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On the structural behaviour of triglycerides with time

Ensio Laine [1], Pirjo Auramo [1] and Paavo Kahela [2]

[1] *Department of Physical Sciences, University of Turku, Turku (Finland) and* [2] *Farmos Group Ltd., Research Center, Turku (Finland)*

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Summary

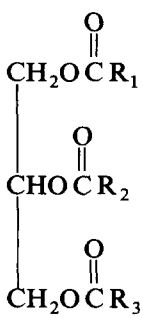
Structural changes in main components of fatty suppository bases, i.e. triglycerides and their binary mixtures were studied during ageing. Experimental measurements were carried out using temperature-controlled X-ray diffractometry and differential scanning calorimetry. These studies have shown that fresh solidified triglycerides have partially amorphous layered structures which gradually crystallize in time. The rate of crystallization depends on the size of the molecule and the mechanical treatment of the sample. During crystallization the suppository base hardens and its melting point increases. No signs of polymorphism were found in triglycerides during ageing, only different degrees of the crystalline arrangement of each triglyceride.

Introduction

Many pharmaceutical properties of fatty suppositories tend to change during storage. Many studies have been carried out over the past decades on the physical state, melting behaviour and release rate of suppositories during storage (Moës and Jaminet, 1976; De Blaey and Rutten-Kingma, 1976; Coben and Lordi, 1980; Liversidge et al., 1981; Yoshino et al., 1981; Voigt et al., 1982b; Thoma and Serno, 1983; Adami and Gatti, 1985; Kahela et al., 1987). Changes in melting point, softening time and drug release rate have caused difficulties in drug bioavailability.

Correspondence: E. Laine, Department of Physical Sciences, University of Turku, SF 20500 Turku, Finland.

Commonly used major components of fatty suppository bases are triglycerides which are esters of the trihydroxy alcohol glycerol:



R_1, R_2 and R_3 are hydrocarbon radicals of fatty acids. Capric, lauric, myristic, palmitic and stearic acids are the most used components in fats (Table 1).

TABLE 1

Commonly used fatty acids and their melting points (according to Handbook of Chemistry and Physics)

Fatty acid	Form	Melting point (°C)
Capric acid	$H_3C-(CH_2)_8-COOH$	31.5
Lauric acid	$H_3C-(CH_2)_{10}-COOH$	44
Myristic acid	$H_3C-(CH_2)_{12}-COOH$	58
Palmitic acid	$H_3C-(CH_2)_{14}-COOH$	63
Stearic acid	$H_3C-(CH_2)_{16}-COOH$	71.5

The main packing features proper to the fatty acids are in all known cases that the molecular axes are parallel and the structures have a marked layered character. All the C atoms in CH_2 groups lie in a plane (or nearly so) and give a more or less flat zigzag. The normal chain period is 0.254 nm and the diameter of the chain including the hydrogen atoms is about 0.4 nm.

The hardening of suppository bases has been examined in many studies, but the published results are at least partially conflicting. In many cases polymorphism has been proposed for the explanation to the changes in mass, in other words the matter has modifications which melt at different temperatures (Voigt et al., 1982a; Thoma and Serno, 1983; Thoma and Serno, 1984; Yoshino et al., 1981; Yoshino et al., 1984). X-ray diffraction and thermal analysis have indicated 2–3 different crystal structures in a suppository base. The hardening occurring during the ageing of the mass is caused by the transformation from the unstable form (called α, β, β', β-prime, or A) to the stable one (called β, β_1 or B).

In some studies it has been proposed that the precipitation is the reason for the noticed changes in the melting behaviour (Voigt et al., 1982b; Eckert et al., 1982; Thoma and Serno, 1983). According to these studies the components melting at different temperatures separate from the suppository base and the total melting point rises. X-ray diffraction and thermal analysis have been used for the traces from the precipitation the researchers observed mainly after storing the specimen above room temperature. Conditions like this, however, may cause partial melting in the fat (Thoma and Serno, 1983; Voigt et al., 1982b).

Crystallization is a third reason for the hardening which has been mentioned in literature (Coben and Lordi, 1980; Bornschein et al., 1980). In those studies the workers could not indicate any traces from different crystal structures, but the results pointed to gradually occurring change from the amorphous state to the crystalline one. Some authors have observed in addition to polymorphic transformations continuous increase in order of fat molecules, too (Thoma and Serno, 1983).

By studying structural changes in fat mixtures used in suppositories different conclusions have been drawn. This may follow from the very complex contents of used materials. In this work we have studied triglycerides and some of their binary mixtures.

Materials and Methods

The triglyceride samples (puriss., Fluga AG) were obtained from Farmos Group Ltd.

In order to destroy any memory of earlier crystals the samples were heated for a short time at a temperature only a few degrees above the melting point. When the sample was completely liquid and transparent it was moulded in different ways:

(1) the sample was solidified in the melting vessel at room temperature (slow cooling)
(2) the sample was cast in the mould at room temperature
(3) the sample was cast in the cold (stored in refrigerator) mould (rapid cooling).

Obviously the triglycerides are very sensitive to treatment because by solidification of a specimen, especially in the case of small molecules, there were considerable variations between separate mouldings. The slow cooling in particular produced surprising and irregular results. The overheating causes difficulties in solidification, too, for it takes a long time before the specimen solidifies and the result is often very irregular.

X-ray diffraction measurements were carried out using a Philips diffractometer equipment with a sample holder that can be heated above room temperature. Nickel-filtered copper radiation was

used in measurements. The reflected radiation was investigated by means of scintillation detector.

Differential scanning calorimetric (DSC) analyses were performed with Mettler TA 3000/DSC 20 system. Samples between 4 and 10 mg were prepared in tightly covered aluminum pans and were tested against an empty, covered pan blank. The test runs were carried out at a heating rate of 2° C/min over the range from room temperature to some degrees above the melting point of each substance.

All samples were studied initially upon moulding and after various storage times up to 3 months. Samples were stored at room temperature (20–23° C). One specimen of each triglyceride was the sample without melting and moulding. This was classified as the 'old' sample.

Results and Discussion

X-ray diffraction measurements indicate that the size of the molecule is of great importance in the behaviour of triglycerides. Samples with relative small molecules, e.g. tricaprin and trilaurin, crystallize by solidification or nearly immediately after that, whereas the crystallization of triglycerides with larger molecules takes remarkable more time. For example, the X-ray diffraction pattern of fresh tricaprin has the Bragg reflections at the same values of 2Θ as tricaprin with long storage time (Fig. 1). In the pattern of fresh sample the peaks are broader than the peaks of aged tricaprin; also, in the pattern of fresh tricaprin not all these peaks are there. This indicates that the crystallization is not complete in the fresh tricaprin.

With mechanical treatment of the sample the crystallization can be accelerated, especially in the case of small molecular specimens. If the tricaprin sample is ground after the solidification there are in its X-ray diffractogram all the same peaks as in the pattern of the aged sample, and the relative intensities are in the same order in both cases (Fig. 1).

The cooling rate slightly affects the structure of the sample. The rapidly cooled triglycerides are more amorphous than the samples with slower

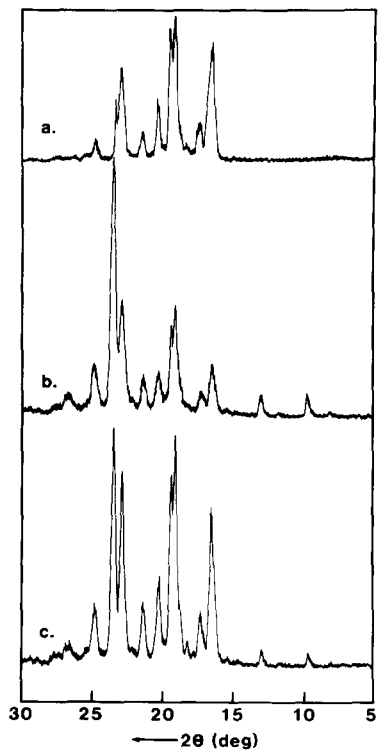


Fig. 1. X-Ray diffractogram of (a) fresh moulded tricaprin, (b) fresh grinded tricaprin, (c) old tricaprin.

cooling. By rapid cooling trimyristin solidifies in disordered form, where the elongated trimyristin molecules have packed together parallel to each other and formed layers. The layered structure appears in the diffractograms, in which there are one relative narrow peak at $2\Theta = 21.0°$ in the pattern of a fresh trimyristin (Fig. 2). The corresponding d-value is 0.423 nm. The fresh solidified trimyristin is transparent but it becomes clouded quite rapidly by itself. The layered structure of trimyristin disappears also gradually. Storage for some days is enough for the molecules to crystallize (Fig. 2). The resulting structure is similar to that of trimyristin stored a long time. The layered structure disappears also by heating a fresh sample of trimyristin to about 30° C (Fig. 3). With rising temperature the kinetic energy of the molecules increases and they can move to their places in the crystal lattice. By heating a crystallized sample no structural changes take place below the melting point. The tripalmitin molecule ($M =$

807.35 g/mol) is more elongated than the trimyristin molecule ($M = 723.19$ g/mol) for the fatty acid chains have two CH_2-groups more, so they are 0.254 nm longer than the trimyristic chains. Tripalmitin also solidifies in a layered amorphous form and it changes to the crystal form after some weeks at room temperature (Fig. 4). The peak in the X-ray diffractogram of fresh tripalmitin is at $2\Theta = 21.6°$, which corresponds to $d = 0.411$ nm.

Tristearin ($M = 891.45$ g/mol) solidifies in layered form and it stays in that form for quite a long time. Ageing for months at room temperature causes noticeable crystallization (Fig. 5). Accord-

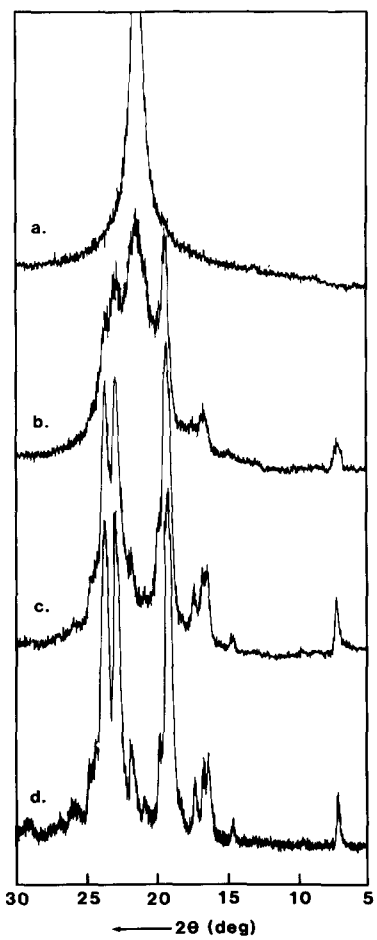


Fig. 2. Changes in the structure of trimyristin during storage. X-ray diffractograms (a) immediately after solidification, (b) after 1 day, (c) after 4 days, (d) of an old sample.

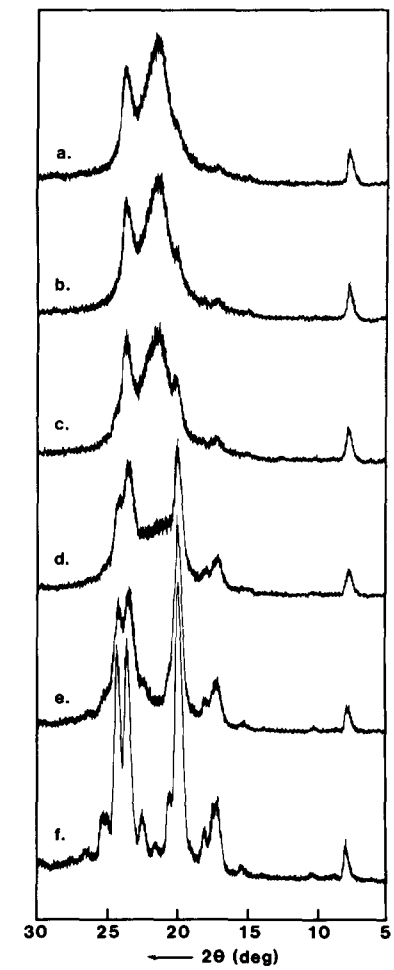


Fig. 3. Influence of heating on the structure of trimyristin. The samples were heated to the measuring temperature and maintained there for half an hour. X-ray diffractograms at temperatures of (a) 22 °C, (b) 25 °C, (c) 28 °C, (d) 30 °C, (e) 33 °C, (f) 55 °C.

ing to the X-ray diffractogram of the layered form the d-value is 0.407 nm ($2\Theta = 21.8°$). The tristearin sample remains transparent much longer than the other triglycerides studied.

Many studies on binary mixtures of triglycerides have been carried out (e.g. Rossell, 1967; Liversidge et al., 1981). In this work we have studied the following mixtures:

50% (w/w) trilaurin + 50% (w/w) trimyristin
50% (w/w) tripalmitin + 50% (w/w) tristearin
20% (w/w) trimyristin + 80% (w/w) tristearin
80% (w/w) trimyristin + 20% (w/w) tristearin.

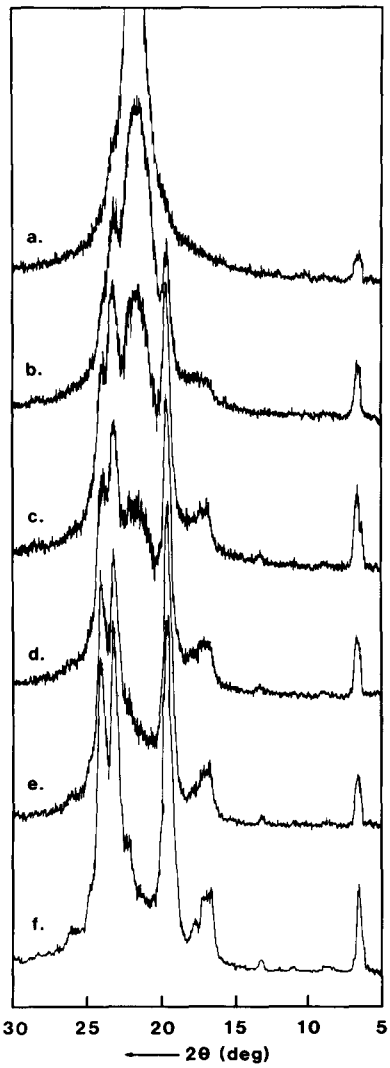


Fig. 4. The effect of storage time on the structure of tripalmitin. X-ray diffractogram (a) immediately after solidification, (b) after 4 days, (c) after 7 days, (d) after 14 days, (e) after 22 days, (f) after 81 days.

As a rule the mixture behaves similar to the component alone. The mixture of trilaurin and trimyristin was slightly amorphous but it crystallized in a few days at room temperature to the stable form (Fig. 6). In the mixture of tripalmitin and tristearin the crystallization takes much more time, like the pure components. Between two various mixtures of trimyristin and tristearin there were no actual differences in structure. The more tristearin the sample contains, the slower the crystallization occurs.

Using thermal analysis the great importance of molecular size was also indicated. In the DSC-curve of tricaprin and trilaurin no changes appear during ageing. The curves have only the endothermal melting peak. In the case of trimyristin there are structural changes during ageing. Fresh trimyristin crystallizes little by little by heating. When the kinetic energy of molecules has increased enough, the layered structure disappears. In the DSC-curve it can be seen as an endothermal peak (Fig. 7). The heating in the DSC-experiment is much faster than in X-ray one and that

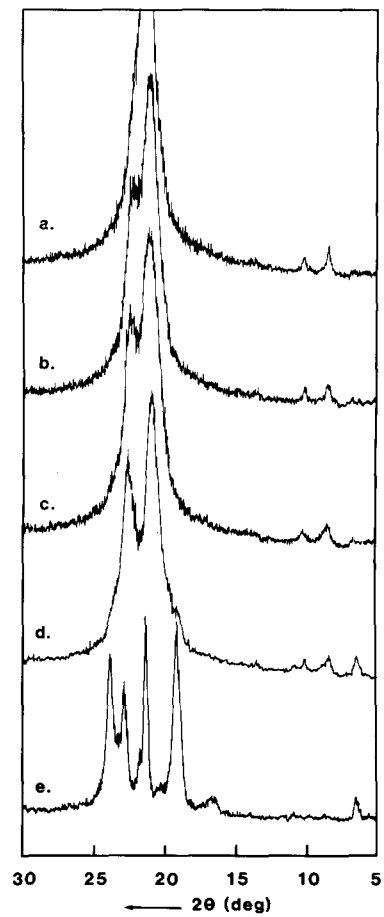


Fig. 5. Changes in the structure of tristearin during the storage. X-ray diffractogram (a) immediately after solidification, (b) after 1 day, (c) after 14 days, (d) after 82 days, (e) of an old sample.

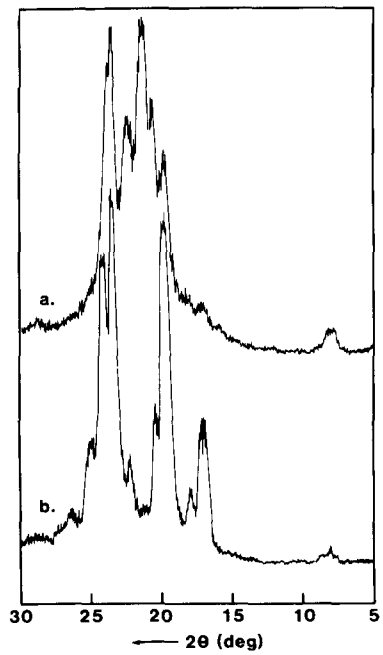


Fig. 6. A binary mixture of trilaurin and trimyristin (1:1). X-Ray diffractogram of (a) fresh sample, (b) 4 days stored sample.

explains the differences in observed temperatures between these two studies. Owing to the higher kinetic energy the molecules are able to move relative to each other and they move to their crystal places because of energy reasons. The sample crystallizes and in the DSC curve there is an exothermal peak. When the heating is continued the sample melts at its melting point. By ageing of trimyristin the peaks of structural falling in and crystallization disappear from the DSC curve and in the curve of aged trimyristin there is only one endothermal peak which is due to the melting.

In the DSC curve of tristearin the peaks caused by falling in and crystallization are more distinct than in curves of other specimens studied and they also stay longest. The DSC curve of aged tristearin has only the melting peak.

Conclusions

We did not observe any changes in 2Θ-values of peaks in our X-ray diffractograms of crystalline

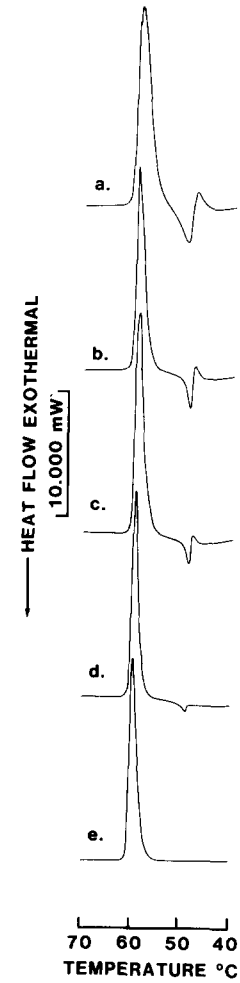


Fig. 7. The effect of ageing on the DSC curve of trimyristin (a) after 1 day, (b) after 2 days, (c) after 3 days, (d) after 8 days, (e) of an old sample.

triglycerides during storage. Thus, we did not find any marks of polymorphism, in the strict sense of this term, during the ageing of triglycerides. On the contrary, it was apparent that the triglycerides solidify in layered form and the crystallization occurs later. The crystallization rate depends clearly from the size of the molecule. The slow crystallization belongs to the general properties of many amorphous matters (Guinier, 1984) and it explains also the noticed changes in triglycerides. The hardening of fatty suppositories follows from the change from the layered form to the crystalline one in the base vehicles.

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