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THE COLORIMETRIC DETERMINATION OF AMMONIUM AND NITRATE IN WOODLAND SOILS

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1. INTRODUCTION

In order to survey woodland soils for quantities of ammonium-N and nitrate-N, and to investigate the possibilities of using such measurements to estimate rates of ammonification and nitrification, we require rapid methods for the quantitative estimation of ammonium-N and nitrate-N in such soils. This paper presents the results of tests in which colorimetric methods were used to determine ammonium-N and nitrate-N in soils from two local woods. These soils represent the extreme humus layer types, mull and mor, found in woods of the southern Lake District.

The problems in this type of work are (a) choosing a suitable extractant, (b) determining ammonium-N in the solution after extraction, and (c) determining nitrate-N in the solution after extraction.

(a) Choice of extractant

In general, the preparation of extracts for colorimetric determination of ammonium or nitrate in soils presents special problems, because it is necessary to obtain clear, colourless extracts which do not contain substances that interfere with the colorimetric methods available. Some colour in the extracts can be tolerated as long as this colour has a low light absorbance at the light frequency used for measuring the absorbance of the reaction colour. There are also problems associated with the nature of the ion to be extracted. Thus, many soils have the capacity to fix ammonium, i.e. to absorb ammonium in such a way that it is not readily exchangeable. The extent to which this ammonium can be removed by cation-exchange methods depends very largely upon the nature of the cation employed. Furthermore, several workers have shown that the release of fixed ammonium is blocked effectively by the presence of a small amount of potassium in the reagent used to effect release. These problems are reviewed by Bremner (1965), who noted that, in practice, most workers have found it convenient to define fixed ammonium in soil as ammonium which is not extractable by 1 N potassium chloride at laboratory temperatures, although Bremner stated that, in his own laboratory, 2 N potassium chloride was used.

In the past, methods employed for the extraction of nitrate from soils were developed on the assumption that nitrate is not held by the soil colloids, and can be readily extracted with water. This assumption may not always be valid, because it has been found that some soils have a significant anion-exchange (or anion-retention) capacity. 1 N or 2 N potassium chloride solution can be used to extract soil nitrate, and would allow both ammonium-N and nitrate-N to be determined on the same extract. A method which has been found very satisfactory for the extraction of nitrate is to shake the soil with water containing calcium oxide or potassium aluminium sulphate (Bremner, 1965).

(b) Determination of ammonium-N

Until recently, ammonium-N was commonly determined by one of the steam-distillation methods (Bremner, 1965). However, these methods are not very convenient to use, particularly if a large number of samples must be processed, and in recent years there has been a trend towards the use of colorimetric methods. This trend has been associated with the use of automatic equipment such as the Pye Unicam AC60 Chemical Processing Unit. In our tests, we used a modification of the Pye Unicam method for determination of ammonium-N in plant extracts. This method depends upon the formation of the blue dye, indophenol. The light absorbance of this dye is measured at 564 nm, and at this frequency light absorbance by colour which might be extracted from soil organic matter is not likely to cause serious interference.

(c) Determination of nitrate-N

Soil nitrate-N was originally determined by steam distillation, as in the case of ammonium-N. Subsequently colorimetric methods were developed, the most widely used of these being the phenoldisulphonic acid method (Bremner, 1965). However, the phenoldisulphonic acid method has certain disadvantages when used with soils, e.g. it is tedious and time-consuming, and it is subject to interference by chloride and nitrite ions. West and Lyles (1960) developed a procedure for the determination of nitrate in water samples using the sodium salt of chromotropic acid (4,5-dihydroxy-2,7-naphthalene disulphonic acid) for colour formation. Sims and Jackson (1971) adapted this method for use with soils, particularly with regard to fertilizer studies in agricultural soils. There does not seem to be any information in the literature on the use of this method for woodland soils, and so we have used this method in the tests described below.

Other problems occur in handling and storage of soil samples, because changes in amounts of ammonium-N and nitrate-N occur in soils during storage (Bremner, 1965). Many of these changes are biological, but storage below $0^{o}C$ is apparently not suitable, because of freezing and thawing effects (Allen and Grimshaw, 1962). It is evident that determinations of nitrate-N and ammonium-N should take place by the day after the samples are collected, or at least the extracts should be prepared by that time. Overnight, soils can be stored at about $2^{o}C \pm 1$ degree.

If it is not possible to determine the nitrate-N and ammonium-N contents of the extracts immediately after extraction, it may be necessary to store them overnight at $2^{o}C \pm 1$ degree, although this should be avoided if possible, as changes may occur in the extracts during storage. Bremner (1965) commented that the possibility that determinations of exchangeable ammonium and nitrate in soils may be invalidated by enzymatic reactions during preparation or storage of extracts appears to have been overlooked. This possibility should not be ignored, because soils are known to contain enzymes capable of transforming nitrogen compounds. Bartholomew (1928) found that oxidation of nitrite to nitrate occurred during aqueous extraction of soils for nitrite analysis. Lewis (1961) found that biological loss of nitrate occurred when aqueous suspensions of soils were stored for a few hours before analysis. He also showed that this loss did not occur with sterilized soils, and it could be prevented in unsterilized soils by use of 0.02 N cupric sulphate for extraction of nitrate.

Various reagents (e.g. toluene, chloroform) known to inhibit or retard microbial growth and activity have been used to supress microbial activity during preparation or storage of soil extracts, but little is known about their efficiency. In their instruction manual for the nitrate ion electrode, Orion Research Incorporated recommend the use of 10 ppm phenyl mercuric acetate as a biological inhibitor in the water used to make test and standard solutions. They state that this is not necessary if solutions are measured within 4 hours of preparation, but, because of the changes which may occur during extraction of soils, it would seem to be advisable to experiment with the use of an inhibitor in soil nitrate determinations.

2. DETERMINATION OF AMMONIUM-N

The colorimetric method used is based on Pye Unicam Automatic Analysis Method 21, for the determination of ammonium-N in acid digests of plant material. The principle of this method is that ammonium ions react with hypochlorite to form chloramine, which combines with phenol to give p-quinonechlorimine. The latter reacts with another molecule of phenol forming the blue dye indophenol. The reaction is catalysed by sodium nitroprusside. The method is extremely sensitive and it is necessary to ensure that all glassware is absolutely clean and that the laboratory atmosphere is free from ammonia. Smoking should not be allowed in the laboratory. Deionized water is probably better than distilled water for making up the solutions. Because this method was designed for use with acid digests of plant material, tests were carried out to find what modifications were necessary for use with soil extracts. Some of these are briefly described below.

Preparation of soil extract

Soils were passed through a 4 mm mesh sieve in their normally moist condition. A sub-sample of sieved soil was taken for determination of moisture content. Preliminary tests showed that results for ammonium-N extracted by 0.2% w/v calcium oxide in water were much greater after 30 minutes shaking than after 15 minutes, whereas with 2 N KCl extractant, results after 15 and 30 minutes shaking were similar. Therefore, only 2 N KCl was used in further tests.

Colorimetric reagents

(a) (Reagents 1 and 2). Phenol-sodium nitroprusside: Dissolve 5 ml of liquified phenol and 20 mg of sodium nitroprusside in 200 ml of deionized water.

(b) Stock hypochlorite solution: Standardize commercially-available sodium hypochlorite by adding excess HCl and titrating with sodium thiosulphate. Dilute the hypochlorite to 0.2 N (equivalent to available chlorine concentration of 0.75 g/100 ml).
It is probably better not to keep this stock solution more than one week.

(c) (Reagent 3). Working hypochlorite solution: Dilute the stock hypochlorite solution with an equal volume of 5 N NaOH.

Standard solutions

(a) Stock ammonia solution (1 mg NH_4^+-N per ml = 1000 ppm): Dissolve 0.3821 g of dry ammonium chloride (3 hr at 105°C) in the extractant solution as used for the soil and make up to 100 ml in a volumetric flask.

(b) Working standards (prepare freshly each day): 1 ml of stock ammonia solution made up to 100 ml with extractant solution, as used for the soil, in a volumetric flask gives a standard solution of 10 µg NH_4^+-N per ml (= 10 ppm). Make up an appropriate range of standards.

Tests of procedure

The method was designed for use with a Pye Unicam AC60 Chemical Processing Unit, which is fully automatic. Light absorbance of the test and standard solutions is determined at 564 nm, reagents being at 25°C. The method can be adapted for use with an ordinary spectrophotometer, provided that it has automatic sample-changing facilities, as the timing of addition of reagents and reading of light absorbance is critical. We found it reasonably easy to adapt this method for use with the Cecil Spectrophotometer with a turntable sample changer, but care must be taken with the timing, and a good stopclock should be used. The sequence given in the Pye Unicam method is as follows:

minutes before absorbance read	to 0.145 ml of sample add
14	2.0 ml of reagent 1
12	2.0 ml of reagent 2
10	0.4 ml of reagent 3

Because the nitrate content of soil extracts is likely to be very low, we initially increased the sample size to 1 ml. To simplify the procedure, reagents 1 and 2, being the same, were combined into a single addition of 20 ml. The volume of reagent 3 was increased to 2.0 ml. Time for development of maximum colour with this combination of reagents was tested on a standard ammonium-N solution

minutes after addition of reagent 3	% light transmission (20 µg/ml NH_4^+-N)
15	48
32	26
40	25
46	25
48	25
63	24.5

A similar test was run with different ammonium-N solutions:

Minutes	% light transmission		
	5 µg/ml	10 µg/ml	20 µg/ml
30	60.5	41.5	20.5
45	60.5	41.5	20.0

With this combination of reagents, there is evidently a plateau in the absorbance-time curve from 40 to 48 minutes, and probably from 32 to 63 minutes.

Tests with soils

For the preliminary tests, soil was collected from Meathop Wood at 0-5 cm depth and was passed through a 4 mm mesh sieve in the moist condition. Fresh soil was collected for each set of tests. The fresh weight of soil used and the ratio weight of soil:volume of extractant varied. Extraction of the soils was carried out with shaking.

Initial tests showed poor agreement between replicates, while recovery of added ammonium-N was generally much greater than 100%. We therefore changed the proportions of the reagents in the colorimetric procedure in order to gain sensitivity. Some of the early tests also showed the difficulty of plotting accurately a calibration curve of percentage transmission against ammonium-N on normal paper, and some of the errors were attributable to this.

In a second series of tests, the reagents were used in the following proportions:

2 ml sample
20 ml reagents 1 and 2
2.5 ml reagent 3

The calibration graph was drawn on log/normal paper, which gave a straight line with standard solutions up to 150 µg/ml. It was subsequently found to be more convenient to set the spectrophotometer to give a log output. Some results of tests using this modified procedure are given in Table 1. The results obtained with the spectrophotometer recorder set to give a log output **show** much better agreement between replicates than was obtained when the calibration graph was drawn on log/normal paper and the results read from this. Values for percentage recovery of added ammonium-N in Table 1 are good.

Further tests were carried out on recovery of added ammonium-N added to Meathop Wood soil and to soil from Low Fell wood, an acid soil under oak. The results are shown in Table 2. Most of the values for percentage of added ammonium-N recovered were in the range 101% ± 4, with two wild values outside these limits.

Conclusions

The results suggest that the Pye Unicam method can be modified **to determine ammonium-N in 1 N or 2 N KCl extracts of soils. With the soils used, the results were similar after 15 minutes.** It is probably better to use 10 g of fresh soil rather than 5 g, because the sieved soil is sometimes very variable, e.g. in organic matter content. A soil:extractant ratio of 1:5 gave similar results to those obtained with a ratio of 1:10. For the method to be completely acceptable, it would need to be compared with a standard distillation method, but time did not allow this in the present project.

Table 1.

Results of second series of tests on extraction and determination of ammonium-N from Meathop Wood soil. Extractant 2 N KCl, shaking time 15 min, replicate determinations on each extract

Extract	Soil fresh wt. (g)	Ratio soil:extractant	NH_4^+-N in unamended soils µg/g OD	Total NH_4^+-N added (µg)	Total NH_4^+-N in extract (µg)	Recovery of added NH_4^+-N (µg)	Recovery %
I							
1a	5	1:5	46.6	-	140.8	-	-
1b	5	1:5	52.1	-	157.5	-	-
2a	5	1:5	-	125	276.6	127.4	101.9
2b	5	1:5	-	125	282.3	133.1	106.5
3a	5	1:10	51.2	-	154.9	-	-
3b	5	1:10	50.5	-	152.8	-	-
4a	5	1:10	-	125	267.2	113.3	90.6
4b	5	1:10	-	125	277.3	123.4	98.7
II							
1a	10	1:5	57.4	-	347.4	-	-
1b	10	1:5	53.5	-	323.7	-	-
2a	10	1:5	53.5	-	323.7	-	-
2b	10	1:5	56.6	-	342.6	-	-
3a	10	1:5	-	250	571.8	237.4	95.0
3b	10	1:5	-	250	571.8	237.4	95.0
4a	10	1:5	-	250	571.8	237.4	95.0
4b	10	1:5	-	250	591.9	257.5	103.0
Stored extracts from II measured with spectrophotometer recorder set to give log printout							
1a	10	1:5	50.4	-	304.8	-	-
1b	10	1:5	49.2	-	297.8	-	-
2a	10	1:5	50.4	-	304.8	-	-
2b	10	1:5	50.4	-	304.8	-	-
3a	10	1:5	-	250	541.2	238.1	95.2
3b	10	1:5	-	250	547.1	244.0	97.6
4a	10	1:5	-	250	623.7	320.6	128.2
4b	10	1:5	-	250	547.1	244.0	97.6

Table 2.

Recovery of ammonium-N added to soils from Meathop Wood and Low Fell Wood. Extracted with N KCl, soil:extractant ratio 1:5

Extract	shaking time (min)	soil fresh wt (g)	NH_4^+-N added (ppm)	NH_4^+-N found (ppm)	added NH_4^+-N recovered (ppm)	Recovery %
I Meathop Wood soil						
1a	15	10	-	13.4	-	-
1b	15	10	-	13.2	-	-
2a	15	10	-	13.9	-	-
2b	15	10	-	14.0	-	-
3a	15	10	14.6	29.6	16.0	109.5
3b	15	10	14.6	28.9	15.3	104.8
4a	15	10	14.6	28.2	14.6	100.0
4b	15	10	14.6	27.9	14.3	97.9
II Meathop Wood soil						
1a	15	10	-	10.9	-	-
1b	15	10	-	10.5	-	-
2a	15	10	34.8	46.0	35.3	101.4
2b	15	10	34.8	44.8	34.1	98.0
3a	30	10	-	11.15	-	-
3b	30	10	-	11.43	-	-
4a	30	10	34.8	45.5	34.2	98.3
4b	30	10	34.8	45.2	33.9	97.4
III Low Fell Wood soil						
A horizon						
1a	30	5	-	5.8	-	-
1b	30	5	-	5.8	-	-
2a	30	5	35.0	37.4	31.6	90.3
2b	30	5	35.0	40.2	34.4	98.3
3a	30	5	70.1	73.6	67.8	96.7
3b	30	5	70.1	76.4	70.6	100.7
H layer						
4a	30	5	-	30.1	-	-
4b	30	5	-	31.8	-	-
5a	30	5	61.0	94.9	63.9	104.8
5b	30	5	61.0	100.4	69.4	113.8
IV Low Fell Wood, H layer						
1a	30	10	-	13.8	-	-
1b	30	10	-	13.5	-	-
2a	30	10	47.0	61.7	48.0	102.1
2b	30	10	47.0	60.1	46.4	98.7
					mean	100.8

Addendum

After this work had been completed, a programming attachment was acquired for the spectrophotometer. This programmer enables repeated readings to be taken on the same solution at predetermined time intervals. Using this equipment, C. B. Benefield plotted the colour development of a standard solution containing 10 ug NH_4^+-N/ml with (a) the reagents in the proportions used in our final series of tests, and (b) the reagents in the proportions used in the Pye Unicam method, based on ≈ 2.0 ml sample. The results, which are shown in Figure 4, show that the sensitivity in (a) is greater than in (b) at this ammonium-N concentration, which is within the range of concentrations found in our extracts. Full colour development was reached after 15 minutes in (a) and 11 minutes in (b). This agrees with the time given in the Pye Unicam method. It appears that our determination of the rate of colour formation probably contained an erroneous reading at 15 minutes. It is clear from Figure 4 that the colour formed 15 minutes after addition of reagents is stable for at least another 10 minutes, and probably longer. This provides an adequate time margin to allow the method to be used in conjunction with a spectrophotometer equipped with an automatic sample changer, as described above.

Comparisons were made by C. B. Benefield of the results obtained with (a) the colorimetric method, and (b) the standard steam distillation method, on N KCl extracts of two soils. The results were as follows:

test solution		μg NH_4^+-N/ml found by colorimetric method	μg NH_4^+-N/ml found by steam distillation
Soil extract (1)		2.0	2.5
		2.0	2.3
		2.0	2.3
		2.0	2.3
		2.0	2.3
	mean	2.0	2.3
Soil extract (2)		4.5	5.0
		4.0	5.0
		4.0	4.5
		5.5	5.0
		5.0	5.0
	mean	4.6	4.9
Standard solution 10 μg NH_4^+-N/ml		-	10.0
			9.8
			9.8

These results show satisfactory agreement between the two methods. The colorimetric method appears to give a result 0.3 μg/ml (i.e. 0.3 parts per million) lower than given by the steam distillation method.

3. DETERMINATION OF NITRATE-N

The method of Sims and Jackson (1971) was used on soil samples from Meathop Wood and Low Fell wood. In this method, chromotropic acid (CTA) forms a yellow complex with nitrate, and the reaction colour is measured at 430 nm. The precise nature of the chemical reaction is unknown. Sims and Jackson used a 0.2% w/v $Ca(OH)_2$ solution as extractant, but as we wished to determine both nitrate and ammonium on the same extract, we also used 1 N KCl solution. We did not learn of the method of Clarke and Jennings (1965) until all our studies had been completed.

Methods

The stock solution of 0.1% w/w chromotropic acid (CTA) was prepared by dissolving 0.184 g of CTA in 100 ml of concentrated H_2SO_4 (SG 1.84). Because this solution darkens with time, fresh stock solution was made up each week. The working reagent, 0.01% w/w CTA, was prepared by diluting the stock solution with concentrated H_2SO_4. Sims and Jackson (1971) prepared the working solution of CTA by adding 10 ml concentrated HCl to 100 ml of CTA stock solution before diluting to 1 litre with concentrated H_2SO_4, as West and Lyles (1960) stated that the inclusion of Cl^- ions in the working solution increases the sensitivity of the method. We omitted the HCl when N KCl solution was used as extractant.

Working standard solutions containing from 1 to 10 ppm were prepared by diluting a stock solution, containing 1 mg NO_3^--N per ml in water, with the appropriate extractant solution.

Soil extracts were prepared by shaking 10 g fresh soil with 50 ml of extractant for 30 min, followed by filtration. 7 ml of CTA reagent were added to 3 ml of soil extract. After cooling the extract to room temperature, light absorption was measured at 430 nm, using 7 ml of CTA reagent plus 3 ml of extractant solution as a reference. For calibration, 7 ml of CTA reagent were added to 3 ml of each standard nitrate solution and light absorption was read as above.

Recovery of added nitrate was examined by adding standard nitrate solution to 10 g soil samples and comparing the nitrate contents of extracts of these samples with those of unamended samples.

In determining the absorption spectra of soil extract + CTA and soil extract + H_2SO_4, 0.4% w/v $Ca(OH)_2$ was used, as Sims and Jackson (1971) found that this concentration eliminated interfering colour in some tests.

Results and discussion

Sensitivity was greater with calcium hydroxide extractant than with potassium chloride extractant, and the slope change noted by Sims and Jackson (1971) and by West and Lyles (1960) was present (Fig. 1). In spite of the lower sensitivity, potassium chloride extractant worked quite well with Meathop Wood soil samples and high recovery of added nitrate was possible (Table 3). However, in some of these samples a pink-purple colour developed after addition of the CTA reagent, and this colour interfered with the measurement of the light absorption due to the normal yellow colour of the CTA-nitrate complex. Sims and Jackson (1971) found that addition of sufficient nitrate to the soils to give 2 ppm in the extracts prevented the development of the interfering colour. In many of our tests, such treatment prevented the development of this colour, but in others the colour persisted.

The calcium hydroxide extracts of all the soil samples were highly coloured, presumably due to extraction of organic substances such as humic acids. Although this colouration could be allowed for, to some extent, by blanks consisting of soil extract with water added instead of reagents, the results were unsatisfactory in that recovery of added nitrate was low (Table 4), and addition of nitrate to the soil samples to give 2 ppm in the extract generally produced a lower light absorbance than was obtained for the 2 ppm standard solution alone.

The difficulties we experienced with interfering colours, both the pink·purple reaction colour and the coloured material extracted from soil by calcium hydroxide, caused us to study the light absorption spectra of the reagents and extracts. The absorption spectra of the yellow CTA-NO_3 complex given by Sims and Jackson (1971) show a peak in absorbance at 430 nm. However, their spectra did not go below 350 nm, and at that frequency, absorbance was still rising. We therefore examined the absorption spectra of Low Fell Wood extracts after reacting with either CTA reagent or concentrated sulphuric acid alone, (Figs. 2 and 3). Low Fell Wood samples were used as these had given us consistent formation of the pink-purple colour. It is clear from Figs. 2 and 3 that the maximum absorbance does not occur at 430 nm (Sims and Jackson, 1971), but at 360 to 320 nm (West and Lyles, 1960, Clarke and Jennings, 1965). It is noteworthy that a very large part of the light absorbance is due to soil extract plus sulphuric acid only, i.e. the background colour is in excess of the reaction colour.

Clarke and Jennings (1965) used the chromotropic acid method on 0.02 N copper sulphate extracts of soils with organic carbon contents of 2 to 6%. They found that colloidal organic matter must be removed from the extract before chromotropic acid is added. They did this by precipitating copper hydroxide from the extracts as recommended by Harper (1924) and Lewis (1961). However, Clarke and Jennings noted that soluble organic materials may interfere by reacting with chromotropic acid.

The above results show that difficulties may be encountered when the chromotropic acid method of Sims and Jackson (1971) is used on woodland soils. Difficulties were encountered due to: (a) background colour in calcium hydroxide extracts, (b) the formation in all the mor samples, and many of the mull samples, of a persistent pink-purple colour which masked the reaction colour, and (c) the anomalous recoveries of nitrate added to the mor samples. These difficulties appear to arise from the greater content of organic matter in these woodland soils than in the agricultural soils for which the method has been used (Sims and Jackson, 1971). The nature of the soil organic matter may also be important. Background colour due to colloidal organic matter can be removed, but reactions between soluble organic matter and chromotropic acid may occur.

Conclusions

The lack of knowledge of the precise chemical reaction(s) involved in this method, together with the difficulties experienced in our tests, indicate that more work is needed on this method if it is to be used on woodland soils. Furthermore, the fact that concentrated sulphuric acid is used as a solvent makes the method unattractive to use on a routine basis. The Orion nitrate electrode seems to be preferable for the purpose of rapidly determining nitrate in soil extracts for nitrification studies.

Acknowledgement

The authors thank Mr. C. B. Benefield for discussion on various aspects of this work.

Table 3.

Recovery of nitrate added to Meathop Wood soil, using N KCl extractant

	NO_3^--N ppm OD basis		
Sample	added	detected	% recovery
1	0	7.1	
	28.1	35.2	100.3
2	0	21.5	
	97.1	115.7	97.0

Table 4.

Recovery of nitrate added to Low Fell Wood soil, using $Ca(OH)_2$ extractant

	NO_3^--N ppm OD basis		
Sample	added	detected	% recovery
1	0	8.2	
	29.2	27.3	65.4
3	0	16.2	
	17.3	20.6	25.4

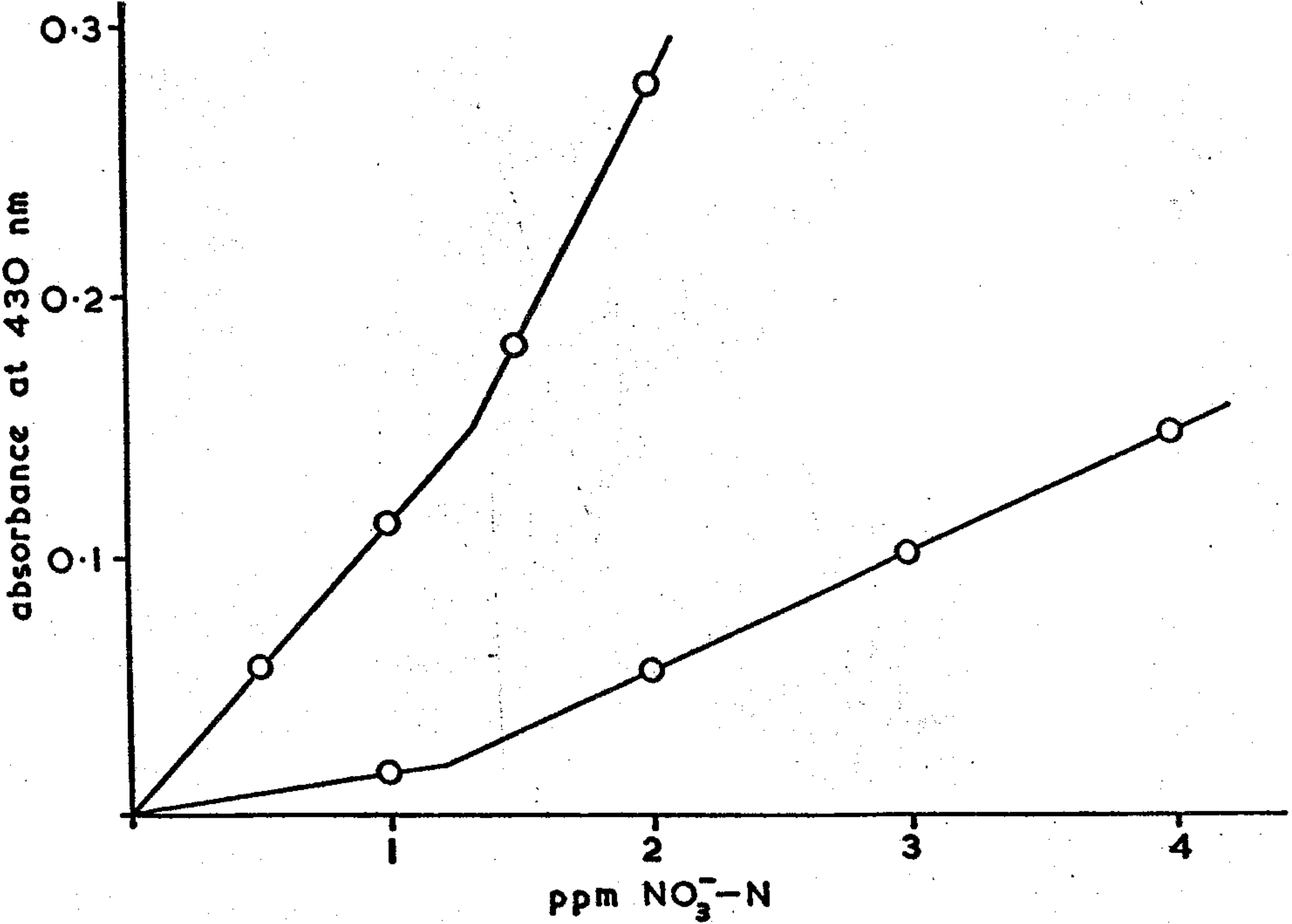

Fig.1 Light absorbance by CTA–NO_3 complex at 430 nm as a function of NO_3 concentration in $Ca(OH)_2$ and KCl solution

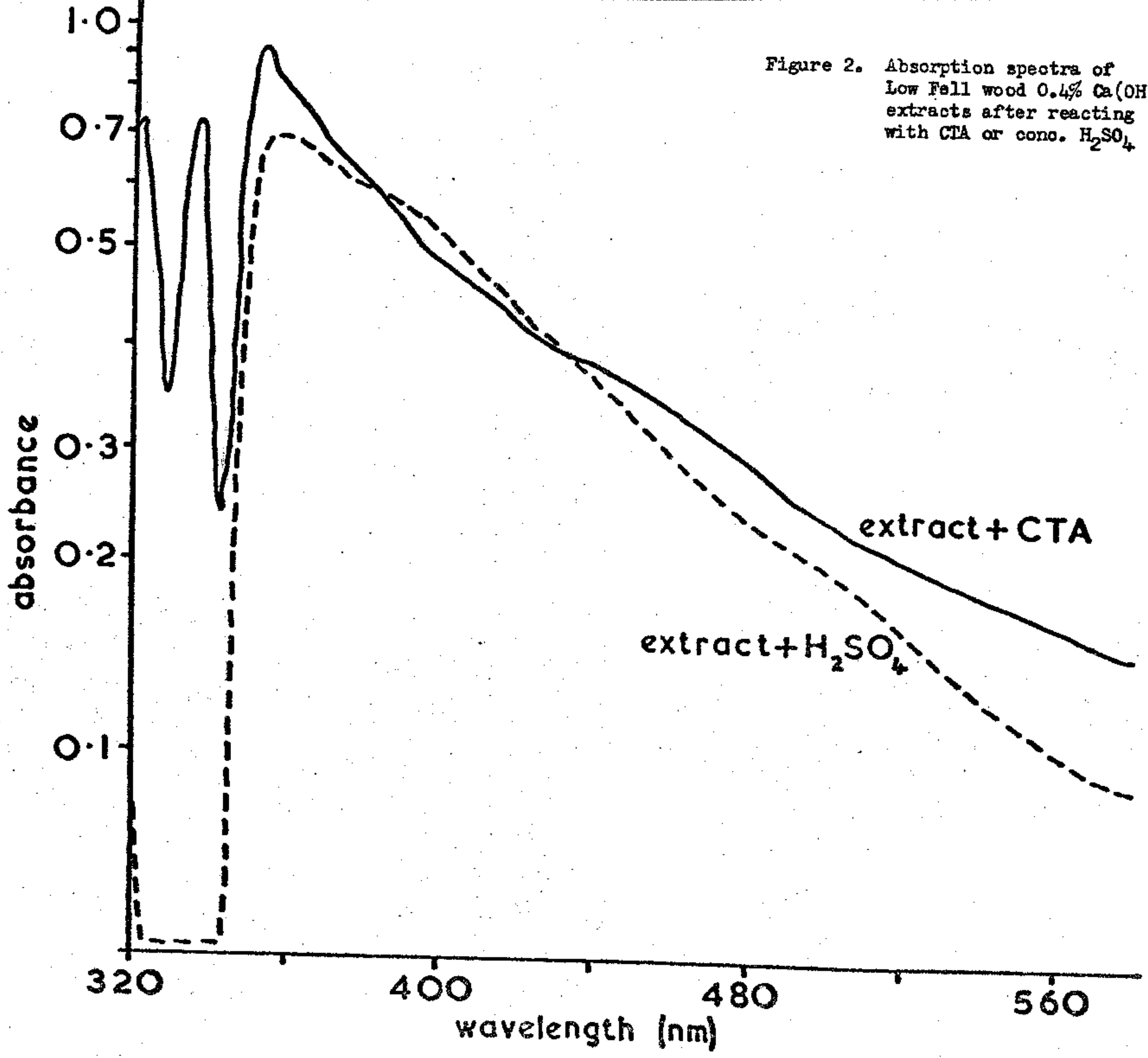

Figure 2. Absorption spectra of Low Fell wood 0.4% $Ca(OH)_2$ extracts after reacting with CTA or conc. H_2SO_4

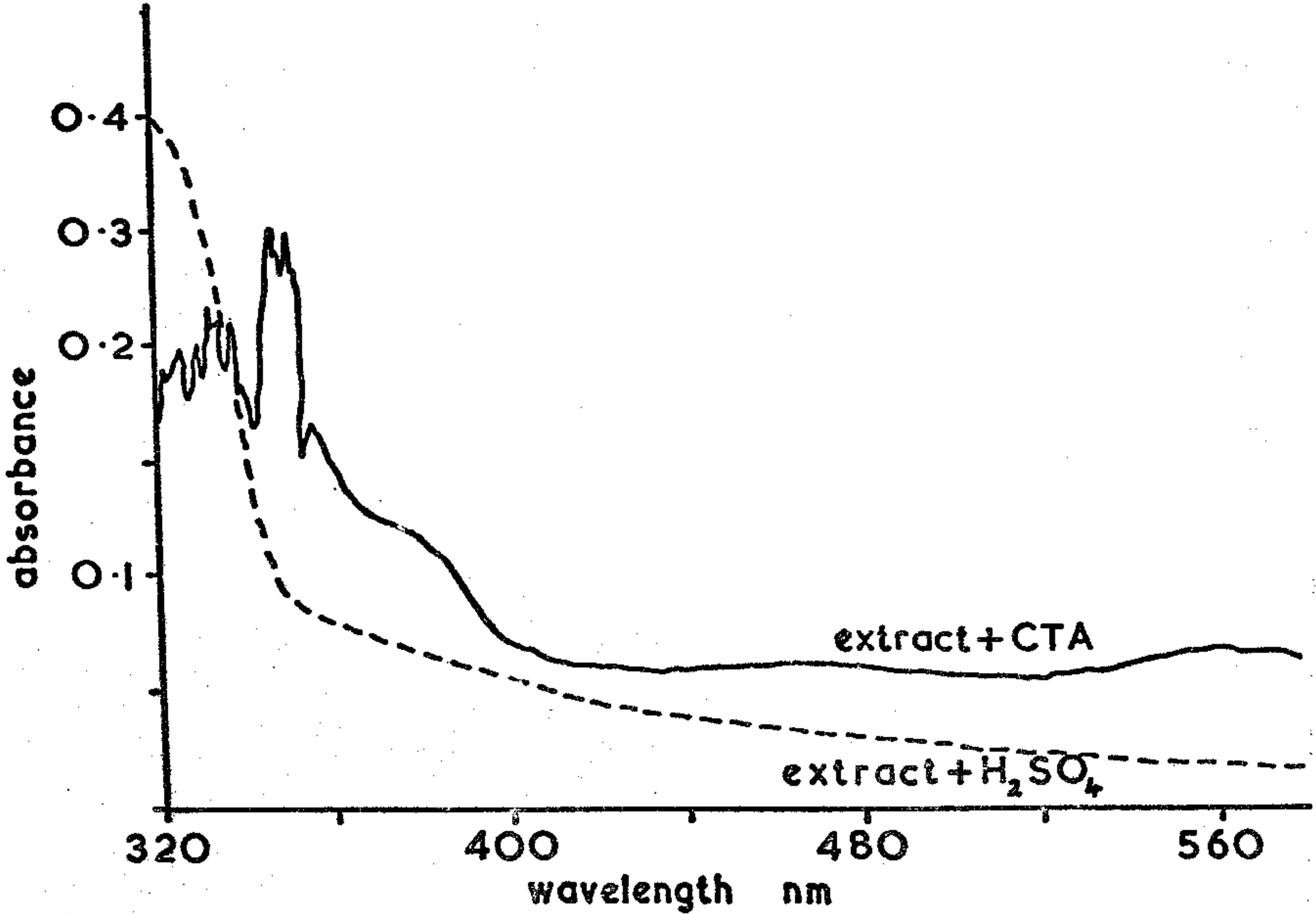

Fig.3 Absorption spectra of Low Fell wood KCl extracts after reacting with CTA or conc H_2SO_4

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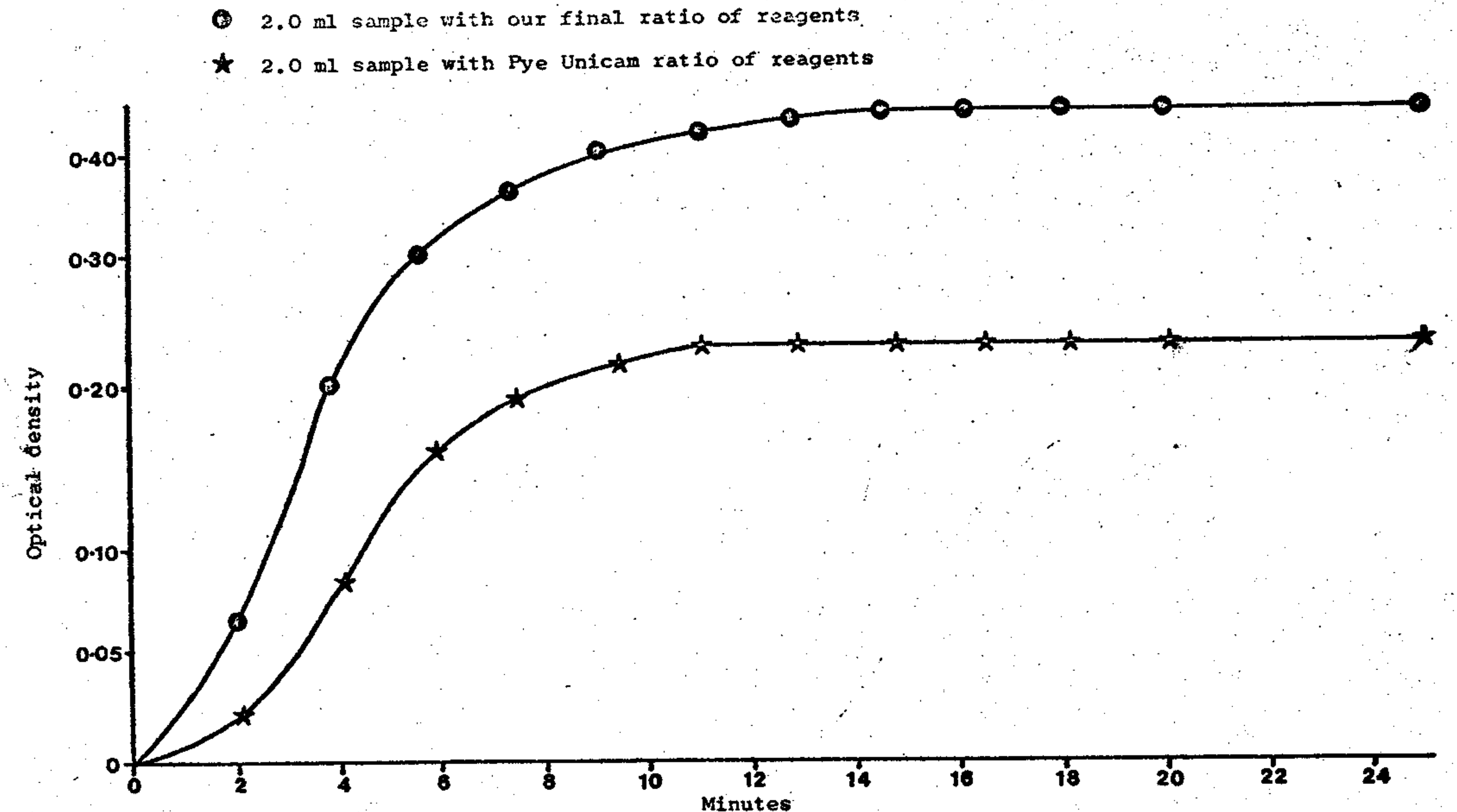

Figure 4. Comparison of colour formation in 2.0 ml samples of standard solution, containing 10 µg NH_4^+-N/ml, with different reagent ratios.