Mechanoactivated Medicinal Preparation of Calcium Gluconate: X-Ray Diffraction, Microscopic, and X-Ray Electron Investigations

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Abstract

Mechanoactivation method has been applied to an officinal medical preparation of calcium gluconate for obtaining its modified form. It was found by X-ray diffraction and microscopic investigation techniques that the mechanoactivated powder constitutes X-ray amorphous, nanodispersed product with particle size from 50 to 500 nm. X-ray electron spectral analysis revealed no basic changes in chemical composition of calcium gluconate upon mechanoactivation.

INTRODUCTION

Mechanoactivation method is widely applied now for modification and mechanochemical synthesis of medicinal preparations [1–3]. It is common knowledge that treatment in high-energy mincing devices makes it possible to obtain instant, assimilable, and more effective medical preparations. For example, one of them is instant aspirin [4–6]. However, it turned out that nobody was engaged until now in mechanochemical modifying of officinal medicinal preparations of calcium. The mechanoactivated calcium gluconate we obtained for the first time has been approved on the clinical base of the Izhevsk State Medical Academy to treat osteoporosis and other bone diseases caused by it with respect to children in the age from several months to 17 years [7–10]. The results have suggested that the modified preparation is capable to exert a favourable influence on the remodelling processes for bone tissue. The analysis of tentative data has demonstrated that there is a need to continue complex research in view of viability of this direction.

The purpose of the present work is to carry out the definition phase studies aimed at disclosing the reasons that cause effective therapeutic action, an increase in bioavailability, and improvement of pharmacokinetic properties of the officinal medicinal preparation of calcium gluconate modified by the mechanoactivation method.

EXPERIMENTAL

To carry out this work, the tabloid of officinal medicinal preparation of calcium gluconate has been chosen. Mechanoactivated powders were obtained by the mechanical grinding method of calcium gluconate tablets in Pulverisette-7 planetary ball mill in tight vessels in a gas medium with argon added. Time of grinding t_{gr} ranged from 15 min to 16 h. The vessels and balls have been made from steel of SHKH15 grade. SHKH15 steel possesses high hardness and wear resistance; it is low-alloy and it contains a minimum quantity of impurities (content of Cr is 1.5 mass %, C – 1 %),

which practically rules out a possible contamination of the obtained powders by the material of grinding bodies (Fe and Cr). The investigations made earlier have demonstrated that at grinding time t_{gr} = 4–8 h, the use of SHKH15 steel does not result in contamination even for Fe–C and Fe–Si metal systems [11], which possess much better abrasive properties relative to calcium gluconate. With the purpose of primary control, gravimetric measuring of vessels, balls, and samples was conducted before and after grinding.

Analysis of chemical composition of tablets and of the grinded powder was conducted by the X-ray photoelectron spectroscopy method (XPES) in ES-2401 spectrometer with MgK_α excitation. The limiting residual gas pressure in the spectrometer was not over 10^{-6} Pa. The procedure of carrying out the measurements and processing the results is given in [12]. The structural-phase analysis of tablets and mechanoactivated powders was conducted by the X-ray diffraction (XD) method in the DRON-3 diffractometer with monochromated CuK_α radiation. The size of powder particles was defined with Auger microprobe JAMP-10S in a raster scanning mode, with Neophot-2 optical microscope, and by the atomic power microscopy method (APM) with the R47 microscope (NT-MDT, Russia).

RESULTS AND DISCUSSION

Gravimetric measurements demonstrated that the powder weight remains invariant up to the maximum grinding time of 16 h. X-ray diffractograms of an initial tablet of calcium gluconate $Ca[CH_2OH(CHOH)_4COO]_2 \cdot H_2O$ and the grinded powders are presented in Fig. 1. The initial state shows a set of structural reflexes that is characteristic of a crystalline state of calcium gluconate [13]. In an interval of t_{gr} from 0.25 to 0.5 h, an increase of amorphous halo and a reduction of intensity for structural reflexes develop. At t_{gr} = 1 h, a complete X-ray amorphous state of powder is attained and a further increase in the treatment time does not result in any changes in the X-ray diffractograms. Consequently, t_{gr} that is equal to 1 h is the time large enough for the translation

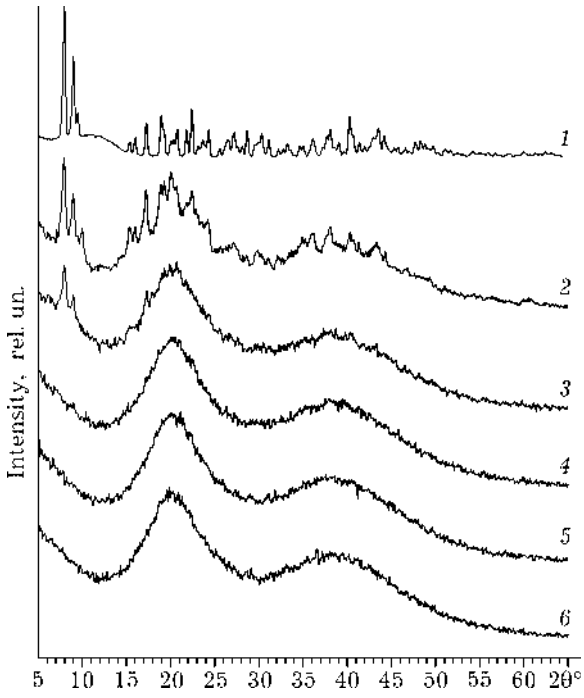


Fig. 1. X-ray diffractograms of calcium gluconate (CuK_α radiation). t_{gr}, h: 0 (*1*), 0.25 (*2*), 0.5 (*3*), 1.0 (*4*), 2.0 (*5*), 16.0 (*6*).

symmetry to disappear and the compositional and topological disorder to form. The freedom from α-Fe structural reflexes testifies that within the limits of XD sensitivity there was no contamination of samples by a material of the applied grinding bodies during mechanical grinding.

An estimate of particle sizes, which has been performed in the Auger microprobe and optical microscope for the samples with t_{gr} = 2 h, has demonstrated that they do not exceed 1 μm. The more detailed investigation has been conducted by the APM method (Fig. 2). Upon 15 min of grinding, the powder mostly consisted of the particles 100–300 nm in size, which formed agglomerates of a size up to 800 nm. Upon 2 h of grinding, the particle size ranged from 50 to 500 nm. Hence, according to APM and XD data, a reasonably definite assertion is possible that the X-ray amorphous state of the medicinal preparation of calcium gluconate is produced at $t_{gr} \geq 1$ h with the particle sizes in the range from 50 to 500 nm.

X-ray electron spectral analysis revealed no Fe and Cr in the produced mechanoactivated powders. Only calcium and carbon lines were present. With an increase in grinding time, 5 %

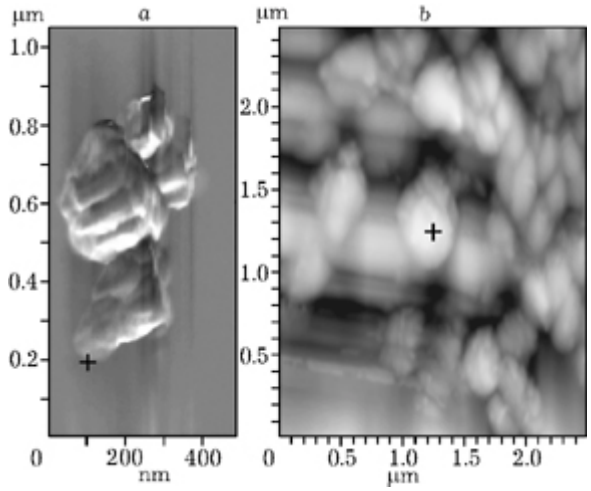
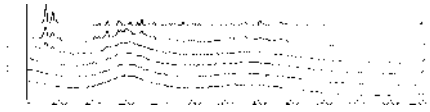


Fig. 2. APM images of calcium gluconate powders. t_{gr}, h: 0.25 (*a*), 2 (*b*).

increase of oxygen concentration and 6 % decrease of carbon concentration occurred as compared with the initial state (Table 1). These changes can be caused by the effect of residual atmosphere in the grinding device and by interaction of mechanoactivated powders with the atmosphere during their extraction from the grinding device. Nevertheless, one can argue that there were no any significant changes in chemical composition of medicinal preparations. The line shape and half-width of Ca2$p_{3/2}$ line remained constant for the initial sample and samples after grinding (see Table 1 and Fig. 3, *a*). The changes that were more essential were observed in C1*s* spectra (see Fig. 3, *b*). According to the structural formula of calcium gluconate [14]



(1)

TABLE 1

Results of XPES investigations of mechanoactivated calcium gluconate

t_{gr}, h	Fraction of atoms on the sample surface, at. % (1 % error)			Half-width of Ca2$p_{3/2}$ line, eV
	C	O	Ca	
0	53	45	3	2.3
0.25	51	45	4	2.3
0.5	51	47	3	2.1
1	49	49	3	2.2
16	47	50	3	2.3

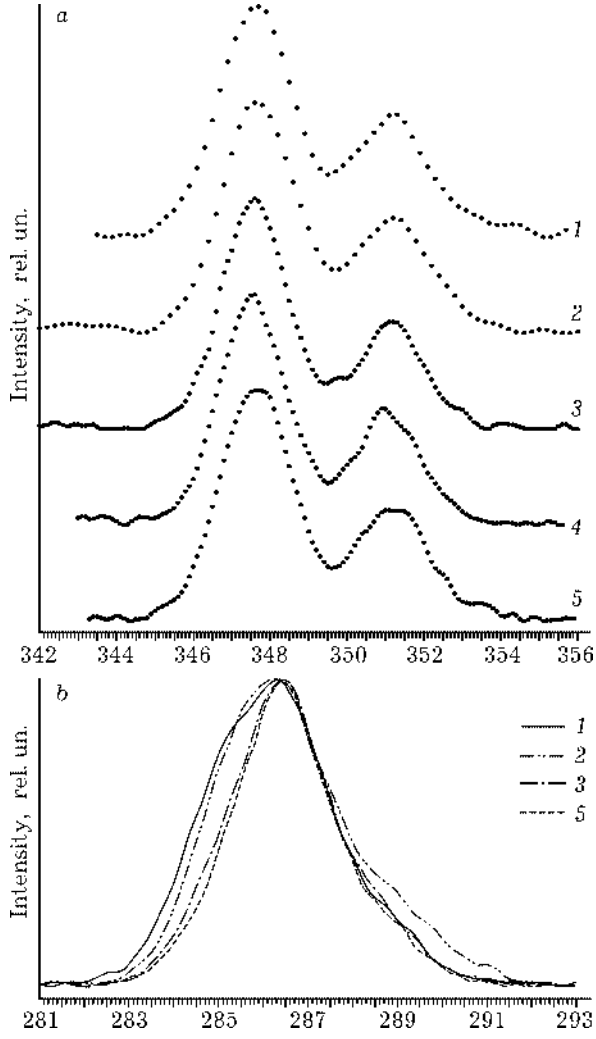


Fig. 3. X-ray electron spectra of Ca2$p_{3/2}$ (*a*) and C1*s* (*b*) for calcium gluconate. t_{gr}, h: 0 (*1*), 0.25 (*2*), 0.5 (*3*), 1.0 (*4*), 16.0 (*5*).

only two lines with binding energies E_b = 286.5 eV (C–OH group) and E_b = 288.8 eV from Ca carboxylate [15] must be present in carbon spectra with the 5 : 1 intensity ratio. However, the line with E_b = 285 eV from CH groups is present in C1*s* spectra, which may result from hydrocarbon contamination of the sample surface during obtaining the spectrum. With increasing t_{gr}, the reduction in comparative intensity of the line with E_b = 285 eV is evidenced. The most intensive change of C1*s* spectrum occurs in *tgr* interval from 0.25 to 0.5 h. During the further increase of t_{gr}, no essential changes are observed. The intensity of the line with E_b = 285 eV in the final state comprises 11 %, of the line with E_b = 286.5 does 71 %, and that with E_b = 288.8 eV does 28 % from

the total area of carbon peak, *i. e.* C1*s* spectrum of the mechanoactivated powder of calcium gluconate better matches the structural formula (1) with the increased time of grinding.

It has been found that pH of a solution of calcium gluconate tablet (0.5 g in 0.25 ml of fresh distilled water) is equal to 6.5 (distilled water showed pH 6.53). Upon 2 and 16 h of grinding, pH of the solution comprised 8.97 and 9.21, respectively.

By and large, from the above investigation results it follows that mechanical grinding does not contaminate the grinded preparation and does not change significantly its elemental chemical composition. The performed treatment results in that the preparation becomes a nanosize amorphous powder with much increased proportion of active surface and with the changed local atomic environment around carbon as a consequence of amorphization. The data acquired make it possible to assert that no critical chemical transformations with the formation of radically new compounds occurred during mechanical grinding of calcium gluconate and the preparation under investigation can be thought of as mechanoactivated amorphized calcium gluconate.

CONCLUSIONS

The modified form of calcium gluconate has been produced by method of mechanoactivation of the officinal medicinal preparation. It constitutes an X-ray amorphous powder with the particle size from 50 to 500 nm. No basic change in chemical composition of calcium gluconate has been revealed during mechanoactivation. The higher than usual biological activity of the amorphized nanodispersed calcium preparation is caused, most likely, by its high reactivity and by a much greater particle surface. The results arrived at are generally of provisional character and further physicochemical, biochemical research, and clinical tests are needed to gain an insight into the nature of elevated biodigestibility of the mechanoactivated calcium preparation.

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