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Pyrolysis Gas Chromatography of Wool

Part I: Detection and Quantitation of Commercially Applied Agents

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ABSTRACT

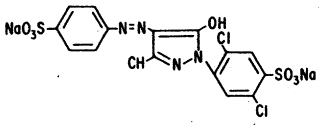
An investigation determined the feasibility of detecting and quantitating commercially applied agents on wool by pyrolysis gas chromatography. Characteristic peaks were generated from benzoylated wool, from wool treated with the insect-resist agent Mitin FF and from wool dyed with Lissamine yellow 2G, thus demonstrating that the technique is suitable for detecting the presence of such agents. Two approaches were used for the quantitative analysis of the treatment and the dyeing using the data from the resulting pyrograms. One approach used peak area response per microgram of treated wool and the other used peaks generated from wool's tyrosine as internal standards. Although the latter approach gave a better linear fit, inverse regression analysis demonstrated that the former approach was most suitable for predicting agent add-on (percent on weight of fiber).

The analysis of mixtures of compounds by chromatography generally is restricted to volatiles in the case of gas chromatography (GC) and solubles in the case of liquid or thin-layer chromatography. The coupling of pyrolysis to GC or mass spectrometry (MS) offers a means to study nonvolatiles such as synthetic and natural polymers. Pyrolytic decomposition under well-controlled conditions leads to the repeatable generation of mixtures of volatiles that may in turn be separated and quantitated by GC.

The earliest work on pyrolysis-GC of wool was concerned with the analysis of volatile and sometimes toxic gases. For example, Kirret and Küllik demonstrated the formation of CO_2, CH_4, H_2S, and CS_2 from various amino acids and noted differences in the pyrograms of wools from different sources and between wool and other proteinaceous fibers (silk, casein fiber) [3]. Urbas and Küllik studied the evolution of HCN, benzene, and toluene for wool, polyacrylic, and polyamide fibers [10]. Sekikawa *et al.* noted characteristic peaks in the pyrograms of leather that were not seen for wool and silk [7]. Perlstein showed that pyrolysis-GC was useful for identifying fiber composition based on the appearance of diagnostic peaks. Wool and silk, for example, gave rise to toluene, pyrrole, and *p*-cresol [6]. Recent work in pyrolysis-GC has benefitted from the use of capillary columns to effect more efficient GC separations. The increased complexity of the resulting chromatograms has necessitated greater sophistication in detection techniques and data analysis. Pyrolysis-GC, for example, has been coupled to mass spectrometry in Munson and Vick's forensic studies of human hair [5]. Wright *et al.* used multidimensional GC techniques (heartcutting, cold trapping, backflushing) to simplify multirun analyses of synthetic fibers [11].

We have been applying pyrolysis-capillary column GC to the study of three aspects of wool chemistry: the detection and quantitation of agents commercially applied to wool, the protein composition of wool and compositional changes induced by chemical treatment, and the comparison of wool pyrograms with pyrograms of other animal hair. This paper reports on an investigation of the first aspect, in particular the quantitation of the insect-resist Mitin FF and the acid dye Lissamine yellow 2G, current procedures for the analysis of which are very time-consuming [2].

(MITIN FF)



(LISSAMINE YELLOW 2G)

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Experimental

MATERIALS AND METHODS

Wool from the Technical Centre of the International Wool Secretariat (IWS) was combed top of 50s quality, and was received already treated with Mitin FF (MFF). Plain weave fabric was obtained from the United States Testing Co., Inc.[1], and had been prepared from Australian Merino 70s, 19.6–21.0 μm fiber diameter, 3.0–3.5 inch staple length. Neat Mitin FF High Conc was obtained from CIBA-Geigy Dyestuffs and Chemicals Division. Lissamine yellow 2G (LY2G) was obtained from ICI Americas, Inc.

Wool was benzoylated with benzoic anhydride in dimethylsulfoxide to give a benzoyl content of 664 μmol per gram of wool [4].

For the Mitin FF treatment, wool top was treated at 40°C for 10 minutes with an aqueous solution containing sodium sulfate (5.0% on weight of fiber [owf]) and 90% formic acid (2.0% owf), with a liquor : wool ratio of 8:1, v/w. MFF (1% owf) was added and the bath temperature was held at 40°C for an additional 5 minutes, then raised to 100°C over 30 minutes, and kept at 100°C for an additional 30 minutes. Samples provided by the IWS with amounts of MFF less than 1.0% owf were prepared by blending the 1.0% owf samples with untreated wool, with thorough blending achieved by multiple gilling operations.

For the dyeing procedure, wool fabric was treated with an aqueous solution containing various amounts of LY2G, sodium sulfate (10% owf), and sulfuric acid (4% owf) at a liquor : wool ratio of 50:1, v/w. The temperature of the dye bath was raised from 50 to 100°C over 45 minutes and kept at 100°C for 1 hour.

Pyrolyses were done with a Pyrofoil Curie point pyrolyzer (model JHP-3S, Japan Analytical Industry Co., Ltd.) coupled to a capillary column gas chromatograph (model Sigma 2000, Perkin-Elmer) equipped with a flame ionization detector. Detector signals were channeled through a recording integrator (model LCI-100, Perkin-Elmer) and then into a laboratory computer (model 7500, Perkin-Elmer) that was equipped with a 28 Mbyte hard disk.

To achieve proper sampling for pyrolysis, treated wool samples (about 50 mg) were pulverized under liquid nitrogen using a mortar and pestle. This step was essential for the blended samples containing MFF, but not for those uniformly treated with LY2G. Pyrolyses were performed on samples sizes of 100 to 200 μg, weighed (± 2 μg) using an electronic microbalance.

[1] Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Preweighed fiber samples were wrapped, according to directions provided by the supplier, in fresh foils of appropriate alloy (9 mm × 12 mm, supplied in 9 mm × 24 mm dimensions by Japan Analytical Industry Co., Ltd.) for pyrolyses at 764°C. Each wrapped sample in a pyrolysis tube was inserted into the pyrolysis apparatus, which then was flushed with GC carrier gas (helium) for 30 seconds. The tube with its contents then was lowered into the heated zone (130°C) for 5 minutes, and finally subjected to radiofrequency irradiation (5 seconds) to achieve pyrolysis at 764°C.

Volatiles from the pyrolysis were swept through a stream splitter (33:1 split ratio) onto a GC capillary column (fused silica, 0.25 mm id, 30 m, with SP2330 cyanopropyl silicone stationary phase, Supelco). The temperature program for the GC oven had to be such that the initial stream of pyrolysate (after stream splitting) was trapped momentarily at the injector end of the column to avoid band broadening during chromatography; an initial oven temperature of 70°C was adequate. The following GC settings were used: He flow, 1.0 ml/minute; He pressure, 69 kPa; He velocity, 36 cm/second; oven at 70°C for first 5 minutes, then raised 5°C/minute to 225°C, then held at 225°C for 34 minutes (total run time 60 minutes, with additional 10 minutes for column conditioning prior to recycling).

DATA ANALYSIS

We found that the automatic electronic integration of GC peak data often was inadequate for the complex peak patterns generated from pyrolysis. Computer assistance using Chromatographics 3 software (version 2.0, Perkin-Elmer) allowed inspection and alteration of the baseline, and then reintegration and generation of a new report. The peak area was determined following the software's interactive reintegration routine for baseline correction. Baselines were redrawn to connect the valleys on either side of the peaks of interest. These peaks included one generated by MFF, which eluted at 32 minutes, and one generated by LY2G, which eluted at 18 minutes. Peaks at 21 and 22 minutes were used as internal standards. These standard peaks (generated by phenol and *p*-cresol, respectively) arise from the pyrolytic decomposition of tyrosine from the wool protein [8, 9].

Statistical analysis of the data was by the method of inverse regression (straight line case) [1], so as to be able to predict both the percent owf and the error for duplicate determinations. The error terms are expressed as upper and lower confidence (or "fiducial") limits at the 95% confidence level.

Results and Discussion

Milligan and Wolfram found that benzoylation of wool modifies the free amino and hydroxyl groups of wool protein [4]. Pyrolysis of the treated wool gave rise to a characteristic GC peak at a retention time of 14 minutes. This same peak also was generated by pyrolysis of benzamide and likely was due to the formation of benzonitrile, with which the peak shared its retention time. The effect of such benzoylation on the rest of the wool pyrogram is the subject of a forthcoming report. However, the observation that characteristic GC peaks are produced by pyrolysis of wool pretreated with aromatic agents led to the current study of investigating the feasibility of detecting and quantitating aromatic agents that are commercially applied to wool. Such detection and quantitation is feasible by pyrolysis GC if the agents give rise to characteristic peaks that are not obscured by peaks from the pyrolysis of wool itself. Agents MFF and LY2G give rise to appropriate peaks (Figure 1).

Pyrolytic decomposition of MFF under the experimental conditions gives rise to a characteristic peak at 32 minutes, and similar decomposition of LY2G gives a peak at 18 minutes (Figure 1). We also detected these peaks in wool that had been treated previously with these agents; the characteristic peaks from MFF and LY2G occur at retention times not characteristic for any peaks generated by wool itself. Pyrograms of proteins, including wool, and of tyrosine show a pair of peaks at 21 and 22 minutes (Figure 1), already ascribed to the formation of phenol and *p*-cresol, respectively [6, 8, 9]. We therefore tested two approaches to determine the amounts of MFF and of LY2G that had been added onto wool. The first approach related the GC detector response (corrected peak area) to the weight of a pyrolyzed wool sample. The second approach relied on the use of peaks generated from the tyrosine of wool as internal standards. Detector response data are given for wool treated with MFF in Table I and similarly for wool treated with LY2G in Table II.

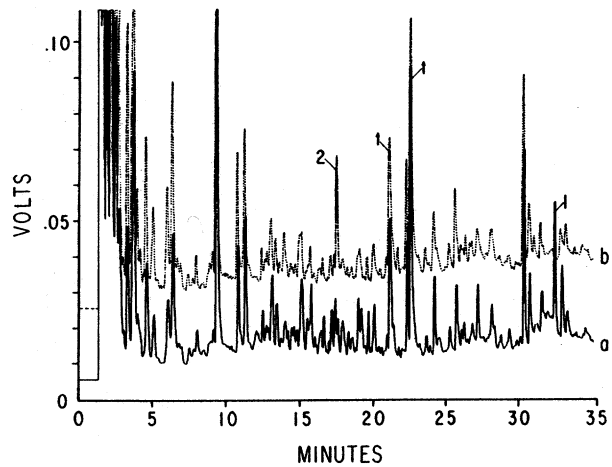

FIGURE 1. Pyrograms of wool, showing 0–35 minute region. Tyrosine-generated peaks (t) are seen at 21 and 22 minutes. Conditions as per Experimental. (a) Solid line = pyrogram of wool with Mitin FF at 1.0% owf, showing resulting peak (1) from MFF at 32 minutes, (b) broken line = pyrogram of wool with Lissamine yellow 2G at 1.4% owf, showing resulting peak (2) from LY2G at 18 minutes.

The peak generated by MFF could be detected reliably as low as 0.3% owf; samples at 0.1% owf gave peaks that often merged with a rough baseline caused by numerous small peaks of similar retention time. The peak generated by LY2G could be detected easily at 1.4% owf, the lowest add-on of LY2G that we investigated.

DATA ANALYSIS

We used two approaches to investigate the repeatability and linearity of response. In the first approach, (corrected) peak areas for MFF or LY2G were adjusted

TABLE I. Detector response data for wool treated with Mitin FF (MFF).

MFF, % owf	Number of samples	Response per μg: Peak from MFF	Response per μg: *p*-Cresol peak	Response per μg: Sum, cresol + phenol peaks	Peak ratios, MFF to: *p*-Cresol	Peak ratios, MFF to: Sum, cresol + phenol
1.0	5	1202 ± 214[a]	4056 ± 1292	7146 ± 815	0.3202 ± 0.0987	0.1806 ± 0.0351
0.60	5	432 ± 51	2351 ± 159	3871 ± 353	0.1846 ± 0.0262	0.1124 ± 0.0171
0.30	6	201 ± 18	2320 ± 216	3841 ± 477	0.0872 ± 0.0111	0.0539 ± 0.0071
Regression analysis						
y intercept (*y* at 0% owf)		−296	–	–	−0.0137	0.0014
Slope		1440	–	–	0.3332	0.1804
Correlation coefficient (*R*)		0.945	–	–	0.9999	0.9993

[a] Standard deviation.

TABLE II. Detector response data for wool treated with Lissamine yellow 2G (LY2G).

LY2G, % owf	Number of samples	Response per μg: Peak from LY2G	Response per μg: *p*-Cresol peak	Response per μg: Sum, cresol + phenol peaks	Peak ratios, LY2G to: *p*-Cresol	Peak ratios, LY2G to: Sum, cresol + phenol
7.6	5	10794 ± 1353[a]	3943 ± 330	7432 ± 889	2.7527 ± 0.3951	1.5914 ± 0.1250
2.7	5	3624 ± 1298	3378 ± 840	5435 ± 1660	1.0715 ± 0.2434	0.5933 ± 0.1038
1.4	5	994 ± 110	3677 ± 430	5047 ± 1112	0.3063 ± 0.0363	0.2023 ± 0.0316
Regression analysis						
y intercept (*y* at 0% owf)		−901	–	–	−0.1068	−0.05629
Slope		1548	–	–	0.3804	0.2185
Correlation coefficient (*R*)		0.998	–	–	0.994	0.997

[a] Standard deviation.

for the weight of pyrolyzed sample, and therefore handled as peak areas per μg. Tsuge and Matsubara reported such an approach for an earlier study of protein pyrolysis [9]. Reliability in that case was linked to accurate weighing and transferring of the sample and repeatable generation of pyrolytic products. The second approach used peaks of wool pyrolysis products as internal standards. Reliability here depended on pyrolysis products unaffected by the commercial wool treatment. The second approach worked best when an internal standard component was selected from midway through the chromatogram. Selecting a component of too high volatility proved unreliable because of the variability in the amounts formed from run to run. We initially selected the peak from *p*-cresol as internal standard. *p*-Cresol is a pyrolysate of tyrosine; since some of the *p*-cresol undergoes secondary decomposition to phenol, we also tested the data using the combined peak areas of phenol and *p*-cresol as an internal standard, an approach Tsuge and Matsubara reported [9] for the quantitation of tyrosine in other proteins.

Tables I and II show that the repeatability of measurements by either approach is the same: the average coefficient of variation is 16% for both. In the internal standard approach, the use of the sum of the phenol and cresol peak areas as the internal standard allows a linear fit to the data with a smaller *y*-intercept than obtained from the cresol peak alone. Nevertheless, results from the cresol peak alone are usable for quantitation, and the extra effort required to integrate the phenol peak does not seem to be justified. Although the internal standard approach produces a better linear fit than does the other approach (response per μg), the following statistical treatment justifies the response per μg approach as the best choice.

The most relevant statistical analysis would be one that demonstrates the predictability of a percent owf value based on a single or duplicate run, and also gives confidence limits at a specified confidence level. The data therefore were processed by the method of inverse regression. Results using the response per μg approach are presented graphically in Figures 2 and 3, in each of which the best linear fit is sandwiched between (nonlinear) plots of confidence limits. Results using the internal standard approach were unsatisfactory; the data produced confidence limits that were unacceptably large. Figure 2 shows the mean linear fit and the 95% confidence limits for duplicate determinations of MFF/μg. Construction lines illustrate the prediction of percent owf based on an average peak area of 600/μg from hypothetical duplicate determinations. Equation 1 may be used to estimate the 95% confidence limits of percent owf for any number of hypothetical replicate determinations of MFF (Table III):

$$x = (y + 295.7)/1439.6 \pm 0.1836[1/q + 1/16 + (((y + 295.7)/1439.6 - 0.6125)^2/1.3375)]^{1/2} \quad , \quad (1)$$

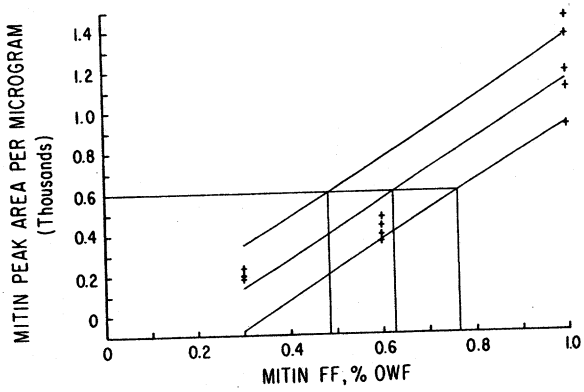

FIGURE 2. Predictability plot for % owf Mitin FF using peak area per microgram of treated wool, showing upper and lower confidence limits at the 95% confidence level and mean linear fit for hypothetical duplicate determinations.

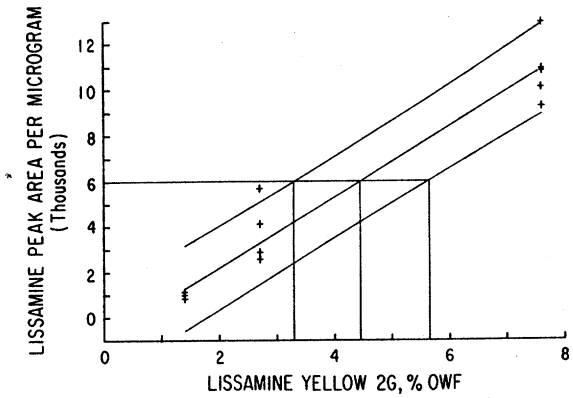

FIGURE 3. Predictability plot for % owf Lissamine yellow 2G using peak area per microgram of treated wool, showing upper and lower confidence limits at the 95% confidence level and mean linear fit for hypothetical duplicate determinations.

where x is the range of predicted % owf, y is the average of the replicate determinations of peak area/μg, q is the number of hypothetical replicate determinations, and 16 is the number of determinations of MFF in this study. Similarly, Figure 3 shows the mean linear fit and the 95% confidence limits for hypothetical duplicate determinations of LY2G/μg. Construction lines illustrate the prediction of percent owf based on an average peak area of 6000/μg from hypothetical duplicate determinations. Equation 2 may be used to estimate the 95% confidence limits of percent owf for any number of hypothetical replicate determinations of LY2G (Table III).

$$x=(y+900.9)/1548.3 \pm 1.526[1/q+1/15 + (((y+900.9)/1548.3-3.9)^2/106.9)]^{1/2} \quad , \quad (2)$$

where x, y, and q are as defined in Equation 1 and 15 is the number of determinations of LY2G in this study.

The confidence limits in Figures 2 and 3 may be constricted by increasing the number of hypothetical replicate determinations (Table III), but the benefits of doing this diminish with each additional run, and the practicality of using the pyrolysis-GC technique for quantitative determinations is compromised.

Conclusions

Benzoyl functionality and commercially applied agents MFF and LY2G may be detected on wool by the pyrolysis-GC technique, which offers the potential for detecting multiple agents on wool. The quantitation of agents MFF and LY2G by this technique is restricted by problems of repeatability, and the user must decide whether the errors are acceptable for the intended application.

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TABLE III. Sample predictions of percent owf from inverse regression analysis.

Agent	Hypothetical average of replicate determinations, peak area/μg wool	Predicted % owf	Predicted range of % owf using q replicate determinations, where q = 1	2	3	4	5	∞	Equation
MFF	600	0.62 ±	0.19	0.14	0.12	0.10	0.09	0.05	1
LY2G	6000	4.5 ±	1.6	1.2	1.0	0.9	0.8	0.4	2

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