DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 21(17), 1953-1964 (1995)

EFFECT OF CRYOGRINDING ON PHYSICO-CHEMICAL PROPERTIES OF DRUGS. I. THEOPHYLLINE: EVALUATION OF PARTICLES SIZES AND THE DEGREE OF CRYSTALLINITY, RELATION TO DISSOLUTION PARAMETERS.

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ABSTRACT

An activation of crystalline form of theophylline was carried out by low temperature grinding (at 78 K). From electron microscopy data, there were determined the form, mean linear sizes of particles and then specific surface was calculated. From X-ray data, a fraction of microcrystalline phase in cryogrinded samples was calculated and it was showed that the intense defect formation process takes place up to partial amorphization at final stage of mechanical treatment. By ultra-violet spectroscopy, the solution behaviour of initial (intact) and activated forms of theophylline was studied. The values of solution rate and solubility for activated forms are 12 and 1.5 times greater accordingly then ones for initial form. On the basis of experimentally determined thermodynamic characteristics of solution process for the initial and activated forms, the thermodynamic characteristics of theophylline activation were calculated.

INTRODUCTION

In many drug forms active substances are in a solid state. As a rule, these are organic compounds (crystals), the therapeutic properties of which are determined by both chemical structure and physical properties, in particular, by

the state of their crystalline structure. The process of grinding widely used in pharmacology allows to influence the substance directly, transforming it to the activated state with thermodynamically and structurally unstable arrangement of crystal lattice elements. Low temperature grinding is one of the most up-to-date types of mechanical treatment which enables to extend the class of drug substances to be activated (owing to thermolabile compounds) and to provide high efficiency of this process. Mechanical treatment, including low temperature one, is accompanied by the change of specific surface, phase state, by partial amorphization and appearance of specific intermolecular interactions. These structural changes lead to the change of biological accessibility of drug compounds and may be the decisive factor influencing upon their therapeutic action.

The objective of the present study was to carry out complex investigation of structural, morphological and thermodynamic features of cryogrinded theophylline and the studying their effect on dissolution parameters of this compound.

MATERIALS

Theophylline (TP), purine derivative, 1,3-dimethylxanthine, $C_7H_8O_2N_4$, molecular weight 180.16, melting point 268-272 °C, white crystalline powder. TP needles from H_2O (1). TP used corresponded to the standards of USSR State Pharmacopoeia.

METHODS

Cryogrinding. TP (20 g) was grinded in a ball cryomill, designed by the authors (2). The grinding was performed in liquid nitrogen ("wet grinding") for 1 hour with the concurrent sampling every 5-10 min.

Determination of Particles Sizes and Shape. Transmission electron microscopy investigations were carried out by microscope JEM-100U (JEOL, Japan), accelerating voltage 100 kV. Samples were prepared by the "wet method" in accordance with standard technique (3). Maximum linear size of particles, L, was determined from photographs taking into account an instrument magnification. Histograms of particle's distribution were plotted versus measured lengths. Histograms for each sample were characterized by the lengths scattering value (the histogram's width), ΔL, 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.

Specific Surface Calculation. Based on electron microscopy data, calculations of specific surface, S, were carried out using the method described in (4). The calculation formulae from this report were modified (5) to take into account the specific character of particle's size distribution and particle's form (spheres and square rods):

$$S^{sph} = \{6/(\rho \bar{L})\}(\sum_i n_i \delta_i^2)/(\sum_i n_i \delta_i^3), \quad \delta_i = l_i / \bar{L}, \qquad (2)$$

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

where ρ is the substance density, l_i is the sphere diameter or 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_α radiation, λ=1.54178 Å, X-ray tube BSV-24, operating voltage 30 kV, current 30 mA, standard 20 mm diameter vessels. Electron microdiffraction patterns of initial and cryogrinded TP samples were obtained with the microscope JEM-100U in the microdiffraction mode. The extent of crystal lattice disorder was estimated by the method described in (6), where the percentage (A) of amorphous phase was determined in a sample, containing the substance both in crystalline and amorphous states:

$$A = \frac{I_a}{I_a + I_c} 100\ \%, \qquad (4)$$

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 2θ=6.52° from the small-angle region of diffractogram was chosen. Its half-breadth was measured and the value of true diffraction broadening ΔB was calculated.

Dissolution Behaviour Study. Kinetics of dissolution was studied by the method (7) in the presence of an excess of solid phase (the quantity of TP being dissolved was 4 times as much as one needed for the solvent saturation). A spectrophotometer SPECORD UV VIS (DDR) was used for the spectrophotometric analysis of solutions. The absorption curves were registered at 270 nm (8). Substance concentrations were calculated in a standard way using the calibration curve.

Calculation of Thermodynamic Parameters of Activation. By means of the following representation of dissolution reaction of TP in cryogrinded (activated) and initial (non-activated) states:

$$B^*_{solid} \rightarrow B_{dissolv} + H^*_T , \quad (5)$$

$$B_{solid} \rightarrow B_{dissolv} + H_T, \quad (6)$$

the thermodynamic parameters of activation (the relative changes of enthalpy ΔH^*_T , free energy ΔG^*_T and entropy ΔS^*_T) were calculated at T=298 K as follows:

$$\Delta H^*_T = H^*_T - H_T , \quad (7)$$

$$\Delta G^*_T = - RT \ln (K^*_s / K_s), \quad (8)$$

$$\Delta S^*_T = (\Delta H^*_T - \Delta G^*_T) / T , \quad (9)$$

where K^*_s and K_s are the equilibrium constants of dissolution reaction of activated and non-activated (initial) substances, respectively.

RESULTS AND DISCUSSION

Shape, Linear Sizes and Specific Surface of Theophylline. The results of particles sizes determination ($\bar{L} \pm$ S.D., ΔL and γ) as well as calculations of specific surface (S) are presented in Table 1. The samples of TP are sequentially numbered 0, 1, 2, 3 and 4 in accordance with increase of the cryoeffect time (including initial sample).

The electron micrographs of TP samples showed the variety of shapes of the particles being observed. The initial sample contains the needles and thin plates. The samples 1 and 2 consist, mainly, of spherical shape particles as well as of their secondary agglomerates in the case of sample 2. In the latter case it is possible to determine the individual sizes of particles from which aggregates are composed. In the sample 3 both isolated ellipsoids with unsmooth surface and their large aggregates (up to 4 μm) are observed. The sample 4 contains the needles, which form large aggregates of different morphology. The spherical particles shape being observed in TP samples seems to be caused by the treatment method (9) and by material's properties (such as TP dissolution to a certain extent under the "wet" method of low temperature grinding).

TABLE 1

Sample number	t_{grind} (min)	Particle shape	$\bar{L}$±S.D. (μm)	ΔL (μm)	S (m^2/g)	γ
0	0	rod	0.87 ± 0.15	3.2	5.2	3
1	10	sphere	0.20 ± 0.06	1.2	9.6	-
2	20	sphere	0.14 ± 0.04	0.6	12.5	-
3	40	sphere	0.26 ± 0.05	0.8	9.4	-
4	60	rod	0.95 ± 0.12	2.8	6.9	3

As can be seen from the Table 1, with increase of the cryogrinding time, the values $\bar{L}$ and ΔL decreased and reached their minima (0.14 and 0.6 μm, accordingly) at t_{grind} = 20 min. Further increase of the cryoeffect time leads to the gradual increase of the mean length of particles observed and to the sharp increase of the granulometric composition heterogeneity.

Such time grinding dependence of particle's size results from the action of two opposite effects: dispersion and formation of secondary aggregates of particles. The relative ratio of these two effects has strongly pronounced dependence upon temperature. Low temperatures used enabled to intensify the process of dispersion and to achieve the particles size of 0.1 μm order. Comminution of TP by heat method has not led to the reduction of particles' sizes of initial samples (formation of aggregates took place already during first period of the process).

Aggregates observed during last stages of grinding were not destructed later on by the various sample preparation methods. Van der Waals and electrostatic forces play the key role in the formation of aggregates (10).

Specific surface reaches its maximum after 20 min of dispersion (2.4 times increases compared to the initial one). Further comminution (20-60 min) is characterized by the decrease of specific surface due to process of cryoparticle's aggregation.

Specific surface of polydispersed systems, which are the TP powders studied, depends on the several parameters: mean length ($\bar{L}$), form of the particle's size distribution, particles shape. S value of a given compound and the character of its change during grinding is determined by the the relative ratio of these parameters contributions.

Structural State of Mechanoactivated Theophylline. It is known that the process of grinding is accompanied by the intense formation of defects in the material to be treated. In order to evaluate the degree of the defect formation in molecular crystals the authors of (11) used such a parameter as the extent of crystallinity: the change of X-ray diffraction patterns of the cryogrinded digoxin (disappearance of characteristic diffraction peaks) took place in the small-angle region ($2\theta = 5^{\circ}$-14°), that corresponds to the true amorphization of crystalline structures (short-range disorder).

Figure 1 shows X-ray powder diffractograms of an initial and two cryogrinded (time of grinding 20 and 60 min) TP samples (Fig.1 A, B and C, respectively). Changes of X-ray diffraction patterns (disappearance of characteristic diffraction peaks), in contrast to ones described in (11), were registered in the region of $2\theta = 16^{\circ}$-30° and was caused by the broadening of X-ray peaks as a result of the microcrystalline phase formation. The wide diffuse background peak indicated by firm line in Figure was formed, as we suppose, due to X-ray scattering from the microcrystalline phase (MCP) fraction of cryogrinded samples. Percentage of MCP (A, %) was calculated according to formula (4) in assumption that the intensity of X-ray scattering from the sample regions without defects of crystalline structure is proportional to the area under the most pronounced peaks, while that from a whole sample is proportional to the total area under the curve. The intensity of X-ray scattering from the microcrystalline regions is proportional to the area of the wide diffuse peak, lying under the pronounced peaks. The calculated values of parameter A are shown in Table 2.

It should be noted that such marked changes in TP crystalline structure was observed at the first stage of grinding (cryoeffect time 0-20 min), the stage of dispersion. At the next stage (20-60 min) MCP fraction had no marked changes.

However, the analysis of time grinding dependence of the broadening ΔB of chosen alone line (in small-angle region) showed that the broadening is observed upon all stages of grinding and has the close orders of magnitudes for samples 1, 2 and 3 (Table 2). In sample 4 the value of ΔB is more then 2 fold increased, indicating the more intense process of the defect formation accompanied by the appearance of the true amorphous regions at this cryoeffect stage.

Electron microdiffraction patterns of TP samples clearly demonstrated the evolution of crystalline state: the formation of amorphous phase under the influence of mechanical treatment (2).

Dissolution Kinetics and Thermodynamics of the Activated Forms of Theophylline. The dissolution behaviour was studied by the analysis of the concentration curves of TP solutions in the presence of an excess of solid phase. The results for dissolution and solubility of TP samples are presented in Table 3.

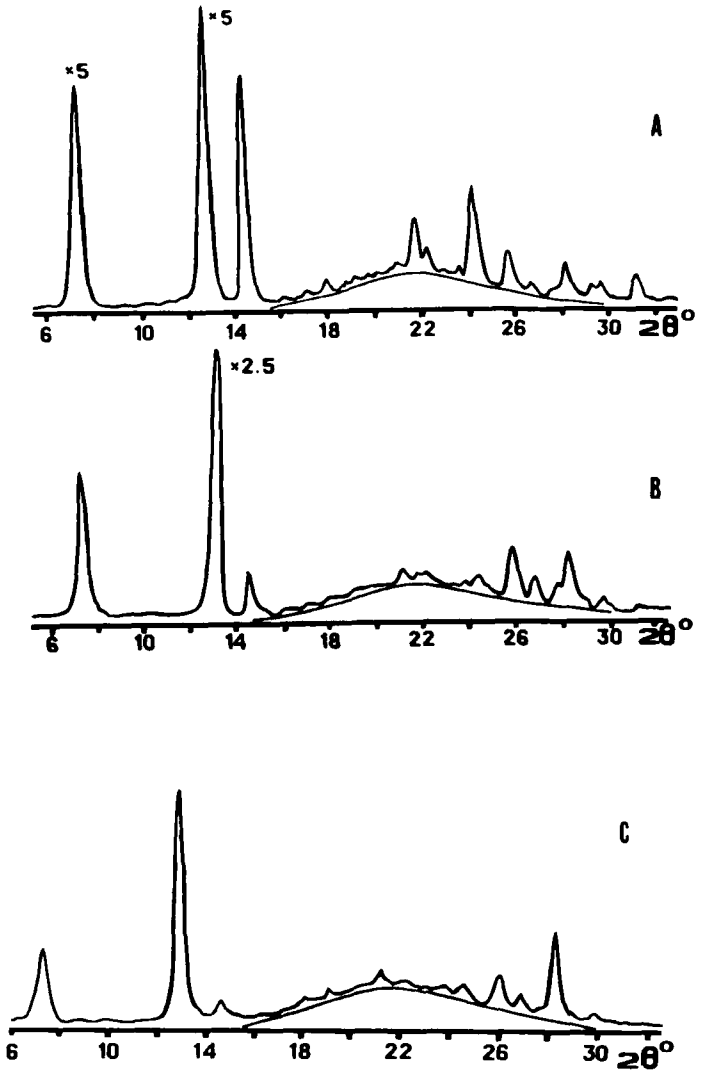

FIGURE 1.

X-ray powder diffraction patterns of theophylline. A - initial (intact) sample; B - t_{grind} = 20 min; C - t_{grind} = 40 min.

TABLE 2

Sample number	t_{grind} (min)	A (%)	ΔB (rad)
0	0	20	-
1	10	66	$3.5 \cdot 10^{-3}$
2	20	70	$3.5 \cdot 10^{-3}$
3	40	69	$2.8 \cdot 10^{-3}$
4	60	69	$7.8 \cdot 10^{-3}$

TABLE 3

Sample number	V $\frac{mg}{ml \cdot min}$	C_s^n $\frac{mg}{ml}$	ΔH $\frac{kJ}{mol}$	ΔG^{*n} $\frac{kJ}{mol}$	ΔH^{*n} $\frac{kJ}{mol}$	ΔS^{*n} $\frac{kJ}{mol \cdot K}$
0	0.27	17.0	38.9	-	-	-
1	1.46	20.5	45.6	0.46	6.69	0.021
2	2.27	21.5	47.7	0.59	8.79	0.029
3	3.00	23.5	51.9	0.79	13.0	0.042
4	3.24	24.2	53.1	0.88	14.2	0.046

The concentration maximum corresponds to solubility, C_s (the concentration gradient controlled process). The greater time of grinding the greater the activated form`s solubility, its maximum value is 1.4 times as large as that of non-activated form. The dissolution rate V was calculated as the tangent to the concentration curve at the point $C_s^n/2$; index n corresponds to the sample number. As shown in Table 3, the dissolution rate of sample 4 was 12 times increased as compared with that of initial TP sample dispersed by a routine thermal method.

Since for saturated solutions at given temperature and pressure $C_s^n = K_s^n$, where K_s^n is the equilibrium constant, the temperature dependence of

concentration C_s^n allows to determine the enthalpy ΔH of TP dissolution by Vant-Hoff plot. The calculated ΔH values (the temperature range 298-328 K was observed) as well as the thermodynamic parameters of TP activation calculated by equations (7)-(9) are presented in Table 3. Maximum values of thermodynamic parameters of activation were observed at grinding time 60 min. The comparison of ΔH^{*n} and ΔG^{*n} values shows, that in the case of mechanical activation of TP the enthropy contribution should not be neglected, in contrast to the cases with a small crystal lattice disorder.

Dependence between Structure and Dissolution Parameters at Different Stages of Cryoeffect. Based on the obtained experimental data, the character of relationship between TP dissolution parameters (V, C_s) and the state of its surface (S) and crystalline stucture (parameters A, ΔB) was analysed.

The process of TP cryogrinding is suggested to consist of two stages; the first is dispersion (sample 0-2), and the second is aggregation (samples 3 and 4). As can be seen from the plots presented in Fig. 2-4, at the stage of dispersion, the increase of dissolution parameters takes place with the increase of both surface parameters and the parameters of crystalline structure. At the stage of aggregation, the increase of dissolution parameters is accompanied by the decrease of specific surface and the increase of parameter ΔB (relative decrease of ΔB values for sample 3 is an exception), parameter A has no marked changes.

It is known, that the broadening of X-ray diffraction peaks (ΔB) is determined by two factors: the crystallite`s sizes and the microstresses within the crystal lattice. It seems likely that the broadening of diffraction peaks is mainly contributed by the decrease of primary crystallites sizes at dispersion stage and by the increase of microstresses at the stage of aggregation. This increase of microstresses is accompanied by the amorphization indicated by amorphous halo in the electron diffraction patterns. ΔB decrease for sample 3 corresponds to the transition from one stage to another.

Thus, the relative ratio of contributions from the surface (specific surface) and structural (MCP fraction and microdisorders of crystal lattice) changes to dissolution parameters of a compound studied is changed in the course of cryoeffect. At the first stage these contributions are approximately equal. At the next stage the change of TP dissolution parameters is determined mainly by the process of defect formation.

CONCLUSIONS

The following conclusions can be derived from the above experimental results.

By means of low temperature grinding, high-dispersed TP powders were obtained. Their degree of homogeneity and mean particle`s sizes are 5-6 times as large as that of pharmacopoeia powders obtained by usual methods.

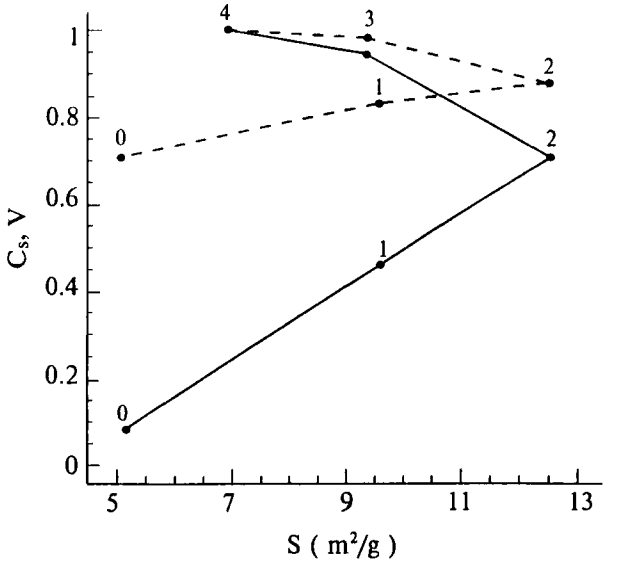

FIGURE 2.

Graphycal plot of dissolution parameters, C_s (broken line) and V (firm line), against specific surface S for initial and cryogrinded theophylline. C_s and V values are normalized to their maxima. Samples are numbered: 0, 1, 2, 3 and 4.

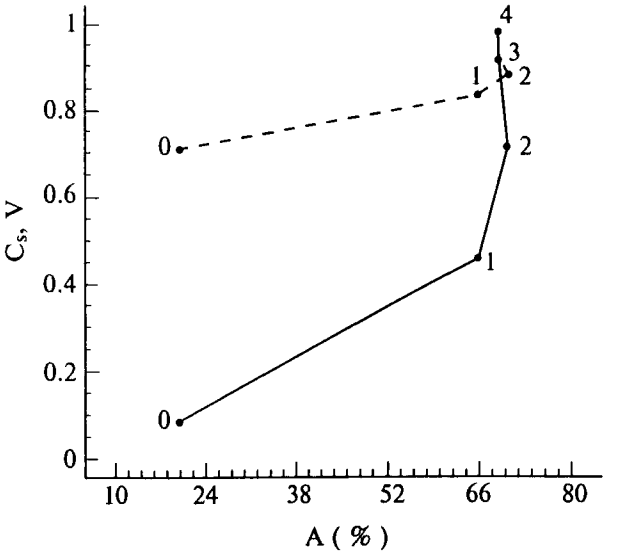

FIGURE 3.

Graphycal plot of dissolution parameters, C_s (broken line) and V (firm line), against A for initial and cryogrinded theophylline. C_s and V values are normalized to their maxima. Samples are numbered: 0, 1, 2, 3 and 4.

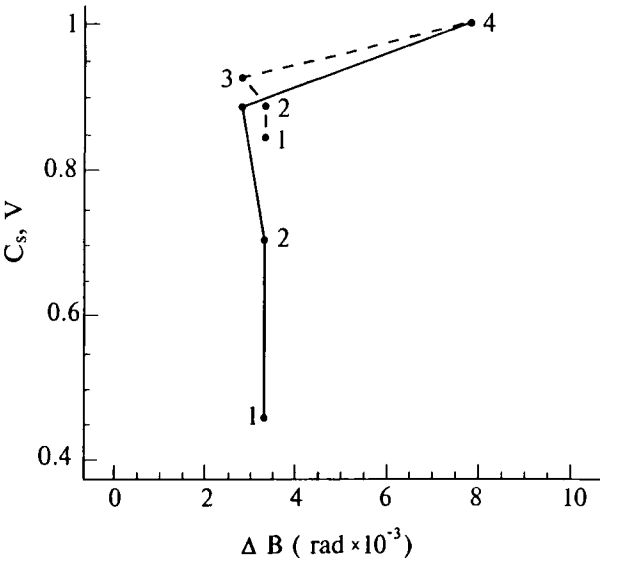

FIGURE 4.
Graphycal plot of dissolution parameters, C_s (broken line) and V (firm line), against ΔB for cryogrinded theophylline. C_s and V values are normalized to their maxima. Samples are numbered: 1, 2, 3 and 4.

As shown by the study of morphological state of cryogrinded powders, the grinding process is the result of action of two opposite effects: dispersion and formation of aggregated secondary particles.

As revealed by the changes in X-ray diffraction patterns, the TP structural state is changed during the cryogrinding process. At the stage of dispersion the microcrystalline phase is formed; its maximum fraction is 55%. Later on, at the stage of aggregation, the marked broadening of the small-angle X-ray diffraction peak is observed, indicating the intense process of the defect formation up to the partial amorphization.

Such change of structural state leads to the increase of dissolution rate and solubility of cryogrinded TP in 12 and 1.4 times, accordingly, compared to that dispersed by usual (thermal) method. The maximum values of these parameters as well thermodynamic characteristics of cryogrinded TP correspond to maximum degree of amorphization.

The analysis of relation between the dissolution parameters and characteristics of surface and crystalline structure allows to conclude that, at the stage of dispersion, the change of TP dissolution parameters is determined by

both surface and structural changes. At the stage of aggregation, the change of dissolution parameters is mainly determined by the process of defect formation (by partial amorphization of the samples).

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