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The Colorimetric Determination of Sodium in Vegetation.

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In a recent issue of the Report of the Director of Veterinary Services and Animal Industry, Malan and Van der Lingen (1931) gave details of the Uranyl-zinc-sodium-acetate method for the determining of sodium in blood and vegetation. It was found, however, in the course of the determinations of sodium in grasses in connection with "The Study of the Feeding Value of South African Pastures" (Du Toit et al 1932) that the method did not give concordant results under all conditions. As a matter of fact, it was soon noticed that the values for sodium were inexplicably high on some occasions, and that they could be made to vary at will by altering the potassium content of the aliquot in question and the temperature at which the precipitation of the triple acetate is effected. It was, therefore, decided to investigate the method for the determination of sodium and to develop a reasonably accurate technique suitable for routine procedure as many thousands of determinations had to be carried out in the course of the study.

Since the completion of this work the author noticed that McCance and Shipp (1931) advise the main modification now included in the present method for the determination of sodium. However, it is considered advisable to supplement Malan and Van der Lingen's article with the present one and for that reason full details will be given.

REAGENTS.

1. Precipitating reagent:—

   (*a*) 10 gm. Uranyl-acetate dissolved in 50 c.c. water containing 6 c.c. 30 per cent. acetic acid.

   (*b*) 30 gm. zinc acetate dissolved in 50 c.c. water containing 3 c.c. 30 per cent. acetic acid.

The salts are brought into solution on the water bath, the solutions mixed while still hot, allowed to stand in a cool place for 24 hours, filtered and kept in a sodium-free glass-stoppered bottle.

2. Powdered calcium hydroxide (sodium free).

3. 20 per cent. $K_4Fe(CN)_6$ solution.

4. Absolute alcohol.

5. 96 per cent. alcohol saturated with $(UO_2)_3$ Zn. Na $(CH_3COO)_9$.

6. Standard solution of NaCl containing 0·1 mg. Na/cc.

7. Standard solution of triple acetate.

The triple acetate $(UO_2)_3$ Zn. Na. $(CH_3COO)_9 \cdot 6\ H_2O$, is prepared by mixing 300 c.c. of the above standard solution of NaCl, 900 c.c. absolute alcohol and 600 c.c. of the precipitating reagent. After 30 minutes the precipitate is collected on a filter attached to a suction apparatus, thoroughly washed with 96 per cent. alcohol saturated with the precipitate, and followed by two or three washings with ether. The precipitate is then dried in an electric oven at 103° C. and allowed to cool in a desiccator. 0·6689 gm. of the salt is accurately weighed, dissolved in 50 c.c. 10 per cent. acetic acid in a 100 c.c. volumetric flask and filled to the mark with distilled water. 1 c.c. of this solution is equivalent to ·1 mg. Na. It has been found to keep well for three months.

EXPERIMENTAL.

(*a*) Elimination of Phosphorus.

Malan and Van der Lingen directed that the elimination of phosphorus should be brought about by absolute alcohol saturated with zinc acetate. By testing the supernatant fluid so obtained for phosphate it was, however, observed that alcoholic zinc acetate eliminated phosphorus in an erratic manner. But the phosphate not removed by this means was never found to be precipitated by Uranyl zinc acetate. However, on trying to determine sodium without eliminating phosphate it was found that the precipitate of uranyl zinc phosphate is insoluble in dilute acetic acid and water. Non-elimination of phosphate would mean a saving of time and it was therefore decided to establish to what extent this insoluble phosphate precipitate would interfere with the determination of sodium.

For the first part of the investigation a pure solution of sodium chloride was accurately made up. To this definite amounts of a solution of tricalcium phosphate were added. The procedure for determining Na was that described further on in this article. Colours were compared within 5-10 minutes after development. Results obtained are given in Table I below. Table II shows the effect of eliminating and not eliminating phosphate with powdered $Ca(OH)_2$ on the sodium content of 1 c.c. of a grass extract. The percentage error is reckoned on the value obtained when phosphate has been eliminated. Column 1 gives the amount of phosphorus actually present in the aliquot extract used.

Table I.

	Mgm. Na Taken.	Mgm. P Added.	Vol. made up to.	0.1 mgm. Na std. at 20 Colorimetric Reading.	Mgm. Na Found.	Percentage Recovery.
1	·04	0	25·0 c.c.	24·8	·0404	101·0
2	·04	·1	,,	24·8	·0404	101·0
3	·04	·2	,,	23·0	·0435	108·7
4	·08	0	50 c.c.	25·0	·08	100
5	·08	·1	,,	24·8	·0807	100·9
6	·08	·2	,,	24·7	·081	101·25
7	·1	0	,,	20·0	·1	100·0
8	·1	·1	,,	19·6	·102	102·0
9	·1	·2	,,	19·4	·103	103·0
10	·2	0	100 c.c.	20·0	·2	100·0
11	·2	·2	,,	20·0	·2	100·0
12	·2	·3	,,	20·0	·2	100·0
13	·2	·4	,,	20·6*	·194	97·0
14	·4	·4	,,	10·1	·396	99·0
15	·8	0	,,	5·0	·8	100
16	·8	·4	,,	5·0	·8	100

* Compared three minutes after development of colour.

Table II.

Extract No.	Mgm. P in Aliquot Used.	Mgm. Na Found in Aliquot without Removing P.	Mgm. Na Found in Aliquot after Eliminating P.	Percentage Error.
1.....	·035	·033	·027	+ 22·2
2.....	·012	·035	·029	+ 20·7
3.....	·20	·02	·01	+ 100·0
4.....	·14	·015	·011	+ 36·3
5.....	·16	·015	·01	+ 50·0
6.....	·15	·014	·01	+ 40·0
7.....	·12	·015	·011	+ 36·3
8.....	·17	·015	·01	+ 50·0
9.....	·14	·042	·032	+ 31·2
10.....	·18	·145	·141	+ 2·8
11.....	·28	·235	·231	+ 1·7
12.....	·33	·43	·41	+ 4·8
13.....	·73	·586	·570	+ 2·8
14.....	·05	·12	·119	+ ·84
15.....	·05	·10	·10	0
16.....	·07	·153	·148	+ 3·4
17.....	·06	·126	·125	+ 0·8
18.....	·09	·204	·204	0
19.....	·09	·114	·114	0
20.....	·21	·17	·167	+ 1·8

A glance at Tables I and II brings out the fact that if the phosphorus content of the specimen to be tested for sodium is not unduly high reasonably accurate values are obtained between the limits ·04—·8 mgm. Na. The interference of phosphorus is limited to a slight turbidity in the developed colour. In Table I the greatest error occurs in No. 3, where ·2 mg. P was present in an aliquot containing ·04 mg. Na and the volume was made up to 25 c.c. The phosphate precipitate was dispersed through the whole solution in a finely divided condition giving the developed colour a distinct milky appearance. If this colour is compared 2 or 3 minutes after development a low value is obtained against a standard of the pure sodium triple acetate, 10 minutes after the colour intensity has appreciably increased, so much so that a result above the theoretical amount of Na is obtained. With the same amounts of phosphorus present but higher quantities of Na and consequently larger volumes in which colours are developed this effect becomes negligible, the error introduced being within the limits usually encountered in colorimetric work.

With all sodium values below ·04 mgm. where the volume is made up to only 10 c.c. the interference of phosphorus becomes more marked. Apart from the fact that even when phosphorus has been eliminated values differing by as much as 30 per cent. for the same test sample have been obtained, the elimination here is advisable especially when the phosphorus content of the sample to be analyzed is high.

The use of $Ca(OH)_2$ in powder form has been found a rapid and reliable method for eliminating phosphorus. For the purpose of routine work on grasses it will seldom be found necessary to resort to elimination. In the present method the potassium content of a grass determines the aliquot allowable in the determination of sodium. In the case of green grasses with high potassium content aliquots as low as 0·2 c.c. have to be used. This at the same time brings the phosphorus content of the sample to be tested for sodium to such a low level that interference from this source will be serious only in those samples having a low sodium content, when, in addition, the volume in which the colour has to be developed is only 10 c.c. However, when the sodium level drops to such a low value that phosphorus interferes under the conditions described an accurate determination is hardly necessary for the difference in intake of sodium by an animal on pasture containing ·01 or ·02 per cent. is negligible as either value gives an exceedingly low intake compared with the requirements of the animal.

(*b*) Interference of Potassium.

Especially on cold winter days a coarse yellow crystalline precipitate as distinct from the fine precipitate obtained from sodium alone was deposited on the sides of the precipitating tube. This precipitate was found to be readily soluble in dilute acetic acid and

water. Results could seldom be reproduced, especially when duplicates were tried on hotter days or when a different aliquot was used in the determination. The following table is given to illustrate the point: —

Table III.

	Precipitating Temperature: 26° C.			Precipitating Temp.: 20° C.
	Mgm. Na per c.c. extract when 0·2 c.c. is used.	Mgm. Na per c.c. extract when 0·5 c.c. is used.	Mgm. Na per c.c. extract when 1 c.c. is used.	Mgm. Na per c.c. extract when 1 c.c. is used.
1	·047	·133	·69	1·08
2	·029	·258	·66	1·05
3	·029	·053	·33	·89
4	·032	·037	·31	·67
5	·024	·046	·33	·68
6	·32	·42	·49	1·11

Sjollema and Dienske (1931) stressed the interference of potassium when the ratio of Na : K exceeds 1 : 20 due to the co-precipitation of a Pot. uranyl salt. To overcome this difficulty the authors resorted to partial elimination of potassium by means of tartaric acid. Apart from the fact that under certain conditions of temperature the residual potassium may still interfere with an accurate determination, the procedure is much too laborious to be of practical value in our work.

Tests were undertaken by the author in order to satisfy himself as to the reliability of Sjollema and Dienske's conclusion on the one hand, and to ascertain the reason for inconsistent results obtained in analysing grass extracts on the other. Numerous tests with known solutions of NaCl and KCl were undertaken, the concentrations and ratios of Na : K being varied within wide limits. K was found to interfere but this interference was seldom found to be the same for any one concentration of K. Further, it was established that this interference was not dependent upon the ratio of Na : K, but upon the absolute concentration of K in the test sample.

The inconsistency of interference was established to be due to difference in temperature in the precipitating medium. The following table illustrates this point while giving at the same time an indication as to the concentration of K at which precipitation commences. 2 c.c. precipitating reagent were added to a mixture of 3 c.c. absolute alcohol and 1 c.c. distilled water containing varying amounts of K as KCl. The precipitates obtained were washed with 96 per cent. alcohol saturated with sodium zinc uranyl acetate, dissolved in 0·5

c.c. 10 per cent. acetic acid and made up to definite volumes with water. Colours developed and matched against a 0·1 mg. Na standard. Results given as mgm. sodium:—

Table IV.

	Mgm. K in 1 c.c. Water.	Precipitation at 26° C. Mgm. Na Found.	Precipitation at 10° C. Mgm. Na Found.
1	0·4	—	—
2	0·5	—	—
3	0·6	—	Trace.*
4	0·7	—	Trace.
5	0·8	—	Trace.
6	0·9	Trace	·009
7	1·0	·007	·11
8	2·0	·23	·40
9	3·0	·33	·66
10	4·0	·53	1·0

* Traces increase from Nos. 3 to 5.

According to this table potassium will begin to be precipitated in the cold at a concentration of only 0·6 mg. in the test sample whereas at the higher temperature interference commences at 0·9 mgm. However, in the presence of sodium, especially at high concentrations, one will expect less potassium to be precipitated, probably due to preferential precipitation of sodium and more of the potassium precipitate dissolving in the diluted reagent. The expectation was borne out by experiment as is clear from the following table of results:—

Table V.

PRECIPITATION AT 20° C.

	Mgm. Na Present.	Mgm. K Present.	Mgm. Na Found.	Percentage Error.
1	·05	3·0	·125	+ 150·0
2	·10	3·0	·164	+ 64·0
3	·20	3·0	·285	+ 52·5
4	·40	3·0	·50	+ 25·0
5	·60	0·6	·60	0
6	·60	3·0	·78	+ 30·0
7	·35	·50	·348	— 0·57
8	·35	2·5	·50	+ 42·8
9	1·00	·40	·98	— 2·0
10	1·00	1·00	1·06	+ 6·0
11	·80	·30	·79	— 1·25
12	·80	·80	·84	+ 5·0

(*c*) Effect of Time and Temperature on the Precipitation of Sodium.

Since the solubility of pot. zinc uranyl acetate in the precipitating medium varies greatly with temperature, it was thought advisable to ascertain the behaviour of sodium zinc uranyl acetate towards temperature and, incidentally, whether the usual 30 minutes was sufficient for complete precipitation of the sodium.

A solution of NaCl containing ·1 mg. Na per c.c. was used to test these points. One series of 1 c.c. test samples were taken through all the stages of the method, allowing precipitation to proceed at the laboratory temperature (26° C) for one hour, while a second series was similarly treated except that the tubes were placed during precipitation in a basin containing iced water registering 2° C. In a third series the sodium was precipitated at room temperature (26° C) for 30 minutes. All the precipitates were dissolved in 50 c.c. water containing 0·5 c.c. 10 per cent. acetic acid, the colours developed and matched against a standard colour from 1 c.c. of a triple acetate solution made up to 50 c.c. with water. 1 c.c. of this triple acetate solution is equivalent to 0·1 mg. Na. The standard was placed at 20 m.m. Colorimetric readings are given in Table VI.

Table VI.

First Series Room Temperature. One Hour.	Second Series 2° C. One Hour.	Third Series Room Temperature. 30 Minutes.
20·2	20·0	20·2
20·3	19·9	20·8
20·3	20·0	20·6
19·9	19·9	20·0
Averages: 20·17	19·95	20·4

The second series is closest to the theoretical reading, viz. 20·0, while the third series shows the highest error. If it is remembered, however, that the present method is intended only for routine analysis of vegetation, there is no reason to prolong the time of precipitation to 1 hour, nor for allowing it to take place at the lower temperature which has in addition the drawback of allowing potassium to interfere at lower concentrations. Tests were also made which show that the same negligible error is made when amounts of Na up to 0·9 mgm. is present in the test sample, allowing precipitation to proceed for 30 minutes at room temperature.

(*d*) Washing the Precipitate.

When all the foregoing points are taken into consideration in the determination of sodium inconsistent results will still be obtained if the precipitate is washed three times with absolute alcohol as

described by Malan and Van der Lingen. This difficulty was overcome by following the procedure of Salit (1931) except that 96 per cent. alcohol saturated with the triple acetate is used instead of his more expensive concentrated acetic acid. The washing mixture is prepared by adding about 2 grams of uranyl-zinc-sodium-acetate to 3 litres of 96 per cent. alcohol, shaking the flask vigorously several times and then putting it away in the ice chest (10° C) for at least a day before being filtered for use.

(*e*) Detailed Description of Method.

Sodium and potassium are determined in the same extract of vegetation. Full details for the preparation of the extract are given by Malan and Van der Lingen (1931). Briefly, the HCl extract of the ash of approximately 10 gm. dry grass is made up to 100 c.c. with distilled water. The sodium estimations follow after the potassium determinations so that the amount of K present in the aliquot for sodium determination will be known. The elimination of phosphorus will be included for the sake of completeness, but in the majority of analyses this step was omitted in the routine procedure employed for the determination of sodium without more than a negligible experimental error.

Into a conical centrifuge tube of 10 c.c. capacity is pipetted about 5 c.c. of the extract and about 0·2 gm. $Ca(OH)_2$ added by means of a small round spoon, which when full contains roughly this amount. Close the tube with a rubber stopper, shake vigorously, and after allowing it to stand for five minutes centrifuge for three minutes. Transfer by means of a pipette graduated to 0·02 c.c. a definite volume of the supernatant, not more than 1 c.c. and containing not more than 0·8 mgm. K, to another conical centrifuge tube containing 3 c.c. absolute alcohol. If less than 1 c.c. of the dephosphated extract has been measured out the difference is made up with doubly distilled water so that the total added to the 3 c.c. absolute alcohol is 1 c.c. Now add 2 c.c. of the precipitating reagent, close the tube with a clean rubber stopper and invert several times. Remove the stopper carefully, wiping the surface that has been in contact with the liquid on the edge of the tube. 1 c.c. Standard solution is treated similarly. After 30 minutes centrifuge for 5 minutes at 2,000 r.p.m. Carefully decant the supernatant fluid, invert the tubes on a piece of filter paper and allow to drain for 10 minutes. With a piece of cloth moistened at one end with alcohol the mouths of the tubes are then wiped clean, and 5 c.c. of the cold washing mixture allowed to run slowly from all round the edge into the tube. Now stir up the precipitate, dispersing it evenly through the whole liquid. Centrifuge for 5 minutes, decant supernatant fluid and allow to drain for at least 15 minutes, after which the mouths of the tubes are again wiped with a dry cloth. The precipitate is dissolved in 0·5 c.c. 10 per cent. acetic acid and a few c.c. of water. The solution is then quantitatively transferred to a glass tube of even bore, bearing a 25 c.c., 50 c.c. and 100 c.c. mark. If the apparent bulk of the precipitate from the test sample is twice that from the 0·1 mg. Na standard its solution is made up to 100 c.c., and if half as much to 25 c.c. The standard is made up to 50 c.c.

A standard is also made up from the standard triple acetate solution by diluting 1 c.c. to 50 c.c. 0·25 c.c. potassium ferrocyanide solution is then added for every 25 c.c. solution. The tubes are inverted to mix and the colours compared in a Duboscq colorimeter after 4 minutes using the triple acetate solution as standard at 20 m.m. The 0·1 mg. Na standard should read at 20·0 mm. When a series of determinations, say 30, are done at the same time, it was found best, especially when phosphorus has not been eliminated, to develop colours in only 10 samples at a time so that the comparisons thereof are finished about 15 minutes after the colours have been developed.

The majority of grasses are very low in sodium content so that even if the volume has been made up to 25 c.c. the readings obtained are much beyond the range of proportionality for accurate results. In these cases 0·5 c.c. 10 per cent. acetic acid and 9·5 c.c. water are accurately pipetted into the tube containing the dry precipitate. After the whole precipitate has gone into solution 0·1 c.c. of the pot. ferrocyanide solution is added, the tube is inverted several times and the colour compared with the standard as before.

The author uses the precipitate obtained from 1 c.c. standard NaCl solution for gauging the bulk of the unknown precipitate and as a check on the standard made up from the triple acetate solution. When small aliquots, e.g. 0·2 c.c. of a grass extract, are used for a determination, and if at the same time the precipitate is so small that the colour volume has to be made up to 10 c.c., a serious error is introduced into the calculations by traces of sodium present as impurity in the reagents used. For this reason it is advisable always to run a blank with a series of determinations and to make, if necessary, corrections when calculating final results.

SUMMARY.

(1) A revision of the method by Malan and Van der Lingen for the colorimetric determination of sodium in vegetation is described in detail.

(2) Evidence is presented to show that:—

(*a*) ·04–0·1 mgm. Na can be determined with reasonable accuracy in the presence of 0·1 mgm. P; when 0·1–0·2 mgm. Na is to be determined 0·2 mgm. P, and when 0·2–0·8 mgm. Na, up to 0·4 mgm. P may be present.

(*b*) The interference of K is dependent upon absolute concentration and temperature; working at ordinary laboratory temperature (± 25° C.) not more than 0·8 mgm. K should be present in the aliquot for a determination.

(*c*) At 25° C. precipitation is complete within experimental error in 30 minutes.

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APPENDIX.

Method for calculating true percentage Na_2O in vegetation when a small precipitate, due to impurities in the reagents, in the blank necessitates a correction.

Example :—

(1) Data :

(i) Weight grass in 100 c.c. extract..... x gms.
(ii) Volume extract used for determination..... t c.c.
(iii) Colour volume for blank..... 10 c.c.
(iv) Colour volume for test sample..... y c.c.
(v) 0·135 mg. Na_2O standard in 50 c.c. at—
(*a*) A m.m., test sample reading..... a
(*b*) B m.m., blank reading..... *b*

(2) Correct value :

$$= \left\{ \frac{A}{a} \times \cdot 135 \times \frac{y}{50} \times \frac{1}{t} \times 100 \times \frac{100}{x} \times \frac{1}{1{,}000} \right\} -$$

$$\left\{ \frac{B}{b} \times \cdot 135 \times \frac{10}{50} \times \frac{1}{t} \times 100 \times \frac{100}{x} \times \frac{1}{1{,}000} \right\}$$

$$= \left\{ \frac{\cdot 135}{50} \times \frac{1}{t} \times 100 \times \frac{100}{x} \times \frac{1}{1{,}000} \right\} \left\{ \frac{Ay}{a} - \frac{10\,B}{b} \right\}$$

$$= \frac{\cdot 027}{tx} \left\{ \frac{Ay}{a} - \frac{10\,B}{b} \right\} \% \ Na_2O.$$