# THE COLORIMETRIC DETERMINATION OF SMALL AMOUNTS OF IODIDE, WITH SPECIAL REFERENCE TO THE DETERMINATION OF IODIDE IN THE PRESENCE OF ORGANICALLY-BOUND IODINE

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A photocolorimetric method has been developed for the determination of small quantities of inorganic iodide in the presence of organically-bound iodine. The iodide-containing solution is treated with a mixed iodate-starch solution at an appropriate pH, when a stable blue colour is produced. The relationship between concentration of iodide and optical density is linear. The effect of various salts on the reaction has been investigated; the analytical application of the method to some iodine-containing drugs and pharmaceutical preparations has been studied. The reason for the failure of the colour reaction to obey Beer's law is discussed.

In a study of the stability of thyroxine and of 3,5,3′-triiodothyronine (liothyronine) in alkaline solution, Maclagan, Bowden and Wilkinson (1957) and Lein and Michel (1959) have shown that these compounds break down with release of inorganic iodide. In an attempt to study these observations quantitatively, an assay for small amounts of inorganic iodide in the presence of iodothyronines and of iodotyrosines was required. The methods already described for the spectrophotometric and photocolorimetric micro-estimation of iodide (Zak, 1958; Armstrong, Gill and Rolf, 1961; Barker, 1962) are inapplicable in the presence of organically-bound iodine. Thus the ceric arsenite (Sandell-Kolthoff) method is not specific for iodide, since iodotyrosines and iodothyronines also catalyse the reduction of ceric ions by arsenious acid. Similarly in the oxidation of iodide to iodate with bromine or with permanganate, followed by determination of iodine liberated by potassium iodide, there is the risk of simultaneous oxidation of bound iodine, resulting in high values.

The iodate-starch method of Lambert (1951) is not applicable in the presence of compounds such as thyroxine, triiodothyronine or iodophthalein, since these alter the pH of the reaction mixture and thereby decrease the rate of formation of the blue colour. It has been found, however, that provided the pH is carefully controlled, treatment of an aqueous solution of iodide with a mixed potassium iodate-starch solution produces a colour which reaches a maximum value after about 12 min. and thereafter remains stable for at least a further 15 min. The method is applicable to the determination of not less than 4 p.p.m. of iodide in solution, for which a 10 ml. sample is required, or of about 0·004 per cent of iodide in an organic iodine-containing compound, assuming that 1 g. of material is examined.

## EXPERIMENTAL

### *Preliminary Experiments*

Preliminary experiments were made by adding to aliquots of an aqueous potassium iodide solution small volumes of the 1:2:1 potassium iodate-starch-N sulphuric acid spray reagent devised by Roche, Jutisz, Lissitsky and Michel (1950) for the detection of iodide on paper chromatograms. The colours obtained were unstable, the rate of fading depending on the acid content of the solution. Excess of iodate or of starch did not affect the colour. Using the extinction at 615 mμ, it was found that colour stability and the times at which maximum colour intensities are attained, are governed principally by the pH of the test solution before the iodate-starch reagent is added. Best results were obtained with a potassium hydrogen phthalate-hydrochloric acid buffer pH 2·2 (Bower and Bates, 1955) to which 0·5 per cent w/v of sodium sulphate was added to promote colour stability. This buffer solution gave excellent results for neutral or near-neutral solutions of iodide, but could not be used when the iodide solution contained sodium salts of organic acids, such as thyroxine, triiodothyronine and iodoxyl, since these substances altered the pH of the iodide-buffer solution. In these cases it was necessary to adjust the pH with a mineral acid, and to filter off any precipitated matter.

To extend the method to a range of organic iodine compounds, three modifications (A, B and C) have been used appropriately. In all instances there is a linear relationship between iodide content and extinction although the calibration line does not pass through the origin. The threshold value (i.e. iodide content for zero extinction) is about 120 μg./25 ml. When smaller amounts of iodide are to be determined, a standard amount of iodide must be added to overcome the "threshold".

### *Reagents and Procedures*

All additions of iodide solution, buffer, 0·2N sulphuric acid, water and of iodate-starch reagent to be made accurately with burettes.

METHOD A. (Used for determination of inorganic iodide in neutral or near-neutral solution in absence of organically-bound iodine).

*Potassium iodide solution* containing about 30 mg. of potassium iodide, Analar, accurately weighed, in 1 litre of water.

*Potassium hydrogen phthalate-sodium sulphate solution.* Dissolve potassium hydrogen phthalate Analar (20·42 g.), sodium sulphate (anhydrous) Analar (10 g.) and chlorocresol B.P. (0·2 g.) in water, and dilute to 1 litre. (This solution is usable for several months.)

*Buffer solution.* Mix equal volumes of potassium hydrogen phthalate-sodium sulphate solution and of 0·1N hydrochloric acid. This buffer solution must be freshly prepared, as phthalic acid crystallises out on standing.

*Iodate-starch solution.* Mix 2 volumes of freshly-prepared 1 per cent starch solution with 3 volumes of 1 per cent potassium iodate (Analar) solution. This solution remains usable for one week.

*Procedure.* Dilute the test-solution, which should contain 150 to 500 μg. of iodide, to 15 ml. with water. Similarly dilute 7·5 ml. and 12·5 ml.

of potassium iodide solution (0·003 per cent) to 15 ml. with water (solutions used for calibration purposes). To all solutions add buffer solution (6 ml.), iodate-starch solution (2 ml.) and dilute to 25 ml. with water. Allow to stand for 15 min., then determine the extinction at 615 m$\mu$ in 1 cm. cells using a similarly prepared blank as reference liquid.

The iodide content (i) in $\mu$g. of the test solution is calculated from the equation

$$i = i_1 + (e_t - e_1)(i_2 - i_1)/(e_2 - e_1)$$

where $e_t$ = extinction of test-solution; $e_1$ and $e_2$ = extinction of standard solutions (7·5 ml. and 12·5 ml. respectively); $i_1$ and $i_2$ = $\mu$g. of iodide contained in 7·5 ml. and 12·5 ml. of potassium iodide solution, respectively.

METHOD B. (Used for determining inorganic iodide in organic iodo-compounds, where the inorganic iodide content of the sample is not less than 350 $\mu$g.).

*Potassium iodide solution* containing about 60 mg. of potassium iodide, Analar, accurately weighed, in 1 litre of water.

*Procedure.* Transfer a suitable quantity of iodo-compound (*cf.* Table IV), accurately weighed, to a 30 ml. beaker and add water (10 ml.). Adjust potentiometrically to pH 2·2 with 0·2N sulphuric acid. Note the volume of acid required (*a* ml.). Rinse the electrodes with 5 ml. of water, add the washings to the contents of the beaker and allow to stand for 10–20 min. Filter through a No. 1 paper* and wash the beaker and filter with 17–*a* ml. of water, so that the volume of filtrate is 32 ml.

Determine the volume of 0·2N sulphuric acid required to adjust 10 ml. of potassium iodide solution (0·006 per cent) to pH 2·2 (about 0·6 ml.). Let this be *b*. Transfer 7·5 ml. and 12·5 ml. of potassium iodide solution to 50 ml. graduated flasks, then add to each flask *b* ml. of 0·2N sulphuric acid and water to 32 ml.

To all solutions add 5 ml. of iodate-starch reagent and dilute to 50 ml. with water. Allow to stand for 15 min., then determine the extinctions at 615 m$\mu$ in 1 cm. cells. The percentage of inorganic iodide (i) in the iodo-compound is calculated thus:

$$i = \frac{1}{w.10^4}[i_1 + (e_t - e_1)(i_2 - i_1)/(e_2 - e_1)]$$

where w = weight (or volume) of iodo-compound taken, in g. (or ml.).

METHOD C. (Used where inorganic iodide content is less than 350 $\mu$g.)

*Procedure.* Process as for Method B, but extract the iodide with potassium iodide solution (0·006 per cent, 10 ml.) in place of 10 ml. water. The percentage of inorganic iodide (i) in the iodo-compound is calculated thus:

$$i = \frac{1}{w.10^4}[i_1 - i_3 + (e_t - e_1)(i_2 - i_1)/(e_2 - e_1)]$$

where $i_3$ = $\mu$g. of iodide contained in 10 ml. of potassium iodide solution.

* Iodoxyl is soluble at pH 2·2 and does not require to be filtered.

## Results and Discussion

The results of recovery experiments in which small amounts of potassium iodide were added to recrystallised *m*-iodobenzoic acid are shown in Table I, while the effects of various ions (generally in 10–100 molar excess) on the colour reaction are shown in Table II. Tables III and IV respectively include results, illustrating the use of the method for assay of small samples of pharmaceutical preparations and for estimation of iodide, when present as an impurity.

TABLE I

RECOVERY OF SMALL AMOUNTS OF POTASSIUM IODIDE IN THE PRESENCE OF *m*-IODOBENZOIC ACID USING METHOD C

Each 10 ml. aliquot of test-solution contained 100 mg. of *m*-iodobenzoic acid (recryst.) and 0·6 mg. of potassium iodide.

| Potassium iodide added (expressed as iodide ion), µg. | Iodide recovered, per cent |
|---|---|
| 20 | 108·5 |
| 40 | 100·5 |
| 60 | 101·6 |
| 80 | 101·9 |
| 100 | 101·8 |
| 120 | 102·1 |
| 140 | 98·95 |

The results for the inorganic iodide content of Thyroxine Sodium B.P. samples 1 and 2 are of interest. In the first place methods B and C gave closely corresponding results. Secondly the relatively high inorganic iodide content found in these samples is in agreement with a report that inorganic iodide detected during paper-chromatography of thyroxine and related compounds may derive from iodide present in the starting-material,

TABLE II

EFFECT OF SOME INORGANIC COMPOUNDS AND OTHER SUBSTANCES ON THE PHOTOCOLORIMETRIC DETERMINATION OF IODIDE BY THE IODATE-STARCH REACTION

| Potassium iodide taken (as I) | Compound added | Amount added | Method | Iodide recovered, per cent |
|---|---|---|---|---|
| 2 µM (254 µg.) | $Na_2SO_4$ | 200 µM (28·4 mg.) | A | 101·9, 101·8, 101·9 |
| " " " | $NH_4Cl$ | " " (10·7 mg.) | " | 99·4, 100·4 |
| " " " | $NH_4NO_3$ | " " (16·0 mg.) | " | 101·3, 102·3 |
| " " " | KCl | " " (14·9 mg.) | " | 99·3, 100·0 |
| " " " | $KClO_3$ | " " (24·5 mg.) | " | 99·0 |
| " " " | KBr | 20 µM (2·38 mg.) | " | 94·2, 94·7 |
| " " " | " | 200 µM (23·8 mg.) | " | 69·05 |
| " " " | $KBrO_3$ | " " (33·4 mg.) | " | 99·0, 99·3 |
| " " " | Sodium acetate (anhyd.) | 20 µM (1·64 mg.) | " | 101·1, 99·9 |
| " " " | " " " | 200 µM (16·4 mg.) | " | 50·3 |
| " " " | Sodium citrate B.P. | 20 " (5·88 mg.) | " | 91·9 |
| " " " | " " " | 200 " (58·8 mg.) | " | Nil |
| " " " | Lactose | " " (72·1 mg.) | " | 99·6, 99·45 |
| " " " | Ethanol | 0·2 g. | " | 99·9 |
| " " " | " | 2 g. | " | 94·65, 94·55 |
| 4 µM (508 µg.) | $Na_2SO_4$ | 400 µM (56·8 mg.) | B | 107·7, 107·5 |
| " " " | KCl | " " (29·8 mg.) | " | 106·3, 106·4 |
| " " " | KBr | 40 " (4·76 mg.) | " | 84·35, 84·3 |
| " " " | Iodine* | 1·10 mg. | " | 98·6 |
| " " " | Ethanol | 2 g. | " | 88·7 |

* Solution acidified to pH 2·2 and extracted with chloroform.

rather than arise as a result of decomposition in the course of chromatography, as is sometimes stated in the literature (Donhoffer, Várnai, Szegvári, Farkas and Járai, 1960).

TABLE III

ASSAY OF SMALL AMOUNTS OF QUATERNARY AMMONIUM IODIDES BY IODATE-STARCH REACTION USING METHOD A

| Compound | Theoretical iodine content, per cent | Loss on drying, per cent | Weight taken for determination, mg. | Recovery, calculated with reference to anhydrous material, per cent |
|---|---|---|---|---|
| Decamethonium iodide— | | | | |
| Sample 1 .. .. .. | 49·56 | 7·29 | 0·5 | 100·0, 99·6 |
| Sample 2 .. .. .. | 49·56 | 6·38 | 0·5 | 99·8, 100·3 |
| Hexamethonium iodide .. | 55·63 | 0·43 | 0·5 | 101·3, 101·0 |
| Dimethyltubocurarine iodide | 28·00 | 5·42 | 1 | 99·3, 99·3 |

Of two samples of Diodone Injection examined, one complied with the B.P. limit test for inorganic iodides and one did not.

Elemental iodine interferes if present before the addition of the iodate-starch solution, but it may be removed conveniently by extraction with chloroform.

TABLE IV

INORGANIC IODIDE CONTENT OF SOME ORGANIC IODO-COMPOUNDS AND INJECTIONS

| Test substance | Amount taken for determination | Method | Inorganic iodide content |
|---|---|---|---|
| | | | per cent w/w |
| Diiodohydroxyquinoline B.P. .. .. | 0·2 g. | C | Nil |
| Ethyl iodophenylundecanoate B.P.C. .. | 0·5 g. | C | Nil |
| Iodoform .. .. .. .. .. | 0·5 g. | C | 0·008, 0·005, 0·008 |
| Iodophthalein .. .. .. .. | 30 mg. | B | 1·455, 1·47* |
| Iopanoic acid B.P. .. .. .. .. | 0·5 g. | C | Nil |
| Liothyronine sodium B.P.—Sample 1 .. | 0·2 g. | C | 0·028, 0·026 |
| Sample 2 .. | 0·2 g. | C | Nil |
| Pheniodol B.P. .. .. .. .. | 0·2 g. | C | 0·020, 0·020 |
| Propyliodone B.P. .. .. .. .. | 0·2 g. | C | Nil |
| Thyroxine .. .. .. .. .. | 0·2 g. | C | 0·028, 0·031 |
| Thyroxine sodium B.P.—Sample 1 .. | 40 mg. | C | 0·30, 0·31, 0·28 |
| " " .. | 0·2 g. | B | 0·285, 0·295 |
| Sample 2 .. | 40 mg. | C | 0·30 |
| " " .. | 0·2 g. | B | 0·275, 0·28 |
| Sample 3 .. | 0·2 g. | C | 0·039 |
| | | | per cent w/v |
| Diodone injection B.P.—Sample 1.. .. | 1 ml. | C | 0·0063, 0·0064 |
| Sample 2.. .. | 1 ml. | C | 0·0026, 0·0019 |
| Iodoxyl injection B.P. .. .. .. | 0·5 ml. | C | 0·022, 0·022 |

* Inorganic iodide content in iodophthalein found by titration with potassium iodate (Lang's method) 1·40 per cent.

In only three instances was the method inapplicable; these were iodoacetic acid (the relatively strong acidity caused a lowering of pH), thyroid and thyroglobulin.

The failure of the calibration line to pass through the origin may be explained as follows: since the hydrogen ion and iodide ion concentrations of the reaction medium are relatively small, some of the iodine produced in the oxidation of iodide by iodate

$$5I^- + IO_3^- + 6H^+ = 3I_2 + 3H_2O \quad .. \quad .. \quad (1)$$

will be hydrolysed until a small but definite concentration of hypoiodous acid is established

$$I_2 + H_2O = HOI + H^+ + I^- \quad .. \quad .. \quad (2)$$

(cf. Allen and Keefer, 1955). This hydrolysis will result in a "threshold" below which no iodine is formed. Support for this hypothesis is provided by the specific extinction coefficients for the iodide-iodate-starch reaction and the iodine-starch reaction. The latter reaction, which is conducted in the presence of a considerable excess of iodide ion (thus suppressing hydrolysis of iodine by mechanisms 1 and 2) has an extinction coefficient of about 1470 with respect to iodine (Ovenston and Rees, 1950). The specific extinction coefficient for the iodide-iodate-starch reaction, on the other hand, is appreciably lower (about 650 for 250 $\mu$g. of iodide made up to a final volume of 25 ml., and 800 for 500 $\mu$g. of iodide in a final volume of 25 ml.). In the absence of excess iodide the iodine-starch colour also fails to obey Beer's law, and exhibits a "threshold" (Müller and McKenna, 1936).

In a recent publication Lambert and Zitomer (1963) attribute the iodide-iodate-starch "threshold" to loss of iodine by deposition in the interior of the starch helices. This explanation, however, is open to the objection that the "threshold" would then vary with the starch content of the reaction mixture, whereas provided the starch is present in excess the actual concentration has no effect on the reaction.

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