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Research paper

Crystallisation of estradiol containing TDDS determined by isothermal microcalorimetry, X-ray diffraction, and optical microscopy

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Dedicated to Prof. Dr. Dr.h.c. Bernd W. Müller on the occasion of his 60th birthday.

Abstract

Transdermal drug delivery systems (TDDS) enable a controlled delivery of drugs to the skin. However, it is still a problem to achieve a stable and prolonged constant drug release. To attain high permeation rates across the skin, the concentrations of the drug dissolved have to be high and often create supersaturated, thermodynamically metastable, or unstable systems that possess a high tendency to crystallise.

In the present study, microcalorimetry as well as polarisation microscopy and X-ray powder diffraction (XRPD) were used to characterise the growing crystal germs of estradiol (E2) hemihydrate. Polarisation microscopy enabled the observation of crystals with two different morphologies of E2 in the polymeric acrylic transdermal patch matrix. Crystal formation and growth were also detected by XRPD. The diffraction pattern corresponded to estradiol hemihydrate. The intensity of the observed reflections was proportional to the crystal quantities and increased during storage. A high supersaturation resulted in high peak intensities caused by a high crystallisation rate.

Since precipitation is generally accompanied by heat evolution, crystal germ formation, and crystal growth could easily be detected early by isothermal microcalorimetry. Much lower amounts of crystal were detected by this method than with the significantly less sensitive XRPD method. Microscopy was equally sensitive to but much more time-consuming than microcalorimetry.

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

1. Introduction

Hormone replacement therapy represents a frequent application for transdermal drug delivery systems (TDDS). TDDS enable a continuous delivery of controlled doses of drugs at constant rates to the human skin. The drugs are mostly dissolved in the adhesive matrix of the TDDS.

Skin represents a strong barrier against the penetration of external drugs. To achieve high permeation rates and to attain therapeutic dose levels, it is necessary to overcome the barrier properties of the stratum corneum. Therefore, it is useful to employ physical or chemical penetration enhancers [1,2]. However, chemical enhancers may have irritant effects to the skin whereas the application of methods, such as ultrasound, iontophoresis, or electroporation often may be too unwieldy and expensive for common use. The increase of the concentration of the dissolved drug is a good and reliable possibility to enhance drug permeation through the skin which does not damage the integrity of the stratum corneum at all.

Mostly a linear relationship exists between drug content and drug permeability resulting in an increased drug flux with increasing thermodynamic activity [2–7]. The concentration of the active drug that is needed to release a therapeutic dose through the skin, therefore, often is close to saturation or even at supersaturated concentrations. However, such transdermal patches are generally thermodynamically unstable because the drug shows the tendency to recrystallise during storage [8-10]. Consequently, transdermal drug absorption may be altered negatively [11,12]. If the drug exists as a suspension or precipitate,

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its flux becomes independent of the administered concentration [2,13] because the flux from saturated systems remains constant [3,4].

In some cases, it is possible to use antinucleant polymers or other additives to stabilise the supersaturated patches [6,7,12–15]. The success of the prevention of the crystallisation process of the dissolved drug by the addition of excipients depends on the ability to inhibit nucleation and crystal growth. Some additives can even accelerate the crystal growth and may act as crystallisation initiators [14], for instance PEG 4000 and 8000 for estradiol [5].

Consequently, for the development of a new TDDS formulation, it is desirable to have an analytical method that can measure the process of crystallisation rapidly and reliably to decide about long-term stability. The often time-consuming test methods for formulation optimisation may be minimised by the employment of thermoanalytical methods.

It is easily possible to measure even small heat quantities with isothermal heat conduction microcalorimetry [16]. Heat evolution or absorption result from an ongoing process, which is mostly caused by the instability of the existing system. Since the crystallisation process is associated with the generation of heat [17–31], it should be possible to measure this in transdermal patches. The energy evolved during the transformation of amorphous material to its crystalline state is sufficiently large enough to be easily detected and can accurately be measured by a microcalorimeter. This may be possible at relatively low temperatures, but often stability studies under accelerated conditions are necessary.

This study was performed to characterise estradiol crystals formed in a transdermal patch during the storage process using and comparing different methods, such as microscopy, X-ray diffraction, and microcalorimetry.

2. Materials and methods

2.1. Materials

Micronized 17β-estradiol hemihydrate (E2–hemihydrate), polyethylene glycol 400 (PEG 400), pressure-sensitive acrylate adhesive dissolved in ethyl acetate (Durotak® 387-2287, National Starch and Chemical B.V., Zutphen, Netherlands), backing membrane (Hostaphan® RN 23), release liner (Hostaphan® RN 100) as well as the semi-permeable membrane Cotran® 9728 were obtained as a gift from Lohmann Therapiesysteme LTS (Andernach, Germany). The Hostaphan foils (Mitsubishi Polyester Film GmbH, Wiesbaden, Germany) consist of polyethyleneterephthalate (PET) and the number accompanying these foils indicate their thickness in micrometers. Hostaphan® RN 100 is siliconized on both sides for better removal. Semipermeable Cotran® 9728-membrane (3M Drug Delivery Systems, Borken, Germany), in contrast, consists of poly(ethylene-co-vinylacetate) containing 19% vinylacetate and having a thickness of 50.8 μm.

The solvents ethyl acetate, methanol, and tetrahydrofurane (THF) were provided by Merck Eurolab (Darmstadt, Germany). Deionized water was made by Ultra-pure Water System Milli-Q plus.

2.2. Preparation of TDDS

The TDDS consists of a self-adhesive acrylic matrix, which contains different concentrations of 17β-estradiol hemihydrate. A small content of 7.1% PEG 400 was incorporated as plasticizer.

Transdermal patches were prepared by the solvent evaporation technique: the acrylic polymer was dissolved in ethyl acetate. E2–hemihydrate and PEG 400 were added and mixed to homogeneity by stirring with a blade stirrer at 700 rpm. The solution was coated onto polyester foil Hostaphan® RN 100 by using a 550 μm casting knife. After coating, the systems were dried at room temperature for 15 min, followed by a 15 min drying period at 80°C in an oven to remove the residual organic solvents. Finally, two dried films were combined to form a two-layer sheet (double layer technique). One Hostaphan® RN 100 foil was removed and the dried film was then laminated by a rubber roller onto the backing membrane Hostaphan® RN 23. The primary coatweight was about 140 g/m^2, meaning that a total coatweight of 280 g/m^2 for double layer technique was achieved. These two-layer laminate films were stored at room temperature.

To determine the saturation concentration of E2–hemihydrate in the acrylic matrix, special two-layer laminates which were separated by a semi-permeable Cotran® 9728 membrane were prepared. One layer contained the drug while the other one consisted of a drug-free acrylic polymer patch. These laminates were also stored at room temperature so that the dissolved drug molecules could diffuse to the drug-free side until saturation was achieved. Excessive drug crystallisation occurred on the drug side confirming supersaturation. After reaching the equilibrium of concentration, the initially drug-free system was removed from the Cotran® 9728 membrane and the concentration of E2–hemihydrate was determined by high-performance liquid chromatography (HPLC).

2.3. Polarisation microscopy

The laminates were examined visually as well as with a polarisation microscope for the presence of E2 crystals at predetermined time intervals. The microscopy was carried out with a Carl Zeiss Standard-microscope (Oberkochen, Germany) at a magnification factor of 80 ×. The polarizer and analyser were adjusted to crossed positions to yield the greatest contrast between the crystals and the matrix. A quartz crystal disc coloured the microscopic picture by a wavelength shift. The semi-crystalline backing membrane

and the release liner were removed before any investigation to determine the presence of drug crystals.

2.4. Microcalorimetry

Small disks of a diameter of 10 mm were punched out immediately of the manufactured laminates for the microcalorimetric examination. They were placed like a rouleau in a 4 ml stainless steel ampoule and weighed. The ampoules were completely filled to avoid any vapour above the patches and a resulting effect of humidity on crystallisation processes. Ampoules were hermetically closed with a screw cap as well as with a Teflon disk and an empty ampoule of the same type was used as reference vessel. The investigations were carried out at 25°C with the isothermal heat conduction microcalorimeter TAM 2277 (Thermal Activity Monitor, model 2277, Thermometric AB, Sweden). The ampoules were equilibrated in the calorimeter for 45 min before slowly lowering into the measuring position. The data collection program DIGITAM was started at the moment when the ampoules reached this position. The heat flow (dQ/dt) was measured and recorded over a 7-day period. To be able to eliminate the inherent baseline drift during the test cycles, a linear drift was assumed. Analysis of the results included baseline correction and normalisation to a polymeric matrix mass of 1 g.

The E2–hemihydrate containing system as well as the placebo were measured against an empty reference ampoule. By subtracting the measured heat flow of the placebo system from that of the examined sample, the drug-associated rate of heat generation was obtained.

The area under the drug-associated heat flow-time curve was referred to the crystallisation process. Exothermic heat flow signals are presented as positive values in this paper. The evolved heat of the crystallisation peaks was averaged over at least three experimental runs.

2.5. X-ray analysis

X-ray powder diffraction (XRPD) method was used to characterise crystals in TDDS. This is possible because characteristic X-ray reflections due to the drug appear above the amorphous background of the polymer [10,12]. Subtraction of this background for all samples was performed using the EVA software program. The background corrected integrated intensities of reflections were used for comparisons of the crystallinity. The quantitative analysis is based on the assumption that changes in peak areas of characteristic reflections are directly proportional to the crystal concentration. It was found that with this method crystalline material could be detected at a concentration of $\geq 2\%$ [32].

The X-ray diffraction patterns were recorded in transmission mode by a Guinier diffractometer (Huber, Germany) using SiO_2-monochromatized Cu $K\alpha_1$-radiation of a wavelength of $\lambda = 1.5406$ Å. The X-ray tube with a copper anode was operated at 40 kV and 25 mA. The 2θ scans were performed between $7° \leq 2\theta \leq 30°$ (stepwidth 0.01° θ in 5 s). For examination, the polymer films were pasted on a sample holder.

2.6. HPLC analysis

HPLC analysis of E2–hemihydrate 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 220 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 was made by threefold injection of a standard solution containing 0.2 mg/ml E2–hemihydrate. The calibration coefficient amounted to 0.3035 ± 0.0154 mV min ml/μg using an injection volume of 20 μl. The retention time of E2–hemihydrate was 30 min. The same method was used to detect possible decomposition products of E2–hemihydrate, i.e. estriol, Δ9(11)-estradiol, estron, and 17α-estradiol, after storage at 25°C. The relative retention times compared to E2–hemihydrate were 0.31, 0.64, 1.06, and 1.16, respectively.

3. Results and discussion

3.1. Polarisation microscopy

The TDDS laminates were amorphous and crystal-free immediately after the manufacturing process. The onset of the crystallisation in the adhesive matrix was observable visually as well as by polarisation microscopy after a few days or after some weeks of storage depending on the drug concentration (Fig. 1). As expected, the time up to the beginning of crystallisation (nucleation time) decreased and the quantity of crystals increased with higher E2–hemihydrate contents. Accordingly, the density of the crystal carpet also increased during storage. Because only a few crystals could be observed microscopically in the 1.5% E2–hemihydrate containing patches, one can assume a saturation concentration near 1.5% in the polymeric matrix.

At low concentration, the E2–hemihydrate formed mainly prismatic crystals (Fig. 1a) whereas at higher concentration, needles were prevalently formed (Fig. 1c–f). At even higher concentrations, these needles formed clusters (Fig. 1e–f) but prismatic forms were still present. The previous work of Variankaval et al. [9] also identified different crystal forms of E2 in the transdermal patches by IR measurements. Therefore, they assumed that

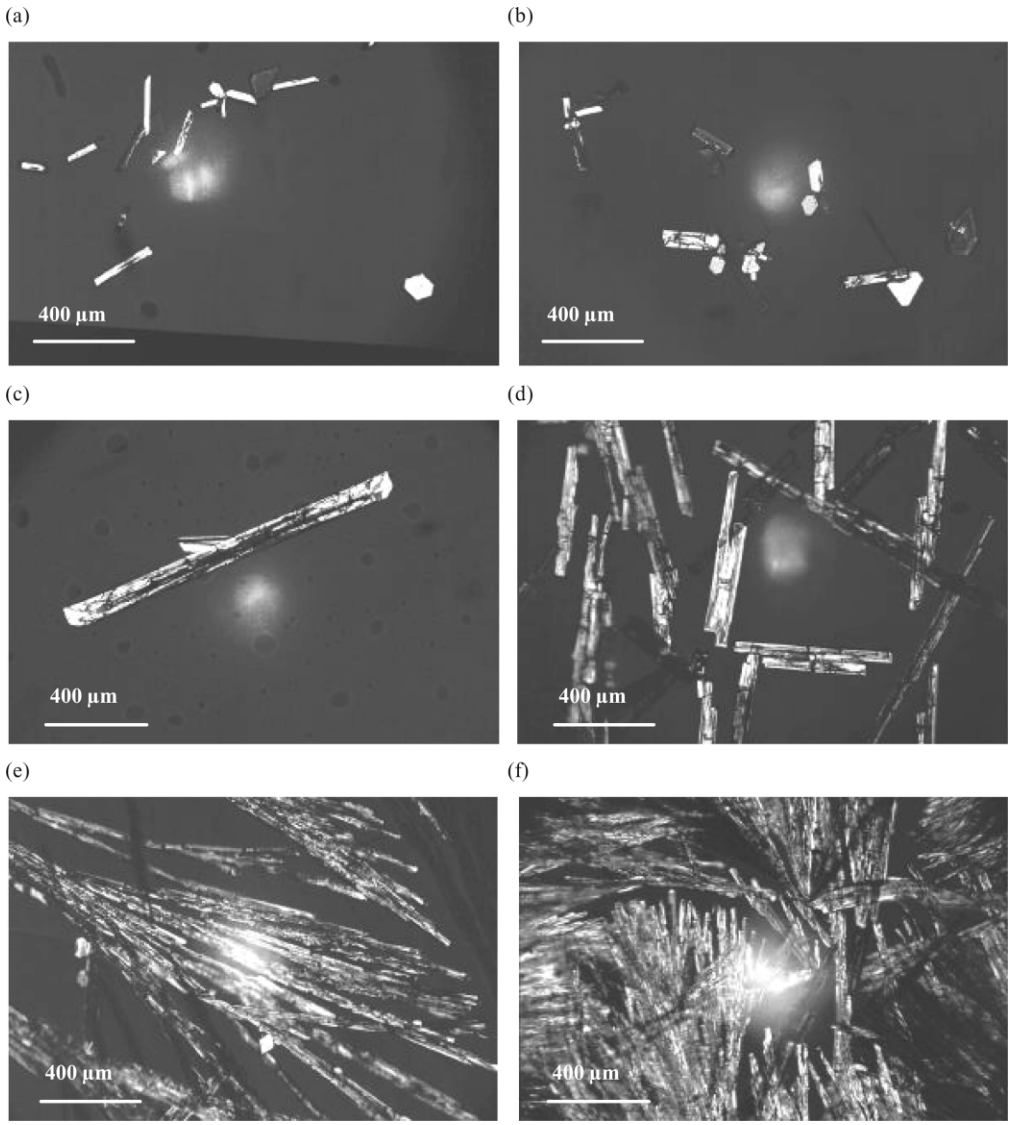

Fig. 1. Polarisation microscopy of estradiol containing TDDS with increasing content of E2–hemihydrate, storage at room temperature: (a) 1.5%, 6 months; (b) 2%, 3 months; (c) 3%, 8 weeks; (d) 4%, 8 weeks; (e) 5%, 8 weeks; (f) 6%, 8 weeks.

the needles were a result of co-crystallisation of estradiol with matrix material.

3.2. XRPD

In the present study, crystals formed in the polymeric matrix during storage showed the same X-ray diffraction pattern as pure E2–hemihydrate (Fig. 2c–h). The polymeric matrix favours the growth of the most stable physical form of crystalline E2–hemihydrate [10]. Therefore, we assume that the same crystal lattice of drug was formed regardless of whether it was in the adhesive matrix or in the pure solvent. We could not detect any evidence that the matrix had an effect on the crystal structure. If the polymer would have been incorporated into the crystal, it would have significantly changed the crystal lattice and altered the reflection positions. This view is supported by the findings of Iervolino et al. [7] who described that hydroxypropylmethylcellulose (HPMC) molecules were not included into the ibuprofen crystals because of their incompatibility in size.

Since the adhesive matrix was amorphous, it did not produce a distinct X-ray pattern and therefore did not interfere with the pattern of E2. Because of the relatively low sensitivity of XRPD to detect small quantities of crystalline material in the amorphous matrix, it was not possible to measure the small amounts of drug that led to the formation of exclusively prismatic crystals. Patches containing 1.5%, respectively 2%, of E2–hemihydrate appeared completely amorphous by XRPD even though some prismatic and quadratic crystals were observable in the microscope after 3–4 weeks or 1 week, respectively, of storage. A prismatic morphology for E2 crystals was described for the anhydrous crystal form [33] as well as

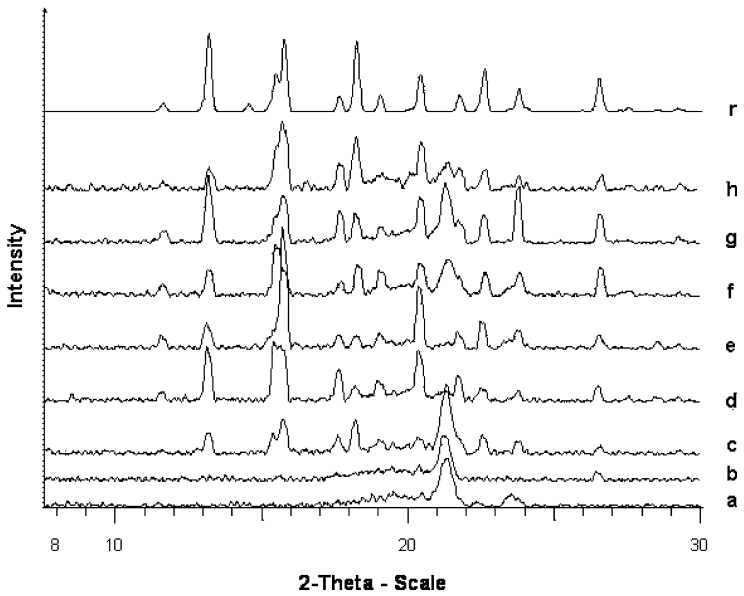

Fig. 2. XRDP pattern (after correction of the amorphous background) of 6% E2–hemihydrate containing TDDS storage at room temperature: (a) 0 day; (b) 1 week; (c) 2 weeks; (d) 4 weeks; (e) 6 weeks; (f) 8 weeks; (g) 3 months; (h) 4 months; (r) pure E2–hemihydrate as reference. The peak appearing at approximately 21.3° 2θ was caused by sample holder foil.

for E2–hemihydrate [34,35]. However, crystal morphology can change within the polymeric matrix by attachment of polymeric molecules to the crystal sides via hydrogen bonding. If the degree of adsorption at different crystal sides is different, crystal morphology changes, since the morphology is determined by the slowest growing faces [15].

In conclusion, X-ray diffraction enables the detection of crystal formation in the laminates at a total drug concentration of approximately 3% and above in the patch. However, since E2–hemihydrate crystals were detectable at a concentration of 1.5% by optical microscopy and, presuming a saturation concentration of 1.5% E2–hemihydrate, it has to be assumed that at this total drug concentration of 3% in the matrix, 1.5% of the E2–hemihydrate remains dissolved (corresponding to 50% of total drug). Hence, the detection limit of XRPD lies much lower than previously assumed [36], i.e. at 1.5% crystallinity relative to total mass of drug and matrix.

However, while microscopy does not allow a quantitative evaluation of the formed crystals, it is possible to determine semi-quantitatively the amount of crystallisation by XRPD on the basis of increasing reflection intensities with ageing [37].

E2–hemihydrate shows some typical reflections that can be used to determine the crystal quantities in the amorphous matrix – for instance 13.1° 2θ, 15.7° 2θ, and 26.5° 2θ. The twin peak at 15.7° 2θ possesses the greatest net peak area of all characteristic E2–hemihydrate reflections (after subtraction of amorphous underground) and therefore the highest sensitivity, respectively, the lowest error (Fig. 2). This twin peak is especially pronounced in the reference, i.e. pure E2–hemihydrate (Fig. 2r).

Fig. 2a–h shows the crystallisation behaviour of one batch of a 6% E2–hemihydrate containing patch at room temperature. The reflection pattern was well reproducible when measuring different cutouts of the same laminate at one time point but the intensity could differ subject to the grade of crystallisation because of the low measuring area. At the beginning of crystallisation, greater differences exist than using completely recrystallised patches. Crystallisation is a random process and the crystallisation behaviour within the polymeric matrix is not equal within all parts of the laminates.

The reflection areas increased continuously with storage time until completion of crystallisation (Fig. 3). The crystallisation process seems to be retarded at lower drug concentration. Taking into account that XRPD shows a detection limit of 1.5%, low supersaturated patches have to be crystallised nearly completely to detect reflections whereas high supersaturations reach the detection limit of crystallinity at a faster rate. Nearly all patches investigated with XRPD except the system containing 8% E2–hemihydrate exhibited a crystallisation inhibition for some weeks after manufacturing until detectable crystals were formed. The induction time, which is the time required to form crystal germs of critical size before the growth can begin, decreases with increasing degree of saturation [7,15]. Nucleation and crystal growth accelerated at high drug concentrations followed by a faster crystallisation cessation. Patches containing $\geq$6% of E2–hemihydrate within the laminate reached the maximum peak area (steady state) after a short time of 2–4 weeks, for concentrations of $\leq$4% a slow and nearly linear increase (slow growth) was observed (Fig. 3). No crystallisation occurred after 3 months or even longer time periods.

The net X-ray reflection areas show strong fluctuations

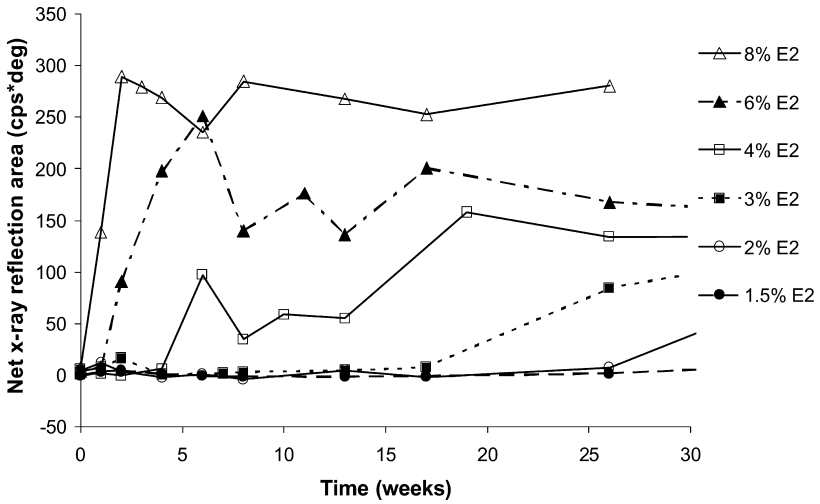

Fig. 3. X-ray net peak area for the characteristic E2–hemihydrate reflection at 15.7° 2θ in dependence of E2–hemihydrate content in the patch and storage time at room temperature (standardisation on matrix area weight of 100 g/m^2, constant layer thickness).

with ageing because of the random location of the crystallisation nuclei in a comparatively small observation area. Therefore, the data evaluation was performed as follows. The theoretical peak area after 13 weeks was calculated by linear, respectively, logarithmic regression analyses of the measured XRPD data (Fig. 4). A nearly linear correlation of the calculated net peak area of the E2–hemihydrate reflections was found with rising estradiol content above a concentration of 2%. The point of intersection with the axis of abscissae lies between 2 and 3% of E2–hemihydrate and represents the saturation concentration. The determined saturation solubility for E2–hemihydrate was higher than that determined by microscopy due to the lower sensitivity of XRPD.

3.3. Microcalorimetry

The results of polarisation microscopy were confirmed by isothermal heat conduction microcalorimetry. The measurable drug-associated heat flow increased with rising drug concentration (Fig. 5). While a content of 1.5% E2–hemihydrate did not show any measurable drug-associated heat flow, a minimal flow of 0.1 μW/g matrix weight was observed for patches containing 2%. The sensitivity of microcalorimetry appears to be useful for the quantification of small amounts of crystal formation, even if only some prismatic or needle-like crystals could be observed with the microscope. Nevertheless, microscopy was more sensitive and specific than microcalorimetry. For TDDS containing 1.5% E2–hemihydrate, the first crystals appeared after 3–4 weeks as observed with the microscope, but no drug-associated heat flow could be detected. Despite the lower sensitivity of microcalorimetry, crystallisation processes at concentrations above 2% are more rapidly detected than by microscopy. Already after 1 day, it is possible to decide about instability at these concentration levels by microcalorimetry, whereas by microscopy crystals often are visible only after 1 or more weeks. Therefore, in the case of

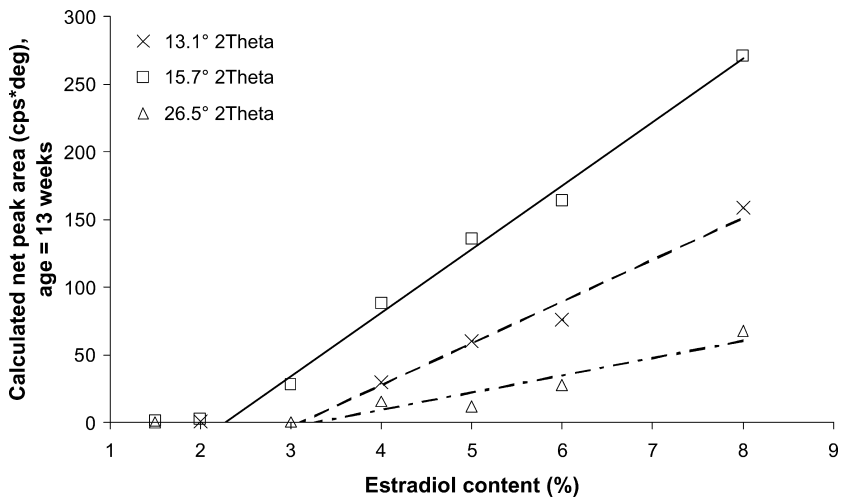

Fig. 4. Dependence of the calculated net peak area after 13 weeks of storage on the E2–hemihydrate content.

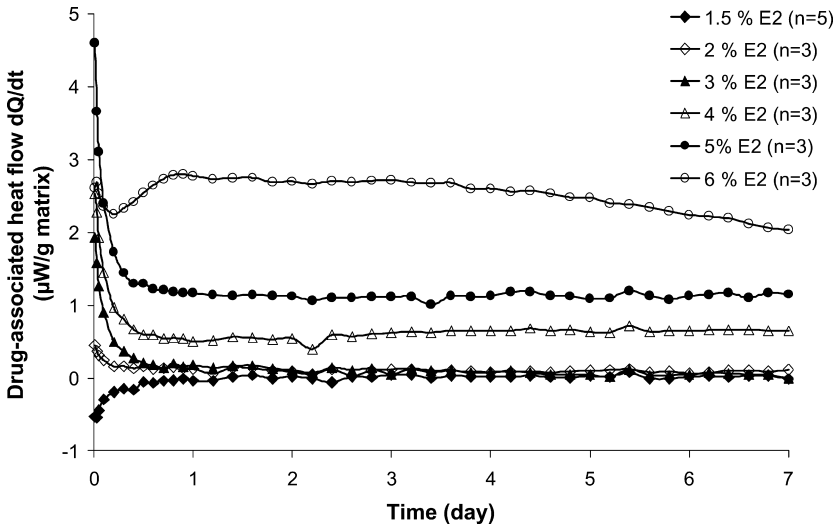

Fig. 5. Concentration-dependent drug-associated heat flow of E2–hemihydrate containing TDDS (25°C).

no measurable heat at low concentration flow, additional microscopical investigations are necessary.

The microcalorimetric data point to a saturation concentration of the E2–hemihydrate in the patch of between 1.5 and 2% due to the lower sensitivity compared to microscopy. Nevertheless, the sensitivity of microcalorimetry is still greater than that of XRPD.

The drug-associated heat flow signals shown in Fig. 5 are very low and correspond to 1 g patch matrix. However, signals measured in the microcalorimeter were considerably higher since the total matrix mass in the measuring cells amounted to 2.5 g matrix and the placebo heat flow was included in the signal.

The drug-associated heat flow is due to crystallisation because there was no significant chemical decomposition detectable over a 1-year period at 25°C. The main decomposition products of E2–hemihydrate, estron, estriol, and 17α-estradiol, were not detectable during this time period. Only the fraction of Δ9(11)-estradiol, however, increased by about 0.1%/year.

For microcalorimetry, discs of 10 mm diameter had to be punched out with a metal punch. As observed by microscopy, crystallisation was stronger around the edges of the resulting cut, which was caused by the shear force during the punching operation [8]. Microscopic analysis confirmed that in areas of strong mechanical force (like cut edges), crystallisation was happening more vigorously resulting in a high initial heat flow. The crystal germs formed by the cutting process began to grow fast. After this, the crystallisation rate and correspondingly the drug-associated heat flow decreased (Fig. 5).

Laminates containing 6% E2–hemihydrate exhibited a broad heat flow maximum at $t = 1$ day (Fig. 5). High supersaturations promote crystal germ formation and following crystal growth. Therefore, the potent formation of crystal germs inside the patch was observed as this separate maximum. The more crystal nucleation took place within the sample and hence the more heat was generated within the first day during nucleation, the larger was the maximum.

The first 24 h were strongly affected by cutting effects. Therefore, the total heat quantity was calculated from correlation functions of the time interval of 1–7 days. Integration of these functions resulted in total heat Q (in joules), and extrapolation to the zero-point yielded to the initial drug-associated heat flow $dQ_{t=0}/dt$ (μW). The values demonstrate an increasing drug-associated heat and a rising drug-associated initial heat flow for patches with increasing drug content in agreement with microscopic pictures and X-ray diffraction measurement (Fig. 6). This increase was disproportionate to E2–hemihydrate concentration.

In theory, a linear correlation between drug content and evolved heat above saturation of polymeric matrix should exist, since a constantly increasing quantity of supersaturated drug should crystallise. However, linearity was not observed over a measuring period of 7 days because crystallisation is not complete within this short time.

Crystallisation was accelerated with rising drug content and also finished earlier. The reason presumably was the increasing thermodynamic activity of the strong supersaturated patches. Within 7 days, a greater quantity of total crystallisation heat occurred to systems with a higher drug content and consequently stronger supersaturation corresponding to a higher crystallisation rate. This may explain the overproportional increase in heat and initial heat flow (Fig. 6).

The theory of linear correlation between concentration and heat, however, was shown by microcalorimetric measurements of supersaturated patches over a long time period of 5 weeks (Fig. 7). Heat flow did not fall to zero within the measuring time but to a constant low heat flow signal. Assuming that all patches after this time delivered the same heat, this constant plateau was taken as the end point. This constant heat flow could be attributed to the

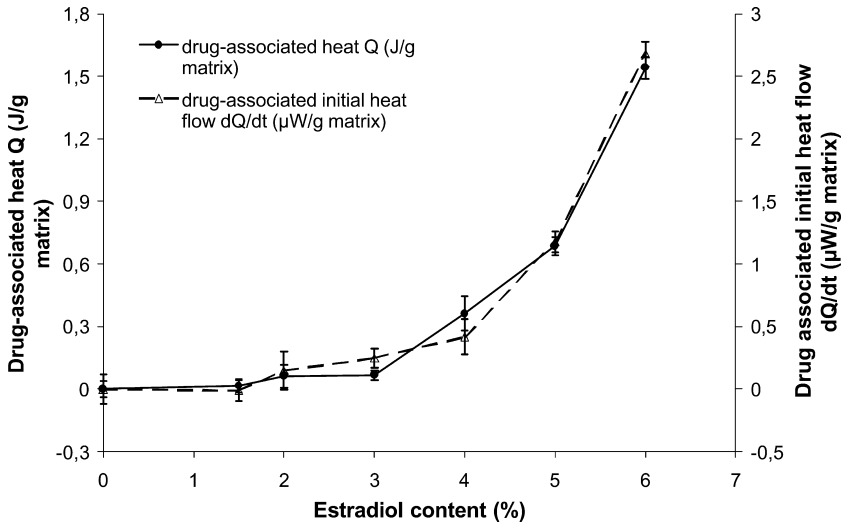

Fig. 6. Correlation between calculated 7-day drug-associated heat and initial heat flow in dependence of E2–hemihydrate content (25°C).

placebo system and was corrected to zero after a measuring time of 35 days by subtraction of the heat flow of the placebo system. However, for a longer measuring time, inaccuracy increased. Low fluctuations in baseline influence the result, and fluctuations are not always linear over a measuring time of some weeks.

Nevertheless, the measured heat values showed a linear correlation. The slope of the straight line resulted in a crystallisation enthalpy of E2–hemihydrate of -23.3 ± 1.2 kJ/mol in the acrylic matrix containing 7.1% PEG 400.

Drug crystallisation started with the formation of small germs. These were not detectable by XRPD but resulted in high heat flow signals. Therefore, no X-ray reflection areas were measured after 1 week of storage, whereas crystallisation generated high heat flow signals that were measurable by microcalorimetry. XRPD possessed a low accuracy when only a low percentage of crystalline material was present. Nevertheless, the reflection area–time functions enable the extrapolation to the theoretical net area of the reflections after 1 week of storage. By comparing the 7-day heat quantity with theoretical X-ray diffraction reflections, a correlation is possible but is not very high. Both techniques seem to record the same incidence of crystallisation, but the sensitivity, accuracy, and beginning of crystallisation differ.

3.4. HPLC

The saturation concentration of E2–hemihydrate in the patches was determined by HPLC after equilibration between a supersaturated patch containing E2–hemihydrate and an initially drug-free patch, mounted together but separated by a semi-permeable membrane. With this technique using a 6% E2–hemihydrate laminate, a saturation solubility of the E2–hemihydrate in the patch of 0.6% was obtained whereas with the 4 and 5% systems, an average solubility of 1.65% was measured (Table 1). The

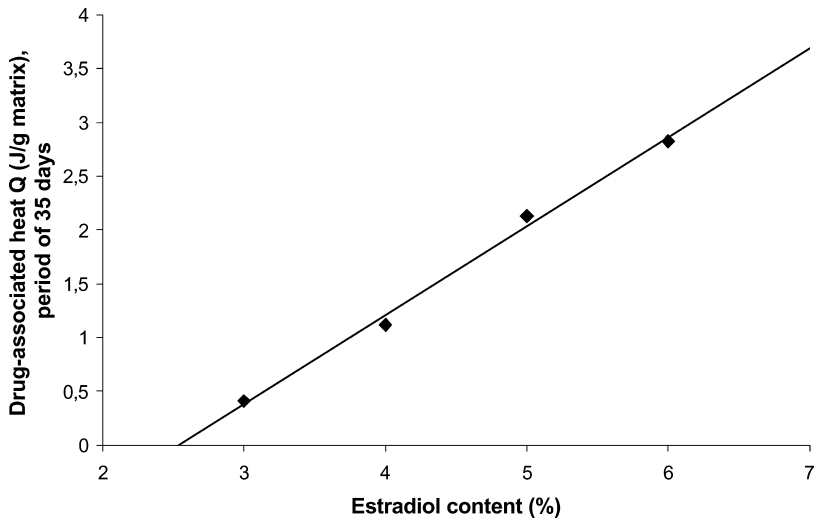

Fig. 7. Linear correlation between drug-associated heat in dependence of E2–hemihydrate content over a long measurement period of 35 days (25°C).

Table 1
Saturation concentration of E2–hemihydrate in the polymeric matrix determined by HPLC (time of storage: 2 months)

E2–hemihydrate content at time zero (%)	E2–hemihydrate content of the placebo layer after storage (%)
4	1.7
5	1.6
6	0.6

6% E2–hemihydrate laminate crystallised very quickly, i.e. much more rapidly, whereas with the 4 and 5% systems, the supersaturated state continues for a relatively long time. As a consequence in the 6% system, the supersaturated state in the matrix disappeared much faster. There are two different methods to determine the saturation solubility – dissolution of an excess of solid drug under solubility conditions (unsaturated solution with crystals) or crystallisation from a supersaturated solution. The second method generally yields values which are larger than the true solubility C_{sat} owing to solution equilibrium [28]. The resulting relative supersaturation $(C - C_{sat})/C_{sat}$ of a factor of about 1.5–2 is called 'dead zone'. The observed differences, i.e. 0.6 vs. 1.65% result in a relative supersaturation of a factor of 1.75 which fits very well into the borders of the dead zone 1.5–2. Since the solubility value of 0.6% for E2–hemihydrate in the patches did not change even after longer storage times, this value can be assumed to represent the true solubility.

4. Conclusion

This study demonstrates that crystallisation processes in TDDS can be detected by isothermal heat conduction microcalorimetry over a 7-day period. In contrast, the microscopical analysis of crystallisation processes as well as X-ray diffraction often requires a much longer time to detect formed crystals.

The measurable drug-associated heat quantity increased with rising drug concentration but was not directly proportional to E2–hemihydrate content. This may be caused by accelerated crystallisation. The higher the drug concentration in the laminate, the stronger was the tendency to crystallise. In addition, formation of E2–hemihydrate crystals and the completion of crystallisation were faster with higher drug concentrations. The sensitivity of microcalorimetry for the detection of crystallisation was somewhat lower than microscopy but better than XRPD.

The XRPD method represents an analytical method with the lowest sensitivity to identify crystals of organic matter in the amorphous matrix of TDDS: while small crystals often could be seen visually in the laminate films, still no reflections were measurable by XRPD. On the other hand, while microscopy and microcalorimetry were more sensitive and able to measure very small quantities of crystalline material, XRPD represented a very crystal-specific semi-quantitative method.

Despite the differences in the analytical methods used to determine the saturation concentration in the polymeric matrix, a good agreement in the determined values under crystallisation conditions existed. The concentration at which crystallisation of E2–hemihydrate started to occur amounted to 1.5–2%. High humidity in the polymeric matrices promotes E2–hemihydrate crystallisation due to its very low aqueous solubility [11]. It is well known that E2 containing patches may be stabilised against crystallisation processes by efficient reduction in water content [10]. Therefore, improved drying conditions can lead to a stabilisation of the patches against crystallisation [8] possibly caused by the formation of an anhydrous crystal form with a greater solubility. Therefore, the determined values only refer to the crystallisation of E2–hemihydrate at ambient humidity.

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