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Research paper

Use of isothermal heat conduction microcalorimetry, X-ray diffraction, and optical microscopy for characterisation of crystals grown in steroid combination-containing transdermal drug delivery systems

Silvia Latsch[a], Torsten Selzer[b], Lothar Fink[c], Michael Horstmann[d], Jörg Kreuter[a,*]

[a]*Institut für Pharmazeutische Technologie, Frankfurt am Main, Germany*
[b]*Baxter Oncology GmbH, Frankfurt am Main, Germany*
[c]*Institut für Anorganische und Analytische Chemie, Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany*
[d]*LTS Lohmann Therapiesysteme, Andernach, Germany*

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Abstract

The combined application of the steroids estradiol (E2) hemihydrate and norethindrone acetate (NEA) is desirable for hormone replacement therapy. Transdermal drug delivery systems (TDDS) enable a controlled delivery of these drugs to the skin. However, in order to attain high skin permeation rates the concentration of the dissolved drugs in the TDDSs has to be high. This often results in supersaturated systems with a high crystallisation tendency.

The combination of NEA and E2-hemihydrate in the acrylic matrix of patches yields crystals that are different from single drug systems. A new crystal phase showing additional X-ray powder diffraction peaks and a new feather-like crystal shape appeared. The crystal formation was considerably accelerated and enhanced by increasing E2 contents in the patches. The new crystal phase seems to be kinetically favoured compared with crystals appearing from pure E2-hemihydrate or NEA. A crystallisation enthalpy of -7.9 ± 0.95 kJ/mol in the matrix containing a 1:3 mixture of E2-hemihydrate and NEA was determined by isothermal microcalorimetry. The crystallisation rate increased with higher drug concentrations. In addition, the influence of patch pre-treatment at 80°C prior to storage on crystallisation was investigated. This treatment enabled a slight reduction of the crystallisation in the TDDSs. Microcalorimetry enabled the classification of various additives according to their influence on the crystallisation process.

Keywords: Estradiol; Norethindrone acetate; Transdermal drug delivery system; Crystallisation; Microcalorimetry; X-ray powder diffraction

1. Introduction

Hormone replacement therapy is a frequent application of transdermal drug delivery systems (TDDS). The combined application of estradiol and one type of gestagen represents an improvement over the single application of an estrogen. TDDS deliver drugs continuously to the skin with drugs mostly dissolved in the polymeric matrix. However, because of the low permeability of the stratum corneum it is often difficult to obtain potent drug concentrations in the body with such systems. Percutaneous absorption depends on the concentration of the drug dissolved in the patches. Therefore, supersaturated patches containing high drug concentrations are often required for obtaining sufficient flux rates [1–5]. A high supersaturation of the drug in an amorphous state in the TDDS may, however, lead to the physical instability of the system, because such amorphous materials tend to crystallise during storage [6–11].

Because the saturation solubility of different steroids in transdermal standard matrices is relatively low [6,10,11], already low drug concentrations result in supersaturated states in the patches and have the tendency to partly recrystallise during storage until a saturated state is achieved. The drug crystals in the transdermal patches

* Corresponding author. Institut für Pharmazeutische Technologie, Johann Wolfgang Goethe-Universität, Marie-Curie-Straβe 9, 60439 Frankfurt am Main, Germany. Tel.: +49-69-798-29682; fax: +49-69-798-29694.
E-mail address: kreuter@em.uni-frankfurt.de (J. Kreuter).

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formed during storage do not contribute to the flux of the drug through the skin due to their low dissolution rate. Only dissolved drug molecules can permeate across the skin. Consequently, the drug activity and absorption decrease as a result of crystallisation [9,12], and the flux of drugs becomes independent of the total drug concentration [1,2,13,14].

Therefore, the limitation of the crystallisation process of drugs is of particular interest in the development of TDDS. Significant efforts were made to stabilise supersaturated solutions by the addition of different antinucleant polymers or other additives [4,5,9,12,14–17]. The effectiveness of crystallisation inhibitors depends on their ability to prevent nucleation and crystal growth. It was assumed that growth inhibition is based on an absorption process at the surface of the growing crystal faces by the formation of hydrogen bonding [3,5,12,15,17–19]. A number of additives was classified according to their interaction with some drugs [16]. In this context it should also be noted that some excipients promote crystal growth and act as crystallisation initiators [15].

The crystallisation behaviour in transdermal patches of 17β-estradiol hemihydrate [10] and of norethindrone acetate [11] using microcalorimetry, polarisation microscopy, and X-ray diffraction was described in two previous publications. The objective of the present experiments was to study the stability and the combined crystallisation process of estradiol and norethisterone acetate in a TDDS. Microcalorimetry is considered to be a very sensitive and non-specific method to detect small heat quantities evolved by different physical and chemical processes. In reference to our previous studies [10,11] the measured heat flow can be attributed to crystallisation processes because the used drugs did not show any significant decomposition at 25°C. Relaxation processes of the measuring ampoules, effects of the punching operation during the sample preparation as well as water adsorption or desorption were eliminated by the subtraction of the analogous placebo curve.

2. Materials and methods

2.1. Materials

Micronised 17β-estradiol hemihydrate (E2-hemihydrate), norethindrone acetate (NEA), polyethylene glycol 400 (PEG 400), octyldodecanol, pressure sensitive acrylate adhesive dissolved in ethyl acetate (Durotak® 387–2287, National Starch and Chemical B.V., Zutphen, Netherlands), backing membrane (Hostaphan® RN 23) as well as a release liner (Hostaphan® RN 100) were obtained as gifts from Lohmann Therapiesysteme LTS (Andernach, Germany). The Hostaphan foils (Mitsubishi Polyester Film GmbH, Wiesbaden, Germany) consist of polyethylene-terephthalat whereby thickness in μm is given by the number. Hostaphan® RN 100 is siliconised on both sides for better removal.

Mannitol was obtained from Cerestar (Krefeld, Germany), and glycerol was purchased from P.F.V. (Burgbernheim, Germany). The solvents ethyl acetate, methanol, and tetrahydrofurane (THF) were provided by Merck Eurolab (Darmstadt, Germany). Deionised water was made by Ultra-pure Water System Milli-Q plus.

2.2. Preparation of TDDS

The TDDS consisted of a self-adhesive acrylic matrix Durotak 387–2287, which contained different concentrations of E2-hemihydrate and NEA. A small content of 7.1% PEG 400 was incorporated as plasticiser. Two different test series were performed using the following compositions: As described in Section 3.2, patches containing a constant concentration of 8.7% NEA and different E2-hemihydrate contents between 0.75 and 3.5% were produced. One of these patches containing 8.7% NEA and 1.5% E2-hemihydrate also was used to examine the crystallisation behaviour after a pre-treatment at 80°C (Section 3.4). In Section 3.3 a fixed ratio of 1:3 (m/m) between E2-hemihydrate and NEA was employed given by the total drug content between 2 and 14%.

The analysis of different additives than PEG 400 (Section 3.5) was performed using patches containing 8.7% NEA and 0.75% E2-hemihydrate. The amount of the different additives was always constant at 7.1%.

The TDDSs were prepared as described before [10] using NEA and E2-hemihydrate instead of E2-hemihydrate and stored at room temperature.

2.3. Polarisation microscopy, microcalorimetry, and X-ray powder diffraction (XRPD)

Polarisation microscopy, microcalorimetry, and X-ray analysis were performed as described before [10].

2.4. High pressure liquid chromatography (HPLC) analysis

HPLC analysis of possible decomposition products of NEA and E2-hemihydrate at 80°C was performed using a Hitachi L-6220 intelligent pump, set at a flow rate of 0.8 ml/min, a LaChrom auto-sampler L-7200, a LaChrom column oven L-7350 maintained at 40°C, a LC-Spectrophotometer Lambda-Max 481 variable wavelength UV detector set at 245 nm and a KONTRON PC-integrator version 3.9. The stationary phase was a Hibar 250-4 C-18 reversed phase packed column. The mobile phase consisted of methanol: THF: water with the percentage of 15:22:63 in the beginning and was switched after 49 min to a ratio of 25:25:50. Calibration and retention times of the main decomposition products are described in the previous publications [10,11].

3. Results and discussion

3.1. Crystallisation of mixtures of E2-hemihydrate and NEA

Transdermal patches containing various percentages of E2-hemihydrate and NEA were examined. The mutual crystallisation behaviour of the drugs was determined by dissolving E2-hemihydrate together with NEA at different percentages in ethyl acetate and evaporating the solvent at room temperature. The formed crystals were analysed by XRPD (Fig. 1).

The reflection pattern of the recrystallised mixture showed not only reflections of E2-hemihydrate and NEA crystals as observable after crystallisation of the single drugs from ethyl acetate but also a variety of different peaks that could not be attributed to those of the respective pure drug crystals (Fig. 1). The formation of the anhydrous lattice of E2 was excluded because under these conditions always the hemihydrate is formed [20,21]. There was no continuous transition of the reflection pattern from the pure E2-hemihydrate via the various mixtures to the pure NEA observed. However, a decreasing concentration of either one of the drugs led to the disappearance of its respective typical reflection pattern. Additional reflections appeared mainly within the range of 20–40% E2-hemihydrate in the recrystallised mixture (Fig. 2). The combination of both steroids presumably led to the formation of at least one new crystal structure containing E2 as well as NEA in the lattice. The determination of the crystal composition of the new structures was not possible. A combined crystal lattice of E2-hemihydrate and NEA was facilitated by the existence of the same orthorhombic unit cell.

Characteristic peaks of the pure drug substances were identified and singled out to determine their respective concentrations in the mixtures. For this purpose it is necessary to select peaks that do not interfere with the new crystal reflections, whereas peaks that increased at some mixture concentration like for instance the peak at 19.7° 2θ (Fig. 2) cannot be used. The selected peaks with characteristic reflections at 13.1° 2θ, 15.7° 2θ, and 26.5° 2θ for E2-hemihydrate and at 14.8° 2θ, 16.5° 2θ, and 20.8° 2θ for NEA show a high intensity and a linear dependence on the contained drug fractions.

3.2. Crystallisation in TDDS containing 8.7% NEA and different E2-hemihydrate concentrations

3.2.1. Polarisation microscopy

The laminates were analysed by polarisation light microscopy for the presence and shape of crystals in the patches containing a combination of the drugs NEA and E2-hemihydrate during storage at room temperature (Fig. 3). It was found that the laminates were amorphous and crystal-free immediately after the manufacturing process. However, depending on the concentration the formation of crystals started after a certain time period. Needle-like crystals of the combined drugs aggregated to clusters. The crystals showed a feather-like structure [6] composed of needles with a diameter of 10–20 μm and a length of approximately 300 μm and were completely different to the observed crystal forms of the single drugs [10,11]. In addition to this structure sometimes the prismatic-like shape of E2-hemihydrate (Figs. 3a,d) and the dendritic crystal series of NEA (Figs. 3b,d,e) appeared [10,11]. At an E2-hemihydrate

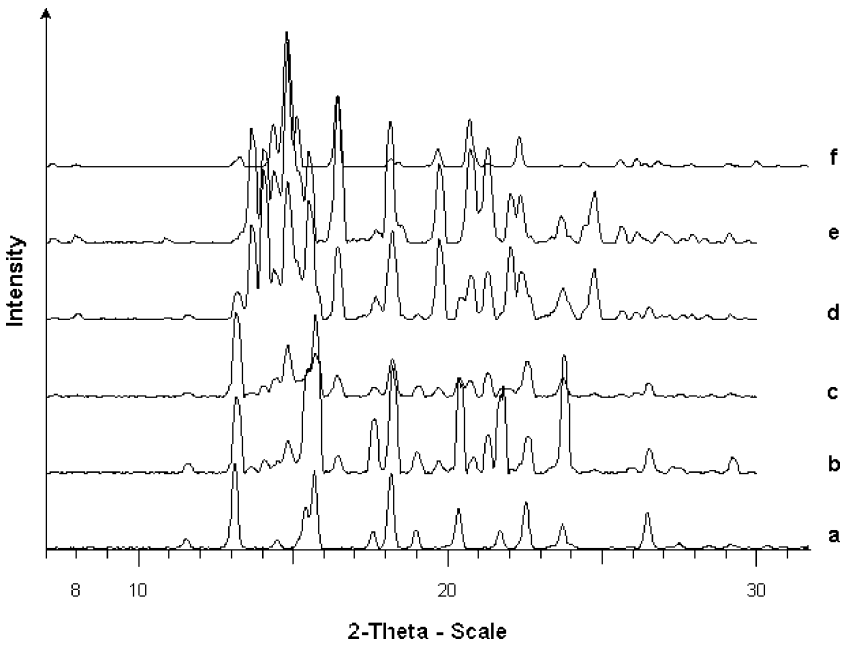

Fig. 1. X-ray diffraction pattern of different mixtures of NEA and E2-hemihydrate recrystallised from ethyl acetate at room temperature. (a) Pure E2-hemihydrate; (b) E2/NEA 80:20; (c) E2/NEA 60:40; (d) E2/NEA 40:60; (e) E2/NEA 20:80; and (f) pure NEA.

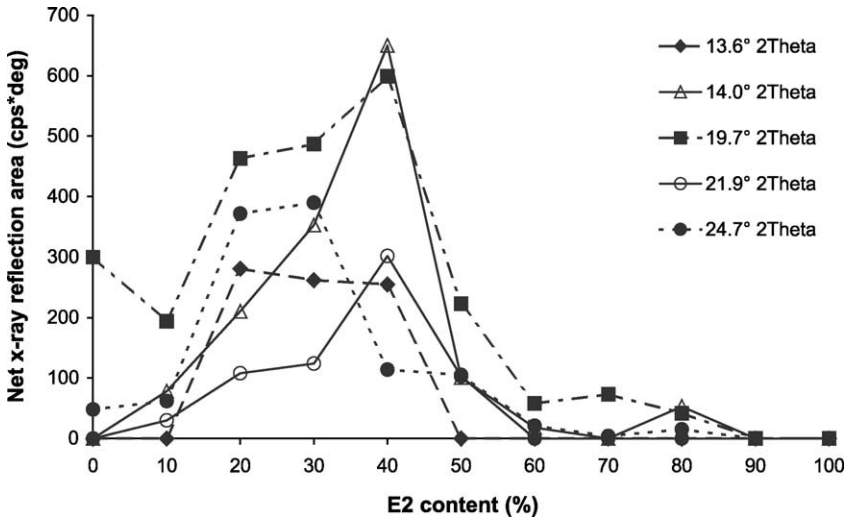

Fig. 2. Net X-ray reflection areas of the newly generated reflections depending on drug percentage of the re-crystallised mixture of NEA and E2-hemihydrate.

fraction of 22% (2.5% E2) and 29% (3.5% E2) of the total drug concentration only feather-like crystals were observed. However, it can be assumed that the feather-like structure was the predominant crystal form for a mixture consisting of E2-hemihydrate and NEA. Stefano et al. [6] isolated feather-like crystals from the polymeric matrix. The analysis proved that these isolated crystals were a combination of NEA and E2, and crystals of different shapes had different ratios of NEA/E2.

By increasing the E2-hemihydrate content at a constant NEA concentration, the crystal carpet became very dense during the storage period. Even a minor increase of the E2-hemihydrate concentration substantially enhanced the crystallisation process. Furthermore, the induction time, i.e. the time until the crystallisation process became visible by polarisation microscopy, decreased with an increasing content of E2-hemihydrate.

3.2.2. X-ray powder diffraction (XRPD)

Similarly to the crystals obtained after crystallisation in ethyl acetate in the TDDS the combined crystallisation of NEA and E2-hemihydrate in the patches led to new crystal structures that also were detectable by XRPD (Fig. 4). While the characteristic main reflections for NEA remained at 14.8° 2θ, 16.5° 2θ and 20.8° 2θ (Fig. 4a) additional reflections appeared at 8.9° 2θ and 11.8° 2θ which were caused by an increasing E2-hemihydrate content but were not typical for this drug recrystallised under the same conditions [10]. The formation of anhydrous E2 was not preferred in the patches. These additional reflections also were different from those observed after crystallisation from an ethyl acetate solution. This means that in the polymeric matrix a different structure of a new crystal phase is formed. A similar appearance of new crystal forms in polymer networks was already earlier observed in poly(butyl cyanoacrylate) nanoparticles [22].

While microscopy does generally not allow a quantitative evaluation of the amount of crystals formed, this determination could be done semi-quantitatively by XRPD based on the increasing reflection intensities during crystallisation. This was also possible for different crystal structures generated in drug mixtures using typical reflections [23–25].

The reflection intensities in the TDDS increased continuously with storage time until the crystallisation process was terminated (Figs. 5 and 6). Although the NEA content was kept constant in all patches tested, the increase of its typical reflection at 14.8° 2θ became flatter with increasing concentration of E2-hemihydrate (Fig. 5). E2-hemihydrate in the presence of NEA inhibited the crystallisation of pure NEA crystals at least during the first months of crystallisation. Nevertheless, after storing these patches for 6 months, they showed the same peak intensities at 14.8° 2θ, except the laminate containing a concentration of 3.5% E2-hemihydrate. This was the case because the crystallisation process was terminated after this time, and the amount of formed NEA crystals was the same for all samples due to the constant NEA content (thermodynamic state of equilibrium). A fraction of 29% E2-hemihydrate in the drug mixture, i.e. 3.5% E2-hemihydrate, strongly inhibited NEA crystal formation.

However, the peaks which appeared at 8.9° and 11.8° 2θ and which are typical for the new crystal phase also showed an increase with increasing E2-hemihydrate concentration (Fig. 6). The higher the E2-hemihydrate concentration the more new phase was formed. Moreover, the new crystal phase seems to be the kinetically favoured crystal form since the equilibrium was achieved faster.

The net X-ray reflection areas show pronounced fluctuations with ageing. This is caused by the fact that at the beginning of the crystallisation process mostly clusters of crystals are formed because of the lower activation energy [26]. Other areas in the laminates were still completely free of crystals. However, only a small area of

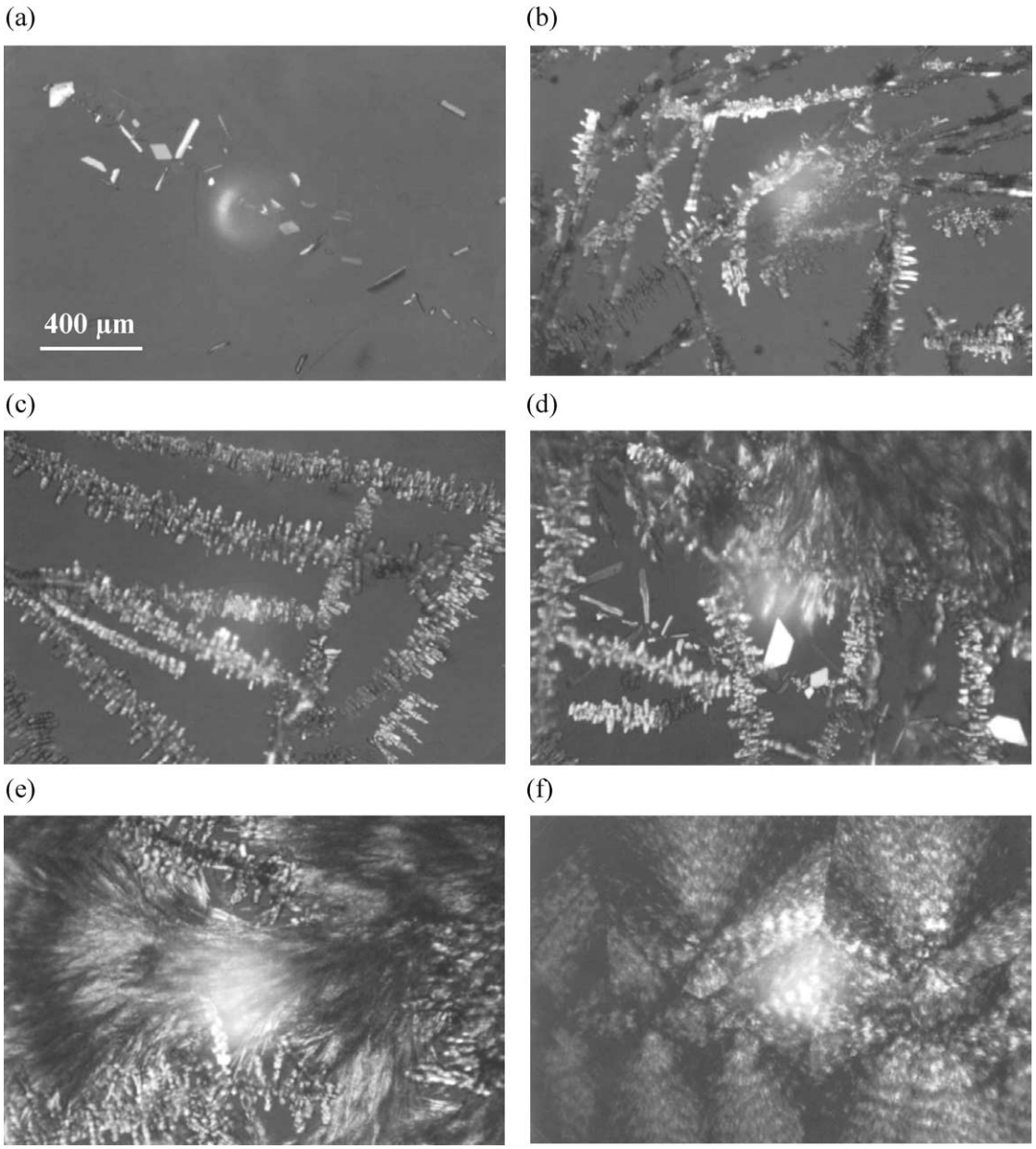

Fig. 3. Polarisation microscopy of NEA containing TDDS with increasing content of E2-hemihydrate after a storage period of 8 weeks at room temperature: (a) 1.5% E2-hemihydrate; (b) 8.7% NEA; (c) 8.7% NEA and 0.75% E2-hemihydrate; (d, e) 8.7% NEA and 1.5% E2-hemihydrate; and (f) 8.7% NEA and 2.5% E2-hemihydrate.

the patches could be observed by a single XRPD measurement. The existing inhomogeneity of the crystals formed in the examined patches and the local differences of the crystal amount in the matrix caused the observed fluctuations. As a result, for the calculation of the theoretical peak area at the test period of 13 weeks a logarithmic regression analysis was used (Fig. 7).

After the selected time point, 13 weeks, the characteristic NEA reflection at 14.8° 2θ decreased with increasing E2-hemihydrate content. The decreasing reflection area of NEA at 14.8° 2θ although the NEA concentration was constant may be explained by the participation of NEA in the formation of the new crystal structure with increasing reflection areas at 8.9° and 11.8° 2θ. The NEA reflection at 16.5° 2θ decreased with increasing E2 contents and increased again at higher concentrations. This reflection possibly overlapped with a peak of the new phase.

3.2.3. Microcalorimetry

The results obtained by polarisation microscopy were confirmed by isothermal heat conduction microcalorimetry. Because the crystallisation process generates a heat flow, the enhanced crystal growth with increased drug concentration observed by microscopy resulted in an increasing drug-associated heat flow (Fig. 8). Because there was no significant chemical decomposition detectable over a 1-year

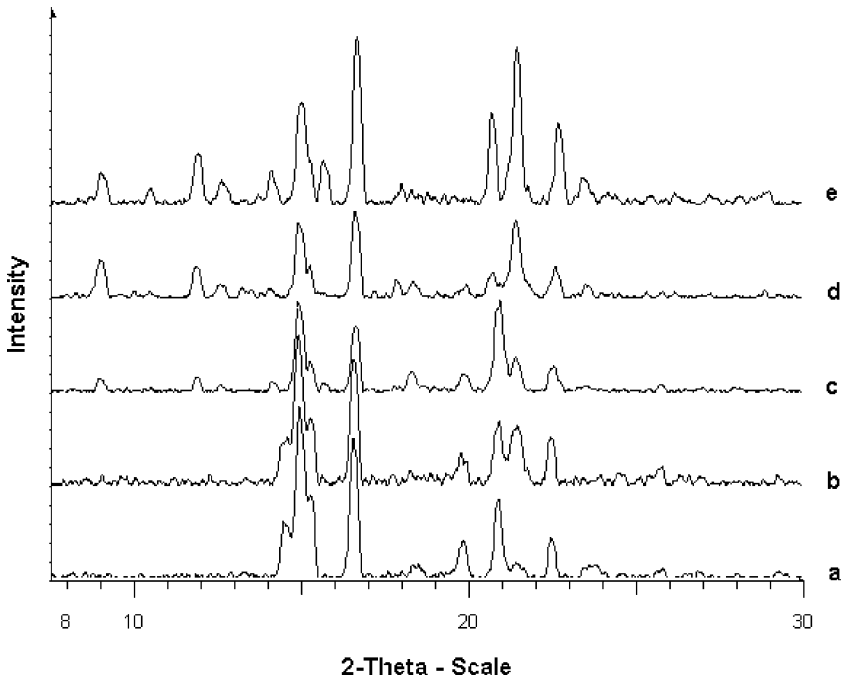

Fig. 4. X-ray diffraction pattern (after correction of the amorphous background) of TDDS containing 8.7% NEA with an increasing E2-hemihydrate content after a storage period of 8 weeks at room temperature: (a) 0% E2-hemihydrate; (b) 0.75% E2-hemihydrate; (c) 1.5% E2-hemihydrate; (d) 2.5% E2-hemihydrate; and (e) 3.5% E2-hemihydrate (standardisation on matrix area weight of 100 g/m^2 was done, i.e. constant layer thickness was applied). The peak appearing at approximately 21.3° 2θ is caused by the sample holder foil.

period [10,11], one can assume that the drug-associated heat flow was only generated by the crystallisation process.

To understand the crystallisation mechanism of these combined systems similar patches containing only a single drug in the respective concentrations, 8.7% for NEA or 1.5% for E2-hemihydrate, were made. While patches with a concentration of 1.5% E2-hemihydrate did not show any and those containing 8.7% NEA only a small drug-associated heat flow during the 7 days measurement period, the laminates with the mixture of the two drugs showed the greatest heat evolution of these three systems (Fig. 8). This heat flow of the combined system was considerably higher than that of the sum of the single systems. Assuming that crystallisation enthalpy of the new phase was not considerably higher than of the single drugs and that the drug-associated heat flow correlates directly with the crystal

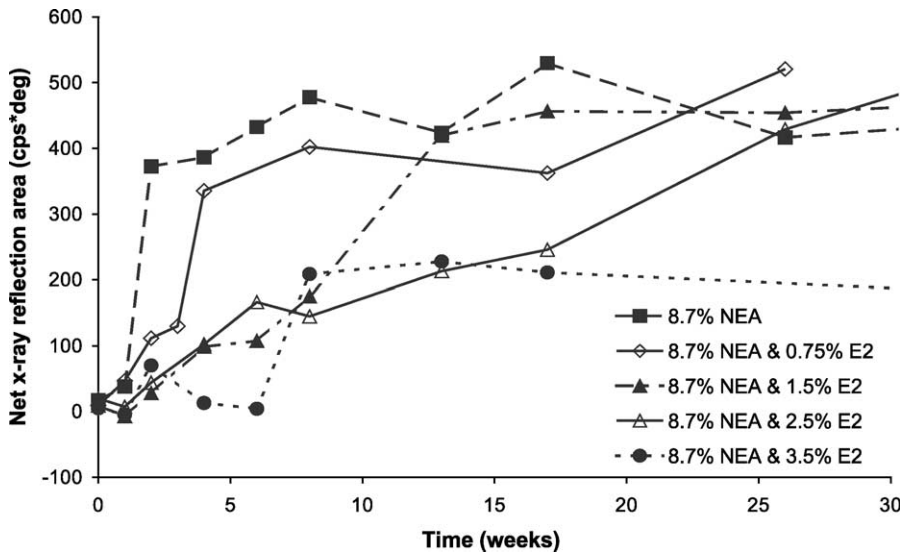

Fig. 5. X-ray net peak area of the characteristic NEA reflection at 14.8° 2θ for combination TDDS samples stored at room temperature containing 8.7% NEA and various E2-hemihydrate concentrations after different storage times (standardisation on matrix area weight of 100 g/m^2, i.e. using a constant layer thickness).

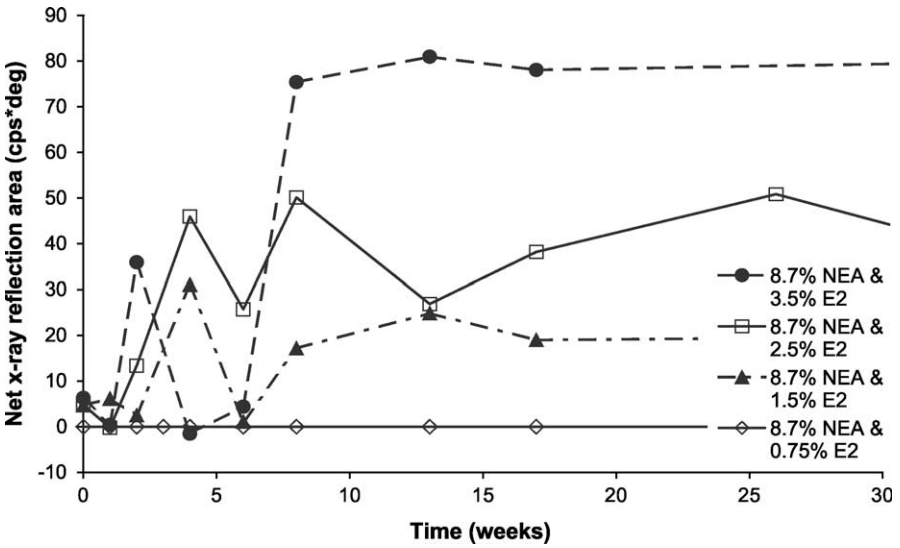

Fig. 6. X-ray net peak area of the new crystal phase at a reflection at 11.8° 2θ for combination TDDSs at room temperature containing 8.7% NEA and various E2-hemihydrate concentrations after different storage times (standardisation on matrix area weight of 100 g/m^2, i.e. using a constant layer thickness).

formation, the crystallisation process, therefore, was either significantly enhanced or accelerated by the addition of E2-hemihydrate. This result demonstrates that the crystallisation within the mixture is not a simple separate process of the drugs but rather is based on a much more complicated physical interaction between the substances applied. The crystallisation process within patches with a mixture of drugs was not only depended on the total hormone concentration but particularly also on that of estradiol as already reported by Stefano et al. [6].

3.3. TDDS containing a constant ratio of NEA and E2-hemihydrate

As described in Section 3.2, the evaluation and analysis of the experimental data obtained from the test series carried out with laminates containing drug mixtures of various percentages is difficult since different crystal structures generate different X-ray patterns and evolve different heats. With microcalorimetry it was impossible to discriminate between the different portions of the crystallised drugs. To facilitate the analysis of the crystallisation process it was decided to continue the experiments using a fixed ratio of 1:3 between E2-hemihydrate and NEA and varying the total drug concentration. This approach should guarantee similar crystal lattices of both drugs in the TDDS for the test series since all examined patches should exhibit the same X-ray diffraction pattern whereas the peak intensities should only depend on the total drug content and not on variation in crystal structures.

Fig. 9 shows the calculated net peak areas after 13 weeks of storage at room temperature. The characteristic

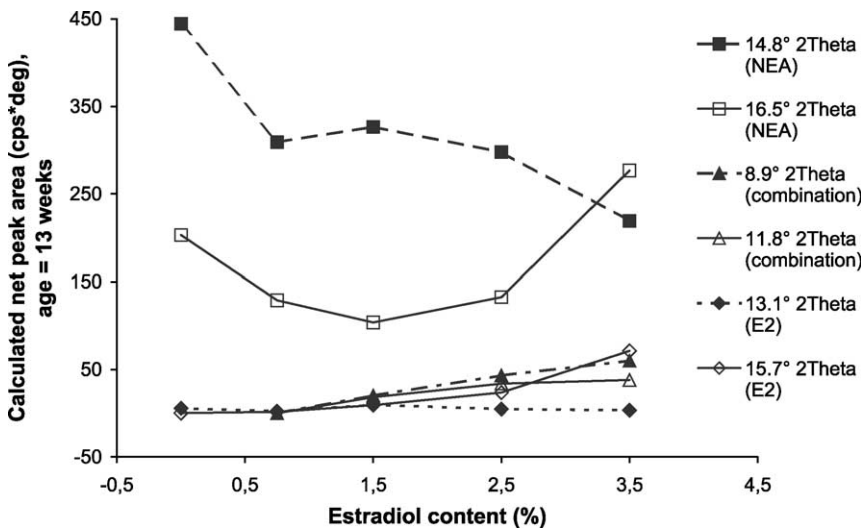

Fig. 7. Dependence of the calculated net peak area of various E2-hemihydrate concentrations (with a constant NEA concentration of 8.7%) after a storage period of 13 weeks.

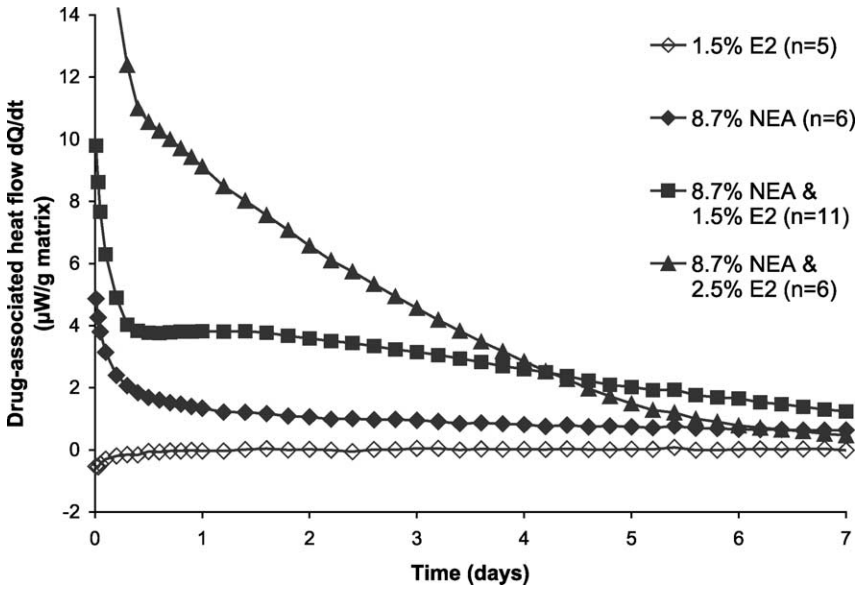

Fig. 8. Concentration dependent drug-associated heat flow at 25°C for a drug concentration of 8.7% NEA and various concentrations of E2-hemihydrate in TDDS.

NEA reflections measured at 14.8° and 16.5° 2θ as well as the new peaks at 8.9° and 11.8° 2θ increased proportionally to the total drug concentration. However, the characteristic E2-hemihydrate reflections did not increase at all. The peaks of the respective preparations versus total drug concentrations were extrapolated to the *x*-axis. The intersection was in the range of 4–5% of total drug concentration which is in good agreement with the first crystals observed at the cut edges by microscopy at 4% of the drug mixture and corresponds to approximately 3.4% NEA and 1.1% E2-hemihydrate. These values are somewhat lower than saturation solubility of the single steroids in the matrix described previously [10,11]. A dense crystal carpet grew at higher concentrations, as for instance in the 6% system. The microscopically observed crystals consisted exclusively of the dense feather-like type.

Patches containing 2 and 4% of drugs examined by microcalorimetry did not evolve any drug-associated heat flow (Fig. 10). An increasing drug-associated heat flow with increasing total drug concentration was observable above 4% total drug which is caused by the more pronounced crystallisation processes.

Microscopic analysis showed a more pronounced crystallisation occurred especially at the cutting edges of the samples [6]. Discs with a diameter of 10 mm for the microcalorimetric examination were punched out of the laminates with a metal punch. The strong mechanical shear force needed for punching puts a large stress on

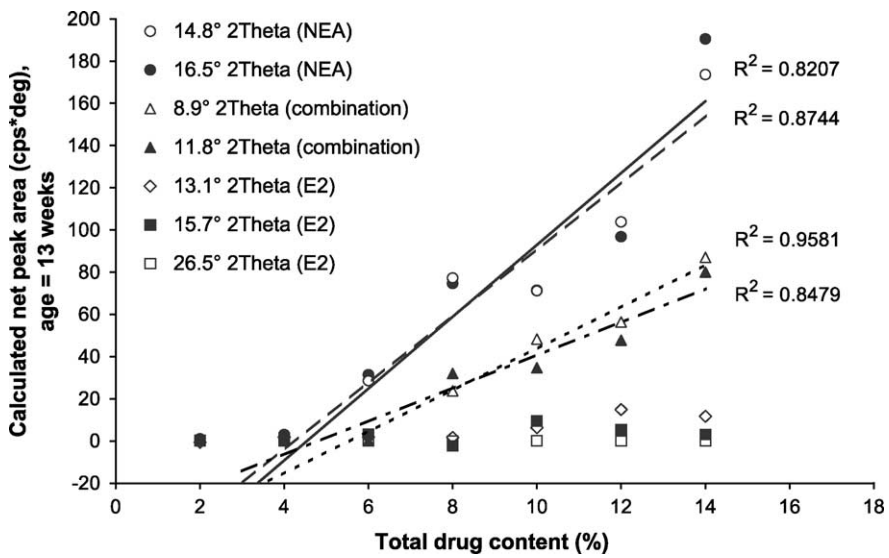

Fig. 9. Calculated net peak area in TDDSs with a constant ratio of 1:3 between E2 and NEA after 13 weeks of storage.

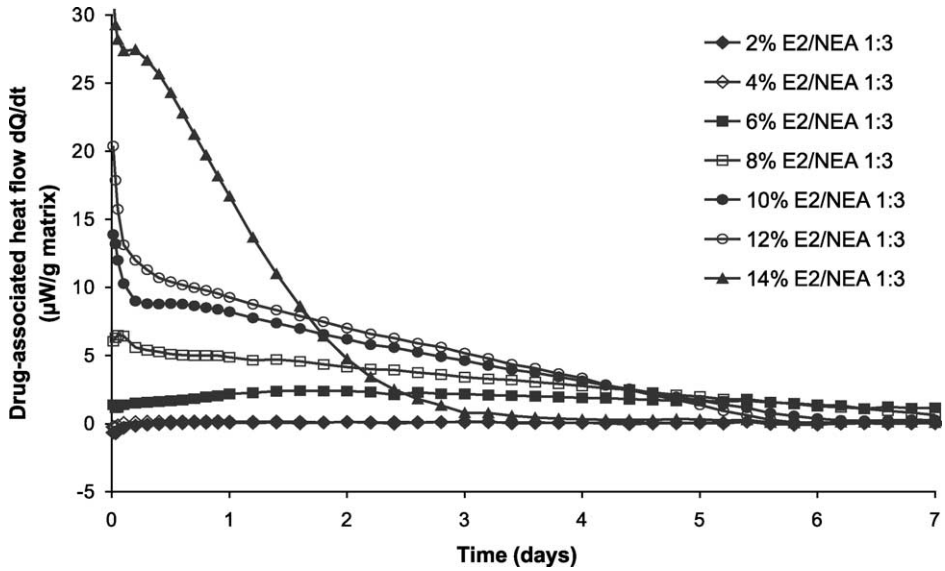

Fig. 10. Concentration dependent drug-associated heat flow at 25°C at a constant E2/NEA ratio of 1:3 in the TDDSs.

the edges of the laminates. This explains the formation of small crystal germs and the high initial heat flow caused by the samples. Following this significant initial heat flow a decreasing drug-associated heat flow caused by the growth of the formed crystal germs was observed (Fig. 10).

The first hours of microcalorimetric examination of the samples consequently were strongly affected by these cutting effects. In order to largely eliminate this effect, the evolved total heat quantity (AUC) was calculated using best fit correlation functions for the time interval between 1 and 7 days. The integration of these functions resulted in the total heat Q (J). Extrapolation to the zero time point yielded the initial drug-associated heat flow $dQ_{t=0}/dt$ (µW).

In agreement with the results of the microscopic examination and the X-ray diffraction measurements (Fig. 9), the microcalorimetric values resulted in an increasing drug-associated initial heat flow as well as an increasing heat for patches with increasing total drug contents above 4% (Fig. 11). A linear correlation between the drug concentration and the heat evolved existed at concentrations above the saturation solubility.

The crystallisation process was accelerated by increasing drug contents and, therefore, finished earlier, presumably due to the higher thermodynamic activity of the highly supersaturated patches. All patches with total drug concentrations of ≥8% already reached a constant heat flow plateau close to 0 µW/g matrix after this short test period (Fig. 10). This points to the termination of the crystallisation processes. Due to the termination of the crystallisation processes within 7 days the slope of the straight line in Fig. 11 resulted in a crystallisation enthalpy of 7.9 ± 0.95 kJ/mol in a 1:3 E2/NEA mixture in the acrylic matrix containing 7.1%

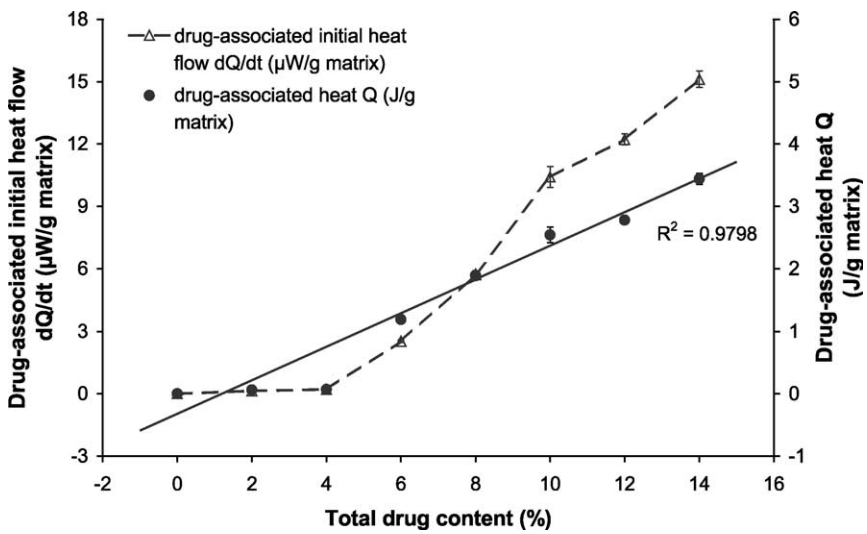

Fig. 11. Correlation between drug-associated heat calculated over the 7 day period and the initial heat flow at 25°C in dependence on the total drug content with a constant E2/NEA ratio of 1:3.

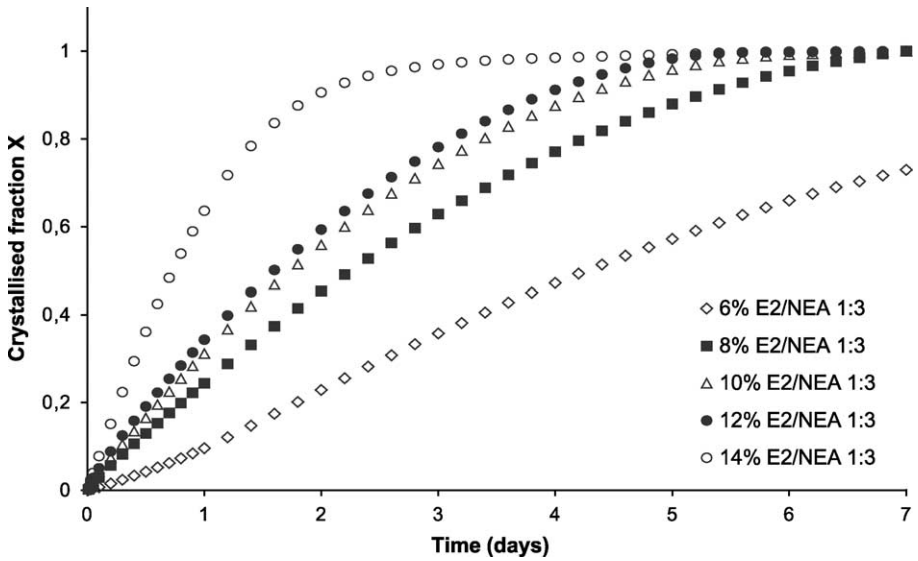

Fig. 12. Degree of crystallisation of the steroids versus time determined by isothermal microcalorimetry at 25°C depending on the total drug content at a constant ratio of 1:3 between E2/NEA.

PEG 400. Although the crystallisation of pure E2-hemihydrate and NEA yielded a greater crystallisation enthalpy of −23.3 and −22.8 kJ/mol, respectively [10,11], and, consequently, should lead to a thermodynamically more stable state, the formation of the new mixed crystal phase from both steroids seems to be kinetically favoured.

The microcalorimetric measurement of the total crystallisation process enables the calculation of the crystallised drug fraction versus time assuming that the recrystallised fraction is proportional to the heat evolution (Fig. 12). It is known that the formation of the crystalline phase generally starts slowly, followed by a faster phase with a constant growth rate [27–34]. The crystallisation process then slows down towards the end until it totally stops when the saturation concentration is reached. The crystallised fraction as function of time, therefore, gives a typical S-shape transformation curve. Substantial energy was added to the laminate samples by punching, which initiated a nucleation at the edges. Hence, the initial slow phase of crystal formation was overlapped by this effect and, therefore, could not be detected. Only the 6% containing patch shows a sigmoid curve.

The half-life period, i.e. the time to reach a crystallised fraction of 0.5, decreased using increasing degrees of supersaturation (Fig. 12), i.e. an advanced nucleation and crystal growth was running according to an increasing rate constant. This proved that the rate of precipitation depends on the degree of supersaturation [15,35].

3.4. Stabilisation of TDDS by a pre-treatment at 80°C

A high instability of the examined patches containing the E2-hemihydrate/NEA mixture was observed after storage at room temperature. It is known that patches containing pure E2 may be stabilised against crystallisation processes by a reduction or the total removal of water [8]. A high water content in the polymeric matrices promotes E2 crystallisation due to its very low aqueous solubility [12] of 0.2–5 μg/ml at 25°C [36,37]. Higuchi et al. [38] also described a similar low aqueous solubility of nearly 6 μg/ml for NEA.

Higher temperatures enable a reduction in water content. Therefore, tests were carried out to determine the influence of an initial storage (pre-treatment) at a temperature of 80°C on the crystallisation process.

Patches containing 1.5% E2-hemihydrate and 8.7% NEA were initially stored at 80°C for 7 days. After this pre-treatment a remarkable stabilisation of the drugs contained within the laminates at the following storage period at room temperature was found (Fig. 13). Compared to the untreated laminates significantly less crystallisation areas were observed after 2 weeks at room temperature (Figs. 13a,b). In addition to the decrease in the amount of crystals the crystal shape also changed, as observed by microscopy (Figs. 13c,d). The needles which were formed became more jagged than previously obtained. Stefano et al. [6] also detected various shapes and amounts of crystals depending on different drying conditions. But they described that the differences were not related to the presence of residual solvents, since matrices that were dried under different conditions but contained similar amounts of residual solvents showed different degrees of crystallisation.

In addition to the microscopic observation, the crystallisation process in the patches pre-treated at 80°C was also analysed by microcalorimetry. In agreement with the above obtained results it was found that crystallisation heat decreased the longer the samples were pre-treated at 80°C (Fig. 14). The lowest crystallisation heat was detected in the patches after pre-treating for 8 weeks at 80°C. However, already a pre-treatment of 1 week showed a strong effect, especially in those patches that were punched out before

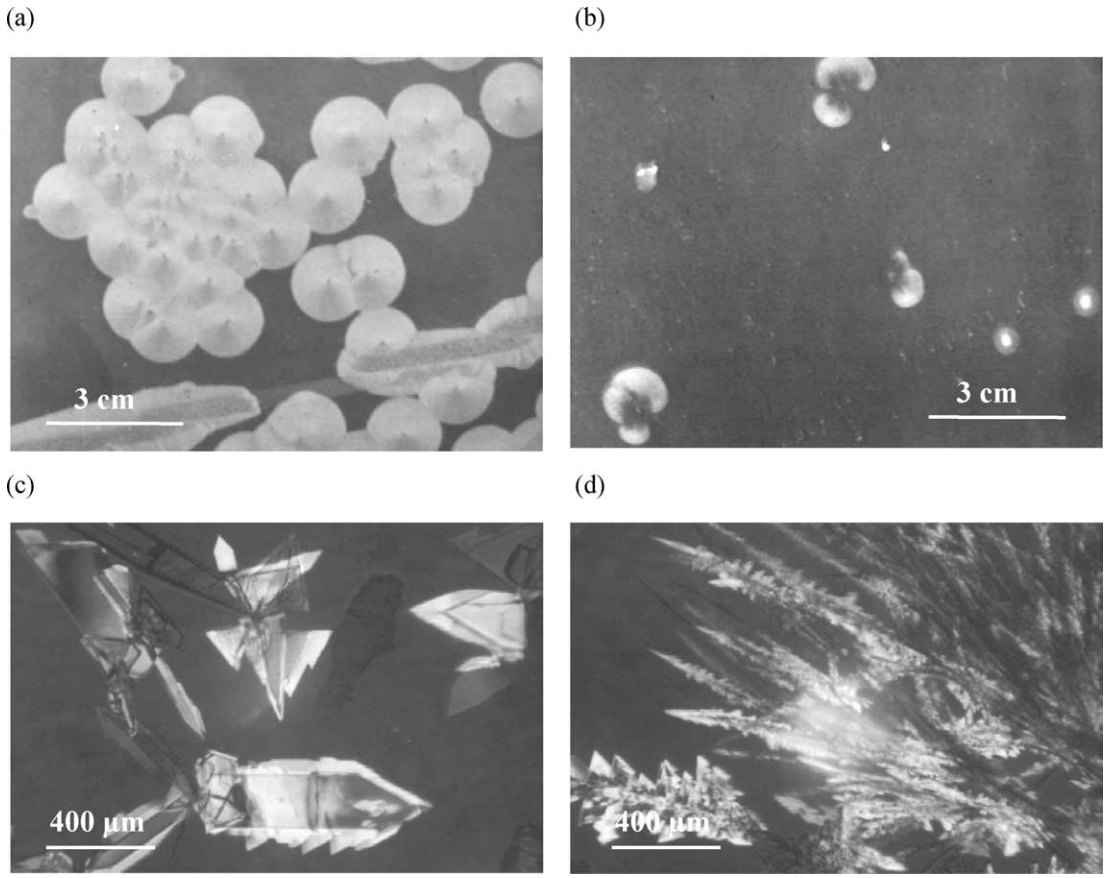

Fig. 13. Crystallisation of a TDDS containing 8.7% NEA and 1.5% E2-hemihydrate: (a) storage for 2 weeks at room temperature without pre-treatment; and (b) 1 week pre-treatment at 80°C followed by 2 weeks storage at room temperature. Polarisation microscopy of different sections of the same system (c, d) 1 week pre-treatment at 80°C followed by 4 weeks storage at room temperature.

pre-treatment. The nucleation process caused by the cutting process seems to be effectively suppressed even by such a short pre-treatment period because the solubility of the drugs at 80°C strongly increases thus impeding the cutting process-induced crystal formation. Stabilisation was slightly but not statistically significant improved by unsealed storage of the patches at 80°C which enhanced the evaporation of water. Pre-treatment at 80°C in closed ampoules largely prevented this evaporation. In conclusion, the greatest improvement in crystallisation prevention can

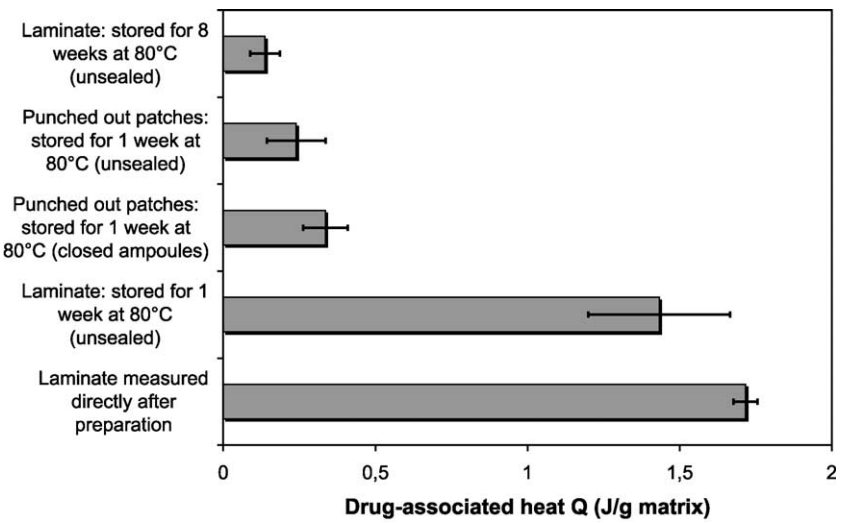

Fig. 14. Microcalorimetric measurement over 7 days of TDDSs containing 1.5% E2-hemihydrate and 8.7% NEA at 25°C pre-treated at 80°C for different periods of storage.

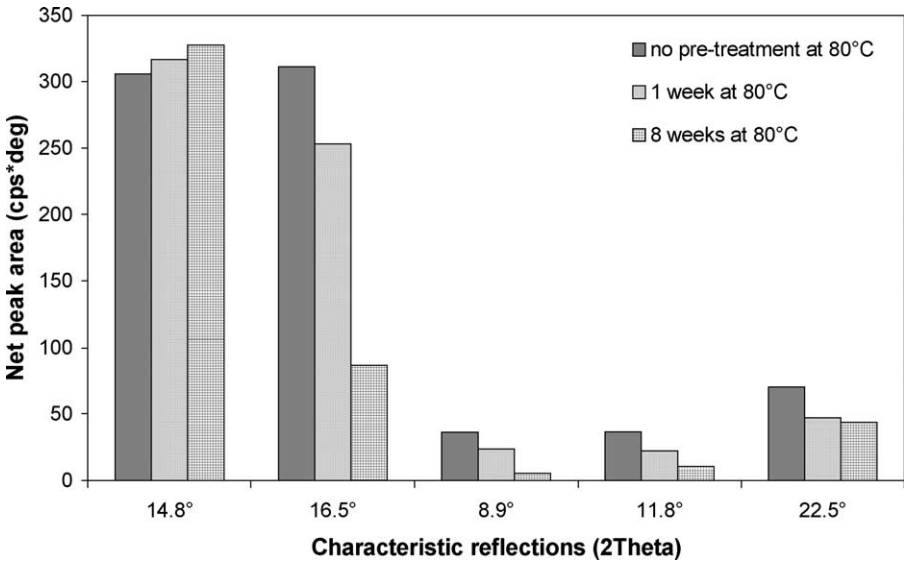

Fig. 15. Results of the examination of patches pre-treated at 80°C by XRPD after a storage period of 21 months at room temperature (standardisation on matrix area weight of 100 g/m^2, constant layer thickness).

be attributed to the storage at the high temperature rather than to water evaporation. This conclusion was supported by measurement of the water content of a pre-treated patch by the Karl Fischer method. After 2 weeks at 80°C the water content of the matrix decreased from 0.75 to 0.68%. The influence that can be expected by this small water loss on the drug solubility is very low: Due to the reduction in water content the polymer concentration in the patch would increase by 0.07%. Assuming an exponential increase in the solubility with a linear increase in polymer concentration [39] an increase in solubility of about 0.02% for NEA and less than 0.01% for E2-hemihydrate was calculated based on the previously determined drug solubility in the matrix [10,11]. This small increase in solubility caused by the water loss is much lower than the increase observed after heating and, therefore, cannot be responsible for the inhibition of crystallisation after annealing.

Visual comparison of the patches pre-treated for 1 week at 80°C to those without 80°C pre-treatment did not show any difference in the crystal formation after storage for 21 months at room temperature, i.e. equally dense crystal carpets were visible. However, a pre-treatment of 8 weeks at 80°C reduced the crystal formation significantly. Only a few star-shaped crystals were formed in the matrix.

The results of this visual test were quantitatively checked by XRPD. It was found that the majority of analysed reflection net areas strongly decreased the longer the pre-treatment period was extended (Fig. 15). However, the degree of this decrease varied for the different peaks. The reflection at 14.8° 2θ did not change its intensity

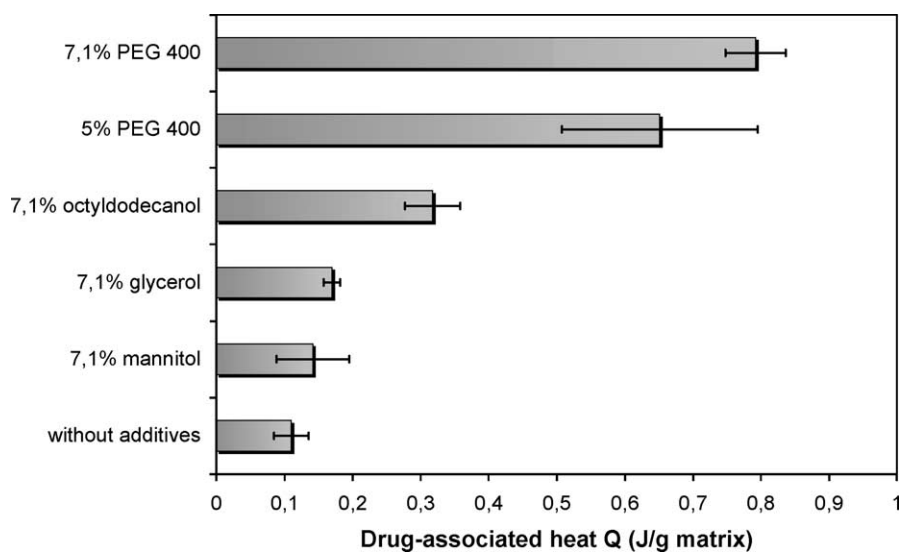

Fig. 16. Microcalorimetric measurement of crystallisation in TDDSs containing 0.75% E2-hemihydrate and 8.7% NEA in using different additives acting as crystallisation initiators at 25°C (time period of 7 days).

whereas that at 16.5° 2θ decreased considerably. The average of the peak area reduction obtained from reflection areas was calculated to amount to 30% for the 1 week pre-treatment at 80°C and to 50% for the pre-treatment period of 8 weeks. In conclusion, even the pre-treatment for one week at 80°C has a low inhibitory effect on the drug crystallisation process in transdermal patches for long time storage at room temperatures.

The use of the high temperature pre-treatment is restricted by the decomposition of the drugs. The main decomposition products were 6β-hydroxy-NEA for NEA and Δ9(11)-estradiol for E2. Formation of these products increased within 1 week at 80°C by about 0.29% for 6β-hydroxy-NEA and 0.14% for Δ9(11)-estradiol, i.e. to a greater extent than after 1 year storage at room temperature [10,11]. After 8 weeks at 80°C an increase of 2.5 and 0.5%, respectively, was measured.

3.5. Effect of additives on the crystallisation of E2-hemihydrate and NEA

As previously observed crystallisation is altered by addition of different excipients [9,12,15]. The required test period needed for determining the induction time for the generation of crystal germs by microscopy could be very long. Therefore, examination periods of up to 10 months are necessary for a reliable determination of the stability [15]. However, with microcalorimetry a drug-associated crystallisation heat was already detectable within 7 days. The measured heat increased with more pronounced crystallisation processes.

Patches containing 0.75% E2-hemihydrate, 8.7% NEA, and different additives were examined over 7 days by microcalorimetry (Fig. 16). PEG 400 was found to be the most potent crystallisation initiator. A higher content of PEG 400 led to a more pronounced crystallisation probably due to its hydrophilic properties which can reduce the saturation solubility of the lipophilic steroid hormones. The order in ability to initiate crystallisation of NEA and E2-hemihydrate was determined to be mannitol < glycerol < octyldodecanol < PEG 400.

The visible observation of the different laminates after 12 months at room temperature confirmed the microcalorimetric results. The PEG 400-containing laminates showed the most dense crystal carpet and the shortest induction time (Table 1), whereas less crystals were seen in the octyldodecanol-containing laminates. TDDS containing no additives exhibited approximately the same crystallisation behaviour as the systems containing mannitol and glycerol.

Table 1
Induction times for the crystallisation process within TDDS containing 0.75% E2-hemihydrate and 8.7% NEA in using different additives observed by polarisation microscopy

Additives	Microscopically determined crystal induction times (weeks)
Mannitol	>12
Glycerol	6
Without	6
Octyldodecanol	2
PEG 400	1

4. Conclusion

Crystal structure and formation in TDDSs containing a mixture of E2-hemihydrate and NEA were qualitatively and quantitatively analysed by microcalorimetry, polarisation microscopy, and XRPD. A mixture of E2-hemihydrate and NEA altered the crystal structure of the generated crystals compared to that of the pure substances and led to the formation of a new crystal structure. Even low E2-hemihydrate concentrations caused pronounced alterations in crystal shape and XRPD reflection pattern. In addition, the crystallisation process was considerably accelerated by the combination of the two steroids in the TDDSs and by increase in supersaturation.

Microscopy represented the most sensitive although a slow method for the detection of crystallisation process of drugs in TDDSs. In contrast, microcalorimetry was found to be a time-saving and effective method to examine the influence of different additives as well as the influence of pre-treatment of the laminates at 80°C on this process. Microcalorimetry enabled the classification of additives for their efficiency to initiate the crystallisation in the patches within 7 days whereas several months would be necessary to obtain similar reliable results with polarisation microscopy. Pre-treatment at 80°C represents a good possibility to stabilise TDDS against crystallisation.

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