ELECTROMETRIC METHOD FOR DETERMINATION OF CHLORIDE FROM INSECTICIDES AND MEASUREMENT OF FREE AND COMBINED CYANIDE IN PLANTS

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CHAPTER I

ELECTROMETRIC METHOD FOR DETERMINETION OF CHLORIDE FROM INSECTICIDES

INTRODUCTION

Over 3 billion dollars worth of pesticides are used each year in the United States to combat some 60,000 species of insects that compete with man for his food supply. Approximately 100 different insecticides are currently in use. Forty of these contain organically-bound chlorine. These compounds often contain one or more aromatic, alicylic or heterocylic groups, and the definite and accurate determination of the compound is complicated and difficult. Such analytical procedures as are available are often not suited for routine analysis of insecticide residues in foods and feeds. In only a few cases, have satisfactory colorimetric procedures been developed (16, 23). Bioassay procedures are valuable in the standardization of other methods (12, 15, 17), particularly when closely related compounds differ in their insecticidal potency, but they also are not suitable for routine examination of residues. The presence of organically-bound chloride, however, in many insecticides does provide a possible routine method for the insecticides of this type. Generally speaking, the total organic chloride is analyzed by extraction of the insecticide by suitable organic solvents, followed by release of Cl^- through

reduction by metallic sodium, oxidation with nitric acid or other means. The inorganic chloride thus produced is then measured (25).

The measurement of chloride may be carried out by direct titration methods (26), by colorimetric means (23) or by electrometric titration methods. Two direct titration methods (The McLean and Van Slyke iodometric titration, 18, 26, 32, and The Volhard procedure 33) are extensively used for determination of chloride in biological materials. In these procedures, however, when very small amounts of chloride are to be determined, serious errors are introduced by large corrections needed to compensate for Cl^- in the large amounts of reagents required. There is also interference by unoxidized material in many insecticide residue studies (6). When sodium reduction methods are used in such analyses, the large quantities of sodium salts resulting from the sodium decomposition complicate the application of determinations in which the end-point is sensitive to high salt concentration.

Electrometric titration methods are well known. The amperometric methods for the determination of the chloride ion (1, 2, 25) are usually quite sensitive, but do not lend themselves to use in routine applications because of their complexity. Potentiometric methods, however, though less precise, offer promise of permitting the determination of Cl^- with sufficient accuracy to make them useful for residue

analyses.

The present study deals with an investigation of a method for the simple and accurate determination of chloride ion by potentiometric titration, using a commercial instrument, of solutions similar to those which would result in the routine determination of insecticides by sodium reduction methods.

The Fisher Titrimeter has been extensively used for the determination of chloride by potentiometric titration. There are, however, problems remaining to be evaluated in the determination of the chloride content of insecticide residues in soils, plants, and biological materials especially in the very low ranges of chloride which would occur in the form of insecticide residues. One of the most significant difficulties is caused by the conversion of the organic chloride into sodium chloride by reduction by metallic sodium. In the conventional bomb combution or by the Carius Method, the organic matter is destroyed by oxidation and the chloride converted into silver chloride by the addition of silver nitrate. Due to other halides involved and to the small amount of sample, this method is usually not satisfactory for insecticidal residues. The reduction by metallic sodium in alcohol (isopropyl alcohol or butyl alcohol) has, therefore, frequently been used. This method requires the neutralization of the resulting alkali by means of HNO_3, and this introduces a high concentration of sodium nitrate

into the final solution. In such conditions, determination of very small amounts of Cl^- is unsatisfactory by the McLean and Van Slyke procedure (24, 26, 32).

During the year 1954-55, in determining insecticide residues in soil, the insecticides were so reduced by decomposition or evaporation over a long period that only traces of insecticide remained. It was not possible to determine Cl^- with sufficient accuracy by use of these methods to show statistically significant differences among the different treatments, although there was evidence from biological assay to believe that differences between treatments actually existed. The insecticide was determined by difference in organic chloride between the treated and untreated soils. Hence variation in the untreated soil, introduced a sizable error in the value of the insecticide. It was also necessary to use a relatively large correction for unavoidable contamination from apparatus and reagents.

The determination of actual insecticidal Cl^- involved the following sources of error:

$$\sigma_A^2 = \sigma_x^2 + \sigma_{B_1}^2 + \sigma_{B_2}^2, \text{ where}$$

A ..... total observed insecticide chloride

X ..... total true insecticide chloride

B_1 ..... untreated sample organic chloride

B_2 ..... contamination chloride from apparatus and reagents (blank)

Ideally $\sigma_{B_1}^2$ and $\sigma_{B_2}^2$ are very small, so that X, the value

obtained from the practical determination of insecticides, is large enough to adequate compensate for other sources of error, and the results are dependable. However, if the value of X is extremely small, the influence of $\sigma^2_{B_1}$ and $\sigma^2_{B_2}$ show up significantly in the results. Practically speaking, in order to interpret the results from many experiments, not only must the action of the treatments be considered, but the extraneous variations, which tend to mask the effects of the treatments, must also be evaluated.

The investigation of methods for increasing the accuracy of experiments has played a prominent part in experimental research (10, 28). These techniques may be classified broadly into three types. The first is to increase the size of the experiment, either through the provision of more replicates or by the inclusion of additional treatments. The second is to carefully select experimental material and to make such additional measurements to predict, at least to some extent, the validity of the experimental results. In an experiment to measure insecticide residue in the soil following different amounts and kinds of insecticidal treatments, the population of microorganism in the soil would be a supplementary measurement of this kind, since insecticidal residue content is probably correlated with the growth of the soil microorganism (3, 20, 31). Third, refinements of experimental technique may be introduced; such improvements are especially important for the chemist. The principal objects of good technique are to secure informity in the

application of the treatments and to exercise sufficient control over external influence so that every treatment produces its effects under comparable and desired conditions. Faulty technique may increase the experimental errors in two ways. It may introduce additional fluctuations of a more or less random nature. Such fluctuations, if they are substantial, should reveal themselves in the estimation of error as calculated in the analysis of variance (29).

It is worthwhile to consider from time to time whether simplifications can be brought into the technique without undue loss of accuracy. Many procedures for chloride determination have been know. The McLean and Van Slyke procedure has been generally applied in this laboratory to the routine determination of the chloride in insecticide analysis (5, 8, 9, 22). In the Van Slyke procedure the excess of silver ion titrated by potassium iodide after precipitation of chloride as silver chloride.

(1) $Cl^- + Ag^+ = AgCl$ (white precipitate)

(2) Ag^+ (excess from reaction 1) + $I^- = AgI$ (yellow precipitate)

(3) $4\ H^+ + 2\ I^-$ (first drop of excess from reaction 2) $+2\ NO_2^- \longrightarrow I_2 + N_2O_2 + 2\ H_2O$ (blue color of iodine with starch)

Despite carefully following the procedures in the micro-chloride analysis, the complexity of the procedure induced substantial errors. For example, in the last step of

titration with potassium iodide, results are affected by the volume of the solution being titrated. The blue color induced by starch-iodine complex which denotes the endpoint is hard to visualize at times, and when very small amounts of dilute solutions are involved, the endpoint may be quite unsatisfactory.

The potentiometric titration for the chloride determination is attractive for this field of work because of its relatively simplicity coupled with acceptable accuracy. An added advantage is the availability of commercial methods of automatic titration (19) equipment available. Application of the Fisher Titrimeter to very low ranges of Cl^- content in the presence of high concentrations of other salts has not been previously attempted in these laboratories and no references were found upon search of the literature. Hence, a study was undertaken to determine its insecticidal residues by determination of organic chloride.

The electrode used for Cl^- measurement was improved by Yeck and Kessin (34). The procedure introduced by Holthoff and Kuroda (21) for determination of trace of chloride, however, fails to show a sharp end point using 1×10^{-3} N normal silver nitrate solution (30). Sanderson investigated this method using 0.1 N silver nitrate solution for determination of chloride in biological materials (28). Recently Jones and Baum (19) used a Beckman automatic titrimeter Model K to determine organic chloride. At this station three years ago, Paul Solomon made extensive investigations

of the chloride procedure by the potentiometric method. It showed good results on samples of fairly high chloride content. Methods developed at that time were not adequate to determine Cl in the low ranges frequently encountered in insecticidal residue analyses. Fahey and Rusk (11) used a Fisher titrimeter for determining the organic chloride of DDT in milk, but only got satisfactory results on milk containing high amounts of DDT.

Further study was deemed justifiable, however, in the hope that meticulous attention to detail would permit the accurate measurements of Cl^- in the concentrations and under the conditions usually encountered in insecticidal residue analyses.

A Fisher titrimeter equipped with a micro burette and Fisher silver electrode (Model No. 9-312-37) and silver chloride electrode (Model No. 9-312-28) were used. The results have shown that difficulty is encountered in elucidation of the endpoint in the range of 0.5 mg. chloride or less per sample. Perhaps within such low ranges, the silver chloride more or less dissociates in solution and affects the potential. This error is eliminated by the addition of purified silver chloride suspension in diluted nitric acid solution to produce saturation of silver chloride in the solution, or by addition of known amounts of chloride in the low ranges of samples before titration. When this was done, the results were more satisfactory.

EXPERIMENTAL

Procedure for Routine Measurement of Cl Using Potentiometric Titration.

Precautions: The concentration of silver nitrate solution used in the titration was extensively investigated. 0.0141 N. standard silver nitrate solution (1 ml. equivalent to 0.5 mg. chloride ion) was adequate for routine titration ranges between 0.5 mg. and 30 mg. chloride. Nitric acid was added in the standard silver nitrate solution to stabilize the silver ion to light and to keep constant acidity in the titrating solution. The titrating solution was acidified with chloride-free nitric acid at the rate of 1.0 ml. of 16 N. acid per 50 ml. - 70 ml. of final solution.

The electrode system was subject to interferences by those ions which form complex or precipitate silver ions in acid solution more efficiently than chloride ions. If these extraneous ions exist in the solution, these ions metathetically react with the solid silver chloride coating and secondary, e.m.f.'s results. A common interfering ion is the carbonate ion. The action of this ion was suppressed or eliminated by making all measurements in acidified solutions and by vigorously stirring the solutions before titration.

Before the measurement, the sample solutions were cooled to room temperature. This eliminated the temperature effect in the electrode system. Not only does carbonate ion

affect the electrode system, but also any substance capable of complexing precipitating silver ion more efficiently than chloride in acid solution. These substances are not merely interferences with the method, but may also spoil the electrodes, Bromide, and iodide, for example, must be absent or the electrode becomes metathesized (4).

Apparatus And Reagents.

1. Fisher titrimeter equipped with Fisher Silver Electrode (Model No. 9-312-37) and Fisher Silver Chloride Electrode (Model No. 9-312-38)
2. Electric stirrer
3. Microburette graduated in 0.01 ml.
4. Standard silver nitrate solution, 0.0141 N., equivalent to 0.50 mg. Cl^- per ml. Weigh out 2.3954 g. reagnet grade silver nitrate. Dissolve in double distilled water and add 125 ml. of chloride-free concentrated nitric acid. Make to a volume 1000 ml. by fresh double distilled water.
5. Standard solution of sodium chloride. Weigh out 0.1648 g. of sodium chloride (National Bureau of Standards) and make to 1000 ml. with double distilled water.

Operational Steps For Potential Measurement: Set the silver electrode on the right. The silver electrode should be clean and wiped with cotton moisened with ammonium hydroxide. Connect the two prong plugs from the stand and

unit into 110 volt A. C., 60 cycle sources. The sample solution is then installed and the instrument is tuned in by turning the Eye Control Knob clockwise. After waiting 5 or 10 minutes for warming up the instrument, set the electric stirrer on adequate speed.

The titrimeter is calibrated each time. Set the volts scale at 0.500 volt, set "Cal-Use" control to "Cal" and adjust the Eye Control to open and close the eye. Set "Cal-Use" control to "Use" and repeat the above calibration procedure 2 or 3 times.

Titration Procedure: Add slowly first 0.10 ml. of standard silver nitrate solution. After two of three minutes stirring, the standard silver nitration solution is added continusouly until 0.550 volts is recorded on the Potentiometer. Further additions are then made drop wise until the end point of 0.572 - 0.573 V. is reached.

When silver nitrate is added to a solution containing chloride ions, the concentration of silver ion in the solutions is at first very low. As more silver ions are added, they increase first slowly then more rapidly as the equivalance point is reached. When this point is passed and all the chloride ions were precipitated as silver chloride, silver ions accumulate very rapidly with resulting rapid change in voltage.

Study of the Effect of Concentrations of $AgNO_3$ Solution

It was deemed necessary to determine the minor concentrations of $AgNO_3$ solution which could be satisfactorily used in the titration of Cl^- by this method. Varying concentrations were, therefore, tested under conditions similar, insofar as possible, to the conditions that would be obtained in an analysis of insecticide residues. For example, in the routine analysis for organic chloride of insecticidal residues by the sodium fusion procedure, substantial amounts of sodium nitrate (about 9.24 gm. per 70 ml.) remain in the final solution. For this reason, an equivalent amount of reagent grade of sodium nitrate was added to each titration aliquot for the following calibration tests.

Varying concentrations of $AgNO_3$ were tested varying from 0.00564 N. to 0.03 N. The results of these investigations are presented separably for each concentration in Table I and V.

Within the limitation of the sensitivity of each instrument, it is desirable to determine the optimum concentration of silver nitrate; therefore, the sensitivities and accuracy as influenced by 0.03 N., 0.0028 N., 0.0056 N. and 0.0141 N. silver nitrate solutions were investigated. Of the concentrations used, 0.0028 N. and 0.0056 N. silver nitrate solutions were unsatisfactory because of difficulty

Table I

Results obtained in calibration with 0.03 N silver nitrate solution.

Known Chloride Mg.	Titration Value Ml.	Endpoint Volt	Mg Cl Recovered	% Recovered
0.5	0.450	0.583	0.479	95.8
0.5	0.425	0.573	0.452	90.4
0.8	0.725	0.578	0.772	96.5
0.8	0.725	0.572	0.772	96.5
1.0	0.975	0.601	1.038	103.8
1.0	0.925	0.590	0.985	98.5
1.0	0.925	0.580	0.925	98.5
1.5	1.517	0.580	1.517	101.1
1.5	1.400	0.579	1.491	99.4

Table II

Results obtained in calibration with 0.0028 N silver nitrate solution.

Theoritical Chloride Mg.	Titration Value Ml.	Endpoint Volt	Mg. Chloride Recovered Mg.	% Recovered
0.5	4.91	0.571	0.491	98.2
1.5	14.30	0.568	1.430	95.2

Table III

Results obtained in calibration with 0.0056 nitrate solution.

Theoritical Chloride Mg.	Titration Value Ml.	Endpoint Volt	Chloride Recovered Mg.	% Recovered
0.2	0.85	0.570	0.17	85.0
0.5	2.50	0.557	0.50	100.0
0.5	2.45	0.559	0.49	98.0
1.0	4.65	0.580	0.93	93.0
2.0	9.40	0.547	1.88	94.0
3.0	14.35	0.541	2.87	95.7

Table IV

Results obtained in calibration with 0.0141 N silver nitrate solution

Theoretical Chloride Mg.	Titration Value Ml.	Endpoint Volt	Chloride Recovered Mg.	% Recovered
0.1	0.125	0.584	0.063	63.0
0.1	0.125	0.578	0.063	63.0
0.2	0.25	0.574	0.13	63.0
0.2	0.25	0.570	0.13	63.0
0.3	0.45	0.570	0.23	63.0
0.3	0.45	0.564	0.23	75.0
0.4	0.55	0.567	0.23	75.0
0.5	0.85	0.567	0.43	85.0
0.5	0.85	0.552	0.43	85.0
0.7	1.25	0.561	0.63	90.8
0.7	1.40	0.562	0.70	90.8
1.0	1.85	0.563	0.93	93.0
1.0	1.85	0.563	0.93	93.0
1.5	3.10	0.563	1.58	104.8
1.5	3.05	0.563	1.53	101.7
2.0	4.25	0.564	2.13	101.7
2.0	4.05	0.572	2.03	101.3
3.0	5.98	0.573	2.99	99.6

Table V

Results obtained in calibration by 0.014 N silver nitrate solution. Solution of Cl^- acidified by 1 ml. of 16 N nitric acid.

Theoretical Chloride Mg.	Titration Ml.	Titration - Blank Ml.	Recovered Mg.	Recovered %
0.0	0.15	-----	-----	-----
0.0	0.15	-----	-----	-----
0.0	0.15	-----	-----	-----
0.1	.24	.09	.045	45.0
0.1	.25	.10	.050	50.0
0.1	.23	.08	.040	40.0
0.2	.49	.34	.170	85.0
0.2	.39	.24	.120	60.0
0.5	1.11	0.96	.480	96.0
0.5	1.12	0.97	.485	97.0
0.5	1.14	0.99	.495	99.0
1.0	2.05	1.90	.950	95.0
1.0	2.22	2.07	1.035	103.5
1.0	2.12	1.97	0.985	98.5
1.2	2.54	2.39	1.195	99.5
2.0	4.13	3.98	1.990	99.5
2.0	4.11	3.96	1.980	99.0
2.0	4.18	4.03	2.015	100.7
3.0	6.25	6.10	3.050	101.6
2.2	4.66	4.51	2.252	102.4
2.2	4.46	4.31	3.153	97.8
2.2	4.55	4.40	2.200	100.0

in elucidation of end point. However, as far as the end point detection was concerned, both 0.03 N. and 0.0141 N. silver nitrate solution were satisfactory, addition of 0.02-0.03 ml. showed a substantial deflection.

The last two experiments showed the effect of acidity of the sample solution. Perhaps, the carbonate ion effect showed on the experiment of Table IV. By the addition of nitric acid, the carbonate ion is eliminated from the electrode system.

Unfortunately in ranges of less 0.5 mg. chlorine the recoveries were unsatisfactory. It maybe that this is due to an effect of solubility of silver chloride. The effect of the added salt (sodium nitrate) did not result in decrease in sensitivity in these tests.

Application of Technique to Insecticide-Containing Materials

Procedure for Soil Samples: The following procedure was finally adapted for the routine determination of Cl^--containing insecticides in soil:

1. Weigh out a 500 grams air dried sample into a wide mouth 1/2 gallon mason jar. Add 500 ml. of reagent grade benzene, place on mechanical shaker, and shake for one hour.
2. Remove the jar from the shaker and drain the benzene through a piece of cloth into a graduate cylinder. Record the volume of benzene extract recovered.
3. Evaporate the solvent until about 5 ml. remains, under an infra red light with ventilation of an electric fan.
4. Add 10 ml. of reagent grade ether and filter into an alkali resistant reflux flask, wash by 10 ml. of ether successfully.
5. Evaporate until about 5 ml. remains; add 25 ml. of isopropanol.
6. Add 2.5 grams of small pieces of metallic sodium to the flask. Reflux the mixture vigorously for 30 minutes.
7. After refluxing, add 15 ml. of a 1:1 mixture of water-isopropanol through the air condenser. Allow the excess sodium to react. After the reaction has ceased, reflux mixture for an additional 10 minutes.
8. Add 10 ml. of water and evaporate the liquid until the alcohol is gone.

9. After cooling, neutralize with 16 N. nitric acid until phenolphthalein end-point, add 10 ml. of 16 N. nitric acid.

10. Filter the solutions. Wash 4 times by water.

11. Titrate by 0.0141 N. silver nitrate solution by Fisher titrimeter.

Procedure for Vegetable, Hay or Grass Samples. The following procedure was finally adapted for plant material samples:

1. Cut the sample of plant material into 3 inch strips, mix well and weigh out a 100 grams sample into a wide-mouth 1/2 gallon Mason jar. Add 100 ml. benzene. Place on mechanical shaker and shake for one hour.

2. Filter the sample through a piece of cloth.

3. Add a misture of attaclay and supercel (2:1 respectively) about 10 % of benzene extract. Shake vigorously for about 20 minutes, let stand 10 minutes, filter the benzene extract, record the volume of benzene recovered.

4. Evaporate until 5 ml. remains. The remainder of the analysis is conducted as previously indicated for soil extracts.

DISCUSSION

The results of the studies made with varying concentration of $AgNO_3$ in the titrating solution were subjected to statistical treatment and presented in Table VI. It can readily be seen that there was relatively little difference in the standard deviation until the lowest concentration of $AgNO_3$ is encountered. When HNO_3 in excess was employed, this concentration could also be employed with no essential decrease in accuracy.

The relative precision between the electrometric method and the Van Slyke method was compared by analysis of insecticide residue in alfalfa hay and soil. The results were as follows (Table VI):

Pooled variance of electrometric method.....0.00081

Pooled variance of Van Slyke method.....0.09316

The data from the study comparing 0.014 N. $AgNO_3$ with and without an excess of HNO_3 was subjected to analysis of variance to determine whether this difference in apparent relative precision was a real difference. The ratio of variation was calculated as F values, thus

$$F = \frac{S_1^2}{S_2^2} = \frac{4320}{61.4} = 7.02$$

S_1^2..... 0.0141 N. $AgNO_3$ with nitric acid

S_2^2..... 0.0141 N. $AgNO_3$ without nitric acid

Table VI

Comparison of Sum of Square and Standard Deviation of Percentage Recovery of Known Amounts of Chloride with Various Concentrations of Silver Nitrate.

Expt. No. / Conc. $AgNO_3$	0.03 N.	0.00282 N.	0.00564 N.	0.0141 N.	0.0141 with HNO_3
1	95.8	98.2	100.0	85.0	97.0
2	90.4	95.2	98.0	85.0	99.0
3	96.5	---	93.0	90.8	95.0
4	96.5	---	94.0	90.8	103.5
5	103.5	---	95.7	93.0	98.5
6	98.5	---	---	93.0	99.5
7	98.5	---	---	104.8	99.5
8	101.1	---	---	101.7	99.0
9	99.4	---	---	101.7	100.7
10	---	---	---	101.3	101.6
11	---	---	---	99.6	102.4
12	---	---	---	---	97.8
13	---	---	---	---	100.0
Mean	99.99	96.7	96.1	95.2	99.5
Variance	112.56	4.5	33.0	432.0	61.4
Std. Deviation*	± 3.71	± 2.1	± 2.8	± 6.3	± 2.4

* Standard Deviation $d_{\bar{x}} = \pm\sqrt{\frac{\Sigma x^2}{N-1} - \frac{(\Sigma x)^2}{N(N-1)}}$

Table VII

Comparison between Van Slyke Method and Electrometric Method

Method / Sample	Van Slyke X(mg. Cl/100 g. Hay)	S.S.	Electrometric X(mg. Cl/100 g. Soil)	S.S.
No. 1	2.55 + 0.64	0.4141	0.051 + 0.0099	0.000098
No. 2	2.04 + 0.01	0.0002	0.173 + 0.0162	0.000265
No. 3	0.68 + 0.04	0.0018	0.230 + 0.0085	0.000072
No. 4	0.67 + 0.06	0.0032	0.903 + 0.0057	0.000032
No. 5	0.16 + 0.21	0.0465	0.652 + 0.0184	0.000338
Pooled Variance	0.4658/5 = 0.09316		0.000805/5 = 0.000161	

Table VIII

Summary of Statistical Data

Conc. $AgNO_3$	0.03N	0.00282N	0.00564N	0.0141N	0.0141N + HNO_3
Mean	99.99	96.7	96.1	95.2	99.5
Variance	112.56	4.5	33.0	432.0	61.4
Degree of Freedom	8	1	4	10	12
F value	1.82	13.64	1.86	7.02	----
Table F(5%)	2.85	19.41	5.91	2.91	----

Then the F value is read from the statistical table under d. f. 10 and 12, F 2.91. It is concluded tha F is significant at the 5% level, for potentiometric determination of chloride. The procedure using the nitric acid addition is definitely more precise than the use of silver nitrate alone.

The comparison of other concentrations of $AgNO_3$ with 0.0141 N. of silver nitrate with nitric acid show no statistically significant difference.

Although the method described was developed for analysis of spray residues, it could also be applied to the estimation of the technical product or to commercial formulations. The procedure outlined for extraction of residue from plant material is intended to serve as a general guide, and should be modified as necessary for application to specific samples. The chloride contain insecticides that have been analyzed by the method described include, D.D.T., Toxaphene and Endrin in alfalfa hay and in soil samples.

SUMMARY

An electrometric method has been developed for the estimation of spray residues of chloride containing insecticides.

Chloride containing insecticides are removed from the plant material and soil by extraction with benzene and decomposed by metalic sodium into sodium chloride. The chloride ion is titrated by a Fisher Titrimeter using silver-silver chloride electrodes with 0.0141 N. standard silver nitrate solution under the condition of strong nitric acid acidity.

Amounts of chloride between 0.5 - 30 mg. in each sample may be readily determined. The method is applicable to the analysis of a wide variety fo plant meterials or to soil samples.

BIBLIOGRAPHY

(1) Agazzi, E. J., Peters, E. D. and Brooks, F. R. Anal. Chem. 25, 237 (1953)

(2) Agazzi. E. J., Thomas, D. P. and Brooks, F. R. Anal. Chem. 26, 1011 (1951)

(3) Anderson, G. R. and Baker, G. O. Agron. J. 42, 456-8 (1950)

(4) Blaedel, W. J., Lewis, W. B. and Thomas, J. W. Anal. Chem. 24, 509 (1952)

(5) Carter, R. H. Anal. Chem. 19, 54 (1947)

(6) Carter, R. H. and Hubanko, R. E. J. Assoc Office Agr. Chemists. 29 111 (1946)

(7) Chanin, M. Science 119, 323 (1954)

(8) Chisholm, R. D. and Koblitsky, L. Agri. Chemicals 7, No. 2 47-49, 119 (1952)

(9) Claborn, H. V. J. Assoc. Official Chem. 29, 330-7 (1946)

(10) Cochron, W. G. and Cox. G. M. "Experimental Designs" Wiley, New York, 1950

(11) Fahey, J. E. and Rusk, H. W. Anal. Chem. 23, 1826 (1951)

(12) Fleming, W. E., Coles, L. W. and Maines, W. E. J. Econ. Ent., 44, 310-15 (1951)

(13) Furman, N. H. Anal. Chem. 26, 84 (1954)

(14) Furman, N. H. and Low, G. H., Jr. J. Am. Chem. Soc. 57, 1585 (1935)

(15) Hallen, H. L. "Advances in Chem. Ser." No. 1 65 (1950)

(16) Hellmkamp, G. K., Gunther, F. A. and Wolf, J. P. J. Agr. and Food Chem. 2, 836 (1954)

(17) Hoskins, W. M. and Messenger, P. S. "Advances in Chem. Ser." No. 1, 93 (1950)

(18) Iwasaki, I., Utsumi, S. and Ozawa, T.
Bull. Chem. Soc. Japan 25, 226 (1952)
Via C. A. 47, 10407f.

(19) Jones, H. and Baum, H. Anal. Chem 27 99 (1955)

(20) Jones, L. W., Soil Sci. 73, 237-41 (1952)

(21) Kolthoff, I. M. and Kuroda, P. K.
Anal. Chem. 23, No. 9 1306 (1951)

(22) Koblitsky, L. and Chisholm, R. D.
J. Assoc. Official Agr. Chem. 32, 781-6
(1939)

(23) Lambert, J. L. and Yasuda, S. K. Anal. Chem. 27,
444, (1955)

(24) McLean, F. C. and Van Slyke, D. D. J. Biol. Chem.
21, 364 (1915)

(25) Peters, E. D., Rounds, G. C. and Agazzi, E. G.
Anal. Chem. 24, 710 (1952)

(26) Peters, J. P. and Van Slyke, D. D. "Quantities
Clinitical Chemistry" Vol II 842-46
(1932)

(27) Phillips, W. F. and Debenedictio, M. E.
Agr. and Food Chem. 2, 1226 (1954)

(28) Sanderson, P. H. Biochem. J. 52, 502-5 (1952)

(29) Snedecor, G. W. "Statistical Methods" Iowa State
College Press, Ames, Iowa (1948)

(30) Spicer, G. S. and Strickland, J. D. H., Anal. Chem.
Acta 6, 493 (1953) via C. A. 46, 7939
(1952)

(31) Tiedjeno, V. A. Agr. Chemicals 7, No. 3, 4507
118-20 (1952)

(32) Van Slyke, D. D. and Donleavy, J. J. J. Biol. Chem.
37, 551 (1919)

(33) Volhard, A. S. J. Anal. Chem. 17, 481 (1878)

(34) Yeck. R. P. and Kissin, G. H. Anal. Chem. 17, 692,
(1945)

II. Measurement of Free and Combined Cyanide in Plants

INTRODUCTION

Cyanogenetic forage plants, especially Johnson grass, sorghum, sudan, and flax, have been extensively investigated by many workers both in this country and abroad during the past fifty years. This interest has been concerned primarily with the cyanogenetic glucosides, which under adverse conditions have a tendency to liberate hydrocyanic or prussic acid (30), a compound wich is extremely poisonous to domestic animals (8,19). The cyanogenetic glucosides contained in these plant materials are not toxic in themselves, but under adverse conditions, such as high temperatures, wilting, frosting of succulent second growth and mechanical injury to the plants, enzymes present in the tissues hydrolyze the glucosides and liberate free hydrocyanic acid (31). It has been generally assumed that after the plant has had an opportunity to recover from such injury, the HCN gradually disappeared.

Early investigators were also of the opinion that when these injuried crops were harvested and dried, the toxic compound also gradually disappeared. Some authorities, however, were of the opinion that the free HCN might remain in the dried forage and produce toxicity (3,28). This later concept has been supported by investigations in this station. In a number of instances, Johnson grass hay, which had been put in the mow has been found extremely toxic even after a year in storage. It has also been

thought that in the process of making silage, the toxic material likewise disappeared. It has been observed during the past year, however, that this may not always occur. On several occasions, a high prussic acid content has been found in silage with reported deaths of animals (2).

A serious handicap in the investigation of this problem has been a lack of satisfactory methods for the analysis of the toxic substance and its non-toxic precursor. A second difficulty has arisen in attempting to distinguish between the total prussic acid liberated during hydrolysis in the laboratory and free prussic acid present at the time the plant was harvested. There is ample evidence that whenever the plant is cut or crushed, enzymatic action follows with liberation of HCN from the glucoside. Thus, since considerable time may elapse between collection of the material and analysis in the laboratory, spuriously high values are frequently obtained.

Various methods have been devised and improved over the years so that it is possible to measure the total HCN content of the plant with accuracy. Several comprehensive bibliographies, dealing with this subject, have been published and are available, so references to previous work only be cited when the data has a direct bearing upon this investigation.

So far as the determination of free CN^- ion is concerned, numerous papers had been published from different

points of view (1,4,25,26,27). The forensic chemist has been particularly concerned in developing methods for free CN^-.

One of the classical methods is based upon the development of prussian blue (13,14,15,20). Thus:

$$3\ FeSO_4 + 18\ NaCN + 2\ Fe_2(SO_4)_3 \rightleftharpoons Fe_4[Fe(CN)_6]_3 + 9\ Na_2SO_4$$

When present in large amounts CN may be determine as follows (24):

$$Ag^+ + 2\ CN^- \rightleftharpoons Ag(CN)_2 \text{ (quantitative at end point)}$$

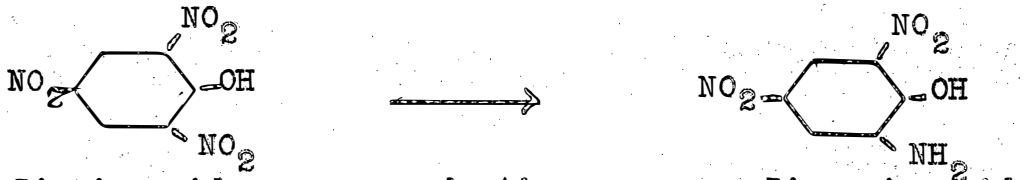
$$Ag^+ \text{ (excess ion)} + I^- \longrightarrow AgI$$

Add 5 to 6 ml. of 6 N. ammonia and 0.2 g. of KI to CN^- containing sample, dilute about 100 ml. and titrate with 0.1 N. silver nitrate soltution until a permanent tubidity of silver iodide is obtained.

Colorimetric procedures have been investigated: for example, in the presence of Cu oxidizes a basic solution of phthalein to colored phthalein (23), alloxan conversion to isopurpurate (13), and others (14).

Of the available colorimetric methods for the determination of CN^- in tissues of plants of the sorghum family, that using alkaline sodium picrate seems best suited.

The major step in this reaction is the formation of the red colored picramate ion from yellow picrate ion, thus:



Picric acid yellow — reduction — Picramic acid red

The reaction (11,17) is not specific for CN^- but also is brought about such compounds as; Na_2S, H_2S, PH_3 and AsH_3. Fortunately, these compounds do not occur in plant tissues in appreciable quantities or do not steam distill with the HCN during its liberation and isolation.

It is recognized, however, that existing methods are not entirely satisfactory in determining cyanides in plants tissues due to the failure to differentiate between the free and combined forms of CN^-.

EXPERIMENTAL

Quantitative Determination of HCN in Plant Materials.

Method for the estimation of total HCN in plant materials have been revived by Melville and others (1940) (20). Routine determination of total HCN has been made in these laboratories by the method of the A. O. A. C. a satisfactory method for the determination of free HCN existing in the plant when collected in the field. Since this is the poisonous materials, it would be advantageous to have a suitable method to permit its quantitative measurements.

It was felt that an approach to the problem might be made through the inhibition of the enzyme responsible for the hydrolysis of the cyanogenetic glucoside. If a satisfactory inactivating procedure could be found, it might be possible to prevent the release of HCN and thus permit the assessment of the free HCN originally present.

Of the possible enzyme inhibitors, mercuric chloride, trichloroacetic acid and phosphotungestic acid were considered. These were discarded since they combine with HCN and interfere with its isolation. Inactivation by means of heat was next considered and a procedure was devised which proved to be satisfactory.

It would be necessary, however, to devise a method to cause the release of CN^- from the glucosidic linkage in

order to measure total cyanide. Combined cyanide could then be calculated by difference. Enzymatic hydrolysis was deemed more satisfactory than strictly chemical means since any treatment effecting the CN^- - carbohydrate linkage would hydrolyze many other linkages with possible interference. There are numerous sources of β- glucosidases capable of hydrolyzing the cyanogenetic β - glucosides.

Development of Method for Free HCN. The following procedure was finally adopted for routine use in the determination of free cyanide:

Sampling.

The sample was collected in the field and placed in a sealed container. The container and sample was placed in a pre-heated autoclave and heated at 18 lbs. pressure for 2 minutes. Autoclaved samples were then cut into 1/2 inch pieces. The sample was well mixed and a sub-sample weighed out and placed in a wide mouth 500 ml. Erlenmeyer flask.

Isolation of HCN.

The HCN was then steam distilled into 10 ml. of 0.2 N NaOH solution. The distillation was stopped when approximately 200 ml. of distillate had been collected. The distillate was accurately made to 200 volume.

Measurement of HCN.

The estimation of HCN was carried out by the following colorimetric procedure. A suitable aliquot was taken from the distillate and placed in a colorimetric tube. 5 ml. of sodium picrate solution was added into the sample solution, and the mixture was heated for 20 minutes in boiling water. The tube was then covered in water for 15 minuted. The resulting color was read in the photometer at 565 mμ . A standard curve was prepared from known concentrations of KCN, starting from the steam distillate with addition of 10 ml. of saturated tartaric acid. The agreement with Beer's Law was excellent.

Reagents.

(1) 0.2 N NaOH solution.

(2) Standard HCN solution 1 ml. ≒ 0.05 mg. HCN. Place stock solution 1.2057 grams of KCN in a 500 ml. volumetric flask and make up to volume with water. Standard solution: Take 50 ml. of the stock solution and make up to 1000 ml. with water.

(3) Sodium picrate solution. Dissolve 5 grams of recrystallized picric acid and 50 grams of sodium carbonate in 1000 ml. of water.

Development of Method for Total Cyanide in Plant Materials. The successful procedure for the determination of total HCN in the plant materials depends on complete hydrolysis fo the glucoside in the sample. In a large number of estimations of HCN in grass samples, the amount of free and combined HCN varied and depended on the treatment of samples. For instance, a sample of old hay contained all the HCN as free HCN. Another hay sample contained higher amounts of cyanogenetic glucoside and low free HCN. Some HCN remained in sillage after 2 months. As far as the presence of glucosidase is concerned, it was assumed that the concentrations of enzyme is variable, as has been shown to be true in other species (5).

The substitution of autolysis for hydrolysis has been suggested in some procedures (24, 29, 31). For these hay samples, such a technique was not satisfactory. To provide successful contact between enzyme and substrate, chloroform was added for cytolysis of the plant cell (9, 18). Glucosidase solution (prepared as described on page 36) is added to the sample to accelerate and complete hydrolysis of glucosides. The sample was incubated at 37° C for two or three hours (after 1 hour 30 % of the glucoside was hydrolyzed, after two hours the maximum HCN was obtained and after three, or four hours, did not show further increase of HCN).

The following procedure was finally adopted for the

determination of total cyanide in plant materials:

Sampling.

Cut grass sample into 1/2 inch, mix the sample, weigh 20 grams into 700 ml. in a wide mouth Erlenmeyer flask.

Release of combined CN^-, steam distillation, development of color by sodium picrate solution.

Preparation of β - glucosidase (linimarase) extract from flax seed.

The enzyme preparation was made from flax seed by an adaptation of the method of (Cook 1940) (5).

Ordinary flax seed, which is the richest available source of the enzyme, was put through the grinder to break the seeds. The product was repeatedly extracted with ether for about forty-eight hours. The oil-free meal was again ground through a fine screen. One hundred grams of the finely ground and defatted meal were shaken vigorously in lots of about 20 g. with 41 ml. of water, and immediately centrifuged. 1,000 ml. of a slight viscous solution resulted containing about 10 % of original weight of meal and nearly all of the enzyme activity. A mixture of 100 ml. sodium acetate and 100 ml. N/5 acetic acid was added to this with stirring. After standing for a few hours, a white precipitate, which was centrifuged off. The supernatant liquid was cooled to below 10° c. and one-half its volume of cold

alcohol added, with stirring. A further precipitate was formed. This precipitation was centrifuged off, discarded, and 2,900 ml. of cold 95 % alcohol, added with stirring to make a 60 % alcohol solution. Almost all the enzyme was precipitated at this stage and was collected by centrifugation. The precipitate was taken up in 100 ml. of distilled water and brought to neutrality by adding dilute NaOH solution. This enzyme is believed to hydrolyze specially β-d-glucosides. The rate of hydrolysis may vary greatly due to different β-d-glucosides but the specificity towards the β-d-glucosides is relatively great. Although the rate of hydrolysis may vary, from plant sample to plant sample, the enzyme is sufficiently active to use for liberation of HCN from cyanogenetic glucosides.

Calculation of bound CN^- Combined cyanide is calculated by difference, subtracting the free cyanide as determined by the method previously described from the total cyanide as determined following enzymatic hydrolysis.

Carbohydrates as Inhibitors of Enzyme System

There have been several observations reported in the literature that glucose, soluble sugars or other readily available carbohydrates counteract the toxic effect of hydrogen cyanide in forages consumed by livestock. (7, 8, 12). There have been suggestions as to the possible mechanism by which these carbohydrates might exert their influence. One of these has been that the presence of large amounts of readily available carbohydrates affects the activity of the hydrolytic enzyme which bring about the hydrolysis of cyanogenetic glucoside.

It was deemed worthwhile in the course of the investigation recorded here to undertake a brief study to determine whether or not the presence of carbohydrates would affect the hydrolysis of the naturally occuring glucosides by the preparation of β- glucosidase from linseed meal.

EXPERIMENTS

The determination of the effect of added carbohydrates was conducted using varying forms of carbohydrates, the naturally occuring glucoside present in Johnson grass, and the enzyme derived as previously described from linseed meal. Free and glucosidic cyanide was determined by the methods previously described in this thesis. The detailed procedure for the tests were as follows:

The source of cyanogenetic glycoside was a sample of

freshly collected macerated Johnson grass which had been autoclaved for three minutes at 18 pounds pressure to destroy any internal glucosidases. This sample of material was analyzed for free and combined cyanide with the following results (three replications): Total HCN, 6.4 mg. % ; free HCN, 3.8: combined HCN, 2.6 mg. %. The reaction mixture consisted of 1 gm. of macerated, autoclaved Johnson grass tissue, 1 ml. of the glucosidase preparation, varying amounts of different carbohydrates, and 25 ml. of water. The samples were incubated at 37° C for a total of eight hours. Qualitative analysis for HCN was carried out using the picric acid paper method of Guignaid (22, 25). The results of the experiment are given in the table below:

Effect of Added Carbohydartes on Hydrolysis of Johnson Grass Cyanogenetic Glucosides by Linseed Meal Beta-glucosidase

Added Carbohydrate	2 hrs. incubation	8 hrs. incubation
Sucrose 0.5 g.	(+)	(-)
Sucrose 5.0 g.	(+)	(-)
Maltose 0.5 g.	(-)	(-)
Soluble starch 0.5 g.	(-)	(-)
Fructose 0.5 g.	(-)	(-)
Galactose 0.5 g.	(-)	(-)
Inulin 0.5 g.	(-)	(-)
Lactose 0.5 g.	(-)	(-)
Lactose 5.0 g.	(+)	(-)

Note. + = detectable inhibition
- = no detectable inhibition

DISCUSSION

The results of this study do not suggest that there is any direct effect of added carbohydrates even in relatively high concentrations upon the hydrolysis of the cyanogenetic glucosides of Johnson grass of linseed β - glucosidase. This does not, of course, preclude the possibility that naturally occurring glucosides and glucodidases from other sources might be so affected. It does, however, provide no support for this view of the mechanism of the effect of carbohydrate on the toxicity of cyanide containing plants to livestock.

There are several other hypothesis as to the mechanism which might account for a protective effect of available carbohydrate in the ration. One of these is that the addition of large amounts of carbohydrate provides a surplus of aldehyde groups. The reaction between cyanide radicals and aldehyde groups is well known resulting the formation of cyanohydrin complexes. Such complexes might not be readily absorbed through the rumen wall and thus might delay the adsorption of cyanide and permit the process of detoxification to take place.

A second possibility might well be that the addition of large amounts of readily available carbohydrates would promote a very active rumen fermentation with the comsumption of nitrogen for bacterial production, including the nitrogen contained in the cyanide radical. Various bacteria

are known to be able to use the cyanogen ion for growth, and it is possible that under these circumstances this is increased by the presence of readily available carbohydrates. Here again the availability of the cyanide to the animal would be eliminated and the amount absorved from the rumen reduced.

A third possibility is that the conversion of readily fermented carbohydrates into acid in the rumen and the absorption of these materials into the blood stream of the animal in some way affects the metabolism of the organism rendering it less sensitive to the toxic effects of cyanide.

SUMMARY

An investigation has been conducted to determine the effects of the addition of large amounts of carbohydrates on the hydrolysis of the naturally occuring cyanogenetic glucoside of Johnson grass by the glucosidase derived from linseed meal. Sucrose, maltose, fructose, gluctose, lactose, starch and inulin, were all investigated and none of these in concentrations of 0.5 to 5.0 percent gave any indication of affecting the rate of hydrolysis of this cyanogenetic glucoside by this particular anzyme.

HYDROLYSIS OF JOHNSON GRASS CYANOGENETIC GLUCOSIDES BY VARIOUS ENZYMES

An investigation of the literature failed to reveal any direct information concerning the type of glucoside present in Johnson grass. Since it seemed important to determine whether or not the naturally occurring glucoside in this particular plant was of the alpha form or the beta form, experiments were set up using naturally occurring enzymes known to hydrolyze either alpha or beta glucosides in order to provide information of this particular point.

EXPERIMENTAL

The following specific anzymes were used in this study, alpha amalysase from saliva, takadiastase, maltase, invertase, intestinal mucosa preparation from pigs. The glucoside investigated was the naturally occurring glucoside of Johnson grass present in the form of a mascerated sample of the grass. The determination of liberated cyanide was by the picric acid qualitative test of Guignard (22, 25). The results obtained in this particular experiment are recorded in the table are known to contain alpha analyses and to be relatively devoid of beta-amalytical activity. On the other hand, taktidiastase, malt diastase, beta-glucosidase from linseed meal and other sources of known beta-glucosidase content brought about the hydrolysis of the naturally occurring glucoside in Johnson grass. This seems

Table

Action of Various Sources of Glucosidases on Hydrolysis of the Cyanogenetic Glucosides of Johnson Grass.

Source of Enzyme	2 hours incubation at 38° C	8 hours incubation at 38° C	20 hours incubation at 38° C
α-amalysase	(-)	(-)	(-)
takadiastase	(+)	(+)	(+)
diastase from malt	(+)	(+)	(+)
salivary fluid	(-)	(-)	(-)
intestinal mucosa	(-)	(-)	(+)
β-glucosidase solution	(+)	(+)	(+)
Blank	(-)	(-)	(-)

+ = detectable hydrolysis

- = no detectable hydrolysis

adequate evidence to suggest that the naturally occurring glucoside in Johnson grass has the beta configuration. This would be in common with a number of other well defined cyanogenetic glucosides which have been isolated from a variety of plants (6).

SUMMARY

A qualitative examination of the hydrolysis of the naturally occurring cyanogenetic glucoside of Johnson grass by a variety of enzymes preparations suggests that the glucoside has the beta configuration with the respect to the linkage between the cyanide and the carbohydrate moiety.

RESULTS AND DISCUSSION

The free and combined hydrocyanic acid content of plants appears to vary depending upon the time of sample collection. In table I are presented the results of analysis of Johnson grass for both free and total hydrocyanic acid. The free hydrocyanic acid is one-half to two-thirds of the total hydrocyanic acid in the Johnson grass (table I). The total amount was quite small in comparison to the results of analysis on mature plants both in these laboratories and by previous workers (3, 12). It would appear justifiable to study, using the methods reported here, the variation in the cyanide content of several species of cyanogenetic plants throughout their normal growing season.

By calculation (19), 5 pounds of Johnson grass would provide enough HCN to be fatal to cows and horses if Johnson grass contained 20 mg. % of free hydrocyanic acid. For sheep, 1.25 pounds of Johnson grass would be fatal under similar circumstances. It can be seen, therefore, that, in theory, sufficient cyanide is present in the plants at the time these samples were taken to cause serious toxicity problems. Further studies of the free CN^- content of cyanogenetic plants being grazed by animals without harmful effects would be justifiable.

Table II shows that free hydrocyanic acid is increased by destruction of cells following freezing of samples. It is probably that the enzyme that will hydrolyze the gluco-

Table I

Hydrocyanic acid content of Johnson grass

Date of sample collected	Free HCN mg.%	Total HCN mg.%	Combined HCN (by direct method) mg.%	(by substraction) mg.%
	4.16	6.36	3.12	----
20 April	3.67	6.22	3.80	2.54
1955	3.56	6.44	2.88	----
$\bar{x}$	3.80	6.34	3.27	2.54
$d_{\bar{x}}$	0.32	0.11	0.47	
	9.50	13.50	----	----
	8.80	----	----	----
28 April	10.10	14.45	----	4.82
1955	8.45	----	----	----
	8.80	----	----	----
	9.28	----	----	----
$\bar{x}$	9.16	13.98	----	4.82
$d_{\bar{x}}$	0.59	0.67		

Table II

Increase of free HCN content by freezing

		mg.%	$\bar{x}$
1.	No treatment free HCN	8.1	
2.	No treatment free HCN	8.3	
3.	No treatment free HCN	8.1	8.17
4.	No treatment total HCN	11.8	
5.	No treatment total HCN	12.5	12.15
6.	1 hour deep freezing and kept 1 hour at room temperature		8.82
7.	1 hour deep freezing and kept 5 hours at room temperature		11.11

side is brought in contact with the glucoside by the freezing and thawing. Nearly complete release of the total HCN is brought about by five hours of incubation at room temperature following freezing. Presumably, other traumatic conditions might bring about a similar response.

SUMMARY

1. An analytical procedure for the determination of both free and combined hydrocyanic acid in Johnson grass was developed. Following inactivation by heat of the enzyme that would hydrolyze the cyanogenetic glucoside, free hydrocyanic acid was separated by steam distillation. Hydrocyanic acid was estimated by a colorimetric method involving reaction with sodium picrate. Total hydrocyanic acid was determined by liberating the combined CN^- by incubation of the macerated plant material for 4 or 5 hours with an active β - glucosidase preparation from linseed meal.

2. Sucrose, maltose, fructose glucose, lactose, starch and inulin in concentrations of 0.5 to 5.0 percent did not give any indication of affecting the rate of hydrolysis of the cyanogenetic glucosides of Johnson grass by enzyme present naturally in the grass.

3. The principal cyanogenetic glucosides of Johnson grass appear to have the beta configuration with the respect to the linkage of the aglycon and carbohydrate moiety.

REFERENCES CITED

1. Bishop, L. R., Biochem. J., 21, (1927).

2. Blakley, R. L. and J. E. Coop, New Zealand J. Sci. Technol., 31A (1949).

3. Brise, R. R. and J. F. Couch, J. Agri. Research 57, No. 2 (1938).

4. Church, F. W. and E. E. Campbell, Am. Ind. Hyg. Assoc. Quart., 11, 109-110 (1950).

5. Coop, I. E., New Zealand J. Sci. Technol. 22, 71b-83b (1940).

6. Corkill, A., New Zealand J. Sci. Technol. Sect., B23, 128 (1942).

7. Corkill, L., New Zealand J. Sci. Technol., 34A, 1-16 (1952).

8. Couch, J. F., J. Agri. Research, 57, 81 (1938).

9. Couch, J. F. and R. R. Brise, J. Wash. Acad. Sci., 30, 413 (1940).

10. Couch, J. F. and R. R. Brise, J. Wash. Acad. Sci., 31, 285-288 (1941).

11. Doak. B. W., New Zealand J. Sci. T ech.20A, 163-166 (1938).

12. Dowell, C. T., Oklahoma A. M. C. Agri. Expt. Station, Bulletin No. 122, (1919).

13. Elsdon, G. S. and J. R. Stubbs, Analyst, 62, 540 (1937).

14. Enstein, J. Anal. Chem., 19, 272-273 (1947).

15. Fulton, R. A. and M. J. Van Dyke, Anal. Chem. 19, 922-923 (1947).

16. Galat, A., Chemist Analyst 30, 81 (1941).

17. Goswami, H. C., A. Shaha and B. Mukerjee, J. Indian Chem. Soc., 11, 773 (1934); C. A. 29, 2886 (1935).

18. Hogg, P. G. and H. L. Ahlgren, J. Am. Soc. Agron. 34, 199-200 (1942).

19. Hopper, T. H., L. M. Roderick and F. W. Christensen, North Dakota Agri. College Circular 136 (1935).

20. Melville, J. and B. W. Doak, New Zealand J. Sci. Tech., 22, 67b-71B (1940).

21. Menaul, R. and C. T. Dowell, J. Agri. Research 18, No. 8 (1920).

22. Nowosad, F. S. and R. M. MacVicar, Sci. Agri. 20, 566 (1944).

23. Nicholson, R. I., Analyst 66, 189-192 (1941).

24. Official Methods of Analysis of the Association of Offical Agricultural Chemists 7th ed., Menasha, Wisconsin, pp. 66 (1950).

25. Steyn, O. G., J. S. African Vet. Med. Assoc. 10, 65-68 (1939).

26. Sullivan, J. T., J. Assoc. Official Agri. Chemists, 22, 781 (1939).

27. Sullivan, J. T., J. Assoc. Official Agri. Chemists, 27, 320 (1944).

28. Swanson, C. O., J. Am. Soc. Agron. 13, 28 (1923).

29. Williams, H. A., Analyst 68, 50-51 (1943).

30. Willamn, J. J., J. Agri. Research, 6, 261, (1915).

31. Winkler, W. O., J. Assoc. Agri. Chem. 34, 541 (1951).

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