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Effect of Mechanoactivation on The Structure, Physical, Chemical and Biological Properties of Calcium Lactate, Calcium Gluconate and Calcium Citrate

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***Abstract*-The influence of mechanical activation on calcium lactate, gluconate and citrate structure, dissolution rate, angle of polarization plane of water solutions and microelectrophoretic mobility of erythrocytes and buccal cells in water solutions was studied. Plate-like particles of mechanically activated powder are collected in aggregates of particles. Thickness and sizes of particles depends on the mechanical activation regimes. Such activation results in amorphization of crystal calcium lactate and crystallization of amorphous calcium lactate. The difference of structural state of initial and activated salts remains in water solutions. Angles of polarization plane of mechanoactivated calcium gluconate and calcium lactate water solutions are less than those of initial salts. The increased amount of active buccal cells in solutions of calcium lactate with the lower angle of rotation of polarization plane was observed. However the percentage of active erythrocytes and their vibration amplitude is conversely decreased. Opposite to calcium lactate, less optically active modification of calcium gluconate shows more bio-activity both to buccal cells and erythrocytes. Biological activity of mechanoactivated samples of calcium lactate and calcium gluconate, caused by optical isomerism, returns to the bio-activity level of initial samples at ambient condition during 12 month. Crystal structure of calcium citrate remained unchanged. Water solutions of calcium citrate are not optically active.**

***Keywords*: mechanical activation, calcium lactate, calcium gluconate, calcium citrate, water solutions, structure, amorphization, biological activity, optical isomerism.**

I. INTRODUCTION

There is strong relationship between the structure and biological properties in most of organic compounds. That is why one of the main trends in the modern pharmaceutical chemistry is increasing of efficiency of drugs known before, adverse reactions of which have been studied and therapeutic benefits have been proved by many years of experience. One of the solutions of this problem is improvement of pharmacological characteristics of known medicines by means of changing of their structure without any change of composition. In order to realize this approach the deformational impacts on a compound are quite efficient, such as hydrostatic pressure and mechanoactivation.

Changes in chemical structure of organic compounds caused by deformation can be divided into two groups. Processes of the first group are connected with breaking and forming intramolecular covalent bonds (molecular dissipation, oxidation and hydrolysis). The second group includes processes with breakage and formation of weaker intermolecular bonds (disordering, amorphization and polymorphic transitions of a crystal lattice of compounds, conformational transformations of the molecules forming lattices) [1-5]. Despite numerous researches in this field, it is not always possible to forecast transformations of particular organic compounds exposed to deformation. There is limited number of works that study physical-chemical properties of such compounds in metastable state formed as a result of deformation. There is little information about life time of metastable states. Therefore, it remains actual to obtain experimental data in this area.

Since dissolution is a limiting stage of an effect of drugs onto an organism, from a practical point of view such factors as the dissolution rate, solubility of preparations and retention of difference between initial and modified state compound in a solution are also very important.

The present research is a part of our studies of deformation stimulated structural and chemical transformations of organic compound which are the active compounds of known drugs, and relationship of their structure with physical-chemical and biological properties. We use hydrostatic pressure, severe plastic deformation by torsion, mechanical activation as an action of deformation.

The authors [6,7] find that the mechanoactivation of calcium gluconate in a planetary ball mill results in significant increase of therapeutic efficiency in treatment of a number of diseases connected with calcium deficiency. The probable reason of such effect is amorphisation and isomerization of calcium gluconate [7]. However there were no researches on isomeric state of calcium gluconate and that question remained open.

The influence of the calcium lactate powder ball milling on such pills characteristics like hardness, thickness was studied in the work [8]. It was concluded that the reason of improvement of pharmaceutical properties of pills is polymorphic transformations caused by dehydration of calcium lactate during milling of initial powder. However the following questions were not studied: which particular polymorphic transformations of calcium lactate occur? Shall these transformations have an effect on biological properties of calcium lactate?

Simple calcium salts of organic acids are widely used as a component of drugs and different biologically active supplements for treatment and prevention of diseases connected with the calcium deficiency. Therefore the researches on the methods of formation of mostly biologically active structure of these salts are interesting.

The aim of the study was, firstly, to study the effects of mechanoactivation on the structural and chemical state of calcium salts (lactate, gluconate and citrate); secondly, the identification of structures with the greatest biological activity.

II. EXPERIMENTAL

A. Materials

To carry out the work we used calcium lactate, calcium gluconate and calcium citrate produced by Sigma-Aldrich without any additional purification.

B. Sampling

Samples were subjected to mechanoactivation in the planetary ball mill AGO-2 and the vortex mill VME-150. The energy intensities were 2 and 0.16 kJ/g respectively.

C. *Instruments and methods*

Elemental analysis was carried out by method inductively coupled plasma atomic emission spectroscopy (ICP-AES) using spectrometer Spectroflame Modula. According to the data of analysis there are no any impurities in the observed mechanoactivated powders by the materials of balls and vials. Solubility of initial and mechanically activated samples was determined at ICP-AES.

Severe plastic deformation of samples is carried out at a pressure of 3.5 GPA at the anvils in the form of discs (d = 9 mm). Method of obtaining curves "torque – shear deformation" we described earlier in [9].

The study of powders particles morphology of after mechanical activation was made by the atomic force microscopy (AFM) by means of scanning probe laboratory Ntegra Prima (NT-MDT).The powders particles were provisionally attached to the polystyrene film. The film was applied to sitall with the further fixation of the powder under UV radiation.

X-ray diffraction patterns were obtained in parallel-beam (CuKα and MoKα) geometry using D8 Advance (Bruker AXS) powder diffractometer: II generation Goebel mirror (PGM2) at initial beam, horizontal Soller slit 0.12 deg. and solid-state Si(Li) detector at the secondary beam. Scanning was performed by the method of the variable count time (VCT), Shankland [10]: counting time increases with the 2θ angle. Powder diffraction data were analysed by the Rietveld method using software package Topas 4.2.

Fourier transform infrared (FT-IR) spectra were recorded with an FSM-1202 (Russia) spectrometer with resolution of 1 cm^{-1}. The measurements were performed on pellets made of the samples and pure KBr powder.

Angles of rotation of polarization plane were measured by the circular polarimeter CM-3 at the temperature 25°C.

The microelectrophoresis method was used to analyze the biological properties of initial and mechanoactivated calcium salts. The essence of this method is measuring of the amplitude of cells movement in the field of microscope. In electrophoresis chamber cells make constrained reciprocating movements during reversal of stress at electrodes (10V, frequency 0.1 Hz). The frequency of such movements equals to the frequency of stress reversal at electrodes, but the amplitude of movements can be different depending on the charge of the cell surface which is the marker of physiological state of a cell [11]. The study was made using the complex Cytoexpert (Russia).

Determination of the amount of calcium lactate was made by titration method with EDTA. Thermal coefficients of volume expansion were calculated by the formula $\alpha = -1/\rho\ (\partial\rho/\partial T)$, where ρ-density (g|$cm^3$), T – temperature of solution (°C). The coefficients were determined within the temperature range 25° - 35°C. The solution densities were measured by pycnometer.

III. RESULTS AND DISCUSSION

A. Mixture "crystal calcium lactate + 3.25% vol. crystal calcium carbonate"

X-ray diffraction pattern of calcium carbonate agree with calcite structure. Mechanical in VME-150 in 1h (dose of mechanical energy 0.56 kJ/g) does not result in any qualitative changes in the X-ray diffraction pattern. Cubic form particles in initial sample were of the size in order 80-100 μm. The particle sizes were reduced to 50÷70 and 1.0÷ 1.5 μm after mechanoactivation in 1h and 2 h with dose of mechanical energy 0.56 and 1.12 kJ/g, respectively. Water solubility of machanoactivated calcite is the same as initial nonmechanoactivated.

Powder particles of the initial mixture "crystal calcium lactate + crystal calcite" were of the size 50÷70 μm. Mechanoactivation was performed in the vortex mill VME-150. In 1 h of mechanical activation (dose of mechanical energy 1.12 kJ/g) the powder consisted of aggregates with the size about 10÷20 μm. In 2 h of activation the sizes of aggregates were decreased to 1.5÷3.0 μm.

X-ray diffraction pattern of the initial powder corresponds to the mixture of pentahydrate of calcium lactate and calcium carbonate (Fig.1). The structure of calcium lactate corresponds to the structure of L-isomer [12]. As a result of mechanoactivation a slight expansion of X-ray lines of diffractogrammes and decrease of their intensity are observed, which indicates the decrease of grain size and growth of microstrains. The crystalline structure of calcium lactate and calcite in mixture doesn't change. After 2 hours of mechanoactivation (1.12 kJ/g mechanical energy induced) additional reflexes on the diffraction pattern appears, which can be related to the formation of DL-structure [12].

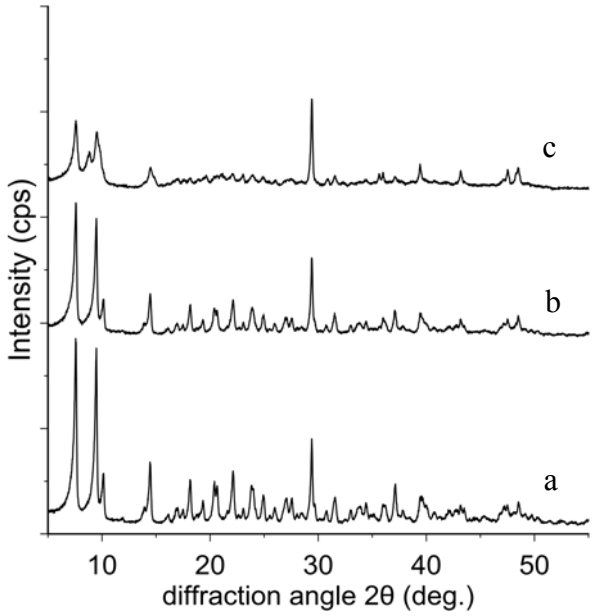

Figure 1. X-ray diffraction patterns of initial (a) and mechanoactivated mixture of crystal calcium lactate and 3.25% vol. crystal calcium carbonate (b) - 1 h, (c) - 2 h

During mechanical activation calcium lactate partially destructed with the formation of calcium carbonate (calcite) (Table 1).

Solubility of amorphous calcium lactate in mechanoactivated mixture increases comparing to the initial crystal calcium lactate: 8.32 and 8.06 g/100 g H_2O respectively (Table 1). Solubility of calcium carbonate in mechanoactivated mixture is changed slightly (~10%).

TABLE 1. Effect of mechanoactivation (in VME-150) on the composition and physical-chemical properties of crystal calcium lactate

Period of mechanical activation, h	Dose of mechanical power, kJ/g	Content of calcium carbonate, vol.%	Solubility of calcium carbonate g/100g H_2O	Volume expansivity rate ($\beta \cdot 10^{-4}$) of water solution (0.02 mol/l, T= 25º÷35ºC)
0	0	3.25	8.06	2.27
1	4.5	14.2	8.55	2.40
2	9.0	15.4	8.74	2.45

Physiological and bio-chemical action of optic isomers is very often absolutely different [13]. In this case the difference is in different microelectrophoretic mobility of live cells in water solutions of isomers. There is observed the increased amount of active buccal cells in solutions of DL-calcium lactate with lower angle of rotation of polarization plane. Whereas the amount of active erythrocytes and their vibration amplitude is conversely decreased (Table 2).

The thermal analysis of pentahydrate of calcium lactate was performed in [14]. Endothermic peaks at DSC thermographs correspond to formation of crystalline hydrates with little amount of water molecules. The conclusion was made that dehydration leads to polymorphic transformations of calcium lactate. However these polymorphic transformations were not studied. We heated the mix of crystalline calcium lactate and carbonate up to 115°C during 30 minutes. Thermal treatment results in amorphization of calcium lactate (Fig.2). The same result was obtained after the thermal treatment at 80°C [14].

TABLE 2. Angle of rotation of polarization plane of water solutions of crystal calcium lactate (0.22 g/100 g of water) and microelectrophoretic mobility of live blood cells (erythrocytes) and buccal cells in water solutions (0.01g/100g of water) of initial, thermally treated and mechanoactivated calcium lactate

Dose of mechanical energy, kJ/g	Angle of rotation of polarization plane,°	Buccal cells				Blood cells (erythrocytes)	
		Amount of active cells, %	Vibration amplitude, μm			Amount of active cells, %	Amplitude of cell vibration, μm
			cells	nuclei	plasmalemma		
0	-15	28	0	0	1.2	100	14.0
2	-10	35	0	1,1	2.2	90	10.0

The crystalline structure of calcite preserved in the mixture under the heating (Fig.2). The percentage of calcite not increased, i.e. there was no destruction of calcium lactate. Thus, initially, pentahydrate of calcium lactate in the work [14] apparently had crystalline structure, and later there occurred its dehydration and amorphization.

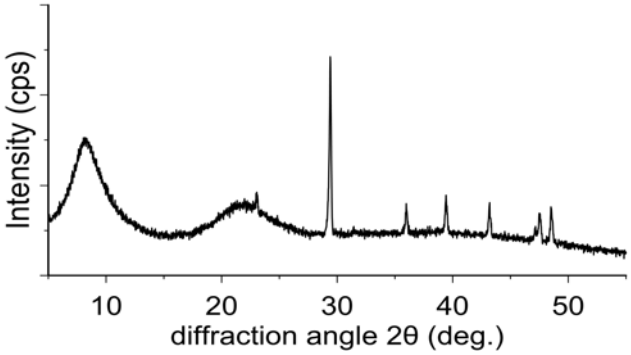

Figure 2. X-ray diffraction pattern of mix "crystal calcium lactate + 3.25% vol. crystal calcium carbonate" after heating up to 115° C

Microelectrophoretic mobility of buccal cells and erythrocytes in the thermally treated calcium lactate solution and in the initial calcium lactate solution are the same. Change in the structure of calcium lactate does not change the biological properties of its water solution.

B. Amorphous calcium lactate

Initial powder of amorphous calcium lactate (without impurities) contains particles of micron-level size - over 50 μm. In 0.5 h of ball milling in AGO-2 (dose of mechanical energy 3.5 kJ/g) the powder consists of aggregates of laminar particles in the form of trapezoidal prism (Fig.3). Particles sizes in aggregates are quite significant: 1.5 ÷ 8 μm. Lamels thickness in a particle are 150÷200 nm. In 1 hour of ball milling (dose of mechanical energy 7 kJ/g) the form of particles is preserved, but the maximum particles sizes in aggregates are decreased to 2 μm. The sizes of aggregates does not exceed 5 μm. Average size of a laminar particle is 1.1 μm. After 3 hour of ball milling (dose of mechanical energy 21 kJ/g) the powder contains aggregates consisting of particles of 250÷500 nm. Aggregates are poorly bonded and destroyed during scanning under the action of cantilever. Average size of the particles is of the order 330 nm. Thickness of lamels also decreases to 25 nm. With the increase in activation time to 6 hours simultaneously with decreasing particle size (140 nm) occur process aggregation and compaction units. The dimensions of the aggregates remain the order of 5 μm.

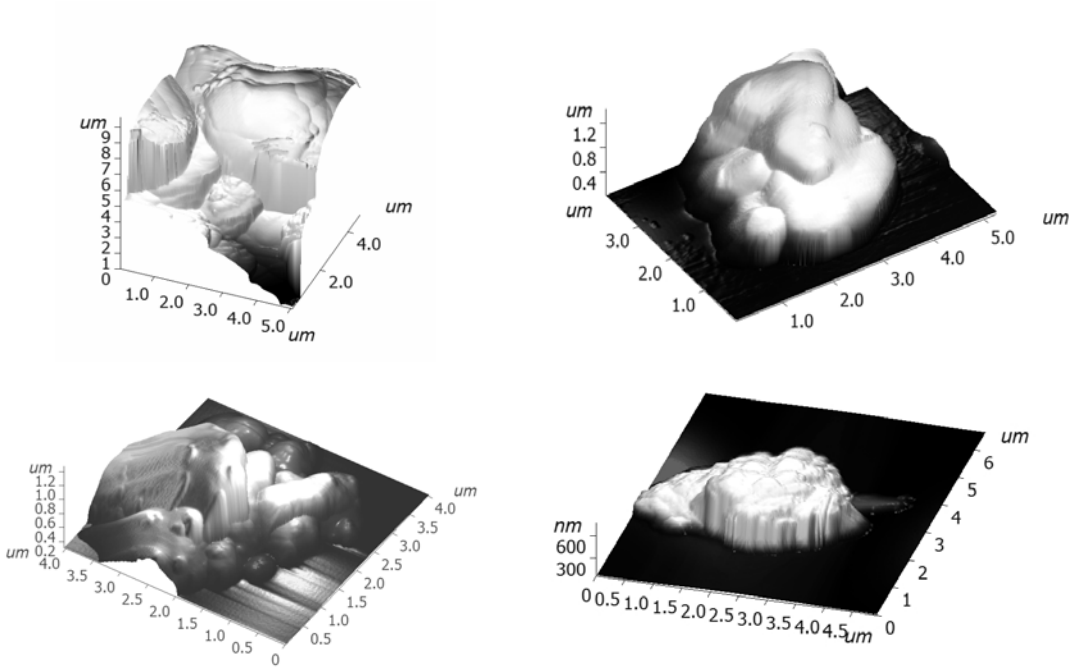

Figure 3. AFM-image of the powder particles of calcium lactate after ball milling 0.5 h (a), 1 h (b), 3 h (c), 6 h (d)

X-ray diffraction patterns of initial and ball milled calcium lactate are reported in Fig.4. Diffraction peaks from calcite and the background halo from amorphous calcium lactate are visible for all ball-milled samples.

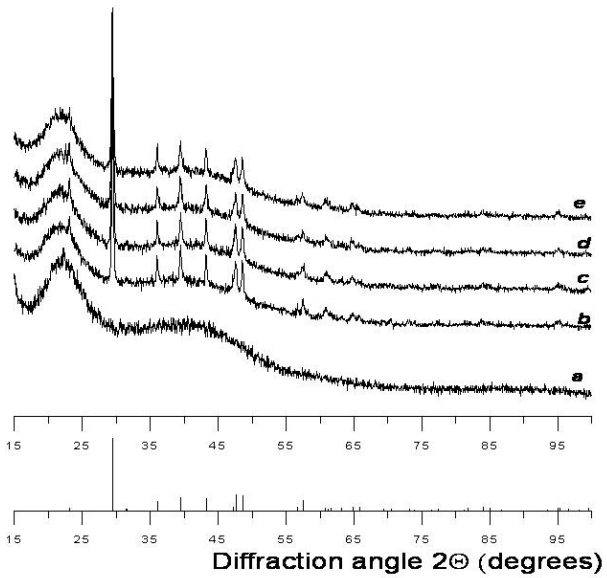

Figure 4. X-ray diffraction patterns of initial (a) and ball milled calcium lactate (b – 0.5; c - 1 h; d - 3 h; e - 6 h)

With the increase of the ball-milling time intensity of the crystalline peaks slightly decrease. This effect is due to grain size decrease and microstrain level growth. During the ball milling, partial decomposition of calcium lactate and calcium carbonate occurs.

IR spectra of ball milled samples (Fig.5) shows a band at ~875 cm^{-1} associated with deformational vibrations of CO_3^{2-} and increasing in intensity of the stretch vibration band of CO_3^{2-} at 1430 cm^{-1}. This is clear evidence of the formation the calcium carbonate after 30 minutes of ball milling.

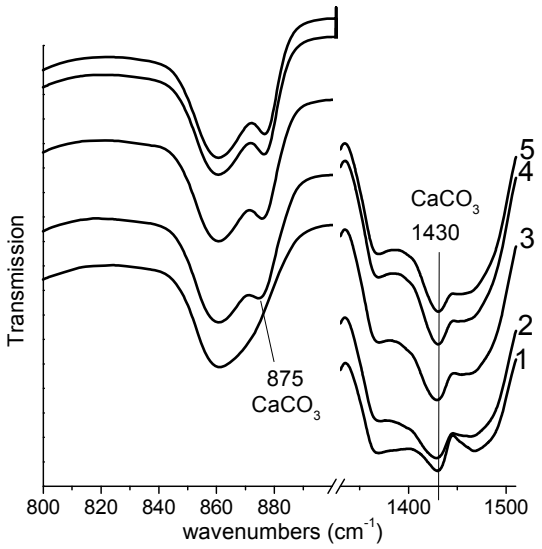

Figure 5. IR-spectra of initial (1) and ball milled calcium lactate: 2- 0.5h; 2 – 1h; 3 – 3h; 4- 6h.

Results of analysis (by titration with EDTA) of calcium lactate content in initial powder and that of ball milling are shown in the Table 3.

Solubility may depend on the particle size of powders. This relationship is expressed in mathematical form as an Ostwald - Freundlich equation [15]. Although this equation was criticized in literature, experimental results show that

solubility really increases for small enough crystallites. For example solubility of griseofulvin in water increases from 11.9 mg/100 g H_2O (microcrystal) to 60.2 mg/100g H_2O (nanocrystal) [16], methylhydroxyprohesteron – from 1.2 mg/100g H_2O to 3.5 mg/100g H_2O [17], nimesulphide - from 11 mg/100g H_2O to 25 mg/100g H_2O [18]. So, even if it is difficult to check the equation in quantitative aspect, it is valid in qualitatively.

TABLE 3. Effect of ball milling on a composition and physical-chemical properties of calcium lactate

Period of mechanical activation, h	Dose of mechanical power, kJ/g	Calcium carbonate content, % vol.	Solubility of calcium lactate, g/100g H_2O	Volume expansivity ratio ($\beta \cdot 10^{-4}$) of a water solution (0.02 mol/l) T= 25º÷35ºC
0	0	1.2	8.36	2.2
0.5	3.5	11.3	8.36	2.4
1	7.0	13.4	8.80	2.4
3	21.0	16.1	9.05	2.4
6	42.0	16.8	9.03	2.2

Sizes of particles of mechanoactivated calcium lactate powders are not less than 180 nm, which can not result in decrease of solubility of calcium lactate. Amorphous structure of calcium lactate remains unchanged during mechanoactivation, and calcium carbonate retains crystalline structure. Thus the increased solubility of mechanoactivated calcium lactate with carbonate impurity is not connected with their crystalline structure or sizes of powder particles. Solubility rate of mechanoactivated calcium lactate is higher than that of initial one in approximately 2.5 times.

The two new bands vibrations of carboxylate-anion (asymmetric $\nu_{as}(COO^-)$ at 1585 cm^{-1} and symmetric $\nu_s(COO^-)$ at 1430 cm^{-1}) are observed in spectra of water solutions of calcium lactate. Additionally a shoulder at the band 1735 cm^{-1} has been attributed to carbonyl vibrations, that occurs in both initial and ball milled calcium lactate. The shoulder is due to impurities of a lactic acid or calcium lactate stereoisomer.

Usually the solubility of enantiomers is the same or slightly differs. However solubility of racemic mix significantly differs from the solubility of pure enantiomers (could be both more or less). Due to this it can be supposed that the possible reason of increased solubility is the changed isomeric structure of calcium lactate and formation of racemic mixture.

There are the results of analysis of optical activity of water solutions of calcium lactate in the table 4. All solutions are of levorotatory isomers. There are shown the results of measurements of rotatory angles for the solutions of the concentration 0.2g/100g H_2O in the Table 4. The decreased optical activity of water solutions of calcium lactate is observed with more dose of mechanical energy.

TABLE 4. Angle of rotation of polarization plane of calcium lactate water solutions (0.22 g/100 g H_2O) and microelectrophoretic activity of live erythrocytes and buccal cells in water solutions (0.01 g/100 g H_2O) of initial and ball milled calcium lactate

Dose of mechanical energy, kJ/g	Angle of rotation of polarization plane, °	Microelectrophoretic activity of live cells					
		Buccal cells				Buccal cells	
		Percentage of active cells, %	Vibration amplitude, μm			Percentage of active erythro-cytes, %	Vibration amplitude of erythro-cytes, μm
			cells	nuclei	plasmalemma		
0	-15	34	0	0	1,2	100	14,0
3.5	-14	40	0	0	0	84	9,0
7.0	-7	73	0	2,1	2,8	82	7,5
21.0	-7	70	0	0,5	1,1	86	7,5
42.0	-6	66	26	0	1,7	84	5,5
In 12 months after ball milling	-14	35	0	0	0.8	92	12.0

Comparison with the results of polarimetric and microelectrophoretic study of crystalline calcium lactate let us suppose that there was L-isomer of calcium lactate in initial powder in amorphous state, but DL-isomer appeared after ball milling. The percentage of DL-isomer increases with increasing of dose of mechanical energy.

C. Calcium gluconate

The particles of test specimens of the powder, ball milling within 1 h in AGO-2 (dose of mechanical energy 7 kJ/g), are of platelet shape. The width, length and thickness of platelets are about 5 nm (Fig.6). Besides there observed agglomerates sized of several hundreds of nanometers (up to 500 nm).

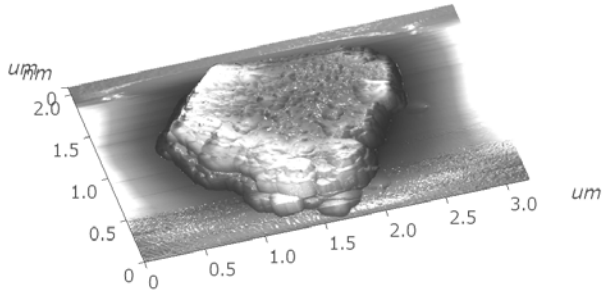

Figure 6. AFM –image of a plate-like particle of ball milled calcium gluconate

Earlier it was determined in [6,7] that mechanoactivation of calcium gluconate in a planetary ball mill results in its amorphisation. The true density of mechanically activated calcium gluconate is less than that of initial crystalline: 1.67 g/cm^3 and 1.91g/cm^3 respectively.

In [19] we studied the effect of ball milling on calcium gluconate by the method of X-ray photoelectron spectroscopy. It was shown that ball milling of a pure calcium gluconate without impurities during 1 h (dose of mechanical energy 7 kJ/g) does not result in destruction and formation of new chemical compounds. In order to perform comparative study of spacial arrangement of OH-groups in the molecules of initial and ball milled calcium gluconate, there was used their chemical marking by trifluoroacetic anhydride [19, 20]. It was found that steric availability of OH-groups in the initial calcium gluconate molecules is higher than in molecules of ball milled sample. It has been suggested that gluconate-anion is "turned off" in non-closed cycle and part of OH-groups becomes unavailable to trifluoroacetic anhydride [19]. However, it should be noted that another possible reason for the change of the steric accessibility of OH-groups of the gluconate anion may be the formation of an optical isomer. However optical activity of calcium gluconate was not studied. [19].

Solubility of calcium gluconate and ball milled (1 h) calcium gluconate differs, being 3.5 and 4.5 g/l respectively. The solubility rate of ball milled calcium gluconate is significantly higher comparing to the solubility rate of initial powder of calcium gluconate in stationary state and while stirring. The period of solution of ball milled activated and initial powders in stationary state is 60 s and 48 h respectively, when stirring – 15 s and 30 s respectively. The solubility rate was measured using Ca-selective electrode. Rotation speed of magnetic stir bar was 130 rpm [19].

pH value of water solutions of initial and ball milled calcium gluconate with concentration 0.16 mol.% is equal 6.75°±0.1.

Researches [19] on concentration dependence of structure-sensitive properties of calcium gluconate water solutions suggest that differences of structural state of initial and ball milled gluconate remain unchanged in solutions. Analysis of structure-sensitive properties of water solutions (temperature and concentration dependence of density, viscosity, refraction indices, conductivity, diffusion rate) indicates decreasing of hydrophilic properties of ball milled gluconate-anion comparing to the initial one.

Angle of rotation of polarization plane of the water solution of ball milled calcium gluconate is almost 2 times larger than for the water solution of initial calcium gluconate of the same concentration (Table 5).

TABLE 5. Angle of rotation of polarization plane of the water solutions of calcium gluconate (1.3 g/100 g of water) and microelectrophoretic mobility of live blood cells (erythrocytes) and buccal cells in water solutions (0.025 g/100 g of water) of initial and ball milled calcium gluconate

Dose of mechanical energy, kJ/g	Angle of rotation of polarization plane, °	Microelectrophoretic mobility of live cells				
		Buccal cells			Blood cells (erythrocytes)	
		Percentage of active cells, %	Vibration amplitude, μm		Percentage of active cells, %	Vibration amplitude of cells, μm
			nuclei	plasmalemma		
0	-29	8.3	0	1.2	0	0
3.5	-12.5	86	2.1	6.4	2.0	56.8
In 12 months after ball milling	-31	6.0	0	1.2	0	0

This indicates that molecules of initial and ball milled calcium gluconate are different diastereomers. Change in mutual arrangement of hydrophilic OH-groups in the structure of gluconate-anion of isomer formed during ball milling leads to decrease of area of hydration. Increased gluconate-anion hydrophobicity is the reason for less capillary viscosity and for growth of diffusion rate in water. Decreasing of molar volume of water in solutions of ball milled calcium gluconate indicates less water H-bonding which is caused by reduction of whole volume of hydration shells [18].

We do not reject possibility of formation of open cycle of gluconate-anion. Moreover both diastereomers can form open cycle. Additional research should be done to make the final confirmation or eliminate the possibility of such transformation of gluconate-anion.

The results of the study of microelectrophoretic mobility of live erythrocytes and buccal cells in water solutions of initial and ball milled calcium gluconate are presented in the Table 5. Unlike calcium lactate, less optically active modification of calcium gluconate shows more bio-activity both to buccal cells and erythrocytes.Thus we can conclude that the reason of high therapeutic effect of ball milled calcium gluconate discovered by the authors [6,7] due to optical isomerism, i.e. due to formation of more bio-active optical isomer.

D. Calcium citrate

Laminar structure of particles and agglomerates is observed in 1 h of mechanoactivation in AGO-2 (dose of mechanical energy 7 kJ/g) of calcium citrate powder. Sizes of agglomerates are up to 10-15 μm, average size of particles is 350 nm; thickness is about 120 nm (Fig.7).

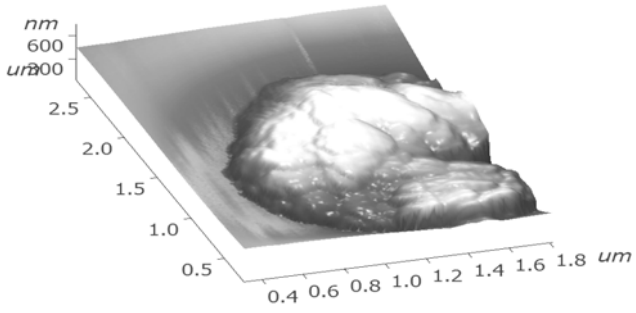

Figure 7. AFM-image of a particle of calcium citrate powder after 1 h of ball milling in AGO-2

Ball milling during 2 h (dose of mechanical energy 14 kJ/g) results in decreased grain size and growth of microstress level without changing of structure and volume fraction of phases. X-ray structural analysis (Fig.8).indicates that the initial powder contains calcium citrate with two different structures [21]: tri-calcium di-citrate tetra-hydrate $[Ca_3(C_6H_5O_7)_2(H_2O)_2]\cdot 2H_2O$ with triclinic structure P-1 and earlandite with monoclinic structure.

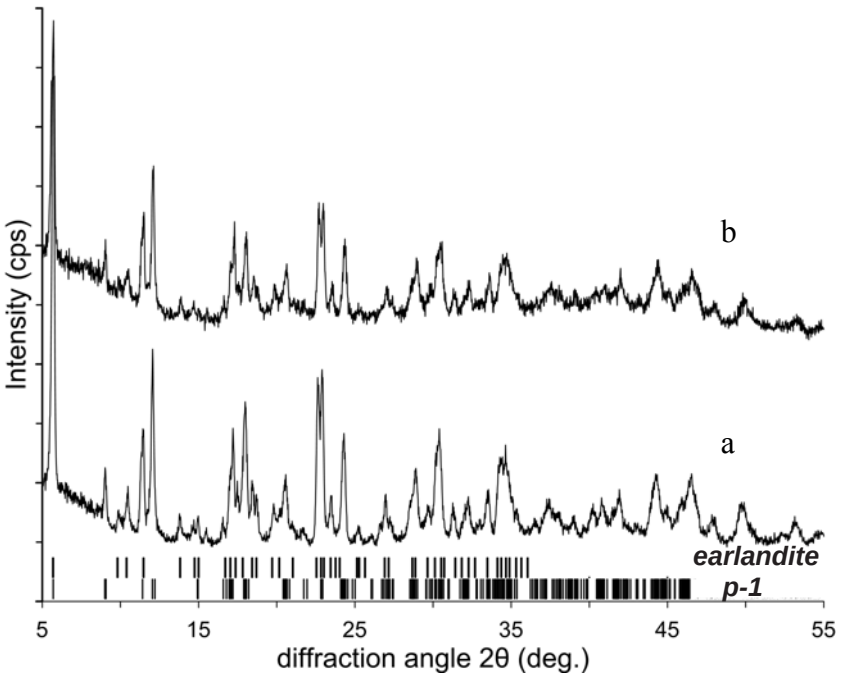

Figure 8. X-ray diffraction patterns of calcium citrate powder in initial state (a) and in 2 h of ball milling in AGO-2 (b)

Figure 9 compares the IR spectra of initial calcium citrate and that after ball milling. All spectra are characterized by a broad band at 3430 cm^{-1} due to the stretching vibration of hydroxyl group ν(OH) involved in the hydrogen bonding. Peaks at 1437 and 670 cm^{-1} associated with deformation vibrations of hydroxyl δ(OH): in-plane and out-of-plane respectively, which is a characteristic of structures like R-O-H. Absorption which is ascribed to vibrations of water molecules is not observed.

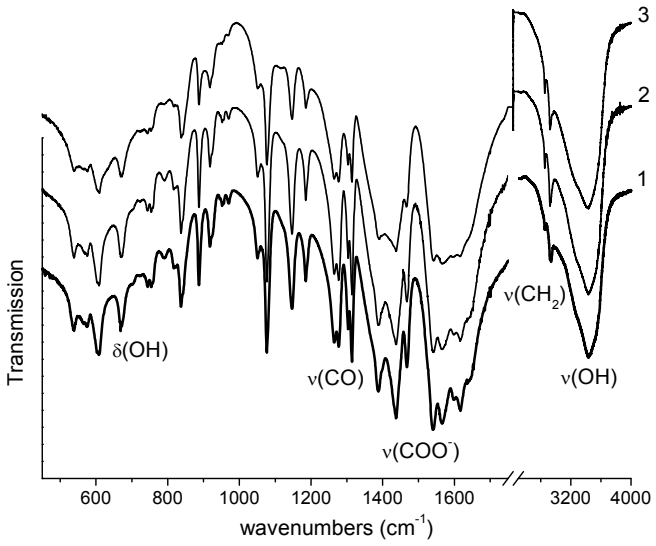

Figure 9. IR spectra of initial (1) and ball milled calcium citrate (2 – 1h; 3 – 2h).

Stretch symmetric $\nu_s(CH_2)$ and asymmetric $\nu_{as}(CH_2)$ vibrations of methylene groups results in two peaks: at 2852 and 2936 $см^{-1}$ respectively. Instead of characteristic band of carbonyl absorption of citric acid occur two bands in range 1550-1610 and 1300-1400 cm^{-1} due to asymmetric and symmetric stretch vibrations of carboxylate ion COO^-. In the region 1300-1400 cm-1, along with the carboxylate anion absorption bands are observed, attributed to other skeletal vibrations (for example, deformation vibrations of methylene groups). Absorption at 1147 and 1185 cm^{-1} can be assigned to stretch vibrations of C-O groups.

Ball milling increases the biological activity of the calcium citrate. The reasons for this effect require additional research.

Calcium citrate bands in the IR- spectrum shift to the region of lower wave numbers for the formation of needle crystals of thickness of several tens nm [22]. In our experiments the position of absorption bands does not change with increased time of ball milling. This confirms the fact that there are no changes of bond lengths and angles of calcium citrate molecule. Thus it can be concluded that ball milling during 1 h (dose of mechanical energy 7 kJ/g) did not change the structure of calcium citrate, no destruction or formation of new compositions was observed.

Solubility of initial and ball milled calcium citrate is close: 0.20 and 0.26 g/l respectively. Solubility rate of calcium citrate slightly increases after ball milling (1.5 times approximately).

TABLE 6. Angle of rotation of polarization plane of water solutions of calcium citrate (0.12g/100 g of water) and microelectrophoretic mobility of live blood cells (erythrocytes) and buccal cells in water solutions (0.025g/100 g of water)) of initial and ball milled calcium citrate.

Dose of mechanical energy, kJ/g	Angle of rotation of polarization plane, °	Microelectrophoretic mobility of live cells				
		Buccal cells			Blood cells (erythrocytes)	
		Percentage of active cells, %	Vibration amplitude, μm		Percentage of active cells, %	Vibration amplitude of cells, μm
			nuclei	plasmalemma		
0	0	5.5	2.5	3.0	64.5	3.2
3.5	0	0	0	0	Hemolysis	Hemolysis
In 12 months after ball milling	0	0	0	0	47.8	1.5

Water solutions of initial and ball milled calcium citrate are not optically active. It was quite expected due to known structure of this compound.

Activity of erythrocytes grows and amount of active buccal cells increases slightly in the solution of initial calcium citrate (Table 6).

E. Effect of severe plastic deformation on the structure of calcium salts

In Fig.10 shows deformation curves M(γ) "moment of torsion – shear deformation". All curves are characterized by the presence of two stages: 1- intense growth of values of deformation curves at low values of shear deformation (γ~400); 2- monotonous increase of values of deformation curves until the end of the experiment.

The initial samples had crystalline structure. Crystalline calcium gluconate become amorphous after action of deformation. Their curves M(γ) are almost coincide. The values of torsion moment are high enough.

The torsion moment of calcium citrate that is not changing its structure under the action of deformation is significantly lower. The lowest value of the torsion moment is of calcium lactate that initially has amorphous structure and later becomes crystalline under the action of deformation.

As for calcium lactate, the sample in initial crystalline state (curve 2) and initial amorphous state obtained by heating at 115 ºC (curve 4) was deformed. Compressibility of calcium lactate depends not only on the bonds type, but also the method of packing of molecules. Crystalline calcium lactate amorphized, and amorphous calcium lactate crystallized as a result of a severe plastic deformation by torsion.

We plan to perform systematic researches on this matter in the future.

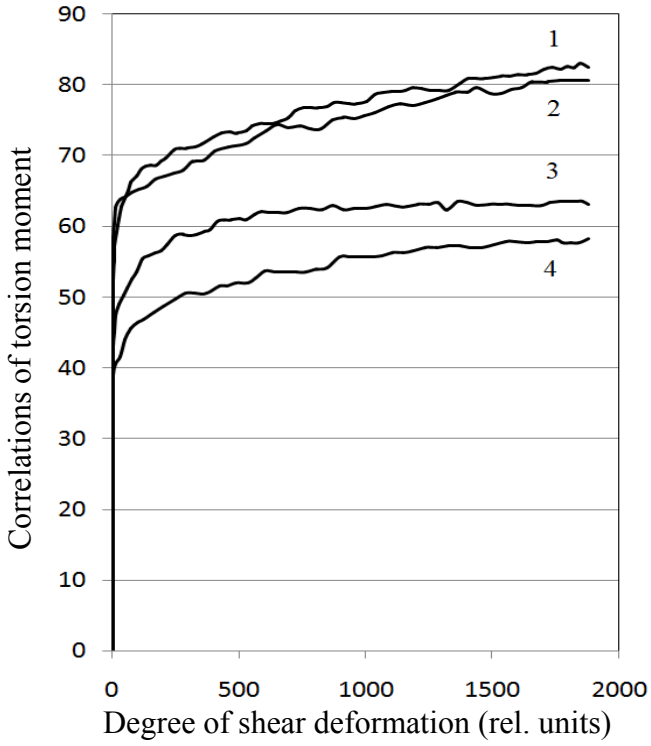

Figure 10. Correlations of torsion moment (M) and degree of shear deformation (γ) for:1 – calcium gluconate; 2 – mix crystalline calcium lactate+ crystalline calcite; 3 – calcium citrate; 4 –calcium lactate (initial amorphous), all obtained as a result of severe plastic torsion deformation at P=3.5 GPa.

IV. Conclusion

1. Plate-like particles aggregates are formed during mechanoactivation of calcium lactate, calcium gluconate, calcium citrate. Particles sizes and thickness depend on conditions of mechanoactivation (dose of mechanical power).

2. Mechanoactivation results in amorphization of crystalline calcium lactate and crystallization of amorphous calcium lactate. In both cases optical isomers are formed. The water solutions have less angles of rotation of polarization plane comparing to isomer that exists (or dominates) in the initial sample. Initial form of calcium lactate had the structure of L-isomer, and DL-structure after mechanoactivation.

3. Calcium gluconate is also an active optic compound. Water solutions of ball milled calcium gluconate have less angle of rotation of polarization plate than solutions of initial calcium gluconate.

4. Increased percentage of active buccal cells is observed in solution of DL-lactate, meanwhile the percentage of active erythrocytes and their vibration amplitude in such solutions conversely decreases. Unlike calcium lactate, less optically active modification of calcium gluconate shows more biological activity both to buccal cells and to erythrocytes.

5. Crystalline structure of calcium citrate remains unchanged during ball milling. Water solutions of calcium citrate are optically not active. Hemolysis of erythrocytes is observed in solutions of ball milled calcium citrate, buccal cells are not active, while active cells are observed in solution of initial calcium. The reason for such effect is not clear yet. It needs additional studies.

6. Biological activity of mechanoactivated samples of calcium lactate and calcium gluconate caused by formation of metastable optical isomers returns to the level of bioactivity of initial samples in 12 months of storage at room temperature. Optical activity of solutions of the studied mechanoactivated samples changed in 12 months of storage, tending to initial samples.

7. Compressibility of calcium lactate depends not only on the bonds type, but also the method of packing of molecules. Crystalline calcium lactate amorphized, and amorphous calcium lactate crystallized as a result of a severe plastic deformation by torsion.

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