BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 41 475—478 (1968)

# Analyses of Organic Soils Extracted from Naturally-soiled Cloths

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(Received April 22, 1967)

The analyses of organic soils extracted from naturally-soiled chloths were carried out by IR spectroscopy, gas chromatography, thin-layer chromatography (TLC), and gel filtration. The fatty-acid constituents involved in organic soil were determined by gas chromatography. The presence of triglycerides, cholesterol and its ester, and such hydrocarbons as paraffin and squalene in organic soil was also found by means of TLC. In gel filtration, two peaks were found in elution diagrams of the detergent solution after washing naturally-soiled cloths. The ultraviolet spectra, which were measured for the effluent corresponding to each peak, indicated that the nitrogen compound involved in each effluent is probably a protein-lipid complex such as lipoprotein. The nitrogen content in each effluent was also determined by the micro-Kjeldahl method. A smaller nitrogen content was given for the effluent corresponding to the first peak than for that of the second peak. The nitrogen compound involved in the effluent for the first peak was harder to remove from cloths than that for the second peak.

Knowledge concerning natural soils adhering on cloths is necessary in order to investigate the detergency mechanism. The natural soils may be divided into two main groups; one is an inorganic soil for which dirt floated in an atmosphere is considered to be the main constituent, while the other is an organic soil consisting of skin fats.

A study has been made by Brown[1] of the amount and composition of the organic soil adhering to domestically-soiled fabrics. However, the constituents have not been shown in detail for each component involved in the organic soils. Bey[2] has reported the results of his analysis of skin-surface lipids which were transferred on to textiles or underwear during wearing. Oldenroth *et al.*[3] have also reported similar analytical results for organic soils extracted from underwear. The composition of human foream sebum and of adult human-hair fat have been determined by Wheatly[4] and Nicolaides *et al.*[5] respectively. Further, the compositions of human surface lipids have been reported in detail by Nicolaides *et al.*[6]

With the recent advance in the separation techniques of lipid mixture, such as gas and thin-layer chromatography, it has become possible to determine the constituents of natural lipids in detail. The purpose of this work is to determine the composition of organic soils extracted from naturally-soiled collars with such techniques as infrared spectroscopy, gas and thin-layer chromatography (TLC), and gel filtration.

## Experimental

Two kinds of organic soils were prepared for analysis. Sample 1 and Sample 2 were prepared by extraction with methylene chloride for the soiled cloths supplied by the Lion Fat and Oil Co. and with petroleum ether for the soiled cloths prepared in our laboratory in a manner similar to that described by Tomiyama *et al.*[7] The two samples were essentially similar.

The composition of free fatty acid involved in the organic soil was determined by a Hitachi F6-D gas

1) C. B. Brown, *Research*, **1**, [7] 46 (1947).
2) K. H. Bey, *Am. Perfumer and Cosmetics*, **79**, [8] 35 (1964).
3) K. Kind and O. Oldenroth, *Wascherei-Tech u-Chem*, **1**, [5] 9, 13 (1948); O, Oldenroth, *ibid.*, **10**, 430 (1957); O. Oldenroth and K. Nettelnstroth, *Melliand Textilber.*, **45**, 79 (1964).
4) V. R. Wheatley, *Seifen-Oele-Fette Wachse*, **81**, 214 (1955).
5) N. Nicolaides and R. C. Foster, *J. Am. Oil Chem. Soc.*, **33**, 404 (1956).
6) N. Nicolaides and R. E. Kellum, *ibid.*, **42**, 685 (1965); N. Nicolaides, *ibid.*, 691; N. Nicolaides and Thomas Ray, *ibid.*, 702; N. Nicolaides, *ibid.*, 708.
7) S. Tomiyama and M. Iimori, *ibid.*, **42**, 449 (1965).

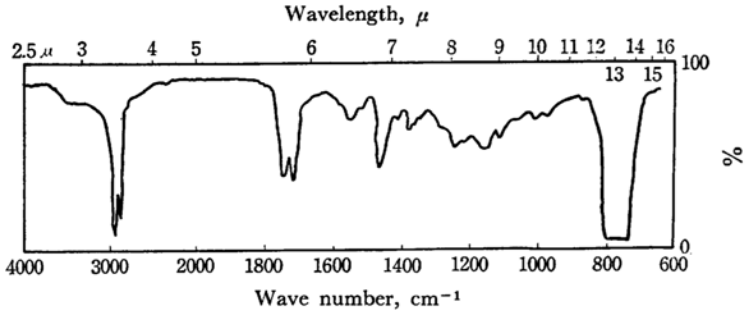


Fig. 1. IR spectrum of $CCl_4$ solution of a organic soil extracted from naturally soiled cloths (Sample 2).

chromatograph (with a flame-ionization-type detector) using a Golay column B. D. S. 45 m in length. The methylation[8] was performed by refluxing Sample 2 with a 5% HCl - methanol solution at 100°C for several hours.

The lipid components were detected by the TLC method. Silica gel (Wako gel B-O, Wako Pure Chemical Ind., Ltd.) was used as the adsorbent. The commercial solvents and reagents were used without purification. The adsorbent layer was produced on a 20×20 cm clean glass plate with a thickness of about 250 $\mu$ and equipped with an applicator (Yamato TL-1, Yamato Sci. Co., Ltd.), and then activated by heating in an oven at 110—120°C for 1—2 hr. After 10 m*l* of a dilute methylene chloride solution of each sample had been applied to the adsorbent layer with a microsyringe, the chromatoplate was dipped up to the depth of 1 cm into some developing solvent in a covered-glass vessel. The identification was carried out by heating the plate at 120—180°C in an oven after spraying it with concentrated $H_2SO_4$.

The Sephadex gel filtration technique was also attempted in order to find the nitrogen compounds involved in natural soils. Completely-swollen Sephadex G-100 gel after been in a 1% NaCl aqueous solution for 2 days was packed in the column 2.5 cm in diameter and 45 cm in height. After 10 m*l* of the sample solution had been added to the column, 0.1% NaCl aqueous solution was poured in from the top of the column. Nitrogen compounds in the effluents were detected by measuring the absorbance at 280 m$\mu$ with a Hitachi Perkin-Elmer spectrophotometer, 139.

## Results and Discussion

**Infrared Spectrum.** The infrared spectrum of Sample 2 in a carbon tetrachloride solution is shown in Fig. 1. (A similar spectrum was obtained for Sample 1.) The IR spectrum exhibited characteristic peaks at 1740 $cm^{-1}$ and 1710 $cm^{-1}$ and a broad peak in the 1300—1100 $cm^{-1}$ region which can not seen in the spectrum of the solvent. The peaks at 1740 $cm^{-1}$ and 1300—1100 $cm^{-1}$ were identical with those of the triolein, but that at 1710 $cm^{-1}$ could not be observed in the triolein spectrum. The peaks at 1740 and 1710 $cm^{-1}$ may be identified as, respectively, the stretching vibration in carbonyl groups in glyceride molecules such as triolein and that in free fatty acid molecules. Since the infrared spectrum shown in Fig. 1 was essentially identical with that of triolein except for the peak at 1710 $cm^{-1}$, it can be estimated that such triglycerides as triolein are present in naturally-soiled cloths. On the other hand, the existence of the peak at 1710 $cm^{-1}$ indicates that free fatty acids are also present in the samples.

**Gas Chromatography.** A gas chromatogram of the fatty acid in Sample 2 is shown in Fig. 2. The results show that most of the fatty acids involved in this organic soil have carbon numbers from 14 to 18 in their molecules. For the $C_{14}$ fatty acids, the peak height of saturated acid was greater than that of unsaturated fatty acids. In the $C_{16}$ fatty acids, both are of equal height. On the contrary,

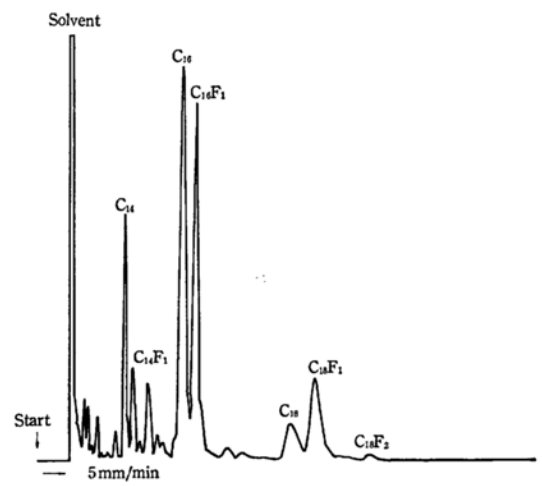


Fig. 2. Gas chromatographic analysis of fatty acids involved in a organic soil extracted from naturally soiled cloths.
Sample 2. 4 $\mu l$, column: Golay B. D. S. 45 m, Oven temp.: 170°C, Injection block: 300°C, Carrier gas: $N_2$, Flow rate: 0.7 kg/cm², Attenuation: 10×4, Chart speed: 5 mm/min, Instrument: No. F6-D.

8) H. Schlenk and J. L. Gellerman, *Anal. Chem.*, **32**, 1412 (1960).

the order of peak height mentioned above was reversed in the case of $C_{18}$ fatty acids. A similar result has been reported by Bey.[2] It is interesting that the ratio of the amount of unsaturated fatty acids to that of saturated acids increases with the increase in carbon number.

**Thin-layer Chromatograph (TLC).** By preliminary experiments it was found that the cyclohexane and a solution consisting of 1-nitropropane and *n*-hexane can be successfully used as solvents for the detection of nonpolar lipids, such as squalene and paraffin, and polar lipids, such as glycerides, cholesterol, and its stearate. It may be seen in Figs. 3 (solvent: cyclohexane) and 4 (solvent: solution consisting of 1-nitropropane and *n*-hexane) that paraffin, squalene, triglycerides, cholesterol, and its esters are involved in natural organic soils.

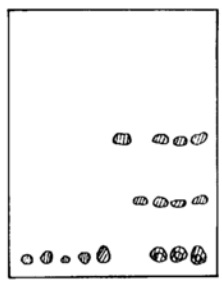


Fig. 3. Thin layer adsorption chromatographic separation of hydrocarbon involved in organic soils.
Solvent is cyclohexane.
a: Stearic acid b: Glycerin monostearate
c: Tristearin d: Cholesterol
e: Cholesterol stearate
f: Paraffin g: Squalene
h: Mixture of a—g lipids
i: Sample 1 j: Sample 2

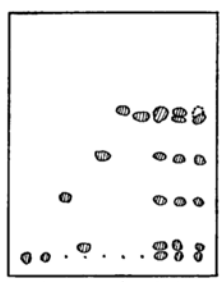


Fig. 4. Thin layer adsorption chromatographic separation of cholesterol, cholesterol ester and triglyceride involved in organic soils.
Solvent is *n*-hexane 180 cc+1-nitropropane 20 cc solution.
a: Stearic acid b: Glycerin monostearate
c: Tristearin d: Cholesterol
e: Cholesterol stearate
f: Paraffin g: Squalene
h: Mixture of a—g lipids
i: Sample 1 j: Sample 2

**Gel Filtration.** Figure 5 shows the elution curves obtained for: (a) the distilled water, (b) the sodium dodecylbenzene sulfonate (NaBS) aqueous solution after washing the naturally-soiled cloths, and (c) a 0.1 wt% egg albumin aqueous solution containing 0.05 wt% NaBS.

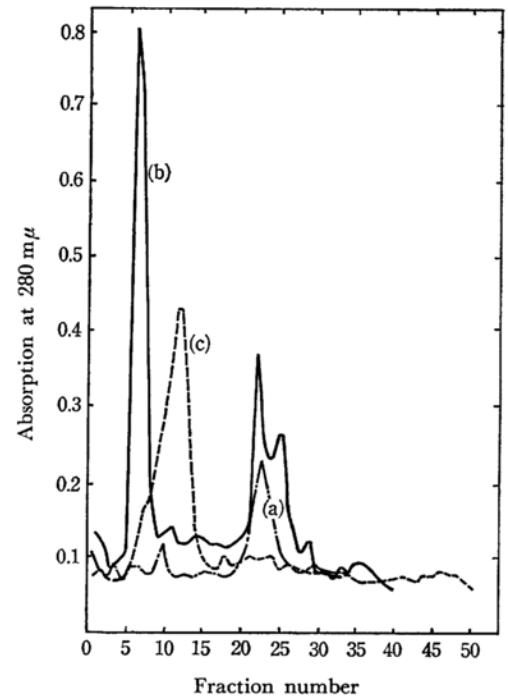


Fig. 5. The elution curve obtained for the distilled water (a), the NaBS aqueous solution after washing the naturally soiled cloths (b) and 0.1% NaBS (c).

A single peak appeared in fraction number 23 in the elution curve (a). On the other hand, two peaks were found in fractions number 6—8 and 22—26 in the elution curve (b). The heights of these peaks increase with the increase in the concentration of NaBS.

From a comparison of (b) with (c) in Fig. 5, we may find that the substance corresponding to the first peak in the curve (b) has a larger molecular size, while the substance corresponding to the second peak has a smaller molecular size, than the egg albumin molecule.

The nitrogen compound involved in the effluents of fractions number 6—8 could be removed from cloths only when a detergent solution was used for washing. Such interactions between proteins and detergents have been reported by Aoki *et al.*[9]

9) K. Aoki, *J. Am. Chem. Soc.*, **80**, 4904 (1958); K. Aoki and J. Hori, *ibid.*, **81**, 1885 (1959); K. Aoki, *Yushi*, **16**, [11] 21 (1963); **17**, [2] 26 (1964); **17**, [3] 12 (1964); **17**, [4] 20 (1964).

and by others.[10]

**The Ultraviolet Spectra.** Figure 6 shows the ultraviolet spectrum for each effluent corresponding to the first and second peaks in the elution curve (b) in Fig. 5, along with those of egg albumin and $\beta$-lipoprotein. The spectrum of egg albumin showed the characteristic absorption due to the polypeptide bond at 280 m$\mu$, but a smaller plateau was found at 270 m$\mu$ in that of $\beta$-lipoprotein. In the spectrum of each effluent, the characteristic absorption found in egg albumin was not observed, but the inflection point near 270 m$\mu$ and the plateau in the region from 240 to 270 m$\mu$ were found for each effluent corresponding to the first and second peaks in Fig. 5, curve (b). The nitrogen compound involved in each effluent is not considered to be pure protein, but a protein-lipid complex such as lipoprotein.

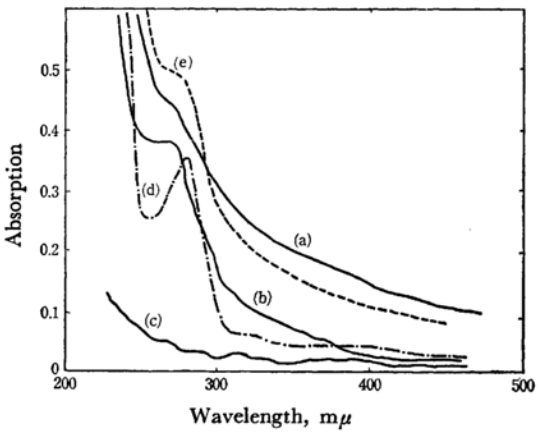


Fig. 6. Ultraviolet spectra.
a : Effluent corresponding to first peak in curve (b) in Fig. 5.
b : Effluent corresponding to second peak in curve (b) in Fig. 5.
c : Effluent corresponding to fraction number 45 in Fig. 5.
d : 1% egg albumin.
e : $\beta$-Lipoprotein.

The nitrogen contents involved in the two effluents corresponding to the first and second peaks and egg albumin, as determined by the micro-Kjelder method, were 5.40, 13.50, and 65.60 $\gamma N/ml$ respectively.

The author wishes to express his hearty thanks to Professor I. Hara of Tokyo Medical and Dental University for his valuable discussions and for supplying the $\beta$-lipoprotein. He is also grateful to Mr. K. Komeji of Tokyo Metropolitan University and Mr. I. Kashiwa of Lion Fat and Oil Co. for supplying the cholesterol stearate and naturally-soiled cloth respectively. Grateful acknowledgment is also made to Mr. N. Ichiki for his gas chromatographic analysis of fatty acids and to Miss M. Imagawa for her assistance in the experimental work.

10) H. P. Lundgren, *J. Am. Chem. Soc.*, **63**, 2854 (1941); F. W. Putnam and H. Neurath, *ibid.*, **66**, 692 (1944); F. W. Putnam and H. Neurath, *ibid.*, **66**, 1992 (1944).