

A physical method for qualitative examination of human sebum

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Synopsis

Total lipids from human scalp have been collected from individuals (both sexes and of different ages), and examined by differential scanning calorimetry.

Melting endotherms have been plotted between -100 and $+50^{\circ}\text{C}$. The curves tend to change with the age of the subject. The shape of the curve is a physico-chemical property of sebum, from which a diagnosis, seborrheic or not seborrheic, may be obtained.

This new parameter has been correlated with other properties of sebum.

Introduction

The film of scalp lipids is composed of: sebum resulting from sebaceous gland excretion; lipids proceeding from the Malpighi (1) layer epidermal cells and waste fatty fractions.

Due to the preponderance of scalp sebaceous glands, these three elements are normally referred to collectively as sebum.

This human sebum has been studied, with special regard to: its composition (1) (2) (3) (4) (5) (6); the factors which influence its production (7) (8) (9) (10) (11); the mechanism of its formation (relation squalene-cholesterol).

Some authors have been interested in composition variations according to age and sex (12) (13) (14) (15) (16).

Our purpose was to define a seborrheic state through consideration of physico-chemical properties corresponding with this state.

If one attempts to define seborrheic state from a cosmetic point of view, one can say that it is characterized by: an exacerbation of the fatty and oily aspect of the scalp; the presence, on hair, of a more or less thick, shiny, greasy lipidic film which fixes dust and gives an unpleasant heavy aspect to the hair.

This subjective evaluation proves to be insufficient to start searches for the anti-seborrheic properties of certain molecules and to measure the evolution of a seborrheic state during a treatment. Our studies on the seborrhea evaluation led us to consider physical and morphological differentiations of sebum. J. L. Burton (17) in 1970, in his study of the acneic phenomenon made some physico-chemical measures of sebum such

as: density; viscosity; surface tension; melting point. The extracted amounts of sebum being small, measurements were delicate. In particular viscosity was calculated from the Washburn's equation from capillarity measurement.

We have tried to measure the melting endotherms of sebum through the Differential Thermal Analysis technique. The results of this investigation were submitted to correlation studies with some physico-chemical parameters and led us to define some properties of sebum corresponding to a non pathogen seborrheic state.

Collection methods

To obtain sufficiently reliable results it was necessary to obtain appreciable quantities and quantitative samplings of sebum. At present the known methods are: direct suction of the sebaceous gland; application to the skin of a sampling rod (7) (8) (9); application of a solvent on the skin surface (18) (19); application to skin of absorbing matter (10); friction of hair followed by washing with solvent (12) (20).

The amounts of sebum obtained are determined by gravimetry when the sample is large enough; other more or less approximate methods have been proposed when the sample is not large enough e.g. turbidimetry (19), sebograph - sebum-meter (7) (9), colorimetry (21), Acid Number - Iodine Number (19), Oxidation (19). The amounts of sebum obtained through point sampling (1 mm²) have poor reproducibility. These variations prove the heterogeneity of the distribution of sebaceous glands. The composition of the mixture collected depends, to a certain extent, on the method of extraction used (22). In the method now to be described the total amount of sebum present on scalp and hair is collected. The principle is based on the quantitative emulsification of sebum by a surfactant mixture whose composition has been devised to effect a predetermined detergency:—

Sodium laurylsulfate	4.3 g
betaine derivative	2.0 g
$\begin{array}{c} \text{CH}_3 \\ \\ \text{R} - \text{N} - \text{CH}_2\text{COO}^\ominus \quad (\text{R} = \text{coprah}) \\ \\ \oplus \text{CH}_3 \end{array}$	
lactic acid	0.0235 g
de-ionized water qs	100 ml

The extraction is achieved in two stages:

(1) Exactly 15 ml of the surfactant solution are poured on to the wet hair; a light massage is used with gloved hands until foam is obtained. It is then rinsed with 1000 ml of de-ionized water and the whole is carefully collected in a measuring vessel.

(2) The preceding operation is repeated with 10 ml of the surfactant solution. It is rinsed and collected again in the measuring vessel and the total volume of aqueous sebum suspension is measured exactly. The whole or a part of the suspension carefully homogenized is extracted by peroxide free diethyl ether. The ether solution carefully washed with water and dehydrated with sodium sulfate, is evaporated. The sebum is evaluated by gravimetry. The amounts of sebum collected from a given person, at constant time intervals, are reproducible within limits of $t \pm 15\%$. A detailed analysis of the sebum so

collected leads to results consistent with those published in the literature and shows that the sebum so collected is not altered.

THERMAL ANALYSIS MEASUREMENTS

Definition—Apparatus—Methodology

Human sebum is in the solid crystalline form at -100°C ; it is wholly liquid at $+50^{\circ}\text{C}$. Between these two temperatures the physical state of sebum is not well defined. Some constituents are in the crystalline form, others in the liquid form. One cannot exclude the presence of other physical forms such as liquid crystals or emulsions. Differential thermal analysis records the curve $\Delta H = f(T)$.

Between -100°C and 50°C , this curve can be called, as a first approximation, the sebum melting curve. This melting signal is very widely spread on the temperature axis and we are therefore led to measure weak ΔH values against units of temperature.

It is therefore necessary to work at high sensitivity and the apparatus, under these conditions, is also sensitive to specific heat (C_p) variations.

From *Fig. 1*, the baseline of the melting curve strongly slopes towards the 'endo' direction. The form of this graph represents the evolution of C_p as a function of temperature. This aspect of the thermal analysis curve has not been exploited.

Measurements have been achieved on a thermal analyser Dupont® 900, fitted with a DSC cell (Differential Scanning Calorimetry).

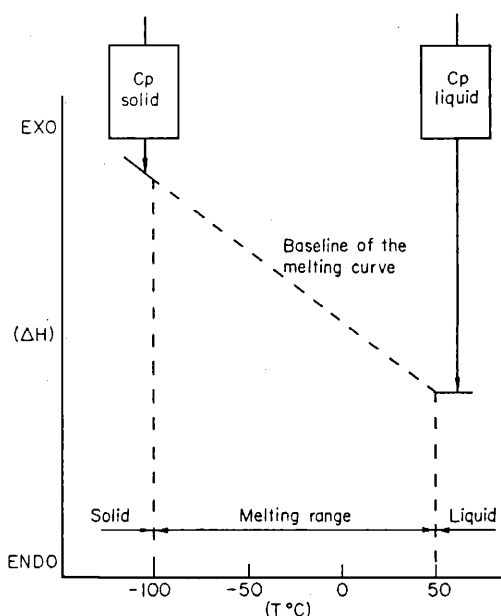


Figure 1. Localization of the melting curve of sebum on the differential scanning calorimetry plot.

The following experimental conditions were adopted

Amount of sample	: 30 mg
Sensitivity on temperature scale	: 50°C inches ⁻¹
Sensibility on T scale	: 0,2°C inches ⁻¹
Sample atmosphere	: dry N ₂
Starting temperature	: -100°C
Final temperature	: +50°C
Heating rate	: 10°C m ⁻¹

Each sample was submitted to two fusions from which only second plots were retained. Indeed, a difference is often observed between the first and second plot which depends on the thermal history of the sample. On the other hand, it was shown that the plot does not change between the second, the third, and subsequent fusions. The crystallization plots were not recorded, because the device does not allow a linear temperature programmed from +50°C to -100°C.

Exploitation of differential scanning calorimetry signals

Fig. 2 shows a typical plot of human sebum melting. The signal is very complex. Within the scope of the present study, we shall not attempt to develop a theoretical interpretation of this curve. In order to obtain from the plot anything other than a purely visual observation, a processing system capable of obtaining comparative numerical results for several individuals must be chosen.

If one tries to achieve from the plot anything other than a purely visual observation one must choose a processing system capable of obtaining comparative numerical results for several individuals.

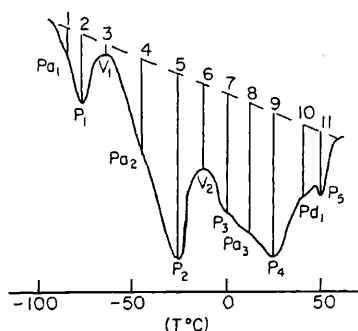


Figure 2. Typical melting curve of human sebum, with the points used for calculations.

It is possible, for example, to integrate the plot (by cutting out and weighing) and exploit the integral plot that would result from it, and which would represent the melted fraction as a function of temperature.

Finally 11 specific points were chosen that could be found again on every plot. The plots were characterized by the ordinate of these points (with respect to the baseline). This is a way of exploiting but not integrating curves. Therefore the chosen points are not equidistant on the temperature axis but they take into account the peculiarities of the plot. As Fig. 2 shows these points are: Slopes up and slopes down (Pa and Pd); peaks (P); valleys (V). The ordinates were normed with respect to their sum; we set up this sum equal to 100.

The validity of the research depends on the reliability of the device giving the plots. The plots in *Fig. 3* were recorded successively starting from the same sample. It is obvious that the apparatus can give the plots with satisfactory reproducibility.



Figure 3. Reproducibility test for the apparatus. Successive plots from the same sample.

SAMPLING

The previously described method of sampling was well accepted by the subjects, allowing us to carry out a large number of operations on heads without any difficulty.

The research was performed on forty-seven subjects of both sexes with natural hair (not dyed, not bleached) whose ages ranged from 5 to 65.

For each subject many samplings were carried out at various time intervals.

Results

The melting plots for human sebum are all in the same temperature range (from -100°C to 50°C).

The same signals are always encountered, but the relative ratios of some of them in respect to the others vary widely from one subject to the other.

In *Fig. 4* the melting curves of three subjects are reproduced. Obviously the plots are typical of each subject. The relationship, subject-appearance of the melting curve is constant in time—for a given subject the same pattern is met with from sebum sampled at several weeks intervals.

Investigations of the plots obtained for the population studied permitted the following conclusions to be drawn: none of the characteristics of the thermal analysis curve

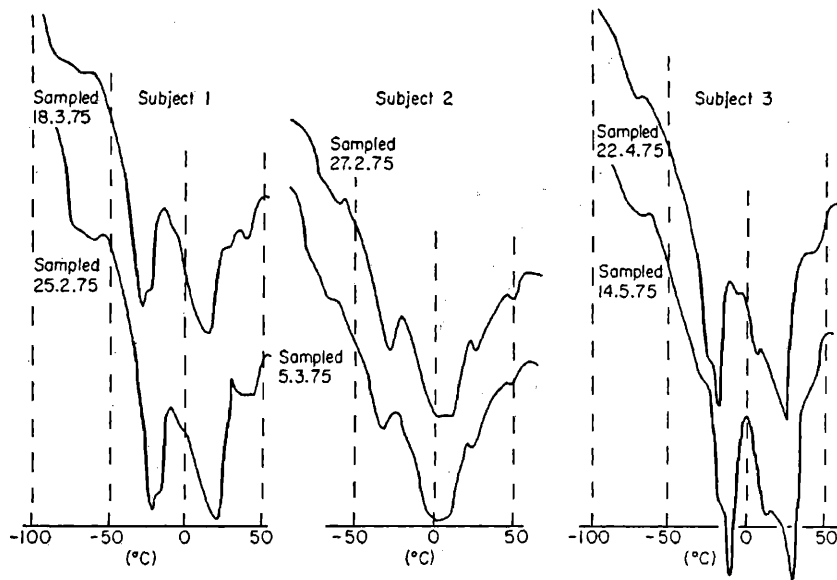


Figure 4. Characteristic melting curves from three different subjects.

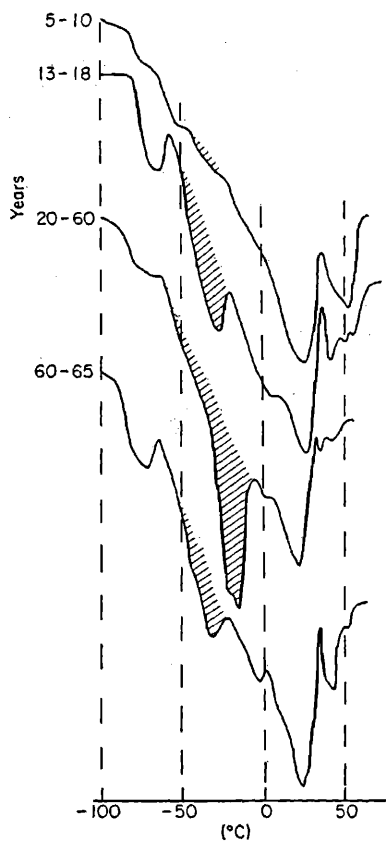


Figure 5. Examples of melting curves showing the evolution of sebum with age.

depended on the sex of the subject; the thermal analysis curves varied with the age of the subject as is shown by the plots reproduced in Fig. 5. Note that the area of the melting signal below -15°C , increases very drastically at puberty, increases less quickly from 20 years on, then decreases gradually with ageing.

This trend is emphasized by the plots of Fig. 5 which are individual cases that we chose in each age group.

A method was then sought to check whether this trend was confirmed within the total population.

The melting plots were characterized by eleven numbers which are the ordinates (normed in respect to their sum) of the previously defined points.

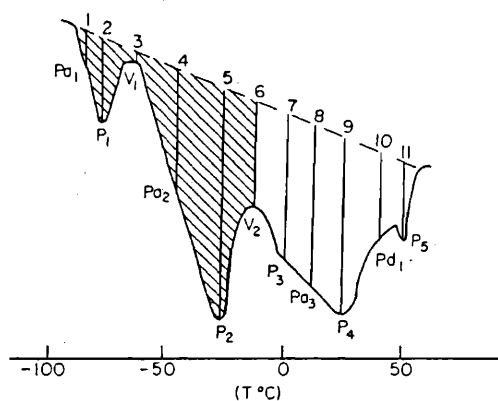


Figure 6. Typical melting curve of human sebum. Calculation of the parameter Σ_6 .

The area of the curve located below the temperature of -15°C is represented by the sum of the first six ordinates. This value will be afterwards symbolized by Σ_6 (Fig. 6)

$$\begin{aligned} \Sigma_6 = & \text{normed ordinate of } Pa_1 \\ & + \text{normed ordinate of } P_1 \\ & + \text{normed ordinate of } V_1 \\ & + \text{normed ordinate of } Pa_2 \\ & + \text{normed ordinate of } P_2 \\ & + \text{normed ordinate of } V_2. \end{aligned}$$

The value Σ_6 has not a definite physical meaning: recourse to this term is an arbitrary way of defining the melting curve. One consequence is that the larger the sebum fraction melted at -15°C , the higher Σ_6 .

The term Σ_6 was determined for the whole population. It varies between 26 and 57. Fig. 7 shows the average values of Σ_6 in the different age groups.

Within the whole population, the trend previously illustrated by individual cases is repeated: a sharp increase of Σ_6 at puberty with a tendency to decrease with increasing age.

Discussion

The population was submitted to a clinical examination. The clinician and the subject answered three questions concerning the observation: of the state of the hair; of the state of the scalp; of the frequency of shampooing required for good set of the hair-styling.

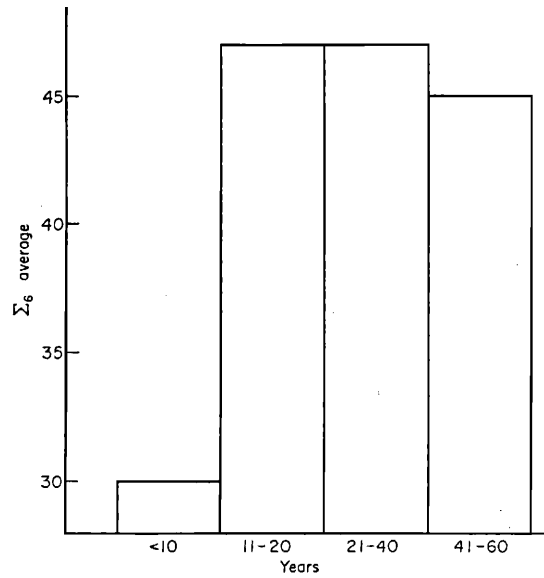


Figure 7. Average values of Σ_6 in the different age groups.

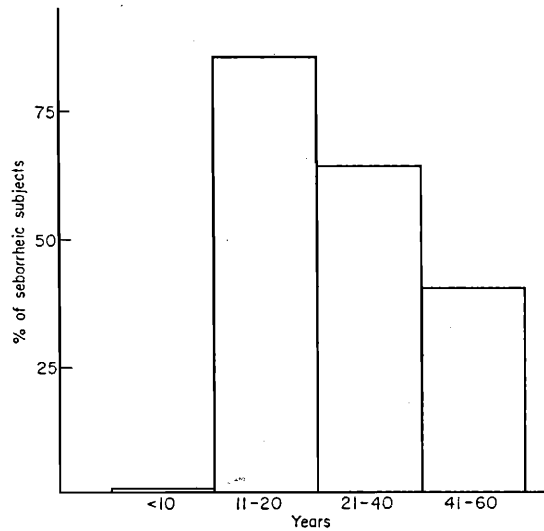


Figure 8. % of seborrheic subjects in the different age groups.

The answers distinguished a certain percentage of seborrheic individuals. The diagnosis of this clinical examination was formulated from a strictly cosmetic point of view. Fig. 8 shows how seborrheic persons were distributed in the different age groups.

It is clear that persons affected with seborrhea are encountered in all age groups, except children under 8 years. Between 13 and 18 years of age 90% of the investigated subjects were affected. The percentage of seborrheic persons then decreased with age and, if this evolution is compared to that of Σ_6 , as shown in Fig. 8, the similarity is evident.

The whole of the panel was classified according to increasing value of Σ_6 . From this classification, the histogram in Fig. 9 was drawn based on the conclusions of the clinical examination.

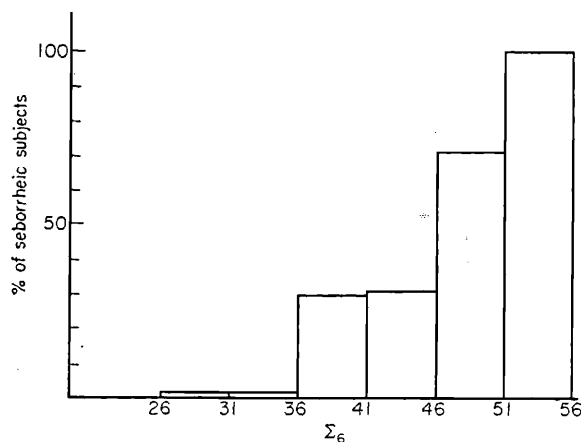


Figure 9. Distribution of seborrheic subjects according to values of Σ_6 .

It is to be noted that the percentage of seborrheic subjects increases with Σ_6 . For Σ_6 values included between 26 and 36 no cases of seborrhea were found.

As a corollary, above $\Sigma_6 = 51$ only seborrheic individuals were found.

These two groups represent 20% of our population. For these subjects the determination of Σ_6 gives a perfect correlation with the diagnosis.

Between these two limits, the probability of finding seborrheic subjects increases with the value of Σ_6 .

This fact shows that for $36 < \Sigma_6 < 51$ there is no absolute and perfect correlation between the conclusion of the clinical examination—which is subjective—and the parameter Σ_6 which is measurable.

This finding is not surprising; the clinical examination includes a complex reality where there is simultaneously: the quantity of sebum secreted per unit time (possibly corrected by a factor related to the mass and to the surface state of the hair), the quality of sebum which is related to its physical properties (spreading, viscosity, melting plot . . .). The method used only included the qualitative parameter.

There are other measurable parameters which define this notion of quality of sebum, especially viscosity. Other parameters directly bound to composition are: percentage of squalene; iodine number; the ratios of the quantities of palmitic and palmitoleic acids.

These parameters were determined for the whole population, to see whether they are in correlation with Σ_6 .

The correlation is positive with iodine number and percentage of squalene, negative with viscosity and the ratio

$$\frac{\text{quantity of palmitic acid}}{\text{quantity of palmitoleic acid}} \quad (\text{Fig. 10})$$

These correlations are readily explicable: a high Σ_6 shows that the melting signal is important in the low temperature range. In other words, at ambient temperature, the larger is the liquid fraction of sebum the higher is Σ_6 . Similarly high Σ_6 values must

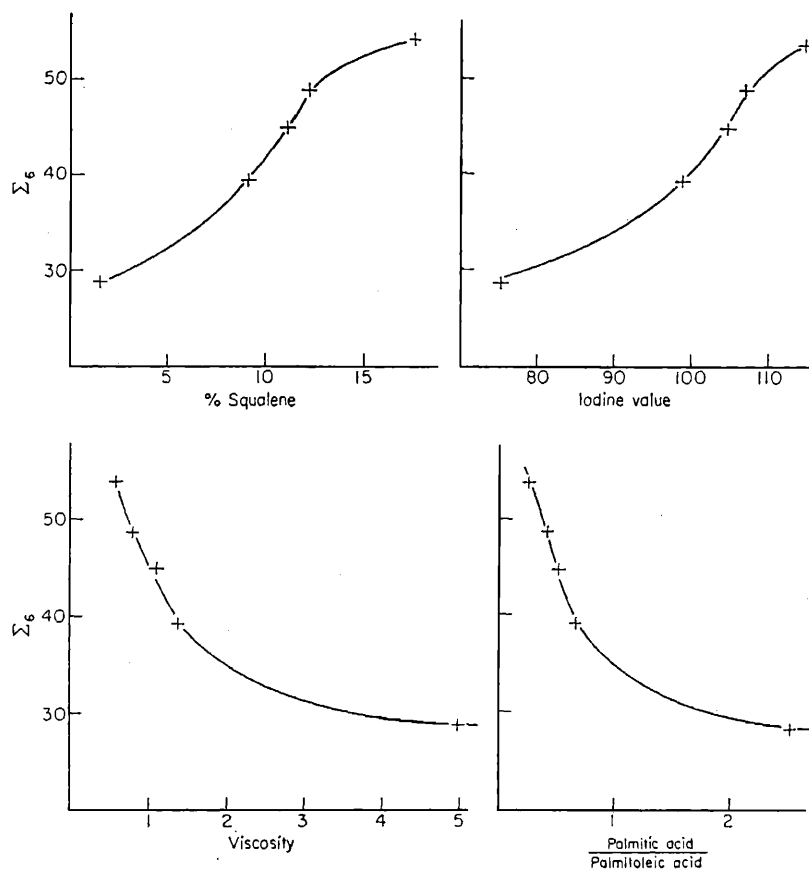


Figure 10. Correlations between Σ_6 and some physico-chemical parameters.

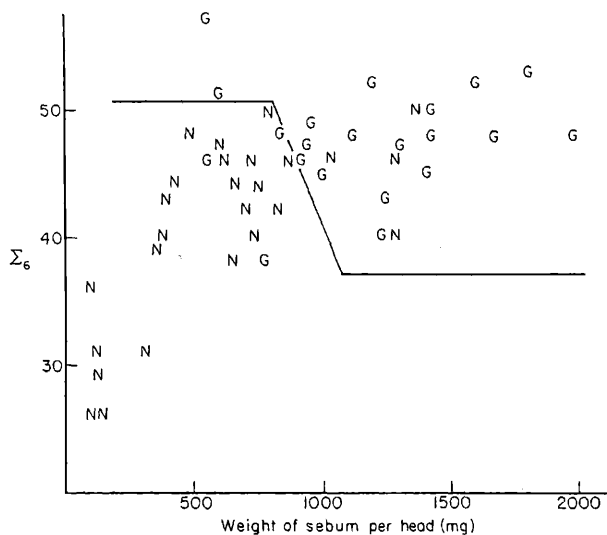


Figure 11. Relation between quantitative and qualitative parameters.

correspond with a low viscosity. Squalene, a polybranched polyunsaturated hydrocarbon is a low viscosity low melting oil, consequently, the richer the sebum is in squalene, the higher is Σ_6 .

The correlations of Σ_6 with the two other variables: iodine number; ratio palmitic acid/palmitoleic acid; illustrate in a similar manner the relation between the fluidity of sebum and the proportion of unsaturated compounds which compose it.

Independently of these qualitative criteria, a quantitative parameter was also determined, this being the mass of sebum extracted from the head. *Fig. 11* shows the relation between the quantitative and qualitative parameters; also indicating by G, seborrheic subjects; N, non-seborrheic subjects.

The results of the differential scanning calorimetry allow us partially to define the borders of this field: if Σ_6 is lower than 36 the quality of sebum is such that the subject is certainly not seborrheic; on the contrary if Σ_6 is higher than 51 the quality of sebum is such that the subject is certainly seborrheic. If Σ_6 lies between these limits, the clinical symptom arises from a joint contribution of the qualitative and quantitative parameters.

Conclusions

The method represents a new approach to the qualitative estimation of human sebum.

Considering the correlations which have been established the following physico-chemical parameters of sebum appear to define the limits of a seborrheic or non-seborrheic state:

Physico chemical parameter	Seborrheic state	Non-seborrheic state
Σ_6	> 51	< 36
% squalene	> 12	< 9
Iodine number	> 102	< 80
Palmitic Acid		
Palmitoleic Acid	< 0.70	> 1
Viscosity 25°	< 0.7*	> 1.5†

* Newtonian

† Plastic or pseudo plastic

In practice these physico-chemical measurements are not all easily accessible. Thus the determination of the percentage of squalene requires the use of a gas-liquid chromatographic procedure. The determination of C_{16} acids requires a preliminary saponification then precise chromatography on a capillary column. The viscosity, when determined by a cone and plate process as used in this work, requires many samples. The applied constraint and the temperature at which the measurement is carried out, are chosen in an arbitrary manner before the measurement and their choice has an effect upon the significance of the observed values.

On the other hand, the iodine number is a parameter easy to determine with good precision but the results lie in a narrow range of values which are difficult to interpret.

The new method supplies more information since by following the thermodynamics of fusion it is possible to measure objectively slow or small changes in sebum, for example in the course of a corrective treatment. This method is easy and can be applied to small amounts of sample (30 mg). One could also consider its application for the study of acne.

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