Thermochimica Acta, 31 (1979) 1–11

AN APPARATUS FOR THERMOANALYTICAL MICROSCOPY AND ITS APPLICATIONS*

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(Received 3 February 1978)

ABSTRACT

A high-sensitivity apparatus for thermoanalytical microscopy under reflected light has been designed and has been applied to studies of (1) the mechanism of the polymorphic transition of stearic acid, (2) the fine structure of the smectic–cholesteric transition of cholesteryl nonanoate and (3) the process of the multiplication of yeast bacteria.

INTRODUCTION

Thermoanalytical microscopy (TAM) means the simultaneous measurement by microscopy and thermal analysis. TAM apparatuses and their applications were developed mainly by the author and her co-workers[1–5], while a similar paper was presented at the 2nd International Conference of Thermal Analysis[6]. This paper describes a high-sensitivity TAM apparatus for observations under reflected light.

APPARATUS

Construction

Figure 1 shows the schematic diagram of the apparatus in which TAM expresses the hot stage and P, A, and L denote the polarizer, analyser and light source, respectively. The digital photo-printer records the temperature given by the digital thermometer on the microphotograph. Figure 2 shows the construction of the hot stage. It is composed of a glass jacket, a copper block, and two aluminum capsules of the same type, in which a thin (60 μm) plate of mica is hung. A Bi–Sb thermopile as the heat sensor is printed radially on the mica plate and the outside junctions are attached to the capsule edge. The sample holder is placed at the center, metal-printed, surrounded by the inside junctions of the thermopile. An electrical resistor is printed on the center of the opposite side of the mica plate, for calorimetry.

Thermal analysis is carried out by detecting the differential voltage between both thermopiles for the sample and reference material. The heating and cooling

* Presented at the 5th International Conference on Thermal Analysis, Kyoto, Japan, August, 1977.

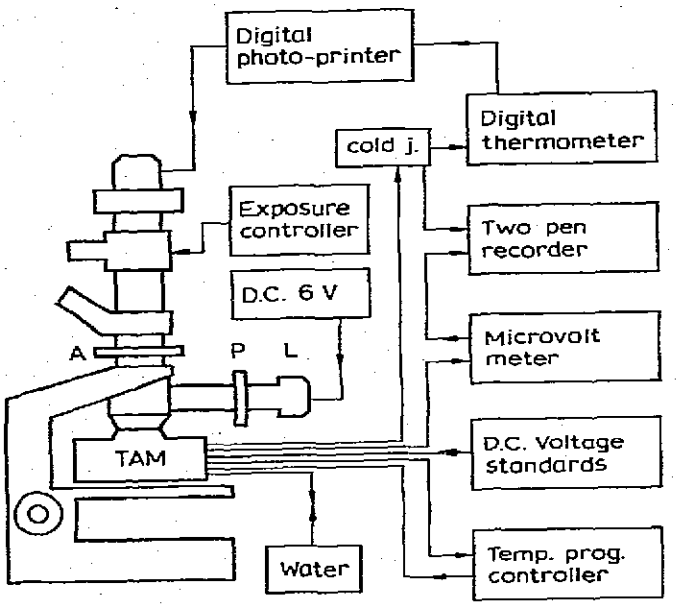


Fig. 1. Schematic diagram of the apparatus. TAM, hot stage for TAM; P, polarizer; A, analyser, L, lamp.

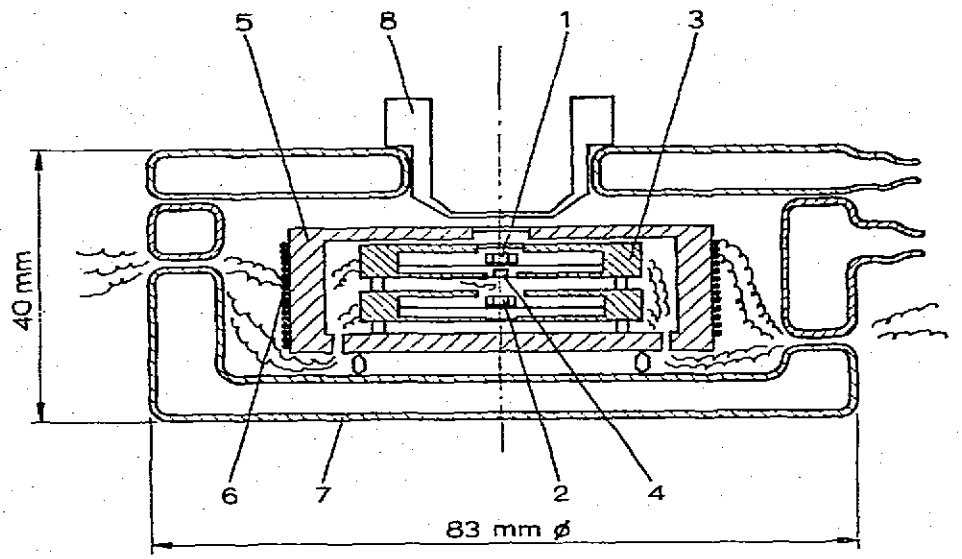


Fig. 2. The construction of the hot stage. 1 and 2, sample holders; 3, thermopile capsule; 4, thermocouple for temperature measurement; 5, copper block; 6, monitoring thermocouple; 7, glass jacket; 8, Teflon adapter.

rates are controlled by a program controller connected to the heater of the copper block. The sample temperature is detected by a Cu–constantan thermocouple located just under the center of the thermopile plate on the sample side.

The sample holder (Fig. 3) is circular and made of copper (0.3 mm thick). It has a small mirror in it for microscopy under reflected light. The cover glass is 0.15 ± 0.02 mm thick.

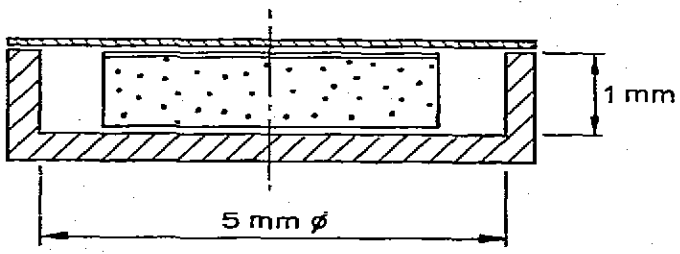


Fig. 3. The sample holder. Hatched area, copper vessel (0.3 mm thick); dotted area, aluminum printed glass disk.

Calorimetric basis

It can be assumed that the heat transfer in the hot stage follows Newton's cooling law. If a set of cylindrical coordinates is applied to the system, the temperature distribution may be symmetrical with respect to the z axis and is expressed by the Bessel function.

For simplicity, the calorimetry is based upon the following assumptions: (1) The temperature of the outside junctions of the thermopile is equal to that of the capsule, which varies linearly when the temperature of the copper block is controlled at a constant rate. (2) The difference between the characteristic thermal constants of the two thermopiles can be neglected in comparison with the difference between the respective thermal conditions, such as the light effects, the heat diffusibilities in the direction of the z axis, etc. (3) The condition of heat transfer of Joule's heat for calibration is similar to that of the heat generated by the sample, since the mica plate is very thin.

Then the heat transfers within the sample and reference capsules may be described by the following equations.

$$C_1 \frac{dT_1}{dt} = k(T_1^0 - T_1) + \frac{dQ}{dt} + q \tag{1}$$

$$C_2 \frac{dT_2}{dt} = k(T_2^0 - T_2) \tag{2}$$

where C, k, T, and t represent the heat capacity, effective heat transfer coefficient, absolute temperature, and time, respectively. Subscripts 1 and 2 denote the sample and reference parts, respectively. In addition, superscript ^{0} denotes the outside junction of the thermopile. dQ/dt expresses the rate of heat generation in the sample and q denotes the heat effect per unit time from the light source for microscopy. From eqns. (1) and (2), eqn. (3) can be obtained.

$$C_1 \frac{d\theta}{dt} + (C_1 - C_2) \frac{dT_2}{dt} = k(\alpha_1 - \alpha_2)t - k\theta + \frac{dQ}{dt} + q \tag{3}$$

where $\theta = T_1 - T_2$, and α_1, α_2 are the coefficients based on assumption (1).

In the stationary state, $dQ/dt = 0$ and

$$T_1^0 - T_1 = \frac{1}{2\pi kl} \log\left(\frac{r_i}{r_0}\right) = T_2^0 - T_2 \tag{4}$$

where r_i and r_o denote the radii of the inside and outside junctions of thermopile and is the thickness of the thermopile. From eqn. (4)

$$T_1 - T_2 = T_1^0 - T_2^0 = (\alpha_1 - \alpha_2)t \tag{5}$$

If θ^* is θ in the stationary state, $d\theta^*/dt = \alpha_1 - \alpha_2$ from eqn. (5). Then, in the stationary state, eqn. (3) can be written as

$$k\theta^* = (\alpha_1 - \alpha_2)(kt - C_1) - (C_1 - C_2)\frac{dT_2}{dt} + q \tag{6}$$

From eqns. (3) and (6)

$$C_1 \frac{d\theta}{dt} = C_1(\alpha_1 - \alpha_2) - k(\theta - \theta^*) + \frac{dQ}{dt} \tag{7}$$

If the heat generation occurs between t_i and t_f

$$Q = \int_{t_i}^{t_f} \frac{dQ}{dt}\,dt = k\int_{t_i}^{t_f} (\theta - \theta^*)dt + C_1[\theta]_{\theta_i}^{\theta_f} - C_1(\alpha_1 - \alpha_2)[t]_{t_i}^{t_f} \tag{8}$$

The first term of the right-hand side of eqn. (8) gives the value of Q, since the second and final terms are practically equal to each other, as described in the next paragraph. Q can be obtained graphically as θ^* corresponds to the base line of the thermogram.

The initial and the end points of the thermogram do not, in principle, coincide with t_i and t_f, respectively. In the case of the apparatus with a short time constant, however, they approximate to t_i and t_f*. The time constant of this apparatus is 14.4 sec with common organic substances. This corresponds to 0.6 K, if the temperature scanning rate is 2.5 K/min.

The characteristic coefficient of the apparatus, k, was obtained empirically with a blank sample holder. Namely

$k_T = k_{273}[1 + 0.0012(T - 273)]$
$k_{273} = 3.01\ \mu J/\mu V$ sec

room temperature $= T = 400$ K
For the calculation, the least square method was used.

The energy effect of the light for microscopy, q, is included in θ^*. In the optimum condition, only q/k may be left as the base line. The light energy effect, however,

* When $t = t_i$ or t_f, $dQ/dt = 0$. In that case, the integration of eqn. (7) gives
$\theta - \theta^* = (\alpha_1 - \alpha_2)[1 - \exp(-t/\tau)]$
where τ, the time constant, is equal to C_1/k. Therefore, if τ is very short compared with the duration of heat generation, $\theta_i = \theta^*(t_i)$ and $\theta_f = \theta^*(t_f)$.

TABLE 1

THE TEMPERATURES AND HEATS OF TRANSITIONS OF STEARIC ACID

	Transition B to C		*Fusion*	
	T(K)	*Q(kJ mole^{-1})*	*T(K)*	*Q(kJ mole^{-1})*
This work	327.2	4.3	342.9	61.3
Ref. 4b	326.7*	4.5*	342.9	63.1
Ref. 7	327		343.9	
Ref. 8			342.6	63.0

* Unpublished values.

TABLE 2

THE TEMPERATURES AND HEATS OF TRANSITION OF CHOLESTERYL MYRISTATE

	Cryst–Smec		*Smec–Chol*		*Chol–Iso*	
	T(K)	*Q(kJ mole^{-1})*	*T(K)*	*Q(kJ mole^{-1})*	*T(K)*	*Q(kJ mole^{-1})*
This work	343.8	45.2	351.6	1.7	356.7	1.1
Ref. 4a	343.8	47.2	351.6	1.4	356.7	0.9
Ref. 9	343.7	46.5	351.0	1.4	356.4	1.0

can in practice be eliminated by supplying the corresponding electrical energy to the resistor on the reference side. The base line deviation can also be adjusted by controlling the power to the resistor of either side. From this standpoint, the apparatus can be easily modified into the DSC type.

Calorimetric results

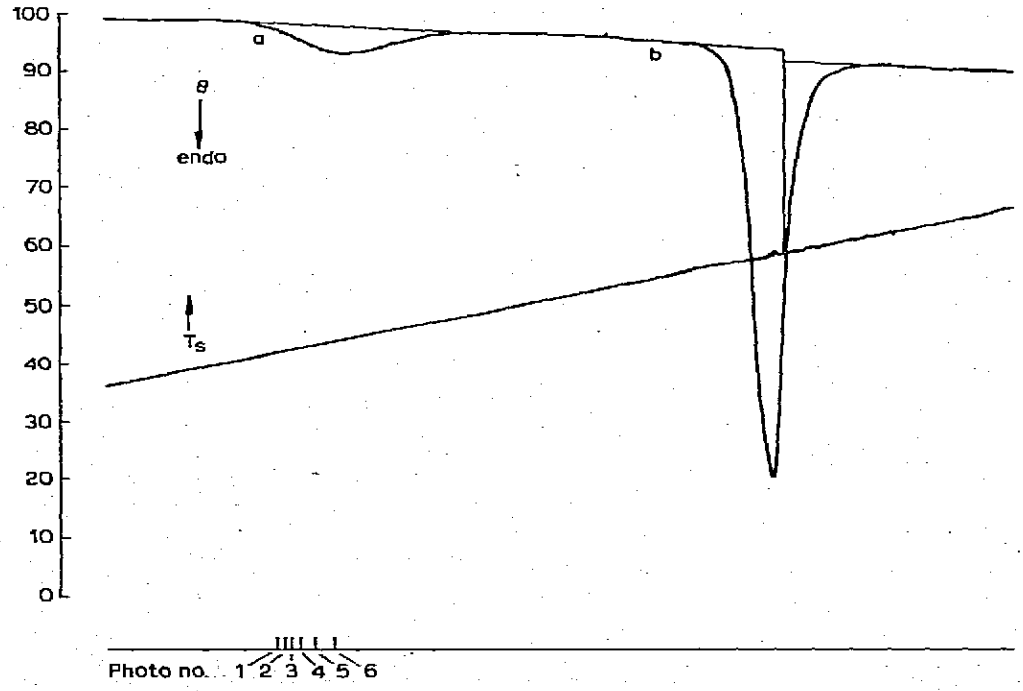
The heat of fusion of stearic acid was measured to test the performance of the apparatus by comparing the result with that obtained by the previous apparatus[5]. Table 1 shows the transitional data of stearic acid. The purity of the sample, determined by a gas chromatograph, was over 99.9%. The value of the heat of fusion was 2.7% less than the previous value. It may not be very important as the values in the published references are very scattered[5]. The standard deviation obtained from eight measurements of the heat was 1.5%.

Table 2 shows the transition data of cholesteryl myristate compared with the previous data and with the published data.

APPLICATIONS

Polymorphic transition of stearic acid

The polymorphic transitions of normal alkanoic acids are very interesting.



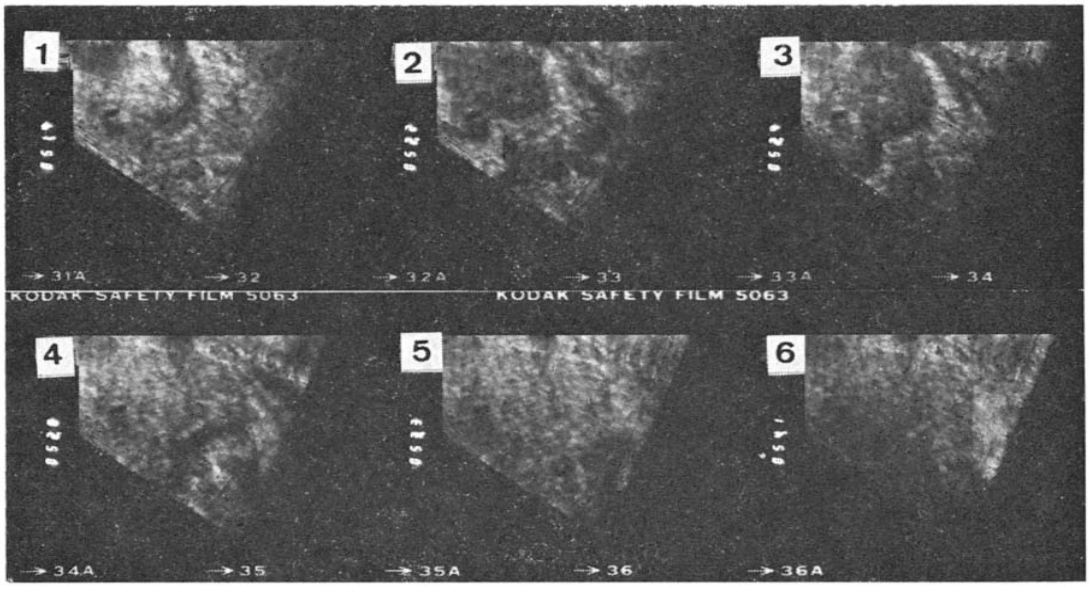


Fig. 4. Thermoanalytical microscopy for stearic acid. Sample weight, 1.11 mg; heating rate, 2.5 K/min; chart speed, 20 mm/min; full scale in θ, a, 1000 μV; b, 2500 μV. Microscopy, × 100, crossed polars, ASA 400, exposure 1/2 sec.

The transitions are irreversible for even-numbered carbon acids, but are reversible for odd-numbered carbon acids[7]. Although the data of the latter are well tabulated[11], those of the former are not.

Figure 4 shows the TAM of a single crystal of stearic acid. The photographs show the situation of the transition from the B-form to the C-form observed on the

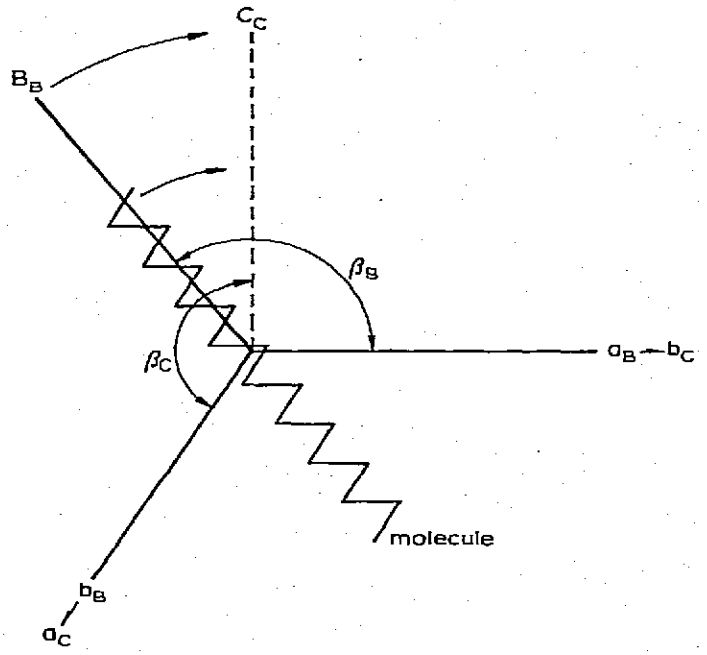


Fig. 5. Mechanism of the transformation from B-form to C-form in stearic acid. a, b, and c, Crystal axes in B- or C-form.

(001) plane. Namely, the change of optical retardation coincides with the appearance of the transition peak and shifts continuously to a definite direction on the crystal. The retardation change disappears up to the top of the transition peak. Any other outstanding changes, such as deformation or volume expansion, are not observed. The transition covers a very wide temperature range and the peak height is low. The entropy change estimated from Table 1 is 13.1 J/mole K. It corresponds to 7% of that of the fusion and makes a good contrast with those of odd-numbered carbon acids[11]. For example, the entropy change of the transition of heptadecanoic acid, the nearest neighbour of stearic acid, corresponds to 13% of that of fusion. The irreversibility may be due to the small free energy change which retains the stable form at high temperatures.

X-Ray diffraction analysis[9] suggests that the a- and b-axes in the C-form take the place of the b- and a-axes in the B-form, respectively, at the transition (Fig. 5). These results suggest as the mechanism of the transition that thermal agitation breaks the lattice of the B-form and modifies the mode of hydrogen bonding of the carboxyl groups. The modification results in a change of direction of the molecular axis which approximately coincides with the c-axis in both forms.

Mesophase transition of cholesteryl nonanoate

The smectic–cholesteric transition of cholesteryl nonanoate is monotropic. But on reheating the sample cooled down to the smectic phase, the transition to the cholesteric can also be observed. In this case, the plane texture of the cholesteric phase, which rarely occurs spontaneously, certainly appears, though the texture

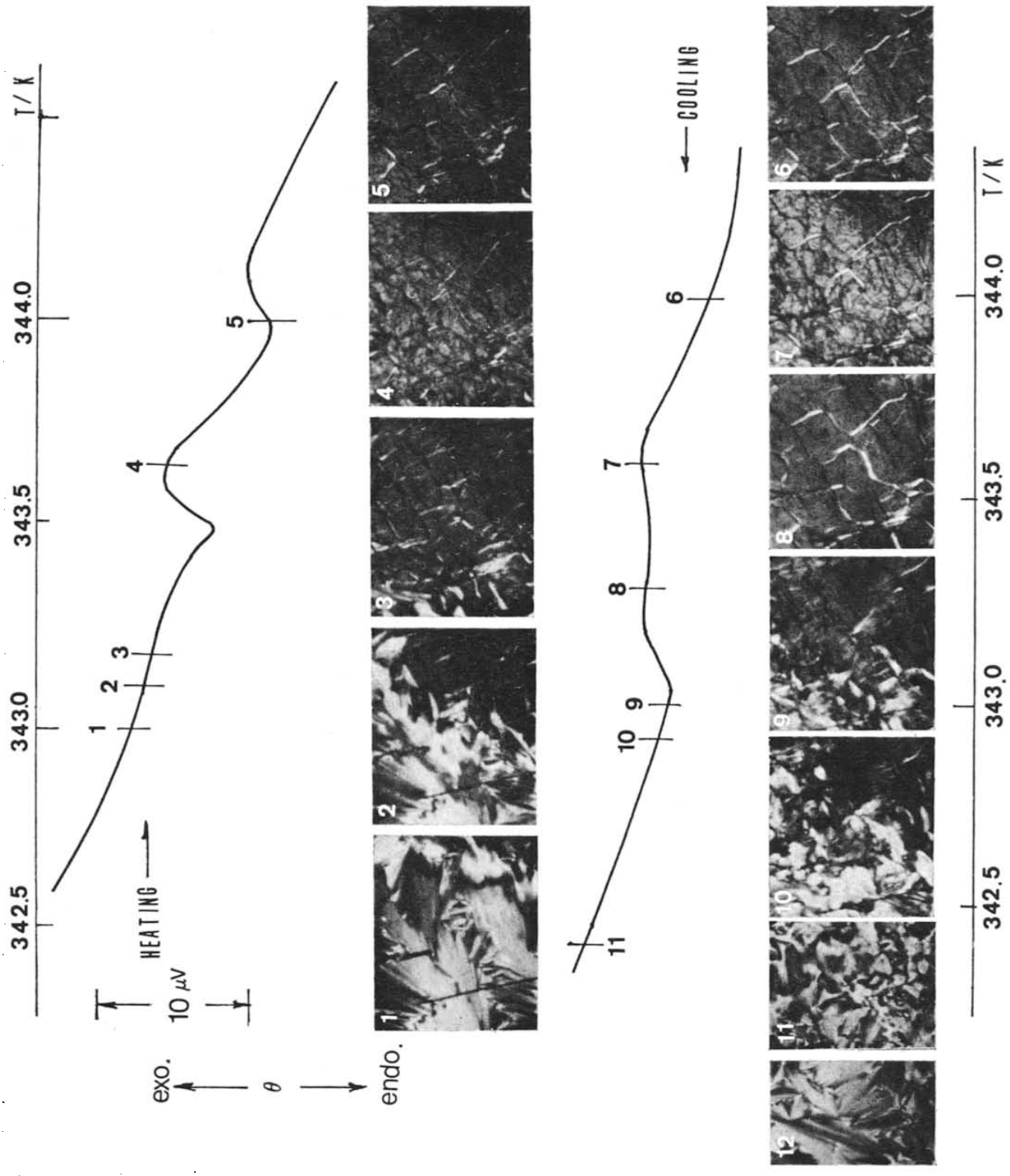
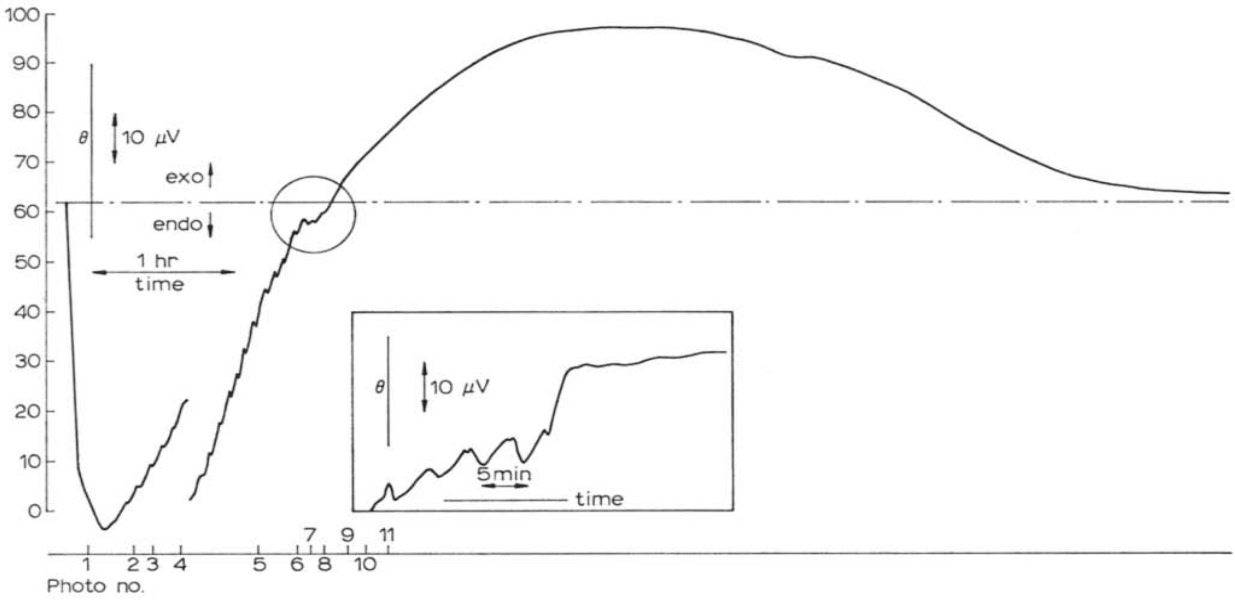


Fig. 6. Thermoanalytical microscopy at the smectic–cholesteric transition of cholesteryl nonanoate. Sample weight, 2.87 mg; heating or cooling rate, 0.3 K/min; chart speed, 20 mm/min. Microscopy, × 100, crossed polars, ASA 100, exposure 1 sec. Photographs: 1,12 smectic; 3,8 cholesteric dark homeotropic; 4,7 cholesteric red homeotropic; 5,6 cholesteric green homeotropic.



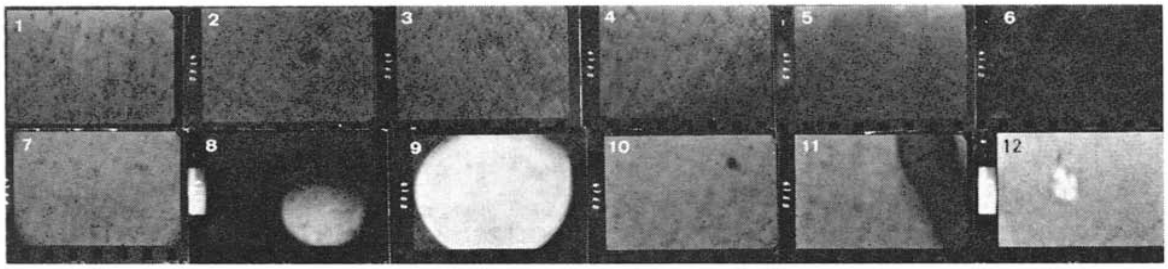


Fig. 7. Thermoanalytical microscopy of yeast multiplication. Sample, dry yeast 1.19 mg; 10% aq. sucrose solution (16.73 mg); temperature, 31.4 ± 0.1°C. Microscopy, × 200, crossed polars, ASA 400, exposure 1/2 sec.

includes the so-called oily streaks. The characteristic colored light scattering starts with red and ends with violet, passing through green and blue on heating. The order of appearance of the colours is reversed on cooling.

The thermogram, on slow heating, shows two peaks at the transition as shown in Fig. 6. The separation might be related to the two processes, namely, one is disentanglement of the smectic structure and another is twisting to the cholesteric state. The inference confirms the appearance of the dark homeotropic texture (photographs 3 or 8) between the smectic texture (photographs 1 or 12) and the red plane texture (photographs 4 or 7) in both heating and cooling series.

The peak area of the transition on the first run corresponds to 0.49 KJ/mole which is 14% larger than the previous value[4]. The peak area decreases 10% with every successive run.

Multiplication of yeast bacteria

Figure 7 shows a TAM of the multiplication of yeast bacteria. No pretreatment was carried out. The sample was made by just putting a granule of dry yeast into a

10% sucrose solution. The temperature was held at around 303 K. The constancy was ± 0.1 K.

The thermogram shows first an endothermic peak and then a broad exothermic peak which takes several hours. Small periodic peaks superposed on the endothermic peak coincide with the movements of the bacteria, i.e. the bacteria do not move for several minutes and suddenly move cooperatively with the appearance of a peak.

With the passage of time, individual bacteria are not going to appear (photographs 1–3). Instead, a homogeneous medium is formed (photographs 7 or 10) and then the fermentation begins. After fermentation becomes constant, the thermogram settles to a smooth exothermic curve. Photographs 8 and 9 show a bubble of CO_2 being generated. The dark part of photograph 11 shows the trace of the removal of a bubble, which suggests that the medium is more solid than liquid. At the end of the exothermic peak, some spherules with isogyres are observed in some places (photograph 12). The spherules may be the small holes made by CO_2 gas enclosed in the cake-like medium. The crossgyres suggest that the medium is optically anisotropic. Since the form of the hole is ellipsoidal, the medium is not only optically anisotropic but also mechanically anisotropic.

If the exothermic peak corresponds to the heat of decomposition of sucrose by yeast into ethanol and carbon dioxide, the heat corresponds to 2000 KJ/mole of sucrose. On the other hand, the calculation of the heat of formation by Hess's law gives approximately 200 KJ/mole. The former is ten times the latter.

Without the light for microscopy, the zigzag thermogram was not observed. In this case, the peak area was 60% of that in the case with the light. The result suggests that there might be a special effect caused by the radiation from the light.

The heat generated in the fermentation process depends on the anaerobic or aerobic conditions of culture. The quantitative analysis of the thermogram requires the establishment of rigid experimental conditions. It has been proved, however, that a quantity of heat generated over a long period, such as in a biological reaction, can be applied to the apparatus.

CONCLUSION

Using two thermopiles vertically, a high-sensitivity apparatus for thermoanalytical microscopy has been constructed. The sample quantity required is one tenth of that of the previous apparatus[4]. A few examples of the application proved the excellent performance but the applicable temperature range is limited below 450 K and the resolving power is limited to that of the objective, below 20 ×, though the magnification can be raised by selecting a suitable eyepiece.

ACKNOWLEDGEMENTS

The author expresses her sincere gratitude to Mr. Y. Takeda of Nikkohm K. K. for his efforts in the design of the thermopile capsule. She is also indebted to Dr.

M. Gotoh for the offer of her highly purified stearic acid and to Dr. A. Katoh for his determination of the purity of the stearic acid.

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