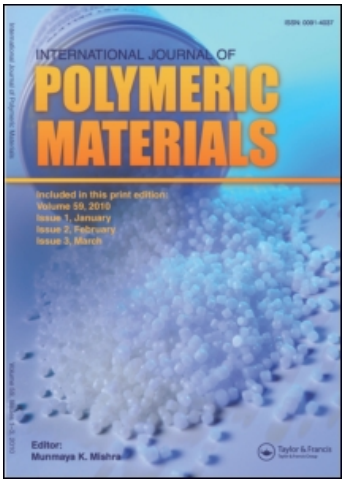

International Journal of Polymeric Materials
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713647664

The Rate of Solution of Hydroxyapatite Reinforced with Collagen as the Criterion of Polymer Implant Materials Quality. 1. The Solution of Bone Tissue Transplanted in HCL

I. A. Ershov[a]; S. D. Litvinov[b]
[a] Pharmaceutical Faculty, I. M. Sechenov Moscow Medical Academy, Moscow, Russia [b] Samara Medical University, Samara, Russia

To cite this Article Ershov, I. A. and Litvinov, S. D.(1995) 'The Rate of Solution of Hydroxyapatite Reinforced with Collagen as the Criterion of Polymer Implant Materials Quality. 1. The Solution of Bone Tissue Transplanted in HCL', International Journal of Polymeric Materials, 28: 1, 83 — 89
To link to this Article: DOI: 10.1080/00914039508012090
URL: http://dx.doi.org/10.1080/00914039508012090

Intern. J. Polymeric Mater., 1995, Vol. 28, pp. 83–89
Reprints available directly from the publisher
Photocopying permitted by license only

The Rate of Solution of Hydroxyapatite Reinforced with Collagen as the Criterion of Polymer Implant Materials Quality. 1. The Solution of Bone Tissue Transplanted in HCL

I. A. ERSHOV† and S. D. LITVINOV‡

†*Pharmaceutical Faculty, I. M. Sechenov Moscow Medical Academy, Moscow, Russia;* ‡*Samara Medical University, Samara, Russia*

(Received July 15, 1994)

Solution kinetics of a mineral component in the bones of humans and animals in solution 0.5–10 M HCL has been studied. Kinetic data permit us to determine the optimum concentration interval of solutions HCL which is necessary for preparing bone prostheses. In addition, this kinetic method of approach permits us to obtain data relating to the bone tissue structures which can serve as a basis for standardizing prostheses.

KEY WORDS Polymer implants, bone prosthesis, bone tissue, hydroxylapatite.

INTRODUCTION

The bone tissue consists of inorganic (c70%) and organic (c30%) components.[1] The inorganic component is mainly hydroxylapatite (c95%) $Ca_{10}(OH)_2(PO_4)_6$, magnesium and iron salts (c5%), as well as other biogenous elements.

The quantitative estimation of the bone tissue behavior under conditions of demineralization calls for exact knowledge of inorganic and organic bone components. We already know the summary formula:

$$Ca_{8.3}(PO_4)_{4.3}(HPO_4, CO_3)_{1.7}(OH)_{0.3}$$

of periosteum, which can initiate the bone tissue growth. In the same work, it has been noted that the formed bone cannot correspond to the stoichiometric composition of hydroxylapatite.[2]

However all the data of the works[3,4] based on the results of the X-ray phase analysis do not corroborate this assumption. For example, according to Reference 3 $Ca_{10}(OH)_2(PO_4)_6$, $Ca_3(PO_4)_2$ (amorphous), $CaCO_3$ (aragonite), SiO_2, H_2O are a part of the bone as separate phases.

In the process of forming bone calcium octaphosphate $Ca_8H_2(PO_4)_6$ [5] can appear which can hydrolyze into $Ca_3(PO_4)_2$ and then into apatite under the influence of the fermentative system.

Apatite is used by the organism as a joint for fastening polypeptide fibers of protein tropocollagen (protein organic matrix). Apatite as a basis salt dissolves in the aqueous solution and the organic component remains as a gel.

The dissolution time depends on the HCL-concentration. The demineralization rate of the bone tissue can be determined by the rate of introduction of calcium and phosphate ions into the solution.

EXPERIMENTAL TECHNIQUE AND RESULTS OF INVESTIGATIONS

Separate fragments of the same bone taken in the diaphysical section of the shin bone were cleaned by chloroform, dehydrated by ethanol (96.4°), dried to a constant mass. Separate portions were powdered and screened; fractions of particles of approximately the same size were selected.

Rabbit, dog and human bones were investigated for the purpose of revealing species peculiarities.

The behavior of both separate fragments of native bone and the bone powder in HCL-solutions was investigated for the purpose of determining the influence of the surface development.

Analytical samples of bone tissue specimens with a mass of 0.5–0.7 g were introduced into a vessel with a capacity of 250 ml which contains the HCL solution; the solution was intermixed continuously at a temperature of 25°C. In the process of demineralization the liquid phase of Ca^{2+} (complexometrically) and of PO_4^{3-} (photometrically) was determined; Ca^{2+}, PO_4^{3-}, and H_2O were determined in samples of the solid phase. The samples were investigated by methods of the X-ray phase analysis and differental thermal analysis as well as infra-red spectroscopy (X-RPA, DTAS, IRS).

In Figure 1(a) we see accumulation kinetics of Ca^{2+}, PO_4^{3-} in 1.2 M HCL. In spite of the similarity of curves, it is difficult to understand the emergence of nonstoichometric amounts of ions ($t < 40$ m) in Figure 1(b). Curve (3) characterizes changes of ratios in the content of Ca/P(mol/mol) in the liquid phase under conditions of demineralization of the powder of the human bone tissue. The diagram shows that, at the first moment of the reaction, the amount of Ca^{2+} is more than PO_4^{3-}. This proves incongruent decomposition of apatite under these conditions. In 90 minutes the ratio Ca/P in the solution becomes c1.7 which corresponds to the full dissolution of apatite.

Our experiment shows that dissolution of fragments of the native bone takes place much slower than the dissolution of the powder, but the rate depends on the type of bone (rabbit, dog, human) and on the HCL-concentration.

In Figure 2(a), concentration oscillation curves of Ca^{2+} in the demineralization solution HCL (1.2–8.4 M) human bones fragments are shown. Analogous dissolution curves of the dog and rabbit bones in HCL solutions (0.1–1.5 M) were obtained.

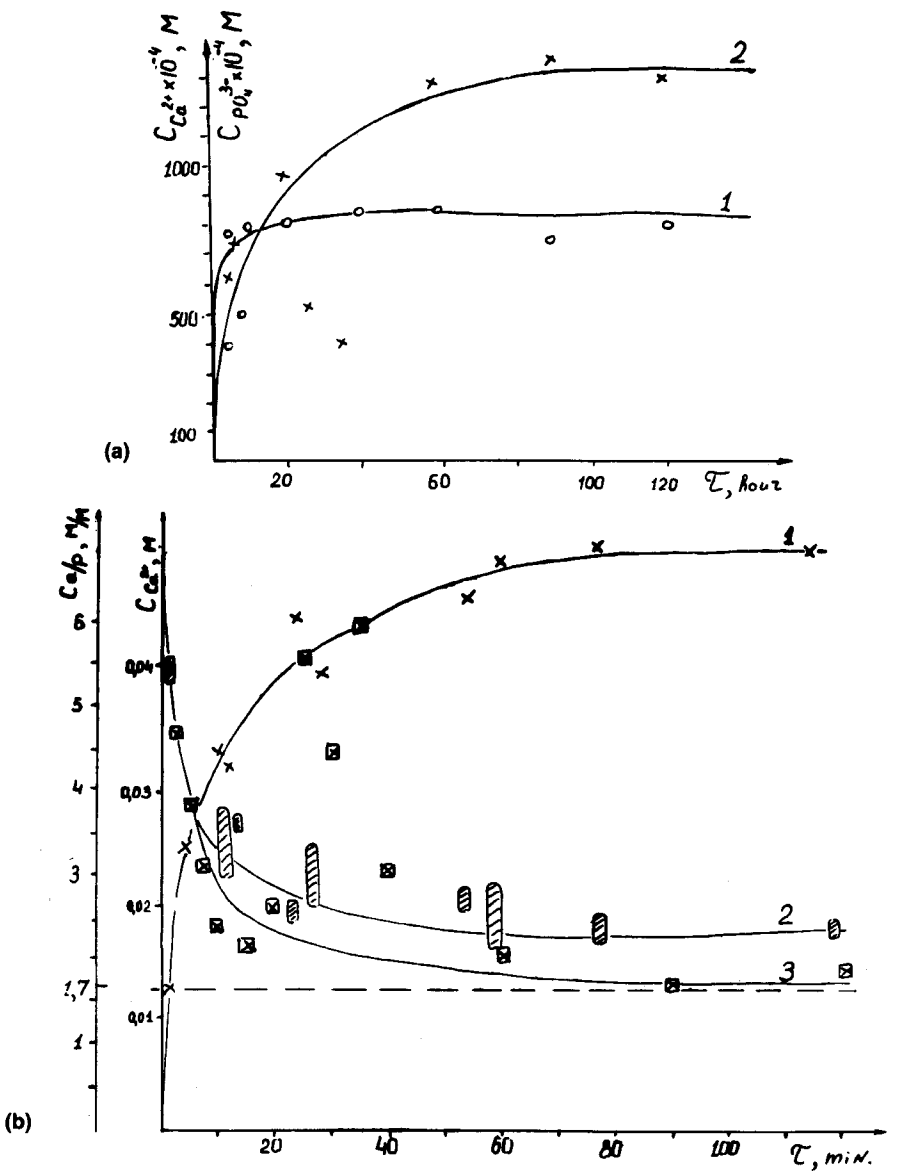

FIGURE 1(a) Concentration change of Ca^{2+} (1), PO_4^{3-} (2) in 1.2 M HCL under condition of demineralization of man bone powder; (b) Concentration change curves of Ca^{2+} (1), ratio of Ca/P (3) and relative mass loss of BE demineralized of man bone powder (2) in 1.2 M HCL.

It was shown that in the 1.2 M HCL solution the rabbit bone tissue demineralized fully in 3 hours, dog bone tissue in 20 hours, human bone tissue in 30 hours. In the more concentrated solution this process came to its close still earlier. It is evident that in any of the used solutions ($C_M > 1.2$) the process of demineralizaton will be complete in less than 2 days. Therefore, strict observance of methods of preparing bone prostheses of carcass (corpse) materials 06b using exactly 1.2 M HCL is hardly reasonable.

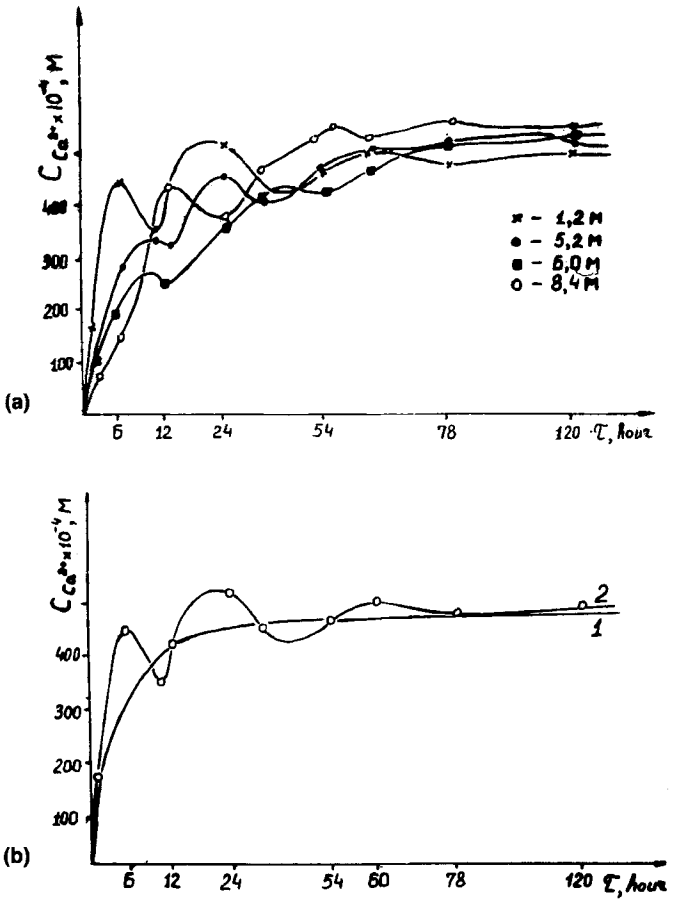

FIGURE 2(a) Concentration change of Ca^{2+} under condition of demineralization of the man bone in 1.2–8.4 M HCL; (b) Concentration change Ca in 1.2 HCL under condition of demineralization of the man bone. 1–common tendency, 2–$C_{Ca}2+$.

Statistical analysis of experimental data draws the conclusion that the common tendency of changing the calcium concentration in demineralization solutions finds expression in the product of function $C_X = F_1(t) \times F_2(t)$. This has been well approximated in the following way as "arctg." The function $F_1(t)$ describes the common tendency (Figure 2(b), N1) and $F_2(t)$ is an periodic component (Figure 2(b), N2).

The oscillation curve of Ca^{2+} in the liquid phase and the relative change in the sample mass (Figure 1(b), curves 1, 2) has a synchronous antisymbatic character. This confirms the correctness of our results.

All the obtained data allow us to calculate the demineralization time of the sample for the purpose of obtaining prostheses with a determined extent of demineralization.

It is necessary to note that, in the case of full demineralization the Ca^{2+} content in the solution makes 25.1% (if we take bone apatite into consideration) which fully corresponds to the theoretical content of Ca^{2+} in the bone tissue 23.9%). A

small divergence can be explained by the availability of non-apatite Ca^{2+} as well as the introduction of other two-valency ions into the bone tissue.

Apart from changes of concentration Ca^{2+} and PO_4^{3-} in a demineralizing solution of bone tissue samples of human, dog and rabbit, we investigated solid phases of demineralization products by X-ray phase analysis and differential thermal analysis methods.

Some heating curves of the rabbit shin bone with diverse demineralization degree are shown in Figure 3(a). We can observe endothermal peaks of molecular water losses and an exothermal peak of bone collagen oxidation on these curves. In the course of demineralization, we can also observe widening the endoeffect range (losses of some molecular water at more elevated temperatures). It is evident that this is determined by the fact that stronger binding the water molecules with the protein matrix at the expense of deblocking collagen hydrophilic areas by dissolved apatite takes place.

In accordance with further decrease of the salt content in the sample, the oxidation character of the bone protein base also changes: the whole exoeffect begins dividing into two very distinguished maximum quantities (t = 330–350°C, t = 450–500°C). The separation of the exoeffect apatite structure which evenly blocked the organic bone base. Furthermore, the specific surface grows. Demineralization heterophases lead to unevenness of collagen release in sample volume, hindering the simultaneous oxidation process of the whole protein base; this fact can explain the dividing of the effect.

The distinction of the oxidation nature of the samples demineralized to a variable degree as native collagen obtained from the solution by acidic dissolution is noteworthy. In case of native collagen, the maximum temperatures of both effects are approximately identical to the demineralized ones.

However, exothermal heat is different: all the demineralized samples have more heat in the second phase. In contrast to this, native samples have other characteristics. This discrepancy between samples can be explained by the lack of any regular structure and less packing density of collagen fibrils of protein from the solution than takes place during bone demineralization.

The data of X-ray phase analysis (Figure 3(b)) of demineralization products of the rabbit shin bone are shown. The character of reflexes of apatite crystallite changes during the course of the decrease of the bone salt content.

Widening and eroding poorly resolved three-strong apatite lines show that there is uneven degradation of the saline phases in the sample. This unevenness enhances some amorphous starting state of the bone crystalline structures. The given observation is compatible with dividing the exothermal effect on heating curves 3(a).

Further investigations of heating products of the bone tissue above 600°C with the help of X-ray phase analysis testify that there is an apatite phase. The thermograms of synthetic apatite and apatite as thermal decomposition of the bone is shown: does not decompose up to 1130°C losing adsorption water (tc120–190°C) for synthetic. The decomposition takes place in temperature range of 1130–1300°C and leads to forming $Ca_3(PO_4)_2$ (β-form, ASTM: card N 9-169).

All the obtained results can be used for creating the nonimmunogenic biodegradation materials having limited (regulated) wetting ability which will ensure con-

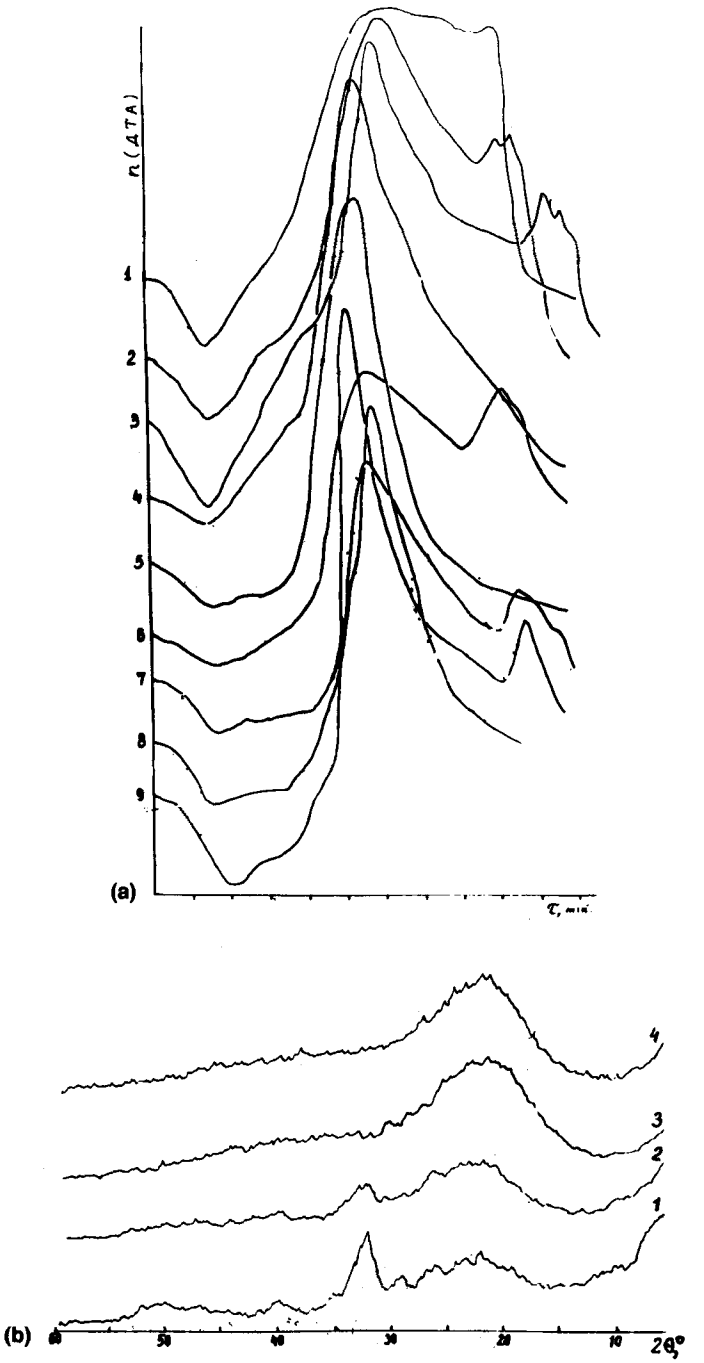

FIGURE 3(a) Heating curves of demineralized samples of the rabbit bone tissue in 0.1 M HCL (1—15′, 2—30′,3—45′, 4—120′, 5—150′, 6—180′, 7—210′, 8—225′, 9—240′); (b) Solid phase X-ray photograph of demineralization products of the rabbit bone in 1 M HCL. 1—native bone, 2—in two hours, 3—in 4 hours, 4—in 6 hours.

servation of the prosthesis form (of this material) under conditions of locating it into a medium of biological liquids.

Acknowledgment

The authors thank Prof. A. S. Trunin, Ph.D., I. K. Garkushin, Eng., V. I. Pavsky (Samara Technic University) for their help in carrying out the research. DTA of apatite was carried out by I. K. Garkushin, senior scientific worker of Samara Technic University.

References

1. B. S. Kasavina and V. P. Torbenko, *Gizn kostnoi tkani. M.: Nauka*, s.21 (1979).
2. G. Bonel, R. Legros and N. Balmain, *Calcified Tissue Int.*, **39**, 66 (1984).
3. S. M. Golubev, *Gurnal obschei biologi*, 784–806 (1987).
4. I. E. Eastoc, *Calcified Tissue Int.*, **35**, 60 (1984).
5. Pei-Tak Chong, *Calcified Tissue Int.*, **40**, 339–343 (1987).
6. V. I. Savelev, *Sbornik nauchnih trudov NITO im. R.R. Vredena*, Leningrad, 3 (1987).