THE USE OF SELE
IN
KJELDAHL NITROGE

THESIS FOR THE

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THE USE OF SELENIUM COMPOUNDS
IN THE
KJELDAHL NITROGEN DETERMINATION

A THESIS

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by

Clark Sherman Chamberlain

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INTRODUCTION

The determination of nitrogen is one of the most common quantitative determinations made in an analytical laboratory. Because of the importance of nitrogen in all matters pertaining to nutrition, the determination of this element is one of the most important, if not the most important, to every food analyst. This determination is also of great importance in Soils and Farm Crops laboratories. The organic chemist is constantly in demand of the exact amount of nitrogen present in synthetic compounds. Since the value of fertilizers is based on nitrogen content, we find that fertilizer laboratories are constantly making use of nitrogen determinations. Due to the universal application of the Kjeldahl-Gunning-Arnold official method, any change which would shorten the length of the process without affecting the accuracy of the determination or increasing the cost more than the amount that the labor item is decreased, would prove a benefit to thousands of analytical chemists.

There has been enough work done in the last few years to prove that the use of selenium compounds, materially shortens the process. With this in mind the author in the work here outlined has endeavored to carry on a detailed investigation using various selenium compounds, in an effort to determine the extent of their efficiency over the official method, and also to determine whether or not the accuracy of the process is affected in any way.

To the knowledge of the author this is the first work available as to the use of selenium dioxide as a catalyst in the nitrogen determination.

Before dealing with the adaptability of new catalysts it will be beneficial to review the history of the original Kjeldahl process and study its chemistry.

HISTORY OF NITROGEN DETERMINATION

In the past there have been three different types of methods used. First, the absolute method of Dumas, which involves the dry combustion and reduction of the gaseous products by a copper foil and measurement of the nitrogen formed. Second, the method of Will and Warrentrapp in which the material is heated with soda lime and the ammonia formed either titrated or weighed as ammonium platinic chloride. Third, the method of Kjeldahl, devised by Kjeldahl in 1883.

The first two methods are the oldest. They are highly accurate, however, very time consuming and laborous. The Kjeldahl method with its modifications is so much more simple and short that it is at the present time almost universally employed by commercial chemists.

ORIGINAL KJELDAHL PROCESS

The process was first introduced in 1883. It consisted of the production of ammonium sulphate by moist combustion of the nitrogenous substance with concentrated sulphuric acid. The salt thus formed was subsequently distilled with an excess of caustic alkali. The free ammonia so produced is absorbed in a known volume of standard acid solution present in excess, the risidual amount being determined by titration. The method as originally introduced was applicable only to nitrogenous organic compounds in the absence of nitrates.

During the moist combustion in the original method, dry powdered potassium permanganate was added little by little to the hot liquid in the digestion flask until a permanent green color was produced; the object being to hasten the process of decomposition. It is extremely interesting to note that while potassium permanganate is a reagent upon which, to a large extent, the Kjeldahl method was originally based, the process has in the course of time been so modified that the desirability of its use is extremely doubtful, and in some cases it has been found to give low results owing to the destruction of a portion of the ammonia.

THEORY OF REACTIONS IN ORIGINAL PROCESS*

I. Sulphuric acid abstracts from the organic matter the elements of water.

II. The sulphur dioxide produced by the action of the risidual carbon on sulphuric acid exercises a reducing effect on the nitrogenous bodies present.

III. From the nitrogenous bodies produced by the above reduction, ammonia is formed by the action of an oxidizing body.

IV. The ammonia thus formed is at once fixed by the strong acid as ammonium sulphate.

$$\text{Nitrogenous Body} + H_2SO_4 \longrightarrow (NH_4)_2SO_4$$
$$(NH_4)_2SO_4 + NaOH \longrightarrow NH_3 + Na_2SO_4 + HOH$$
$$NH_3 + HCl \longrightarrow NH_4Cl$$

THE GUNNING MODIFICATION

Since Kjeldahl's article** first appeared, a number of investigations have been made by various chemists and a seemingly endless number of articles have been written on the subject. A great deal of good work has been done, al-

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*Volumetric Analysis 11th. Ed., Sutton, pp. 85.
**Zeitschrift für analytische Chemie - 23, 366 (1883)

though the results reported in some instances would indicate that the experimental work was not of a satisfactory nature. In these articles many modifications are suggested and the use of a great number of chemicals has been investigated, however, only a few of these have introduced features which are of importance to the Kjeldahl method. Of all these the modification of I. W. Gunning introduced in 1889 deserves first mention*.

This modification was based upon the observation that in the ordinary Kjeldahl process the excess sulphur trioxide in the beginning of the operation soon escapes or unites with water in a form not easily decomposed. During this process the acid diminishes in strength and in this diluted condition the oxidation takes place more slowly. To remedy this difficulty Gunning proposed the use of potassium sulphate. The salt forms with the acid, acid salts which on heating lose water more easily than the acid alone; the acid salts supplement the decomposing and oxidizing power of the sulphuric acid in a valuable manner.

Upon heating the mixture of sulphuric acid and potassium sulphate with organic matter, not only the water

*Agricultural Analysis Vol. II - Wiley - pp. 370.

originally present, but also that which is formed during the oxidation, is driven off without loss of the acid. For this reason instead of the oxidizing mixture becoming weaker, the acid becomes stronger, the boiling point of the mixture rises and this combined with the fluidity of the mass, favors the decomposition and oxidation of the organic matter in a constantly increasing ratio.

REACTIONS OF THE GUNNING MODIFICATION

The various reactions which take place during the combustion as tabulated by Van Slyke follow*.

The first reaction to take place is the union of sulphuric acid and potassium sulphate in accordance with the following equation:

$$(1) \quad K_2SO_4 + H_2SO_4 \longrightarrow 2KHSO_4$$

When heated the potassium acid sulphate decomposes forming potassium disulphate and water, thus:

$$(2) \quad 2KHSO_4 \longrightarrow K_2S_2O_7 + H_2O$$

The potassium disulphate at higher temperatures decomposes forming normal potassium sulphate and sulphur trioxide, thus:

$$(3) \quad K_2S_2O_7 \longrightarrow K_2SO_4 + SO_3$$

*Division of Chemistry, Bulletin 35, 1892:68

At a sufficiently high temperature the two succeeding reactions may take place as one, thus:

$$2KHSO_4 \longrightarrow K_2SO_4 + SO_3$$

At the temperature at which these reactions take place the water that is set free does not recombine with the sulphur trioxide nor with the sulphuric acid present in excess, but is expelled from the mixture, hence the mixture becomes more concentrated. The sulphur trioxide acts on the organic matter in a powerful manner and the potassium sulphate formed in the last reaction above unites with another molecule of sulphuric acid, and the same round of reactions is repeated continuously as long as there is an excess of sulphuric acid present.

The credit for the introduction metallic oxides into the process to serve as catalysts is shared jointly by Arnold* and Wilfarth**; copper and mercury oxides have been used commonly. When mercuric oxide or metallic mercury is used it is necessary to add sufficient potassium sulfide solution to precipitate the mercury before distillation. This prevents loss of ammonia by formation of non-volatile mercuro ammonium compounds.

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*J. A. O. A. C. - 10, 507 (1927).
**Agricultural Analysis, Vol. II, Wiley, pp. 356.

REVIEW OF WORK WITH SELENIUM

It was first discovered by Lauro* that selenium and its compounds might be used to advantage as catalysts in the Kjeldahl nitrogen determination. In 1931 Mr. Lauro was working on an entirely different project; the chemistry of the rare earths, when he stumbled upon the fact that metallic selenium might be a good catalyst. He found that it was possible to shorten the period of digestion to one-fifth of the time required in the original method. Also this eliminated the necessity of precipitating the mercury before distillation of the nitrogen. Lauro recommends the use of finely powdered metallic selenium rather than selenium oxychloride, as it is much more pleasant to work with.

Mr. C. E. Rich** in working with cereals found that the best combination of catalysts for cereal work was metallic copper and fuming selenium oxychloride (dispensed by means of a medicine dropper, calibrated to deliver .2 c.c.). There is a two fold reason for using both catalysts; the combination accelerates the reaction more than when either is used alone, and for the indicator properties of the copper in turning the solution a deep blue when an excess

*Ind. and Eng. Chem. Anal. Ed. - 3, 401-2 (1931)
M. F. Lauro
"Modification to the Kjeldahl Method with Selenium".

**Cereal Chemistry - 9, 118-120 (1932)
C. E. Rich
"$SeOCl_2$ in the Kjeldahl Determination".

of alkali has been added prior to distillation. Mr. Rich found that with this method the solutions were clear in fifteen minutes and digestion was complete in thirty minutes. This constitutes a time saving of thirty-five minutes per sample over the original method.

Mr. R. M. Sanstedt* carried out an investigation as to the catalytic properties of metallic selenium, as compared to copper and mercury, working with wheat and bran flour. He made the following conclusions: "Metallic selenium acts more rapidly than metallic copper, and about the same as mercuric oxide. The digestion with selenium and with mercuric oxide is complete in thirty minutes, while with copper sixty minutes is necessary. It appears that there is greater danger in losing nitrogen by extremely long digestion with selenium than with the other catalysts. The cost of catalyst per determination was found to be .15 cents as against .48 cents for .7 gram mercuric oxide or about .003 for .1 gram copper".

Mr. Harry C. Messman** working in an elevator company in Enid, Oklahoma conceived the idea of using a flux in the

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*Cereal Chemistry - 9, 156-157 (1932)
R. M. Sandstedt
"Selenium as a Catalyst in the Kjeldahl Method".

**Cereal Chemistry - 9, 357 (1932)
Harry C. Messman
"Metallic Selenium in the Kjeldahl Method".

Kjeldahl determination made up as follows: 90 parts Na_2SO_4, 7 parts $HgSO_4$, 1½ parts $CuSO_4$, 1½ parts powdered Se. He used 8 grams of this flux in each determination and found that constant and accurate results were obtainable after fifteen minutes digestion.

The selenium modification has also found favor in the laboratories of the New York State Hospital* where it is necessary to determine the nitrogen content of various aqueous extracts as a method of standardization. By use of powdered metallic selenium they were able to do the same amount of work in one-half the length of time originally consumed. Results were good.

R. A. Osborn and Alexander Krasnitz** working in the Bureau of Chemistry and Soils in Washington, contributed additional work on the use of selenium and its compounds. They did considerable work on the effects of the use of selenium on the Kjeldahl determination. Their work was done with 400 watt, calibrated burners, working on the time required to heat the samples after the time of clearing. They concluded that if the samples were heated for a period

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*Ind. and Eng. Chem. Anal. Ed. - 4, 410 (1932)
J. Tennant, H. L. Harrell and A. Stull
"Selenium in the Kjeldahl Method".

**J. A. O. A. C. - 16, 110 (1933)
R. A. Osborn and Alexander Krasnitz
"Comparison of Selenium, Mercury and Copper as Catalysts".

of ten to fifteen minutes after clearing it was sufficient to obtain good results. Periods of digestion were tried all the way from twenty-five minutes to three hours and it was found that results after three hours were the same as after twenty-five minutes. This disproved the old belief that long periods of heating with selenium resulted in losses in the nitrogen content. They concluded that the use of $SeOCl_2$ had a slight advantage over $CuSO_4$ but no advantage over HgO. Metallic selenium was found to be more suitable and economical than the $SeOCl_2$. They found that a combination of Se and HgO and $CuSO_4$ had an advantage over any of the three when used alone.

Mr. L. V. Taylor* of the Missouri Department of Agriculture, carried out an investigation on the efficiency of metallic selenium, mercuric oxide, and a combination of the two. His work was done on animal feeds. He found that accurate results were obtained after thirty minutes digestion in the presence of the mercuric oxide-selenium combination. However, with mercury alone accurate results were not obtained until after sixty minute digestion. He reports that there is no significant differences between the relative efficiencies of mercuric oxide and selenium when each is used alone.

*Ind. and Eng. Chem. Anal. Ed. - 5, 363 (1933)
L. V. Taylor, Jr.
"Use of a Selenium-Mercuric Oxide Combination in Determination of Nitrogen in Feed Materials".

With this brief but comprehensive survey of the work done with selenium compounds, we have all the information that is available. Thus, we find that the use of selenium compounds is a comparatively new subject and is still a matter to be carefully investigated. In the work that is to follow a new compound of selenium, which has, here-to-fore never been reported as a catalyst in the nitrogen determination, is used. It is well to study the chemistry of selenium and its compounds before outlining the work.

CHEMISTRY OF SELENIUM

The element selenium was discovered by Berzelius in 1817, in the flue dust of a sulphuric acid plant. Selenium belongs in the chemical family of sulphur and tellerium; it is a non-metallic element and exists, as does sulphur, in several allotropic forms: amorphous, crystalline and metallic. A red form may be obtained from reduction of selenious acid (H_2SeO_3); the black form is obtained by melting the red. Selenium compounds in general resemble those of sulphur in composition as well as in properties. Frequently metallic selenium is found in small quantities in natural sulphur, and quite often in various metallic ores. It may be separated from a pulverized ore by treating with hydrochloric acid to dissolve earthy carbonates. The washed and dried residue is then ignited with potassium carbonate and charcoal, this treatment converts the selenium to potassium selenide which upon treatment with boiling water is dissolved away from the oxides formed at the same time. The water solution when exposed to the oxygen of the air yields selenium as a grey deposit which may be purified by washing.

Another source is in the combustion of seleniferous pyrites in sulphuric acid manufacture. Selenic oxide (SeO_3) being thereby formed and reduced by sulphurous acid to free selenium which may be recovered and purified.

The most common source of selenium in America is its recovery from the sludge of copper slimes. In 1935 the annual yield was 125,000 pounds. Much more could have been produced had there been sufficient demand for it.

During the late World War an important commercial use was found for selenium. Due to the war American importation of manganese was cut off, selenium replaced manganese dioxide (MnO_2) as a glass decolorizer, and it has now been adopted as a standard by the glass industry of the world. Selenium colors glass a rose red and so may be used to neutralize the green tint of ferrus iron impurities.

PREPARATION OF SELENIUM DIOXIDE USED AS CATALYST IN THIS WORK

Selenious oxide (SeO_2), the compound featured as a catalyst in this work, is a white crystalline solid. This compound was prepared by dissolving selenium in boiling HNO_3 and evaporating the solution to dryness beneath an inverted funnel. The selenious oxide sublimes on the funnel as a white solid, in needle-like crystals. It may also be prepared by burning selenium in a stream of oxygen. Selenium dioxide dissolved in boiling water yields selenious acid.

$$SeO_2 + \text{hot } HOH \longrightarrow H_2SeO_3$$

EXPERIMENTAL RESULTS

One of the first things considered at the beginning of this work, was the proper indicator to use in the titration of the excess acid, thus determinging the dissolved ammonia present. After careful consideration methyl red was chosen to be the most applicable. Methyl red is red in acid solution and lemon yellow in alkaline solution. One small drop will effect a distinct change of color. In this work an alcoholic solution of the indicator was used and it was made of such strength that two or three drops were sufficient for each determination.

Since in the standard method the use of either sodium sulphate or potassium sulphate is optional, it was desirable to note whether either in its action brought about more speedy digestion than the other. Using the standard method* and working on samples of wheat flour, varing the procedure only as to the use of 10 grams of K_2SO_4 or 10 grams of Na_2SO_4 the following results were obtained:

K_2SO_4	clear	45 minutes
Na_2SO_4	clear	60 minutes

These figures give average results of duplicate samples of same weight and same rate of heating. Thus it was found advisable to use K_2SO_4 in all work throughout this investigation.

Throughout this work all digestions were carried out on digestion racks in the nitrogen room pictured on the following page. The system of drawing off fumes did not work as efficiently as might be desired.

The first pre-requisite for the use of selenium compounds is the fact that their use materially shortens the time required to clear a solution. Results follow giving a comparative study of the efficiencies of various catalysts in clearing solutions of equal weight samples of flour.

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*Official Methods of Analysis, 1930, pp. 21.

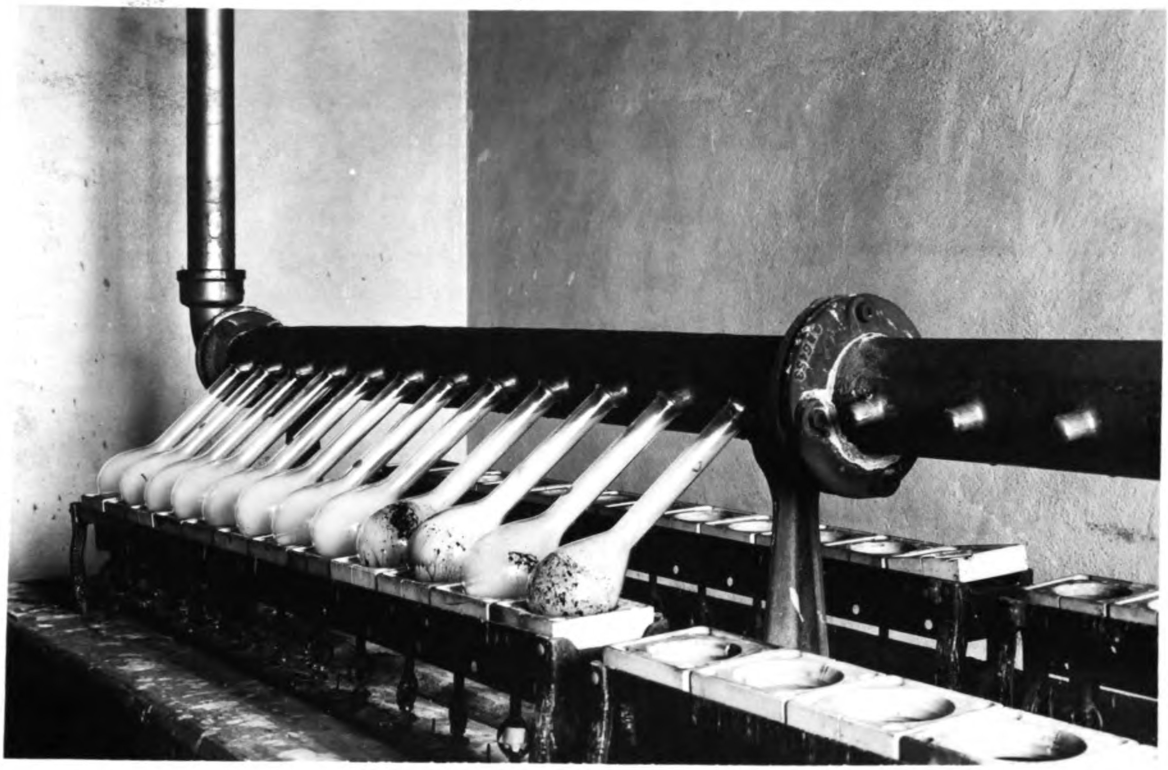


Figure 1 - Digestion Rack Used in the Digestion of All Samples.

Table I - Efficiency of Various Catalysts

Catalysts	Number of samples	Average clearing time (min.)
.2 gram Metallic Copper	2	45.0
.5 gram $CuSO_4$	8	31.5
.1 gram Powdered Se	5	16.0
.1 gram Powdered Se .5 gram $CuSO_4$	3	13.0
.3 gram SeO_2	5	10.0
.5 gram Rare Earth	2	47.5
.5 gram V_2O_5	2	44.0
.5 gram SeO_2 .5 gram $CuSO_4$	2	12.0
.5 gram Powdered Se	3	11.0
Equivalent Quantities SeO_2 and $CuSO_4$	2	9.5
Equivalent Quantities $CuSO_4$ and Powdered Se	2	14.5
Equivalent Quantities $CuSO_4$ and SeO_2 and Se	1	17.0
.2 c.c. $SeOCl_2$ .2 gram Metallic Copper	9	15.7
.2 c.c. $SeOCl_2$ .5 gram $CuSO_4$	9	17.7

10 grams K_2SO_4 used in each sample.

The results indicate that .3 gram SeO_2 reduce the time required to bring solutions clear, more than any of the other combinations tried. More results throughout this paper will tend to substantiate this primary conclusion.

In the case of the use of .5 gram of powdered selenium we find that the time is less than with a .1 gram sample, however, later investigation demonstrated that it was impractical to use so large an amount.

Running determinations on 2 gram samples of wheat flour, it was first noted that there was a tendency to obtain results slightly lower where selenium was used than in the standard method. Distillation of these samples was carried out in the nitrogen laboratory with the block tin condensers.

Table 2 - Results on Flour Analysis

Number of samples	Weight of sample	Weight of catalyst	Average clearing time (min.)	Average per cent nitrogen
10	2 grams	.3g. SeO_2	12.0	1.30
10	2 grams	.2g. SeO_2 .5g. $CuSO_4$	15.2	1.32
10	2 grams	Standard method .5g. $CuSO_4$	32.6	1.32

From Table 2 we find that the results with the selenium method are lower by .02 per cent than those with the standard method.

$$1.32 - 1.30 = .02$$

The following figures represent the percentage loss over the standard method:

$$.02 \times 100 / 1.32 = 1.51\%$$

Before attempting an explanation for this error, we will study results on the analysis of a milk sample.

In the experimentation with milk samples they were delivered from a calibrated 10 c.c. pipette and the weight obtained by multiplying the specific gravity, as determined by lactometer, by the corrected volume of milk delivered. The pipette was allowed to drain for one minute each time it was used thus a uniform volume was always delivered.

Table 3 - Results on Milk Sample Number 1

Number of samples	Weight of sample	Weight of catalyst	Average clearing time(min.)	Average per cent nitrogen
8	10.30 g.	.3g. SeO_2	13.5	.4652
8	10.30 g.	.3g. SeO_2 .5g. $CuSO_4$	14.9	.4713
8	10.30 g.	.5g. $CuSO_4$	30.6	.4727

From Table 3 we find that the results with the selenium method are lower by .0075 per cent than the results with the standard method:

$$.4727 - .4652 = .0075$$

This represents a percentage loss of 1.58 per cent over the standard method:

$$.0075 \times 100 / .4727 = 1.58\%$$

Without explanation at this point we look at the results on another sample of milk.

Table 4 - Results on Milk Sample Number 2

Number of samples	Weight of sample	Weight of catalyst	Average clearing time(min.)	Average per cent nitrogen
10	10.30 g.	.3 g. SeO_2	12.0	.4700
9	10.30 g.	.3 g. SeO_2 .5 g. $CuSO_4$	15.0	.4779
9	10.30 g.	.5 g. $CuSO_4$	30.0	.4776

From Table 4 we find that the results with the selenium method are lower by .0076 per cent than those with the standard method:

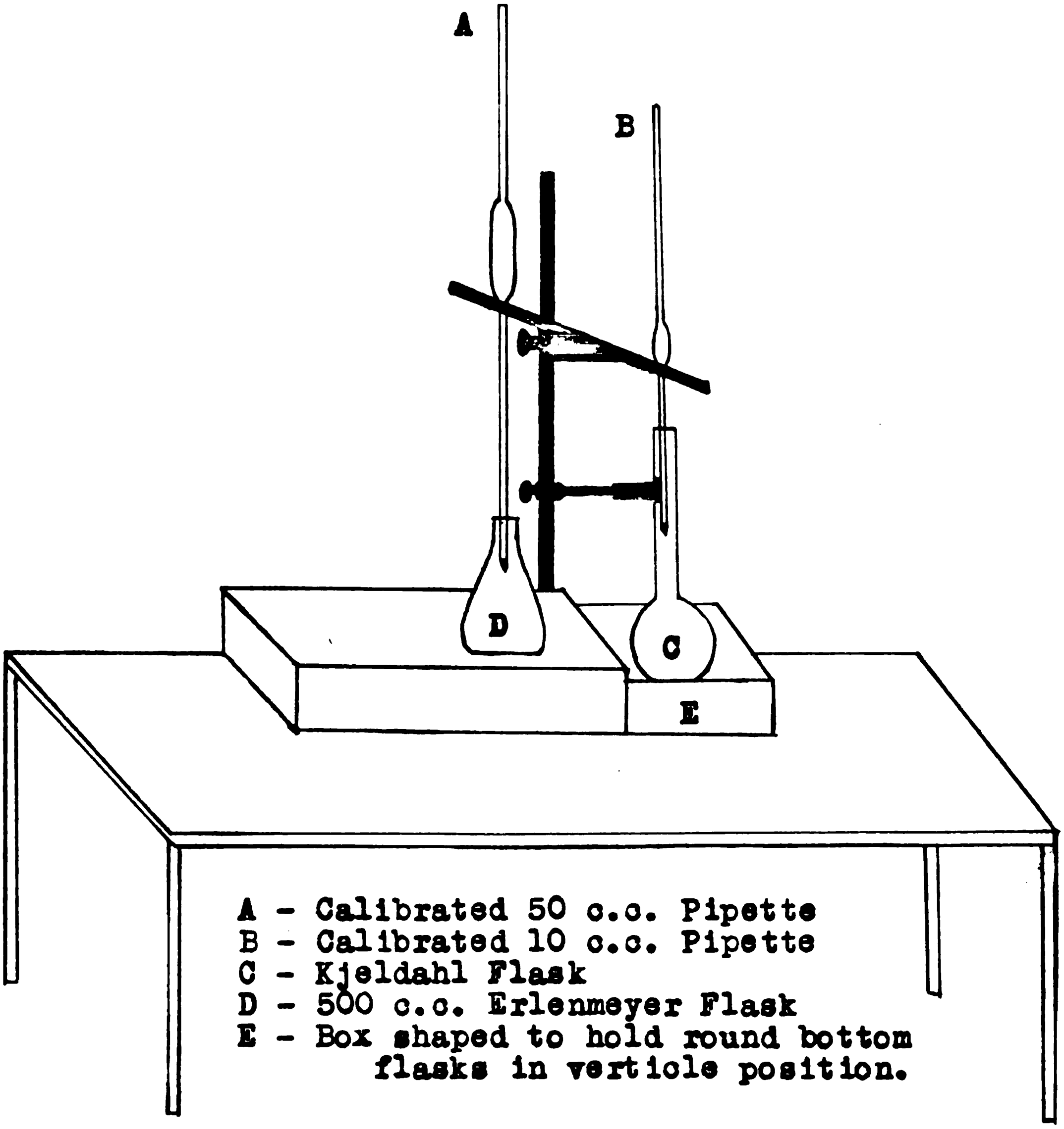
.4776 - .4700 = .0076

This represents a percentage loss of 1.59 per cent over the standard method:

.0076 X 100 / .4776 = 1.59%

Tables 2, 3 and 4 all show that low results were obtained when selenium was used. This did not seem to be in keeping with results reported by other workers, hence in an effort to determine if this was really an unavoidable error when using selenium the author started working with a standard solution of ammonium chloride.

A solution of ammonium chloride (NH_4Cl) was made up by carefully weighing out 10 grams of the dried salt and dissolving it in a clean beaker and transferring it into a calibrated 1000 c.c. graduate flask. Care was taken to wash the beaker at least five times so that there would be no danger of loss of ammonium chloride in transferring to the flask. It was then made up to 1000 c.c. and in determinations a 10 c.c. sample was used.



A - Calibrated 50 c.c. Pipette
B - Calibrated 10 c.c. Pipette
C - Kjeldahl Flask
D - 500 c.c. Erlenmeyer Flask
E - Box shaped to hold round bottom flasks in verticle position.

Fig. 2 - Apparatus for delivery of NH_4Cl sample and for measure of standard acid.

The samples were always measured from a calibrated pipette and allowed to drain into the Kjeldahl flasks from a vertical position. A funnel rack was especially constructed to hold the pipette in a vertical position inside the neck of the flask, so that it might drain each time from a stationary position without touching the edge of the flask. The pipette was allowed to drain for sixty seconds on each sample then the tip was touched to the side of the flask neck and any solution collected in the tip would thus drain to the same point each time. Figure I illustrates the apparatus used for pipette delivery of NH_4Cl samples and also for standard acid.

In these samples 10 grams of K_2SO_4, roughly weighed, was added with the catalyst which was also weighed into the Kjeldahl flask; the 10 c.c. of NH_4Cl, and 25 c.c. concentrated H_2SO_4 were added and the mixture was gently warmed to bring-about a clear solution. When properly cooled the samples were diluted with 260 c.c. of tap water; a pinch of granulated zinc was added; 80 c.c. 40% NaOH was carefully added, allowing it to flow slowly down the side of the flask so that it forms a layer in the bottom of the flask without mixing with the acid layer. Flasks were immediately transferred to distillation racks and 225 c.c. of distillate was collected in Erlenmeyer flasks containing 50 c.c. of carefully measured standard hydrochloric acid. These distillations were carried out through the block tin condensers in the nitrogen room. The stills were carefully cleaned before each distillation by washing with boiling distilled water. During the distillation the condenser tips were allowed to dip below the surface of the acid in the receiving flask. Blank determinations were run in exactly the same manner.

The results which follow are not as concordant as might be desired, however, sufficient numbers were run so that the average results give a true picture of the conditions. During later work it was found that more or less erratic results were due to the use of the block tin condensers in the nitrogen room. These condensers were being used by many students; the processes involved by each was slightly different, hence it was difficult to know just when the condensers were clean enough to give accurate results. Some students used paraffin in distilling flasks to eliminate frothing. Upon distillation paraffin collects on the inner surfaces of the block tin;later due to negligence on the part of operators the content of Kjeldahl flasks boils over through the condenser, and into the receiving flasks resulting in strong alkali accumulating on the paraffin which makes it impossible to obtain true results.

Table 5a - Distillation of NH_4Cl Solution Without Digestion Using SeO_2 as a Catalyst

Number of samples	Weight of catalyst	Acid (c.c.) added	Alkali (c.c.) used	Milligrams nitrogen recovered
1	.3 g. SeO_2	50.00	20.13	25.78
2	"	"	20.13	25.78
3	"	"	20.26	25.48
4	"	"	20.04	25.99
5	"	"	19.90	26.32
6	"	"	19.92	26.28
7	"	"	20.05	25.97
8	"	"	20.00	26.09
9	"	"	18.92	25.85
10	"	"	18.90	25.90
11	"	"	18.76	26.34
12	"	"	18.79	26.27
13	"	"	18.85	26.13
14	"	"	18.75	26.36
15	"	"	18.78	26.29
16	"	"	18.81	26.22
17	"	"	18.76	26.34
18	"	"	18.76	26.34

Average milligrams nitrogen recovered - 26.096

Each sample contained 26.183 milligrams of nitrogen.

Standard HCl used, samples 1-8 incl. - .10405 Normal
" 9-18 " - .10071 "

Standard NaOH used, " 1-8 " - .16710 "
" 9-18 " - .16813 "

Table 5b - Distillation of Blank Samples Without Digestion Using SeO_2 as a Catalyst

Number of samples	Weight of catalyst	Acid (c.c.) added	Alkali (c.c.) used	Milligrams nitrogen
1	.3 g. SeO_2	50.00	31.05	.18
2	"	"	31.05	.18
3	"	"	31.03	.23
4	"	"	31.07	.14
5	"	"	29.88	.16
6	"	"	29.87	.18
7	"	"	29.85	.23
8	"	"	29.86	.21
9	"	"	29.84	.16
10	"	"	29.88	.16

Average milligrams nitrogen - .193

No NH_4Cl solution added.

Standard HCl used, samples 1-4 incl. - .10405 Normal
" 5-10 " - .10071 "

Standard NaOH " " 1-4 " - .16710 "
" 5-10 " - .16813 "

Tables 6a and 6b will show results of the same procedure when $CuSO_4$ is used as a catalyst instead of SeO_2.

Table 6a - Distillation of NH_4Cl Solution Without Digestion Using $CuSO_4$ as a Catalyst

Number of samples	Weight of catalyst	Acid (c.c.) added	Alkali (c.c.) used	Milligrams nitrogen recovered
1	.5 g. $CuSO_4$	50.00	19.70	26.79
2	"	"	19.90	26.32
3	"	"	19.75	26.67
4	"	"	19.93	26.25
5	"	"	18.73	26.32
6	"	"	18.69	26.50
7	"	"	18.63	26.65
8	"	"	18.68	26.53
9	"	"	18.67	26.55
10	"	"	18.41	27.16
11	"	"	18.30	27.42
12	"	"	18.70	26.48
13	"	"	18.80	26.25
14	"	"	18.80	26.25
15	"	"	18.78	26.29
16	"	"	18.90	26.01

Average milligrams nitrogen recovered - 26.527

Each sample contained 26.183 milligrams of nitrogen.

Standard HCl used, samples 1-4 incl. - .10405 Normal
" 5-16 " - .10071 "

Standard NaOH " , " 1-4 " - .1671 "
" 5-16 " - .16813 "

Table 6b - Distillation of Blank Samples Without Digestion Using $CuSO_4$ as a Catalyst

Number of samples	Weight of catalyst	Acid (c.c.) added	Alkali (c.c.) used	Milligrams nitrogen
1	.5 g. $CuSO_4$	50.00	31.05	.18
2	"	"	31.05	.18
3	"	"	31.00	.30
4	"	"	30.95	.42
5	"	"	29.87	.30
6	"	"	29.80	.35
7	"	"	29.75	.47
8	"	"	29.78	.40
9	"	"	29.70	.58
10	"	"	29.85	.23

Average milligrams nitrogen - .341

No NH_4Cl solution added.

Standard HCl used, samples 1-4 incl. - .10405 Normal
" 5-10 " - .10071 "

Standard NaOH " , " 1-4 " - .16710 "
" 5-10 " - .16813 "

A study of Tables 5a and 5b, 6a and 6b, reveals the following figures:

Average gross nitrogen recovered with SeO_2 - 26.026 mg.
Average blank with SeO_2 - .193 "
Actual net recovery nitrogen - 25.903 mg.

Average gross nitrogen recovered with $CuSO_4$- 26.527 mg.
Average blank with $CuSO_4$ - .341 "
Actual net recovery nitrogen - 26.186 mg.

Thus, we find further proof that results with selenium dioxide are lower than results of the standard method. This is in keeping with results as previously reported in Table 2 on flour analysis, and in Tables 3 and 4 on milk analysis. Remembering the equation for the reaction between selenious oxide and hot water, it was thought that perhaps selenious acid was being distilled.

$$SeO_2 + \text{hot } HOH \longrightarrow H_2SeO_3$$

Since selenious acid is a strong acid resembling sulphurous acid in its reactions, if any was to be passed over into the receiving flask; it would add to the standard acid already present; upon titration more than the amount of standard alkali actually necessary to titrate the excess acid not used in absorbing the ammonia liberated, would have to be used. This would make the number of mil-equivalents of NaOH used abnormally high and upon deduction from the number of mil-equivalents of HCl present, it would give a figure for the number of mil-equivalents of standard acid used in absorbing the ammonia, which would be abnormally low, hence upon multiplying by the mil-equivalent weight of nitrogen the results would still be abnormally low.

It did not seem possible that selenious acid could volatilize from a solution strongly alkaline with caustic soda, hence further investigations were made.

In an effort to determine the amount of selenious acid distilling over the following experiment was outlined: Reagents including 10 grams K_2SO_4 - 25 c.c. H_2SO_4 and catalyst as indicated in Table 7, were mixed together and digested for twenty minutes; they were cooled, and diluted with 260 c.c. HOH and made alkaline with 80 c.c. NaOH and distilled into receiving flasks containing 50 c.c. distilled HOH and 10 c.c. standard acid.

Table 7 - Distillation of Blank Samples

Number of samples	Weight of catalysts	Acid (c.c.) added	Alkali (c.c.) used	Excess (c.c.) acid	NaOH ≎HCl used	Acid used
1	None	10.00	5.73	---	.26	.434
2	.5 g. $CuSO_4$	"	5.65	---	.34	.567
3	.5 g. SeO_2	"	5.90	---	.09	.150
4	.5 g. SeO_2	"	5.90	---	.09	.150
5	3. g. SeO_2	"	6.04	.083	---	---

10 c.c. HCl ≎ 5.99 NaOH

There is always a certain amount of nitrogen present in the H_2SO_4, tap water, and other reagents, hence the amount indicated by a blank determination is a measure of the nitrogen present in reagents used. Table 7 indicates that where selenium dioxide is used it is necessary to use more NaOH in titration and the amount of HCl used in dissolving the ammonia is lower than it actually should be. In sample 5, 3 grams of SeO_2 was used to see if this would further increase the amount of NaOH used. It was found necessary to use more NaOH in titration than was equivalent to the amount of acid added in the beginning. This was not surprising, however, but further confirmed previous data that perhaps H_2SeO_3 was distilling into the standard acid. In this case no acid was used in absorbing the nitrogen and there was an excess equivalent to .083 c.c. acid more than was added in the beginning.

It was thought advisable to run a more detailed investigation along this line. Tables 8a, b, c, and d, were carried out on exactly the same procedure as Table 7.

Table 8 - Demonstrating the Volatilization of Selenious Acid

	Number of samples	Weight of catalysts	Acid (c.c.) added	Alkali (c.c.) used	NaOH ≎ HCl used	Acid (c.c.) used	Milligrams nitrogen
a -	1	.5 g. SeO_2	10.00	8.44	.174	.202	.306
	2	"	"	8.38	.234	.272	.412
	3	"	"	8.33	.284	.329	.498
	4	"	"	8.50	.114	.132	.200
	5	"	"	8.49	.124	.144	.218

Average acid used - .2158 c.c.
Average nitrogen - .3268 mg.

b -	1	.5 g. Se	10.00	8.60	.014	.016	.024
	2	"	"	8.56	.054	.063	.095
	3	"	"	8.45	.164	.190	.287
	4	"	"	8.55	.064	.074	.112
	5	"	"	8.42	.144	.225	.245
	6	"	"	8.48	.134	.155	.235

Average acid used - .1099 c.c.
Average nitrogen - .166 mg.

c -	1	.5 g. $CuSO_4$	10.00	8.25	.464	.538	.815
	2	"	"	8.27	.444	.515	.780
	3	"	"	8.27	.444	.515	.780
	4	"	"	8.25	.464	.538	.815

Average acid used - .526 c.c.
Average nitrogen - .798 mg.

d -	1	None	10.00	8.34	.274	.318	.481
	2	"	"	8.33	.284	.329	.498
	3	"	"	8.45	.164	.190	.287
	4	"	"	8.22	.394	.457	.692
	5	"	"	8.30	.314	.364	.551

Average acid used - .331 c.c.
Average nitrogen - .502 mg.

10 c.c. acid ≎ 8.614 c.c. alkali

Results of Tables 8a, b, c and d may be summarized in the following succinct manner.

.5 g. SeO_2 Average acid used - .2158 c.c.
Average nitrogen - .3268 mg.

.5 g. Se Average acid used - .1099 c.c.
Average nitrogen - .166 mg.

.5 g. $CuSO_4$ Average acid used - .526 c.c.
Average nitrogen - .798 mg.

No Catalyst Average acid used - .331 c.c.
Average nitrogen - .502 mg.

Here, again, we find that results in samples where selenium or selenious oxide were used, are lower than in the samples where copper sulphate or no catalyst were used. The apparent amount of acid used in the case of selenium is found to be lower than that in the case of selenious oxide. This is due to the fact that .5 grams of SeO_2 is not equivalent to .5 grams of selenium.

$$SeO_2 \text{ --- } \begin{array}{lr} \text{selenium} & 79.2 \\ \text{oxygen} & \underline{32} \\ & 111.2 \end{array}$$

$$79.2 \;.\; 100 \;/\; 111.2 = 71.2\% \text{ Se}$$

$$.712 \;.\; X = .5 \;.\; 1$$

$$X = .702 \text{ grams}$$

Thus it would take .702 grams of SeO_2 to be equivalent in selenium to .5 grams of powdered selenium. This will account for the difference between the apparent amount of acid used in samples of SeO_2 and Se. As usual the amount of acid used in samples where $CuSO_4$ was used is greater than in those samples where no catalyst was used. This may be attributed to the fact that the copper sulphate has a small nitrogen content which necessitates the use of slightly greater amount of acid. These figures may be explained the same as all previous data. Since much difficulty had been experienced in getting good results by using the distilling racks in the nitrogen room, it was decided to make another attempt to obtain more accurate results before drawing final conclusions.

At one time during this work it was observed that the use of an excessive amount of zinc, results in erratic results in samples where $CuSO_4$ was used. It was decided that from this point on, all reagents used in determinations should be weighed. One-tenth gram of granulated zinc was used in each sample. At this point use of the distilling racks and block tin condensers was abandoned and a set-up of glass condensers was arranged in my laboratory. The photo on the following page shows details of the distilling set-up which accommodated four distillations at one time. An effective trap was used to prevent a spray of NaOH from passing

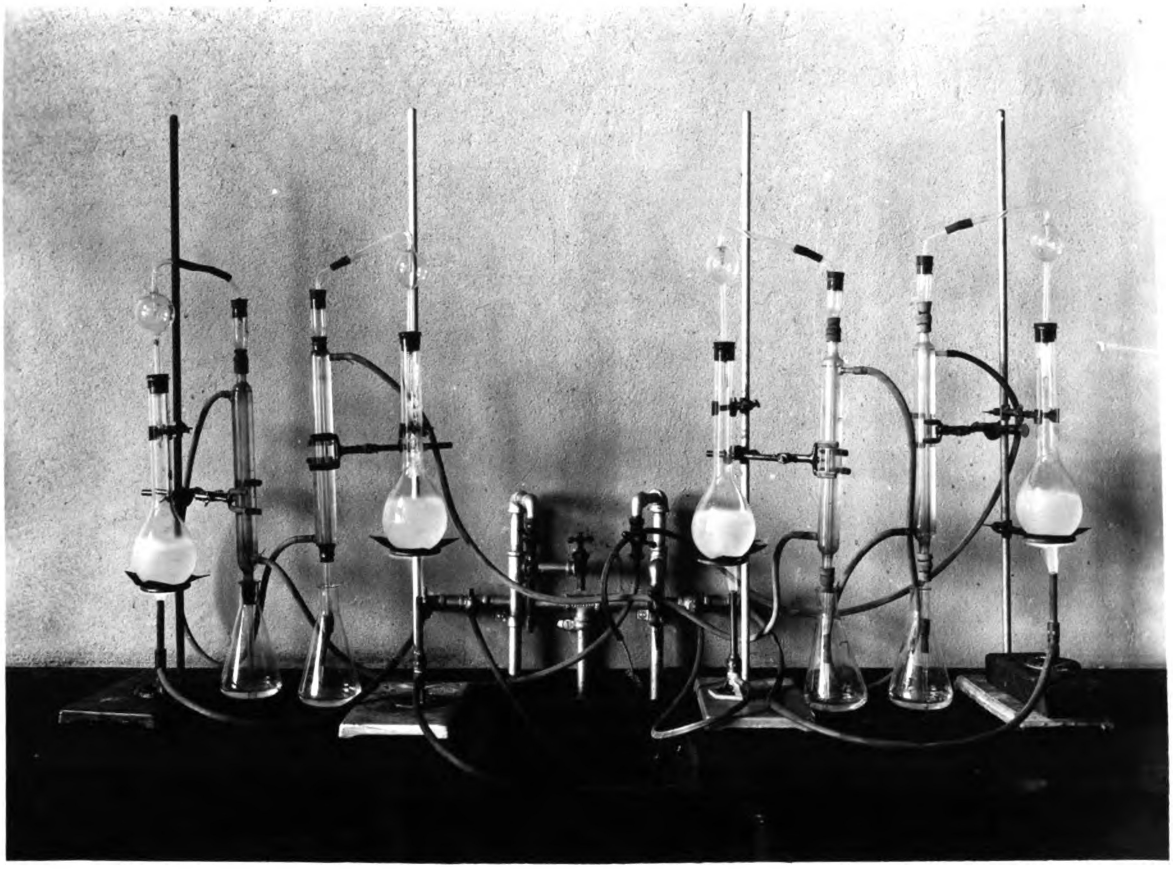


Figure 3 - Glass Condensers Used to Replace Block Tin Condensers for all Distillations in the Latter Part of Experimental Work.

into the receiving flask. A down-slope from this trap into the condenser was used; thus there was no excess liquid allowed to condense and to be trapped before reaching the condenser. Connection from the tip of the condenser dipped below the surface of the liquid in Erlenmeyer flasks. After distillation was complete the stopper connecting distilling flask and condenser was disconnected and the entire apparatus was lifted and a rest inserted below the base of the standard; this permitted the tip of delivery tube to be well above the surface of the liquid. A stream of distilled water from a wash bottle was played over this tip then the inside of the condenser was rinsed with distilled water and allowed to drain into receiving flask. After thus making sure to get all dissolved ammonia in contact with standard acid, flasks were removed and inside sprayed with distilled water just prior to titration. Standard alkali was added from a calibrated burette and all corrections were made in readings before recording in the data.

Many results on determinations carried out in the nitrogen room were not of sufficient concordance to receive mention in this paper. For a time mossy zinc was used in flasks to prevent bumping during distillation; the pieces were so large that it is quite probable that as much as one gram was used per determination; this did not affect the selenium results but in the case of copper sulphate, results seemed to vary quite extensively. This is in keeping with the finds of O. M. Shedd*. He reported, that where large amounts of zinc were used, sodium hydroxide was carried over as a spray in the excessive hydrogen gas generated. This error will occur even when an effective trap is used. He concluded that when cupric sulphate was in solution there was a greater tendency to carry over sodium hydroxide, because in each instance when it was precipitated by potassium polysulfide, less NaOH was found in the distillate. Shedd recommends the use of not to exceed 100 milligrams of zinc per determination and also the precipitation of either mercury or copper with potassium polysulfide, previous to distillation.

In the final work before drawing conclusions as to the reason for persistant low results where selenium or selenious oxide were used, a solution of ammonium chloride was prepared exactly twice as strong as had been used in foregoing determinations. Thus theoretically each sample contained 52.181 milligrams of nitrogen.

Tables 9a, b, c and d are results on distillation of samples without any digestion; reagents were just dissolved then diluted, made alkaline and distilled. An amount of selenium equivalent to .5 grams of SeO_2 was used in selenium samples.

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*J. A. O. A. C. - 10, 507 (1937)

Table 9 - Distillation of NH_4Cl Solution Without Digestion

	Number of samples	Weight of catalyst	Nitrogen added	Acid added	Alkali used	Milligrams recovered
a -	1	.5 g. SeO_2	52.181 mg.	49.94	22.50	51.62
	2	"	"	"	22.42	51.74
	3	"	"	"	22.40	51.76
	4	"	"	"	22.35	51.81
	5	"	"	"	22.35	51.81
	6	"	"	"	22.45	51.66
	1	"	Blank	"	57.03	.182
	2	"	"	"	56.92	.28

Average - average blank - 51.50 mg.

b -	1	.3561 g. Se	52.181 mg.	49.94	22.48	51.65
	2	"	"	"	22.46	51.67
	3	"	"	"	22.41	51.76
	4	"	Blank	"	57.00	.224

Average - Blank - 51.47 mg.

c -	1	.5 g. $CuSO_4$	52.181 mg.	49.94	22.32	51.86
	2	"	"	"	22.28	51.93
	3	"	"	"	22.25	51.97
	4	"	Blank	"	56.99	.182

Average - Blank - 51.74 mg.

d -	1	.7 g. Hg	52.181	49.94	22.18	52.07
	2	"	"	"	22.21	52.19
	3	"	"	"	22.20	52.20
	4	"	"	"	56.80	.462

Average - Blank - 51.69 mg.

Standard acid - .12176 N

Standard alkali - .1065 N

Tables 9a, b, c and d may be summarized by the following averages:

SeO_2 Average - Average - Blank - 51.50 mg.
Se Average - Blank - 51.47 "
$CuSO_4$ Average - Blank - 51.74 "
Hg Average - Blank - 51.69 "

From these tables it is observed that when equivalent amounts of selenium and selenious oxide are used the results are alike in that they are both lower than the standard method by the same amount.

In Tables 10a, b, c and d, .5 gram of sugar was added to each sample. This made it necessary to digest the sample until clear. This digestion was carried out at a moderate temperature and samples were all heated for a period of fifteen minutes after they were clear. If nitrogen were being lost in the selenium and selenious oxide samples during the digestion, we would expect the results in Tables 10a and 10b to be considerably lower than the results in Tables 9a and 9b. If, however, they are not lower we are forced to conclude that losses occur during distillation rather than during digestion.

Table 10 - Distillation of NH_4Cl Solution With Digestion

Number of samples	Weight of catalyst	Nitrogen milligrams added	Acid (c.c.) added	Alkali (c.c.) used	Milligrams nitrogen recovered
a - 1	.5 g. SeO_2	52.181	49.94	22.55	51.52
2	"	"	"	22.70	51.30
3	"	"	"	22.38	51.77
4	"	"	"	22.42	51.70
5	"	"	"	22.44	51.68
6	"	"	"	22.44	51.68
1	"	Blank	"	57.00	.168
2	"	"	"	56.93	.252

Average - Average - Blank - 51.40 mg.

b - 1	.3561 g.Se	52.181	49.94	22.38	51.77
2	"	"	"	22.44	51.68
3	"	"	"	22.37	51.78
4	"	"	"	22.46	51.65
5	"	"	"	22.44	51.68
6	"	"	"	22.42	51.70
1	"	Blank	"	57.07	.350
2	"	"	"	57.00	.168

Average - Average - Blank - 51.45 mg.

c - 1	.5 g. $CuSO_4$	52.181	49.94	22.30	52.04
2	"	"	"	22.18	52.08
3	"	"	"	22.35	51.82
4	"	"	"	22.18	52.07
5	"	"	"	22.30	52.04
6	"	"	"	22.30	52.04
1	"	Blank	"	57.00	.168
2	"	"	"	56.97	.196

Average - Average - Blank - 51.83 mg.

d - 1	.7 g. Hg	52.181	49.94	22.01	52.32
2	"	"	"	22.09	52.21
3	"	"	"	22.05	52.26
4	"	"	"	22.01	52.32
5	"	"	"	21.98	52.36
6	"	"	"	21.85	52.50
1	"	Blank	"	56.87	.350
2	"	"	"	56.75	.532

Average - Average - Blank - 51.89 mg.

Standard acid - .12176 N.

Standard alkali - .1065 N.

The following comparison of averages of results from Tables 9 and 10 will show the effect of digestion and distillation as compared with distillation only.

Catalyst	Average Net Nitrogen Recovery	
	NO DIGESTION	DIGESTION
.5 g. SeO_2	51.50 mg.	51.40 mg.
.3561 g. Se	51.47 "	51.45 "
.5 g. $CuSO_4$	51.74 "	51.83 "
.7 g. Hg	51.69 "	51.89 "

The differences as shown here between samples digested and samples not digested are so small and insignificant that they may be taken as conclusive evidence that losses occur during distillation rather than during digestion. They also prove conclusively that results in samples containing selenium and selenium dioxide are considerably lower than results obtained by the standard methods.

Working with cotton seed meal it was decided to try a more lengthy digestion period to see if this would overcome this tendency for low results. A three hour digestion was carried out on the samples summarized in the following Table.

Table 12 - Three Hour Digestion of Cotton Seed Meal

	Number of samples	Weight of catalyst	Weight of sample	Time to clear	Acid (c.c.) added	Alkali (c.c.) used	Per cent nitrogen
a -	1	.5 g. SeO_2	1.0460	15'	49.94	2.68	6.02
	2	"	1.0155	15'	"	2.74	6.19
	3	"	1.0095	15'	"	2.90	6.21
	4	"	.8768	12'	"	7.90	6.28
	5	"	.8581	12'	"	11.10	5.85
	6	"	.8887	10'	"	9.64	5.90

Average clearing time - 13'
Average per cent nitrogen - 6.07%

b -	1	.356 g. Se	.9990	18'	49.94	1.80	6.44
	2	"	1.0065	20'	"	1.30	6.46
	3	"	.9734	20'	"	4.88	6.12
	4	"	.8533	12'	"	9.91	6.09
	5	"	.8111	15'	"	8.87	6.01
	6	"	.8058	15'	"	12.70	5.93

Average clearing time - 16.6'
Average per cent nitrogen - 6.17%

c -	1	.5 g. $CuSO_4$	.9335	25'	49.94	2.88	6.71
	2	"	1.0034	30'	50.94	----	6.81
	3	"	.9878	35'	50.22	.10	6.81
	4	"	.8170	25'	49.94	7.14	6.88
	5	"	.8222	28'	"	6.89	6.88
	6	"	.8105	28'	"	7.38	6.89

Average clearing time - 28.5'
Average per cent nitrogen - 6.83%

Standard acid - .09585 N.

Standard alkali - .1084 N.

The following averages were obtained from Table 12.

SeO_2 Average per cent nitrogen - 6.07%
Average clearing time - 13.0'

Se Average per cent nitrogen - 6.17%
Average clearing time - 16.6'

$CuSO_4$ Average per cent nitrogen - 6.83%
Average clearing time - 28.5'

These results indicate that more lengthy digestion does not overcome the tendency for low results in the case of selenium and selenious oxide, but that it increases the amount by which they are lower than the standard results. This confirms the findings of other workers on the selenium modification, who have reported that lengthy digestion of selenium samples has a tendency to lower the results.

Conclusive evidence, that selenious acid is being carried over into the standard acid in the receiving flask, may be seen in comparing sample 3 in Table 12a, with sample 3 in Table 12c.

In sample 2 Table 12c, we find that with a slightly less weight of cotton seed meal, 49.94 c.c. of standard acid is not sufficient to dissolve the ammonia liberated and instead of adding standard alkali to neutralize the excess acid which is not present, it is necessary to add more acid, until 50.94 c.c. were used. In sample 3 Table 12a, the weight of cotton seed meal is slightly more hence there is present a slightly larger amount of nitrogen, however, after distillation there was still present an excess of standard acid equivalent to 2.90 c.c. of standard alkali. Since it is absolutely impossible for the ammonia liberated from 1.0095 grams of cotton seed meal to be dissolved in less standard acid than the ammonia liberated from 1.0034 grams of cotton seed meal, the only conclusion to follow is that more acid must have been added to the standard acid in the receiving flask. The most logical assumption to be made is that selenium in the form of the dioxide has been dissolved in hot water and carried over as selenious acid.

$$SeO_2 + \text{hot water} \longrightarrow H_2SeO_3$$

After digestion is complete there is always a red covering of selenium around the inside of the neck of the Kjeldahl flasks; this is in the form of a scum. It is insoluble in cold water, as it is not washed down into the flask upon dilution. It can be wiped out on a piece of absorbant paper and imparts a red color to the paper. Always when the strong alkali was added to flasks prior to distillation it was added carefully down one side of the flask; with this manner of addition, strong alkali did not contact all portions of the neck of the flask; however, it was noticed that the portion which it did contact was left colorless, making a break in the red scum just where this alkali had passed.

It was known that in these experiments much more selenium and selenium dioxide was being used than was necessary to produce the characteristic marked catalytic effect. This was being done intentionally for the purpose of determining the effect of excessive amounts of selenium compounds upon results. Also previous results have shown the effect of lengthy periods of digestion.

In all preceeding work selenium compounds have been used under adverse conditions. From this point on the experimental work was outlined in such a manner that the effect of selenium compounds might be studied under favorable conditions. In samples containing selenious oxide, not to exceed .15 gram per determination was used, and in the samples containing powdered selenium not to exceed .1 gram per determination was used. The strong alkali added immediately before distillation was carefully poured down the neck of the flask, which was being slowly rotated. In this manner strong alkali contacted all portions of the flask neck. Different lengths of digestion were tried so that we would be able to see how long a digestion was necessary to obtain maximum results. This would give an exact comparison of the efficiency of the selenium modification as compared to the standard method, providing the accuracy of the method was not impared.

The first material, on which the efficiency of selenium compounds was tested under favorable conditions, was a sample of Brookston loam soil. The Official Method for Soil* was used as a check. Approximately 10 gram samples were used, and 35 c.c. sulphuric acid was added per determination. It was impossible to prevent the samples from bumping around during the digestion as well as during the distillation.

Table 13 - Determination of Soil Samples

Number of samples	Weight of sample	Time to clear	Time of digestion	Acid (c.c.) added	Alkali (c.c.) used	Per cent nitrogen
a - 1	11.7926	18'	30'	49.94	26.29	.231
2	9.9870	18'	"	"	28.34	.241
3	10.6299	18'	"	"	27.28	.241
4	10.7069	18'	"	"	27.51	.236
Average per cent nitrogen - .237%						
b - 5	9.9770	18'	60'	49.94	28.94	.232
6	10.9929	18'	"	"	27.15	.235
7	8.5408	20'	"	"	30.68	.240
8	10.4221	16'	"	"	29.14	.220
Average per cent nitrogen - .231%						
c - 9	12.2803	18'	120'	49.94	26.20	.222
10	10.3208	16'	"	"	28.60	.229
11	11.2156	18'	"	"	27.10	.231
12	9.8760	20'	"	"	28.78	.237
Average per cent nitrogen - .229%						

Weight of Catalyst - .15 gram SeO_2

Average clearing time - 18 min.

Standard acid - .09585 N.

Standard alkali - .1082 N.

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*Official Methods of Analysis - 1930 - pp. 7.

Table 13 (Cont.) - Determination of Soil Samples

	Number of samples	Weight of sample	Time to clear	Time of digestion	Acid (c.c.) added	Alkali (c.c.) used	Per cent nitrogen
d -	1	10.3979	20'	30'	49.94	29.62	.213
	2	10.6085	22'	"	"	29.22	.214
	3	10.6238	22'	"	"	29.10	.216
	4	11.4335	20'	"	"	27.80	.218

Average per cent nitrogen - .215%

e -	5	11.8320	22'	60'	49.94	26.63	.225
	6	10.4510	21'	"	"	28.50	.228
	7	9.2404	21'	"	"	29.97	.234
	8	9.9148	20'	"	"	29.28	.228

Average per cent nitrogen - .229%

f -	9	9.7178	20'	120'	49.94	29.65	.227
	10	9.0951	20'	"	"	30.60	.228
	11	10.4615	22'	"	"	28.50	.227
	12	9.9159	22'	"	"	29.40	.226

Average per cent nitrogen - .227%

Weight of Catalyst - .10 gram Se

Average clearing time - 21 min.

g -	B	10.0323	50'	60'	49.94	30.60	.206
	C	10.9027	50'	"	"	29.00	.234
	D	10.5209	50'	"	"	29.16	.217

Insufficiently concordant to average

h -	1	8.7496	55'	120'	49.94	31.00	.228
	3	9.3958	55'	"	"	30.11	.228
	4	9.6885	55'	"	"	29.90	.224

Average per cent nitrogen - .227%

i -	A	9.5680	50'	180'	49.94	30.30	.221
	B	9.7185	55'	"	"	29.81	.225
	C	10.8261	60'	"	"	27.81	.229

Average per cent nitrogen - .225%

Weight of Catalyst - .70 gram Hg

Average clearing time - 55 min.

Standard acid - .09585 N.

Standard alkali - .1082 N.

Before discussing the preceding tables on the soil determination it is best to summarize the results in the following concise manner.

Catalyst	Time to clear	% N. 30' digestion	% N. 60' digestion	% N. 120' digestion	% N. 180' digestion
.15 g.SeO_2	18'	.237	.231	.239	----
.10 g. Se	31'	.215	.239	.237	----
.7 g. Hg	54'	----	Not concordant	.237	.235

The first feature to be noticed is that the results in samples where selenium and selenium dioxide were used are as high as the standard method; they also check well with each other. Thus from the work on soil samples it is apparent that selenium dioxide may be used to advantage without affecting the accuracy of the determination.

It will also be noted that the maximum results are obtained with the selenium dioxide samples after a digestion period of only thirty minutes. The samples determined by the standard method required a period of one-hundred-twenty minutes before maximum results were obtained. Thus by the use of the selenium dioxide modification under controlled conditions as outlined it is possible to complete the digestion in one-quarter of the time previously required. Results on the standard method after sixty minutes digestion were not of sufficient concordance to average. This is due to insufficient length of digestion.

In samples where powdered selenium was used it is found that maximum results were not obtained until sixty minutes digestion. This is perhaps a bit misleading as it is thought that the optimum period of digestion is between thirty and sixty minutes. The selenium dioxide samples were clear in eighteen minutes while the selenium samples required twenty-one minutes for clearing, thus it would be expected that a longer time of digestion would be necessitated, however, it seems most logical that the optimum time be around forty-five minutes rather than sixty. It is unfortunate that time did not permit a more accurate assertation of the optimum time of digestion. The results on samples determined with selenium dioxide as a catalyst, show a tendency to decrease upon long periods of digestion, however, this is not a very marked decrease.

The next step in the outlined procedure was to test the accuracy of the controlled use of selenium compounds on the determination of cotton seed meal. Blank determinations were made to test for the amount of nitrogen in the reagents used, and it was found that .0003 gram nitrogen was present per sample. However, the amount of nitrogen added in the cotton seed meal per sample was about .067 gram thus the blank is sufficiently small to become negligible in the dealing with these figures.

Table 14 - Determination of Cotton Seed Meal Samples

	Number of samples	Weight of sample	Time to clear	Time of digestion	Acid (c.c.) added	Alkali (c.c.) used	Per cent nitrogen
a -	1	.9465	17'	30'	49.94	7.32	6.738
	2	.9232	17'	"	"	8.39	6.732
	3	.9537	17'	"	"	7.12	6.726

Average per cent nitrogen - 6.732%

b -	5	.9796	17'	60'	49.94	6.30	6.668
	6	.9969	17'	"	"	5.39	6.691
	7	.9136	17'	"	"	9.16	6.675

Average per cent nitrogen - 6.678%

c -	9	.9524	17'	120'	49.94	7.75	6.627
	10	.9293	17'	"	"	8.68	6.640
	11	.9430	17'	"	"	8.38	6.592

Average per cent nitrogen - 6.619%

Weight of Catalyst - .15 gram SeO_2

Average clearing time - 17 min.

d -	1	.9955	25'	60'	49.94	4.87	6.780
	2	1.0162	25'	"	"	4.88	6.640
	3	1.0073	25'	"	"	4.70	6.720
	4	.9578	25'	"	"	7.40	6.645

Average per cent nitrogen - 6.696%

e -	5	.9552	25'	120'	49.94	7.50	6.648
	6	.9630	25'	"	"	6.90	6.689
	7	.9554	25'	"	"	7.08	6.713
	8	.9969	25'	"	"	5.64	6.653

Average per cent nitrogen - 6.676%

Weight of Catalyst - .70 gram Hg

Average clearing time - 25 min.

Standard acid - .10711 N.

Standard alkali - .10845 N.

Table 14 (Cont.) - Determination of Cotton Seed Meal Samples

	Number of samples	Weight of sample	Time to clear	Time of digestion	Acid (c.c.) added	Alkali (c.c.) used	Per cent nitrogen
f -	1	.9486	20'	30'	49.94	7.81	6.645
	2	.9332	20'	"	"	7.48	6.808
	3	.9160	20'	"	"	9.66	6.574
	4	.9687	20'	"	"	8.22	6.443

Insufficiently concordant to average

g -	5	.8780	20'	60'	49.94	10.45	6.723
	6	.9017	20'	"	"	9.30	6.739
	7	.9007	20'	"	"	9.23	6.758
	8	.9519	20'	"	"	7.25	6.711

Average per cent nitrogen - 6.7327%

h -	9	.9456	20'	120'	49.94	7.51	6.714
	10	.9277	20'	"	"	8.24	6.724
	11	.8025	20'	"	"	14.30	6.624
	12	.9732	20'	"	"	6.56	6.672

Average per cent nitrogen - 6.683%

Weight of Catalyst - .10 gram Se

Average clearing time - 20 min.

i -	1	.9708	50'	60'	49.94	6.00	6.760
	2	.9223	45'	"	"	8.19	6.771
	3	.9704	40'	"	"	6.40	6.716
	4	.9765	45'	"	"	5.80	6.767

Average per cent nitrogen - 6.753%

j -	9	.9686	45'	120'	49.94	6.30	6.744
	10	.9325	45'	"	"	7.70	6.777
	11	.8537	45'	"	"	11.70	6.691
	12	.8854	45'	"	"	10.30	6.692

Average per cent nitrogen - 6.733%

Weight of Catalyst - .50 gram $CuSO_4$

Average clearing time - 45 min.

Standard acid - .10711 N.

Standard alkali - .10845 N.

The following summary of the four methods used in Table 14, again shows the advantage of the selenium dioxide modification.

Catalyst	Time to clear	% N. 30' digestion	% N. 60' digestion	% N. 120' digestion
.15 g. SeO_2	17	6.732	6.678	6.619
.1 g. Se	20	not concordant	6.732	6.683
.5 g. $CuSO_4$	45	---	6.753	6.733
.7 g. Hg	25	---	6.696	6.676

In samples where selenious oxide was used as a catalyst, maximum results were obtained after a thirty minute digestion. In all other cases maximum results were obtained after a digestion period of one hour. Thus the use of selenium dioxide has increased the efficiency of the method 50 per cent without affecting the results in any way.

The samples in which selenium was used as a catalyst required but three minutes longer to clear, however, results after thirty minutes digestion were not sufficiently concordant to average. It is believed that the optimum period of digestion in these cases is much less than sixty minutes. Time did not permit a more accurate determination, however, it is known to lie between thirty and sixty minutes.

Slight differences between duplicate samples determined in the same manner may be due to the heterogenicity of the cotton seed meal sample.

Table 15 - Determination of Rice Flour Samples

Number of samples	Weight of sample	Time to clear	Time of digestion	Acid (c.c.) added	Alkali (c.c.) used	Per cent nitrogen
a – 1	2.1248	17'	30'	49.94	35.15	1.0120
2	2.2948	17'	"	"	34.25	.9975
3	1.9829	17'	"	"	36.33	.9944
4	2.0738	17'	"	"	35.88	.9843

Average per cent nitrogen - .9975%

b – 5	2.0420	17'	60'	49.94	35.74	1.0100
6	1.9673	17'	"	"	36.46	.9927
7	2.2378	17'	"	"	34.40	1.0120
8	2.1703	17'	"	"	34.70	1.0230

Average per cent nitrogen - 1.011%

c – 9	1.7281	17'	120'	49.94	38.20	.9754
10	2.1392	17'	"	"	48.35	.068
11	2.0213	17'	"	"	49.20	.080
12	2.5957	17'	"	"	49.20	.070

Insufficiently concordant to average

Weight of Catalyst - .15 gram SeO_2

Average clearing time - 17 min.

d – 1	2.5355	32'	60'	49.94	32.23	1.024
2	2.1694	31'	"	"	34.97	1.004
3	1.9212	31'	"	"	36.61	1.005
4	2.4730	32'	"	"	32.70	1.021

Average per cent nitrogen - 1.013%

e – 5	2.0538	32'	120'	49.94	35.35	1.032
6	1.9790	32'	"	"	36.01	1.021
7	2.1630	32'	"	"	34.65	1.030
8	2.4018	30'	"	"	33.30	1.013

Average per cent nitrogen - 1.024%

Weight of Catalyst - .5 gram $CuSO_4$

Average clearing time - 31.4 min.

Standard acid - .10711 N.

Standard alkali - .10845 N.

Since all results thus far obtained have proven selenium dioxide to be a better catalyst than powdered selenium, the use of selenium was discontinued throughout the remainder of the experimental work. A review of the work on rice flour follows:

Catalyst	Time to clear	% N. digestion	% N. digestion	% N. digestion
.15 g. SeO_2	17'	.9875	1.011	not concordant
.5 g. $CuSO_4$	31.4'	---	1.013	1.024

It is evident that a thirty minute digestion of the rice flour is not sufficient to give maximum results when selenium dioxide is used, therefore, the optimum time of digestion is between thirty and sixty minutes. The time required to obtain maximum results with the standard method is about two hours or perhaps slightly less. Even though the time required for maximum results with selenium dioxide is more than it has been on other materials tested it is, however, still 50 per cent more efficient than the standard method.

In the one-hundred-twenty minute digestion of rice flour the results appear very bad. Referring to Table 15c it is observed that sample 9 is slightly lower in nitrogen content than the maximum results. This may be attributed to the general tendency for loss of nitrogen on prolonged digestion where selenium dioxide is used. Samples 10, 11 and 12 all were so reduced in volume of acid that they crystalized on the inner surface of the flasks the instant the heat was turned off. This crystalized mass was rather insoluble in cold water; so to bring about a solution after dilution it was necessary to heat them again. They were heated in an upright position over a bunson burner, the mouths of the flasks being open. Although all this took place before samples had been made alkaline, the nitrogen was lost in some manner. It is unnecessary to carry the digestion anywhere near two hours in the selenious oxide modification thus this danger will never be encountered in actual determinations.

It was thought advisable to test the applicability of the selenious oxide modification on a series of fertilizer samples. Table 16 will give results of determinations carried out on the commercial fertilizer selling under the name of "Loma". The certified analysis as given on the box gives the nitrogen content as 5 per cent.

Table 16 - Determination of Commercial Fertilizer Samples

Number of samples	Weight of sample	Time to clear	Time of digestion	Acid (c.c.) added	Alkali (c.c.) used	Per cent nitrogen
a - 1	.9630	13'	30'	49.94	17.35	5.040
2	.8386	13'	"	"	21.02	5.124
3	1.3670	13'	"	"	11.01	5.117
4	.8188	13'	"	"	22.06	5.054
	Average per cent nitrogen - 5.084%					
b - 5	.7802	13'	60'	49.94	23.30	5.068
6	1.0738	13'	"	"	13.66	5.043
7	.8866	13'	"	"	19.65	5.081
8	.9856	13'	"	"	16.50	5.057
	Average per cent nitrogen - 5.062%					
c - 9	.9456	13'	120'	49.94	17.47	5.114
10	1.2726	13'	"	"	7.09	5.058
11	1.0046	13'	"	"	16.83	4.911
12	1.0218	13'	"	"	14.85	5.121
	Average per cent nitrogen - 5.051%					
	Weight of Catalyst - .15 gram SeO_2					
	Average clearing time - 13 min.					
d - 1	1.0346	21'	60'	49.94	15.26	4.999
2	1.0135	21'	"	"	16.07	4.981
3	.9667	21'	"	"	17.50	4.998
4	1.1342	21'	"	"	12.20	4.970
	Average per cent nitrogen - 4.987%					
e - 5	1.0306	21'	120'	49.94	15.18	5.030
6	.8440	21'	"	"	21.40	5.023
7	.9297	21'	"	"	18.61	5.016
8	.8575	21'	"	"	21.12	4.993
	Average per cent nitrogen - 5.0155%					
	Weight of Catalyst - .7 gram Hg					
	Average clearing time - 21 min.					

Standard acid - .10711 N.

Standard alkali - .10845 N.

Table 16(Cont.)-Determination of Commercial Fertilizer Samples

Number of samples	Weight of sample	Time to clear	Time of digestion	Acid (c.c.) added	Alkali (c.c.) used	Per cent nitrogen
f - 9	.9283	27'	60'	49.94	18.87	5.009
10	1.0767	27'	"	"	13.77	5.014
11	1.0361	27'	"	"	15.21	5.000
12	.9856	27'	"	"	16.80	5.060

Average per cent nitrogen - 5.021%

g - 1	.8140	27'	120'	49.94	22.40	5.022
2	1.1474	27'	"	"	10.90	5.089
3	1.0402	27'	"	"	14.91	5.023
4	1.2308	27'	"	"	8.30	5.061

Average per cent nitrogen - 5.049%

Weight of Catalyst - .5 gram CuSO$_4$

Average clearing time - 27 min.

Standard acid - .10711 N.
Standard alkali - .10845 N.

The experimentation on commercial fertilizer is succinctly reviewed in the following table:

Catalyst	Time to clear	% N. 30' digestion	% N. 60' digestion	% N. 120' digestion
.15 g. SeO$_2$	13'	5.084	5.062	5.0510
.7 g. Hg	21'	---	4.987	5.0155
.5 g. CuSO$_4$	27'	---	5.021	5.049

It is evident that the use of selenious oxide is applicable, and highly efficient in fertilizer determination. Maximum results are obtained after thirty minutes digestion while in the standard mercury and standard copper sulphate methods the maximum results are not obtained until after a two hour digestion. Thus the use of the selenious oxide modification permits completion of digestion in 25 per cent of the time required by the standard methods.

SUMMARY

The use of potassium sulphate is recommended over sodium sulphate as a salt to raise the boiling point of acid solution. It was found the use of 10 grams of potassium sulphate per determination would bring about a more speedy digestion than an equal amount of sodium sulphate. It was found advisable to use 10 grams per determination.

A study of the efficiency of various catalysts and combinations of catalysts, revealed selenium dioxide to be the most efficient and best adapted to the purpose. It is recommended that never more than .15 gram be used. With the use of .15 gram the length of digestion time required to give maximum nitrogen results is only 35 to 33 per cent of that required with the standard copper sulphate, or standard mercury methods. The cost of this amount of selenium dioxide is much less than the cost of .7 gram of mercury, required in the standard method. The use of selenium dioxide permits completion of determinations in one-third to one-half the time originally required.

All experimental data on the use of excessive amounts of selenium dioxide portrays the fact that low results are obtained. A possible explanation as gathered from experimental results, hinges on the fact that during digestion a red scummy layer of selenium collects on the neck of the Kjeldahl flask. The more selenium dioxide that is used the more of this red deposit will be formed. Upon distillation hot water in the form of steam passes over this selenium dust and a certain amount of it is dissolved and carried over into the standard acid in the receiving flask. The selenium dioxide reacts with hot water forming selenious acid, an acid resembling sulphurous acid in its reactions and nature.

$$SeO_2 + \text{hot } H_2O \longrightarrow H_2SeO_3$$

This adds to the acid already present thus upon titration the amount of standard sodium hydroxide used will be more than the amount actually necessary to titrate the excess acid not used in dissolving the ammonia liberated. This causes results to be lower in samples where excessive amounts of selenium dioxide were used.

In order to avoid this error it is recommended that not to exceed .15 gram of selenium dioxide, or if selenium powder is used, not to exceed .1 gram, be used. As the samples are made alkaline just prior to distillation, the strong caustic soda should be added carefully down the neck of the flask; as it is being added the flask should be carefully rotated so that all portions of the neck of the flask have been in contact with the strong alkali. If these precautions are followed no selenious acid will be formed. The results obtained by the selenium dioxide modification were found to check as well as did the results with standard methods. All experimental work performed under favorable conditions as above outlined gave results comparable to standard method results.

It is recommended that no paraffin be used during distillation of samples. If paraffin is used to prevent frothing some of it is carried over into the condenser and solidifies on the inside. If any samples are then allowed to boil over through condenser or if any acid from receiving flask is drawn back into the condenser, foreign matter in the form of alkali or acid is incorporated in the paraffin; this is hard to clean especially where block tin condensers are used. Repeated distillations may wash off enough of this foreign matter to make results non concordant.

Where excessive amounts of zinc were used during distillation, poor results were obtained especially in samples determined with copper sulphate as a catalyst. This confirms the work of O. M. Shedd whose work was referred to on page 34. Even though an effective trap is used small amounts of sodium hydroxide are carried over into the receiving flask in the excessive hydrogen gas generation. Thus it is recommended that not to exceed 100 milligrams of zinc be used per determination.

All experimental data has proven selenium dioxide to be a better catalyst than powdered metallic selenium. The time required to clear solutions is less and the total time of digestion required to obtain maximum results is considerably less. Hence, since it is no more expensive, its use is advocated in preference to powdered selenium.

In the selenium dioxide modification it is never necessary to carry the digestion longer than sixty minutes. A long digestion tends to decrease the amount of nitrogen, hence lengthy digestions with selenium dioxide must not be made.

There is an optimum length of digestion time for each substance tested, at this point maximum results are obtained. Regardless of the catalyst used this point should be carefully determined for the material to be tested before a series of samples are run. It will be found that this optimum digestion time where selenium dioxide is used as a catalyst will be as small as 20 to 33 per cent of the time required in the standard method. Hence it is recommended, that (1) in the use of selenium dioxide the optimum time of digestion for each substance tested be carefully determined, and then (2) that digestion never be continued longer than this time. The time saving will be great enough to more than make up for the extra trouble of locating the optimum digestion time.

After completion of experimental work in connection with the use of selenium dioxide in the Kjeldahl Nitrogen Determination, its use under favorable conditions is most heartily recommended. Distillations may be carried out more evenly with less bumping or frothing, and liquid during distillation is clear and transparent; much less time is consumed in a complete determination and the cost of the catalyst is so small that it may be considered negligible. It was found that determinations were more pleasantly performed with the selenium dioxide modification than with the standard method.

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