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Anisotropic properties of trabecular bone. Conductometric and ultrasonic studies

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Bones are materials characterized by anisotropic mechanical and structural properties. The main directions of anisotropy of such materials are related to mechanical loads to which they are subjected in physiological conditions. Studies of mechanical properties show that bones are mostly orthotropic or transversally isotropic materials. The aim of the present paper is to investigate parameters of anisotropic structure of trabecular bone such as formation factor and tortuosity using measurements of electric impedance. Moreover, the qualitative correlation between the measured parameters of structure and velocity of propagation of ultrasonic waves in these materials is established. To determine the impedance of the materials the two-electrode method with RLC bridge or direct technique are used. The cubic specimens (1 cm × 1 cm × 1 cm) of trabecular bovine bone were cut from the distal epiphysis of fresh bovine femora and measurements of impedance were made both for material containing bone marrow and physiological fluid. The results show a good correlation between tortuosity measured by electric spectroscopy and velocity of longitudinal waves.

Key words: trabecular bone, anisotropy, formation factor, tortuosity, velocity of ultrasonic waves

1. Introduction

The diagnostic techniques used in medicine and based on e.g., ultrasonic or radiological methods, permit growing recognition of structure of biomaterials and as a result more appropriate evaluation of some diseases. In particular this refers to studies of the microstructure of bones, the evolution of which may indicate pathological changes in organism, like development of osteoporosis [7].

It is commonly recognized that bones are anisotropic materials in which directions of principal axes of anisotropy depend on mechanical loads, which act on bones in physiological conditions (see, e.g., [2]). The studies showing anisotropy of bones refer mostly to mechanical properties, such as modulus of elasticity and strength of bones [10, 13]. There are also some results concerning anisotropy of structural properties of the materials (see, e.g., [3, 7]).

In this paper, the three pore structure parameters: volume porosity, formation factor, and tortuosity are studied. The formation factor, FF, is defined as the ratio of conductivity of electrolyte, λ, and conductivity of porous material saturated with the electrolyte, λ_p, i.e., $FF = \lambda / \lambda_p$. The tortuosity, T, is equal to the product of formation factor and porosity, f_v, i.e., $T = FF \cdot f_v$ [1].

Since the said structure parameters are important both for flow of fluid and migration of chemicals through permeable materials [5] they are of primary significance for natural processes of transport within bone tissues. Although, based on the existing literature, it has been proved that from the mechanical point of view trabecular bones are orthotropic or transversally isotropic materials, still little is known about the directional properties of pore structure parameters (formation factor and tortuosity) of the bone material and distribution of the properties within particular bones. On the other hand, in spite of the fact that there are some results showing that mechanical properties of trabecular bones depend on the scalar parameter of volume porosity [4] or the fabric tensor describing internal structure of the solid skeleton [3, 8], there is no deeper understanding of relationship between the anisotropic properties of structure of pore space and mechanical properties of skeleton.

The aim of this work is to study two parameters characterizing anisotropic pore structure of trabecular bone, i.e., formation factor and tortuosity, from different places of heads (epiphysis) of femoral bovine bones, and examining the correlation between the measured parameters of pore structure and the velocity of propagation of ultrasonic waves. The method of electric spectroscopy was used to determine values of structure parameters. The samples used in the study were obtained from three layers of head of the femoral bones and tested intact (saturated with marrow) or saturated with physiological liquid.

2. Materials and methods

Three sets of samples of trabecular bone were obtained from bovine femoral bones. From the distal part of the bones three parallel layers of about 1 cm in thickness were cut out and cubic samples were chosen, in the way shown in figures 1, 2, and 3. One of the directions of the axes of samples was identical with the direction of the long axis of stem (diaphysis) and the other axes were determined by the radial (circumferential) direction or the direction of the forehead of bone.

A diamond saw was used to cut out the layers of material to preserve their parallelism. Cubic samples were extracted using manual saw and then polished with fine abrasive paper.

In the case of the two sets of samples the measurements of conductivity were done in axial, circumferential and radial directions (see figures 1 and 2), and for the third set the layer with the greatest homogeneity was chosen (the layer nearest to the stem of bone) and samples were cut out in direction parallel to the forehead of the bone (see figure 3). The liquid filling the pore space of trabecular bones in conductometric studies was marrow or isotonic physiological liquid (0.25 mole/liter of NaCl). The samples filled with physiological liquid were vacuum saturated.

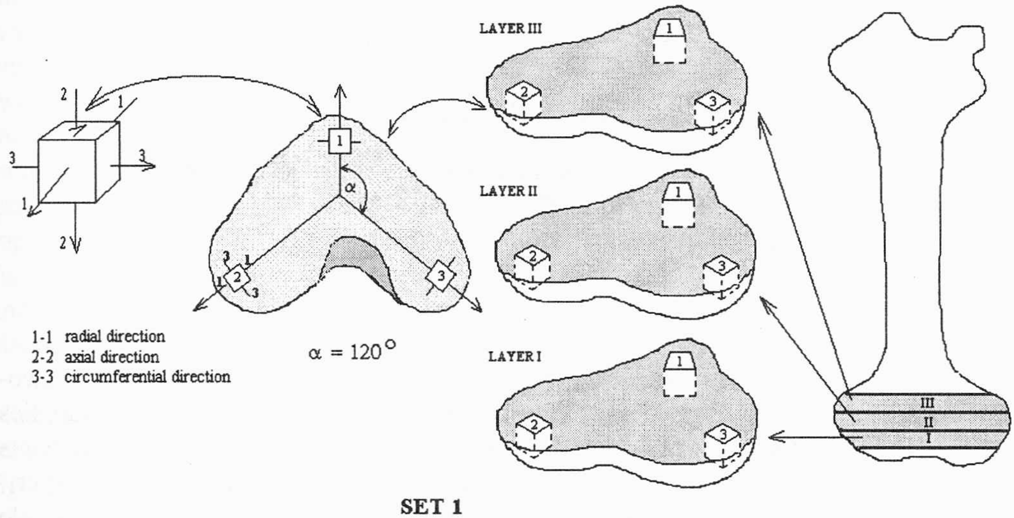


Fig. 1. Distribution and orientation of samples of trabecular bone for set 1

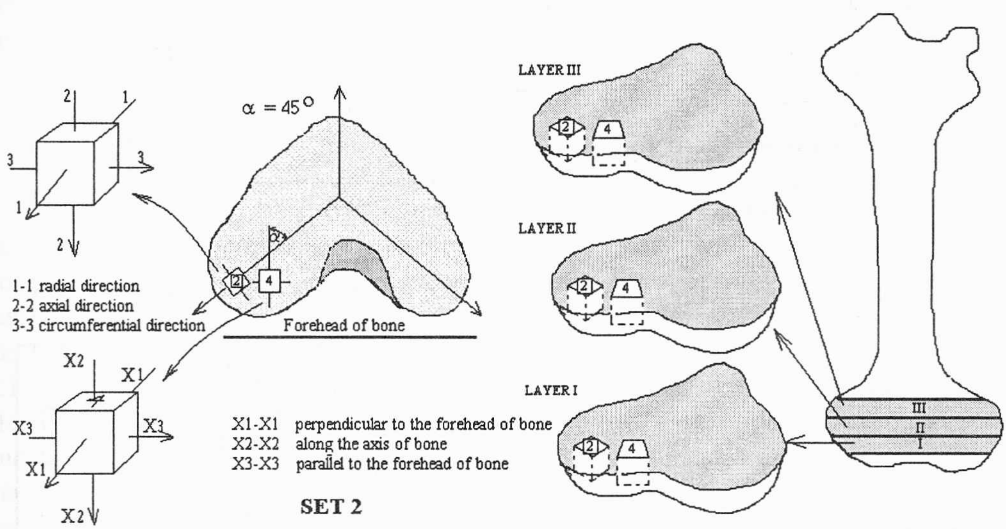


Fig. 2. Distribution and orientation of samples of trabecular bone for set 2

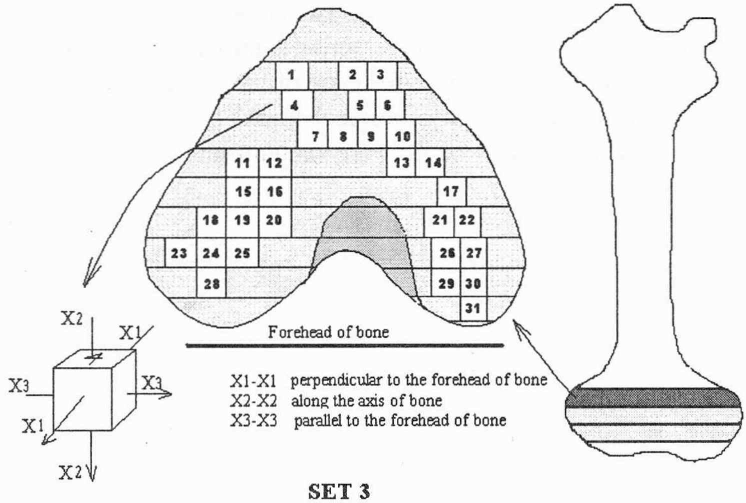
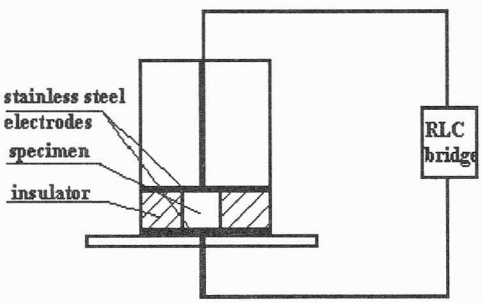
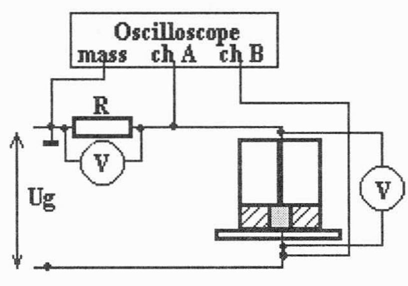


Fig. 3. Distribution and orientation of samples of trabecular bone for set 3

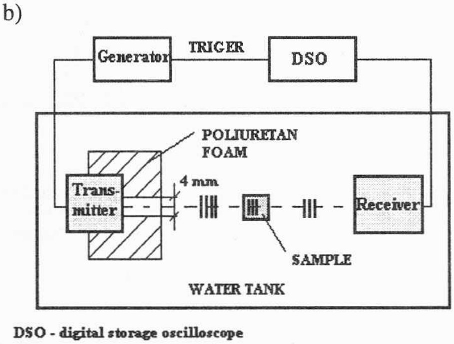
In the conductometric studies the RLC bridge and direct method with two-electrode system were applied [12] (see figure 4a, b). The bridge method supplies results for fixed frequency of 1 kHz while the direct technique allows measurements in the frequency range from 100 Hz to 100 kHz. The application of the two experimental methods was to substantiate the results and give a possibility to check the role of electrode polarization effects [9].



a)



b)



c)

Fig. 4. Arrangement of measuring systems:
a) bridge method; b) direct technique
and c) ultrasonic setup

The experimental setup for measurement of conductivity includes a chamber with two electrodes made from stainless steel. The sample is placed between electrodes in the opening of a layer of insulating material. The insulator limits the active area for ionic current to the sample of trabecular bone. The chamber made from transparent PVC material is of cylindrical shape with internal diameter of about 70 mm. While the lower electrode is fixed, the upper electrode can be removed from the chamber to insert the sample. In order to ensure a good contact between electrodes and a sample when investigating materials filled with marrow, the removable electrode is loaded with a weight and a blotting-paper saturated with physiological liquid is placed between the electrodes and the sample. The experimental setup in the bridge method includes RLC bridge model E 318, which measures conductance and capacity of the sample (see figure 4a). In the direct technique the experimental setup consists of the generator Metex MS-9150 giving sinusoidal signal of prescribed frequency, two analog multimeters V-640, digital oscilloscope and standard decade of resistance (see figure 4b). The procedure for determination of the values of electrolyte conductivity and of the system – sample saturated with electrolyte is based on a model of parallel electric system of resistance and capacitance.

The porosity of individual samples of trabecular bone was determined by the gravimetric-volumetric method. Measurements of the velocity of propagation of longitudinal ultrasonic waves were performed using the experimental setup shown in figure 4c. The system consists of the 1 MHz Panametrics transducers, the generator Model 5058 PR and Link Instruments digital storage oscilloscope. Initially, a series of several samples from the sets 1 and 2 was selected for ultrasonic measurement. Due to the obtained perfect correlation between the velocity of propagation of ultrasonic waves in these samples and the inverse of tortuosity, the ultrasonic tests were next performed for all samples of the set 3. The samples for ultrasonic studies were vacuum water saturated.

3. Results

The determination of structure parameters, i.e., formation factor and tortuosity using conductometric measurements on bone samples filled with conducting liquid, is possible in the case when the volume conductance of the pore solution dominates over the surface conductance [6]. In order to establish the role of surface conductance the measurements for samples filled with solution of physiological liquid in ratios of 1:1; 1:2; 1:5; 1:10; 1:20 to water were done. The results of measurements proved clearly that the use of physiological liquid allows one to assume that in the ionic conductance of electrolyte saturated bones the volume conductance is much higher than the surface conductance and thus conductometric measurements for bone structure can be applied.

Since the samples of bones are relatively thin, the effects of electrode polarization may influence the measured electric parameters. To study the role of polarization the conductivity of several samples was determined at various frequencies from 100 Hz to 100 kHz. The results of measurements in axial direction for samples from the set 2 saturated with physiological liquid are shown in figure 5.

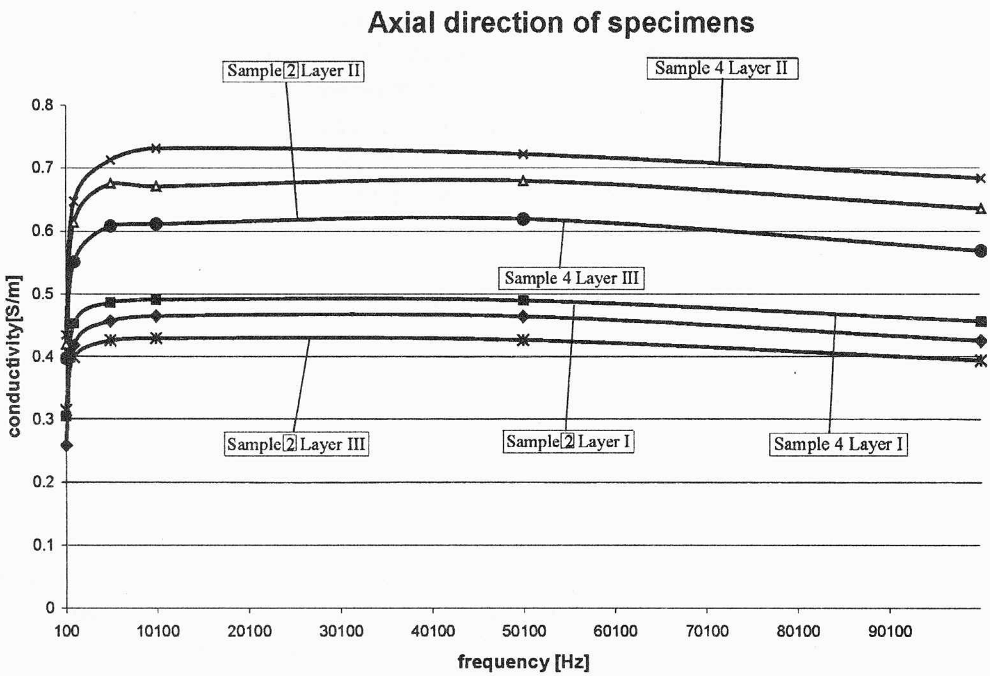


Fig. 5. Conductivity of specimens for set 2 in axial direction versus frequency

In the range from 100 Hz to 10 kHz a considerable increase of conductivity is visible indicating the significance of electrode polarization [6]. Next, in the range from 10 kHz to 50 kHz, the values of conductivity are almost constant and for frequency from 50 kHz to 100 kHz one can notice a slight decrease in conductivity of bone samples. The latter effect may be related to the polarization of the interphase between pore fluid and skeleton [6]. Similar changes of conductivity versus frequency were obtained for samples studied in circumferential and radial directions as well as filled with marrow. Taking into account the results obtained, the frequency at which conductivity was measured to determine the structural parameters was assumed to be equal to 10 kHz.

Studies of porosity of bone samples (see figure 6) show significant differences in values of volume porosity both for samples from the same layer as well as for samples from different layers. The porosity of specimens from sets 1 and 2 shows changes in the range from 65% to 71%. The greatest values of porosity of the samples from set 3 are exhibited by samples obtained from the central part of the layer. Samples situated near the forehead of the bone have the lowest porosity. Variations of porosity for the set 3 range from 59% to 83%.

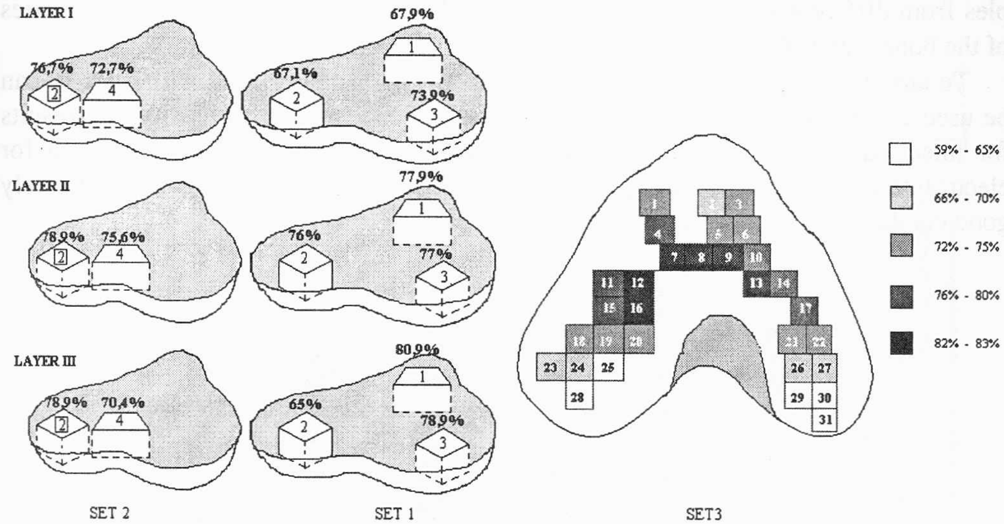


Fig. 6. Values of porosity for all samples of trabecular bone under study

In figure 7, the values of formation factor for samples of trabecular bone of set 1 saturated with physiological liquid are presented.

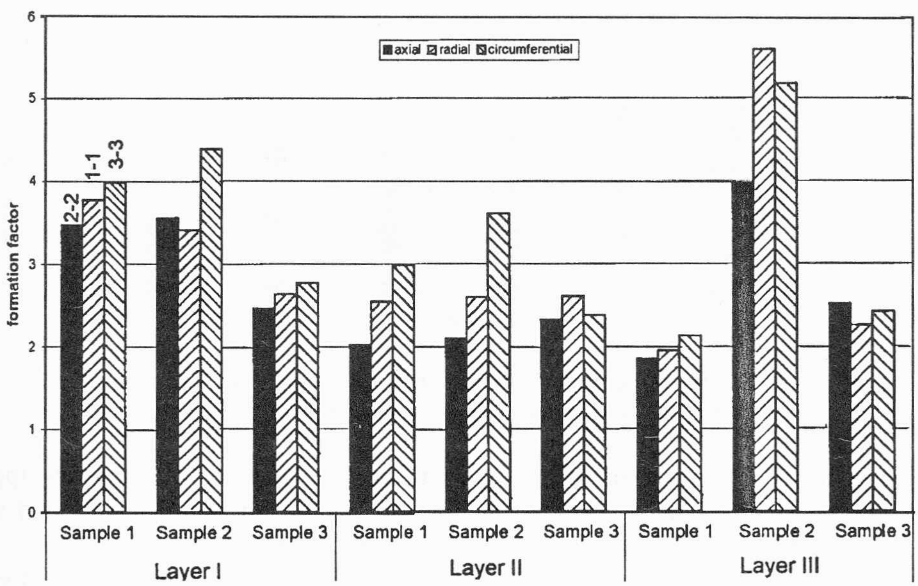


Fig. 7. Formation factor of bone specimens (set 1) saturated with electrolyte

Comparison of the formation factor in axial, radial and circumferential directions show that for most samples the pore structure of the trabecular bone is anisotropic. Considerable differences in values of formation factor in the same direction, for samples from different sites, indicate significant inhomogeneity of directional properties of the bone material.

To answer the question whether measurements on samples filled with marrow can be used to determine structure parameters: formation factor or tortuosity, the results for intact samples saturated with marrow were compared with those obtained for electrolyte saturated samples, figure 8. The studies assume that marrow is a relatively good conductor as compared to the bone matrix [11].

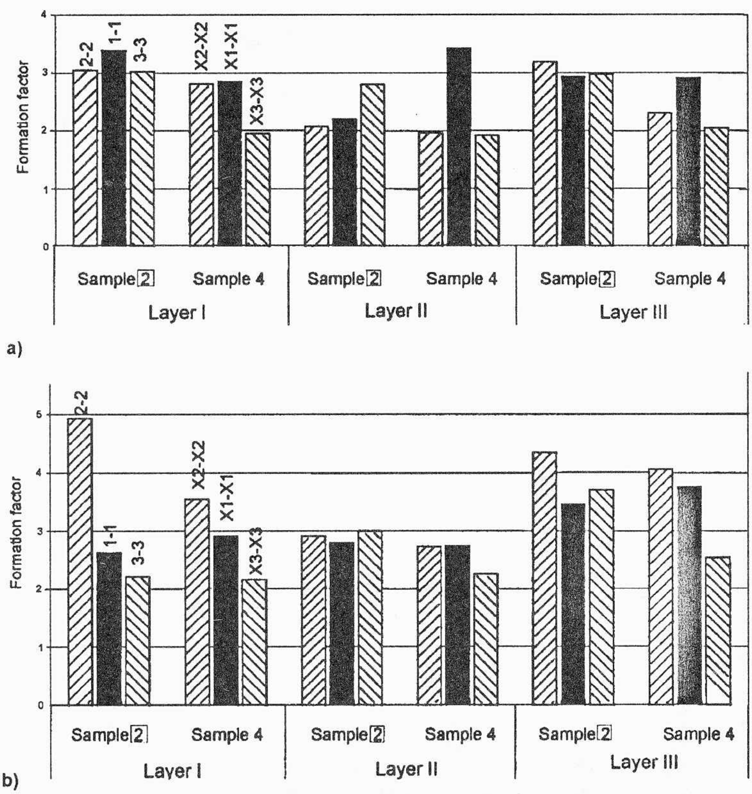


Fig. 8. Formation factor for bone specimens (set 2):
a) samples saturated with electrolyte, b) samples filled with marrow

The values of the formation factor obtained for samples filled with marrow appear to be essentially different in comparison with the results for samples saturated with physiological liquid. Given that physiological liquid is perfectly homogeneous and isotropic, divergence of the results for formation factor indicate that there must exist the inhomogeneity of electric properties of marrow. It results also that the intact samples cannot be used to determine parameters of pore structure.

In order to compare the distribution of parameters of structure determined by conductometric method with data for velocity of propagation of longitudinal ultrasonic waves the results for all samples of set 3 are presented in figures 9 and 10.

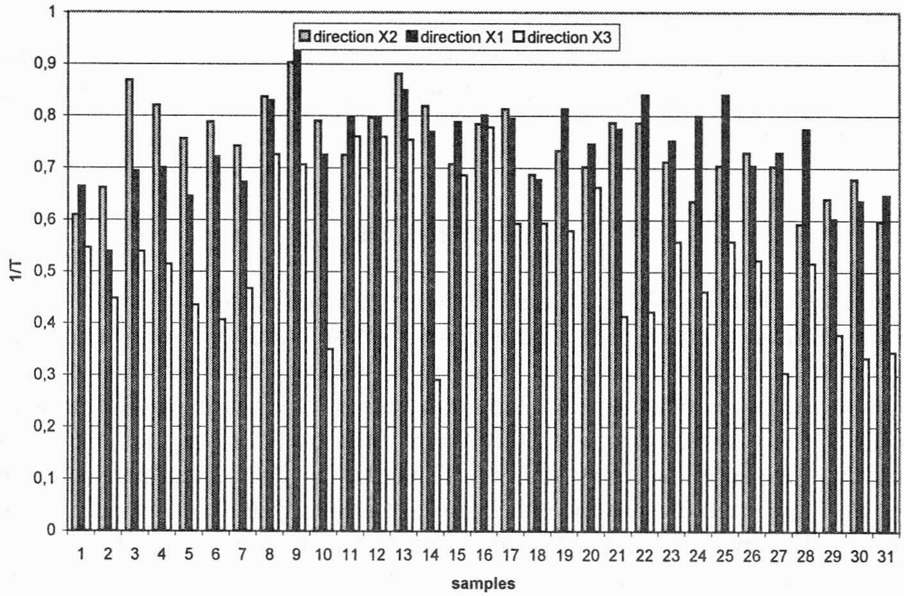


Fig. 9. Inverse of tortuosity of specimens (set 3)

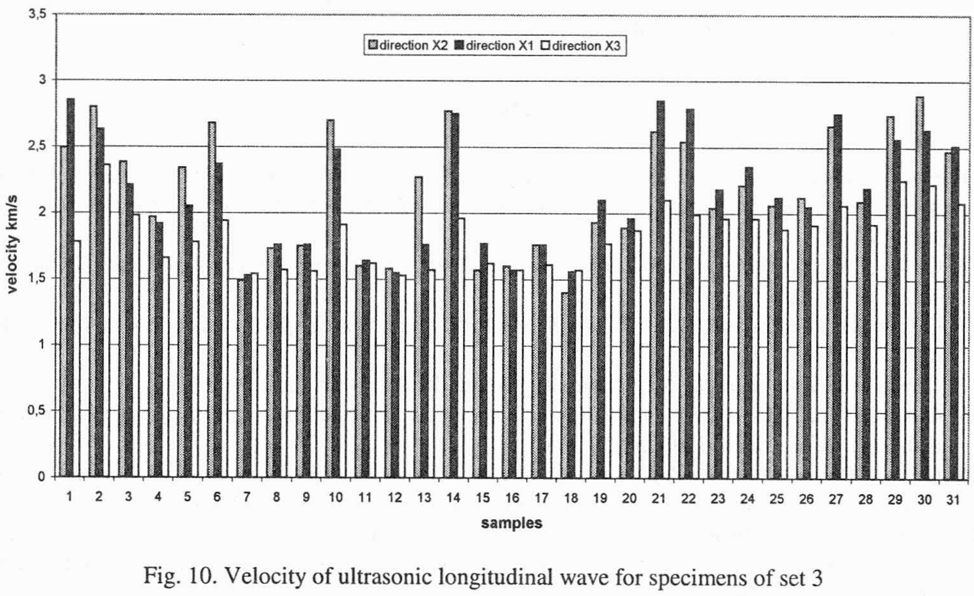


Fig. 10. Velocity of ultrasonic longitudinal wave for specimens of set 3

The data show excellent correlation between values of velocity of ultrasonic waves (measured in the three perpendicular directions) and values of the inverse of tortuosity of the samples. This effect is very interesting and promising in establishing links between formation factor and some mechanical properties of porous bone. Since the velocity of ultrasonic waves is connected with the stiffness of bone skeleton and that is determined by the structural arrangement of plates and rods composing the skeleton (see [3, 4]), the relationship between parameters describing pore structure and parameters of skeleton structure seems to be natural. Since a closer quantitative relation between the parameters has not been studied the problem should be investigated in the future.

4. Conclusions

The results obtained show strong inhomogeneity and anisotropy of pore structure of bovine trabecular bones. The inhomogeneity of structural properties concerns volume porosity, formation factor, and tortuosity of samples from different parts of the head of bovine bone. For the whole head of bone or any larger part of the material, considering the parameters of structure, there does not exist any simple symmetry (orthotropy, transversal isotropy) of bone. The materials containing marrow cannot be used to evaluate structure parameters because of inhomogeneity of electric properties of marrow. The measurements of velocity of propagation of longitudinal ultrasonic waves prove that there is a very good correlation between values of wave velocity and the inverse of tortuosity. This fact should be further explained in order to find a quantitative relationship between the pore structure parameters and mechanical characteristics of bone material.

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