ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Nanocrystalline Doped Calcium Carbonate-Phosphates as a Biomaterial for Osteogenesis.

LF Koroleva*.

Institute of Engineering Science of the Russian Academy of Sciences, Ural Branch, 34 Komsomolskaya St., Ekaterinburg, 620049, Russia.

ABSTRACT

A mechanism of the transport of nanocrystalline doped calcium carbonate- phosphates through the membrane on the model of interaction with phospholipids is considered. Nanocrystalline doped calcium carbonate – phosphates are perspective to transport of the cations in a living cell. Kinetics of the synthesis of nanocrystalline doped calcium carbonate- phosphates are non-linear kinetics. Biomaterial based on Nanocrystalline doped calcium carbonate – phosphates has a high degree of resorption: it can be used for the osteogenesis in a living organism with the restoration and hardening of bone and dental tissue. Heat treatment of the doped calcium carbonate phosphates produces calcium hydroxyapatite containing cation vacancies, which can be used as an active for osteogenesis bioceramics.

Keywords: Calcium carbonate-phosphate; Phospholipids; Membrane cell, Transdermal; Osteogenesis

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The world has been experiencing a growing interest towards the calcium phosphate biomaterial for regenerative orthopedics obtaining bone grafts. If previously for reconstruction of defects arising from accidents, diseases in bone surgery and dentistry only used car dealership-or allotransplanted, now at replacement of bone defects are increasingly applying calcium phosphate biomaterials [1-9]. However, synthetic calcium hydroxyapatites lack transdermal activity, and they have relevant slowly current reparative processes from 6 months to several years. Hydroxyapatite is known to be very similar in composition to the inorganic component of bone tissue: the dry residue of bone tissue consists of 70% hydroxyapatite and 30% organic component of collagen. The bone tissue should be characterized as an organic matrix impregnated by amorphous $Ca_3(PO_4)_2$ and crystals of calcium hydroxyapatite synthesized in bone tissue osteoblast cells. The content of anion CO_3^{2-} in calcium hydroxyapatite of the bone material can be from 5 to 8% by weight: they can replace OH- or PO_4^{3-} - group. Therefore, in view of the introduction of anion CO_3^{2-} in calcium hydroxyapatite structure likely formula is the following: $Ca_{10-x}(CO_3)_x(PO_4)_{6-x}(OH)_{2-2x}$ [10-17].

A characteristic feature of bone, tooth growth, is the accumulation of calcium. If the organism cannot absorb calcium, that is associated with age and hormonal changes, the limitation of calcium promote of the calcium hydroxyapatite bone dissolution. Therefore, one important issue is the emerging osteoporosis is as progressive decline in bone mass and density [18-20]. Medication with effect of transdermal delivery are extremely promising, especially to restore the bone which take to the nanocrystalline doped calcium carbonate-phosphates [21-24].

The penetration mechanism of cations and anions through the skin is a complex process involving the structure of the skin, which among other functions, constitute an obstacle to the entry of micro-organisms, viruses, toxins. Transdermal medication with activity penetrates the layer of keratin or through the hair follicles and sebaceous glands. Keratin layer acts as a depot, where are concentrated the substance. All substances get into the cell through membrane. Since all the substances fall within the cell membrane, which is a lipid bilayer containing phospholipids, the study of interaction with phospholipids nanocrystalline doped calcium carbonate-phosphates can confirm their penetration through the skin. The mechanism of ions transit through the membrane is known: the first is dissolution of the ions in the lipid phase membrane, diffusion and subsequent transition from membranes into the solution the cells; the second is ion movement in hydrophilic channels of the membrane; third - transport involving carriers, which most often is the intracellular cyclic adenosine monophosphate. The same mechanism is typical for natural biological and artificial lipid membranes [25-28].

The study of interaction nanocrystalline doped calcium carbonate-phosphates with phospholipids can confirm their penetration through the skin.

MATERIALS AND METHODS

Since 50% of lipids of cellular membranes are derivatives of phosphatidic acid, which is a product of the etherification for the primary alcohol group of diacylglycerol phosphoric acid, we were used in the experiment, mainly phospholipids phosphatidylcholine (1.2-diacil-glicero-3- phosphatidylcholine). When dispersing the phosphatidylcholine in water phase generate vesicles and liposome, which are widely used to as model biological membranes, as well as for the transport of biologically active substances in the body of animals. To determine the mechanism of transport through membrane doped carbonate-calcium phosphates used reactions interact with phospholipids doped calcium carbonate phosphate solution containing ions Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, PO_4^{3-}. For preparation of the phosphatidylcholine solution was use ethanol extract of egg yolk. The extraction of the phosphatidic concentrate of ethanol with ratio alcohol/phosphatidic concentrate as 6/1 at three levels of extraction gives two fractions: the first, enriched by phosphatidylcholine, the second is enriched other phospholipids. In the experiment was used a first fraction at the same time derived from egg yolk. The ratio between fraction nanocrystalline doped calcium carbonate-phosphates solution and phosphatidylcholine is 5:1 [29].

The object of the study was the solution of nanocrystalline doped calcium carbonate-phosphates [21-24].

ISSN: 0975-8585

Control over the interaction between phosphatidylcholine with doped cations calcium carbonate-phosphates by applying the method of mass spectrometry with inductively coupled plasma by ICP-MS (spectrometer ELAN-9000). Samples neutralize nitric acid, purified by a method "sub-boiling distillation" in the BSB-939-JR firm Berghof, in accordance with the requirements for sample preparation for ICP-MS. Integrating spectral interference carried out by software. Data analysis of samples submitted to the detection limit. To build gauging dependencies used certified according ISO 9001 multielement standard solutions (PerkinElmer Instruments). Drift correction device using the internal standard-^{115}In (certificate № 9300124, prepared on the basis of standard, US SRM 3124a, PerkinElmer Instruments).

The samples thus obtained were characterized by X-ray diffraction (XRD) (DRON-2 diffractometer, CuKα radiation; STADI-P diffractometer, software for diffraction peak identification using JCPDS–ICDD PDF2 data). The chemical composition (Ca, P, Fe, Mg, Zn, Mn, K, Si) was determined by standard techniques of X-ray fluorescent analyses with the use of the EDX-900HS energy dispersion spectrometer (Shimadzu, Japan).

RESULTS AND DISCUSSION

As a research result revealed that the cations Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+} и PO_4^{3-} interact to with phospholipids. In Figure 1 represent according to changes in the concentration of cations in solution after interaction with phospholipids.

Note that elements such as cations iron, manganese, zinc and copper interact with phosphatidylcholine. The absorption degree of calcium and magnesium with phosphatidylcholine is 98-99.9%, phosphorus is 98%. Association of lipids interact with alloying elements cations (Fe^{2+}, Zn^{2+}).

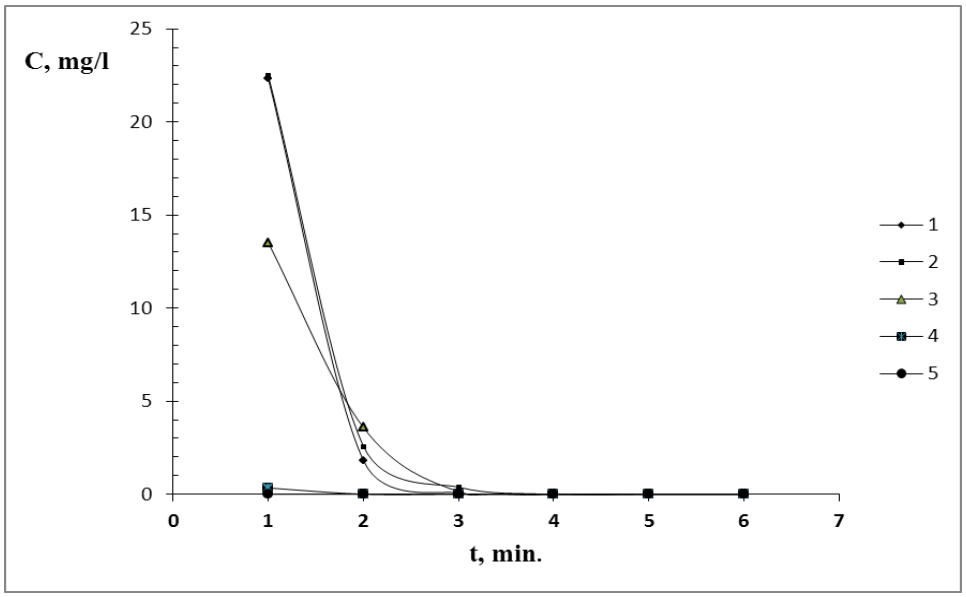


Figure 1: Dependence to changes of the concentration (mg/l, C) in time (t) in the reaction solution in interaction with the phospholipids of the following ions: 1– Ca; 2– P; 3– Mg; 4– Fe; 5– Zn

Improvement of biological activity of nanocrystalline doped calcium carbonate-phosphates is become clear due to the formation of crystal phase $Ca_{4.905}(PO_4)_{3.014}Cl_{0.595}(OH)_{1.67}$. As a result of the synthesis occur the embedding of ammonium cation in crystalline structure of the calcium hydroxychlorapatite that provides fast interaction with the cell membrane $Ca_{4.905}(PO_4)_{3.014}Cl_{0.595}(OH)_{1.67} \cdot H^+$, for example, by the reaction:

$$NH_4^+ + Ca_{4.905}(PO_4)_{3.014}Cl_{0.595}(OH)_{1.67} \rightarrow NH_3 + Ca_{4.905}(PO_4)_{3.014}Cl_{0.595}(OH)_{1.67} \cdot H^+ \quad (1)$$

As one of the possible mechanisms for transporting nanocrystalline doped calcium carbonate-phosphates can be assumed that in the process of transport occurs membrane pH gradient within the existing structure. The ammonium cation and proton-containing phosphate on the surface of the membrane form as a

result of the ammonium cation dissociation. A similar scheme to transport drugs through the liposome by ammonium cation dissociation and hydrolysis with the formation of a proton is discussed in [30].

In [31] also shows interaction of nanocrystalline doped calcium carbonate-phosphates with the membranes cells. The effect of microelements migration with nanocrystalline doped calcium carbonate-phosphates may be used in the development of transdermal drug delivery.

It is also known that elements such as platinum, gold in the form of colloid previously used as effects in vivo against rheumatoid arthritis, tumors. However applied dose produced side effects [32, 33]. In the event of a potential transfer of cations Au and Pt on molecules of nanocrystalline doped calcium carbonate-phosphates dose can be reduced significantly that will reduce side effects to a minimum. At the same time preserve the therapeutic effect. In the synthesis of doped gold and platinum calcium carbonate- phosphates concentrations of these elements make up the thousandths mole rate. Received samples of nanocrystalline doped phosphate calcium carbonate-containing cations of gold and platinum were tested for antibacterial effects with positive result of the possible use of purulent surgery.

Biomaterial based on nanocrystalline doped calcium carbonate-phosphates has a high resorption degree; it applies for activation of osteogenesis in humans of any age to as recovery and hardening of bone and dental tissue hardening, including migrating through the skin. The results are illustrated in Figure 2 showing the transverse mechanical strength (shear stress) of the bone tissue (femur) and the dental enamel as a function of the duration suspension introduction into animals. As a result, it has been found that there is a 13% increase in the mechanical strength (shear stress) of the bone tissue and a 7 % increase in the strength of the dental tissue (enamel), and this enables us to make an assumption of strengthened osteogenesis in a living organism. With the introduction of nanocrystalline doped calcium carbonate-phosphates in a living organism are increasing of the calcium concentration in the bones to 1-2%, and in human blood plasma is observed stabilization at the age of 55 years or more [22, 23, 34].

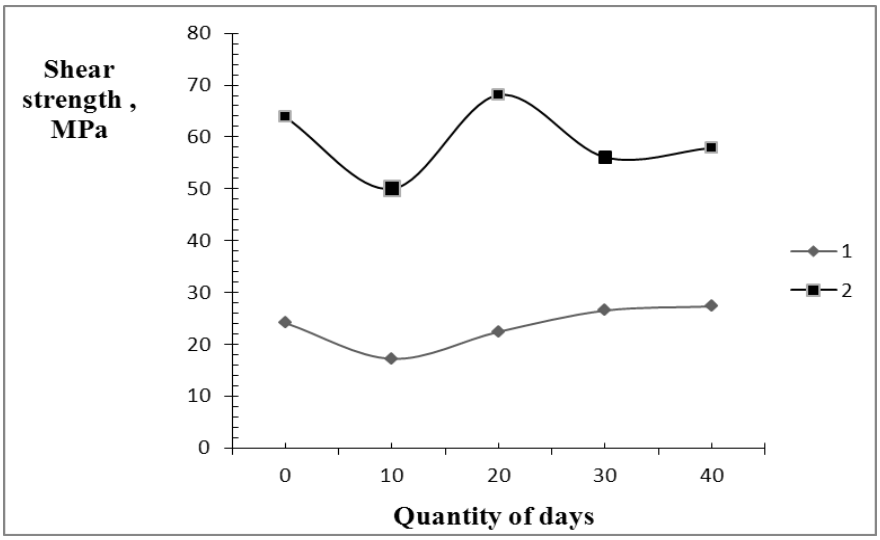
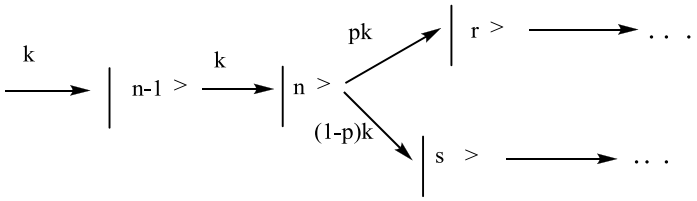


Figure 2: Influence of doped calcium carbonate-phosphate at shear strength bone tissue (curve 1) and of dental (curve 2) enamel from days quantity

Previously a mechanism of the synthesis reactions of nanocrystalline doped calcium carbonate-phosphates is considered, and an oscillating type of model is proposed for these reactions [24]. Synthesis of nanocrystalline doped calcium carbonate-phosphates includes a number of transformations: from the source connection calcium carbonate in the form of three crystalline forms (calcite, aragonite, vaterite) under the action of phosphoric acid in the presence of chloride, ammonium hydroxide and carbonate and trace elements of living organism: K^{+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+}. System kinetics relate to non-linear kinetics [35]. It should be noted that the process of forming primary particles doped calcium carbonate- phosphates may represent the wave model, in which primary particles are generated and escalated by the wave equation. Chemical transformations occur in areas where intersect the potential surfaces of the electron states, the called in quantum dynamics of conical intersections. Reaching the conical intersections, a wave packet is split: one part

ISSN: 0975-8585

continues to move in the same state, and the second goes to a different state, which could in the future lead to different processes: dissociation, isomerization or other conversions. The splitting of the wave packet is modeled parallel reactions with rate constants are determined by the electronic transition probability p:



(2)

The movement of the wave packet on this surface is described by a set of consecutive first-order reactions [36, 37].

In the case of carbonate- phosphate system navigation scheme can be represented as follows:

$CaCO_3 \rightarrow NH_4CaCO_3OH \rightarrow CaHPO_4$
$CaCO_3 \rightarrow NH_4CaCO_3OH \rightarrow Ca_8H_2(PO_4)_6$
$CaHPO_4 \rightarrow Ca_{4.905}(PO_4)_{3.014}Cl_{0.595}(OH)_{1.67}$
$Ca_8H_2(PO_4)_6 \rightarrow Ca_{9,70}P_{6,04}O_{23,86}Cl_{2,35}(OH)_{2,01}$
$CaHPO_4 \rightarrow CaCO_3$ (3)

It is important in this system receive to initial reaction products: different crystalline carbonates (calcite, aragonite, and vaterite). The formation of three types of crystalline calcium carbonate structures and their mutual transition has also been observed in vivo.

In Figure 3 show the dependence of the kinetic study of the reaction products in synthesis of nanocrystalline doped calcium carbonate-phosphates. Sequence of education is as follows: brushite, hydroxychlorapatite, and calcite.

Basic substances are obtained: crystal $CaHPO_4 \cdot 2H_2O$, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ and to complement the crystalline phases: $Ca_{9,70}P_{6,04}O_{23,86}Cl_{2,35}(OH)_{2,01}$ or $Ca_{4,905}(PO_4)_{3,014}Cl_{0,595}(OH)_{1,67}$. Crystal structure of these compounds has two types of channels with different positions, which are cations calcium and trace elements: Fe^{2+}, Mg^2, Zn^{2+}, Mn^{2+}. In the synthesis process observe to the possible introduction of cations NH^{4+}.

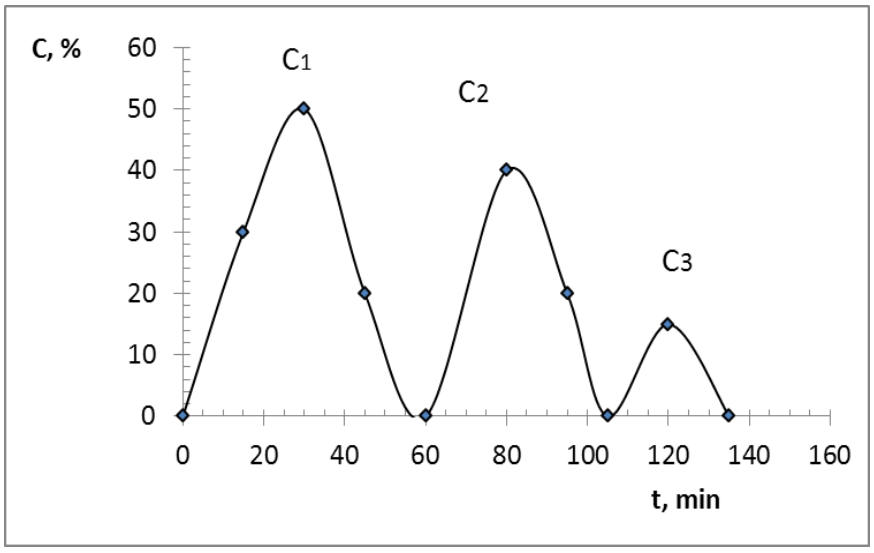


Figure 3: Dependence to changes of the concentration in the reaction synthesis of nanocrystalline doped calcium carbonate-phosphates: brushite (C_1), hydroxychlorapatite (C_2) and calcite (C_3)

The experimental data shows a possibility of the production of active bioceramics based on calcium hydroxyapatite. Difference of bioceramics is the presence of cation vacancies in the crystal structure. Heat treatment of samples doped carbonate-calcium phosphates in the temperature range 900-1100^{o}C leads to the formation of calcium hydroxyapatite with calcium channels, as evidenced by X-ray analysis and chemical analysis. This fact can be explained by the presence of free channels in the crystal structure of the heat treatment process. The general formula can be represented with the cation vacancy $Ca_{10-x-u}V_u(PO_4)_{6-y}(OH)_{4+z}$, where x=0,131; y=0,414; u= 1,028; z=0,006, V_u is cationic vacancy. The cation vacancies in the structure, which was formed by the removal of ammonium cations, provides for calcium hydroxychlorapatite active interaction with amine groups of proteins in living organisms [38].

CONCLUSIONS

Studies confirm the active transport mechanism of nanocrystalline doped calcium carbonate-phosphates across cell membranes due to the interaction with phospholipids. Nanocrystalline doped calcium carbonate-phosphates are perspective for the transport cations inside a living cell. Kinetics of the synthesis of nanocrystalline doped calcium carbonate-phosphates is non-linear kinetics. Biomaterial based on nanocrystalline doped calcium carbonate-phosphates has a high degree of resorption: it can be used for strengthening bone in a living organism with the restoration and hardening of bone and tooth. Thus, the development of the biomaterial based on nanocrystalline doped calcium carbonate-phosphates is allows to create new osteoplastic materials with unique biological properties.

REFERENCES

[1] Champion E. Acta Biomater 2013;9(4):5855-5875.
[2] Samar J Kalita, Abhilasha Bhardwaj, Himesh A. Bhatt. Mater Sci Eng C 2007;27(3):441-449.
[3] Brigitte Wopenka, Jill D. Pasteris. Mater Sci Eng C 2005;25:131–143.
[4] Yuri D. Tretyakov. Russian Chem Rev 2004;73:831-846.
[5] Fomin AS, et al. Doklady Chemistry 2008;418(1):22–25.
[6] Safronova TV, Putlyaev VI. Nanosystems: Physics, Chemistry, Mathematics 2013;4(1):24-47.
[7] Bouyer E, Gitzhofer F, Boulos MI. J. Mater Sci: Mater Med 2000;11:523-531
[8] Hong L, Min Ying Zhu, Li Hua Li, Chang Ren Zhou. J Mater Sci 2008;43:384–389.
[9] Rui-Xue Sunl, Yu-Peng Lu. Front Mater Sci China 2008;2:95-98.
[10] FCM Driessens, JGC Wolke, JA Jansen. J Australian Ceramic Soc 2012;48(3):144 – 149.
[11] Hermann Ehrlich, Petros G. Koutsoukos, Konstantinos D. Demadis, Oleg S. Pokrovsky. Micron 2009;40:169–193.
[12] Jennifer H Shepherd, David V Shepherd, Serena M Best. J Mater Sci: Mater Med 2012;23(10):2335-2347.
[13] J Brandt, S. Henning, G. Michler, W. Hein, A. Bernstein, M. Schulz. J Mater Sci: Mater Med 2010;21:283–294.
[14] H Imaizumi, M Sakurai, O Kashimoto, T Kikawa and O Suzuki. Calcified Tissue Int 2006;78(1):45-54.
[15] VK Tsuber, et al. Pharm Chem J 2006;40(8):455-458.
[16] Sherina Peroos, Zhimei Du, Nora henriette de Leeuw. Biomater 2006;27(9):2156-2161.
[17] JP Lafon, E Champion, D Bernache-Assolant. J European Ceramic Soc 2008;28(1):139-147.
[18] Zairin Noor. J Osteoporosis 2013:Article ID 679025.
[19] EL Duncan, MA Brown. Arthritis Res Ther 2008;10(5).
[20] JB Richards, F Rivadeneira, M Inouye, TM Pastinen and other. Lancet 2008;371:1505–1512.
[21] Koroleva LF. Inorganic Mater 2010;46(4):405–411.
[22] Koroleva LF, Larionov LP, Gorbunova NP. Doped calcium carbonate-phosphate - based biomaterial for active osteogenesis. Chapter 5. In Book Osteogenesis. Edited by: Yunfeng Lin. Croatia: InTech, 2012. ISBN 978-953-51-0030-0
[23] Koroleva LF, Larionov LP, Gorbunova NP. J Biomater Nanobiotechnol 2012;3:226-237.
[24] Koroleva LF. Nanotechnol Russia 2010;5(9–10):635–640.
[25] Elliott William H, Elliott Daphne C. Biochemistry and molecular biology. Oxford New York Melbourne: Oxford University Press, 1997. JSBN 5-7846-0036-2.
[26] Monteith GR, Roufoganis BD. Cell Calcium 1995;18:459-470.
[27] Bernhard Schuster. Nano Biotechnol 2005;1(2):153-164.

[28] Michael R Blatt, Carlos Garcia-Mata, Sergei Sokolovski. Membrane Transport and Ca^2+ Oscillations in Guard Cells. In book Rhythms in Plants: Phenomenology, Mechanisms, and Adaptive Significance/ Eds. S. Mancuso and S. Shabala. Springer-Verlag Berlin Heidelberg. - 2007.- P. 115-133
[29] Demidov IN, Kramarenko AA. Problem Chem Chemical Technol 2008;2:58-63.
[30] Tazina EV, Kostin KV, Oborotova NA. Pharm Chem J 2011;45(8):481-490.
[31] Koroleva LF, Cherednichenko NV, Dobrinskay MN. Doped Nanocrystalline Calcium Carbonate-Phosphate - Biomaterial with Transdermal Activity for Osteogenesis. Chapter 14. In Book: Nanotechnology. Biomaterials. Vol. 11. Edited by Naveen Kumar Navani and Shishir Sinha, USA-India: STUDIUM PRESS LLC, 2014. ISBN 1-626990-11-5
[32] Taukumova LA, Mouravjoy YV, Gribakin SG. Adv Exp Med Biol 1999;455:367-373.
[33] Ornat Sia. Sov Med 1991;7:82-83.
[34] Larionov LP, Koroleva LF, Gaysina EF, Dobrinskaya MN. Biomed 2011;4:102-103.
[35] Enrique Peacock-López. Chem Educator 2001;6:202-209.
[36] Eremin VV, Glebov IO. Theor Math Physics 2004;153(1):1463–1475.
[37] Møller KB, Zewail AH. Chem Phys Lett 2002;351:281-288
[38] Hailong Zhou, Tao Wu, Xiuli Dong, Qi Wang, Jiawei Shen. Biochem Biophys Res Comm 2007;361:91-96.