Available online at www.sciencedirect.com

SCIENCE DIRECT®

European Journal of Pharmaceutics and Biopharmaceutics 60 (2005) 407–412

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejpb

Research paper

Use of CP/MAS solid-state NMR for the characterization of solvate molecules within estradiol crystal forms

Jeong-Sook Park[a], Hye Won Kang[b], Sung Jean Park[b], Chong-Kook Kim[b,*]

[a]*College of Pharmacy, Chungnam National University, Yuseong-gu, Daejeon, Republic of Korea*
[b]*College of Pharmacy, Seoul National University, Kwanak-gu, Seoul, Republic of Korea*

Received 23 January 2004; accepted in revised form 10 January 2005
Available online 8 April 2005

Abstract

To investigate the solvate molecules within estradiol crystal forms, four crystal forms of estradiol (EM, ET, EP and EC) were obtained by recrystallization from various organic solvents such as methanol, ethanol, isopropanol and acetone, and their physicochemical properties were characterized using XRD, TGA, DSC and solid-state NMR. The XRD patterns showed that the estradiol crystal forms were composed of high crystallinity and their degrees of crystallinity were nearly identical. The solvent phases and solvate molecules within the estradiol crystal forms were detected by thermal analysis and the solvate molecular structures were identified by further observation using solid-state NMR. Estradiol typically tends to crystallize in form of its hemihydrate by the solvate molecules from the mother solvents used for recrystallization. The residual solvent might be incorporated into the estradiol molecules to change the local chemical environment and cause the observed ^{13}C CP/MAS NMR spectral changes. These results suggest that the thermal analysis and CP/MAS solid-state NMR spectroscopy could be useful at the dried solid state to characterize the solvate molecule within the recrystallized estradiols.

Keywords: Estradiol; Crystal form; Solvate molecule; CP/MAS solid-state NMR

1. Introduction

Small molecules in the amorphous state are thermodynamically metastable compared with the crystalline phase. Therefore, this property results in crystallization during storage and handling of pharmaceutical products, such as postcompression hardening of tablets [1], particle aggregation in dry powder inhalers [2], and structural instability in transdermal parches [3]. Moreover, recent work on crystallization of estradiol in transdermal patches has revealed that the drug tends to crystallize in more than one polymorphic form in the patch when it is stored for extended periods of time [4]. Polymorphism, which is produced when a compound crystallizes in a various solid phases that differ in crystal packing, is common among pharmaceutical compounds [5,6]. Due to multiplicity in molecular packing, the direct influence of these polymorphs on drug release, solid-state stability, hygroscopicity and bioavailability of pharmacological compounds is frequently dependent upon crystal forms [5–7]. For the effective clinical use of drugs, determination of polymorphism is a necessary feature.

Estradiol (Fig. 1) was chosen as the model drug for this work due to its widespread use in hormonal replacement therapy for the treatment of postmenopausal symptoms and the protection against long-term consequences of estrogen deficiency (osteoporosis and cardiovascular disease) [8]. However, due to the low solubility of estradiol, supersaturated patches containing high drug concentrations are often required for obtaining sufficient therapeutic efficiency. A high saturation of the drug in the transdermal drug delivery systems may lead to supersaturated states in the patches which have the tendency to partly recrystallize during storage until a saturated state is achieved [9]. The most characteristic property of estradiol is its tendency to adopt the hemihydrated form, in which state it precipitates,

---

[*] Corresponding author. National Research Lab for Drug and fene Delivery, College of Pharmacy, Seoul National University, San 56-1, Shillim-Dong, Kwanak-gu, Seoul 151-742, Republic of Korea. Tel.: +82 2 880 7867; fax: +82 2 873 7482.
E-mail address: ckkim@plaza.snu.ac.kr (C.-K. Kim).

0939-6411/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2005.01.010

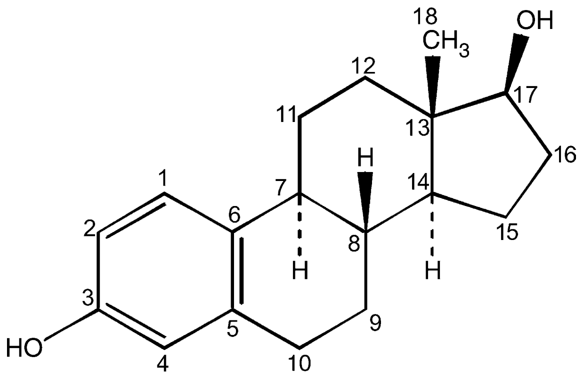

Fig. 1. Schematic representation of the estradiol molecules. The numbers represent the naming system of the carbon atoms in the molecule.

not only from partially aqueous solutions, but also from ethyl acetate, chloroform, absolute ethanol, and other anhydrous solvents [10].

The existence of diverse solid phases reflects the complex balance of intermolecular interactions and molecular conformational changes of direct crystal packing [11]. Unfortunately, solid phase diversity complicates the development of solid pharmaceutical products, which must be manufactured with a consistent phase composition. The use of organic solvents in pharmacy crystallizes a variety of solid phases, which may include polymorphs, hydrates, or solvates [12,13]. However, traces of residual mother liquor can induce a solvent-mediated polymorphic transformation during storage to ensure polymorphic purity, and of course, sometimes bring toxicity [14–16]. Therefore, the identification of the present residual solvent as a solid state within the drug can provide practical information.

The crystallization behavior in transdermal patches of 17β-estradiol hemihydrate [17] and of norethindrone acetate [18] using microcalorimetry, polarization microscopy and X-ray diffraction was described in previous publications. Previously, it was also shown by thermal analysis, Raman microscopy, X-ray analysis and solid-state NMR that β-estradiol formed a hemisolvate with methanol [4]. However, in terms of pharmaceutical application, interaction between the crystal structure of estradiol and the present impurity, has not yet been fully characterized.

Therefore, this study was performed to characterize various solvate molecules within estradiol crystal forms recrystallized from methanol, ethanol, isopropanol and acetone using and comparing different methods, such as X-ray diffraction, thermal analysis and CP/MAS solid-state NMR.

2. Materials and methods

2.1. Materials

Estradiol (1,3,5,(10)-Estratriene-3,17β-diol) was purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of estradiol crystal forms from organic solvents

Estradiol was dissolved in various organic solvents such as methanol (3.3 g/100 ml), ethanol (4 g/100 ml), isopropanol (9 g/100 ml) and acetone (3.3 g/100 ml). After estradiol was completely dissolved by stirring at 35 °C, the solution was cooled at room temperature and then crystallized at 4 °C using a cold chamber for 3 days. The final crystal form was obtained by drying in a vacuum oven at 60 °C for 3 days.

2.3. X-ray powder diffractometry (XRD)

X-ray diffraction patterns were measured on a Scintag XDS 2000 Diffractometer (Scintag Inc., Cupertino, CA, USA) using Cu Kα radiation. The diffractometer was equipped with Ge(Li)-diode detector. The samples were scanned for 27 min from 3 to 30° 2θ in the stepscan mode with a step size of 0.03° and 1.8 s counting time.

2.4. Thermal analysis

Gravimetric and calorimetric changes accompanying thermal decomposition were measured using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC, Du Pont 9900, Du Pont Instr., DE, USA) calibrated with Al_2O_3. The TGA and DSC measurements were carried out using 15 mg of fine powdered sample in an aluminum pan with a heating rate of 1.25 °C/min.

2.5. Solid-state NMR measurement

The ^{13}C solid-state NMR spectra were obtained using a Bruker DSX-400 NMR spectrometer with an Oxford wide-bore (89 mm) magnet. The static field of the superconducting magnet was 4.7 T and a multi-nuclear cross-polarization magic angle spinning (CP/MAS) technique was employed. All ^{13}C spectra were acquired under proton decoupling and all chemical shifts were made with reference to external tetramethylsilane (TMS) at 0.0 ppm.

3. Results

3.1. X-ray powder diffraction analysis

XRD analysis was carried out to examine crystallinity of four forms of recrystallized estradiol from different organic solvents. Different patterns of XRD, particularly in position and relative peak intensity in the range of $2\theta = 3–20°$, were obtained as shown in Fig. 2. The XRD patterns of all

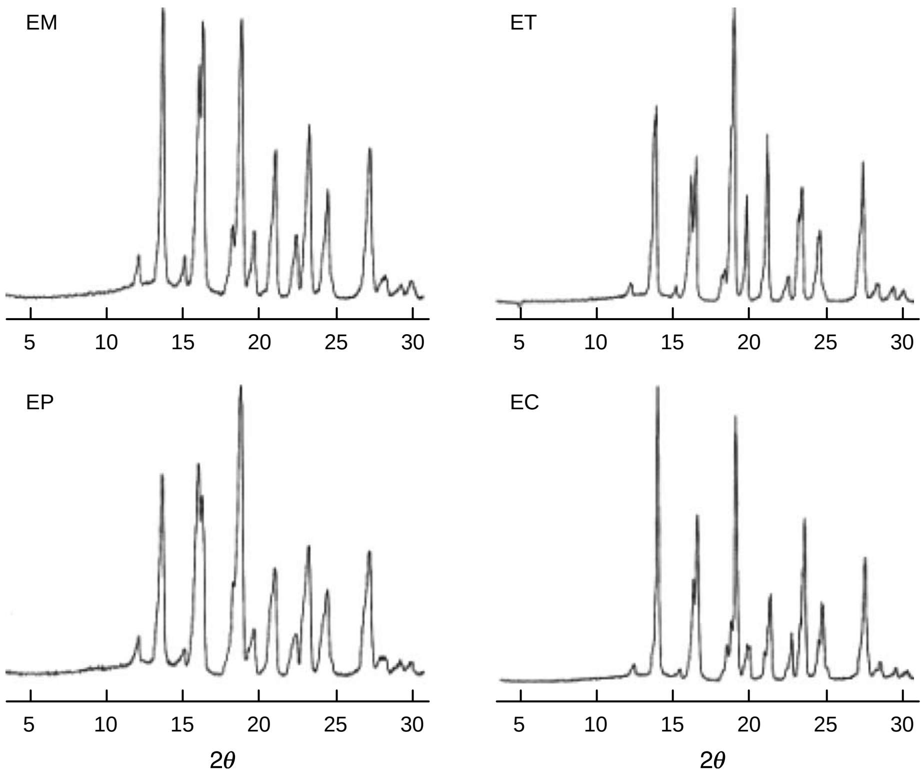

Fig. 2. X-ray powder diffraction patterns of the crystal forms of estradiol.

samples were nearly identical with defined diffraction peaks and significant baseline separation, indicating that the solids were composed of high crystallinity. The position of the main diffraction peaks in this range for estradiol are listed in Table 1. The XRD patterns of materials featured neither shifted diffraction peaks to indicate changes in the unit crystal dimensions nor significantly different degrees of crystallinity among the samples, demonstrating a change in crystal form.

Table 1
Position of the main diffraction peaks at the range of $2\theta = 3–20°$ for recrystallized estradiol

EM	ET	EP	EC
11.5925	–	11.5594	–
13.1781	13.2612	13.1266	13.2341
14.5538	–	–	–
15.5175	15.5972	15.4619	15.5463
15.7531	15.8709	15.6903	15.7762
17.6778	17.7431	–	17.6950
18.2306	18.3384	18.2103	18.2919
19.0547	19.1747	19.0075	19.0122
	–	–	20.1000
20.4188	20.5209	20.3716	20.4597
21.7722	21.8478	21.7053	21.8538
22.6181	22.7184	22.5797	22.6938
23.8053	23.8747	23.7566	23.8319
26.5462	26.6663	26.5144	26.5947
	27.6381	–	27.5797

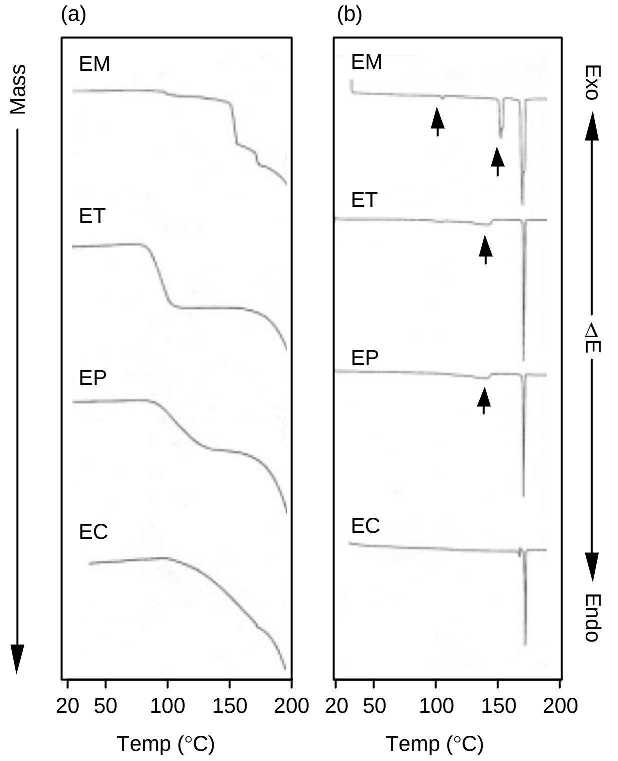

Fig. 3. Thermogravimetric analysis (a) and different scanning calorimetry scans (b) of EM, ET, EP and EC forms of estradiol. The arrows represent the endotherms under 150 °C due to desolvation. All DSC scans shown were carried out at a heating rate of 1.25 °C/min.

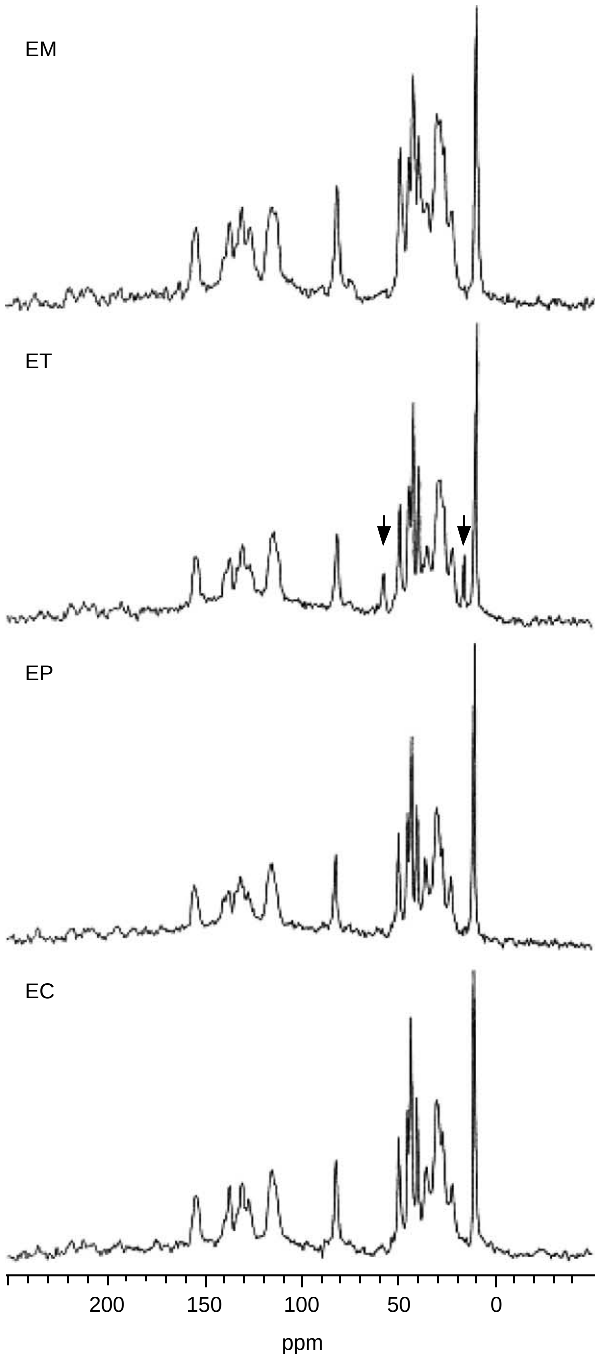

Fig. 4. CP/MAS solid-state ^{13}C NMR spectra of EM, ET, EP and EC forms of estradiol. The arrows represent the newly appeared peaks.

3.2. Thermal analysis

The weight changes during the heating of the estradiol crystal forms were monitored by open pan TGA and sealed pan DSC as shown in Fig. 3. In order to detect precise changes in the sample weight, the slow heating rate of 1.25 °C/min was employed. The DSC of estradiol crystal forms shows an endothermic shape at around 178 °C associated with the melting point. The TGA curves indicate that there were different weight changes among the estradiol crystal forms, especially the distinct weight change which was observed in the EM crystal form. The TGA curve of EM shows that the first weight loss occurred at around 100 °C and then continuously at 155.6, 156.4, 170.67 and 174.2 °C. The DSC curve of EM consists of three sharp endotherm peaks at 101.4, 154.8 and 175 °C, indicating that there were three different types of phase including a solid phase and somehow, a liquid phase. On the other hand, broad peaks were also observed in ET and EP crystal forms. However, it is difficult to point out the weight change in the DSC curve of EC crystal form, which was shown by TGA scan as a shoulder.

3.3. CP/MAS solid-state NMR

The ^{13}C CP/MAS solid-state NMR spectra of estradiol crystal forms recrystallized from organic solvents are shown in Fig. 4 and their tentative peak assignments are listed in Table 2. The assignments were based on the comparison with the ^{1}H and ^{13}C NMR spectra of dissolved estradiol [19]. The spectra of estradiol crystal forms generally have peak per carbon except for C-3, which is consistent with one crystallographically inequivalent site in the unit cell, indicating that it has a symmetric crystal structure [4,20]. The resulting spectra of the different forms of estradiols showed similar patterns, although noticeably different peaks were detected. The peaks of EP between 126–131 ppm are split as more than a doublet, which tendency appears a little bit in ET, while peaks at 57 and 16 ppm were observed only in the ET crystal form. In addition, the sharpness of peaks at about 30 ppm, which are assigned as C-7, C-10, and C-17, decreases in the crystal forms of ET, EP, and EC. The peaks at 126–131 ppm were assigned to C-1 and C-6 from the aromatic resonance. However, the peaks observed at 57 and

Table 2
Chemical shifts of ^{13}C solid-state NMR spectra of EM, ET, EP and EC forms of estradiol

δ (ppm)	EM	ET	EP	EC
C-3	154.46	155.17	154.87	154.22
C-5	137.25	137.45	137.26	137.30
C-6	131.01	130.99	131.22	130.44
C-1	126.47	127.32	127.47	127.34
C-4, C-2	115.34	114.88	115.11	115.04
C-17	82.04	81.94	82.14	82.13
	–	57.75[a]	–	–
C-14	49.81	49.86	49.76	49.79
C-9	45.09	45.11	45.06	44.94
C-13	43.14	43.29	43.26	43.26
C-8	40.16	40.18	40.24	40.19
C-12	35.75	35.66	35.80	35.59
C-10, 16	30.82	29.66	30.43	30.30
C-7	29.89	29.48	29.98	30.02
C-11	27.01	27.02	27.14	27.07
C-15	22.94	22.65	22.57	22.46
	–	16.55[a]	–	–
C-18	10.79	10.79	10.88	10.78

[a] The different peaks resulted from solvent.

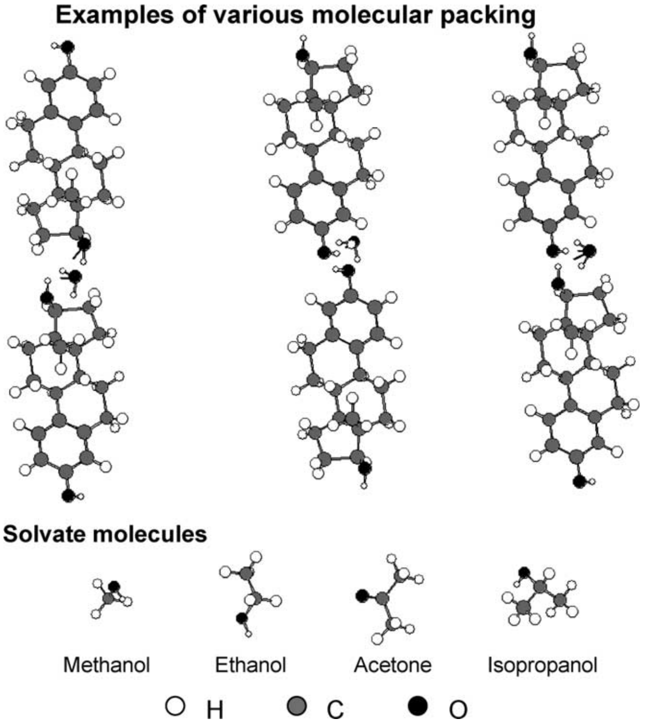

Fig. 5. Molecular packing conformation of estradiol hemihydrate and solvent. The dotted lines represent hydrogen bonds between the hydroxyl groups of estradiol and the water molecule. The drawing is not to scale and is meant for visualization only.

16 ppm were most likely associated with ethanol used for recrystallization. The common peaks at 16 and 57 ppm are associated with a CH_3 group. It is interesting to note that the CH_3 has resonances with different chemical shifts despite similar crystallographic packing. Thus, it is proposed that different molecular structures or further packing of crystal forms may be attributed to the residual solvent or solvate molecule within the crystal forms. These observations suggest a detailed study of the relation between solvated estradiol and its packing.

4. Discussion

It is known that commercial estradiol contains water as hydrated or solvated form [4,20]. The solvated OH exhibits closely to the OH of estradiol. The bond distances of C–C and C–O in the solvate ethanol are considerably shorter than the normal values for such bonds. This is most likely attributed to the thermal vibration executed by the ethanol. However, it was found that the bond lengths and angles involving hydrogen atoms were within the usual range [4,20]. Fig. 5 illustrates a possible molecular conformation of estradiol containing solvate molecules other than OH. The hypothesis evokes the possibility that solvate molecules are not only to connect O(33)–H(83) but also O(97)–H(37) due to the orientation of C-13 and symmetry of estradiol molecules.

The present work demonstrates that the usual solvated OH can be substituted by the solvate molecules from the mother solvents used for recrystallization. Although no significant differences were observed by XRD, the ^{13}C CP/MAS NMR spectra proved the conformational differences of the molecule and of structures in the estradiol crystal forms. Moreover, TGA and DSC show some differences between EM, ET, EP and EC forms of estradiol because the heating rate was much slower than other experiments [9]. With general heating rate of 10 °C/min, it is easy to pass over the minute changes. However, the peak by desolvation could be detected by slowing the heating rate down. The residual solvent might be incorporated into the estradiol molecules to change the local chemical environment and cause the observed ^{13}C CP/MAS NMR spectral changes [4]. Similar results were obtained for erythromycin A, where pseudopolymorphs were distinguishable by solid-state NMR spectroscopy and not by XRD [21]. The differences in the spectra of estradiol crystal forms prove that the solid-state NMR can be used to identify these polymorphs, particularly at solid dry state. Very subtle changes in the crystal composition that may impact physical properties, such as solvated molecules, may be readily detected using a solid-state NMR spectroscopy.

5. Conclusion

The different solvate molecules can be produced by altering the solvents used for recrystallization, which lead to a variety of molecular packing. The solvate molecules within the estradiol recrystallized from organic solvents were detected using the thermal analysis and CP/MAS solid-state NMR spectroscopy.

Acknowledgements

This research was partly supported by the grant from the National Research Laboratory Program (Lab No. 2000-N-NL-01-C-171) in the series of MOST-NRDP in the Ministry of Science and Technology, Republic of Korea.

References

[1] A. Burger, R. Ramberger, W. Schmidt, The influence of the polymorphism of the active substance on the properties of tablets. Part 3. Compressional behaviour of meprobamate, Pharmazie 36 (1981) 41–46.
[2] H.K. Chan, I. Gonda, Development of a systematic theory of suspension inhalation aerosols. II. Aggregates of monodisperse particles nebulized in polydisperse-droplets, Int. J. Pharm. 41 (1988) 147–157.
[3] X. Ma, J. Taw, C.M. Chiang, Control of drug crystallization in transdermal matrix system, Int. J. Pharm. 142 (1996) 115–119.

[4] N.E. Variankaval, K.I. Jacob, S.M. Dinh, Crystallization of beta-estradiol in an acrylic transdermal drug delivery system, J. Biomed. Mater. Res. 44 (1999) 397–406.

[5] J. Bauer, S. Spanton, R. Henry, J. Quick, W. Dziki, W. Porter, J. Morris, Ritonavir: an extraordinary example of conformational polymorphism, Pharm. Res. 18 (2001) 859–866.

[6] S.L. Price, The computational prediction of pharmaceutical crystal structures and polymorphism, Adv. Drug Deliv. Rev. 56 (2004) 301–319.

[7] W. Zhang, R.A. Laursen, Artificial antifreeze polypeptides: alpha-helical peptides with KAAK motifs have antifreeze and ice crystal morphology modifying properties, FEBS Lett. 455 (1999) 372–376.

[8] G.R. Kraemer, R.R. Kraemer, B.W. Ogden, R.E. Kilpatrick, T.L. Gimpel, V.D. Castracane, Variability of serum estrogens among postmenopausal women treated with the same transdermal estrogen therapy and the effect on androgens and sex hormone binding globulin, Fertil. Steril. 79 (2003) 534–542.

[9] S. Latsch, T. Selzer, L. Fink, M. Horstmann, J. Kreuter, 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, Eur. J. Pharm. Biopharm. 57 (2004) 397–410.

[10] E.G. Salole, The physicochemical properties of oestradiol, J. Pharm. Biomed. Anal. 5 (1987) 635–648.

[11] T. Ishida, M. Doi, M. Shimamoto, N. Minamino, K. Nonaka, M. Inoue, Physicochemical properties of crystalline forms of ethinylestradiol solvates: comparison of thermal behavior with X-ray crystal structure, J. Pharm. Sci. 78 (1989) 274–280.

[12] S. Mirza, I. Miroshnyk, J. Heinamaki, L. Christiansen, M. Karjalainen, J. Yliruusi, Influence of solvents on the variety of crystalline forms of erythromycin, AAPS Pharm. Sci. 5 (2003) E12.

[13] M.T. Ledwidge, S.M. Draper, D.J. Wilcock, O.I. Corrigan, Physicochemical characterization of diclofenac N-(2-hydroxyethyl)pyrrolidine: anhydrate and dihydrate crystalline forms, J. Pharm. Sci. 5 (1996) 16–21.

[14] T. Ishida, Y. In, M. Doi, M. Inoue, Y. Hamada, T. Shioiri, Molecular conformation of ascidiacyclamide, a cytotoxic cyclic peptide from Ascidian: X-ray analyses of its free form and solvate crystals, Biopolymers 32 (1992) 131–143.

[15] R. Lipp, A. Müller-Fahrnow, Use of X-ray crystallography for the characterization of single crystals grown in steroid containing transdermal drug delivery systems, Eur. J. Pharm. Biopharm. 47 (1999) 133–138.

[16] G.F. Needham, R.R. Pfeiffer, G.L. Engel, B.S. Rutherford, D.J. Allen, Effect of impurities on estradiol crystallization in a sustained release implant, J. Pharm. Sci. 81 (1992) 1012–1014.

[17] S. Latsch, T. Selzer, L. Fink, J. Kreuter, Crystallisation of estradiol containing TDDS determined by isothermal microcalorimetry, X-ray diffraction, and optical microscopy, Eur. J. Pharm. Biopharm. 56 (2003) 43–52.

[18] S. Latsch, T. Selzer, L. Fink, J. Kreuter, Determination of the physical state of norethindrone acetate containing transdermal drug delivery systems by isothermal microcalorimetry, X-ray diffraction, and optical microscopy, Eur. J. Pharm. Biopharm. 57 (2004) 383–395.

[19] V. Boucheau, M. Renaud, M.R. de Ravel, E. Mappus, C.Y. Cuilleron, Proton and carbon-13 nuclear magnetic resonance spectroscopy of diastereoisomeric 3- and 17β-tetrahydropyranyl ether derivatives of estrone and estradiol, Steroids 55 (1990) 209–221.

[20] G. Precigoux, P. Marsau, F. Leroy, B. Busetta, 17β-Hydroxymethyl-1,3,5(10)-estratrien-3-ol monohydrate, Acta Crystallogr. B 36 (1980) 749–751.

[21] G.A. Stephenson, J.G. Stowell, P.H. Toma, R.R. Pfeiffer, S.R. Byrn, Solid-state investigations of Erythromycin A Dihydrate: structure, NMR spectroscopy, and hygroscopicity, J. Pharm. Sci. 81 (1992) 1012–1014.