	1460	No	Yes
Talisman	—	550		
Improved Sovereign (sprayed with Rogor)	—	40	No	No
Improved Sovereign (not sprayed)	—	390		
Talisman (fruit)	—	60	No	Yes
Talisman (stalks)	—	10		

TABLE II

SAND CONTENTS OF CANS OF STRAWBERRIES

The samples tested were supplied by members of the Fruit and Vegetable Canning and Quick Freezing Research Association

Cannery	Size of can	Number of cans sampled	Sand content found, p.p.m.	Treatment of fruit before canning
A	A2	3	13 to 67 (mean 34)	Not stated
	No. 1 (Tall)	3	11 to 30 (mean 24)	
B	No. 1 (Tall)	6	127 to 328 (mean 174)	
C	A2	5	29 to 65 (mean 49)	Spray-washed
	E1	1	18	
D	E1	6	24 to 97 (mean 52)	Washed; method not stated
E	E1	4	68 to 326 (mean 142)	Washed "only if dirty"
	Picnic	2	80, 227	Not stated
F	No. 1 (Tall)	7	42 to 85 (mean 52)	Spray-washed
	A2	6	31 to 86 (mean 50)	
G	Picnic	3	8 to 35 (mean 20)	Washed in shaker fitted with sprays
	E1	3	18 to 33 (mean 27)	
H	E1	4	2 to 18 (mean 12)	Not washed
	E1	4	7 to 12 (mean 9)	Washed; method not stated
	A2	4	625 to 948 (mean 787)	Unwashed dirty fruit
I	A1	8	7 to 112 (mean 70)	Filled cans immersed in water, inverted and allowed to drain
	A1	8	28 to 166 (mean 83)	Not washed
	A2	2	24, 36	Filled cans immersed in water, inverted and allowed to drain
	A2	2	82, 104	Not washed
J	E1	12	40 to 111 (mean 78)	Washed; rod washer
		12	138 to 290 (mean 217)	Not washed
K	A2	14	7 to 53 (mean 25)	Washed by immersion
		2	157, 169	Not washed
L	A2	14	11 to 248 (mean 86)	Not stated
M	No. 1 (Tall)	8	90 to 530 (mean 301)	
N	A2	10	9 to 91 (mean 46)	Washed; method not stated

Washing the strawberries and the early and efficient placing of straw beneath the fruit are the two most important factors in the prevention of excessive residues of sand in the canned product. It is significant that the raw fruit used for determinations of soil (see Table I) was picked from plants that were strawed late, after most of the fruit had formed. The results for soil and

sand in the first half of Table I are all high because the fruit was picked before the straw was laid. The apparently anomalous values for Talisman and Royal Sovereign in the middle of the Table are explained by the fact that the fruit had formed and been splashed with soil before straw was laid, so that the fruit, although picked after the plants had been strawed for some time, was still dirty. The figures in the lower part of Table I are for fruit picked late in the season, *i.e.*, fruit formed after straw had been laid; these values are low. The other anomaly in Table I is the low sand content of the Improved Sovereign variety. These plants were not strawed, but had not been cultivated after being hoed by hand in the early Spring, and it is possible that weeds surrounding the plants fulfilled the same function as straw in preventing the fruit from being splashed with soil during rain.

The results in Table II show that, although fruit may appear clean when packed, it can still contain as much as 90 p.p.m. of sand; this emphasises the fact that strawberries should be washed whether or not they appear to be dirty. The various methods of washing are not equally efficient in removing soil. Most of the spray-washed fruit contained little sand, but the best method of washing appears to be that combining gentle agitation with spraying.

In Table III the results have been arranged approximately in order of the type of soil. It can be seen that, in general, light sandy soils favour high residues of sand and that medium loam soils favour low residues.

TABLE III

INFLUENCE OF TYPE OF SOIL ON SAND CONTENTS OF CANS OF STRAWBERRIES

Type of soil*	Number of cans tested	Mean sand content, p.p.m.
Very light sand	2	88
Light sand	10	112
Fenland	9	104
Light medium	44	65
Medium loam	5	41
Medium-heavy loam	3	32
Heavy loam	2	73

* Canner's description.

CONCLUSIONS

Our results clearly show that the residues of sand in cans of strawberries can be adequately controlled by washing the raw fruit in water. Control of the amount of soil splashed on to the raw fruit is more difficult, but growers can do much to assist by laying straw early in the season. However, strawing increases the risk of damage by frost,[1] and must therefore not be carried out too early. The average sand content of all cans except those containing excessively dirty fruit was 60 p.p.m.; even experimental cans packed at Campden with fruit that had been carefully washed contained as much as 50 p.p.m. Efficient washing is not always practicable because of the condition of the fruit, and, of the cans containing washed fruit, almost one in ten had a sand content greater than 100 p.p.m.

A logical conclusion, therefore, is that if a pack contains less than 100 p.p.m. of sand, on average, and 100 to 200 p.p.m. in not more than 10 per cent. of the cans sampled, the canner has probably taken reasonable precautions in washing and selecting fruit. Such sand contents are unlikely to be noticed by the consumer.

This conclusion is based on the results reported in this Note, which were obtained by examining the contents of cans filled during two seasons (1957 and 1958). Both seasons were rather wet, but it is not known whether, on balance, continued wet weather will increase or decrease the soil load on strawberries. The conclusion may therefore need modification in the light of experience. Results obtained during the dry 1959 season generally agreed with those reported here, the level of contamination of unwashed strawberries being the same for the very wet season of 1958 and the very dry season of 1959.

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THE FRUIT AND VEGETABLE CANNING AND QUICK FREEZING RESEARCH ASSOCIATION
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THE USE OF ETHYLENEDIAMINETETRA-ACETIC ACID IN THE SEPARATION OF RADIOACTIVE BARIUM AND STRONTIUM

During the development of methods for determining the principal radioactive fission products in samples of aquatic origin, existing precipitation techniques for the simultaneous determination of radioactive barium and strontium were found to be not entirely satisfactory. The subject was critically examined by Sunderman and Meinke,[1] who reported yield and contamination data for several precipitation reactions of calcium, strontium and barium.

In the method described here, which was designed for application to the precipitate of mixed nitrates customarily obtained from 75 per cent. nitric acid, the difference between the solubility products of barium and strontium chromates is reinforced by the different stabilities of the complexes formed by these elements with ethylenediaminetetra-acetic acid (EDTA).

A high yield of barium chromate, almost free from strontium, is obtained by adding an excess of chromate ions to an ammoniacal solution of the complexes of the metals with EDTA, and strontium is subsequently recovered, as sulphate, by displacement with copper.

EDTA was used by Ballczo and Doppler[2] to improve the separation of barium from strontium at pH 5, and studies of the precipitation of barium salts from solutions containing EDTA in the presence of strontium, calcium and a displacing metal were made by Gibson and Wilkinson,[3] Tockstein and Novák[4] and Firsching.[5] EDTA has also been used as eluting agent in an ion-exchange method for separating barium from strontium.[6]

Experimental

Separations were normally carried out by heating for 10 to 15 minutes at 100° C, although somewhat lower temperatures were used for strongly ammoniacal solutions. The tracers used were barium-140, freed from strontium-89 and lanthanum-140 just before use, and strontium-89 containing a few per cent. of strontium-90 but freed from yttrium-90; 10 mg of each element were used as carrier.

Precipitation of barium chromate—

This step is best carried out by using an amount of EDTA just equivalent to the amounts of barium and strontium present. Ammonium salts do not interfere, and the amount of potassium chromate solution added is not critical. Substitution of sodium sulphate for potassium chromate led to much less satisfactory separation. The yields of barium sulphate and chromate and the results for contamination of the precipitates with strontium are shown in Table I.

Table I

Yields and contamination of barium salts precipitated under various conditions

Conditions of precipitation*	Activity added, as strontium-89, counts per minute	Yield of barium salt precipitated, %	Activity found in precipitate, counts per minute	Decontamination factor†
A	10,080	95	38	300
A	25,200	98	73	400
B	10,080	95	239	48
C	22,725	92	46	535
D	22,725	85	473	49
E	8415	98	54	160
F	10,080	67	16	530

* The conditions used were—

A. Solution 0·023 *M* in Sr^{2+}, 0·0145 *M* in Ba^{2+}, 0·0375 *M* in EDTA, *M* in ammonium hydroxide and 1·5 *M* in ammonium chloride; 0·155 *M* potassium chromate as precipitant; 15 minutes at 100° C.
B. As in A, but with 0·06 *M* sodium sulphate as precipitant.
C. As in A, but 50 per cent. more EDTA and potassium chromate.
D. As in B, but 50 per cent. more EDTA; 0·11 *M* sodium sulphate as precipitant.
E. As in A, but 25 per cent. deficiency of EDTA.
F. As in A, but set aside for 1 hour at room temperature.

† Calculated by dividing the initial activity by the recovered activity (corrected for self-absorption, self-scattering and chemical yield).

The barium chromate can conveniently be purified by solution in dilute nitric acid and re-precipitation in presence of EDTA.

PRECIPITATION OF STRONTIUM SULPHATE—

The conditions for obtaining strontium in a form suitable for counting depend on the efficiency with which the residual barium (2 to 3 per cent.) and its daughter, lanthanum-140—troublesome in all experiments involving barium-140—are removed. This lanthanum is readily co-precipitated with most strontium salts in spite of the stability of its complex with EDTA, and precipitation of strontium as carbonate, sulphite, oxalate or fluoride was rejected for this reason. The use of nickel or manganese as displacing metal was rejected for the same reason. Strontium sulphate precipitated from ammoniacal solution in the presence of a cupric salt carries down only a small proportion of the lanthanum, contamination by which can finally be reduced to 0·3 per cent. by re-precipitation. The amount of lanthanum carrier used in the separation is 2 mg (added as its complex with EDTA); larger amounts interfered with the precipitation of barium chromate. The activity of the residual barium can readily be reduced by scavenging.

METHOD

REAGENTS—

EDTA solution—Dissolve 19·51 g of hydrated disodium ethylenediaminetetra-acetate in a mixture of 50 ml of ammonia solution, sp.gr. 0·880, and 250 ml of water, and dilute to 500 ml.

Lanthanum - EDTA reagent solution—Mix 96 ml of the EDTA solution with 4 ml of a 7·8 per cent. solution of lanthanum nitrate hexahydrate.

Potassium chromate solution, 10 per cent.

Ammonium nitrate solution, 2 per cent.

Calcium nitrate solution—Dissolve 1·456 g of calcium carbonate in hot *N* nitric acid, and dilute to 100 ml.

Ammonium sulphate solution, 20 per cent.

Barium chloride solution—Dissolve 1·78 g of barium chloride dihydrate in water, and dilute to 100 ml.

Copper sulphate solution—Dissolve 6·50 g of cupric sulphate pentahydrate in water, and dilute to 100 ml.

Ammonium acetate buffer solution—Dissolve 58 ml of glacial acetic acid and 37 ml of ammonia solution, sp.gr. 0·880, in water, and dilute to 1 litre. Adjust the pH to 5·0 $\pm$ 0·1.

PROCEDURE—

To the neutral or faintly acid solution containing 10 mg each of barium and strontium in approximately 2 ml add 2 ml of lanthanum - EDTA reagent solution. Heat to 100° C, add 1·5 ml of potassium chromate solution, stir, and maintain at 100° C for 15 minutes. Allow to cool, spin in a centrifuge, wash the precipitated barium chromate twice with small portions of ammonium nitrate solution, and combine the washings and supernatant liquid.

Treatment of combined washings and supernatant liquid—Heat to 100° C, add 0·2 ml of ammonium sulphate solution, 0·5 ml of calcium nitrate solution and 1 ml of barium chloride solution (in that order), with stirring, and maintain at 100° C for 25 to 30 minutes. Allow to cool, spin in a centrifuge, and wash the precipitate once with ammonium nitrate solution. Combine the washings and the supernatant liquid, and discard the precipitate. Add 0·5 ml of ammonia solution, sp.gr. 0·880, and 1 ml of ammonium sulphate solution to the combined supernatant liquid and washings, warm to 90° C, and add 0·5 ml of copper sulphate solution, with stirring. Maintain at 90° C for 10 minutes, allow to cool, spin in a centrifuge, and wash the precipitated strontium sulphate with two small portions of water. Discard the supernatant liquid and washings, dissolve the precipitate in 1·2 ml of EDTA solution, and heat at 100° C for 5 minutes to expel excess of ammonia. Add 1 ml of ammonium sulphate solution and 4 ml of ammonium acetate buffer solution, and continue to heat for 5 minutes. Allow to cool, spin in a centrifuge, wash the precipitate with small portions of 2 *N* ammonium hydroxide and water (twice), mount, and weigh for counting.

Treatment of precipitated barium chromate—Dissolve in 2 ml of *N* nitric acid, add 2 ml of EDTA solution, and heat for 15 minutes at 100° C with 3 ml of potassium chromate solution. Allow to cool, spin in a centrifuge, wash the precipitate with 2 *N* ammonium hydroxide and water (twice), mount, weigh, and count without delay (see Note).

NOTE—If it is not practicable to count the barium-140 source immediately after separation, the activity due to lanthanum-140 present at any time may be calculated from the decay constants of the two isotopes or measured directly by determining the ratio of gamma to beta *plus* gamma counting rates for the source and comparing it with the corresponding ratios for pure sources of barium-140 and lanthanum-140. Corrections for self-absorption and self-scattering must be applied when measuring these ratios.

Results

The proposed method was tested with barium-140 and strontium-89 tracers; the results are shown in Table II, together with the decontamination factors for lanthanum-140 and some other important fission products. Although these contaminants are normally almost completely removed by the scavenges with ferric hydroxide and separations in nitric acid that are customary

Table II

Decontamination factors for various isotopes

The mean chemical yields of barium chromate and strontium sulphate were 85 ± 1·7 and 76 ± 4·0 per cent., respectively

Isotope	Decontamination factor* for— barium chromate precipitate	Decontamination factor* for— strontium sulphate precipitate
Barium-140	—	$1·2 \times 10^3$
Strontium-89	$1·2 \times 10^4$	—
Lanthanum-140	36	270
Caesium-137	10^4	$2·7 \times 10^3$
Ruthenium-106†	120	3×10^3
Yttrium-90	530	560
Cerium-144	100	$1·3 \times 10^3$
Zirconium-95 - niobium-95	65	45

* Calculated as described in Table I.

† Ruthenium tracer (0·2 ml) was previously treated with 0·4 ml of fuming nitric acid to simulate the conditions of the usual separation of strontium from calcium.

in procedures for determining radioactive strontium and barium, it was of interest to determine the behaviour of fission products surviving such treatment. Zirconium-95 and niobium-95 were added as an equilibrium mixture, and carrier-free tracers were used throughout. It was found that the precipitated barium chromate and strontium sulphate underwent a small loss in weight when gently ignited, possibly as a result of the co-precipitation of EDTA; allowance was made for this in calculating chemical yields.

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THE DETERMINATION OF SODIUM SULPHIDE AND MERCAPTANS IN THE PRESENCE OF EACH OTHER

The work described, which is a continuation of research on the analytical chemistry of sulphur compounds, deals with determining sulphide and mercaptans in the same sample. The sulphide and aliphatic mercaptans can be accurately determined by titration with *o*-hydroxymercuribenzoic acid[1,2] (obtained, as the anhydride, from Polskie Odczynniki Chemiczne, Gliwice, Sowińskiego 11, Poland), with thiofluorescein or dithizone as indicator. The method described below makes use of the rapid reaction between mercaptans and acrylonitrile[3]; sulphide reacts in the same conditions, but only slowly. The determination consists in titrating sulphide *plus* mercaptan in one sample and sulphide only in a second sample after the mercaptan has been removed with acrylonitrile. The mercaptan content is calculated from the difference between the two titrations.

Method

Procedure for sulphide plus mercaptan—

To a solution of the alkali sulphide and mercaptan add 5 ml of *N* ammonium hydroxide and 1 ml of 0·02 per cent. w/v thiofluorescein, and dilute to 100 ml with water containing about 0·1 per cent. of sodium sulphite. Titrate with 0·001 to 0·05 *N* *o*-hydroxymercuribenzoic acid until the blue colour sharply disappears.

Procedure for sulphide—

To a solution of the alkali sulphide and mercaptan add 5 ml of *N* ammonium hydroxide and a methanolic solution of acrylonitrile (about 2 moles for each mole of mercaptan). Set aside for 2 to 15 minutes depending on dilution and the mercaptan present (2 minutes are sufficient for between 2 and 40 mg of methyl mercaptan in 20 ml of solution), dilute to 100 ml, and titrate with *o*-hydroxymercuribenzoic acid, thiofluorescein being used as indicator.

Results

Some results obtained by the method are shown in Table I.

Table I

Determination of sodium sulphide and methyl mercaptan

Sulphide present, as H_2S, mg	Sulphide found, as H_2S, mg	Methyl mercaptan present, mg	Methyl mercaptan found, mg
5·89	5·95	10·8	10·9
5·89	5·85	21·7	21·5
5·89	5·90	32·5	32·9

Ethyl mercaptan, thioglycollic acid and cysteine can be determined similarly in the presence of sulphide. If the sample contains sulphide, mercaptan and cyanide, the cyanide and sulphide *plus* mercaptan are determined as previously described for sulphide *plus* cyanide,[4] and in a third sample, after removal of mercaptan by acrylonitrile and cyanide by formaldehyde, the sulphide alone is titrated with *o*-hydroxymercuribenzoic acid, a solution of dithizone in sodium hydroxide being used as indicator.

Note that the presence of SO_3^{2-}, $S_2O_3^{2-}$ or CNS^- ions does not interfere in either titration.

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AN IMPREGNATION TEST FOR DETECTING TRACE AMOUNTS OF ORTHOPHOSPHATE

The detection of trace amounts of anions, especially phosphates, is important in biological investigations. Of the methods applicable to such investigations, those involving use of impregnated paper are sensitive, rapid and relatively exact.[1]

The proposed procedure permits the detection of $1·5 \times 10^{-9}$ g of phosphorus and is based on a reaction previously used for the titrimetric determination of phosphate.[2] Drops of the sample solution are applied to a strip of chromatography paper suitably impregnated[3] with insoluble quinoline molybdate. The strip is then dried and dipped into a suitable developer. If phosphate is present in the sample, blue spots appear at the points of application of the sample solution; these spots are produced by reduction of the initially formed yellow quinoline molybdophosphate.

Method

Preparation of test papers—

Dip a strip of Whatman No. 1 or 4 chromatography paper into an aqueous 15 per cent. solution of ammonium molybdate, allow to dry, and then dip into a 4 per cent. solution of quinoline in 0·6 *N* hydrochloric acid. Wash the strip three times with distilled water, and allow to dry. Test papers so prepared are stable if stored in the dark.

Procedure—

If the sample solution is alkaline, acidify with sulphuric, hydrochloric, nitric or perchloric acid; if it is strongly acid, *e.g.*, the product of a Kjeldahl digestion, suitably dilute with water; if it is neutral, spray the paper with a 1·25 per cent. solution of sulphuric acid, and allow to dry immediately before the test. (The best results are obtained when the sample solution contains 1·25 per cent. of sulphuric acid.)

Place 1 drop of solution on the test paper, heat to between 98° and 100° C for not longer than 1 minute, and allow to dry. If the sample contains phosphate, a yellow spot appears at the point of application; the spot is faintly perceptible in daylight, but more so in ultra-violet light. (Spots can be made visible in daylight by spraying the paper with Ilford ID2 developer or immersing it in the developer for 30 seconds to 1 minute; the benzidine test described by Feigl[1] can also be applied.)

Discussion of the test

The proposed simple test is more rapid and sensitive than are spot tests.[4,5] Salts and organic compounds present in concentrations greater than 100 times that of phosphate do not interfere, but the spots are obscured by the presence of silicate and arsenate in amounts, respectively, 20 and 100 times greater than that of phosphate. However, these anions are usually absent from biological material.

Under the optimum conditions we have detected the presence of $1{\cdot}5 \times 10^{-9}$ g of phosphorus in 2 μl of solution; however, if a micropipette is used, several portions of solution can be applied to the same place on the impregnated strip, thereby increasing the amount of phosphate present. Alternatively, a large volume of solution can be applied by using an apparatus of the type described by Antoszewski.[6]

The details of this work have been described elsewhere.[7]

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THE DETERMINATION OF SMALL AMOUNTS OF BROMIDE IN PRESENCE OF MERCURIC IONS

Small amounts of bromide ion in solution can be conveniently determined by electrometric titration with silver nitrate. This was originally shown on the macro scale by Clark,[1] who used several alternative electrode systems, and was adapted to the microgram scale for the analysis of chlorides by Cunningham, Kirk and Brooks.[2] These workers used a simple electrode system, first recommended by Clark, consisting of a silver-wire electrode and an amalgamated silver-wire electrode dipping in the same electrolyte. The e.m.f. of such a couple is not highly reproducible, but, in a given titration, is said always to show a sharp increase at the true end-point. We wished to analyse samples containing small amounts of mercuric and bromide ions, between 100 and 200 μg of bromide being present in each sample. The possibility of using Cunningham, Kirk and Brooks's method for bromide was tested with solutions free from mercuric ions, but although

a sharp increase in e.m.f. was almost always observed, this did not invariably give reliable indication of the true end-point. Results with these solutions were entirely satisfactory when the silver-wire electrode was replaced by an ammonium nitrate - agar bridge leading to an external reference electrode (calomel), but, in presence of mercuric ions, the sharp increase in e.m.f. at the end-point was not observed, and a preliminary separation of the mercury was clearly essential. This was achieved by extracting the aqueous phase with a solution of dithizone in chloroform. The aqueous layer was washed several times with chloroform, and the bromide in a weighed sample was then determined as before. After extraction, the solutions gave sharp end-points, detected by a sudden change in the e.m.f. of the electrode system, and the concentration of bromide so determined was an accurate measure of the concentration of bromide in the original solution (see Table I).

TABLE I

RECOVERY OF BROMIDE FROM SOLUTIONS CONTAINING MERCURIC IONS

In each original solution the ratio of Br^- to Hg^{2+} was about 2 to 1

Weight of bromide in sample, μg	True molality of solution, mole of bromide per kg of water	Molality found by titration, mole of bromide per kg of water
87·5	$1{\cdot}97 \times 10^{-4}$	$1{\cdot}95 \times 10^{-4}$
115	$1{\cdot}97 \times 10^{-4}$	$1{\cdot}97 \times 10^{-4}$
182	$4{\cdot}09 \times 10^{-4}$	$4{\cdot}07 \times 10^{-4}$
132	$4{\cdot}09 \times 10^{-4}$	$4{\cdot}10 \times 10^{-4}$
193	$4{\cdot}09 \times 10^{-4}$	$4{\cdot}12 \times 10^{-4}$
146	$4{\cdot}22 \times 10^{-4}$	$4{\cdot}20 \times 10^{-4}$
136	$4{\cdot}67 \times 10^{-4}$	$4{\cdot}64 \times 10^{-4}$
113	$4{\cdot}67 \times 10^{-4}$	$4{\cdot}67 \times 10^{-4}$
93	$4{\cdot}67 \times 10^{-4}$	$4{\cdot}66 \times 10^{-4}$

All the solutions examined were *N* in perchloric acid, as this problem arose during a study of complex equilibria in which a high constant acidity had to be maintained with a non-complex-forming acid. There is no reason to believe that the method would be any less satisfactory if this component were omitted.

EXPERIMENTAL

Laboratory-reagent grade sodium bromide was thrice recrystallised from water and then dehydrated over sodium hydroxide in a vacuum desiccator. Solutions of this sodium bromide in *N* perchloric acid and of mercuric perchlorate in *N* perchloric acid were mixed to give a solution of known molality in bromide (between 2×10^{-4} and 5×10^{-4}) and with a Br^- to Hg^{2+} ratio of about 2. Approximately 10 ml of this mixture were shaken with 20 ml of a solution of dithizone in chloroform, also containing 1 per cent. of 6 *N* acetic acid to prevent decomposition of the mercury - dithizone complex and so to assist in extracting mercury from the aqueous layer[3]; a moderate excess (about 50 per cent.) of dithizone was used. After extraction and separation, the aqueous layer was washed several times with chloroform, and a weighed sample (2 to 7 g) was analysed by adding silver nitrate solution from a Greiner ultra-micro burette of total capacity 0·1 ml.

When analysing halides electrometrically it is usual to acidify with sulphuric acid to increase the conductivity of the solution, but in this determination such an addition was unnecessary because of the presence of perchloric acid. The silver nitrate solution used was 0·0671 *N* except for the last three results in Table I, for which it was diluted to 0·0255 *N*. End-points were satisfactory even when 0·01 *N* silver nitrate was used, and, had it been necessary, bromide solutions having appreciably lower concentrations than those reported here could have been determined with reasonable accuracy.

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