

Bulletin of  
The Central Leather  
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Madras



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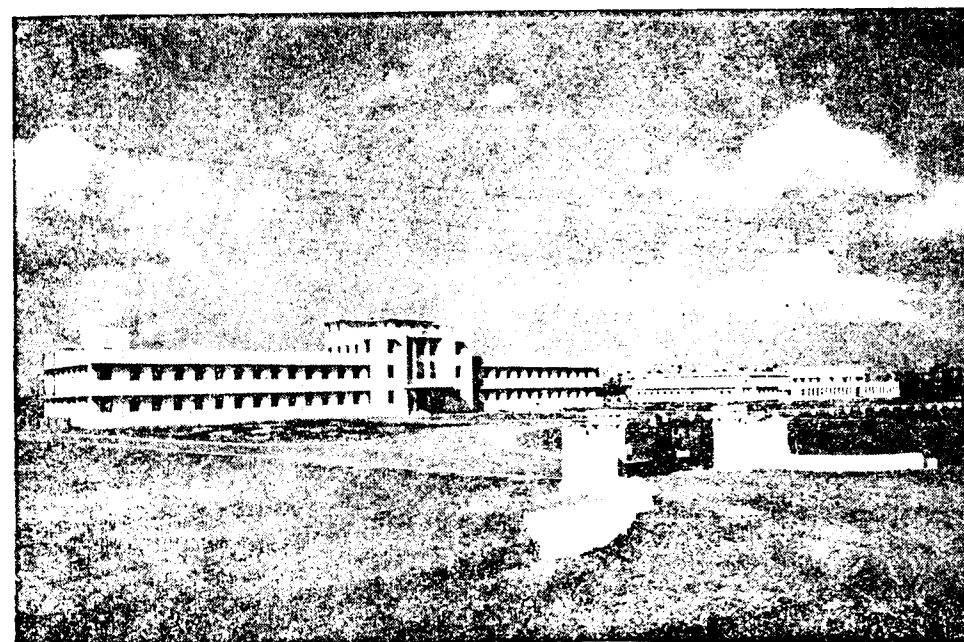
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# Bulletin of The Central Leather Research Institute

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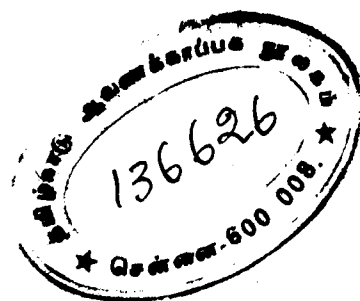
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**EFFECT OF SALTS AND SULPHONYL CHLORIDE  
IN FORMALDEHYDE TANNAGE\***

**BY**

**Y. NAYUDAMMA, K. S. JAYARAMAN & D. RAMASWAMY**

*Central Leather Research Institute, Madras*

**SUMMARY**

It has been shown earlier that the addition of salts affected the fixation of formaldehyde by the protein and that this effect would depend on the nature of the cation and/or the anion of the salt. Further work in this direction has shown that the ammonium salts increase the fixation of formaldehyde as contrasted to other inorganic salts; the effect of charge or oxidation reduction system as obtained in the addition of ferrous and ferric sulphate on the fixation of formaldehyde by the protein is negligible; the increase in the number of carboxyl groups and the aromatic nature have a slight adverse effect on the fixation of formaldehyde; the R group attached to the carboxyl ion has a specific effect as demonstrated in the case of amino-acetic, chloroacetic, acetic, fumaric, malonic, maleic, succinic, salicylic, sulphosalicylic salts; *cis*-and *trans*-configuration have an important role on the fixation of formaldehyde, *cis* salt giving a pronounced fixation of formaldehyde as contrasted to the *trans* system. The pretreatment of the protein with sulphonyl chloride activates and fixes approximately double the quantity of formaldehyde as compared to the protein at all pH ranges from 4 to 10. This abnormal increase is attributed to the ready reactivity of the sulphonamides formed with acid amide groups at low pH values and with amino and guanidino groups at higher pH values.

*Introduction*

In an earlier work,<sup>1</sup> it was shown that the presence of salts affected the fixation of formaldehyde and shrinkage temperature of the protein and that this effect was influenced by both the cation and anion of the salt. A further study was made to determine the specific effect of the cation, anion, carboxyl and hydroxyl groups, aliphatic and aromatic nature, chain length and unsaturation. The salts under study were ammonium chloride,

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\* A paper presented at the 6th Biennial Conference of IULCS held at Munich during Sept. 7-12, 1959.

ammonium sulphate and ammonium acetate, and ferrous sulphate and ferric sulphate, and sodium salts of acetic, chloroacetic, aminoacetic, salicylic, sulphasalicylic, phenolsulphonic, oxalic, succinic, fumaric, maleic and malonic acids. The results are reported in Part I of this paper.

A preliminary study has shown that the sulphonyl chloride pretreatment of the protein increased the fixation of bound formaldehyde. This result is unexpected. Systematic work has, therefore, been conducted to study this reaction mechanism and the results have been reported in Part II of this paper.

#### Experimental

A known weight of dehydrated pelt (dehydrated with repeated changes of alcohol and acetone and air dried) was thoroughly wetted back with water and tanned with 50 ml. of 1% formaldehyde for every 10 g. of pelt, in the presence of salt using a concentration of 100 mg. equivalent of the salt per 100 gms. of the hide substance. The pH of the system was adjusted to the desired value and the material tanned for three days at the end of which it was washed with three changes of water, previously adjusted to a pH equal to that of tanning equilibrium, air dried, ground and analysed for hide substance and bound aldehyde.

In case of the sulphonyl chloride experiment, 10 gms. of hide powder was soaked in distilled water for two hours and water filtered off. The moist powder was further treated with 25% Immergan for a period of three to four days at different pH values. The tanned samples were dried and the excess uncombined Immergan was removed by extraction with acetone for six hours. These samples were retanned with formaldehyde (1% solution using 50 c.c. of the solution for every 10 gms of hide powder) at the same pH of the pretanning. After attaining the equilibrium, the samples were washed with distilled water, adjusted to the pH of the tannage. Samples were then air dried, ground and analysed for hide substance, sulphur and bound aldehyde. A blank experiment was conducted with hide powder, tanning straight with formaldehyde under the conditions outlined above.

The bound aldehyde was determined by the usual method of hydrolysing the tanned material with sulphuric acid, distilling it into sodium bisulphite solution and determining the aldehyde iodimetrically. Sulphur was determined by oxidation of the sample with a mixture of nitric and perchloric acids and precipitating

as  $\text{BaSO}_4$  and expressing as mg. of sulphur per 100 gms. hide substance.

The isoelectric points were determined by electro-osmosis using the leather as a diaphragm in a specially constructed electro-osmotic cell.

#### Data and discussion

##### Part I. Effect of salts.

##### A. Effect of ammonium salts:

The data obtained are given in Table I below.

TABLE I

Fixation of formaldehyde in presence of ammonium salts.

| Nature of salt.      | pH  | Fixed HCHO<br>m. eqt./100 g. of H.S. |                        |
|----------------------|-----|--------------------------------------|------------------------|
|                      |     | Experimental                         | Blank<br>without salt. |
| Ammonium chloride .. | 4.7 | 44.8                                 | 30.9                   |
| Ammonium sulphate .. | 5.4 | 44.9                                 | 37.0                   |
| Ammonium acetate ..  | 5.2 | 43.0                                 | 32.3                   |
| Ammonium chloride .. | 6.0 | 63.6                                 | 40.3                   |
| Ammonium sulphate .. | 6.0 | 65.3                                 | 40.3                   |
| Ammonium acetate ..  | 6.0 | 68.3                                 | 40.3                   |

From the table, it is observed that (1) the ammonium salts increase the fixation of formaldehyde as compared to the control and other inorganic salts investigated earlier; (2) at a uniform pH 6.0 the order of increase in formaldehyde fixation is ammonium acetate > sulphate > chloride. This is probably due to the buffering capacity of the salt. (3) However, at natural pH, the order is reversed.  $\text{NH}_4\text{Cl}$  permits greater fixation of formaldehyde as compared to other salts. The reasons for this behaviour are not quite clear. It may be possible that ammonium chloride may react with formaldehyde to form  $\text{NH}_2 \cdot \text{CH}_2\text{OH} \cdot \text{HCl}$  or hexamethylene tetramine, which in contact with collagen may not be easily removed by washing. A further intensive study on this problem would be of interest.

B. *Effect of ferric and ferrous salts:*  
The data are given in Table II below.

TABLE II

Fixation of formaldehyde in the presence of iron salts

| Nature of salt.  | pH     | Fixed HCHO m.eq./100 g. of H.S. |                     |
|------------------|--------|---------------------------------|---------------------|
|                  |        | Experimental.                   | Blank without salt. |
| Ferric sulphate  | .. 3.5 | 26.7                            | 22.6                |
| Ferrous sulphate | .. 3.5 | 27.2                            | 22.6                |

The pH of the tanning was uniformly at 3.5 both in the experiment and in blank.

From the data, it is seen that the iron salts increase the fixation of formaldehyde only to a very small extent. It is further observed that there is no specific effect of the ferric or the ferrous ion both giving the same formaldehyde fixation. Evidently, the difference in charge or oxidation reduction systems has no effect in this case.

C. *Effect of salts of organic acids:*

The results obtained for the fixation of formaldehyde are given in Table III.

TABLE III

Fixation of formaldehyde in presence of salts of organic acids at pH 7.0

Salt added: 100 mg. eq./100 gm. of H.S.

| Nature of salt.          | Fixed HCHO mg. eq./100 g. of H.S. |
|--------------------------|-----------------------------------|
| Without any added salt   | .. 45.1                           |
| Sodium monochloroacetate | .. 41.9                           |
| Sodium acetate           | .. 54.0                           |
| Sodium glycinate         | .. 73.3                           |
| Sodium benzoate          | .. 41.7                           |
| Sodium salicylate        | .. 51.0                           |
| Sodium phenol sulphonate | .. 54.3                           |
| Sodium succinate         | .. 43.8                           |
| Sodium fumarate          | .. 41.3                           |
| Sodium maleate           | .. 73.8                           |
| Sodium malonate          | .. 44.7                           |

a. *Effect of carboxylic group:*

From the data reported in a previous paper<sup>1</sup> and from the table above, it is clear that monocarboxylic acid salts increase the fixation of formaldehyde as compared to the di- or tri-carboxylic acids. While sodium acetate increases the fixation of formaldehyde, sodium succinate, fumarate and malonate do not affect to any great extent the fixation of formaldehyde as compared to the control. It was noted earlier that oxalate does increase the fixation but to an extent lower than acetate.

b. *Effect of aromatic and aliphatic nature:*

While sodium acetate increases the fixation of formaldehyde, sodium benzoate slightly decreases formaldehyde fixation, indicating the effect of aromatic ring on the carboxyl group.

c. *Effect of hydroxyl group:*

While sodium benzoate decreases the fixation of formaldehyde to a slight extent, sodium salicylate increases the fixation of formaldehyde indicating the effect of the hydroxyl group present in the system.

d. *Effect of side groups:*

Comparing the systems of acetate, monochloroacetate and aminoacetate, it is observed that aminoacetate increases the fixation of formaldehyde to a very great extent, whereas monochloroacetate decreases it as compared to the acetate addition. Chloroacetic acid is a stronger acid compared to acetic and hence it behaves like a strong acid salt which decreases the fixation of formaldehyde, whereas the amino group attached will readily react with formaldehyde accounting for the abnormal increase in the formaldehyde fixation.

In the case of dicarboxylic acids also, the effect of the side group is noticed. While oxalic acid does give an increase in the fixation of formaldehyde, any additional weightage given to the side group will reduce the fixation of formaldehyde as is the case with succinate, fumarate and malonate.

The addition of sulphonic group to salicylic acid also has not affected the fixation. Both sulphasalicylate and salicylate give the

same effect. It would, however, be interesting to see how other reactive groups like carboxyl and nitro groups will affect this system.

Some of these salts like sulphosalicylate have a tendency to break up the cohesive forces of the protein resulting in a higher reactivity towards formaldehyde.

e. *Effect of unsaturation and cis-trans-configuration:*

Unsaturation does not evidently influence the fixation of formaldehyde as seen from the succinate and fumarate systems.

A remarkable difference has been observed in regard to the fixation of formaldehyde in the presence of *cis* and *trans* type salts. Whereas the *cis*-system (maleate) increases the fixation enormously, the *trans*-system decreases the fixation of formaldehyde slightly. Fumaric acid differs from maleic acid in that maleic has a tendency to form an anhydride and to react with active hydroxyl groups and with mono- or dimethylol compounds, if present in the system. But such a reaction might not be possible under the set of experimental tanning conditions at pH 7, where it is present as a maleate. Fumarate and maleate as such do not react with formaldehyde. The only possibility is that maleate and fumarate might in some way vary in their reaction with the protein and in making the protein more reactive to formaldehyde by activating the available group or by increasing the number of reactive groups of the protein. The fumarate with its *trans*-form, is very similar to a monocarboxylic acid in its effect with the two carboxyl groups separated out. In case of maleate with the *cis*-form, both the carboxyl groups will have a pronounced effect on the  $\text{NH}_3^+$  of the protein to rob it of its H, making  $\text{NH}_2$  available for further reaction with formaldehyde. When once this process sets in, more and more  $\text{NH}_2$  will be made available resulting in higher fixation of formaldehyde as observed. Sodium phthalate also does increase the fixation of formaldehyde whereas benzoic acid with one carboxyl group reduces the fixation of formaldehyde. Maleate increases the fixation to a greater extent as compared to phthalate showing again the effect of the aromatic ring.

Further work in regard to the *cis-trans* systems on the fixation of formaldehyde should prove immensely useful for further theoretical interpretation and practical utilisation.

## PART II

### *Effect of pre-treatment with sulphonyl chloride.*

The values of the fixed formaldehyde and sulphur are given in Figure I.

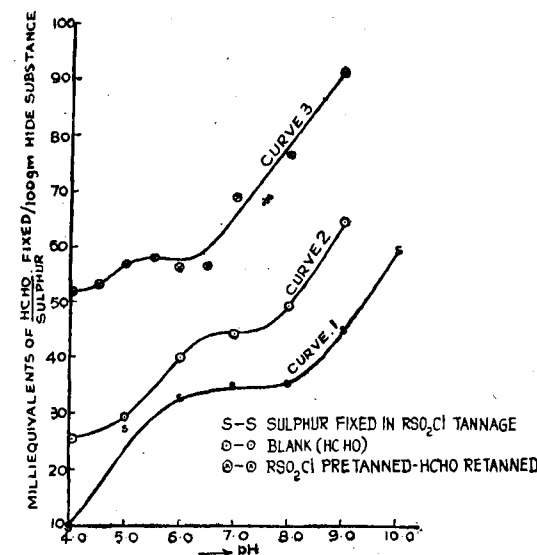
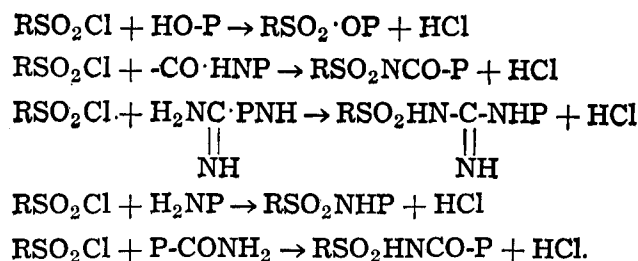


FIG. I

From the figure, it is observed the curves for fixation of formaldehyde and sulphur over a wide range of pH values follow the same trend—an increase in formaldehyde fixed with an increase in pH upto pH 6, a plateau between 6 and 8 and a further rapid increase with the increase in the pH. This may indicate that the same reactive groups of the protein may be involved in both the cases.

The fixation of formaldehyde by the sulphonyl chloride pre-treated protein also follows the same trend as that of sulphur and formaldehyde except that the values are considerably high. This abnormal increase may be due to the increase in the number of reactive groups, or the increase in the reactivity of the available groups of the protein when pretreated with sulphonyl chloride.

The reactions that are likely to take place between the sulphonyl chloride and the protein are as follows:



From the above it may be seen that the compounds formed by the reaction of the sulphonyl chloride with the hydroxyl or the peptide group of the protein may not permit further fixation of formaldehyde.

The compound formed by the reaction of the sulphonyl chloride with guanidino group is likely to react further with formaldehyde. Ordinarily, the guanidino group comes into play only at about a pH 10.0. To check whether such a reaction is possible even in the neutral and acidic regions, a separate experiment was run reacting guanidine carbonate with formaldehyde, with and without the addition of the sulphonyl chloride to the system. The results so obtained are shown in Figure II.

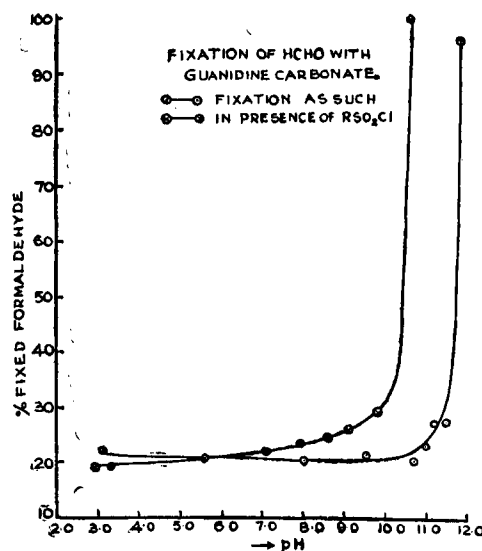


FIG. II

It is observed from this figure that in the presence of sulphonyl chloride, the formaldehyde fixed is equal or slightly lower below pH 7, but with a further increase in pH, the fixed formal-

dehyde is larger and the maximum obtained at pH 10, whereas in the blank experiment, the same value is obtained at pH 12. This clearly suggests that the sulphonyl chloride does activate the guanidino group, but its reaction will take place only at pH 7 and above. The abnormal increase in the fixation of formaldehyde by the sulphonyl chloride pretreated protein in the acidic and neutral regions must, therefore, be accounted for by some other reaction mechanism.

In the presence of the sulphonyl chloride, as in the presence of formaldehyde, the basic amino groups of lysine may be available even at lower pH values for reaction with formaldehyde. The sulphonamide formed by the reaction of sulphonyl chloride with amino group can also further react with one mole of formaldehyde. An amino group can react with one or two moles of formaldehyde, but under the conditions of tanning, one amino group is likely to react with one mole of formaldehyde. In view of the reactivity of the sulphonamide, the formaldehyde fixed by the sulphonyl chloride pretreated pelt could be the same as that of the blank, but the doubling in the value even at lower pH values could not be explained.

This necessitates the study of the reaction of the sulphonyl chloride with the acid amide groups, with the product so formed further reacting with formaldehyde. It is known that acid amide groups do react with formaldehyde in the acidic and neutral regions. It is probable that the product formed by reaction of sulphonyl chloride with acid amide is further reactive towards formaldehyde. The pretreatment with sulphonyl chloride might activate and increase the number of sites for fixation of formaldehyde, because of its reaction with acid amide groups accounting for the observed abnormal increase in the fixation of formaldehyde even in acid region.

The steady increase in the formaldehyde fixation as seen in curves 2 and 3 (Fig. I) at pH 7 and above is probably due to the further reaction of the guanidino group.

Whether sulphonyl chloride pretreatment has any tendency to break down the protein itself even in small quantities resulting in a higher number of reactive groups may have to be considered. Yet another consideration is the possibility of the sulphonyl chloride forming a sulphonate  $\text{RSO}_3\text{Na}$  or  $\text{RSO}_3\text{H}$  during the reaction and this in turn, giving the effect of the salt on the fixation of formaldehyde. A simple mineral oil sulphonate neutralised with

alkali and added to the system of protein-formaldehyde has not shown any increase in the fixation of formaldehyde. However, all these factors may have to be taken into consideration.

*Isoelectric point data:*

The data are given in Table IV below.

TABLE IV  
Effect of different tannages on iso-electric points of collagen.

| Tannage                                         | Iso-electric point |
|-------------------------------------------------|--------------------|
| Untanned collagen                               | .. 5.0             |
| Formaldehyde tanned                             | .. 4.5             |
| Sulphonyl chloride tanned                       | .. 2.6             |
| Sulphonyl chloride tanned—formaldehyde retanned | 2.6                |
| Fish oil tanned                                 | .. 2.8             |

These data indicate that (1) both formaldehyde and sulphonyl chloride treatment decrease iso-electric point, but the decrease is more pronounced in the case of  $\text{RSO}_2\text{Cl}$  treatment; (2) further reaction of formaldehyde with  $\text{RSO}_2\text{Cl}$  pretreated pelt does not further decrease iso-electric point.

$\text{RSO}_2\text{Cl}$  treated protein has iso-electric point 2.6, while formaldehyde treated protein has 4.5, and the iso-electric point of protein itself is 5.0. This very low value for  $\text{RSO}_2\text{Cl}$  treated material may be due to (a) its ready reaction with all the available basic groups as compared to formaldehyde; (b) the sulphonamide so formed particularly that formed with the acid amide groups may be quite acidic in nature, resulting in low iso-electric point. For example, the low iso-electric point (2.8) of fish oil tanned leather may be due to the fatty acids formed and oriented in such a way as to give low iso-electric point and not necessarily involving the reaction of basic groups.

The further interesting observation is that pretreating the protein with sulphonyl chloride and then retanning with formaldehyde does not further reduce the iso-electric point. It may mean

that no further basic groups are involved in formaldehyde fixation when once it is pretanned with sulphonyl chloride limiting the reaction only to sulphonamide already formed.

These data go to prove that sulphonyl chloride is very likely to react with acid amide groups in the acid region and its contribution has to be accounted for in explaining the reaction mechanism. It is also established that the pretreatment with sulphonyl chloride will increase the fixation of formaldehyde at all pH ranges because of the sulphonamide product formed with a high reactivity towards formaldehyde. These findings have significant theoretical and practical implications.

It will be quite interesting to study the physical and chemical characteristics of the leathers tanned by these combination sulphonyl chloride and formaldehyde tannages as well as to study the pretreatment of the pelt with formaldehyde and retanning with sulphonyl chloride.

REFERENCE

1. Nayudamma, Y., Jayaraman, K. S. & Krishnan, T. S. *J.A.L.C.A.* 52, 238 (1957).

## RESEARCH BEARS FRUIT

### Practical Demonstration No. 3

(Fourth Series)

#### Manufacture of Leather Boards from vegetable tanned leather shavings\*

BY

K. RADHAKRISHNAN

Leather boards, used in footwear industry, as insoles, stiffeners, midsoles, heel lifts, etc., are made from tannery and shoe factory waste materials like shavings and trimmings, for which formerly there was no use except as manure, in small quantities. Leather boards are manufactured in India only to a limited extent. A process for the manufacture of leather boards for use as insoles, as worked out at the Central Leather Research Institute on a pilot plant scale, with indigenously available materials was demonstrated to interested parties in June 1960. The details of the process are given below.

The leather shavings (vegetable tanned) are shredded into fibres in a dry condition in the Hammer mill and soaked in water with 1% sodium carbonate. The fibres are then ground in a Condux toothed disc mill in wet condition. A thoroughly defibred, knot-free pulp is obtained by this means. Then the pulp is washed free of excess tannins, soluble salts, etc., and well beaten in the Hollander with addition of other reagents such as wetting agent, basic dyes, plasticizer (sulphated pungum oil) and the binder which in this case, is rubber latex. The amounts of adhesive and oil depend on the use for which the resulting boards are meant. 41.4% of 50% solids content latex and 5% oil on the weight of leather shavings taken give a satisfactory board for insoles. The slurry is kept under agitation in the Hollander and the latex is

precipitated around the leather fibres by 10% aluminium sulphate solution which is the coagulant used. The pH of the pulp is tested periodically and when it is 4.0 addition of aluminium sulphate is stopped. Addition of preservatives like formaldehyde completes the process.

Measured quantities of the pulp are lifted over rectangular sieves for filtration and the wet cakes are pressed in a hand press, dried in shade for a day and finally pressed in a hydraulic press at a pressure of 250 kg./sq. cm. The boards are buffed before being stored. The physical properties such as tensile strength, stitch tear strength, etc., have been tested and found to compare favourably with those of foreign boards.

\* Process demonstrated in June, 1960.

# A PROCESS\* OF UNHAIRING SKINS AND HIDES BY ENZYMES FROM GERMINATED "RAGI" (ELEUSINE CORACANA) FOR THE MANUFACTURE OF LEATHER

BY

S. M. BOSE, W. MADHAVAKRISHNA & (late) B. M. DAS

## SUMMARY

A process for the unhairing of skins and hides making use of the enzymic extract of germinated "Ragi" has been developed. The process consists in immersing soaked and drained skins or hides in an unhairing bath comprising of the enzyme extract from germinated "ragi" and common salt at pH 4.5 for unhairing and bating for a period of three to four days.

## Introduction

Unhairing by liming is the traditional process. However, it is known that certain proteolytic enzymes derived from animal, mold and bacterial sources and certain mucolytic enzymes, also could be used for unhairing. While the use of papain or the latex from madar plant (*Calotropis gigantea*) for unhairing has been patented, the use of enzymes from germinated "ragi" is not, yet known.

The liming process is generally time-consuming and several operations are involved in the process. The object of the present study is to cut short the time and also the operations involved in the unhairing process without affecting the quality of the final leathers. The method described makes use of the enzyme extract from germinated "ragi". Ragi, the raw material for this unhairing process is a cheap millet abundantly cultivated in the states of Mysore, Madras, Hyderabad and Maharashtra. The advantage in using enzymes from sprouted "ragi" is that the process can be applied with great success for getting dual action, viz., the effects of the two processes of liming and bating are achieved in one process alone. This process can be used for the production of different types of both light and heavy leathers. The hair recovered is also quite clean and strong and not damaged, unlike in the liming process.

\* This process, which was originally covered by Indian Patent No. 52013, 1954 is now released to the trade, free of cost.

The following is a detailed description of the process for both skins and hides. The reference in the description to "skins" should be taken to apply to "hides" also.

## Preparation of the enzyme extract from germinated "ragi"

"Ragi" is washed well and steeped in water for 24 hours. Fresh water is circulated through the entire bulk of the "ragi" so that there is constant change of water. The grains are then spread well over a piece of wet cloth covering moist sand and couched for germination for about 72 hours. When the grains are sufficiently germinated, they are mashed well into a fine paste by means of stone mortar and pestle. The paste is thoroughly mixed with the required volume of water and the mixture is stirred well periodically. After about two hours, it is filtered through a fine cloth or through a suitable filter-press. If the filtrate is found to contain an appreciable amount of the suspended solids, it is allowed to settle for about six hours and the supernatant liquid is decanted off. The liquid contains the enzymes required for de-hairing skins. It is essential that as far as possible a clear filtrate should be obtained so as to achieve the best results in the unhairing process.

## Unhairing process

The skins are soaked in water. If the stock is wet salted, soaking for about two hours is quite enough; if dry salted or dried, soaking overnight in water containing 0.1% (on the volume of soak liquor) of a disinfectant like bleaching powder will prevent bacterial action. If fresh skins are available, only a few changes of water are needed.

When the stock is thoroughly soaked, it is washed well and 5% common salt based on the soaked weight of the skins is dissolved in the enzyme extract from germinated "ragi"; while preparing the enzyme extract, the volume of water used should be such that the extract gives just enough float for the skins in the unhairing bath. The extract prepared from 1 lb. of dry "ragi" has been found to unhair quite easily about 4-5 lbs. (soaked weight) of skins. The required amount of acetic acid is added to the unhairing bath to adjust its pH to 4.5 which has been found to be the best for the unhairing action of the enzymes used. The skins are handled in the liquor for some time and left completely immersed under it. Periodical stirring of the liquor and handling of the skins are necessary for uniform unhairing. The time when

the hair comes off quite easily should be carefully observed; too much delay in observing this leads to several unwanted results. All other factors being identical, the time required has been found to be inversely proportional to the atmospheric temperature. In the course of the present investigation, it has been observed that at room temperatures of 26-32°C, the entire process of unhairing and bating is completed in 3-4 days. If the room temperature is lower, more time, and if the room temperature is higher, less time is required. After the required period, the skins are unhairing, fleshed and scudded to remove the short hairs and thoroughly washed in several changes of water. If any short hairs are still not removed by scudding, the skins are put back in the unhairing bath for a few hours more after which the skins are scudded well and washed thoroughly in water. The unhairing pelt after thorough washing has a bated feel and appearance and hence no further bating is required. The pelt prepared by this process is also quite clean in both the grain and flesh sides.

The skins are then pickled if they are to be chrome tanned or pickled and depickled if they are to be vegetable tanned. The tanning and finishing operations are carried out by following the customary procedures as reported in a previous communication.<sup>1</sup> Five types of leather, viz., glaze kid, box sides, glove leather, E. I. tanned kips and skins and sole leather have been manufactured in the present study to indicate how different types of leather can be produced making use of the new process of enzymic unhairing; these do not in any way limit the scope of the application of this process.

If the process is applied for removing only the major portion of wool or hair without taking care to remove the short hairs and to bate the pelts simultaneously, much less quantity of enzymes will be sufficient. For example, it has been observed that the enzyme extract prepared from one lb. of dry "ragi" is capable of dewooling about 25 lbs. of soaked weight of sheep skins. As the short hairs are not removed with lower concentration of enzyme, the skins after enzymic dewooling are subjected to the traditional process of lime and sulphide treatment to remove the short hairs and the pelts are processed in the customary way for the manufacture of leather.

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## LETTER TO THE EDITOR

### Isolation of a glucoside from myrobalans

Myrobalan (*Terminalia chebula*) is an abundantly available indigenous tanning material. The chemistry of its tannins has been much studied and it has been possible to account for more than 50% of the constituents occurring.<sup>1,2</sup> In recent studies carried out at the Institute with fresh myrobalans, a glucoside has been isolated from the ethyl acetate extract of fresh myrobalan nuts.

The ethyl acetate extract had 97.76% tannins and 2.32% non-tannins. A solution of 5 g. of this extract in 30 ml. water when allowed to stand at room temperature began to deposit a white crystalline substance after two days. The precipitation of crystals stopped after ten days. The crystals were recrystallised from water and were pure white and needle shaped. They decomposed at 230°C. The acetyl derivative decomposed at 240°C. The elementary analysis was as follows.

C 44.20; H 6.20; O 49.60.

Suggested molecular formula:  $C_{13}H_{22}O_{11}$ .

The purified compound after hydrolysis with N  $H_2SO_4$  and following the procedure of Gottschalk and Ada, gave a precipitate identified as glucose.<sup>3</sup> The glucosidic nature was inferred from the infra-red and ultraviolet spectral studies.

Further studies are in progress.

C.L.R.I., Madras,  
Aug. 14, 1959.

J. B. RAO & K. N. S. SASTRY.

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## TECHNICAL SEMINAR

### Nature of the linkage of glutamic acid in collagen\*

BY

K. THOMAS JOSEPH

The polypeptide chains forming the triple helix in collagen are assumed to be linked together by a number of hydrogen and electrovalent bonds. However, certain anomalies in the chemical reactions of collagen and also the fact that collagen is insoluble in concentrated solutions of lithium bromide which dissolves straight hydrogen bonded proteins like silk fibroin, has led investigators to speculate about the possibility of collagen containing at least a limited number of covalent crosslinks. Recently, the presence of ester linkages in collagen and gelatin has been reported, based on the reaction with hydroxylamine. The  $\gamma$ -carboxyl group of glutamic acid in collagen could be a site wherein new crosslinks are formed with aging. Experiments were therefore carried out to find out whether the  $\gamma$ -carboxyl groups of glutamic acid were involved in the formation of crosslinks with other groups in collagen and in elastin.

Collagen and elastin were partially digested with trypsin and elastase respectively and the resulting peptide mixtures were tested for the presence of  $\gamma$ -glutamyl peptides by oxidising with sodium hypobromite. Succinic acid was removed by ether extraction and the succinyl peptides insoluble in ether, were hydrolysed by hydrochloric acid and the succinic acid liberated was estimated. The amounts of succinic acid obtained by this procedure indicated the presence of a small number of  $\gamma$ -glutamyl bonds in collagen and elastin. The nature of the bonds in elastin was unknown, but in collagen certain experimental evidences indicated that the  $\gamma$ -carboxyl group of glutamic acid was linked to some of the  $\epsilon$ -amino groups of lysine and the guanidino groups of arginine.

\* Summary of talk given on June 3, 1960.

## TECHNICAL SEMINAR

### Complexometric titrations with special reference to chrome-EDTA system\*

BY

S. ANANTANARAYANAN

The term 'complexones' was first applied by Schwarzenbach to a group of compounds which were essentially derivatives of imino diacetic acid. These compounds possess the remarkable property of chelating with a large number of ions. The chelating effect was traced to the presence of the powerful COO groups and positively charged imino groups. Disodium salt of ethylene diamine tetraacetic acid is known as complexone III. Complexometric titration—a technique for estimation of metal cations as well as anions—was developed by Schwarzenbach, because of the easy formation of complexes of EDTA with metal cations in the stoichiometric ratio 1:1. After reviewing the theory of complexometry the work done so far on chrome-EDTA complexes was briefly traced. The difficulties in the existing methods for determination of trivalent chrome were explained. The procedure successfully used for nickel and vanadium was adopted and standardised for chromium also using Redoxon as well as hydroxylamine as substitutes for ascorbic acid used in the original method. The method was found to be applicable even in the presence of hexavalent chromium. Comparison of the existing methods depending upon oxidation of trivalent chromium to the hexavalent state has shown that the standardised EDTA method was better, because the end point was sharp. The presence of salts like oxalate, citrate, formate and tartrate does not interfere. It is also possible to carry out the estimation spectrophotometrically at 395 m $\mu$ ; EDTA has little or no absorption at this wavelength.

The effect of disodium salt of EDTA on basic chrome liquor has been studied. The addition of EDTA in 1:1 molar ratio to chrome liquor (sulphate as well as chloride), results in the forma-

\* Summary of talks given on June 10 & 17, 1960.

tion of a soluble violet complex and insoluble greenish complex which gets precipitated. The precipitate, after washing to free it from EDTA has been found to contain nitrogen. An empirical formula ( $\text{Cr}_2\text{OH EDTA}$ ) was given to the precipitated complex. The elementary analysis confirmed the empirical formula arrived at. Probable molecular structures for the precipitated complex were proposed. The effect of basicity (with or without aging) on the precipitate formation was also studied. The precipitate formation was found to be uninfluenced by pH. The effect of solvents like alcohol, ethylene glycol and acetone on the precipitate formation also was studied.

## TECHNICAL SEMINAR

### Surface active materials from indigenous oils\*

BY

V. R. KISHORE

In leather manufacture, several surfactants are used in the various processes from curing of raw hides and skins to the finishing of leathers. Emulsifiers—one important sub-group of surfactants—are employed for the preparation of fatliquors and finishes used in leather making. Emulsifiers can be classified as anionic, cationic and nonionic and all these varieties are required for the manufacture of different leathers.

The anionic emulsifiers prepared in this Institute, apart from the sulphated oils, are (i) sulphated fatty acids, (ii) sulphated fatty acid amides and (iii) sulphated monoglycerides, the raw materials being various indigenous fatty oils. A few of them have been found to act as good emulsifiers for fatty oils.

Cationic emulsifiers and nonionic ones have been made using triethanolamine and polyethylene glycol respectively and fatty oils. These are useful in the fatliquoring of combination tanned leathers like semi-alum and chrome retan leathers.

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\* Summary of talk given on June 24, 1960.

### C. L. R. I. NEWS

Dr. Y. Nayudamma, Director, Central Leather Research Institute, attended (1) the meeting of the High Power Committee for the development of tanning and leather industry in Andhra Pradesh, held at Tirupati from May 31 to June 2, 1960 and (2) the 8th Conference of Directors of National Laboratories, held at the National Chemical Laboratory, Poona, on June 10 & 11, 1960.

Dr. Y. Nayudamma toured Vizianagaram, Elluru, Vijayawada and Guntur in connection with the work of the High Power Committee, Andhra Pradesh.

Dr. Y. Nayudamma has been nominated as a member of the Regional Selection Committee, Madras, of the United States Educational Foundation.

Dr. K. Wolf, the UNESCO Expert, assigned to the Central Leather Research Institute, has joined the Institute on July 2, 1960.

## BULLETIN OF THE CENTRAL LEATHER RESEARCH INSTITUTE

Volume VI, 1959-60

### AUTHOR AND SUBJECT INDEX

The abbreviations used in the author and subject index indicate as follows:

|                |                         |
|----------------|-------------------------|
| (A): Abstract; | (N): Notes and news;    |
| (P): Patent;   | (T): Technical Seminar. |

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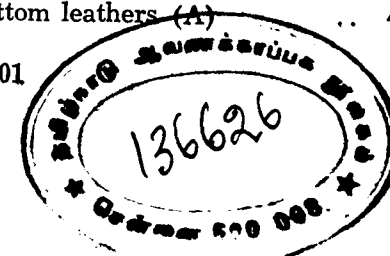
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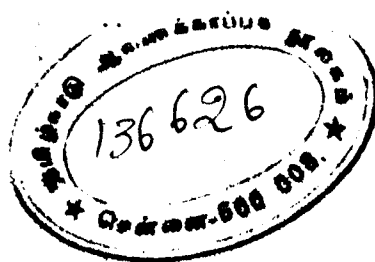
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