ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 47 (1999) 133–138

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

Use of X-ray crystallography for the characterization of single crystals grown in steroid containing transdermal drug delivery systems[1]

Ralph Lipp*, Anke Müller-Fahrnow

Schering AG, Berlin, Germany

Received 2 March 1998; accepted 13 May 1998

Abstract

Target of the study was to characterize crystals which had grown in steroid-containing matrix patches during short-term storage and to thereby establish a rationale for the inhibition of crystal formation in those patches in general. Matrix type transdermal drug delivery systems (TDDS) containing either 2.2% gestodene or 3.3% estradiol were free of crystals directly after their production. However, crystals of up to 800 μm in length grew during 3 months of storage at ambient temperature. The application of several analytical methods did not help to identify the crystals. This was mainly due to the fact that the adhesive matrix surrounding the crystals could not be fully removed in the course of sample preparation with routine laboratory methods and thus impaired DSC, FTIR microscopy and hot stage polarized microscopy. However, within X-ray diffractometry, the residual amorphous patch matrix did not hamper the measurement of the crystals. Thus, they were identified as estradiol hemihydrate and gestodene form I, respectively. These results suggest that steroid-containing matrix TDDS should be stabilized against drug recrystallization e.g. by the addition of suitable crystallization inhibitors. Furthermore, systems containing estradiol may be stabilized by efficient removal water.

Keywords: Estradiol; Gestodene; Transdermal drug delivery system; X-ray crystallography

1. Introduction

Transdermal drug delivery systems (TDDS) have been getting increasingly popular for the application of various drugs during the last decade. This is especially true for TDDS of the matrix type which basically consist of three main components, backing, matrix and release liner [1] (Fig. 1). The matrix in general comprises a medical-grade pressure sensitive adhesive [2] and the drug substance. Pharmaceutically acceptable excipients such as penetration enhancers and co-solvents [3] may be added as well.

Following application of matrix TDDS, the drug substance reaches the systemic circulation via passive diffusion. Therefore, the concentration gradient of the drug between the TDDS and the skin is the driving force for the flux of the drug through the skin [4]. Hence, high concentrations of the drug in the matrix lead, in general, to high drug fluxes and thus allow the administration of high dosages from relatively small, and thereby attractive, systems.

The formulation of supersaturated systems for transdermal drug application is, therefore, highly favourable from the viewpoint of system size. However, crystallization of initially dissolved drugs may occur during the storage of supersaturated systems. This general problem regarding the formulation of TDDS with drug substances bearing pronounced crystallization tendency, is especially true for matrix TDDS containing high concentrations of sex steroids, and has been reported several times in the recent past [4–6].

Such a physical instability could strongly limit the shelf-

* Corresponding author. Schering AG, Drug delivery Systems, D-13342 Berlin, Germany. Tel.: +49 30 46811692; fax: +49 30 46812435; e-mail: Ralph.Lipp@Schering.de

[1] Presented in part at the 11th AAPS meeting, San Diego, CA, USA, November 6–10, 1994.

0939-6411/99/$ - see front matter
PII: S0939-6411(98)00055-1

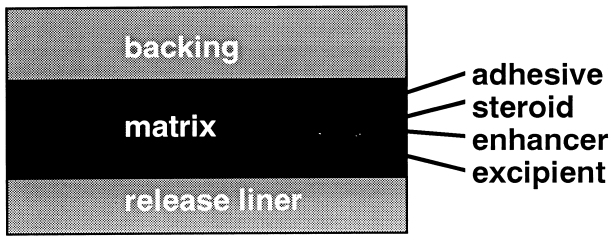

Fig. 1. Cross section of a matrix-TDDS consisting of an impermeable backing film, a removable release liner and, in between, a matrix comprising a pressure sensitive adhesive, the respective steroid and, optionally, a penetration enhancer e.g. 1,2-propanediol, as in the present study.

life of affected TDDS, since transdermal drug absorption may be altered, even after short term storage, especially if major quantities of the drug substance crystallize within the patch.

Therefore, the purpose of the present study was to identify crystals grown in prototypes of polyacrylate based matrix TDDS containing either 3.3% estradiol or 2.2% gestodene. Identification of these crystals should aid in establishing a rationale for stabilization of TDDS containing steroids at stages of supersaturation.

2. Materials and methods

2.1. TDDS formulation and production

The TDDS matrices consisted of 22% 1,2-propanediol (Hüls, Witten, Germany), 3.3% estradiol (Schering AG; Fig. 2) or 2.2% gestodene (Schering AG; Fig. 3) and polyacrylic adhesive Gelva® 788 (Solutia, Springfield, MA, USA) to make up 100%. A Saran®-Hytrel®-coextrudate was used as backing material whereas siliconized PET served as release liner (both from Bertek, St. Albans, VT, USA).

All TDDS were manufactured by applying a continuous process on a Bertek laboratory coater (Bertek, S.A.) as described before [7]. The primary coatweight was 50 g/m and, by means of double layer technique, a total coatweight of 100 g/m was achieved. From the three layered rollstock which was obtained as an intermediate, patches were diecut using 10 cm^2 dies of circular shape. Final patches were packaged into composite foil bags, heat sealed and stored at ambient temperature.

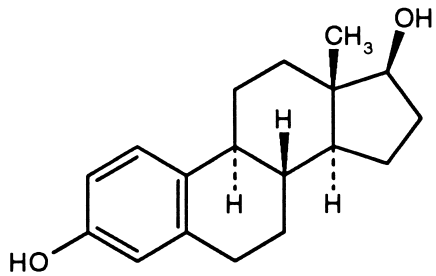

Fig. 2. Structural formula of estradiol.

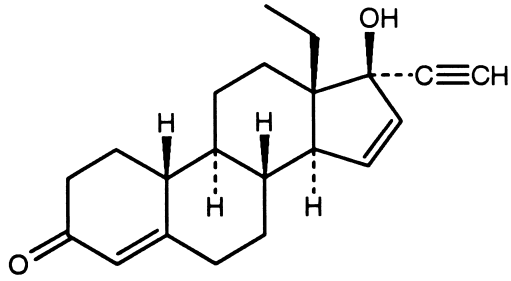

Fig. 3. Structural formula of gestodene.

2.2. Polarized microscopy

Microscopical evaluation in order to search for crystals was carried out using a Leitz Laborlux S, (Leitz, Bensheim, Germany). An entire 10 cm^2-patch was removed from the liner and applied onto an object slide of sufficient width. The examination was carried out through the slide thereby focusing the on TDDS matrix. In order to check for the absence of drug crystals (freshly manufactured TDDS), the examination was carried out with $n = 3$, scanning the whole TDDS area in each case. Polarized microscopy was also used to identify crystal-containing sections of intact, stored TDDS prior to the application of additional analytical methods to those sections.

2.3. Hot stage polarized microscopy

In the first step, polarized microscopy was used in order to search for crystals in the respective TDDS (see Section 2.2). Then, a crystal-containing circular section 4 mm in diameter was diecut from the patch, the liner was removed and the section was applied onto an object slide. A Leitz DM RB microscope (Leica, Wetzlar, Germany) equipped with a Newtronics heating device (Novocontrol, Hundsaugen, Germany) was used. The samples were heated from 30°C to 210°C at an increase of 10°C/min.

2.4. Differential scanning calorimetry (DSC)

In a first step, crystal-containing TDDS sections were identified and prepared as described in Section 2.3. Subsequently, the liner was removed and three of the circular segments 4 mm in diameter were placed in a standard aluminium container for the DSC experiment. A Mettler TA 4000 (Mettler-Toledo, Gießen, Germany) was used and the specimen described above was subjected to the same heating program as given in Section 2.3.

2.5. FTIR microscopy

In the first place, a 10 cm^2 TDDS was removed from the liner and applied onto a slide. After microscopical localization of crystals of sufficient size within the TDDS matrix, IR spectra of crystals were measured with a Nicolet 710 instru-

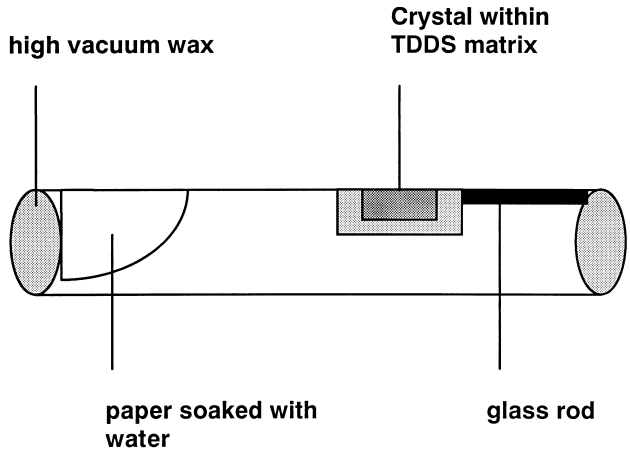

Fig. 4. Crystal mounting in a thin-walled glass capillary for the single crystal structure determination. Due to the adhesive nature of the matrix, it was not possible to remove it completely; the crystals are therefore still embedded within the matrix. To prevent slipping of the crystals during data collection, their positions have been fixed with a glass rod glued inside the capillary. The paper soaked with water used to prevent dehydration in the case of estradiol hemihydrate was not present in the experiments with gestodene.

ment (Madison, WI, USA) combined with a Spectra Tech IR-Plan instrument (Stanford, CT, USA).

2.6. Single crystal structure determinations

Among the predominantly small, cracked or intergrown crystals formed in the TDDS, suitable specimens with volumes of $0.5 \times 0.2 \times 0.2$ mm^3 (estradiol-TDDS) and $0.4 \times 0.3 \times 0.1$ mm^3, respectively, were identified and selected for single crystal structure determinations. Small TDDS segments containing these crystals were cut off using a razor blade and, subsequently, the embodied crystals were disengaged from most of the surrounding matrix in order to allow for their introduction into glass capillaries. To mount the crystals for data collection, they were inserted into the thin-walled glass capillaries and their positions were fixed by glass rods glued inside the capillaries. Since it was presumed that estradiol had crystallized as hemihydrate (see Section 4), special care had to be taken to prevent potential dehydration during data collection. Therefore, a small piece of filter paper soaked with water was placed inside the capillary which was then sealed with wax (Fig. 4). Three reference reflections (h, k and l) measured periodically after each 100 reflections throughout the data collection did not show significant variations. Hence, the crystals proved to be stable during the measurements.

X-ray intensity data were measured on a Siemens P4 diffractometer using MoKα-radiation (λ = 0.71073 Å) and the 2θ-θ scan technique. The structures were solved with direct methods and refined anisotropically [8]. The hydrogen atoms of the hydroxyl groups were located from difference Fourier maps, the remaining hydrogen atoms were included in calculated positions.

3. Results

Both types of steroid-containing patches were free of crystals directly after manufacturing, according to polarized microscopy, which is the method of choice in the search for crystals within matrix TDDS. However, the patches showed crystals of up to 800 μm in length after 3 months of storage (Figs. 5 and 6).

In order to identify those crystals grown in both types of TDDS, several analytical methods were applied. However, the analysis of these crystals was severely hampered by the

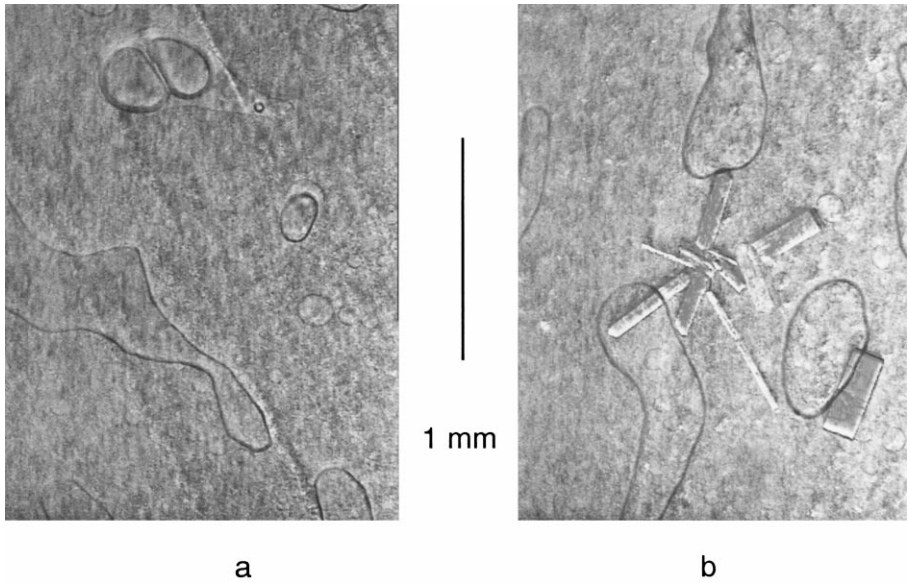

Fig. 5. Microscopic view of estradiol-TDDS. It was not possible to detect any crystals within the TDDS directly after manufacturing (a). Hence (a) shows only the amorphous TDDS-matrix which contains dissolved estradiol. During storage of the TDDS, crystals grew (b) which could be easily detected by means of polarized microscopy.

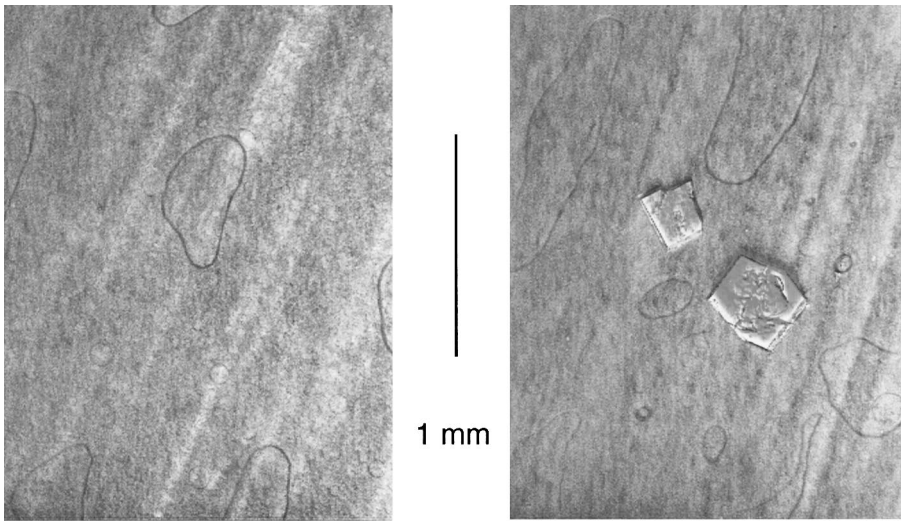

Fig. 6. Microscopic view of gestodene-TDDS which were crystal-free directly after manufacturing (a) but contained gestodene crystals after storage (b).

strong adhesive character of the surrounding TDDS matrix. For practical reasons, different sample preparation techniques were applied (for details see Section 2) but, using standard laboratory methods, it was not possible to completely remove the sticky matrix from the crystals. Therefore, the results of some experiments differ from those obtained with the pure steroids used as references.

Hot stage polarized microscopy (HSPM) was applied to circular, crystal-containing sections of the respective TDDS liberated from the liner using a heating program from 30°C–210°C with an increase of 10°C/min. During the course of this experiment, the crystals did not exhibit a sharp melting point, but a slow dissolution process which started at approximately 110°C in both cases. Whereas the crystals in the estradiol-containing TDDS were completely dissolved at 166°C (m.p. estradiol: 178°C [9]), the crystals within the gestodene-containing TDDS disappeared at 161°C (m.p. gestodene, form I: 200–202°C [10]). Thus, identification of the crystals was not feasible by HSPM.

Differential scanning calorimetry (DSC) was carried out using three crystal-containing circular 4 mm samples of both TDDS types after removal of the release liner. The samples were heated from 30°C to 210°C with an increase of 10°C/min as in the HSPM experiment described above. Since no significant endothermal heatflow could be detected, the DSC-curves did not help to identify the crystals in these special cases.

Attempts to identify the crystals still embedded in the surrounding TDDS matrix by means of FTIR microscopy were carried out after removal of the release liner from the patch. They failed due to interfering signals of the matrix components. In essence, it was not possible to distinguish between the IR spectra derived from crystal-free matrix which contained the respective steroid in dissolved form and derived from the crystals surrounded by parts of the matrix.

As most of the commonly used methods for the identification of drug crystals proved to be ineffective, X-ray diffraction experiments were considered for which no interference with the TDDS matrix was expected. However, the sample preparation for powder diffraction was so tedious that these experiments were stopped in favour of single crystal structure determinations. This method has a major advantage over all previously applied methods. It is not hampered by the presence of small amounts of TDDS matrix since the latter is amorphous and therefore does not interfere with X-rays as the highly ordered crystalline material does.

The results of the single crystal structure determinations

Table 1

Crystal data for estradiol hemihydrate and gestodene

	Estradiol hemihydrate	Gestodene
Crystal habit	Needle	Platelet
Space group	$P2_12_12$	$P2_12_12_1$
Cell constants		
a (Å)	12.037(3)	6.620(2)
b (Å)	19.269(6)	12.650(3)
c (Å)	6.630(3)	20.677(5)
α (°)	90	90
β (°)	90	90
γ (°)	90	90
Volume (Å^3)	1538(1)	1731.6(7)
Z	4	4
D_c (g/cm^3)	1.21	1.19
Reflections measured	1384	1776
Independent reflections	1235	1743
R_{int} (%)	1.74	–
Observed reflections	1013 ($F > 3\sigma(F)$)	882 ($F > 2\sigma(F)$)
Final R-factor (%)	4.91%	4.40%
Final R_w-factor (%)	4.89%	4.24%
Data:parameter	5.1:1	4.2:1

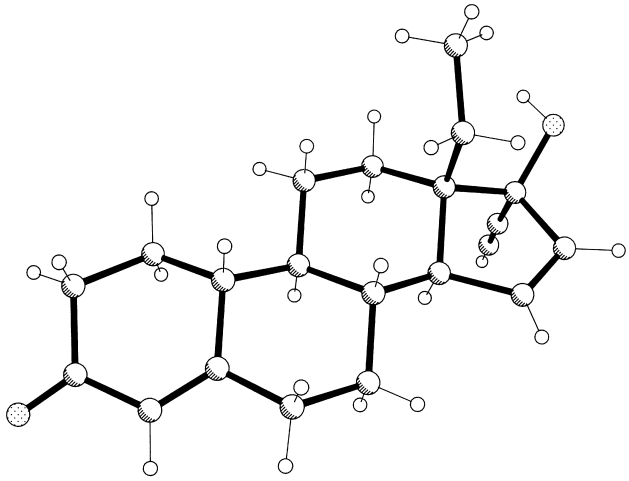

Fig. 7. Crystal structure of gestodene (polymorph I). Oxygen atoms are depicted as circles with a regular dot pattern.

are summarized in Table 1. A comparison of the unit cell dimension derived from the refined positions of approximately 25 reflections with literature data already indicated that the crystals consisted of the steroids from the respective TDDS. This assumption was confirmed by the results of the structure determinations with direct methods (see above) and the subsequent refinement to *R*-values better than 5% (see Table 1).

Two polymorphic modifications of gestodene have been described in the literature [11]. The main difference between polymorphs I and II is the orientation of the ethyl groups attached to C13 with respect to the steroid skeleton. Since even the best among the gestodene crystals had small cracks, the quality of the diffraction data is only moderate, but is good enough to unambiguously determine the positions of all carbon and oxygen atoms. A comparison of this structure with the two known modifications of gestodene showed that in the TDDS, crystals belonging to the thermodynamically more stable form I have been formed (Fig. 7).

In contrast to 17α-estradiol, which has been crystallized

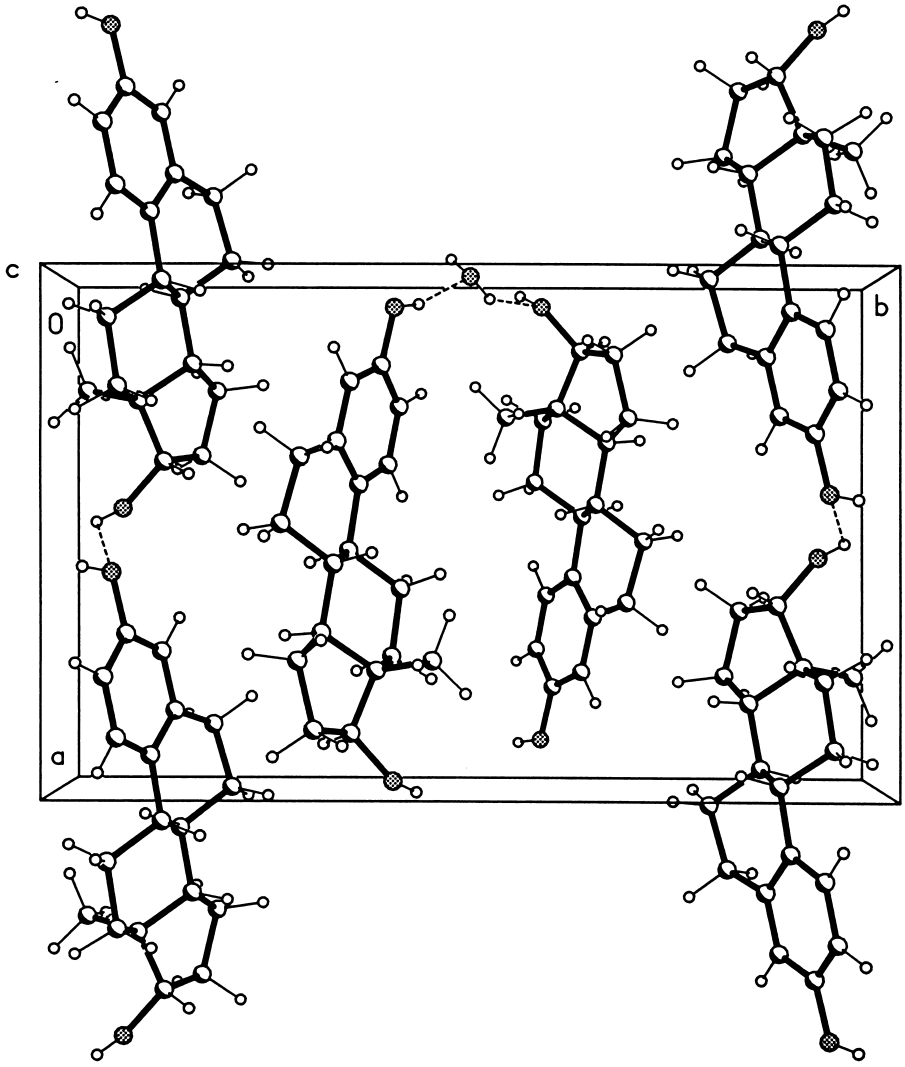

Fig. 8. Packing of estradiol within the unit cell shown along the crystallographic *c*-axis. The intermolecular hydrogen bonds are indicated by dashed lines.

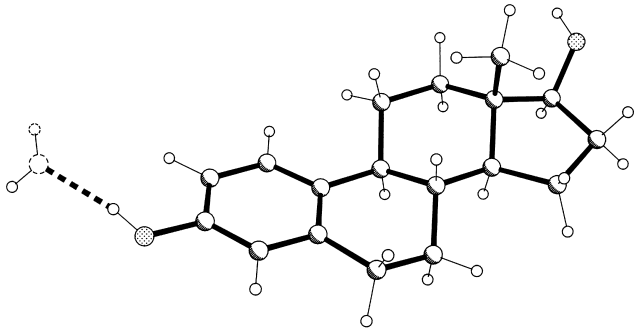

Fig. 9. Crystal structure and molecular conformation of estradiol hemihydrate. The hydrogen atom of the water molecule generated by a crystallographic symmetry operation is indicated by broken lines. The hydrogen bond is indicated by a solid dashed line.

without solvent [12], only solvate structures have been published for the biologically active 17β-estradiol [9,13]. Therefore, it had been assumed, and later confirmed, that the crystals grown in the water containing estradiol-TDDS consisted of estradiol hemihydrate. A superposition of the published estradiol hemihydrate structure with the structure determined in the course of this study showed that estradiol crystallized from the TDDS adopts the same conformation (Fig. 8.) The hydrogen bonding network described in the literature is also present in our structure (Fig. 9).

4. Discussion

Polarized microscopy was used to detect crystals in gestodene- and estradiol-containing matrix TDDS after a few months of storage. In order to determine the nature of these crystals, the affected TDDS sections were isolated and subjected to further analytical methods including hot stage polarized microscopy, differential scanning calorimetry, and FTIR microscopy. However, the latter methods were applied to the crystal-containing patch segments without success. This was mainly due to the fact that, by means of routine laboratory methods, it had not been possible to fully remove the adhesive matrix from the crystals. Therefore, the surrounding TDDS matrix exhibited a negative impact onto the analytical results which otherwise most likely would have been conclusive. In our study, even methods like DSC that have been effective in other cases [14] did not provide sufficient evidence for the identification of the crystals. Similar difficulties with the application of DSC to solid dispersions of drugs have been reported more recently [15]. However, by application of X-ray diffractometry to crystals still imbedded in a significant amount of TDDS matrix it was possible to identify the crystals as estradiol hemihydrate and as gestodene polymorph I, respectively. Thus, both steroids crystallized in their respective most commonly observed solid state form, facilitated by the presence of water in the case of estradiol.

Since the TDDS were crystal-free directly after manufacturing, it is evident that the drug substances crystallized during storage. Therefore, the described polyacrylate based TDDS prototypes containing either 3.3% estradiol or 2.2% gestodene are supersaturated systems. Supersaturation of the systems was achieved during the manufacturing by rapid evaporation of the process solvent ethyl acetate from freshly coated subsaturated wetmixes within the continuous drying process.

These findings suggest that the physical stability of steroid-containing matrix TDDS should be carefully monitored during their storage. Furthermore, the application of specific crystallization inhibitors for estradiol and gestodene may be useful to stabilize the examined TDDS, as well as other supersaturated steroid containing systems. In addition, TDDS containing estradiol may be stabilized by efficient removal of water.

References

[1] Y.W. Chien, Systemic delivery of pharmacologically active molecules across the skin, in: R.L. Juliano (Ed.), Targeted Drug Delivery, Springer-Verlag, Berlin, Heidelberg, New York, 1991, pp. 181–230.
[2] M.C. Musolf, Pressure-sensitive adhesives for transdermal applications, in: Y.W. Chien (Ed.), Transdermal Controlled Systemic Medications, Marcel Dekker, New York, 1987, pp 104–112.
[3] B.W. Barry, Dermatological Formulations: Percutaneous Absorption, In: J. Swarbrick (Ed.) Drugs and the pharmaceutical sciences, Marcel Dekker, New York, 1983, pp. 160–204.
[4] J. Yu, T. Chien, Y.W. Chien, Transdermal dual-controlled delivery of testosterone and estradiol: (I) impact of system design, Drug Dev. Ind. Pharm. 17 (1991) 1883–1904.
[5] X. Ma, J. Taw, C.M. Chiang, Control of drug crystallization in transdermal matrix systems, Int. J. Pharm. 142 (1996) 115–119.
[6] F.J.E. Stefano, F.I. Biali, A.F. Scasso, Crystallization in NETA-17ß-E2 transdermal patches, Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 24 (1997) 703.
[7] R. Lipp, G. Heimann, Statistical approach to optimization of drying conditions of a transdermal delivery system, Drug Dev. Ind. Pharm. 22 (1996) 343–348.
[8] SHELXTL + , Version 5, Siemens Analytical Instruments, Inc.
[9] B. Busetta, M. Hospital, Structure cristalline et moleculaire de Íoestradiol hemihydrate, Acta Cryst. B28 (1972) 560–567.
[10] E. Eckle, G.-A. Hoyer, H. Hofmeister, H. Laurent, D. Schomburg, R. Wiechert, Die Kristall- und Molekülstruktur von Gestoden, Liebigs Ann. Chem. (1988) 199–202.
[11] V.J. van Geerestein, A.J.M. Duisenberg, M.H.G. Duits, J.A. Kanters, J. Kroon, Structure of modifications (I) and (II) 13-ethyl-17β-hydroxy-18,19-dinor-17α-pregna-4,15-dien-20-yn-3-one (gestodene), Acta Cryst. C43 (1987) 2402–2405.
[12] B. Busetta, Y. Barrans, G. Precigoux, M. Hospital, 1,3,5,(10)oestratrien-3,17α-diol, Acta Cryst. B32 (1976) 1290–1292.
[13] W.L. Duax, Crystal structure of the estradiol urea complex, Acta Cryst. B28 (1972) 1864–1866.
[14] M. Bock, G. Depke, U. Egner, A. Müller-Fahrnow, G. Winter, Detection and characterization of polymorphic modifications of the anxiolytic drug ABECARNIL, Tetrahedron 50 (1994) 13125–13134.
[15] G.R. Lloyd, D.Q.M. Craig, A. Smith, An investigation into the melting behavior of binary mixes and solid dispersions of paracetamol and PEG 4000, J. Pharm. Sci. 86 (1997) 991–996.