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Determination of Salicylanilide in Leather Moldproofing Mixtures and in Treated Leather Goods

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Summary

Spectrophotometric methods have been developed for determination of salicylanilide in leather moldproofing mixtures and in treated leather goods. The du Pont color reagent, freshly diazotized p-nitro-aniline-o-sulfonic acid (sodium salt), is used.

Introduction

Leather exposed to warm, highly humid conditions is subject to mold growths which render it unsatisfactory for service and frequently result in

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damage to valuable instruments. To protect leather and leather equipment used by the Army in tropical areas, a mold- and moisture-resistant treatment was developed by the Hides, Tanning Materials and Leather Division of the Eastern Regional Research Laboratory. The moldproofing solution, which contains Stoddard solvent (dry cleaning solvent), isopropyl alcohol, paraffin wax, and the fungicide salicylanilide, gave effective protection against mold growth in both laboratory and field tests and was adopted by the Ordnance Department of the United States Army as "Tentative Specification AXS-1416 Compound, Moisture and Moldproofing (for Leather)."

The concentration of salicylanilide in treated leather goods must be high enough to be effective against mold growth but not high enough to be deleterious to health. To keep the concentrations within these rather narrow limits, it was necessary to develop a method of analysis for determining salicylanilide in both moldproofing solutions and in the treated leather. Of the three methods for determining salicylanilide—the E. I. du Pont de Nemours and Company, Inc.;[1] the Forster, Gandhi and Venkataraman;[3] and the Fancutt and Twiselton[2]—the du Pont method appeared to be the most applicable and was adapted to this work. This colorimetric method is based on the reaction of salicylanilide with freshly diazotized p-nitro-aniline-o-sulfonic acid (sodium salt), which produces a soluble colored compound.

Experimental

Salicylanilide to be determined by the du Pont method must be in aqueous solution. It is soluble in many organic solvents such as isopropyl alcohol, cyclohexanol and diethyl ether, and although only slightly soluble in water it is sufficiently soluble in dilute aqueous alkali to permit easy extraction from the organic solvents used in the moldproofing solution. Three successive extractions with 25 ml. portions of 1 per cent aqueous potassium hydroxide extract the fungicide quantitatively from approximately 5 grams of the solution. The salicylanilide in an aliquot of the aqueous extract is then measured colorimetrically.

In determining salicylanilide in leather, the problems involved were its extraction from the leather and obtaining it in a clear, aqueous solution, practically colorless and free from materials which would interfere with the color reaction. Benzene, ethyl alcohol, water, trichlorethylene, chloroform, ether, and other solvents were tried. Ethyl ether was the most satisfactory, since it gave a practically colorless extract, which on dilution with a large volume of dilute aqueous alkali formed a nearly homogeneous mixture except when paraffin wax was present. Since the wax collected at the surface of the solution, it was readily removed by dissolving it in Stoddard solvent. This formed the upper layer, which was drawn off by suction, leaving a nearly homogeneous aqueous solution of the extracted salicylanilide.

The aqueous solution, however, was often slightly colored or turbid, preventing an accurate visual comparison of the color in the test solution with that in the standard solutions. Consequently, a spectrophotometric method was developed which uses the same reagents and method of color development used in the du Pont method. The spectrophotometric method compensates for any color or turbidity in the test solution by use of an appropriate blank, and it also gives a more accurate determination of clear solutions.

Procedure

Determination of Salicylanilide in Moldproofing Mixtures

Salicylanilide is measured by comparison of the color of the test solution with that of standard solutions.

Prepare the following reagents: (a) *Standard salicylanilide solution.* Dissolve 50 mg. of pure salicylanilide, dried at 110° C. in an air oven, in 50 ml. of 1 per cent aqueous potassium hydroxide in a liter volumetric flask and dilute to the mark with distilled water. (b) *Buffer solution.* Dissolve 161.5 g. of disodium hydrogen phosphate-dodecahydrate and 41.5 g. of sodium dihydrogen phosphate-monohydrate in water and dilute to 1 liter. (c) *du Pont color reagent.* Diazotize 0.1 g. of p-nitro-aniline-o-sulfonic acid (sodium salt) in 50 ml. of water in a 100 ml. volumetric flask by adding 2.5 ml. of 2 *N* hydrochloric acid and 0.30 ml. of 2 *N* sodium nitrite, shaking well and allowing to stand for 10 to 15 minutes before diluting to the mark with water.

Prepare four standard color solutions by placing 5, 10, 15, and 20 ml., respectively, of the standard solution in 100 ml. volumetric flasks and adding 25 ml. of the phosphate buffer solution and sufficient water to make the volume approximately 50 ml. Add exactly 10 ml. of the freshly diazotized color reagent to each of the salicylanilide solutions, shake well, allow them to stand for 10 to 15 minutes, then dilute to 100 ml. with water. Allow the solutions to stand for 2 hours for maximum color development, after which the color is stable for at least 24 hours.

Measure the percentage transmittancy of these standard color solutions, in 2 cm. matched cuvettes or test tubes approximately 15 mm. in diameter, relative to a blank containing 25 ml. of buffer and 75 ml. of water, with a spectrophotometer using light having a wave length of 500 mμ. At this wave length, fixed changes in concentration produce maximum differences in percentage transmission. To minimize calculations, adjust the spectrophotometer so that it will give 100 per cent transmission through the blank solution. The instrument will then show directly the percentage transmittancy of the colored solution. Construct a standardization curve by plotting the logarithm of the percentage transmittancies of the solutions against concentrations of

salicylanilide in mg. per 100 ml. of colored solution. Although nearly linear, this curve shows slight variation from Beer's Law.

For the analysis of the moldproofing mixtures, transfer 15 to 20 g. of the well-mixed material to a 30 ml. dropping bottle equipped with an eye dropper. If the material contains solidified wax, it is first warmed on a waterbath until the wax is melted, insuring a homogeneous mixture. By means of the eye dropper transfer a 4 to 6 g. sample, weighed by difference to the nearest 0.01 g., to a 125 ml. separatory funnel containing 25 ml. of 1 per cent aqueous potassium hydroxide. Avoid loss of organic solvent by volatilization during the sampling and weighing of the moldproofing solution. Add 25 ml. of Stoddard solvent and shake until all the wax is dissolved. Allow the aqueous and Stoddard solvent layers to separate and then draw off the aqueous layer into a liter volumetric flask. Repeat this extraction twice. Collect the three extracts in the liter flask and make to volume with water. Transfer 5 ml. of this solution to a 100 ml. volumetric flask, buffer, and develop the color as previously described. Prepare a blank by placing a second 5 ml. aliquot and 25 ml. of buffer solution in a 100 ml. volumetric flask and dilute to volume with water. Since the blank contains the same volume of sample as used in the test, any error caused by turbidity or color in the salicylanilide solution will be compensated for; otherwise high results will be obtained.

Determine the percentage transmittancy of the colored test solution relative to the blank and by means of the standardization curve determine the weight of salicylanilide in the 5 ml. aliquot taken for color development.

Determination of Salicylanilide in Leather

Salicylanilide in treated leather is determined by extracting it with ethyl ether, preparing an aqueous alkaline solution of the ether extract, and determining the fungicide in an aliquot of this solution. The reagents are the same as those used for determining salicylanilide in moldproofing mixtures.

Prepare four standard color solutions in 100 ml. volumetric flasks, using 2, 5, 10, and 15 ml. of the standard solution. Develop the color as previously described, but add 30 ml. of ethyl alcohol to each flask just before the final dilution with water. These standards cover a lower concentration range than that previously used, since the addition of alcohol decreases the transmittancy. Determine the percentage transmittancy of the standard color solutions relative to a blank containing 25 ml. of buffer, 30 ml. of alcohol and 45 ml. of water and construct a standardization curve.

Cut into strips sufficient leather to yield a representative sample and mix well. Pack a paper or perforated glass extraction thimble of 30 mm. x 80 mm. with a weighed sample of approximately 10 g. and cover with a loose plug of cotton. Extract for at least 8 hours with ethyl ether, using a 125 ml

extraction flask with either a Soxhlet or Smalley type of extraction apparatus. If the volume of ether extract is greater than 75 ml., evaporate to this amount on a steambath, transfer to a 1000 ml. volumetric flask, and rinse the extraction flask with two 25 ml. portions of 1 per cent potassium hydroxide solution. Add the two washings and 750 ml. of water to the flask and shake vigorously to dissolve the ether and salicylanilide. Rinse the extraction flask with approximately 50 ml. of petroleum ether and add this to the solution in the volumetric flask. Dissolve the floating wax in the petroleum ether layer by stirring gently with a glass rod. To minimize emulsification avoid vigorous mixing of the two layers. Using a pipette and suction, remove the petroleum ether-wax layer. Add water to bring the solution into the neck of the flask and repeat the petroleum ether extraction. After removal of the ether layer, dilute to the mark with water and mix thoroughly.

Transfer a 5 ml. aliquot of salicylanilide solution to a 100 ml. volumetric flask, develop the color as previously directed, add 30 ml. of ethyl alcohol and dilute to the mark with water. Prepare a blank containing a duplicate 5 ml. aliquot, 25 ml. of buffer solution, 30 ml. of ethyl alcohol, and enough water to make 100 ml. Determine the percentage transmittancy of the colored test solution in terms of the blank. From the standardization curve determine the weight of salicylanilide in the 5 ml. aliquot taken for color development and calculate the percentage of the fungicide in the treated leather.

Results

The accuracy of the method for determining salicylanilide in moldproofing mixtures was measured by preparing test solutions of known concentrations containing from 1.24 to 2.06 per cent salicylanilide in a mixture of isopropyl alcohol, Stoddard solvent and paraffin wax. Aliquots of each of these solutions were analyzed by the procedure described. Table I shows the results.

The recovery values indicate that the method has an accuracy of 100 ± 2 per cent for salicylanilide concentrations within the range likely to be encountered in moldproofing mixtures for leather.

TABLE I

Determination of Salicylanilide in Moldproofing Mixtures

Sample No.	Sample	Salicylanilide in Mixture	Salicylanilide Found	Recovery
	g.	%	%	%
1	5	1.24	1.24	100
	10	1.24	1.24	100
2	5	1.54	1.53	99
3	5	2.00	1.97	98.5
4	5	2.06	2.08	101

The accuracy of the method for determining salicylanilide in leather was tested by adding definite weights of moldproofing mixtures containing known amounts of the fungicide to approximately 12 g. pieces of leather. To simulate leathers treated by large-scale processes, the test leathers were dried at 70° C. in an air oven to remove organic solvents. Each piece was cut into strips and extracted with ethyl ether, and the extracts were analyzed for salicylanilide. Table II shows the results. For comparison, some data obtained by methods other than the one proposed are included in Table II.

The recovery values indicate that an accuracy of 100 ± 2 per cent may be expected in the determination of salicylanilide in leather. The data for sample 6 show that extraction of the fungicide may be incomplete unless the leather is cut into narrow strips. An attempt was made to obtain an aqueous solution of salicylanilide free from the interfering oils and fats by extracting the leather with Skellysolve "B" before the ether extraction. The data for

TABLE II

DETERMINATION OF SALICYLANILIDE IN LEATHER TREATED WITH MOLDPROOFING MIXTURES

Sample No.	Extraction Solvent	Salicylanilide Added[1]	Salicylanilide Found	Recovery
		g.	g.	%
A	Ether	0.100	0.100	100
B	Ether	0.085	0.084	99
C	Ether	0.102	0.104	102
3	Ether	0.165	0.165	100
4[2]	Ether	0.091	0.089	98
5[2]	Ether	0.117	0.124	106
6[3]	Ether	0.088	0.080(a) 0.011(b)	102
13	Ethyl alcohol	0.104	0.052	50
16	Benzene	0.098	0.082	84
11[4]	Skellysolve "B" Ether	0.099	0.031 0.069	101
12[4]	Skellysolve "B" Ethyl alcohol	0.095	0.033 0.065	103

[1]Salicylanilide was added to approximately 12 g. of leather.

[2]Ether extract was evaporated, wax and salicylanilide were dissolved in isopropyl alcohol and Stoddard solvent, and salicylanilide was extracted with dilute alkali. Stable emulsion resulted.

[3](a) Extracted from uncut sample; (b) obtained by reextracting sample after it was cut. Per cent recovery is based on the combined weights.

[4]Sample extracted with two different solvents, and each extract was analyzed separately. Per cent recovery is based on the combined weights.

samples 11 and 12 show that this was unsuccessful, since salicylanilide was appreciably soluble in this solvent.

In an effort to obtain a clear, homogeneous, aqueous solution of salicylanilide from treated leather, the ether solution containing the fungicides, waxes and oils extracted from samples 4 and 5 was evaporated to dryness, and the solids were dissolved in a mixture of isopropyl alcohol and Stoddard solvent. The salicylanilide was extracted with aqueous alkali, as in the analysis of moldproofing solutions. This also was unsuccessful, owing to the fact that the resulting emulsions were stabilized by material extracted from the leather. Neither alcohol nor benzene was a satisfactory solvent, since the former gave highly colored extracts and the latter produced stable emulsions with dilute alkali, preventing the extraction of salicylanilide from the benzene solution.

Moldproofing mixtures containing more than 2 per cent salicylanilide were not analyzed, since such mixtures probably would not be encountered in leather moldproofing treatments. However, the results of the analysis on 10 g. of sample 1 (Table I) indicate that larger amounts could be determined satisfactorily.

The standardization curve prepared for determination of salicylanilide in leather can be used also for analysis of the processing solution, provided the same amount of alcohol is added to the test solution after development of color.

If a spectrophotometer is not available, a colorimeter or Nessler comparator tubes can be used with this method, provided the aqueous test solutions are sufficiently clear and colorless. The addition of ethyl alcohol will often clear turbid solutions sufficiently to permit the use of visual comparators.

Acknowledgment

The authors acknowledge the help of the du Pont Company who so kindly made available their visual colorimetric method for determination of salicylanilide and suggested the reagents used in this work.

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