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DISSOLUTION BEHAVIOR OF 17β-ESTRADIOL (E_2) FROM POVIDONE COPRECIPITATES. COMPARISON WITH MICROCRYSTALLINE AND MACROCRYSTALLINE E_2

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SUMMARY

The dissolution rates of macrocrystalline 17β-estradiol (E_2), microcrystalline E_2, and E_2–povidone coprecipitates and physical mixtures varying in weight ratio from 1 : 1 to 1 : 49 were determined at 37°C. E_2 dissolution from the coprecipitates was markedly faster than that from either macro- or microcrystalline forms of the drug and was found to increase with decreasing E_2-to-povidone weight ratio. Based on the results of X-ray diffraction, differential scanning calorimetric, Raman spectroscopic, and thermal gravimetric analysis studies, it is concluded that the formation of a more water soluble, high energy state of E_2 is responsible for the increased dissolution rate of this natural estrogen from low weight ratio povidone coprecipitates. These findings suggest that the use of E_2–povidone coprecipitates may increase the systemic availability of E_2.

INTRODUCTION

The natural estrogen, 17β-estradiol (E_2) possesses limited aqueous solubility in gastrointestinal fluids and is subject to extensive pre-systemic metabolism (first-pass liver and/or intestinal epithelia metabolism) after oral administration to man (Ryan and Engel, 1953; Fishman et al., 1969; Yen et al., 1975). As a result, the systemic availability of orally administered macrocrystalline E_2 is too low to elicit estrogenic activity (Krantz and Carr, 1958; Botella-Llusia, 1973; Yen et al., 1975).

Pre-systemic drug metabolism is usually a capacity limited process (Gibaldi and Perrier, 1975; Riegelman and Rowland, 1974). Hence, a substantial increase in the dissolution rate of E_2 in intestinal fluids may, by increasing luminal E_2 concentrations, increase the

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chances of saturating the enzyme systems at one or both of the pre-systemic biotransformation sites for part of the absorption process. Enzyme saturation for the majority of the absorption process would maximize the systemic availability of orally administered E_2.

Particle size reduction of a poorly soluble drug by mechanical or recrystallization techniques can increase its effective surface area and, thereby, enhance its dissolution rate in gastrointestinal fluids (Bates and Gibaldi, 1970). Using this approach, Martin et al. (1972) recently reported that a tablet containing 2 mg of microcrystalline E_2 (particle size: $>80\% < 20$ μm in diameter) was orally effective in patients with estrogen deficiency and associated menopausal symptoms, resulting in symptomatic relief in over 85% of the 112 patients treated. Serum concentrations of E_2 after oral administration of the 2 mg microcrystalline E_2 tablet to 9 postmenopausal women were appreciably lower than those of its unconjugated metabolite estrone, but were sufficient to produce some estrogenic activity (Yen et al., 1975). Unfortunately, a quantitative assessment of the systemic availability of the microcrystralline E_2 tablet cannot be made, since the time course of E_2 in the serum after intravenous administration or after an oral dose of macrocrystalline E_2 was not determined (Yen et al., 1975).

We have shown that the poorly water-soluble drugs reserpine (Stupak and Bates, 1972; Stupak et al., 1974) and digitoxin (Stupak and Bates, 1973) are dispersed *molecularly* in low drug-to-povidone (a high molecular weight, pharmacologically inert, water soluble polymer) weight ratio coprecipitates and that drug dissolution and absorption from these systems are enhanced. Thus, the possibility exists that the dissolution rate and systemic availability of E_2 from povidone coprecipitates may exceed those of microcrystalline E_2.

The present study was undertaken to determine the rate and mechanism(s) of E_2 dissolution from various weight ratio E_2-to-povidone coprecipitates.

MATERIALS AND METHODS

Materials

Pharmaceutical grade E_2 (macrocrystalline) [1] and povidone [2], and special enzyme grade sodium deoxycholate [3] were used as received. Commercial tablets containing 2 mg of microcrystalline E_2 ($>80\%<20$ μm in diameter) [4] were purchased on the open market and were pulverized [5] prior to use. All other chemicals were of reagent grade.

Preparation of test systems

E_2—povidone coprecipitates, in weight ratios of 3 : 1, 1 : 1, 1 : 3, 1 : 4, 1 : 9 and 1 : 49, were prepared by dissolving both components in absolute ethanol and subsequently removing the solvent, in vacuo. The residue was then dried in vacuo at 37°C to constant weight. Precipitated E_2 was prepared by treating pure E_2 in a similar manner.

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[1] Schering Corporation, Bloomfield, N.J.

[2] Polyvinylpyrrolidone; Plasdone C, K-30, average molecular weight, 40,000, General Aniline and Film Corporation, New York, N.Y.

[3] Schwarz Mann, Orangeburg, N.J.

[4] Estrace tablets, Mead Johnson, Inc., Lot No. MM 011.

[5] Pulverized tablets of microcrystalline E_2 were used rather than pure substance because of the potential influence of formulation factors on the wettability and effective surface area of E_2.

E_2–povidone physical mixtures at 3 : 1 and 1 : 9 (w/w) were also prepared by mechanically mixing the two substances in a mortar with a pestle. The test systems were sized through standard screens and the 100–200 mesh (125–149 μm in diameter) particle size fraction was used in all studies.

Equilibrium solubility determinations

The equilibrium solubility of E_2 was determined at 37°C in pH 7.4 Clark-Lubs phosphate buffer (0.150 ionic strength), pH 7.4 phosphate buffer containing 0.020 moles of sodium deoxycholate per liter, and pH 7.4 phosphate buffer containing 0.020 moles of sodium deoxycholate and 2.25 g of povidone per liter. Excess quantities of E_2 were placed into 25 ml glass-stoppered flasks together with 10 ml portions of these solvents. All flasks were closed securely and mechanically shaken at 37°C ± 0.1°C until equilibrium was attained. Equilibrium was established by repetitive sampling and was found to occur within 24–48 h. The equilibrated samples were filtered (Millipore, 0.45 μm pore size) [6] and the filtrates were assayed for drug content using the spectrophotometric procedure subsequently described.

Dissolution rate studies

The dissolution characteristics of E_2 from 100–120 mesh particles of the test preparations were studied in Clark-Lubs pH 7.4 phosphate buffer (0.150 ionic strength) made 20 mM with respect to sodium deoxycholate. The concentration of the bile salt was sufficient to lower the surface tension of the dissolution medium to 45 dynes/cm which is similar to that of intestinal fluids. The dissolution apparatus consisted of a 1.0-liter, 3-necked round bottom flask containing 1.0 liter of dissolution medium maintained at 37 ± 0.1°C and agitated at 100 rpm by means of a half-moon shaped stainless steel paddle (5 cm wide and 1.9 cm deep at widest part of arc) placed 3.2 cm above the bottom of the flask and connected to a constant-speed stirring apparatus [7]. After introducing a quantity of test preparation equivalent to 25 mg or 250 mg of E_2, 5.0 ml samples were removed from the flask at predetermined intervals and replaced with fresh dissolution medium. Each sample was filtered (Millipore, 0.22 μm pore size) [6] at 37°C prior to extraction of E_2 into 10 ml of anhydrous ether. An 8.0 ml aliquot of the organic phase was evaporated to dryness and the residue dissolved in 1.0 ml of ethanol. The absorbance of the reconstituted sample was measured with a spectrophotometer [8] at 280 nm. The bile salt and povidone in the assay samples did not interfere with the determination of E_2.

Physical measurements

To characterize E_2 (untreated and precipitated from alcohol) and the E_2–povidone physical mixture and coprecipitates, solid samples were analyzed by differential scanning

[6] Millipore filter was pre-saturated with sample.
[7] Servodyne, Cole-Parmer Co., Chicago, Ill.
[8] Beckman DB-G, Beckman Instruments, Inc., Fullerton, Calif.

calorimetry (DSC) [9], thermal gravimetric analysis (TGA) [10], X-ray powder diffractometry [11] and Raman spectroscopy (lattice vibrations) [12].

RESULTS AND DISCUSSION

Equilibrium solubility studies

The equilibrium solubility of E_2 at 37°C in pH 7.4 buffer was increased almost 8-fold by sodium deoxycholate at a concentration (20 mM) whitin the concentration range of total bile salts in human intestinal fluids (Table 1). This result can be attributed to the ability of bile salt micelles to solubilize poorly water soluble drugs (Bates et al., 1966a, b). The solubility of E_2 in the sodium deoxycholate-containing buffer was unaffected by the addition of 0.225% povidone (Table 1).

Dissolution rate studies

The mean dissolution–time profiles for particulate samples (equivalent to 25 mg of E_2) of macrocrystalline E_2 (untreated and precipitated from alcohol; 125–149 μm), microcrystalline E_2 (>80% < 20 μm), and various ratio E_2-to-povidone coprecipitates and physical mixtures (125–149 μm) at 37°C are shown in Figs. 1 and 2. The times required for 50% dissolution ($T_{50\%}$) were obtained from such plots and are summarized in Table 2. The data reveal that the dissolution rate of E_2 is strongly dependent on particle size, the $T_{50\%}$ value for macrocrystalline E_2 being approximately 56 times longer than that for microcrystalline E_2 (Fig. 1 and Table 2). The microcrystalline E_2 used in this study and in the systemic availability studies of Martin et al. (1972) and Yen et al. (1975) was obtained from the same source.

E_2 dissolution from the povidone coprecipitates was appreciably faster than from either macro- or microcrystalline material and increased with decreasing E_2-to-povidone weight ratio (Figs. 1 and 2; Table 2). Partial recrystallization of E_2 observed during the preparation of the 1 : 1 w/w E_2–povidone coprecipitate best accounts for its relatively slow dissolution. In contrast, E_2 appeared to form a *glass solution* with povidone in lower weight ratio coprecipitates. The longer $T_{50\%}$ value for the 1 : 49 w/w coprecipitate relative to the 1 : 9 w/w coprecipitate (4.4 min vs 0.9 min; Table 2) may have resulted from an increase in the viscosity of the aqueous diffusion layer due to the presence of higher povidone concentrations.

The times for complete dissolution (τ) of macrocrystalline E_2, microcrystalline E_2 and the 1 : 9, w/w E_2–povidone coprecipitate particles were estimated to be >480 min, 180 min and 20 min, respectively (Fig. 1). These results coupled with the fact that τ for small

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[9] Thermograms were obtained using a Perkin-Elmer DSC-2 calorimeter (Perkin-Elmer Co., Norwalk, Conn. 06856) and a scanning rate of 10°/min. The system was constantly flushed with dry nitrogen.

[10] Weight changes for each sample as a function of temperature were recorded using a Perkin-Elmer TGS-2 unit (Perkin-Elmer Co., Norwalk, Conn. 06856), in an atmosphere of dry nitrogen.

[11] The power diffraction patterns were recorded on a Toshiba model ADG-301 X-ray diffractometer (Tokyo Shibaura Electric Co., Tokyo, Japan) using Ni filtered Cu Kα radiation.

[12] The Raman spectra were observed photoelectrically with a Spex model 14018 double monochromator equipped with holographic gratings (Spex Industries, Inc., Metuchen, N.J. 08840). Excitation was provided by an argon ion laser at 0.2 W on the 5145 A line (Bellows et al., 1977).

TABLE 1

SOLUBILITY OF 17β-ESTRADIOL IN AQUEOUS MEDIA AT 37°C

Solvent	Solubility mg/liter
pH 7.4 Phosphate buffer [a]	5.12
0.02 M Sodium deoxycholate in pH 7.4 phosphate buffer [a]	39.2
0.225% Povidone and 0.02 M sodium deoxycholate in pH 7.4 phosphate buffer [a]	40.6

[a] Clark-Lubs, 0.150 ionic strength

drug particles is related to particle size by the equation, $\tau = \rho a_0^2/2DS$ (where ρ is the particle density, a_0 is the initial particle radius, D is the aqueous diffusion coefficient of the drug, and S is the aqueous solubility of the drug; Ho et al., 1977), suggest that the particle size of E_2 in low weight ratio coprecipitates is significantly smaller than that of microcrystalline E_2.

The formation of a water soluble E_2–povidone complex during the dissolution process does not appear to play any significant role in the mechanism underlying the enhanced dissolution of E_2–povidone coprecipitates. This conclusion is supported by the observation that the $T_{50\%}$ value for the 1 : 9 w/w E_2-povidone physical mixture was approximately 750 times longer than that for a similar ratio coprecipitate (Table 2). Additionally, since 125–149 μm particles of untreated and alcohol-precipitated E_2 dissolve at comparable rates (Fig. 1 and Table 2), the enhanced dissolution of the coprecipitates is probably not due to the formation of a more soluble ethanol solvate or polymorph during its preparation.

The phase solubility method was used to determine whether a high energy form of E_2

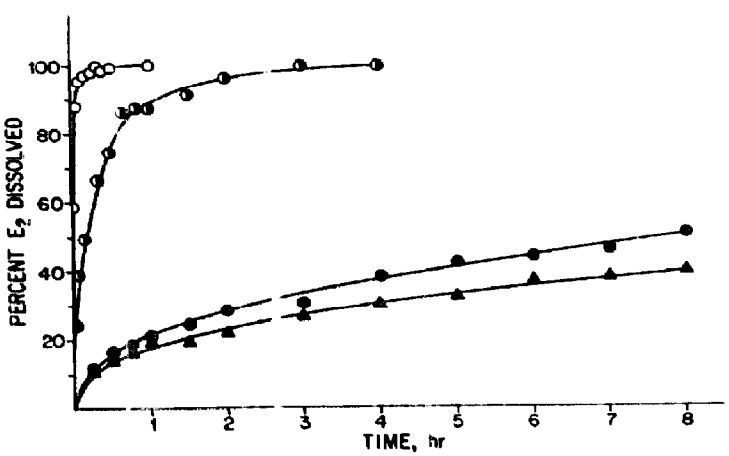


Fig. 1. Dissolution rates of 17β-estradiol (E_2) test preparations (amount equivalent to 25 mg of estrogen) at 37°C. Key: •, untreated E_2 (125–149 μm); ⊕, microcrystalline E_2 (>80% < 20 μm); ▲, 1 : 9 w/w E_2–povidone physical mixture (125–149 μm); ○, 1 : 9 w/w E_2–povidone coprecipitate (125–149 μm).

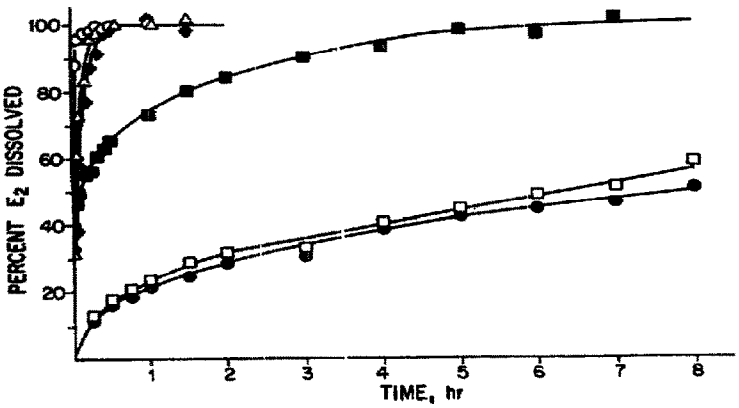


Fig. 2. Dissolution rates of 17β-estradiol (E_2) test preparations (amount equivalent to 25 mg of estrogent; gross particle size, 125–149 μm) at 37°C. Key: •, untreated E_2; ○, precipitated E_2; ■, 1 : 1 w/w E_2–povidone coprecipitate; △, 1 : 4 w/w coprecipitate; ○, 1 : 9 w/w coprecipitate; and ◆, 1 : 49 w/w coprecipitate.

was formed during its coprecipitation with povidone. For these dynamic solubility studies, the amount of E_2 exposed to the dissolution medium was increased 10-fold. The mean time courses of E_2 in solution from 125–149 μm particles of untreated E_2 and the 1 : 9 w/w E_2–povidone coprecipitate are depicted in Fig. 3. The dissolution of E_2 from the coprecipitate was rapid and supersaturation was observed (complete dissolution of the

TABLE 2

EFFECT OF DRUG-TO-POLYMER RATIO ON THE DISSOLUTION RATE OF 17β-ESTRADIOL (E_2) FROM E_2–POVIDONE COPRECIPITATES AND PHYSICAL MIXTURES AT 37°C

Test system [a]	Time required for 50% dissolution ($T_{50\%}$) [b] min
E_2 (untreated)	468 (435–513) [c]
E_2 (precipitated) [d]	387 (345–426)
E_2 (microcrystalline) [e]	8.4 (7.5–9.3)
E_2–povidone	
Physical Mixture	
1 : 9 (w/w)	678 (600–744)
Coprecipitates	
1 : 1 (w/w)	7.0 (6.5–7.5)
1 : 4 (w/w)	1.8 (1.6–1.9)
1 : 9 (w/w)	0.9 (0.8–1.0)
1 : 49 (w/w)	4.4 (3.5–5.0)

[a] Particle size fraction employed was 125–149 μm in diameter.
[b] Mean of three dissolution runs.
[c] Range of values in parenthesis.
[d] Sample prepared by precipitating E_2 from an alcoholic solution.
[e] Greater than 80% of the particles were less than 20 μm in diameter.

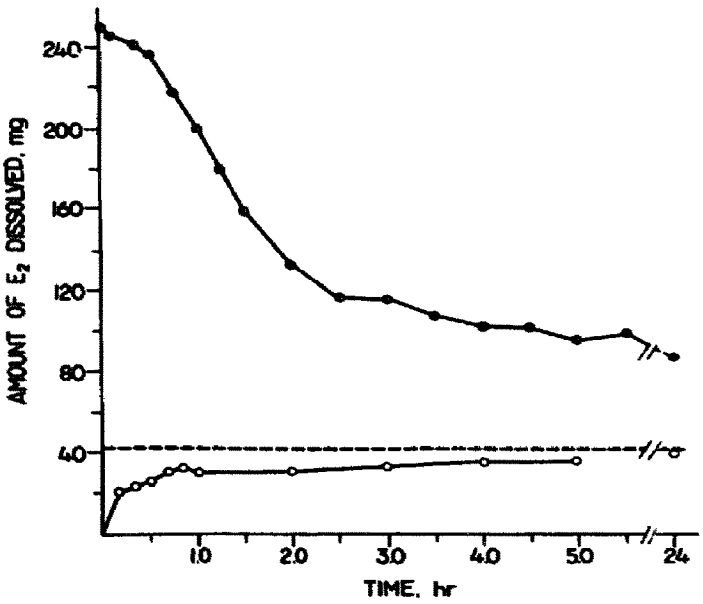
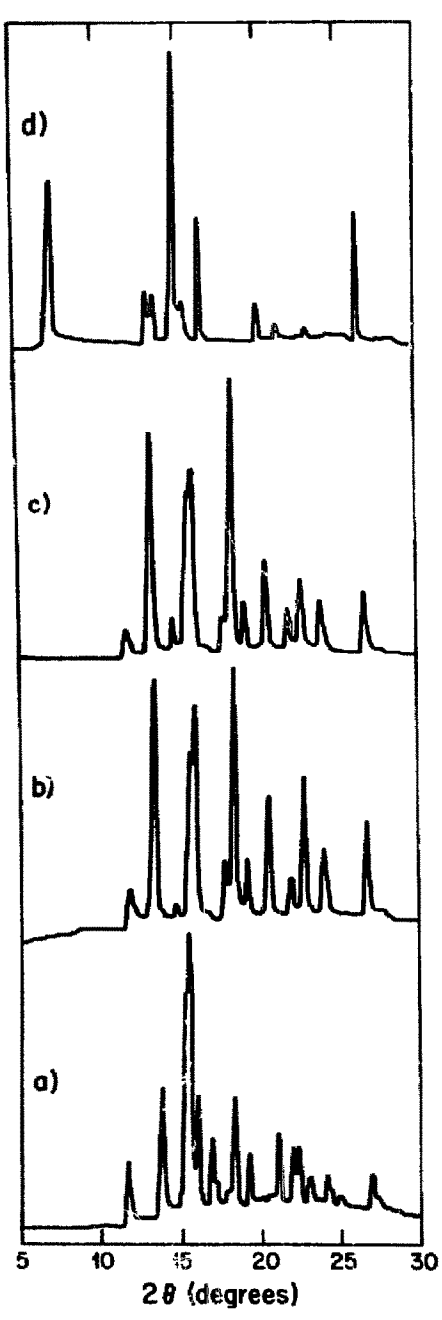


Fig. 3. Dissolution rates of 17β-estradiol (E_2) test preparations (amount equivalent to 250 mg of estrogen; gross particle size, 125–149 μm) at 37°C. Key: ○, untreated E_2; ●, 1 : 9 w/w E_2–povidone coprecipitate; and ------, equilibrium solubility of E_2 in dissolution medium containing 0.225% povidone.

250 mg of E_2 in the coprecipitate sample occurred within 2 min; Fig. 3). After reaching a peak concentration of 250 mg/liter, a value which greatly exceeds the equilibrium solubility of crystalline E_2, the concentration of estrogen in solution from the coprecipitate slowly declined toward the equilibrium solubility value. However, even after 24 h, the amount of E_2 in solution from the coprecipitate was twice the equilibrium solubility value. In striking contrast, exposure of an excess quantity of crystalline E_2 to the aqueous medium resulted in a slow increase in the amount of estrogen dissolved with no supersaturation (Fig. 3). These findings suggest that a form of E_2 possessing a high thermodynamic activity was produced during the preparation of the povidone coprecipitates and that its aqueous solubility is appreciably higher than the most stable crystalline form of this natural steroid. The results of subsequent physical studies, described in the next section, indicate that E_2 exists as a highly energetic form in low ratio E_2–povidone coprecipitates.

Physical measurements

The X-ray diffraction patterns, DSC thermograms and the Raman spectra (lattice vibrations) for untreated E_2 (hemihydrate form), precipitated E_2 (hemihydrate form of E_2 precipitated from absolute ethanol in vacuo at 37°C in the manner used to prepare E_2–povidone coprecipitates), the anhydrous form of E_2 (prepared by heating the hemihydrate at 170°C for several hours), and the ethanol solvate of E_2 are reproduced in Figs. 4–6, respectively. The E_2 used to prepare all of the test systems was the hemihydrate. On heating, these crystals lose their water of hydration between 170 and 180°C; a sharp



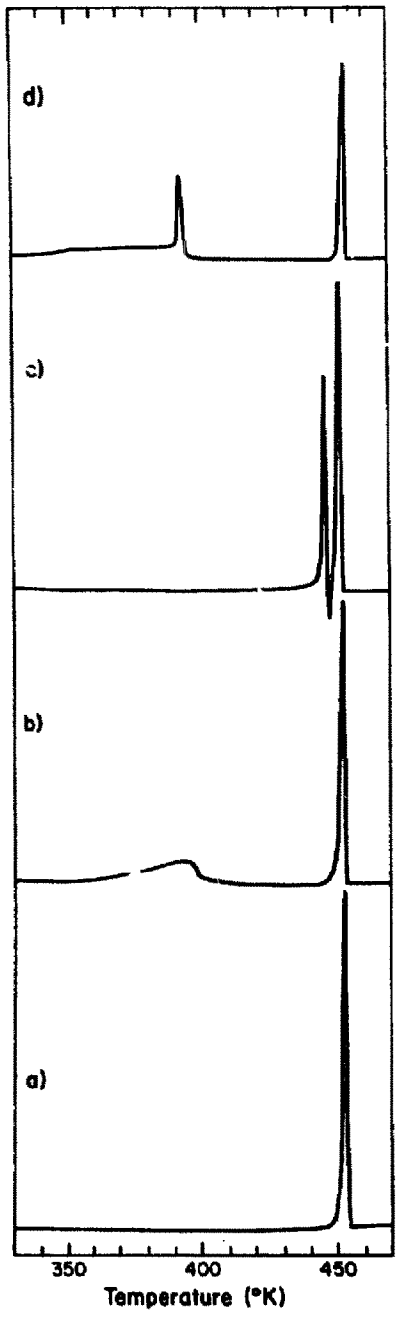
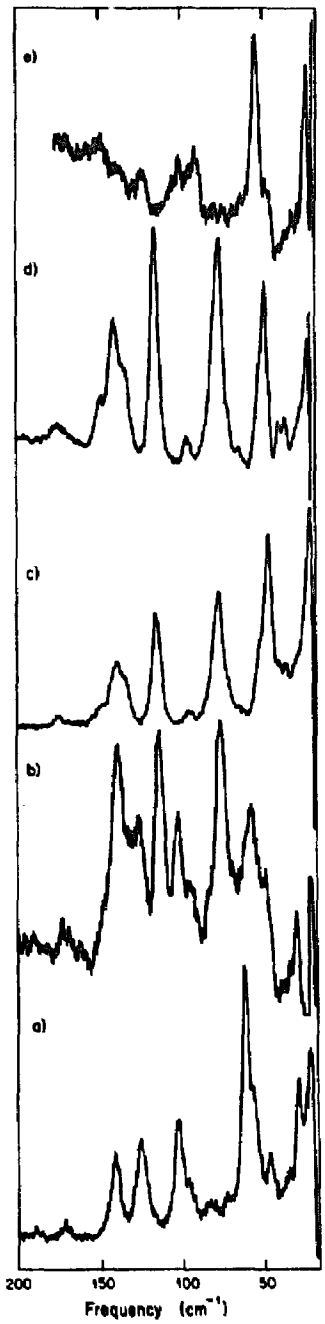


Fig. 4. X-ray powder diffraction patterns for (a) anhydrous 17β-estradiol (E_2), (b) precipitated E_2, (c) untreated E_2 (hemihydrate), and (d) the ethanol solvate of E_2. Ni-filtered Cu Kα radiation was used.

Fig. 5. DSC thermograms obtained for (a) anhydrous 17β-estradiol (E_2), (b) precipitated E_2, (c) untreated E_2 (hemihydrate), and (d) the ethanol solvate of E_2. All peaks shown are endothermic.



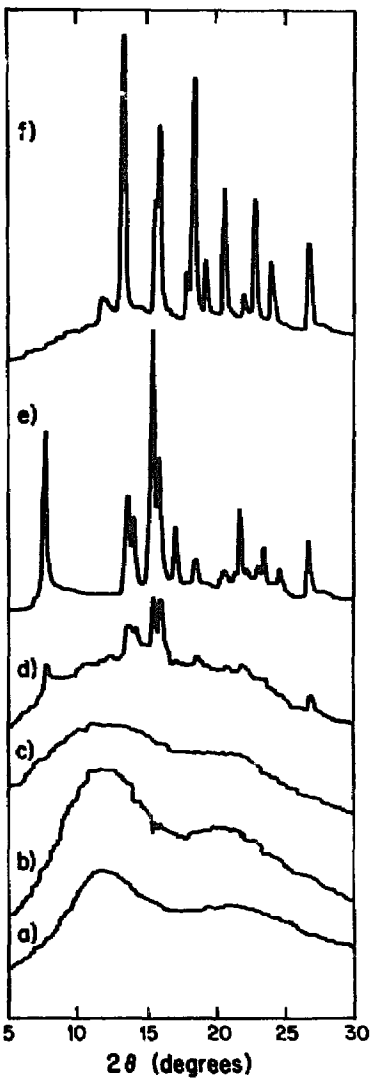


Fig. 6. Lattice vibrational peaks obtained by Raman spectroscopy for (a) the ethanol solvate of 17β-estradiol (E_2), (b) 3 : 1 w/w E_2–povidone coprecipitate, (c) precipitated E_2, (d) untreated E_2 (hemihydrate), and (c) anhydrous E_2.

Fig. 7. X-ray diffraction patterns for (a) untreated povidone, (b) 1 : 9 w/w 17β-estradiol (E_2)–povidone coprecipitate, (c) 1 : 3 w/w coprecipitate, (d) 1 : 1 w/w coprecipitate, (e) 3 : 1 w/w coprecipitate and (f) 3 : 1 w/w physical mixture.

endotherm is observed in the DSC thermogram. The water is bound so strongly in these crystals that recrystallization from a number of non-aqueous solvents or from hydroalcoholic solutions produces only the hemihydrate.

When the hemihydrate of E_2 was *slowly* recrystallized from absolute ethanol at 20–25°C under normal atmospheric conditions an ethanol solvate of the estrogen was formed. DSC and TGA analysis of this material showed it to be a 1 : 1 solvate. However, precipitated E_2 which was prepared by a procedure involving relatively *rapid* recrystallization of the E_2 hemihydrate from absolute ethanol at 37°C and under reduced pressure gave a Raman spectrum and an X-ray diffraction pattern similar to those obtained for the hemihydrate, but it loses its solvent of crystallization at a much lower temperature (TGA and DSC measurements show that desolvation of precipitated E_2 starts taking place near 100 vs. 170–180°C for E_2 hemihydrate). Also, the endotherm associated with desolvation of precipitated E_2 was much broader than that observed for the original hemihydrate. It is believed that significant disorder in the crystals of precipitated E_2 accounts for these observations.

The X-ray diffraction patterns for untreated and precipitated E_2 (Fig. 4), and E_2-povidone physical mixtures containing 10% or 75% (Fig. 7) of E_2 by weight show sharp diffractional peaks derived from estrogen crystals. In striking contrast, E_2 was found to exist in a molecularly dispersed state in E_2–povidone coprecipitates containing 25% of E_2 by weight or less. The X-ray diffraction patterns for these low weight ratio coprecipitates were similar to that for pure povidone (Fig. 7). Crystalline E_2 begins to appear in the isolated coprecipitates when the percentage of estrogen exceeds 25. The X-ray diffraction patterns (Fig. 7) and Raman spectra (lattice vibrations) (Fig. 6) of crystalline coprecipitates containing 50% and 75% of E_2 by weight indicate that E_2 is present as both the ethanol solvate and the hemihydrate. Thermal analysis of the crystalline coprecipitates by TGA and DSC show that the desolvation is diffuse and starts near 100°C, which is suggestive of substantial disorder in the solvate crystals.

Aside from recording the lattice vibrations for the test systems, the internal vibrational spectra were also examined by Raman spectroscopy. If E_2 formed molecular association complexes with povidone to a significant extent, one would expect the internal vibrational modes of the E_2–povidone coprecipitates to be different from those of different crystal forms of E_2. Such was not the case, and we conclude that there is no evidence that E_2 and povidone form definitive complexes in the solid state.

The results of this investigation show that E_2 dissolution is particle size-dependent and that coprecipitation of this natural steroid with povidone at low weight ratios produces a more water soluble, high energy form of the drug. As a result, the dissolution rate of the E_2–povidone coprecipitate is appreciably higher than that of the thermodynamically stable, microcrystalline form of E_2. This may cause more rapid absorption of E_2 from the povidone coprecipitate and thereby increase the systemic availability of E_2 beyond that previously reported for microcrystalline E_2. Support for this hypothesis must await the results of comparative systemic availability studies currently being conducted in postmenopausal women.

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