THE ANALYSIS OF MIXTURES OF HYDROCARBONS, BEESWAX, AND SPERMACETI*

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THE COMBINATION of hydrocarbons, beeswax, and spermaceti is frequently encountered in cosmetic creams but the chemical methods of analysis available for such mixtures are unsatisfactory. This paper is a report of studies on the use of chromatography as a new approach to the problem. We have found that after the saponification of the mixture, we can chromatographically separate the unsaponifiable hydrocarbons from the unsaponifiable beeswax and spermaceti alcohols. Furthermore, from solubility studies on the unsaponifiable beeswax and spermaceti alcohols, it is possible to calculate the beeswax and spermaceti. An outline of the procedure is given in Charts 1, 2, and 3.

It is, of course, necessary to know the behavior of each of the components to apply the data obtained by the above procedure to the analysis of a mixture. Accordingly, the samples of beeswax, spermaceti, and hydrocarbons used were individually examined. The results indicate that these substances behave as follows:

Hydrocarbons. The mineral oil used as the *hydrocarbons* contains no saponifiable material and will be almost entirely recovered in the material not adsorbed by the Al_2O_3 from the petroleum benzin solution. The data are presented in Table 1.

Spermaceti. The saponification constants (Table 2) of spermaceti

TABLE 1—CHROMATOGRAPHY OF HYDROCARBONS

Sample	Gm.
U.S.P. White Mineral Oil (Heavy)	5.230
First petroleum benzin fraction*	5.225
Second petroleum benzin fraction	0.001
First hot alcohol fraction	0.007
Second hot alcohol fraction	0.000

* Rate 4 ml. p.b./minute.

TABLE 2—THE SAPONIFICATION OF SPERMACETI

Sample U.S.P. Spermaceti, Gm.	Unsaponifiable Matter, Gm.	Fatty Acids, Gm.
3.825 (a)	1.894 (49.5%)	2.041 (53.4%)
3.823 (b)	1.903 (49.8%)	2.040 (53.4%)
	Av. (49.7%)	Av. (53.4%)

* Presented at the December 3, 1947, Meeting, New York City.

CHART 1—PREPARATION OF MIXTURE FOR CHROMATOGRAPHY

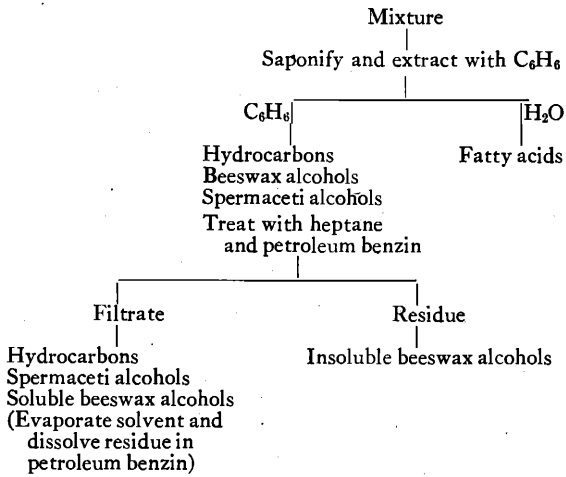


CHART 2—CHROMATOGRAPHY OF HYDROCARBONS, SPERMACETI ALCOHOLS, AND SOLUBLE BEESWAX ALCOHOLS

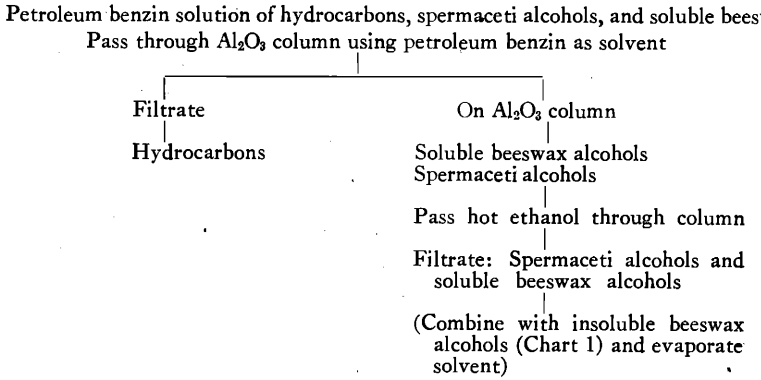
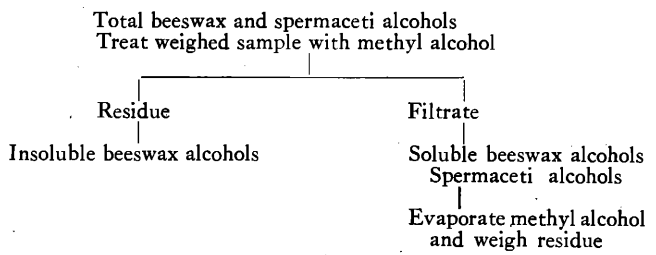


CHART 3—SOLUBILITY OF COMBINED TOTAL BEESWAX AND SPERMACETI ALCOHOLS IN METHYL ALCOHOL



are 49.7% unsaponifiable matter and 53.4% fatty acids. On chromatographing the unsaponifiable matter, it will be adsorbed by the Al_2O_3 from the petroleum benzin solution and stripped from the Al_2O_3 by hot ethanol (Table 3). The spermaceti alcohols obtained from the hot ethanol filtrate will be almost completely soluble in cold methyl alcohol. Although Table 7 indicates that 0.008 gm. out of 0.500 gm. of the spermaceti alcohols is insoluble, the value of 0.008 gm. includes the errors of the manipulative procedure all of which tend to make the result high.

TABLE 3—CHROMATOGRAPHY OF SPERMACETI UNSAPONIFIABLE MATTER

	Sample "a," Gm.*	Sample "b," Gm.
Unsaponifiable matter	1.894	1.903
Unsaponifiable matter insoluble in heptane and petroleum benzin	Nil	Nil
Unsaponifiable matter soluble in heptane and petroleum benzin (by difference)	1.894	1.903
First petroleum benzin fraction*	0.004	0.003
Second petroleum benzin fraction	0.001	0.002
First hot alcohol fraction	1.871	1.879
Second hot alcohol fraction	0.006	0.004

* Sample "a"—rate 4 ml. p.b./minute.
Sample "b"—rate 3.6 ml p.b./minute.

TABLE 4—THE SAPONIFICATION OF BEESWAX

Sample U.S.P. White Beeswax, Gm.	Unsaponifiable Matter, Gm.	Fatty Acids, Gm.
3.147 (a)	1.696 (53.9%)	1.516 (48.2%)
3.134 (b)	1.688 (53.9%)	1.511 (48.2%)
3.139 (c)	1.687 (53.7%)	1.515 (48.3%)
3.179 (d)	1.705 (53.6%)	1.534 (48.3%)
	Av. (53.8%)	Av. (48.3%)

Beeswax. The saponification constants of beeswax are 53.8% unsaponifiable matter and 48.3% fatty acids (Table 4). On chromatographing the beeswax unsaponifiable matter soluble in heptane and petroleum benzin, 13.5% (based on original weight of beeswax sample) will not be adsorbed by the Al_2O_3 from the petroleum benzin solution. The adsorbed material will be subsequently stripped by hot ethanol (Table 5). With the solubility procedure used the combined beeswax alcohols (the ones insoluble in heptane and petroleum benzin added to the ones stripped from the column with hot ethanol) will vary in solubility in cold methyl alcohol from 12% for a 0.5-gm. sample to 27% for a 0.1-gm. sample (Table 6 and Fig. 1).

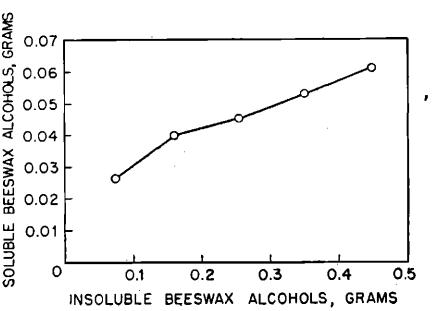


Figure 1

It is known that hydrocarbons (1) are readily washed through an Al_2O_3 column with petroleum benzin and the value of 13.5% unadsorbed material falls within the range of hydrocarbons reported for beeswax

TABLE 5—CHROMATOGRAPHY OF BEESWAX UNSAPONIFIABLE MATTER

	Sample "a," Gm.	Sample "b," Gm.	Sample "c," Gm.	Sample "d," Gm.
Unsaponifiable matter	1.696	1.688	1.687	1.705
Unsaponifiable matter insoluble in heptane and petroleum benzin (insoluble beeswax alcohols)	0.987	1.014	0.983	0.928
Unsaponifiable matter soluble in heptane and petroleum benzin (by difference)	0.709	0.674	0.704	0.777
First petroleum benzin fraction*	0.427 (13.6%)	0.423 (13.5%)	0.422 (13.4%)	0.423 (13.3%)
Second petroleum benzin fraction	0.001	0.000	0.000	0.000
First hot alcohol fraction	0.276	0.246	0.275	0.325
Second hot alcohol fraction	0.003	0.003	0.003	0.021

* Av. 13.5%.
Sample "a"—rate 5 ml. p.b./minute.
Sample "b"—rate 4 ml. p.b./minute.
Sample "c"—rate 4 ml. p.b./minute.
Sample "d"—rate $3^1/_3$ ml. p.b./minute.

TABLE 6—SOLUBILITY OF BEESWAX ALCOHOLS IN COLD METHYL ALCOHOL

Beeswax Alcohols, Gm.	Soluble Material, Gm. Expt. 1	Expt. 2	Av.	Insoluble Material (by Difference) Av., Gm.
0.100	0.026	0.028	0.027	0.073
0.200	0.040	0.039	0.040	0.160
0.300	0.046	0.044	0.045	0.255
0.400	0.054	0.054	0.054	0.346
0.500	0.061	0.062	0.062	0.438

(12–14%) (2). The unsaponifiable material insoluble in heptane and petroleum benzin is also beeswax alcohols which are removed to decrease the amount of material necessary to chromatograph.

THE ANALYSIS OF BEESWAX-SPERMACETI ALCOHOL MIXTURES

An empirical method based on the solubility differences in cold methyl alcohol can be used to analyze mixtures of the two alcohols. The procedure is to treat a weighed sample with methyl alcohol, chill the solution, and filter off the insoluble material. Evaporate the methyl alcohol from the filtrate and weigh the residue. Calculate the insoluble material, mostly beeswax alcohols, by difference. By use of Fig. 1, compute the weight of soluble beeswax alcohols associated with the insoluble beeswax alcohols. The sum of the insoluble material and the soluble beeswax alcohols is approximately equivalent to the total beeswax alcohols in the sample.

TABLE 7—ANALYSIS OF MIXTURES OF BEESWAX AND SPERMACETI ALCOHOLS

Sample	Gm.	Soluble Material, Gm. Expt. 1	Expt. 2	Insoluble Material (by Difference), Gm. Expt. 1	Expt. 2	Beeswax Alcohols (Found), Gm. Expt. 1	Expt. 2	Spermaceti Alcohols (Found), Gm. Expt. 1	Expt. 2
Spermaceti al.	0.500	0.492	...	0.008	...	...	...	0.492 (98%)	...
Spermaceti al.	0.400	0.415	0.419	0.085	0.081	0.114 (114%)	0.109 (109%)	0.386 (97%)	0.391 (98%)
Beeswax al.	0.100	...	...	...	...	...	...	...	...
Spermaceti al.	0.300	0.326	0.333	0.174	0.167	0.215 (108%)	0.207 (104%)	0.285 (95%)	0.293 (98%)
Beeswax al.	0.200	...	...	...	...	...	...	...	...
Spermaceti al.	0.200	0.243	0.247	0.257	0.253	0.302 (101%)	0.298 (99%)	0.198 (99%)	0.202 (101%)
Beeswax al.	0.300	...	...	...	...	...	...	...	...
Spermaceti al.	0.100	0.146	0.151	0.354	0.349	0.409 (102%)	0.403 (101%)	0.091 (91%)	0.097 (97%)
Beeswax al.	0.400	...	...	...	...	...	...	...	...

Subtract the weight of the beeswax alcohols from the weight of sample taken; the remainder is spermaceti alcohols. The results of a series of experiments employing this method are given in Table 7.

All the results were in the range 100 ± 14%. It will be observed that the determinations of the major component of the mixture were accurate to ±5%. The minor component was determined with less accuracy. It will also be noted that with but one exception the recoveries of spermaceti alcohol were somewhat low while those of the beeswax alcohols were high. In analyzing mixtures of this type, it might be well to remember this and weigh the results accordingly.

ANALYSIS OF A MIXTURE OF HYDROCARBONS, BEESWAX, AND SPERMACETI

Saponify and chromatograph the mixture of hydrocarbons, beeswax, and spermaceti according to the described experimental procedure. Combine the unsaponifiable alcohols and treat a 0.5-gm. aliquot by the given solubility procedure. The data presented in Table 8 show the results obtained in a typical experiment by following procedure described.

Estimation of Beeswax and Spermaceti

The soluble alcohols (Table 8) from a 0.5-gm. sample were found to be 0.261 gm.

The insoluble alcohols (by difference) = 0.500 gm. − 0.261 gm. = 0.239 gm.

From Fig. 1, the soluble beeswax alcohols which will be associated with the 0.239-gm. insoluble beeswax alcohols = 0.044 gm.

∴ Total beeswax alcohols in 0.5-gm. sample = 0.239 gm. + 0.044 gm. = 0.283 gm.

But total alcohols = 0.906 gm. (Table 8).

∴ Beeswax alcohols in whole sample $= \frac{0.906}{0.5} \times 0.283 = 0.513$ gm.

From Tables 4 and 5 beeswax alcohols = 53.8% (total unsaponifiable material) − 13.5% (hydrocarbons) = 40.3% of the beeswax.

∴ Beeswax $= \frac{0.513}{0.403} = 1.273$ gm. (103.6% recovery).

Total spermaceti alcohols = total alcohols − beeswax alcohols = 0.906 gm. − 0.513 gm. = 0.393 gm.

and spermaceti alcohols = 49.7% of spermaceti (Table 2)

∴ Spermaceti $= \frac{0.393}{0.497} = 0.791$ gm. (96.5% recovery).

Calculation of Non-Beeswax Hydrocarbons

Total hydrocarbons = 5.693 gm. (Table 8).

Beeswax hydrocarbons = 1.273 × 0.135 = 0.172 gm.

∴ Non-beeswax hydrocarbons = 5.693 gm. − 0.172 gm. = 5.521 gm. (99.5% recovery).

Experimental Procedure

Saponification of Sample and the Chromatography of the Unsaponifiable Fraction. Saponify sample by refluxing for two hours with 25 ml. of absolute alcohol, 1 gm. KOH, and 50 ml. of benzene. Transfer the saponified mixture to a separatory funnel, add 75 ml. of hot water, shake well and draw off the aqueous layer.* Continue the extraction with two additional 50-ml. portions of hot benzene. (Reserve the extracted aqueous solution.) Combine the benzene extracts, wash with three 30-ml. portions of 30% alcohol, and add the washings to the reserved extracted aqueous solution. Filter the washed benzene extract through a cotton plug into a 250-ml. tared beaker, evaporate the benzene on the steam bath, dry the residue in an oven at 100°C for 15 minutes, cool in a vacuum desiccator, and weigh as unsaponifiable matter. Repeat the heating of the unsaponifiable matter in the oven until the weight is constant to 1–2 mg.

Table 8—Analytical Data of Mixture of Hydrocarbons, Beeswax, and Spermaceti

	Gm.
Sample:	
U.S.P. Mineral Oil	5.549
U.S.P. Beeswax	1.229
U.S.P. Spermaceti	0.820
Fatty acids	1.034
Unsaponifiable matter	6.606
Unsaponifiable matter insoluble in heptane and petroleum benzin (insoluble beeswax alcohols)	0.314
Unsaponifiable matter soluble in heptane and petroleum benzin (by difference)	6.292
First petroleum benzin fraction*	5.690
Second petroleum benzin fraction	0.003
First hot alcohol fraction	0.586
Second hot alcohol fraction	0.006
Solubility of 0.5-gm. sample of combined alcohols in cold methyl alcohol	0.261

* Rate 4 ml. p.b./minute.

* Troublesome emulsions can be broken by fastening the stopcock of the separatory funnel with a rubber band and partially immersing the separatory funnel in a steam bath until the contents begin to boil.

Acidify the reserved aqueous solution with HCl, extract with three 30-ml. portions of chloroform, and wash the combined chloroform extracts with water. Filter the washed chloroform extract through a cotton plug into a tared beaker, evaporate the chloroform on the steam bath, dry the residue in an oven at 100°C. for 15 minutes, cool and weigh as fatty acids. Repeat the heating of the fatty acids until the weight is constant to 1–2 mg.

Dissolve the unsaponifiable fraction in 50 ml. of boiling heptane and cool the solution to room temperature with stirring. Filter the resulting mixture through filter paper, wash the residue well with petroleum benzin, and reserve the filtrate. Dissolve the residue on the filter paper by pouring hot chloroform through the filter, evaporate the chloroform solution on the steam bath, dry the residue in an oven at 100°C. for 15 minutes, cool in a vacuum desiccator, and weigh as unsaponifiable material insoluble in heptane and petroleum benzin. Repeat the drying in the oven until the weight is constant to 1–2 mg.

Evaporate the reserved petroleum benzin–heptane filtrate on the steam bath, take up the residue in 50 ml. of warm petroleum benzin (b.p. 30–75°C.), cool the solution to room temperature, transfer solution with the aid of 25 ml. petroleum benzin to a chromatograph tube containing a 9 × $^3/_4$-in. activated Al_2O_3 column ("Alorco" grade F-20 mesh 80–200), and allow the solution to flow through the column at a rate of 3.3 to 5 ml. per minute. Follow this solution with 175 ml. of petroleum benzin, combine the filtrates, and label as first petroleum benzin fraction. Pass another 50 ml. of petroleum benzin through the column and label filtrate as second petroleum benzin fraction. Remove the petroleum benzin by passing 50 ml. of 95% alcohol, under air pressure, through the column. Follow this with 125 ml. of boiling alcohol which is also forced through the column at a rapid rate (4–7 minutes) with air pressure. Mark the combined two filtrates as the first hot alcohol fraction. Pass another 50 ml. of boiling alcohol through the column and label as second hot alcohol fraction.*

Evaporate the various fractions on the steam bath. Dry the two petroleum benzin fractions in an oven at 100°C. for 15 minutes, cool in a vacuum desiccator, and weigh. Repeat drying in oven until weight is constant to 1–2 mg. Redissolve each of the hot alcohol fractions in 10 ml. of chloroform, evaporate chloroform on steam bath, and dry and weigh residues in the manner described for the petroleum benzin fractions.

Solubility Procedure. Weigh sample into a 150-ml. beaker, add 80 ml. methyl alcohol, cover beaker with watch glass, and heat on hot plate until solution is complete. Transfer beaker to ice bath and stir solution vigorously with thermom-

* The second fraction obtained in each case above should contain negligible amounts of non-volatile material.

eter until the temperature is 5°C. Pour resulting mixture through a Büchner funnel containing a 11-cm. No. 595 S&S filter paper turned up with a 1/2-in. edge, and wetted with methanol. Filter by gravity or gentle suction. Do not allow the material on the filter to dry or cake. Wash residue with 20 ml. of ice-chilled methanol and drain dry with strong suction. Transfer the filtrate to a tared beaker with the aid of chloroform, evaporate to dryness on the steam bath, heat in an oven at 100°C. for 10 minutes, cool in a vacuum desiccator, and weigh. Repeat drying in oven until weight is constant to 1–2 mg.

Summary

Studies have been made on a new analytical method for the analysis of mixtures of hydrocarbons, beeswax, and spermaceti. Chromatography plays an important role in the procedure. It is believed that the outlined chromatographic procedure can be used to detect adulterants; particularly, hydrocarbons in beeswax and spermaceti. Work in this field is contemplated.

Bibliography

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