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Investigation of Textural And Surface Chemical Properties of Some Animal Bones

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Abstract

The aim of the study is to examine the textural structure and organic functional groups of horse-donkey, cow, dog, and sheep bones by nitrogen gas adsorption-desorption and Fourier Transform Infrared (FT-IR) spectroscopic methods, respectively. For this purpose, textural properties such as BET (Brunauer-Emmett-Teller) surface area, total pore volume, average pore diameter, and surface organic functional groups were determined by nitrogen gas adsorption/desorption at -196 oC and FT-IR spectral analysis methods, respectively. It was observed that the nitrogen gas adsorption isotherms obtained from the BET analysis results were similar to Type-V, which indicates that they have a mesoporous and/or macroporous textural structure, in the IUPAC nitrogen gas isotherm classification. In addition, it was determined from the FT-IR analysis results that they mainly contain organic functional groups such as amine, alcohol, carboxylic acid, ester, ether.

Keywords: Animal bones,Textural properties, Surface chemical properties

Introduction

Animal bones are generally composed of organic (30%) and inorganic compounds (70%). The mineral parts of the bones provide their hardness and suitable mechanical properties. The model compound corresponding to a mineral phase of bones is non-stoichiometric hydroxyapatite (HAp), i.e. HAp with a molar ratio of calcium to phosphorus different from [1.67]. Biological apatites are components of bones, as well as pathological tissue (urolith, dental scale, and mineralized soft tissue)[1,2,3]. Due to its chemical and structural similarity to bone minerals, hydroxyapatite is a promising candidate for bone substitutes. Hydroxyapatite is not only a biocompatible, osteoconductive, non-toxic, non-inflammatory, and non-immunogenic agent, but also bioactive, i.e. capable of forming direct chemical bonds with living tissues[1,4].

The average crystal size of bone minerals increases throughout the individual's growth period and becomes the same in size at maturity. In some degenerative bone diseases, changes in mineral crystal size occur[5]. In Paget disease, where the bone is reabsorbed and formed at a higher rate than normal, the average size is smaller than unaffected (normal) bone. In osteoporosis, a disease of aging in which resorption is greater than bone reformation, the mean crystal size is larger than in normal bones. Ingestion of fluoride at physiological or pharmacological dosage, the substitution of fluoride (F) for hydroxyl (OH), and increased mean crystal size; both effects tend to stabilize the bone crystal by reducing its solubility. It has been reported that the solubility of bone crystals varies significantly in size[5,6].

The strength of the bone varies according to the direction of the applied load, gender, age, and calcium content. The most important difference between cortical and cancellous

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bone is the porosity ratio. While the porosity of cortical bone increases from 1% to 30% with increasing human age, this rate increases from 70% to 95% in cancellous bone. With the increase in porosity, the strength and density values decrease[7].

Measurability in terms of porosity, volume content, size, interconnectivity, and surface properties determines the effect of porosity on mechanical strength. Thus, it is possible to process and characterize biphasic bone substitutes with a wide variety of micro-and macro-scale pores[8].

The surface of a particle is its interface with the phases that surround it. When the particles are large enough, we can measure their size and shape visually or with a light microscope and calculate the size of the surface. Electron microscopy is used for direct observation of microscopic particles with highly specific surfaces. One of the most frequently applied methods for the direct measurement of the textural properties of powdered solids, such as BET surface area (SBET, m2/g), total pore volume (VT, cm3/g), and average pore diameter (DP, nm), is the BET (Brunauer-Emmett-Teller) method based on low temperature (-196 oC) nitrogen gas adsorption6.

FTIR microspectroscopy (FTIRM) is an important technique for addressing site-to-site variation in mineral quality. It has been successfully used to examine the change in mineral properties in histological sections with a spatial resolution of 20 txm[9]. This technique has recently been placed on a quantitative basis by a combination of second derivative spectroscopy and curve generation[10].
In addition, this study provides scientific arguments for choosing the right bone substitute in terms of osteoconduction and mechanical strength for a specific clinical indication in surgical studies[8].

The aim of this study is to examine the textural structure and organic functional groups of horse-donkey, cow, dog, and sheep bones by nitrogen gas adsorption-desorption and FT-IR spectroscopic methods.

Materials And Methods

Material

The examined animal bones were procured from The examined animal bones were obtained from the bone archive in the anatomy department of ~~xxxx~~ University. In our study, one horse, cow, dog and sheep bone each was used. Before spectral analysis, it was washed with hot water to remove unwanted dust and impurities and then rinsed with ultrapure water many times until a stable neural pH was reached, and then dried at room temperature. Then, the dried bone samples were ground to 80 mesh and made ready for analysis.

Instrumentation

Textural structure properties such as SBET, VT, and DP were determined from nitrogen gas adsorption-desorption isotherms and pore size distribution curves measured with a Micromeritics-TriStar II Plus 2.03 version model surface area and pore meter device. FT-IR spectra for the determination of surface organic functional groups were recorded with a Perkin Elmer spectrum 100 model FTIR spectrophotometer through attenuated total reflectance (ATR) using 4 cm-1 resolution in the wavelength range of 4000-450 cm-1.

Results

Textural structure analysis

Fig. 1 shows the nitrogen gas adsorption-desorption isotherms and pore size distribution curves of the examined animal bones with a surface area and pore meter (Micromeritics-TriStar II Plus 2.03 version) at -196 oC (77 K). When these curves are examined formally, they all resemble Type-V in the isotherm classification determined by IUPAC (International Pure Applied Chemistry)[11]. Accordingly, it indicates that the examined animal bones have a mesoporous and/or macroporous textural structure. This is supported by the pore values such as BET (Brunauer-Emmett-Teller) surface area (SBET, m2/g), total pore volume (VT, cm3/g), and average pore diameter (DP, nm) determined from the curves in Figure 1, given in Table 1. It is seen from Table 1 that they are in the order according to their pore surface area values SBET, horse/donkey > SBET,sheep > SBET,cow > SBET,dog, and according to their pore volume values VT,horse/donkey > VT,sheep > VT,dog > VT,cow. In addition, according to the average pore diameter from the same table, it is seen that they are in the order of DP,dog > DP,sheep > DP,cow > DP,horse/donkey. In addition, it is seen from the same table that they are in the order of DP,dog > DP,sheep > DP,cow > DP,horse/donkey according to the average pore diameter values. In addition, solid materials have been classified by IUPAC as microporous, mesoporous, and mesoporous and/or macroporous solids based on their pore diameter values greater than 0.0-2.0 nm, 2.0-5.0 nm, and 5.0 nm, respectively[11]. Accordingly, it is supported that the examined animal bones have a mesoporous and/or macroporous textural structure. These differences between the textural properties of the examined animal bones may be due to their chemical composition, crystallinity, thickness, hardness, and porosity.

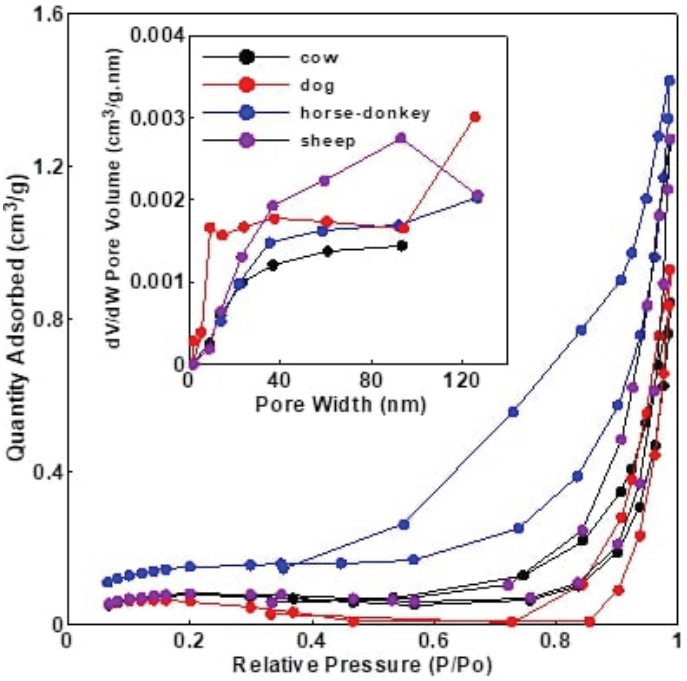

Figure. 1. Nitrogen adsorption/desorption isotherms and pore size distribution curves (inside) of some animal bones.

Table 1. Textural properties of various animal bones.

Animal	BET surface area (m^2/g)	Pore volume (cm^3/g)	Average pore diameter (nm)
Horse /Donkey	0.5864	0.00216	14.76
Cow	0.3110	0.00125	16.03
Sheep	0.3387	0.00187	22.06
Dog	0.2472	0.00137	22.09

Surface functional group analysis

Figure. 2 shows the FT-IR spectrum corresponding to the functional groups on the surface of the examined bones. From FT-IR spectra in this figure, it can be seen that there is no significant difference in peak positions and intensities and that they contain the same organic functional groups. The positions and assignments of the FT-IR spectral vibration bands are listed in Table 212.

Figure. 2. FT-IR spectrums correspond to the functional groups on the surface of the examined bones.

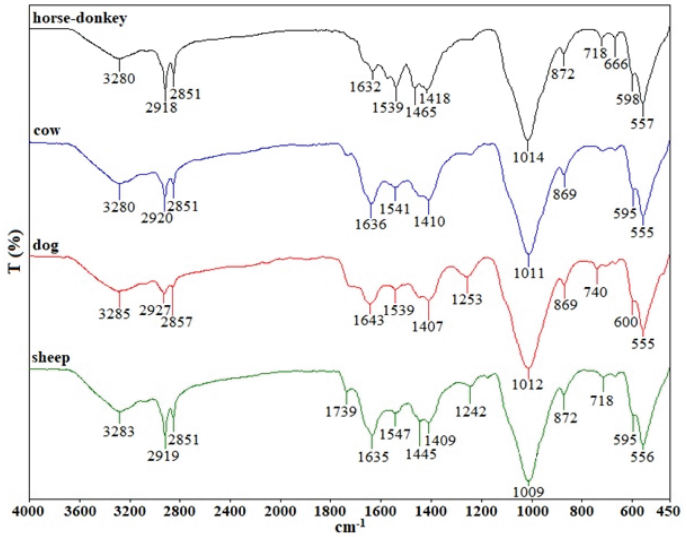

Table 2. Positions and assignments of the FT-IR spectral vibration bands of examined animal bones.

Peak position (cm^{-1})	Intensity	Assignments	Compound class
3550-3200	medium	O-H stretching	alcohol
3000-2840	weak	C-H stretching	alkane
2140- 2100	weak	C ≡ C stretching	alkyne
1650-1580	medium	N-H bending	amine
1440-1395	medium	O-H bending	carboxylic acid
1250-1020	medium	C-N stretching	amine
1220-1000	strong	C–O stretching	alcohols, carboxylic acids, esters, ethers
980-960	medium	C=C bending	alkene
850-550	weak	C–Cl stretching	alkyl halides

Discussion and Conclusion

In this study, the textural structure, surface morphology and elemental composition and surface chemical properties of horse, cow, dog and sheep bones were investigated. It was determined that the examined animal bones had a low BET surface area and a mesoporous and/or macroporous textural structure. The amount, size, and interconnectivity of macropores have a significant effect on bone growth. Often, the interconnectivity of commercial synthetic bone substitutes is not measured. The high porosity content favors bone growth as the empty macroporosity volume can be immediately colonized by bone. A smaller amount of material needs to be absorbed to replace the host bone. However, it has been reported that increasing the porosity content and/or size significantly reduces the mechanical properties13. The morphology of the porosity influences cell proliferation and cell growth in bone substitutes. Macro-links are necessary to promote cell growth, and microporosity seems to affect cell suspending on the material. However, porosity has a detrimental effect on mechanical strength, directly affecting the usefulness of the device when surgeons need to process blocks. It is generally accepted that mechanical strength is not an important parameter for bone substitutes. The mechanical strength of bone substitutes is of equal importance due to their macro-linkage and surface physico-chemical properties, and none should be neglected to guarantee clinical success8. It has been reported that the chemical properties (eg, chemical composition, crystallinity), structural properties (eg, thickness, porosity) and mechanical properties (eg, stiffness, elastic modulus) of bone change with age[14,15]. In our study, it was determined that these parameters changed in

different animal species. In the study of Shaochen et al.16, the structural properties of porous carbon based on porcine bone were stated as 703-2157 m2/g, pore volume 0.57-2.26 m3/g, between the activation temperature of 650 °C and 950 °C. In our study, it differed according to animal species and at 200 °C; surface area was calculated as 0.24-0.58 m2/g and pore volume was calculated as 0.0012-0.0021 cm3/g. Increasing the activation temperature from 650 °C to 950 °C causes a gradual expansion of the adsorption at low relative pressure, indicating the expansion of the micropore size distribution and the formation of larger micropores. As the temperature remains low, the pore size distribution does not expand. In the study[17]; In the BET analysis, the mean pore diameter was 20.54 nm at 650 °C and 7.53 nm at 950 °C. It varies between 14.76-22.09 according to the results of the inter-species analysis. In the study conducted in chickens18, the surface area was reported as 316.05 m2/g. Again, in a study conducted between 300 and 500 °C [6], it was observed that the bone mineral density per surface area decreased as the temperature increased.

Consequently, In this study the textural structure and surface chemical properties of horse-donkey, cow, dog, and sheep bones were investigated. It was determined that the examined animal bones had a low BET surface area and a mesoporous and/or macroporous textural structure. In addition, it was observed from the FT-IR analysis results that they mainly contain organic functional groups such as amine, alcohol, carboxylic acid, ester, and ether. In tissue engineering, regeneration of osteonecrotic bone defects in clinical interventions is seen as a potential alternative to the traditional use of bone grafts. Because it is available in unlimited supply, without the handicaps such as fear of disease transmission, additional donor site morbidity, immune rejection, or pathogen transfer. For these reasons, there is currently an extremely high interest in functional biomimetic biomaterials. A suitable mold design can be realized for the samples to be produced by imitating the structure of the bone (high porosity spongy bone on the inside, dense cortical bone on the outside). In this way, the inside of the samples can be obtained with a porosity of 70-90%, and the outer layer of a porosity of 1-30%, similar to the bone. A material such as hydroxyapatite, which provides biocompatibility, can be used for the exterior of this layered structure, and an alloy that meets the strength requirements for the interior can be used. Also, some organic components, such as proteins, can be degraded with bone calcification. Clarifying which proteins are degraded, how they degrade and what role they play in bone calcification remains a challenge for research. Further investigation of the calcification process may provide a clinical approach to understand the pathogenesis of abnormal calcification and develop treatments for bone regeneration.

References

1. Knychalska-Karwan Z, Ślósarczyk A. Hydroksyapatyt w stomatologii, Krakmedia, Kraków, 1994.
2. Orlovskii VP, Komlev VS, Barinov SM. Hydroxyapatite and hydroxyapatite-based ceramics. Inorganic Materials, (38)10: 973–984, 2002.
3. Vallet-Regi M, Gonzalez-Calbet JM. Calcium phosphates as substitution of bone tissues. Progress in Solid State Chemistry, 32: 1–31. 2004. DOI: https://doi.org/10.1016/j.progsolidstchem.2004.07.001
4. Fathi MH, Hanifi A, Mortazavi V: Preparation and bioactivity evaluation of bone-like hydroxyapatite nanopowder. Journal of Materials Processing Technology, 202: 536–542, 2008.DOI: https://doi.org/10.1016/j.jmatprotec.2007.10.004
5. Thompson DD, Posner AS, Laughlin WS, Blumenthal NC: Comparison of bone apatite in osteoporotic and normal Eskimos, Calcified Tissue International, 35: 392-393, 1983. DOI: 10.1007/BF02405064
6. Posner AS: The structure of bone apatite surfaces, Journal of Biomedical Materials Research, 19(3): 241-250, 1985.
7. Shade DM, Johnson AT. Standad Handbook of Biomedical Engineering and Design, 2004.
8. Bignon A, Chouteau J, Chevalier J, Fantozzi G, Carret JP, Chavassieux P, Hartmann, D. Effect of micro-and macroporosity of bone substitutes on their mechanical properties and cellular response. Journal of Materials Science: Materials in Medicine, 14(12): 1089-1097, 2003.DOI: 10.1023/b:jmsm.0000004006.90399.b4
9. Paschalis EP, Jacenko O, Olsen BR, Mendelsohn R, Boskey AL. FT-IR microspectroscopic analysis identifies alterations in mineral properties in bones from mice transgenic for type X collagen, Bone 19(2): 151-156, 1996. DOI: 10.1016/8756-3282(96)00164-0
10. Paschalis EP, DiCarlo E, Betts F, Sherman P, Mendelsohn R, Boskey AL. FTIR microspectroscopic analysis of human osteonal bone. Calcified Tissue International, 59(6): 480-487, 1996.
11. Sing KSW, Everett DH,. Haul RAW, Moscou L, Pierotti RA, Rouquerol J, Siemieniewska T. Reporting physisorption data for gas/solid systems with special reference to the determination of surface area and porosity. Pure and Applied Chemistry, 57: 603-619, 1985. DOI: https://doi.org/10.1351/pac198557040603.
12. Solomons TG, Fryhle CB, Snyder SA. Solomons' Organic Chemistry, Binder Ready Version Study Guide

& Student Solutions Manual, John Wiley & Sons, 2016.
13. Flautre B, Anselme K, Delecourt C, Lu J, Hardouin P, Descamps M: Histological aspects in bone regeneration of an association with porous hydroxyapatite and bone marrow cells. Journal of Materials Science: Materials in Medicine, 10(12): 811-814, 1999.DOI: 10.1023/a:1008923625599
14. Donnelly E, Boskey AL, Baker SP, van der Meulen MC. Effects of tissue age on bone tissue material composition and nanomechanical properties in the rat cortex, Journal of Biomedical Materials Research Part A, 92(3):1048–1056, 2010. DOI: 10.1002/jbm.a.32442
15. Miller LM, Little W, Schirmer A, Sheik F, Busa B, Judex S: Accretion of bone quantity and quality in the developing mouse skeleton. Journal of Bone and Mineral Research, 22:1037–1045, 2007. DOI: 10.1359/jbmr.070402
16. Wei S, Zhang H, Huang Y, Wang W, Xia Y, Yu Z. Pig bone derived hierarchical porous carbon and its enhanced cycling performance of lithium–sulfur batteries, Energy & Environmental Science, 4(3): 736-740.
17. Sobczak A, Kowalski Z, Wzorek Z: Preparation of hydroxyapatite from animal bones, Acta of Bioengineering and Biomechanics, 11(4): 23-28, 2009.
18. Oladipo AA, Ifebajo AO: Highly efficient magnetic chicken bone biochar for removal of tetracycline and fluorescent dye from wastewater: two-stage adsorber analysis, Journal of Environmental Management, 209: 9-16, 2018. DOI:https://doi.org/10.1016/j.jenvman.2017.12.030