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DETERMINATION OF THE TRIAZINE RING IN
MELAMINE-TANNED LEATHER BY INFRARED
ANALYSIS

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ABSTRACT

Spectrophotometric methods were investigated as possible means of determining the triazine ring in melamine-tanned leather. Ultraviolet techniques for estimation of melamine produced negative results when applied to leather. A method is described for infrared examination of leather samples by the potassium bromide disk procedure, using potassium thiocyanate as an internal standard. An approximate relationship was found between added methylolmelamine and infrared absorption at 815 cm.^{-1} relative to absorption at 2085 cm.^{-1} by the internal standard. However, all precautions to maintain reproducible conditions were unable to remove the large amount of variability inherent in the nature of the tanning process and of the leather itself.



INTRODUCTION

It is well known that in an alum tannage the aluminum is only loosely combined with the collagen and may be easily removed by washing with water. In our studies to produce a more permanent alum tannage we have investigated the use of agents to fix the aluminum more thoroughly in the leather. Polymers such as polyacrylates (1) and methylolmelamine were studied.

In our work with melamine we felt that the investigations would be greatly assisted by a method for measuring the amount of melamine combined with the collagen. As far as we could learn, the literature and patents contain no quantitative data on this combination or procedures for its determination. It was therefore necessary to devise a method for determining combined melamine. Several approaches to the problem were explored.

PRELIMINARY EXPERIMENTS

The combined melamine could not be quantitatively extracted from the finely ground leather with organic solvents capable of dissolving the mela-

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mine polymer. This result was not unexpected since the melamine polymer undergoes further condensation and cross-linking upon combination with the hide protein.

Melamine (2,4,6-triamino-s-triazine) contains theoretically 66.64% nitrogen, and its purity can be determined by the Kjeldahl nitrogen method. However, the analysis of the melamine leather by this procedure is not satisfactory, since it does not identify melamine or differentiate between melamine nitrogen and hide substance nitrogen or nitrogen from other sources. Total nitrogen values for melamine leathers tanned by three different methods are given in Table I. A more specific analytical method was desired, and spectrophotometric techniques were considered.

TABLE I
ANALYSIS OF MELAMINE-TANNED LEATHERS

Equivalent of Trimethylol- melamine Used in Tanning (on pickled-skin weight)	Windus %	Total Nitrogen in Leather by Kjeldahl Method (on dry-weight basis)	Tanak MRX %
%		EURDD %	
1.75	17.6	18.1	17.7
3.50	17.7	18.7	18.6
7.00	20.3	20.8	20.2
14.00	23.1	27.0	24.0

Melamine has a strong absorption band (absorptivity of 81.0) in the ultra-violet spectrum near 235 $m\mu$ (millimicrons). Hirt, King, and Schmitt (2) have developed a highly sensitive spectrophotometric method for the detection and estimation of melamine polymers in wet-strength paper based on this characteristic absorption. Refluxing the paper samples with 0.1N hydrochloric acid extracts the polymer and hydrolyzes it to melamine, which is measured spectrophotometrically. A modification of the above method to determine combined melamine in leather was investigated.

A finely ground sample of melamine leather was hydrolyzed for one hour with 0.1N hydrochloric acid. Examination of the filtered hydrolysate with a Cary Quartz Spectrophotometer showed no absorption peak at 235 $m\mu$. We also found that when leather was hydrolyzed with 2% hydrochloric acid the hydrolysate showed no absorption peak at 235 $m\mu$ and was negative when tested by the alizarin blue dye staining technique of Stafford *et al.* (3). A methylolmelamine polymer control, hydrolyzed and examined under the same conditions, exhibited a strong absorption peak at 235 $m\mu$.

Infrared spectra are more specific and characteristic than the ultraviolet. Melamine has a characteristic sharp absorption at 815 cm^{-1} in the infrared spectrum due to the triazine ring (Fig. 1). Miller and Shreve (4) developed an infrared method for the quantitative estimation of melamine polymer in admixture with urea-formaldehyde and alkyd resins in a paint vehicle.

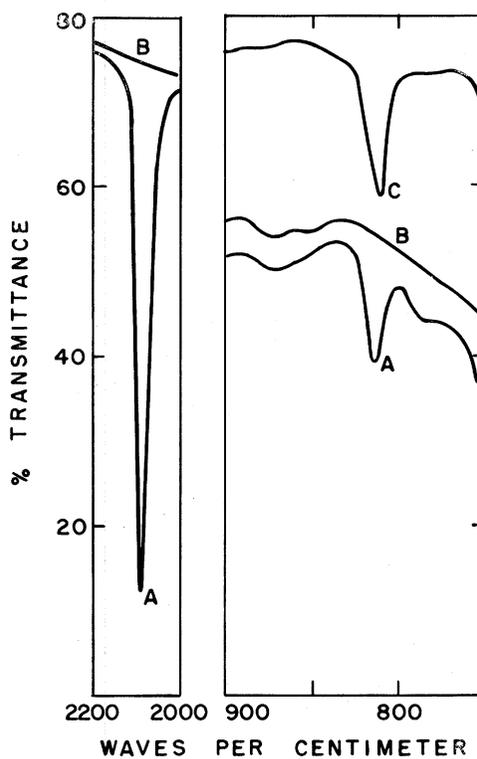


FIGURE 1.—Infrared absorption bands in potassium bromide disks.
(A) Leather tanned with 7.0 % methylolmelamine containing potassium thiocyanate as internal standard.
(B) American standard hide powder, Lot No. 28.
(C) Methylolmelamine polymer (no selective absorption in the 2000–2200 cm^{-1} region).

We investigated the use of infrared absorption spectra for determining melamine in leather and the extent to which the Beer-Lambert law is obeyed for varying amounts of melamine. The potassium bromide disk method of Schiedt (5) and Stimson (6) was used in which satisfactory spectra can be produced with 1 mg. or less of sample. Preliminary experiments indicated that the analytical results had a serious amount of scatter. Therefore, numerous precautions were introduced in an attempt to make conditions as

reproducible as possible. Special attention was given to sampling and grinding the leather in such a way as to produce representative samples of the required small size. Potassium thiocyanate was used for an internal standard, as recommended by Wiberley (8), and operations were carried out in a dry atmosphere to avoid effects such as those described by van der Maas and Tolk (9). One single stock mixture of potassium bromide–potassium thiocyanate was prepared by freeze-drying to assure uniformity, and this one stock mixture was used as the imbedding medium for all the leather samples in this study. An infrared spectrum was also run on American standard hide powder, Lot No. 28 (Fig. 1), as a collagen blank to determine possible background interference with the melamine absorption.

EXPERIMENTAL

Raw stock.—Pickled Amritsar goatskins were used in all the experimental work.

Tanning procedures.—The goatskins were tanned by the following three methods:

(a) *Windus method* (7).—Tannage was carried out in a small laboratory-size drum at room temperature (about 85°F.), using a whole skin for each of the following solutions, based on the drained pickled weight: Water, 100%; NaCl, 5%; and formaldehyde (36% by weight) and melamine (Eastman Kodak Company)* in the ratio of 3:1 moles respectively, in amounts calculated to be equivalent to 1.75, 3.5, 7.0, and 14.0% trimethylolmelamine. The free formaldehyde and free melamine were added to the drum at the start of tanning. The melamine dissolved gradually at room temperature as it reacted with the formaldehyde. The skins were drummed continuously during the day and intermittently overnight. The pH was adjusted to between 4.0 and 4.5 during tanning by addition of sodium acetate or lactic acid as needed. After being tanned for 24 hours, the skins were washed for one hour, horsed overnight, and dried at room temperature without fatliquoring. Shrinkage temperatures ranged from 67° to 91°C., depending upon the amount of melamine and formaldehyde used.

(b) *EURDD method.*—The tanning procedure was the same as in method (a) with the following exception: The formaldehyde and melamine were added to 20% of the water and heated at 70°C. for 10 minutes to dissolve the melamine, then cooled quickly to room temperature. Shrinkage temperatures ranged from 62° to 84°C., depending upon the amount of melamine and formaldehyde used.

(c) *Commercial methylolmelamine (Tanak MRX)*.*—The tanning procedure was the same as in method (a) with the following exception: The

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skins were depickled with water (100%), sodium chloride (5%), and sodium acetate, anhydrous, (2.5%), based on the drained pickled weight, for 45 minutes. The Tanak MRX was dissolved in a portion of the depickle solution by warming on the steam bath and added to the drum. Shrinkage temperatures ranged from 68° to 93°C., depending upon the amount of Tanak MRX used.

Preparation of stock mixture of potassium bromide and potassium thiocyanate.—Approximately 250 g. of Harshaw* infrared quality 200-mesh potassium bromide and 2.0 g. of reagent-grade potassium thiocyanate were dissolved in 500 ml. distilled water. The solution was divided into five 100-ml. portions and freeze-dried under vacuum for 24 hours. The white, fluffy, dried powder from the lyophilization flasks was combined to form one stock mixture from which all leather disks were prepared. A gloved box, continuously purged with dry nitrogen, was used for all handling and storage of the dried potassium bromide–potassium thiocyanate mixture, from the breaking of the vacuum in the lyophilization flasks to the removal of portions of the stock mixture in capped weighing bottles for use in imbedding leather samples. This was necessary since freeze-dried potassium bromide–potassium thiocyanate mixtures turn yellow within a few days after being opened to the atmosphere.

Infrared analyses of melamine leathers.—The melamine-tanned leather, air-dried to about 10–12% moisture, was sampled along the backbone at the shoulder and butt. At 10-g. sample of the leather was ground three times in an intermediate-model Wiley laboratory mill using 10-, 20-, and 30-mesh sieves in succession. Total nitrogen was determined by the Kjeldahl method. Approximately 150 mg. of the leather sample was then further ground for one minute in the stainless steel capsule of a Wig-L-Bug* amalgamator. After exposure to room atmosphere overnight, approximately 25 mg. was used for a moisture determination, and 2 to 3 mg. was weighed into a stainless steel Wig-L-Bug capsule on a microbalance, together with 200 to 275 mg. of the potassium bromide–potassium thiocyanate mixture. All weights were calculated on a moisture-free basis. After being ground together for one minute in the Wig-L-Bug, the mixture was pressed as a disk approximately 13 mm. in diameter by 1 mm. in thickness, using an evacuated die and a force of 10 tons for 10 minutes. The die used was designed in this laboratory and can be directly inserted into the microcell space of a Perkin-Elmer Model 21 spectrophotometer* without the necessity of removing the disk from the die. The disks varied widely in appearance because of the wide variation in melamine concentration. Duplicate disks were made from different portions of the 150-mg. sample of ground leather.

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The die containing the disk was placed in the microcell space of the spectrophotometer and scanned from 2400 to 1900 cm^{-1} and 900 to 725 cm^{-1} . The die was then rotated 30° twice, rescanning both absorption bands at each position of the die, so that triplicate readings were obtained on different areas of each disk to average out inhomogeneities. Instrument settings were as follows: Slit programmed, resolution 930, response 1, gain approximately 6, speed approximately 0.4 cm^{-1} per second, no suppression, scattering filter not used, 100% knob maximum clockwise to remove the trimmer comb from the sample beam. The instrument's automatic chart-gear shift was turned off so that 2.5 cm^{-1} occupied 1 mm. of chart in both regions of the spectrum.

Since the hide powder without melamine showed only a gradually sloping background in the regions of interest (Fig. 1), a straight-line baseline was used in calculating the melamine-tanned leather samples. The ratio

$$R = \frac{A_k W_s}{A_s W_k}$$

was then calculated, where

A_k = absorbance at 815 cm^{-1}

A_s = absorbance at 2085 cm^{-1}

W_k = weight of leather

W_s = weight of potassium bromide-potassium thiocyanate stock mixture

This ratio should be proportional to the concentration of triazine ring in the ground melamine-tanned leather from which the disk was made, if Beer's law is obeyed. The proportionality constant contains absorptivities of potassium thiocyanate and triazine as well as the concentration of potassium thiocyanate in the stock mixture; but the thickness of the disk and lack of optical homogeneity should cancel out.

DISCUSSION

Figure 2 shows the ratio R as a function of methylolmelamine added to the skins in the tanning liquor as a percent of the drained pickled weight. As can be seen from the figure, the general trend of the data follows Beer's law, although the scatter of the points is too great to rule out a small amount of curvature. A least-squares line calculated from the points and passing through the origin has a slope of 3.363, so that unknown samples of leather could be approximately analyzed for percent melamine by measuring R and dividing by 3.363. This constant applies only to disks prepared from the same potassium bromide-potassium thiocyanate stock mixture and would have to be redetermined for each new batch of stock mixture. A statistical

treatment indicated that the scatter is too great to determine whether there is a difference in the constant for the three different methods of tanning.

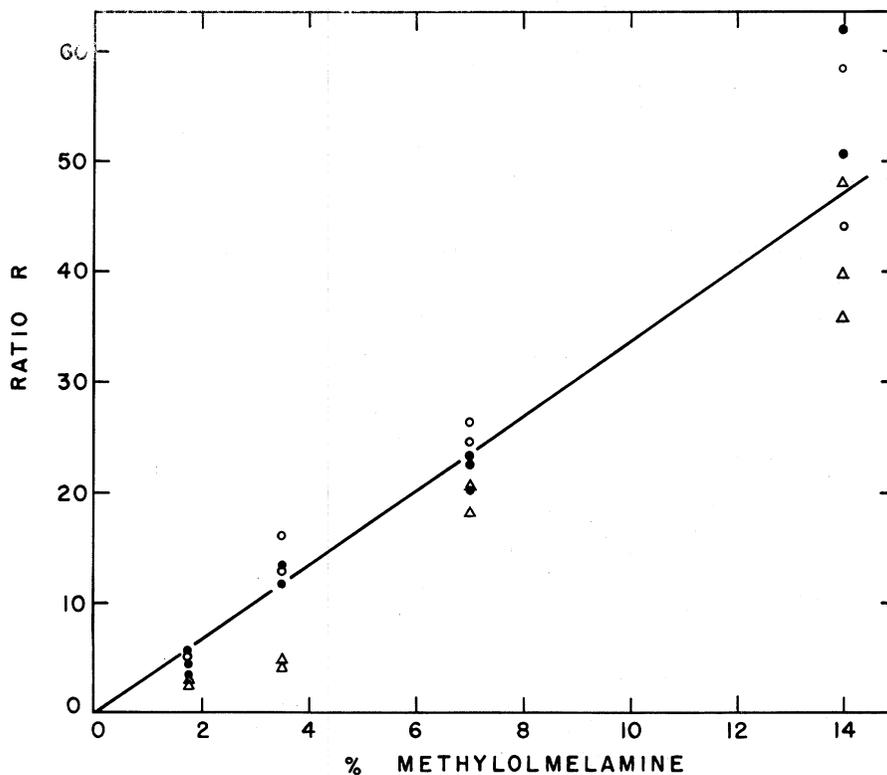


FIGURE 2.—Absorbance of 815 cm^{-1} band of melamine-tanned leather relative to 2085 cm^{-1} band of potassium thiocyanate, calculated to unit weight ratio, as a function of methylolmelamine used in tanning liquors (percent based on drained pickled weight of skins). Calculations were made on the basis of trimethylolmelamine although it is recognized that several methylolmelamines are formed.

○ Tanned by Tanak method, △ tanned by Windus method, ● tanned by EURDD method, straight line calculated by least squares.

It is not certain what chemical species are contributing to the 815 cm^{-1} absorption band. Portions of the added methylolmelamine may be uncombined, combined without change in its infrared absorption, or combined in such a way that its infrared absorption is altered. Since the absorption band is found at the same frequency in the leather as in the uncombined melamine, it seems safe to assume that only triazine rings whose vibrational properties are relatively unchanged are indicated by the infrared analysis. Variations in the proportion of combined to uncombined melamine may be

partly responsible for the variability of the results as well as variations in the amount of melamine lost in the wash water after tannage. The physical state of the methylolmelamine polymer in the leather and the number and spatial configuration of the triazine ring-collagen cross linkages may also influence its infrared absorption. All these sources of variation may differ for the three methods of tannage used, although electron microscopic studies of the melamine-tanned leathers at 29000 magnification showed no observable differences in the appearance of the fibrils.

SUMMARY

This method may be used for an approximate quantitative determination of melamine in leather within the limitations of the variability shown in Fig. 2. The leather may be advantageously examined directly in the infrared without need of an extraction process and without decomposition or chemical modification of the desired constituent.

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