

LXIII.—*On the Determination of Nitric Acid by means of Indigo, with special reference to Water Analysis.*

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My earlier experience of the application of indigo to the determination of nitric acid has been already published (*Chem. News*, **35**, 45, 57). During the last two years a good deal of additional experience has been acquired, and the nitric acid in a large number of drainage waters has been determined by means of indigo in the Rothamsted Laboratory. As the use of indigo affords the speediest means of determining the nitrates present in water, and the method is likely to become generally employed for this purpose, it may be of service to describe the conditions which have been found necessary for its successful application.

The literature of the subject is very voluminous, and has been partially considered in the earlier paper just referred to. The results yielded by indigo are so dependent on the conditions of the experiment, that very various conclusions have been arrived at by different chemists and numerous modifications of method suggested. All that can be done here is to notice occasionally the agreement or disagreement of other experimenters with the plans here suggested.

The form of indigo commonly employed is the sulphindigotate of sodium, known in commerce as "indigo carmine;" it will be found, however, far better to employ a solution of pure indigotin. When the commercial sulphindigotate of sodium is oxidised by nitric acid the

resulting solution is of a deep reddish colour, and the presence of a small excess of unoxidised indigo can be with difficulty ascertained. A solution of sublimed indigotin, on the other hand, yields when oxidised a far paler sherry-coloured solution in which a small excess of indigo is readily perceived, and can be accurately estimated by the eye. Indigotin has been employed both by Tiemann and by Fischer.

The strength of the indigo solution suitable for water analysis is about one-fourth that of the normal solution recommended by Sutton. The normal solution of indigo is equivalent to a solution of nitrate of potassium containing 0.14 gram of nitrogen per litre. If the indigo is to be used in large quantities, it is perhaps most convenient to prepare it in the first instance of the normal strength, and to dilute it as required.

Four grams of sublimed indigotin will furnish more than 2 litres of normal indigo solution. The indigotin is digested for some hours with five times its weight of Nordhausen oil of vitriol, then diluted with water, filtered, and brought to the volume of 2 litres. The strength of the indigo is next ascertained with a normal solution of nitrate of potassium by the method presently described. The indigo solution, which will be found too strong, is finally diluted, so that 10 c.c. correspond with the same volume of the normal nitre solution. There is no object in bringing the indigo absolutely to the normal strength; a near approximation will suffice. To preserve the indigo solution it is well to add to it 4 per cent. by volume of pure oil of vitriol; indigo so fortified undergoes no change when kept in the dark. The use of sulphuric acid is recommended by Sutton.

The normal indigo is diluted, as required, to four times its volume for the purpose of water analysis, oil of vitriol being added in such quantity as to maintain the proportion of 4 per cent. The indigo solution is to be used in a burette graduated to tenths of a cubic centimetre. The solution is too dark to be seen through. The height of the liquid may be read from its upper surface, or an Erdmann's float may be employed. The solution being slightly viscid, the burette should not be read till the level of the liquid becomes constant.

A series of standard solutions of pure nitre will be required to determine the value of the indigo in nitric acid, as this is not uniform throughout the scale. A normal solution of nitre is first prepared by dissolving 1.011 gram of pure nitrate of potassium, gently fused over a spirit lamp before weighing, in 1 litre of water. From this normal solution other solutions of $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, and $\frac{1}{64}$ the normal strength are prepared, and preserved for use.

In water analysis it is most convenient to work with 20 c.c. of the water; when this is done it is necessary to standardise the indigo with 20 c.c. of the $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, and $\frac{1}{64}$ nitre solutions. If 10 c.c. of

water are to be employed the indigo must be also standardised with 10 c.c. of the $\frac{1}{4}$ and $\frac{1}{8}$ nitre solutions. It is most useful to standardise both for 10 c.c. and 20 c.c. of water, as waters of a greater range of strength can then be analysed without dilution. It is unnecessary to standardise with 10 c.c. of the weaker nitre solutions; if the amount of nitrate in a water is no greater than that in the $\frac{1}{8}$ nitre solution, 20 c.c. of the water may be at once taken for analysis.

A large supply of pure distilled oil of vitriol will be required for extensive water analysis. It should be colourless, quite free from nitrous compounds, and contain as little as possible of sulphurous acid, and must be of nearly full gravity. As any variation in the acid affects the determination, it is best to mix the contents of all the bottles purchased before proceeding to standardise the indigo. The oil of vitriol is measured for use in a tolerably wide burette provided with a glass stop-cock.

A further requisite is a chloride of calcium bath, provided with a thermometer; the bath is conveniently made in a porcelain basin. The temperature to be maintained is 140° . As the temperature keeps rising from the evaporation of the solution, it is necessary to bring it down to the required point by the addition of a little water, or chloride of calcium solution, immediately before each experiment. The chloride of calcium bath is not required when strong solutions of nitrate are analysed by a normal solution of indigo, the reaction in such cases being almost immediate. With weak solutions of indigo and nitrate the reaction may take some time, in extreme cases as much as five minutes, and it becomes essential for accuracy that the temperature should be maintained throughout at the normal point.

The standardising of the indigo solution is performed as follows:— 10 c.c. or 20 c.c. of the standard nitre solution are placed in a wide-mouthed flask of about 150 c.c. capacity, as much indigo solution is measured in as is judged sufficient, and the whole mixed. Oil of vitriol is next run from the burette into a test-tube, in quantity exactly equal to the united volumes of the nitre solution and indigo. The contents of the test-tube are then poured as suddenly as possible into the solution in the flask, the whole rapidly mixed, and the flask at once transferred to the chloride of calcium bath. It is essential for concordant results that the oil of vitriol should be uniformly mixed with the solution as quickly as possible. This is especially necessary in the case of strong solutions of nitrate in which the action begins immediately after the addition of the sulphuric acid; with such solutions it is more difficult to get duplicate experiments to agree than with weaker solutions in which the action does not begin at once, and in which therefore time is afforded for mixing. The operator should not attempt to drain the test-tube; the oil of vitriol adhering to the

tube is a fairly constant quantity, and after the first experiment the tube will deliver the quantity measured into it.

It is well to have the flask covered by a watch-glass while holding it in the chloride of calcium bath. The progress of the reaction should be watched, and as soon as the greater part of the indigo has been oxidised the contents of the flask should be gently rotated for a moment. With very weak solutions of pure nitre no change is observed for some time, and it may be necessary in some cases to keep the flask in the chloride of calcium bath for five minutes. If the colour of the indigo is suddenly discharged it is a sign that the nitric acid is in considerable excess, and that a considerably larger amount of indigo must be taken for the next experiment. If some of the indigo remains unoxidised, a little experience will enable the operator to judge its probable amount, and so decide on the quantity of indigo suitable for the next experiment.

The amount of indigo which corresponds to the solution of nitrate is found by a series of approximating experiments made as just described with varying quantities of indigo, the oil of vitriol used being always equal in volume to the united volumes of the nitrate solution and indigo. The determination is finished when a quantity of indigo is left unoxidised not exceeding 0.1 c.c. of the indigo solution used; this amount can be readily estimated by the eye. It is well, until considerable experience has been gained, to check the result by making a further experiment with 0.1 c.c. less indigo, when the colour should be entirely discharged. The tint produced by a small excess of indigo is best seen by filling up the flask with water. The estimated excess of indigo is of course deducted from the reading of the burette.

To reduce the number of experiments required to obtain the result it is well to proceed with some boldness, and ascertain as soon as possible what are the limits between which the quantity of indigo must fall. Seven experiments will be a maximum rarely exceeded; four experiments is about the average required at Rothamsted, where, however, the history of the waters examined is already known.

An attempt has been made to determine the excess of indigo present in the final experiment by means of a solution of permanganate of potassium. This plan, if successful, would greatly shorten the process, as only one experiment made with an excess of indigo would then be required. It was found, however, that the permanganate attacked the oxidised indigo as well as the unoxidised; the quantity of permanganate required to discharge the blue colour was thus greater with strong solutions of nitre, for which much indigo had been employed, than with weak solutions, for which little indigo had been required.

The direction has been given to employ oil of vitriol equal in volume to the united nitrate and indigo solutions. It is most important that

the same proportion of oil of vitriol should be used in every experiment, as the quantity of indigo oxidised is much affected by the proportion of acid present, being considerably greater when the acid equals the volume of the other solutions than when the acid is used in larger proportion. In all the published directions I am acquainted with the oil of vitriol is used in double the proportion here indicated. Not only, however, is the quantity of indigo oxidised much greater with the smaller proportion of acid, but the prejudicial effect of organic matter is less under these conditions, as shown in my earlier paper.

When the indigo solution has been standardised with the series of nitre solutions already mentioned, it will be found that the quantity of indigo consumed is not strictly in proportion to the nitric acid present, but diminishes as the nitrate solution becomes more dilute. In round numbers, a diminution of the amount of nitre present to $\frac{1}{5}$ is accompanied by diminution of the indigo oxidised to $\frac{1}{10}$; or, in other words, if 20 c.c. of the $\frac{1}{5}$ normal solution of nitre require 10 c.c. of indigo, 20 c.c. of the $\frac{1}{10}$ normal solution will require only 1 c.c. of indigo. This is a very important fact, and necessitates the standardising of the indigo with solutions of graduated strength, so that the value of the indigo may be known for all parts of its scale.

In my earlier paper attention was confined to solutions of indigo of normal strength. It was shown that when using a double volume of oil of vitriol, the quantity of indigo oxidised per unit of nitric acid was distinctly less in weak solutions of nitre than in strong; but when using a single volume of oil of vitriol the proportion of indigo oxidised was nearly constant for considerably varying strengths of nitre solution. Experience has since shown that the last statement does not hold good for weaker solutions of indigo and nitre. With a half-normal solution of indigo, 10 c.c. of a $\frac{1}{4}$ normal solution of nitre will oxidise distinctly less than half the indigo required by 10 c.c. of a $\frac{1}{2}$ normal nitre solution; and with the quarter-normal solution of indigo employed in water analysis, the differences produced by dilution become still more considerable.

In consequence of these facts it becomes necessary to form a table of the value in nitrogen corresponding to each part of the indigo scale, and by the help of this table every analysis subsequently made is calculated. Below is given an ideal table of this description. It is assumed, which is very near the truth, that a diminution to one-eighth in the strength of the nitrate solution is accompanied by a diminution to one-tenth in the indigo consumed. It is further assumed, which is also near the truth, that the alteration in the relation of the indigo to the nitrate proceeds at a uniform rate between the limits actually determined. The following will be the results arrived at when using 20 c.c. of the nitrate solution for each experiment:—

Value of the Indigo in Nitrogen for different strengths of Nitre Solutions.

Strength of nitre solution used.	Indigo required.	Difference between amounts of indigo.	Nitrogen corresponding to 1 c.c. of indigo.	Difference between the nitrogen values.	Difference in the nitrogen values for a difference of 1 c.c. in the amount of indigo.
	c.c.	c.c.	gram.	gram.	gram.
$\frac{8}{64}$ normal ...	10·00	—	·000035000	—	—
$\frac{7}{64}$ „ ...	8·71	1·29	·000035161	·000000161	·000000125
$\frac{6}{64}$ „ ...	7·43	1·28	·000035330	·000000169	·000000132
$\frac{5}{64}$ „ ...	6·14	1·29	·000035627	·000000298	·000000231
$\frac{4}{64}$ „ ...	4·86	1·28	·000036008	·000000381	·000000298
$\frac{3}{64}$ „ ...	3·57	1·29	·000036764	·000000756	·000000586
$\frac{2}{64}$ „ ...	2·29	1·28	·000038209	·000001445	·000001129
$\frac{1}{64}$ „ ...	1·00	1·29	·000043750	·000005541	·000004295

The mode of using this table is very simple. Supposing that 20 c.c. of a water have required 5·36 c.c. of indigo, this amount is seen to be 0·5 c.c. above the nearest point (4·86 c.c.) given in the table. We learn from the right-hand column that ·000000149 must consequently be subtracted from the unit value in nitrogen (·000036008 gram) belonging to 4·86 c.c. of indigo. We thus find that the 5·36 c.c. of indigo should be reckoned at ·000035859 gram of nitrogen per cubic centimeter: the water, therefore, contains 9·6 parts of nitrogen as nitric acid per million. If 20 c.c. of the water have required less than 1·0 c.c. of indigo, the unit-value corresponding to 1·0 c.c. of indigo is employed for calculating the result; this will give accurate results if the oil of vitriol employed is quite pure.

It is not necessary in practice to form so complete a table as that now given, it will suffice to determine the indigo with nitre solutions of $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, and $\frac{1}{64}$ normal strength: if another solution is used, that of $\frac{3}{64}$ normal strength seems the most important. It may be sufficient, even, in some cases simply to determine the indigo with the $\frac{1}{8}$ and $\frac{1}{64}$ nitre solutions, and to find all the intermediate values by calculation; examples will be presently given showing the amount of agreement between such calculation and experiment. If 10 c.c. of water are to be employed as well as 20 c.c., separate tables should be formed giving the values of the indigo in each case.

There is one important advantage that follows from this troublesome necessity of standardising the indigo with various nitrate solutions: it tends to remove the errors due to impurities in the oil of vitriol. As the volume of the oil of vitriol used depends far more on the volume of water taken than on the quantity of nitric acid it contains, all

errors due to oxidising or reducing matter contained in the oil of vitriol fall far more heavily on determinations of small quantities of nitric acid than on determinations of larger quantities. Unless, therefore, the oil of vitriol used was absolutely pure, it would always be necessary to standardise the indigo as now recommended, even if the relation of indigo to nitric acid were exactly the same in weak and strong solutions. By standardising as now described, the errors due to the sulphuric acid affect the figures of the table, but not the result of the analysis. Such standardising has also the effect, as far as it goes, of calibrating the burette, any inequalities of capacity falling on the table, and not on the analyses calculated from it.

In standardising the indigo solution, and in the subsequent experiments with it, some regard must be paid to the initial temperature of the solutions. A rise in the initial temperature will be attended by a diminution in the quantity of indigo oxidised; this is most perceptible in the case of the stronger solutions of nitrate. The following table shows the results obtained by standardising the same indigo solution at two temperatures, representing nearly the extreme limits at which the solution would be used in practice. The temperature of the room during the experiment was 10° ; for the trials at the higher temperature, the flask containing the indigo and nitrate, and the test-tube containing the oil of vitriol, were placed for some time in a water-bath at $22\text{--}23^{\circ}$ previously to being mixed.

Indigo Solution Standardised with 10 c.c. of Nitre Solution.

Strength of nitre solution.	At 10° .		At 22° .	
	Indigo required (actual).	Indigo calculated from two extremes.	Indigo required (actual).	Indigo calculated from two extremes.
$\frac{1}{4}$ normal...	10.28	c.c. 10.28	c.c. 9.76	c.c. 9.76
$\frac{1}{8}$ " ...	4.97	4.97	4.78	4.73
$\frac{1}{16}$ " ...	2.30	2.32	2.18	2.22
$\frac{1}{32}$ " ...	0.99*	0.99	0.96	0.96

* It must not be supposed that it was possible to work to one-hundredth of a cubic centimeter; in all the figures given the errors shown by calibrating the measuring vessels employed have been taken account of, and minute fractions thus introduced.

Standardised with 20 c.c. of Nitre Solution.

$\frac{1}{8}$ normal ...	10·26	10·26	9·74	9·74
$\frac{1}{10}$ " ...	4·84	4·96	4·65	4·74
$\frac{1}{32}$ " ...	2·21	2·31	2·21	2·24
$\frac{1}{64}$ " ...	0·99	0·99	0·99	0·99

It is seen that a rise of 12° in temperature diminishes the indigo consumed by about 5 per cent. in the case of the stronger solutions of nitrate; it is evident, therefore, that the indigo solution should be standardised at nearly the same temperature at which it is to be used.

In the table the quantity of indigo corresponding to the intermediate strengths of nitre solution has been calculated from the determinations made with the two extreme strengths, to show the amount of accuracy to be attained by this plan; the agreement of calculation with the results actually obtained is sometimes very close, but in others the difference is somewhat greater than the errors of experiment.

If the indigo solution has been standardised with 20 c.c. of the nitre solutions recommended, the operator will be able to analyse waters containing nitric acid up to 17·5 parts of nitrogen per million. If the indigo has also been standardised with 10 c.c. of the $\frac{1}{4}$ and $\frac{1}{8}$ nitre solutions, the operator can, by employing 10 c.c. of the water, extend his range of analysis to waters containing 34 parts of nitrogen per million. Waters stronger than this should be diluted for analysis. The natural error of the determination, shown by the performance of duplicate experiments, will not exceed 1 per cent. of the nitric acid present in the case of waters containing 17 of nitrogen per million, but may amount to 5 per cent. with waters containing as little as 2 of nitrogen per million.

Chlorides are of course generally present in waters containing nitrates, they are not altogether without effect on the determination with indigo. In my earlier paper it was stated that chlorides somewhat diminish the amount of indigo required. This is true in the case of moderately strong solutions, but with weak solutions a contrary effect is observed. The following results were obtained with 20 c.c. of nitre solution, with and without the addition of chloride of sodium:—

Strength of nitre solution.	Initial temperature.	Chlorine per million.	Indigo required.	Increase or dimi- nution of indigo by chlorine.
$\frac{1}{8}$ normal	10°	None	c.c. 10·15	c.c. —
$\frac{1}{16}$ "	"	100	10·00	-0·15
$\frac{1}{32}$ "	22°	None	9·65	—
$\frac{1}{64}$ "	"	100	9·53	-0·12
$\frac{1}{128}$ "	10°	None	10·25	—
$\frac{1}{256}$ "	"	50	10·10	-0·15
$\frac{1}{512}$ "	"	None	2·25	—
$\frac{1}{1024}$ "	"	100	2·35	+0·10
$\frac{1}{2048}$ "	"	50	2·35	+0·10

The presence of an abundance of chlorides thus occasioned an error of deficiency of about 1·5 per cent. in the case of a $\frac{1}{8}$ normal solution of nitre, and an error of excess of 4·4 per cent. in the case of a solution of one-quarter this strength. At some point between the two the error was probably nil. The different direction of the error is apparently due to the effect of chlorides on the duration of the reaction. As nitre solutions become weaker the time occupied by the reaction with indigo is lengthened, and the proportion of indigo consumed diminished. In the presence of chlorides the duration of the reaction in weak solutions is much shortened, and the quantity of indigo consumed is consequently increased. In the preceding experiments the time of the reaction in the weak solution was reduced by chlorides from $2\frac{1}{2}$ minutes to half a minute, while with the stronger solution the period (about half a minute) was but little affected.

The error introduced by chlorides is for many purposes insignificant. Thus the two nitre solutions employed above contained 17·8 and 4·3 of nitrogen per million; analysed in the presence of excess of chloride they yielded 17·5 and 4·5 per million. As in water analysis the amount of chlorine is always determined, it would be well to make this part of the analysis preliminary to the nitric acid determination. The indigo might also be standardised with nitre solutions containing excess of chloride, and a small quantity of pure chloride added to all waters deficient in this constituent. Finkener has already recommended the addition of chlorides to the standard nitre solutions.

Nitrites are unfortunately not capable of being determined by indigo; the amount of indigo oxidised is much less than in the case of nitrates, and the reaction is far from being sharp. When a dilute solution of a nitrite is mixed with an appropriate quantity of indigo solution, and oil of vitriol added, an immediate reaction takes place; whereas with a corresponding solution of a nitrate an appreciable time is occupied before the indigo is decolorised. When we next endea-

your to find the quantity of indigo equivalent to the nitrite, it is found that several different quantities of indigo yield almost the same final tint, and this tint is not discharged by heating in the chloride of calcium bath.

Nitrites may be easily converted into nitrates by treatment with permanganate of potassium, and may then be determined by indigo. A solution of commercial nitrite of potassium, which contained by the Crum-Frankland method 31.5 parts of nitrogen per million, gave only 22.1 parts of nitrogen when tested by indigo previously standardised with a nitrate. The same quantity of nitrite, in a more concentrated form, was treated with solution of permanganate, acidified with sulphuric acid, and permanganate slowly added till decolorisation no longer occurred. The solution was finally brought to the same volume as in the first experiment and tested with indigo; it now yielded 32.4 parts of nitrogen per million.

If chlorides are present as well as nitrites, free chlorine may be produced during treatment with permanganate, and the determination with indigo consequently come out too high; in this case it may be well to make the liquid slightly ammoniacal after the treatment with permanganate, and raise the temperature for an instant to boiling. This mode of proceeding, according to Boussingault, completely removes free chlorine.

The nitrites in a water may generally be converted into nitrates by simply leaving the water for some days in a half-filled stoppered bottle, in a dark place; this plan will of course be unsuitable in the case of a water containing ammonia, as the ammonia would under these conditions be converted into nitric acid.

The weakest point in the indigo method is its behaviour in the presence of organic matter. Boussingault showed long ago that the presence of sugar, gum, dextrin, and gelatin led to very low results, while oxalic, acetic, citric, and tartaric acids were without effect. He also found that the organic matter of rain-water had a marked influence on the result. In Boussingault's method the concentrated nitrate was boiled for some time with hydrochloric acid and a solution of indigo; it did not therefore certainly follow that organic matter would have as great an effect in the far speedier reaction here adopted. I found, however, as mentioned in my earlier paper, that a small quantity of cane-sugar greatly reduced the quantity of indigo oxidised by a nitrate, the effect of the sugar being greater in dilute solutions, in which the reaction was naturally more prolonged. The use of a large proportion of oil of vitriol also increased the effect of the sugar. I have since found that carbolic acid is an energetic reducing agent, while tartaric acid has been proved by several experiments to be without effect.

The first determinations of nitric acid in the watery extract of soils

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made by the indigo method gave results closely agreeing with those obtained by the Crum-Frankland method, and I was led to conclude that the soluble organic matter of soils had little influence. The first soils examined were both of them extremely rich in nitric acid, a small quantity of soil thus sufficed for the analysis, and the proportion of soluble organic matter to nitrate in the extract was consequently low. In subsequent analyses of ordinary samples of soil, the indigo method has always given results below those yielded by the Crum-Frankland method, which itself errs on the side of deficiency. The following are determinations of nitrogen existing as nitric acid in surface soils by the two methods; the results are expressed as per million of soil:—

Crum-Frankland method.	Indigo method.
729·3	730·4
222·4	224·6
8·5	6·6
4·5	3·5
3·1	2·2

There can be no doubt, therefore, that the soluble organic matter of soils has an injurious effect on the determination by indigo.

The indigo method cannot be employed with safety for the analysis of waters distinctly contaminated with organic matter. Unfortunately there appears to be no method of determining small quantities of nitric acid that is free from suspicion under these circumstances.

In the case of ordinary drainage waters the agreement between the indigo method and that of Frankland is very good. The following analyses of drainage waters refer to waters collected from unmanured arable land. The figures represent nitrogen per million:—

Crum-Frankland method.	Indigo method.
22·7	23·0
16·6	16·9
13·3	13·8

A series of comparative analyses, by the same two methods, of nitrified solutions of ammonium chloride, has been already published (p. 448 of this volume); the agreement between the results was generally good.

The general conclusion respecting the indigo method for determining nitric acid will be that it is excellently adapted by its simplicity, rapidity, and delicacy for general use in water analysis, but that accuracy can be secured only by working under the same conditions which

obtained when the indigo solution was standardised. In the presence of organic matter, the indications obtained with indigo must be accepted as probably below the truth.
