Journal of Thermal Analysis and Calorimetry, Vol. 74 (2003) 723–728

X-RAY DIFFRACTION/CALORIMETRY COUPLING
A tool for polymorphism control

C. Allais [1], *G. Keller* [1], *P. Lesieur* [3], *M. Ollivon* [1] *and F. Artzner* [4*]

[1]UMR 8612 (CNRS), 5 rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France
[2]L.U.R.E., Université Paris XI, 91898 Orsay Cedex, France
[3]Laboratoire de Physico-Chimie des Colloïdes, UMR 7565 CNRS, F-54506 Vandoeuvre-lès-Nancy France
[4]GMCM, UMR 6226(CNRS), Université Rennes1, F-35042 Rennes Cedex, France

(Received March 11, 2003; in revised form July 22, 2003)

Abstract

Polymorphism of trilaurin mixed with 4% of cholesterol was studied with a setup coupling calorimetry and phase characterisation by in-situ X-ray diffraction (Microcalix). Four polymorphic forms were identified. Monotropic and enantiotropic transitions were identified from the reconstruction of Gibbs free energy diagram which allows the control of trilaurin polymorphism.

Keywords: calorimetry, cholesterol, lipid, polymorphism, X-ray diffraction

Introduction

Solid lipid dispersions are common in pharmaceutical, cosmetic and food industry: Solid Lipid Nanoparticles (SLN) are currently developed as drug carriers [1]; in cosmetic and food industry, solid lipid products exhibit pleasant taste according to their textures and their availability to produce cool sensation. Lipids are known to exhibit complex polymorphism, i.e. to crystallize in different crystalline forms. Industrial applications require a perfect control of this lipid polymorphism. Indeed, in pharmaceutical industry, distinct crystalline forms should exhibit distinct release properties and consequently change the efficiency/toxicity of the formulation. On the other hand, in cosmetic [2] and food industry, appearance, texture and taste of solid dispersion are strongly related to the lipid crystalline forms. Indeed, these organoleptic characteristics are relative to different crystal size, softness and melting temperature of dispersion. These relationships between the required industrial properties and the molecular structures force to develop tools and strategies to control crystal polymorphisms. An X-ray/calorimetry setup was built recently with this aim [3]. In this letter, after a short lipid polymorphism introduction, the advantages of coupling thermal and structural

* Author for correspondence: E-mail: franck.artzner@univ-rennes1.fr

1388–6150/2003/ $ 20.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

techniques to investigate and to control lipid polymorphism are demonstrated on trilaurin (LLL) cholesterol mixture which exhibit four crystalline forms.

Lipid polymorphism

Matrix lipids of dispersions are either triglycerids, waxes, fatty acids or alcohols. These molecules or mixtures of molecules are known to exhibit many crystalline species. Such polymorphism is observed because alkane chains are locally organized in α, β', β packing [4]. The hexagonal α packing is the most disordered, orthorhombic β' is less disordered, and the well organized β exhibit a triclinic packing [5]. Generally, thermodynamic stability increases with crystal order, on the contrary to the crystallization kinetics. These packings (3–5 Å) are characterized by Wide Angle X-ray Scattering (WAXS). Moreover lipid chains can exhibit various tilts and molecular conformation in the layers. Layers thickness (20–50 Å) is characterized by Small Angle X-ray Scattering (SAXS).

Materials and methods

Trilaurin (glyceryl tridodecanoate) and the cholesterol were purchased from Sigma with purity >99%. Fast thermal treatment was performed by differential scanning calorimetry using a DSC-7 apparatus operated at 10°C min^{-1}. Other experiments were carried out with coupled time-resolved synchrotron X-ray diffraction as a function of temperature (XRDT) and high-sensitivity differential scanning calorimetry Microcalix [3]. X-ray diffraction was performed using the high-flux of synchrotron beam at L.U.R.E. (Laboratoire pour l'Utilisation du Rayonnement Electromagnétique) D22 station. Beam energy was 10 keV (λ=1.24 Å). Two linear position sensitive detectors allowed XRDT detection simultaneously at small and wide angles. Crystalline β form of high-purity tristearin (SSS) was used as reference wide angles or short spacing (4.59, 3.85, 3.70, ±0.01 Å), and crystalline silver behenate for small angles or long spacing (58.38, 29.19, 19.46 ±0.05 Å), at 20°C. Samples were loaded in thin Lindeman glass capillaries (GLAS, Muller, Berlin, Germany) with a 1.4±0.1 mm diameter (especially designed for X-ray diffraction) using a small glass funnel and capillary tubing. Capillaries were heated at 65°C for 10 min in order to melt all existing crystals. Then, they were cooled at 0°C for 10 min. First, they were tempered at 20°C for 10 min in order to grow β' form of trilaurin. Afterwards, X-ray and DSC patterns were recorded from 0 to 65°C at 1°C min^{-1}. Second, they were tempered at 40°C for 10 min in order to grow β_1 form of trilaurin. In the same way, X-ray and DSC patterns were recorded from 0 to 65°C at 1°C min^{-1}.

Trilaurin polymorphism

Cholesterol had been added to trilaurin in order to simplify the observation of trilaurin polymorphism. The most metastable α phase was crystallized at 7°C by fast cooling at 10°C min^{-1} (Fig. 1) indicating that nucleation of the most stable phases re-

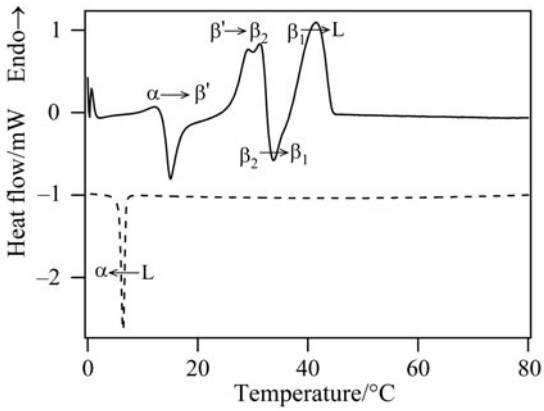

Fig. 1 Calorimetry of trilaurin with 4% (mol/mol) of cholesterol, using a DSC7 apparatus operated at 10°C min^{-1}: —— – heating, - - - – cooling

quires time. Heating DSC at 10°C min^{-1} (Fig. 1) exhibits the very complex pattern of LLL. All peaks correspond to LLL monotropic transitions. The cholesterol solid–solid transition, occurring at 38.9±1.4°C, is a very low energy transition (ΔH=9.8 J g^{-1} [4]) and is consequently not detected by DSC analysis. The first peak corresponds to α form melting and crystallization of LLL liquid (L) in β′ form as shown by an endothermic peak followed by an exothermic crystallization peak. Afterwards, two near endothermic peaks correspond to transition from a β′ form to β form: $\beta' \rightarrow \beta_2$, then $\beta_2 \rightarrow \beta_1$. These two β forms had been described by Precht [6]. LLL melting point of β_1 occurs at 44.8°C and its melting enthalpy ΔH is 170 J g^{-1}. A supercooling larger than 30°C is observed during fast cooling.

Polymorphism control by tempering

Polymorph transitions can be controlled by the use of a thermal treatment: tempering. First, tempering was used in order to avoid α formation and to let growth β′ form. Second, an other one was used in order to obtain a β_1 stable form without any other metastable form α, β′ or β_2. All experiments were conducted with the instrument allowing simultaneous time-resolved XRDT, at small (SAXS) and wide (WAXS) angles, and high-sensitivity DSC. This instrument allows to delimit existence domains of crystalline structures. This technique is able to relate thermal events to structural changes observed.

The first tempering was realized at 20°C (Fig. 2). α form melting temperature is at 15°C [4] and β′ form melting temperature is at 35°C [4]. 10 min at 20°C allows melting of α form and to control the unique formation of β′ crystals. Figure 2 shows DSC and XRDT patterns between 0–60°C. A fusion–recrystallisation exothermic transition at 28°C, and a fusion endothermic transition at 45°C are observed by DSC. By SAXS, the β′ form is characterized by a peak at 0.193 Å^{-1}. A new peak at 0.199 Å^{-1} indicates the $\beta' \rightarrow \beta$ transition at 28°C. The $\beta_2 \rightarrow \beta_1$ transition can be identi-

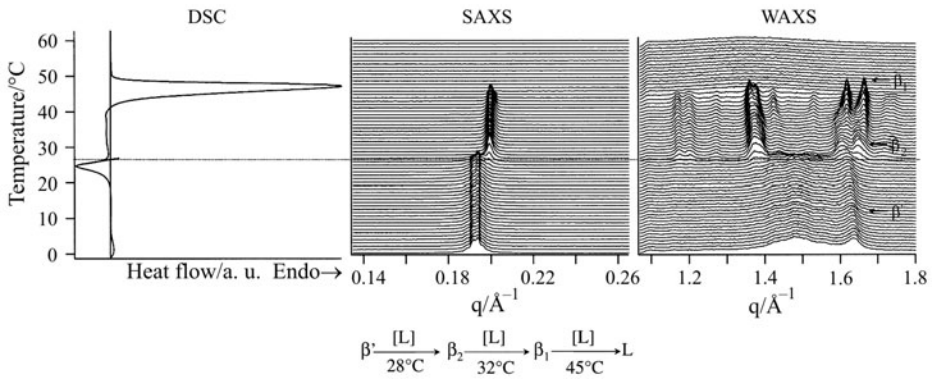

Fig. 2 Trilaurin/Cholesterol 96/4 (mol/mol) sample tempered at 20°C, investigated with the new instrument of calorimetry coupled with XRDT. Left: heat flow upon temperature; center: SAXS (q=2π/d, d is the repeat distance) upon temperature; right: WAXS upon temperature. Note that measurement performed simultaneously at 1°C min^{-1}. Phase sequence is indicated below

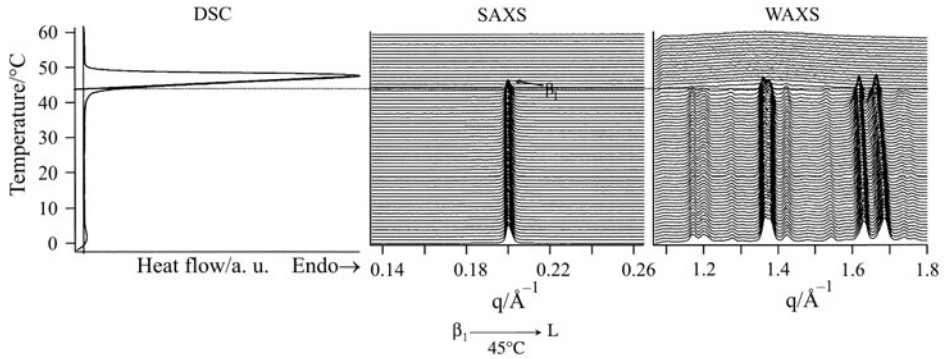

Fig. 3 Trilaurin/Cholesterol 96/4 (mol/mol) sample tempered at 40°C, investigated with the new instrument of calorimetry coupled with XRDT. Left: heat flow upon temperature; center and left: SAXS and WAXS, respectively, upon temperature. Measurement performed simultaneously at 1°C min^{-1}. Phase sequence is indicated below

fied only thanks to simultaneous WAXS measurements because β_2 form exhibits 1.60 and 1.65 Å^{-1} peaks, whereas β_1 shows 1.62 and 1.665 Å^{-1} peaks. The three crystalline forms β', β_2 and β_1 are easily characterized by SAXS/WAXS coupling and the higher monotropic transition temperature is 32°C.

The second tempering (Fig. 3) was realized at 40°C in order to obtain the stable form of trilaurin β_1 by melting every unstable species. One unique enantiotropic transition is characterized by DSC at 45°C. By WAXS at 0°C, the two-peak positions of β_1, are 1.63 and 1.685 Å^{-1} and move with temperature increase to 1.615 and 1.66 Å^{-1} at the melting temperature, corresponding to normal thermal expansion.

Gibbs energy discussion

Figure 1 shows that phase sequence in heating is different from the one obtained in cooling. This behavior indicates that thermodynamic path followed during thermal cycle does not correspond to the equilibrium. Gibbs diagram allows to represent free enthalpies of different phases upon temperature [7], and thereby to understand what happens. Each phase has its own molar Gibbs free enthalpies G_i where i is α, β', β_2, β_1. $G_i(T)=h_i-Ts_i$, with h_i molar enthalpy and s_i molar entropy, is quite linear for small temperature variations. A schematic representation of such diagram is shown in Fig. 4. The negative slope increases with the disorder from the β form to the liquid. By entropy definition, the most stable phase is the one which has the lowest free enthalpy. At temperatures lower than 45°C, β_1 is the most stable, and at higher temperature liquid phase (L) is the most stable. At working temperatures the crystal stability is $\alpha<\beta'<\beta_2<\beta_1$. The four melting temperatures of each crystalline form i are defined by $G_i=G_L$ and correspond to the filled circle on Fig. 4.

Fast cooling experiments hinder well organized crystalline form nucleation and only α phase crystallizes. The thermodynamic path of the first heating experiments (Fig. 1) corresponds to the heating of the α phase (Fig. 4 ①). The three metastable crystalline forms melt successively and the liquid recrystallizes immediately to the next metastable crystalline form. Consequently, four melting transitions observed by DSC in Fig. 1 can be interpreted.

Tempering the sample at 20°C after a fast cooling produces pure β' phase which is long time stable at temperatures lower than 28°C. The three transitions observed in Fig. 2 correspond to the heating of the β' phase (Fig. 4 ②). Tempering the sample at 40°C after a fast cooling produce pure β_1 phase which is long time stable at tempera-

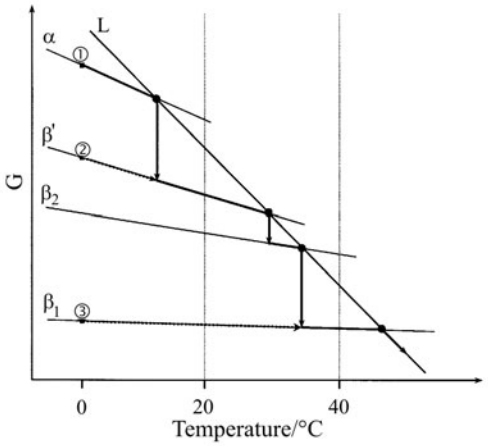

Fig. 4 Free enthalpy of different phases in function of temperature. Thermodynamic ways followed are: ① – path of Fig. 1; ② – path of Fig. 2; ③ – path of Fig. 3; ● – solid phase melting temperatures

tures lower than 45°C. The heating (Fig. 4 ③) exhibits only the melting transition of the β_1 phase, and consequently explains the infinite physical stability of this form.

Conclusions

This example illustrates the large potential of X-ray/DSC coupling experiments to investigate and control polymorphism. The Gibbs free energy diagram of a molecule should be reconstructed from a fine analysis of the results obtained by coupling the three techniques. Then, tempering temperatures should be deduced from the Gibbs diagram. This classical approach for pure compounds, was here applied successfully to a binary mixture. In the future, this approach will be generalized for complex mixtures and dispersion thanks to the coupled X-ray diffraction/DSC techniques, and is promising to control polymorphism.

* * *

We are grateful to Daniel Kalnin for careful reading of the manuscript.

References

1 K. Westesen and B. Siekmann, Biodegradable Colloidal Drug Carrier Systems Based on Solid Lipids, In: Microencapsulation, Ed. by E. S. Benita, Marcel Dekker, New York 1996, p. 213.
2 A. Dingler, R. P. Blum, H. Niehus, R. H. Müller and S. Gohla, J. Microencapsulation, 16 (1999) 751.
3 G. Keller, F. Lavigne, L. Forte, K. Andrieux, M. Dahim, C. Loisel, M. Ollivon, C. Bourgaux and P. Lesieur, J. Therm. Anal. Cal., 51 (1998) 783.
4 D. M. Small, Handbook of lipid research, The Physical Chemistry of Lipids, Ed. Plenum Press, New York 1986.
5 A. I. Kitaigorodskii, Organic Chemical Crystallography, Springer, New York 1984.
6 D. Precht and E. Frede, Milchwirtsch. Forschungsber., 29 (1977) 265.
7 P. W. Atkins, Physical Chemistry, 2nd Ed., Oxford University Press, 1982.