ON THE USE OF $ZnCl_2$ TOGETHER WITH HCl IN LIGNIN DETERMINATIONS

L. PALOHEIMO and K. A. VAINIO

Department of Animal Husbandry, University of Helsinki

Received October 21, 1958.

As early as 1852 Barreswil (ref. STAMM 5, p. 160) found that hot concentrated zinc chloride solution swells and dissolves paper. The first to recommend the use of $ZnCl_2$ in lignin determination was J. KÖNIG (1, p. 65). König claimed that a mixture of 40 g $Zncl_2$ and 100 ml concentrated hydrochloric acid was a convenient hydrolysator to be used in lignin determinations. As the formation of HCl gas is very intense he suggested the use of effective cooling. However, with the addition of 5 ml water to the mixture a temperature of 50° C should be appropriate. König, however, did not present any analytical data obtained from the use of this principle.

In 1938 POPPOFF (2, p. 245) published a treatise on the use of König's principle in lignin determination. His mixture was: 40 g $ZnCl_2$, 100 ml concentrated hydrochloric acid, and 5—10 ml water. 20—30 ml of the mixture was used for 0.5—1 g air dry matter to be analysed, the recommended temperature being 25° C, and the duration of the treatment 10 h. A 2 hours pretreatment with 2.5 per cent hydrochloric acid was used. Protein correction was omitted.

Experimental

In our preliminary experiments filter paper was used as test material. It appeared that water solutions of $ZnCl_2$ as well as solutions of $ZnCl_2$ in diluted HCl decomposed the paper very slowly. The use of mixtures of $ZnCl_2$ and concentrated (37 p.ct.) hydrochloric acid caused a more rapid decomposition of cellulose. A mixture of 150 g $ZnCl_2$ and 100 ml 37 per cent. HCl appeared to be most efficient. In the following this mixture is called the V-solution. At 20° the V-solution dissolves 1 g filter paper in less than 1 hour, and the solution stays clear after dilution with water. Using the V-solution as hydrolysator the lignin determinations were made in the following way.

Table 1. Comparison of results obtained by the 72 per cent H_2SO_4 method and the V-method ($ZnCl_2$ in HCl). Percentages on the dry matter basis.

	72 per cent H_2SO_4 method		V-method	
	Crude lignin[1])	Lignin	Crude Lignin[1])	Lignin
Spruce wood	23.4	—	23.2	
Rye straw	13.2	12.7	13.1	12.6
Timothy	8.1	7.6	8.5	8.0

2 g samples of the matter to be analysed were used. After a pepsin digestion the material was treated with boiling 2 n. hydrochloric acid, and then extracted with ethanol-benzene. The details of the pretreatments are described by SALO (3, p. 185). The residue is transferred into an 100 ml Erlenmeyer flask which is provided with a glass stopper. 50 ml V-solution is added in two portions. After the first addition the material is stirred thoroughly with a glass rod. The flask is put in a 20° water bath and is gently shaken at 15—30 minutes intervals. Towards the end of the treatment it can be shaken more vigorously. After 3 hours the contents of the flask are transferred into a 600 ml beaker and diluted with water up to a volume of 350 ml. The solution is heated to boiling point and then filtered according to the indication of SALO (l.c.). A protein correction is made. If woods are to be analysed the pepsin digestion as well as the protein correction are omitted.

For the sake of comparison some determinations were made both with the method described above and by using 72 per cent H_2SO_4 in the way the latter method is used in our laboratory (described by SALO l.c.). In both cases the pepsin digestion was applied. Table 1 shows that the two methods give, at least as regards the test materials used, highly uniform results. The lignin preparations received by the V-method are appreciably lighter in colour than those from the H_2SO_4 treatment. It is noticeable that the difference in colour does not reflect in the percentages.

Table 2. Comparison of results obtained from the use of König's solution with those obtained from the use of V-solution. Dilution after hydrolysis was omitted. Percentages on the dry matter basis.

	König's solution		V-solution	
	Crude lignin	Lignin	Crude lignin	Lignin
Spruce wood	24.2		21.8	
Birch Wood	14.9		9.4	
Rye straw	12.6	12.1	11.0	10.6
Timothy hay	9.4	9.0	7.6	7.1
Red clover hay	7.1	6.3	6.8	6.1

[1]) Without protein correction.

Table 3. Influence of the duration of treatment with the V-solution. Dilution after hydrolysis omitted. Percentages on the dry matter basis.

Duration of hydrolysis, hours	Spruce wood	Rye straw		Timothy hay	
	Residue without protein correction	Residue without protein correction	Residue with protein correction	Residue without protein correction	Residue with protein correction
1	22.5	11.0	10.5	9.8	9.3
2	21.6	10.7	10.3	8.0	7.5
3	21.8	11.0	10.6	7.6	7.1
5	21.2	11.0	10.6	8.0	7.5
7	21.0	11.2	10.8	7.6	7.1

Poppoff (l.c.) did not dilute with water after the treatment with the mixture of $ZnCl_2$ and HCl. For the sake of comparison we made some determinations with the V-method omitting the dilution. In Table 2 the results are compared with those obtained with the use of König's solution (including 5 ml water). The pretreatments were in both cases similar to those described above. The duration of the hydrolysis was 3 hours, and the temperature 20° C. It should be noted that the terms »Crude lignin» and »Lignin» in Table 2 have not the same meaning as in Table, 1 because the figures in Table 2 are from a treatment without dilution with water. Table 2 shows that the residues received from the use of the V-solution are smaller than those with König's solution. It is not, however, possible to decide whether the greater efficiency of the V-solution is directed only to the cell wall carbohydrates or perhaps also to the lignin to a greater extent than the efficiency of the König's solution. However, our experiments with filter paper have shown the superiority of the V-solution as a hydrolysator of cellulose. As for the behaviour of lignin in acid solutions which are used for decomposition of cell wall carbohydrates we refer to a treatise of Salo (4, p. 206).

The hydrolysis time was fixed at 3 hours after a series of experiments the results of which are shown in Table 3. The materials were pretreated as before, and the dilution after the treatment with the V-solution was omitted. If the probability of experimental errors is taken into account one may conclude that for rye straw and timothy hay a hydrolysis time of 1 hour should be convenient. For spruce wood a longer time seems to be needed. To allow a margin of safety a period of 3 hours was fixed.

Summary

As König's proposal on the use of $ZnCl_2$ together with hydrochloric acid in lignin determinations seems, after Poppoffs experiments, to have been neglected some experiments based on this principle were performed. A mixture of 150 g $ZnCl_2$ and 100 ml concentrated hydrochloric acid appeared to be the most effective for the decomposition of the cell wall carbohydrates. The results obtained were

highly comparable to those obtained from the use of 72 per cent H_2SO_4 as hydrolysator. The lignin preparations were lighter in colour than those obtained by the H_2SO_4 method.

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SELOSTUS:

SINKKIKLORIDIN KÄYTÖSTÄ YHDESSÄ KLOORIVETYHAPON KANSSA LIGNIINIMÄÄRITYKSISSÄ

L. Paloheimo ja K. A. Vainio

Yliopiston kotieläintieteen laitos, Helsinki.

Königin ehdottama prinsiippi sinkkikloridin käytöstä yhdessä konsentroidun kloorivetyhapon kanssa ligniinimäärityksissä näyttää sitten vuoden 1938, jolloin Poppoff julkaisi eräitä tätä prinsiippiä noudattaen saatuja tutkimustuloksia, jääneen kokonaan unohduksiin. Periaatteen käyttökelpoisuutta on nyttemmin kokeiltu kotieläintieteen laitoksessa. Soluseinämähiilihydraattien hydrolysaattorina osoittautui tehokkaimmaksi seos, joka oli kokoonpantu 150 grammasta sinkkikloridia ja 100 millilitrasta väkevää (37 %) kloorivetyhappoa. Saadut tulokset ovat varsin yhtäpitäviä niiden tulosten kanssa, joita saatiin käyttämällä 72-prosenttista rikkihappoa hydrolysaattorina. Saadut ligniinipreparaatit olivat vaaleampia kuin viimeksi mainitun menetelmän antamat.