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THERMAL ANALYSIS OF POLYMORPHISM*

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The combined techniques of thermomicroscopy, differential scanning calorimetry (DSC), thermomicrophotometry (TMP), and micro-Fourier transform infrared spectroscopy (FTIR) are essential for the unequivocal detection of polymorphism. The polymorphism of *p*-hexadecylaminobenzoic acid (HABA), a pharmaceutical intermediate, is presented to illustrate the need to use a multitechnique approach.

While DSC can record thermally induced processes, it is not a specific technique and it alone cannot distinguish polymorphic transformations from other events connected with enthalpy changes. Thermomicroscopy usually provides confirmation of polymorphism by direct observation of polymorphic forms and transformations, but not all transformations result in visual changes in structure. Micro-FTIR, combined with thermomicroscopy, confirms the chemistry of these thermal changes and provides more specific information on changes in molecular structure.

Introduction

Polymorphism is the capacity of a substance to occur in two or more solid forms. Polymorphism is of particular importance to pharmaceuticals because it can affect a drug's activity, stability, solubility, and production. Although polymorphs are chemically identical, they can be identified and characterized because polymorphic forms of the same substance have different physical properties. X-ray diffraction patterns, densities, melting points, enthalpies of phase transitions, optical properties, dissolution rates, and infrared spectra are usually different for different polymorphs.

Polymorphism was first reported in 1821, when Mitscherlich [1] found sodium phosphate to have two different crystalline forms. In 1832, the polymorphs of benzamide were discovered by Woehler and Liebig [2], the first example of a polymorphic organic compound. In 1942, Deffet published physical data for the polymorphs of more than 1200 organic compounds [3].

The importance of polymorphism to pharmaceuticals has been known for the last forty years. Kofler and Kuhnert–Brandstätter [4] found that many phar-

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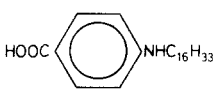
maceutical substances were polymorphic. Often these substances exhibit more than two polymorphic forms: Phenobarbitol has a total of at least eleven forms [5], while several other barbiturates have at least four or five. Of the barbiturates in common use today, about 70% have been reported to be polymorphic. The common steroids are all polymorphic [6, 7]. Of the commercial sulfonamides, 65% have been shown to be polymorphic [8]. Physical properties of polymorphs can be quite different: for example, commercial sulfathiazole in Form II melts at 175°, while Form I melts at 202 °C. On heating Form II, some crystals transform to Form I, but most do not and melting begins at 185 °C. The Form I crystals do not melt until the sample is heated to 202 °C. Without understanding the polymorphic behavior of sulfathiazole, its melting might be interpreted as an impure mixture with a broad melting range.

Polymorphic behavior can be established by a combination of thermoanalytical methods: Enthalpy changes which accompany transformations of polymorphic forms are recorded by DSC. However, observation of enthalpy changes is only an indication of polymorphism; the existence of polymorphism cannot be firmly established by this non-specific technique alone. On the other hand, thermomicroscopy and thermomicrophotometry (TMP) can detect morphological and phase changes that accompany the enthalpy changes observed by DSC. Thermomicroscopy is the most useful technique to confirm the nature of a thermal transformation, but it is not always possible to detect polymorphic changes by direct observation. TMP is a thermoanalytical technique that records changes in light intensity as a function of temperature. TMP complements thermomicroscopy by providing greater sensitivity for detection of subtle phase changes. The combination of thermomicroscopy and micro-FTIR provides the necessary confirmation that chemical composition was retained, thereby establishing the change as a truly polymorphic transformation. Therefore, DSC, TMP, micro-FTIR, and thermomicroscopy are a powerful combination of techniques to establish the existence of polymorphs and to characterize their transformations.

As an example of the application of these thermoanalytical techniques, we have selected *p*-hexadecylaminobenzoic acid (HABA).

HexadecylAminoBenzoic Acid - HABA



HABA, a pharmaceutical intermediate, has three solid state forms and at least one liquid crystalline state, in addition to an amorphous liquid state. The various transformations of HABA can be used to illustrate the necessity for using a combination of techniques in studying polymorphism.

Experimental Details

Materials

All materials were research samples from American Cyanamid Company.

Procedures

Thermomicroscopy system: a polarized light microscope, Olympus BHA Pol or equivalent, fitted with a model FP–5 Mettler Instrument Corporation heating stage. The temperature scale of the instrument was calibrated using melting point standards according to the manufacturer's specifications.

Tandem FTIR/thermomicroscopy instrumentation: a Digilab FTS–15E with microsampling accessory. This accessory was modified to accept the Mettler FP–5 micro heating stage. All glass windows of the FP–5 and the cooling fan were removed. Temperature calibration with melting point standards was necessary.

Thermomicrophotometry equipment: a photodiode, HAV 1000 by EG&G, and a dual channel X–Y recorder, in addition to the thermomicroscopy equipment listed above. This instrumentation has been previously described in detail [9, 10].

DSC equipment: Model TA–3300 DSC by Mettler Instrument Company. The temperature and heat flow scales were calibrated according to the manufacturer's recommended procedures. Samples were hermetically sealed in a dry nitrogen atmosphere. A dry nitrogen atmosphere purged the system during heating and cooling.

Results and Discussion

1. Differential Scanning Calorimetry

The DSC thermal curves of HABA are shown in Figure 1. Three major endothermic transitions are found, when precipitated HABA is first heated. When the melt is cooled and then reheated, DSC again records three endothermic transitions, but these occur at temperatures different from the thermal events observed during the first cycle. Although the transitions overlap, the individual enthalpies of transition can be estimated by peak integration using standard techniques. Estimates were obtained for the precipitated HABA (first heat cycle) and for melt crystallized material. Data for the melt crystallized material are grouped into two sets: data obtained immediately after the first heat cycle, i.e. (second heat cycle), and data obtained 24 hours later (third heat cycle). These enthalpy estimates, along with the extrapolated onset and peak temperatures, are tabulated below.

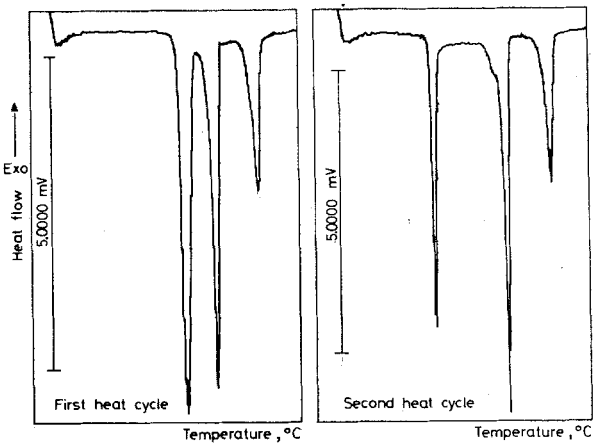


Fig. 1 DSC analysis of precipitated HABA (first heat cycle) and melt recrystallized HABA (second heat cycle). Heating rate: 10 deg/min

Table 1 DSC: Enthalpy values

Heat cycle	Transformation forms*	Enthalpy, J/g	Transformation temps	
			onset temp.	peak temp.
1	I →III	79	90	92
	III →LC	48	105	107
	LC →Melt	34	123	126
2	II →III	38	71	73
	III →LC	54	104	106
	LC →Melt	33	124	126
3	II →III	40	71	73
	III →LC	55	104	106
	LC →Melt	32	124	126

* I, II, III = solid state forms; LC = liquid crystal

The enthalpy for conversion of the room temperature thermodynamically stable form to the solid phase stable above 90° (I →III), i.e. 78 J/g, is larger than the enthalpies of the two other transformations observed in heat cycle 1. For the solid-solid conversion of the metastable Form II to Form III (II →III), the enthalpy change should be smaller than the enthalpy for the I →III transition, since Form II is thermodynamically less stable than I. The experimental result of 39 J/g for the II →III transition substantiates this expectation.

Different transition enthalpies are observed for the three heat cycles for the III →LC transformation. As seen in Figure 1, the III →LC transition appears to be

a single process. However, for subsequent heat cycles, i.e. 2 and 3, the transition involves at least two transformations, as evidenced by a shoulder on the main endotherm. This could be a small amount of residual II →LC or an intermediate (IV) crystal phase between III and LC, but no visual indications of another phase were observed by polarized light microscopy. Enthalpies for the major transitions are included in Table 1. On heat cycle 1, the enthalpy of the III →LC transition, i.e. 48 J/g, is smaller than the values for the comparable transition on heat cycles 2 and 3; the endotherm for this transition overlaps the previous endotherm and cannot be completely resolved. For each heat cycle, the LC →Melt transition has an enthalpy of 33 J/g. This low enthalpy is to be expected, since the LC →Melt transition requires only the breaking of weak crystalline forces.

The enthalpies for these transformations are represented schematically in an energy level diagram—Figure 2. Note that the enthalpy values were obtained only during heating, which was followed by melt crystallization. The double arrows indicate that the transition is observed both on heating and cooling. As will be shown later, the transition from the room temperature metastable form to the room temperature thermodynamically stable form (II →I) can only be effected by crystallization from solution. Therefore, the enthalpy for this transition was estimated from the energy level schematic diagram.

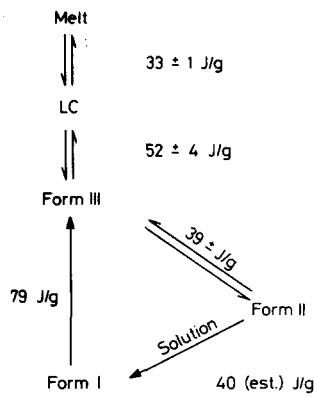


Fig. 2 Energy level schematic diagram for HABA transitions

2. *Thermomicroscopy*

Thermomicroscopy shows that the enthalpic change in the 104–107 °C range is a crystalline solid to liquid crystal transformation (III →LC) and that the final melting, i.e. the disappearance of the anisotropic phase, is the liquid crystal to isotropic liquid phase change. However, the transformation of I →III on first

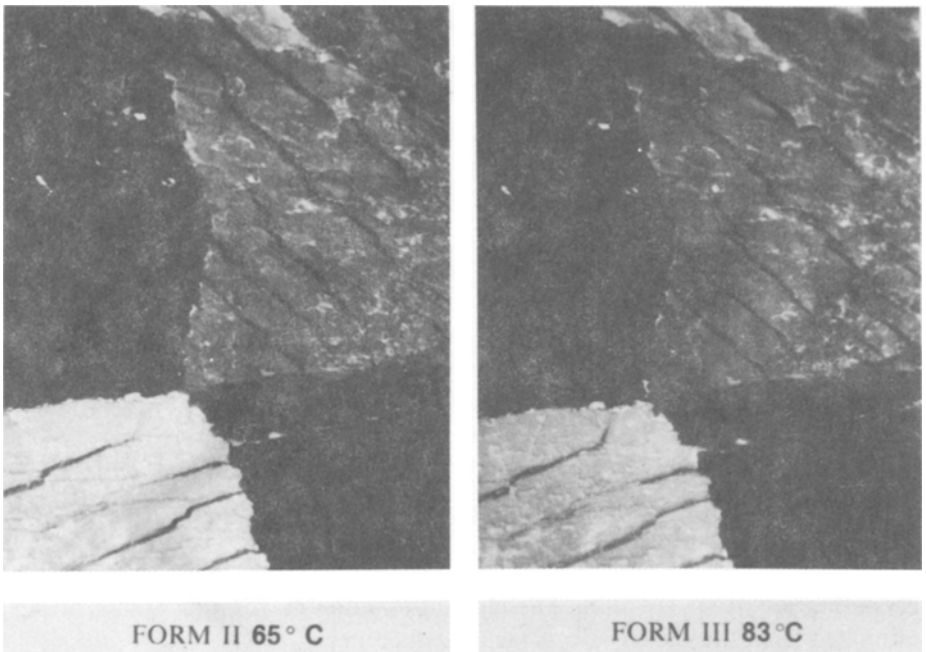


Fig. 3 Polarized light micrograph at 100× showing the morphology of HABA before and after the solid-solid phase transformation II →III at 74 °C. There is no apparent change in morphology

heating and the transformation of II →III on the melt recrystallized material were not observed in initial thermomicroscopy experiments.

Thermomicroscopy alone cannot easily detect the II →III transformation found by DSC. In DSC experiments, this transformation occurs reproducibly when melt recrystallized HABA is reheated. Figure 3 shows the morphology of the recrystallized HABA before and after this transformation; no differences in crystal morphology are seen by conventional light microscopy. Only by careful polarized light microscopical examination can this change be observed. During this solid-solid transformation (II →III), only the birefringence of the HABA crystals, observed as a change in retardation color, changes, while there is no change in the crystal shape or texture. Therefore, it is unlikely that this transformation would have been detected by thermomicroscopy without the information gained by DSC experiments.

3. Thermomicrophotometry

By measuring the intensity of transmitted polarized light as a function of temperature, the II →III phase change is detected. These thermomicrophotometry

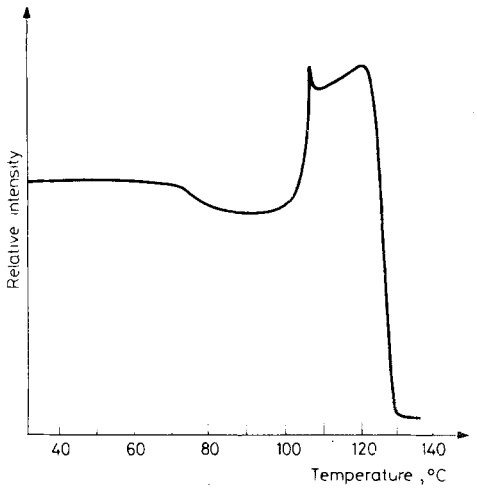


Fig. 4 TMP analysis of melt recrystallized HABA. Heating rate: 10 deg/min. Decrease in polarized light transmission in the 74 °C region confirms the polymorphic transformation

(TMP) data are shown in Figure 4. The decrease in the transmitted light intensity in the 74 °C temperature range corresponds to the II→III transformation. Detecting this change solely by visual observation is difficult.

4. FTIR/Thermomicroscopy

The FTIR spectra of single crystals of Forms I and II, which are the room temperature thermodynamically stable phase and the room temperature metastable phase respectively, can be obtained by conventional infrared techniques. However, the other HABA phases only exist at elevated temperatures. To obtain spectra of the high temperature phases, a thermal stage was adapted to the FTIR microspectrophotometer. The resulting IR spectra are shown in Figures 5, 6, 7, 8 and 9. Major IR absorption bands of all the HABA phases are similar, confirming that HABA undergoes polymorphic transformations, not chemical changes.

While the IR spectra can be interpreted in terms of HABA's chemical structure using group frequency correlations [11], minor spectral differences exist that distinguish each phase. The absorption maxima are listed in Table 2. In Form I, the N—H band at 3400 cm^{-1} is 20 cm^{-1} lower than all other phases; the carboxylic acid carbonyl band is split into a 1660 and 1682 cm^{-1} doublet. The energies of absorption and relative intensities in the 1100 to 1550 cm^{-1} region, shown in Figure 10, are particularly useful for phase identification. For example, the band at 1175 cm^{-1} is found in all phases, but the intensity of this band relative to the band at 1275–1290 cm^{-1} is different for each phase.

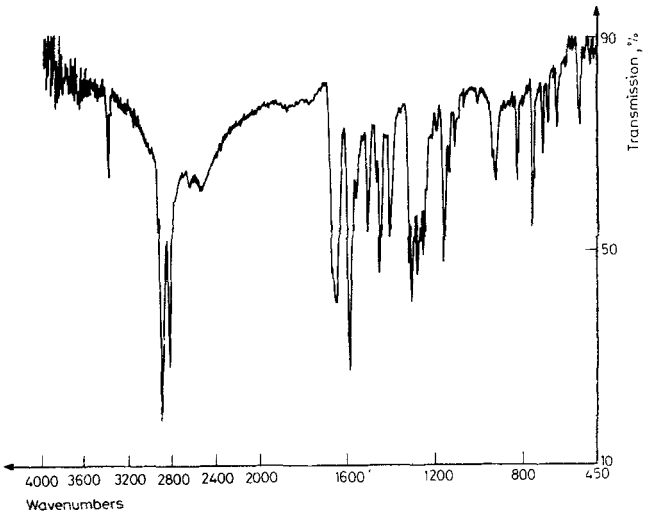


Fig. 5 Infrared spectrum of Form I at room temperature

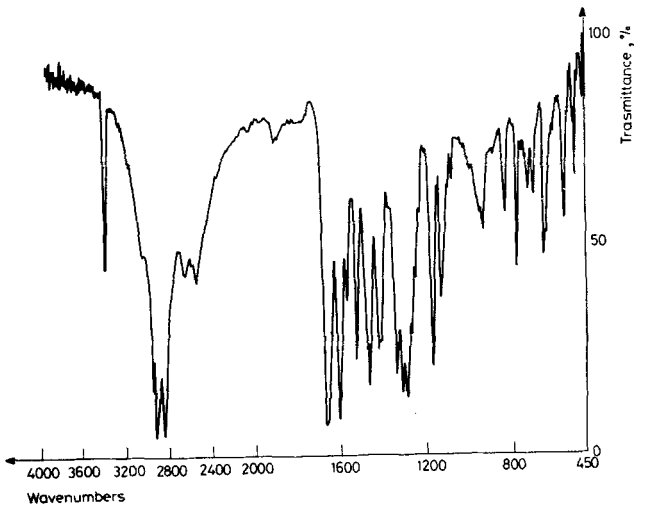


Fig. 6 Infrared spectrum of Form II at room temperature

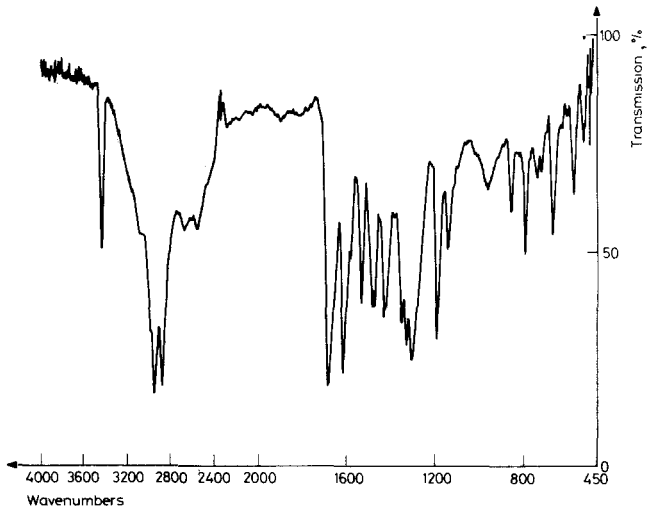


Fig. 7 Infrared spectrum of Form III at 90 °C

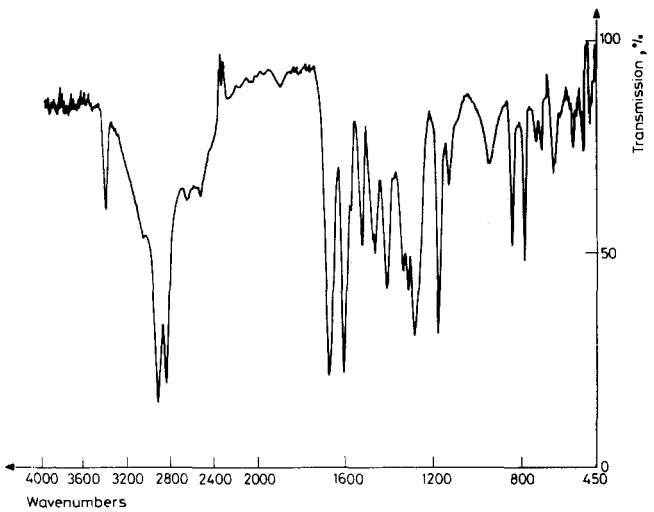


Fig. 8 Infrared spectrum of liquid crystal at 112 °C

Table 2 Summary of infrared absorption spectral data of HABA polymorphic phases

Phase I	Phase II	Phase III	LC	Melt
502s	502m	502m	502m	505m
		510w		
548s	548s			548m
			550m	
		555s		
				615m
	632m	632w		
		645s	640m	640m
652m	652s			
695m	695w	695w	695m	695m
720m	720m	720m	720w	720w
770s	770s	770s	770s	
				775s
		800w		
810w	810w			810w
			835s	835s
840s	840s	840s		
				930m
		940m	940m	
945s	945s			
955m	955m			
990w				
	1005w	1005w		
1020w				
1080w	1080w	1080w		1085w
	1105w			
1110w				
	1125m	1125m	1125m	1125w
1130m	1130m			
1155m				
1175s	1175s	1175s	1175s	1175s
1210w	1210w			
1235w	1235w			
	1250w			
1255w				
1265m	1265w			
				1275s
1280w			1285s	
1290m		1290s		
	1293s			
		1315s	1315m	1315m
1320s	1320s			
1335m			1337m	1337m
	1341m			

Table 2 Cont.

Phase I	Phase II	Phase III	LC	Melt
		1344m		
		1380w	1380w	
				1407s
			1412s	
	1420w	1420s		
1422s				
	1425s			
1465s			1465m	1465m
	1470s	1470s		
			1475m	1475w
	1477m	1477m		
1482m	1482w	1485w		1484m
1520s				
	1526s	1526s	1526s	1526s
1575m	1575m	1575m	1575m	1575m
1605s	1605s		1605s	1605s
		1610s		
1660s				
	1665s			
		1670s		
			1673s	
				1677s
1682s				
			2535m	2535m
		2550m		
2560m	2560m			
				2600w
2665m	2665w	2665m	2660m	2665m
2845s	2845s			
		2850s	2850s	
				2860s
2870w				
2915s				
	2920s	2920s	2920s	
				2925s
2975w		2975w	2975w	
	2980m			
3400s				
	3420s	3420s	3420s	3420m

s = strong, w = weak, m = moderate

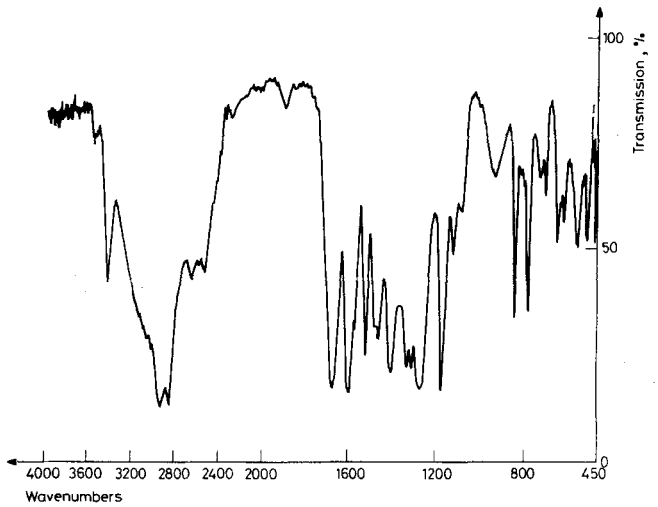


Fig. 9 Infrared spectrum of molten HABA at 135 °C

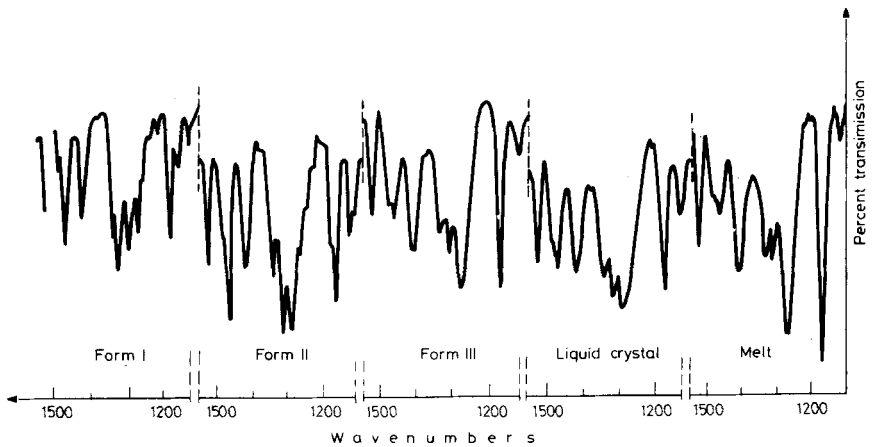


Fig. 10 Infrared spectra of HABA solid. liquid crystal, and molten states in the region of 1100 to 1550 cm^{-1}

In the micro–FTIR of the solid phases, there is a possibility that sample orientation may cause variation in the infrared absorption spectra. Since Forms I and II are available at room temperature, it is possible to compare the spectra obtained by micro and conventional methods. KBr pressed pellets and diffuse internal reflectance spectra (DIRFTS) of Forms I and II, i.e. spectra of randomly oriented material, were compared with spectra of thin films of pure solid obtained with the microscope. No significant orientation effects were found.

5. Discussion of phase behavior of HABA

On the first DSC heating cycle of HABA, there is an endothermic transition with a peak at 92 °C, confirmed by thermomicroscopy to be a solid-solid transformation; this transition is found only on the first heating cycle. On subsequent DSC heating cycles, a new solid-solid transformation occurs with a peak transition temperature of 73 °C; no transition is observed at 92 °C. These observations suggest that crystallization of the melt produces a metastable polymorphic form which does not readily transform even at room temperature. When a film of melt recrystallized HABA existing in its metastable phase II is exposed to an organic solvent, e.g. xylene, at ambient temperature, transformation to the thermodynamically stable form I is observed. Figure 11 illustrates this transformation. The original starting material had been recrystallized from an organic solvent medium for purification. The recrystallization process produced the stable form I, whereas cooling of the melt resulted in the metastable form II.

When HABA is melt crystallized and then recrystallized from xylene, the solid-solid polymorphic transformation (I→III) at 92 °C is observed by DSC. The 92 °C

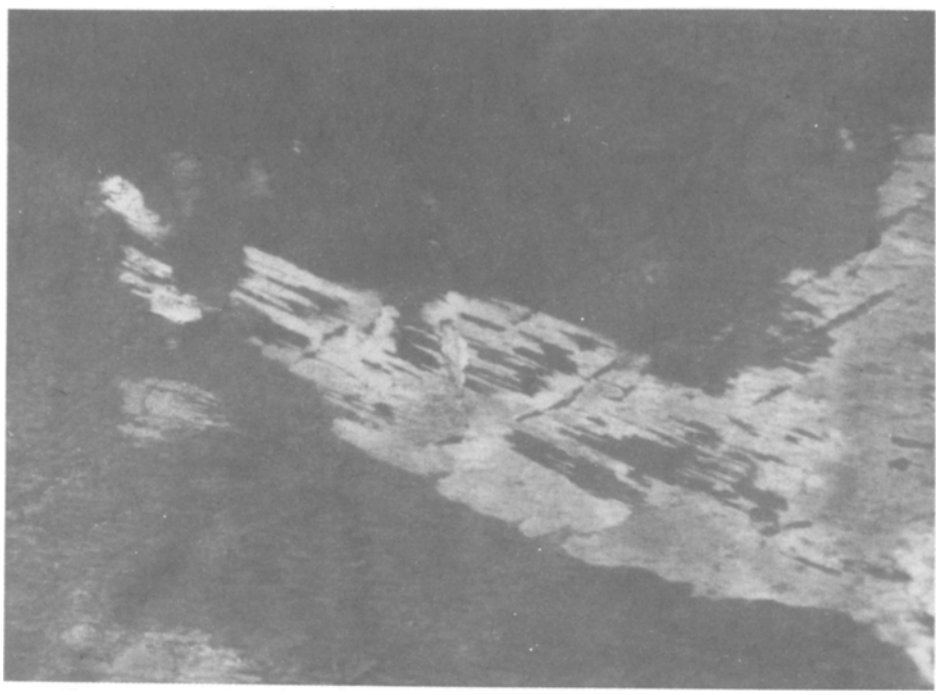


Fig. 11 Polarized light micrograph at 100 × showing the polymorphic transformation II →I in xylene at 27 °C

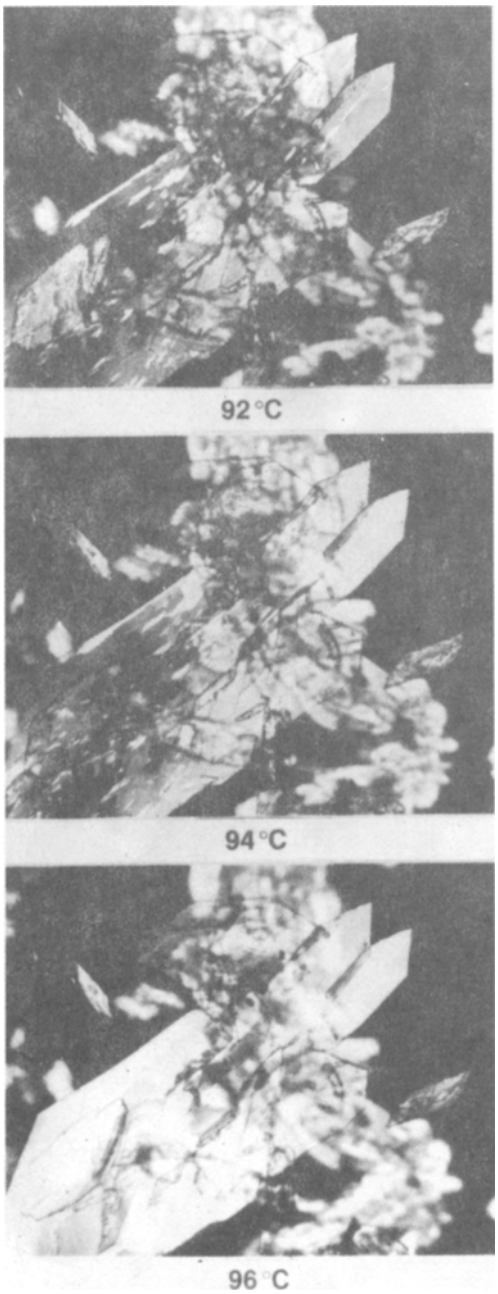


Fig. 12 Polarized light micrograph of xylene-recrystallized HABA at 100× showing the solid-solid transformation I →III

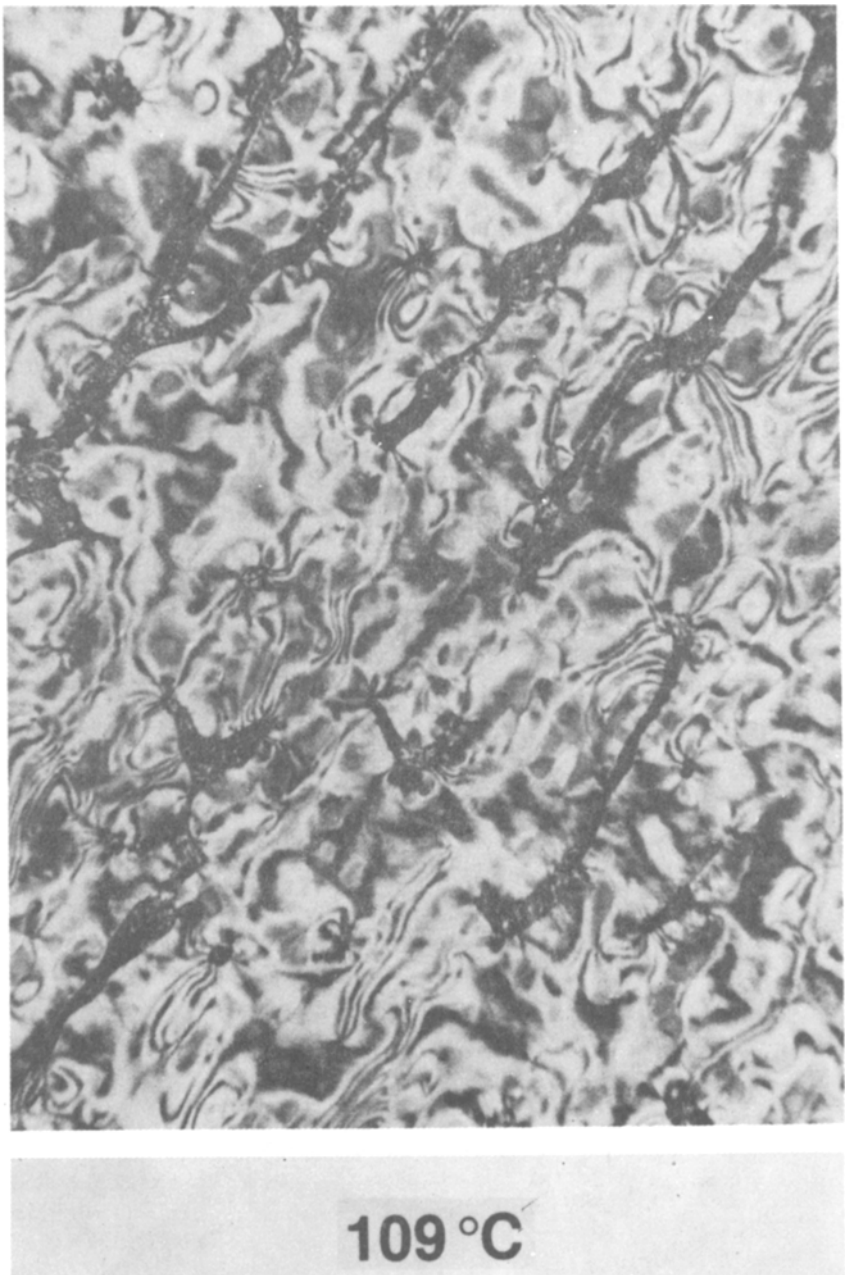


Fig. 13 Polarized light micrograph at 100 × showing the nematic texture of the liquid crystalline phase at 109 °C

transformation of xylene-recrystallized HABA is subtle in nature: In polarized light, it is seen only as a change of birefringence, not as a change in external crystallographic form, Figure 12. Since the 92 °C transformation fails to appear on cooling, the transformation is not reversible, i.e. it is monotropic.

The melting of HABA is further complicated by the formation of an anisotropic liquid crystal phase. The transformation of solid to liquid crystal occurs at 107 °C. The liquid crystal phase is nematic, Figure 13, and melts to an isotropic liquid at 126 °C. During heating of the liquid crystals, minor changes in texture are seen but cannot be positively assigned to a particular type of liquid crystal phase.

The thermal behavior of HABA is summarized in the schematic phase diagram, Figure 14: HABA I is the thermodynamically stable phase at room temperature. HABA I transforms monotropically to HABA III at 92 °C. Form III is stable up to 107 °C where it "melts", forming the nematic liquid crystal phase. At 126 °C, the liquid crystal phase melts to an isotropic liquid. On cooling, the liquid crystal phase forms with little supercooling. Form III crystallizes from the liquid crystal and remains transformed to the 73 °C region. Form III then transforms to Form II. The Form III to Form II transformation is enantiotropic, i.e. reversible on heating or cooling. Form II is metastable at room temperature, but can be transformed to Form I by a solution transformation.

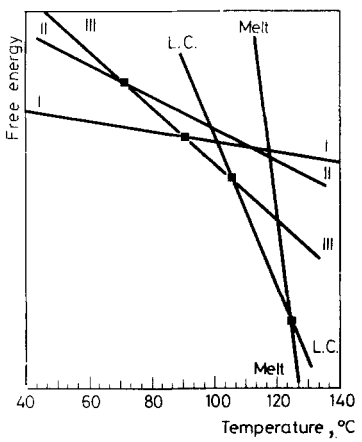


Fig. 14 Schematic free energy diagram for HABA phases

Conclusion

Understanding polymorphism requires the concerted investigation by DSC, TMP, FTIR, and thermomicroscopy. These techniques complement each other and must be used together in polymorphism studies. In the HABA example,

thermomicroscopy detects the formation and melting to a liquid crystalline phase that DSC can detect but is unable to characterize. However, DSC and TMP established a solid-solid transformation not seen by direct microscopical examination.

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Zusammenfassung — Die Kombination von Thermomikroskopie, DSC, Thermomikrophotometrie (TPM) und Mikro-Fouriertransform-Infrarotspektroskopie (FTIR) ist erforderlich um Polymorphie eindeutig nachzuweisen. Die Notwendigkeit der Anwendung einer Kombination von Techniken wird an Hand der Polymorphie von *p*-Hexadecylaminobenzoesäure (HABA), eines pharmazeutischen Zwischenproduktes, illustriert. DSC kann zur Registrierung thermisch ausgelöster Prozesse verwendet werden, es handelt sich aber nicht um eine spezifische Technik, die allein nicht geeignet ist, polymorphe Umlagerungen von anderen mit Enthalpieveränderungen einhergehenden Vorgängen zu unterscheiden. Thermomikroskopie erbringt gewöhnlich die Bestätigung bezüglich der Polymorphie durch direkte Beobachtung der polymorphen Formen und Umwandlungen, aber nicht alle Umwandlungen führen zu visuellen Veränderungen in der Struktur. Mit Thermomikroskopie kombiniert ermöglicht die Mikro-FTIR Aussagen über die Chemie dieser thermischen Veränderungen und liefert spezifische Informationen über Veränderungen in der Molekularstruktur.

Резюме — Комбинированные методы термомикроскопии, дифференциальной сканирующей калориметрии, термомикрофотометрии и микро-инфракрасной фурье-спектроскопии являются ценными для однозначного определения полиморфизма. С целью показа необходимости

использования таких комбинированных методов, представлен полиморфизм *n*-гексадециламинобензойной кислоты, как промежуточного фармацевтического препарата. Например, ДСК может регистрировать термически наведенные процессы, но не является специфической и не может отделить полиморфные превращения от других процессов, связанных с изменением энтальпии. Термомикроскопия обычно подтверждает полиморфизм прямым наблюдением полиморфных форм и превращений, но не все превращения сопровождаются визуальным изменением структуры. Микро-инфракрасная фурье-спектроскопия в комбинации с термомикроскопией подтверждает химию таких термических изменений и представляет более специфическую информацию об изменении молекулярной структуры.