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# EFFECT OF CRYOGRINDING ON PHYSICO-CHEMICAL PROPERTIES OF DRUGS. II. CORTISONE ACETATE: PARTICLES SIZES AND POLYMORPHIC TRANSITION.

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## ABSTRACT

The low temperature grinding (at 78 K) of cortisone acetate was carried out. From electron microscopy data, there were determined a form, mean linear sizes of particles and then specific surface was calculated. From X-ray data, a part of microcrystalline fraction in cryogrinded samples was calculated. As was indicated by X-ray data, the mechano-induced phase transition from "monoclinic" phase (form $F_I$) to "orthorhombic" one (form $F_{II}$) takes place as a result of 10-min grinding. The transition is confirmed by IR-spectroscopy results too. The local pressures induced by the mechanical stress seems to be the main cause of the phase transition observed.

## INTRODUCTION

Many organic compounds, including those used for obtaining the solid forms of drugs, may exist in several polymorphic modifications, having different physico-chemical properties and being stable under certain thermodynamic conditions. Some technological operations (grinding, drying, pressing), as a rule, are accompanied by a change of these conditions and, in some cases, lead to the transition of one polymorphic modification to another. The possible polymorphic transitions of compounds used for preparing of drug forms may be



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one of the reasons of therapeutic nonequivalence of the latters. On the other hand, mechanical effect may be considered as one of ways for the directed changing of drug compound properties. It seems to be the most perspective to use for this aim the low temperature grinding technology which allows to achieve non-standard thermodynamic conditions for a grinding process and to provide its high efficiency: rapid attainment of the high degree of dispersion as a result of the crystalline material brittleness.

This paper deals with the study of structure-morphological state of cryogrinded cortisone acetate, one representative of a wide class of drug compounds, existing in several polymorphic forms.

## MATERIALS

Cortisone acetate (CA), 21-β-acetoxy-17-α-hydroxy-4-pregnen-3,11,20 trione, $C_{23}H_{30}O_6$ , molecular weight 402.0, melting point 238 - 241 °C (USSR State Pharmacopoeia) (1,2). It may be obtained in five polymorphic modifications by means of recrystallization from various solvents (3,4).

## METHODS

Cryogrinding. A vibrational ball cryomill was used designed at B.Verkin Institute for Low Temperature Physics and Engineering of the National Academy of Sciences of the Ukraine (5) and modified by the authors. The grinding of CA was carried out in $N_2$ vapour under external cooling of the sample chamber by liquid nitrogen. Samples were successively taken out at time intervals 10-20 minutes.

Determination of Particles Sizes and Shape. Transmission electron microscopy investigations were carried out by a microscope JEOL-100U (JEOL, Japan), accelerating voltage 100 kV. Samples were prepared by the "dry method" in accordance with standard technique (6). Maximum linear size of particles, L, was determined from photographs taking into account an instrument magnification. Histograms of particles distribution were plotted versus measured lengths. Histograms for each sample were characterized by the lengths scattering value (the histogram's width), $\Delta L$, and the average particles length $\bar{L}$, determined as

$$\bar{L} = \sum_i l_i n_i / \sum_i n_i , \qquad \sum_i n_i = N; \tag{1}$$

where $l_i$ is the midpoint of the i-th interval of histogram, $n_i$ is the number of particles that fall within i-th interval, N is the total number of particles.

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Specific Surface Calculation. Based on electron microscopy data, calculations of specific surface, S, were carried out using the method described in (7). The calculation formulae from this report were modified (8) to take into account the specific character of particle's size distribution and particle's form (square rods):

$$S = \{2/(\rho\ \bar{L})\}(2\gamma +1)\ (\sum_i n_i \delta_i^2) / (\sum_i n_i \delta_i^3),$$

$$\gamma = l_i / a_i,\ \delta_i = l_i / \bar{L}, \qquad (2)$$

where $\rho$ is the substance density, $l_i$ is the rod length, $a_i$ is the side of square of the rod section.

X-ray Powder Diffractometry. A DRON-3 diffractometer was used. The measurement conditions were as follows: Ni-filtered Cu $K_\alpha$ radiation, $\lambda$ =1.54178 Å, X-ray tube BSV-24, operating voltage 30 kV, current 30 mA, standard 20 mm diameter vessels. In a phase analysis, the interplanar distances (d, Å) were calculated by the Bragg equation and compared with ones from the publication (9).

The extent of crystal lattice disorder was estimated by the method described in (10), where the percentage (A) of amorphous phase was determined in a sample, containing the substance both in crystalline and amorphous states:

$$A = \frac{I_a}{I_a + I_c}\ 100\ \%, \qquad (3)$$

$I_c$ and $I_a$ were the integrated intensities of crystalline peaks and of amorphous region, respectively. In order to estimate the broadening of the X-ray diffraction peaks the only alone peak at $2\theta$=7.23° from the small-angle region of diffractogram was chosen. Its half-breadth was measured and the value of true diffraction broadening $\Delta B$ was calculated.

Infra-Red (IR) Spectra of CA Polymorphic Forms. Spectral data were obtained with a UR 20 double-beam spectrometer (Carl Zeiss, Iena, DDR), with registration at wave numbers scale. Registration was in near infrared-region: 700-1800 $cm^{-1}$, NaCl prism. Samples were prepared by standard method (11) in a form of pressed tablets with KBr.

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## RESULTS AND DISCUSSION

Shape, Linear Sizes and Specific Surface of Cortisone Acetate. The results of particle's sizes determination ( $\bar{L} \pm$ S.D., $\Delta L$ and $\gamma$) as well as calculation of specific surface (S) are presented in Table 1. The samples of CA are sequentially numbered 0, 1, 2, 3 and 4, in accordance with the increasing of cryoeffect time (including initial sample). The particles of CA were needle-shaped (sample 1), rod-shaped (samples 0, 2, 3) and plate-shaped (sample 4), that allowed to simulate their shape by parallelepipeds with various ratio of linear sizes ($\gamma$). Sample 3 shows the first indications of particles aggregation, which leads to the appearance of plate-like formations upon further treatment (sample 4).

As shown in Table 1, $\bar{L}$ decreases and reaches minimum value after 20 min of grinding; with further increase of the cryoeffect time, the mean length of particles increases gradually up to the value of that for initial (intact) particles. The maximum heterogeneity of granulometric composition ($\Delta L$) was observed for initial samples. The lowest $\Delta L$ value was indicated after 20 minutes of grinding. Increase of $\Delta L$ for the next samples is attributed to formation of various aggregates of the activated particles. As in the case of theophylline, such time grinding dependence of particle's size results from the action of two opposite effects: dispersion and formation of secondary aggregates of particles (see Part I). Specific surface of cryogrinded CA particles shows a four-fold increase as compared with one for initial sample and reaches the maximum value after 20 min of dispersion (that corresponds to minimum sizes of particles). Further grinding (20-60 min) leads to decrease of specific surface due to the particles aggregation. The value of S and the character of its change during grinding are determined by the relative ratio of such parameters contributions as the mean length, the form of particle's sizes distribution and the particles shape (see equation 2).

Study of Phase and Structural State of Mechano-Activated Cortisone Acetate. For all samples the X-ray diffraction patterns were obtained and analyzed, the relative intensities ($I/I_o$) and interplanar distances ( $\bar{d}$ ) were calculated. The comparison of crystallographic data of CA samples: initial (sample 0), cryogrinded (samples 1-4) and recrystallized from $CCl_4$ (sample 5), showed that a difference in $\bar{d}$ values of cryogrinded and recrystallized samples for the most reflexes did not exceed an experimental error (0.5%), but the marked differences were observed in the interplanar distances of initial and cryogrinded samples. Figure 1 shows the X-ray powder diffraction patterns of initial, cryogrinded (grinding time 10 and 20 min) and recrystallized from $CCL_4$ samples of CA, respectively.

We compared the obtained values of $\bar{d}$ and $I/I_o$ with ones reported by Callow and Kennard (3) for the two CA polymorphic forms, $F_I$ and $F_{II}$, prepared

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TABLE 1

| Sample number | $t_{grind}$ (min) | $\bar{L} \pm$ S.D. (μm) | ΔL (μm) | S ($m^2/g$) | γ |
|---|---|---|---|---|---|
| 0 | 0 | 0.50± 0.10 | 1.2 | 31.4 | 7 |
| 1 | 10 | 0.15± 0.04 | 0.7 | 92.0 | 10 |
| 2 | 20 | 0.09± 0.02 | 0.4 | 131.0 | 7 |
| 3 | 40 | 0.13± 0.03 | 0.5 | 92.0 | 7 |
| 4 | 60 | 0.30± 0.04 | 0.8 | 26.0 | 3 |

by recrystallization from different solvents. It was found that in our case two CA polymorphic forms are observed, $\bar{d}$ values of initial sample and recrystallized form of CA being identical ($F_I$, monoclinic cell, space group P $2_1$ ). According to (3), all cryogrinded samples are identified as $F_{II}$ (orthorhombic cell, space group P $2_1$ $2_1$ $2_1$ ). Thus, the differences in the X-ray diffraction patterns of initial and cryogrinded samples are due to the difference in their phase composition.

The difference in the phase composition of initial and cryogrinded CA samples are also confirmed by IR spectra of samples 0 and 1, shown in Fig.2. The spectrum of initial sample (Fig.2A) is characterized by five peaks, corresponding to the wave numbers ν=1620, 1655, 1701, 1730 and 1750 $cm^{-1}$. The band at ν=1655$cm^{-1}$ has clearly marked shoulder. The hydroxyl band was observed at 3420 $cm^{-1}$. Infrared spectrum of the second sample (Fig.2B) is identical to one of recrystallized sample and characterized by four peaks at ν=1610, 1613, 1710 and 1740 $cm^{-1}$. A small peak, which is absent in reference sample, appears in the spectrum of second sample at ν=1760 $cm^{-1}$. The hydroxyl band was observed at 3370 $cm^{-1}$. The spectra of the two different CA phase have no marked differences in the "finger-prints" region, except the band at 1271 $cm^{-1}$. Its intensity in the cryogrinded sample is three times as high as in the initial one.

Based on the phase analysis data, it can be concluded that the cryogrinding of CA for 10 min resulted in a complete polymorphic transition from form $F_I$ to form $F_{II}$ (form nomenclature from (3)), as confirmed by the two independent physical methods. We observed the indications of this

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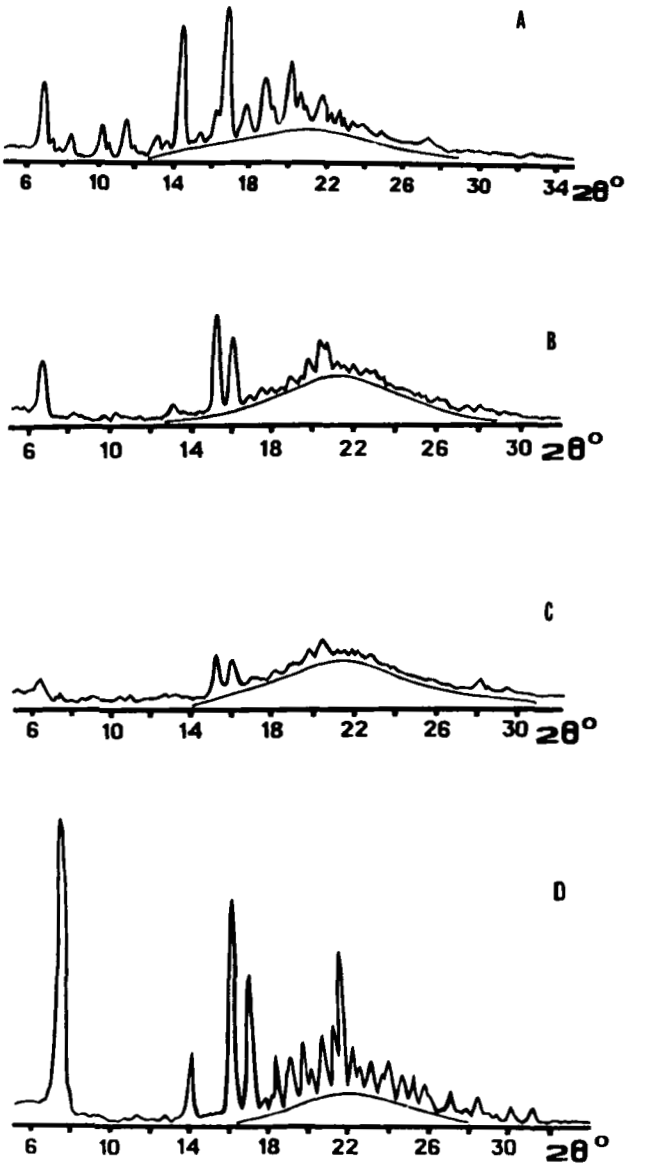


FIGURE 1.

X-ray powder diffraction patterns of cortisone acetate. A - initial (intact) sample ($F_I$); B - $t_{grind}$ = 10 min ($F_{II}$); C - $t_{grind}$ = 20 min ($F_{II}$); D - recrystallized from $CCL_4$ ($F_I$).

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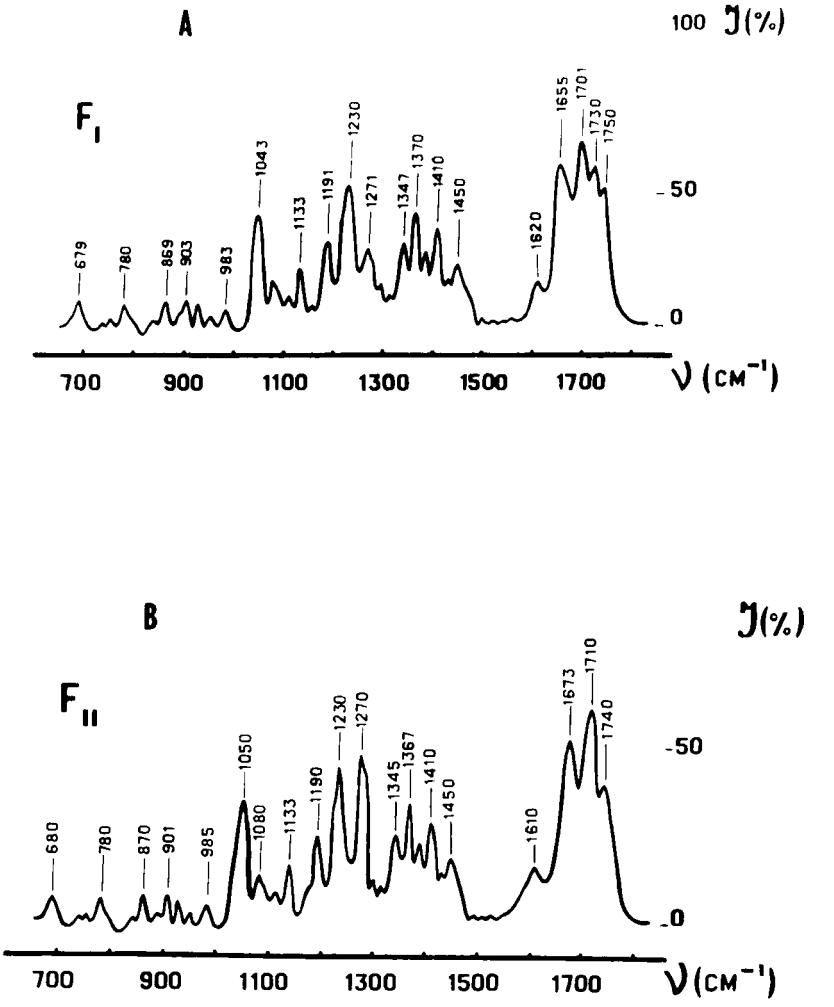


FIGURE 2.

Infrared spectra of cortisone acetate: A - initial (intact) sample ($F_I$); B - $t_{grind}$ = 10 min ($F_{II}$).

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transition in cryogrinded CA earlier (8). But then, in addition to the reflexes of phase $F_{II}$ , $F_I$ phase reflexes were observed at X-ray diffraction patterns; consequently, the both modifications were present in the sample. Such heterogeneity of the phase composition of all cryogrinded samples was probably attributable to the low power of equipment used for the cryoactivation (12).

Mechano-induced polymorphic transitions may occur due to both the local increase of temperature and the high local pressures (13). Phase diagram of all possible CA crystal states is presented and the conditions of their interconversion are discussed in report of Carless et al.(4). According to these data, the crystal form $F_I$ to be of our interest (named by the authors as $F_{III}$ ) may be transfered to the form $F_{II}$ by the prolonged heating in the temperature range 100 °C < T < 200 °C (one of the diagram stages: $F_V \rightarrow F_{III} \rightarrow F_{II} \rightarrow F_I$ , described in (4)). Mechanical treatment of different CA forms, carried out by the authors in agate ball mill during 15 min, did not allow them to realize any of conditions, presented in (13). The low temperature grinding used in our studies excludes the possibility of sufficient local heating. So the action of local pressures sufficient for the realization of phase transition seems to be the main cause of the transition being observed. This conclusion is substantiated by the fact that cryogrinding results in the conversion to relatively high-symmetrical orthorhombic form $F_{II}$ Such scheme of transition ( $F_I \rightarrow F_{II}$ ) may be realized only in systems inclined to polymorphic transformations in a given range of temperatures and pressures, i.e. when it is possible to get into equilibrium by an inclusion of the certain internal degrees of freedom in crystal. In opposite case, the crystal will be undergone to the usual brittle destruction by mechanical stress.

As shown in Fig.1A-D, the changes of X-ray diffraction patterns are observed in the region $2\theta = 16^\circ - 30^\circ$. As in the case of theophylline (see Part I), these changes appear to be caused by the broadening of X-ray peaks as a result of the formation of microcrystalline phase (MCP) fraction. The calculated values of MCP fraction, A, for cryogrinded CA samples are presented in Table 2. In the calculations the method described in (10) was used. The results showed a considerable increase in MCP fraction A at the stages of 0-40 min grinding, whereas at the following stage it had no more changes.

The broadening $\Delta B$ of the only alone diffraction peak (see Methods) in X-ray diffraction patterns for the cryogrinded samples was estimated using the sample 5 (recrystallized one) as reference standard. The broadening was observed at all stages of grinding and had the close order of magnitudes for all samples (see Table 2). The sample 4 (after 60 min grinding) showed a slight increase $\Delta B$. In order to clear, whether this increase to be attributable to the accumulation of defects, leading to the formation of a true amorphous fraction, the additional investigations are needed. It may be supposed, that in a case of molecular

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TABLE 2

| Sample number | $t_{grind}$ (min) | A (%) | ΔB (rad) |
|---|---|---|---|
| 0 | 0 | 15 | $2.8 \cdot 10^{-3}$ |
| 1 | 10 | 30 | $3.0 \cdot 10^{-3}$ |
| 2 | 20 | 38 | $3.2 \cdot 10^{-3}$ |
| 3 | 40 | 55 | $2.8 \cdot 10^{-3}$ |
| 4 | 60 | 54 | $3.8 \cdot 10^{-3}$ |

crystals, consisting of large molecules, the formation and accumulation of the packing defects of a "shifted lamels" type is the most probable, where each lamel is a few molecular layers. The lamel thickness may approach 200-300 Å and crystallites of less than 1000 Å may give line broadening leading to the appearance of amorphous halo.

## CONCLUSIONS

By means of low temperature grinding, the high-dispersed CA powders were obtained, their degree of homogeneity and average particles sizes surpass the according characteristics of powders obtained by usual methods.

As a result of the cryomechanical effect, the X-ray powder diffraction patterns of CA samples were changed due to formation of microcrystalline fraction at the dispersion stage, and due to the intense process of the defect formation at the stage of aggregation.

Low temperature grinding led to the mechano-induced phase transition in CA crystalline fraction: $F_I$ (space group $P\,2_1$) $\rightarrow$ $F_{II}$ (space group $P\,2_1\,2_1\,2_1$), that was indicated by X-ray powder diffraction and IR-spectroscopy. The local pressures induced by the mechanical stress seems to be the main cause of the phase transition observed.

Thus, the low temperature grinding allows both to obtain the high-dispersed drug`s powders and simultaneously to influence upon their crystal structure, that is to obtain the compounds with definite physico-chemical properties that may be of great importance in pharmacology.

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