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Pattern of X-Ray Diffraction Test for Quality Control of the Components That Resembling the Bone Tissue Constituents

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Abstract

Biomedical application represents great interest, in order to develop materials that must be biocompatible and suitable for use in the living tissue recovery or replacement when individual requires it. These materials must have characteristics similar to the tissue that will be replaced, in order to repair or improve living bone damaged tissue. In the current study, components resembled the bone constituents and collagen, casein, polymethylmethacrylate and mineral mixture were evaluated for their crystallinity properties suitable to certify the biomedical applications such as on bone damaged, using materials and supplies of alimentary grade. In the present work, results demonstrated a powder with high purity characteristics of powders according to X-ray diffraction patterns of the powders obtained comparing to the standard of pure HA hydroxyapatite. Diffractogram patterns of samples compared to hydroxyapatite and rat bone powders correspond to that of pure hydroxyapatite phase used as reference. These findings suggest that the used mixtures present adequate properties to guarantee their use of the materials, for medical applications, the selected components offer an interesting option to mixture for recovering of living tissues, such as bone damaged. The X-ray test is an excellent test for quality control. Non-impurity other than that was observed, ratifying the purity of these prepared samples, which were confirmed to be crystalline powder.

Keywords

Protein, Minerals, X-Ray Diffraction, Polymer, Materials

1. Introduction

The X-ray diffraction (XRD) is an analytical technique that allows determining the chemical composition of a material through the structure of its crystals. XRD are diffracted as they pass through the atomic or molecular layers of a crystal, and the intensity of the diffracted rays is evaluated to generate a diffraction pattern. The diffraction pattern provides information about the spacing between the planes of the material, which is specific for each crystalline element. XRD is used in several industrial applications, such as cement manufacturing, metallurgy and the pharmaceutical industry. XRD is a non-destructive and versatile technique that can be used to analyze solid (powders) and liquid [1].

1.1. Bone Tissue

Bone is made up of 65% minerals and 35% organic matrix, cells, and water. The rigid bone matrix encompasses 90% of the tissue volume and the rest is occupied by cells, cellular mechanisms and blood vessels. The organic matrix is composed of 90% collagen and the rest of various proteins [2]. Bone tissue is a structure made up of two phases: an internal part called bone marrow that is covered by an external part, called bone matrix. Bone marrow is a kind of viscous tissue, found in the center of long and axial bones, mainly made up of hematopoietic tissue (responsible for the formation of red blood cells, white blood cells and platelets), adipose cells and vascular sinuses [3].

1.2. Proteins

Within these natural components, we will highlight the proteins, these form an average of 3.4% of the components of milk, and in turn, 80% of the proteins are casein. Casein is classified within the phosphoproteins.

Casein is made up of different fractions, which are called α-casein (which is further divided as αs1-casein and αs2-casein), β-casein, γ-casein and κ-casein. The various types of caseins have molecular weights between 19,000 and 23,900 and vary in amino acid composition. Casein molecules are found in the form of micelles, due to the amorphous calcium phosphate fragments that give them stability, in addition to κ-casein, which is found on the surface of the micelle [4]. On the other hand, the structure of casein depends on the surrounding physical-chemical environment, protein is produced by means of acid or enzymatic precipitation [5]. Casein is used in different industries, of which it is worth mentioning its use in the production of makeup, as glue and within the construction industry, since it is added to make cement. In addition, casein can be used in the production of hard and flexible plastics, this depends on the process that is carried out. It participates in the absorption of calcium, iron and zinc, which is the reason why it is currently used as a biomaterial in dental interventions. It has also been used in the form of amorphous calcium phosphate in the preparation of oral health products, for their fixation of calcium ions, and also is added to toothpaste, for clean dental prostheses together with other components [6]. Another study demonstrates that casein has been used as part of the bone tissue and cartilage replacement material, however, it is used as a composite together with some inorganic biomaterial [7].

Biomaterial based on collagen can be found in the medical area, which uses them as a filling material for bones and teeth (this is because it serves to regenerate bone tissue) and in the pharmaceutical industry, where it has recently been studied as a drug transport material. In addition, collagen has been one of the materials of greatest interest in the cosmetics industry [8].

Collagen structure includes three different polypeptide chains, which are joined in a triple, and the latter is a derivative of proline. 10.5% of the collagen structure follows the configuration Proline-Hydroxyproline-Glycine (Pro-Hyp-Gly), being the triplet that is repeated the most collagen fibers that can be obtained under environmental optimum conditions, temperature between 62˚C and 65˚C for inhibit it denaturalization in collagen from mammals, according to the source used [9]. It is mainly extracted from skin, cartilage, tendons and animal bones, especially from pigs and cattle [7].

For biomaterials, not all types of collagens will be used, type I collagen is the one of greatest interest in this area. Type I collagen has two great advantages, one is the ease of obtaining it, because it is one of the most abundant collagens, and the other one is its biocompatibility. Collagen can form intertwined chains that generate compact solids, in the same way, when taking another configuration, it can form gels [10].

1.3. Polymers

Polymers are materials used in biomedical applications due to the potential for their high stability and maintain their structure and physico-chemical properties. The main properties of polymeric biomaterials, such as biodegradability, biocompatibility, processability, and mechanical properties, can be tailor-made to the specific tissue and application [11].

Recent approaches in creating novel protein materials are aimed at engineering proteins to elaborate structures of increasingly complex properties. Overall, researchers are introducing new experimental parameters at each level of protein structure to birth an endless array of engineering possibilities. One of the most interesting and perhaps challenging area for protein-based materials is targeted drug delivery. Targeted drug delivery offers the potential of a local, increased dose of the drug while lowering systemic and non-specific drug administration. In many cancer applications, for example, off-target delivery of extremely cytotoxic chemotherapeutics causes extreme side effects. Current efforts include combining multiple modalities for stimuli-controlled drug delivery. pH-sensitive nanoparticles can passively accumulate in tumor environments where the acidic environment causes the nanoparticles to release their pay load. Current endeavors aim for increased user-controlled drug release upon the application of external stimuli, such as light, MRI, and even photoacoustic. As protein technology continues to become more advanced and complex, the design and implementation of such proteins must be spear headed by an interdisciplinary effort comprised of (but not limited to) molecular biologists, chemists, protein and tissue engineers, biomedical imagers, and clinicians [12].

1.4. Polymethylmethacrylate (PMMA)

The PMMA is an amorphous and synthetic polymer, discovered in Germany, used for different purposes within the industry, due to its mechanical properties, which make it resistant, in addition to being a transparent plastic. PMMA can be synthesized by bulk, solution, suspension, and emulsion polymerization.

It is used in the automotive industry, as well as to make lighting and cosmetic products, and it also has uses as a biomaterial, specifically as bone cement and for replacement in areas with mechanical load, in addition to being used in the fixation of hip implants. Being transparent, it is used to make contact lenses and artificial eyes. Among the advantages of PMMA as bone cement, we find that a donor is not required, it is malleable, light, strong, inert, non-ferromagnetic and stable [13], which facilitates its use as an implant, it has a density of 1.19 gr/cm^3.

It exhibits excellent toughness and chemical stability; the internally absorbed water decreases the tensile strength and the Young's modulus until it stabilizes over time at a constant value. In addition to having an impact resistance similar to that of impact resistant styrene copolymers. In its optical properties, its transparency stands out and the fact that it transmits lighter than glass, has a glass transition temperature of 120˚C and can be easily molded from 100˚C. As disadvantages, this presents complications at the time of polymerization, since it can reach 80˚C and in therapeutic use this is associated with necrosis, when used as cement in prostheses, if it loosens, the organism can reach have an inflammatory response at the site of the prosthesis [10] [14].

Quality control testing is a bulk of tests, measurements, verifications and tests carried out to determine whether a product meets established specifications. The objective of these tests is to identify if corrective actions need to be taken in the manufacturing process.

1.5. Quality Control of Materials

The quality control is any planned and systematic activity carried out in production in order to guarantee that the goods produced, the services and, in general, any process carried out meet the established requirements and certain standards. According to the ISO 9000:2000 standard [15], which defines this type of standards, quality is a set of specific characteristics and properties of a good product or service to satisfy specific needs.

The bone matrix can take two different structural arrangements, which gives rise to compact or cortical bone and cancellous or trabecular bone. These differences in their mechanical function are reflected in their structure, since cancellous bone has larger pores than compact bone, however, the chemical composition of both is the same. The main objective of the present study was to evaluate, using the X-ray test, the crystallinity test as a quality control test of the substances that resembled the bone composition.

2. Materials and Methods

2.1. Samples

The samples of collagen, casein, and polymer samples are commonly used to obtain fibers, sheets, chains, gels, and three-dimensional polymers. These compounds mixed by a sol-gel synthesis originate generally oxides, however, we can obtain mixed solids, composed of organic and inorganic components [16].

Bone powders used were from five male rats sacrificed with carbon dioxide aspiration, both left and right femurs were removed, cleaned of surrounding soft tissue and cartilage. All animal management was performed following the guidelines formulated by the National Institute of Health publication no. 96-23, revised 2003, 1996. The bones were dried at 40˚C, crushed, and subsequently incinerated at 600˚C during 24 hours. The ash was ground to a fine particle, then X-ray patterns were recorded in a diffractometer.

2.1. X-Ray Test

X-ray test was carried out for the all-powder's samples, since it is traditionally used for the characterization of materials such as minerals, alloys, metals, among others. X-rays are radiation capable of passing through bodies that are considered opaque [17]. All samples were evaluated thought X-ray test using a diffractometer Rigaku Ultima IV, for verified the crystallinity as a quality control test of the substances that mimic the elements of a bone constituents (**Figure 2**, **Figure 3**).

To calibrate the X-ray equipment was used a standard powder reference material certified standard by the National Institute of Standards and Technology was used, with the standard 1400 designed for evaluation of analytical methods for the determination of bone tissue components, and in a similar matrix material. Using the powder samples, of collagen, casein, polymethylmethacrylate (PMMA), food grade mineral mix, synthetic hydroxyapatite and Wistar bone rat of 15 age-days which were used as reference.

3. Results and Discussion

X-Ray Tests

As we can see, the structures of collagen and casein and PMMA show diffraction patterns similar to each other, in addition to proteins being poorly crystalline materials, noise is generated in the diffractograms (**Figure 1**). When compared the samples, the X-ray diffractograms, bone was compared with hydroxyapatite, to which we can see that their main spikes of diffractograms were similar, despite the fact that hydroxyapatite is a crystalline solid and the pattern for bone shows an interference because this is amorphous (**Figure 2**). In addition, we can observe that both bone and hydroxyapatite) have amorphous structures and that showed the mixture of minerals (**Figure 3**), they generate bands similar to those produced by hydroxyapatite suggesting that findings are due to they have similar composition, reinforces the possible use for induce bone recovering or replacement.

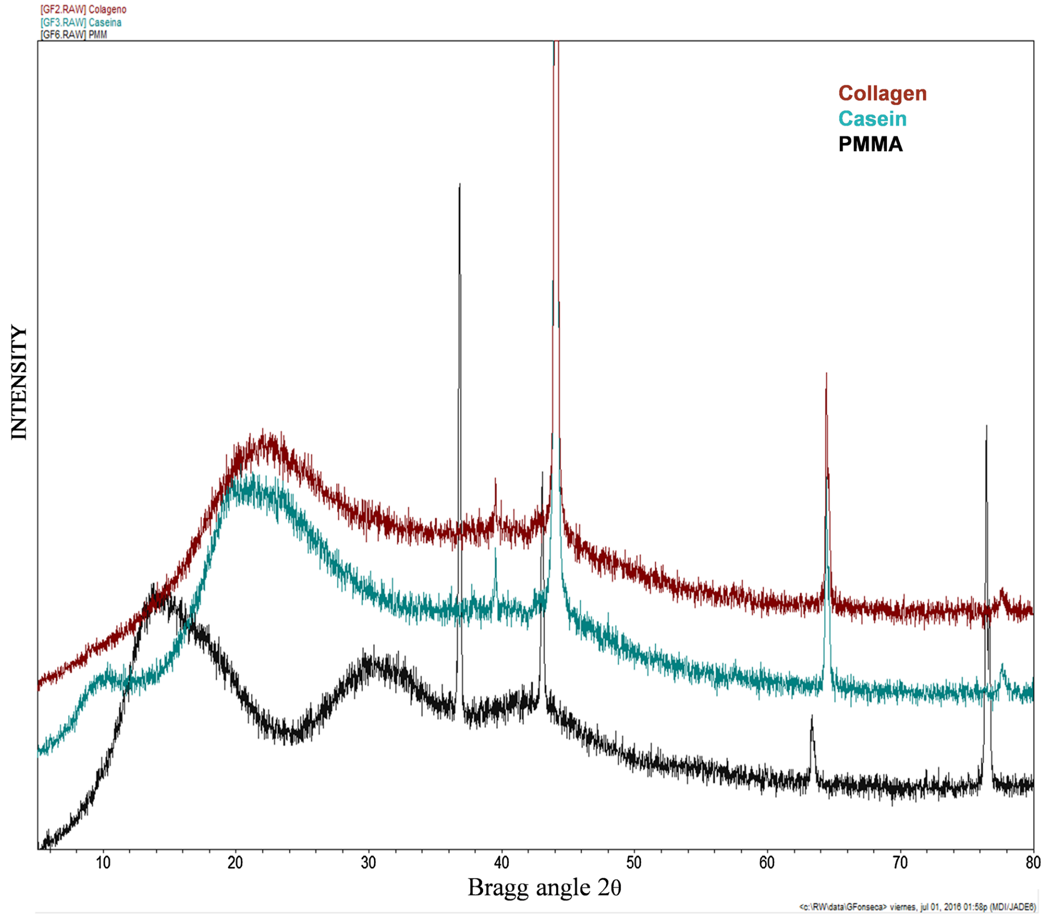

Figure 1. Diffractograms of collagen, casein and polymethylmethacrylate. As we can see, the collagen and casein structures show similar diffraction patterns, and since they are poorly crystalline materials, noise is generated in the diffractograms.

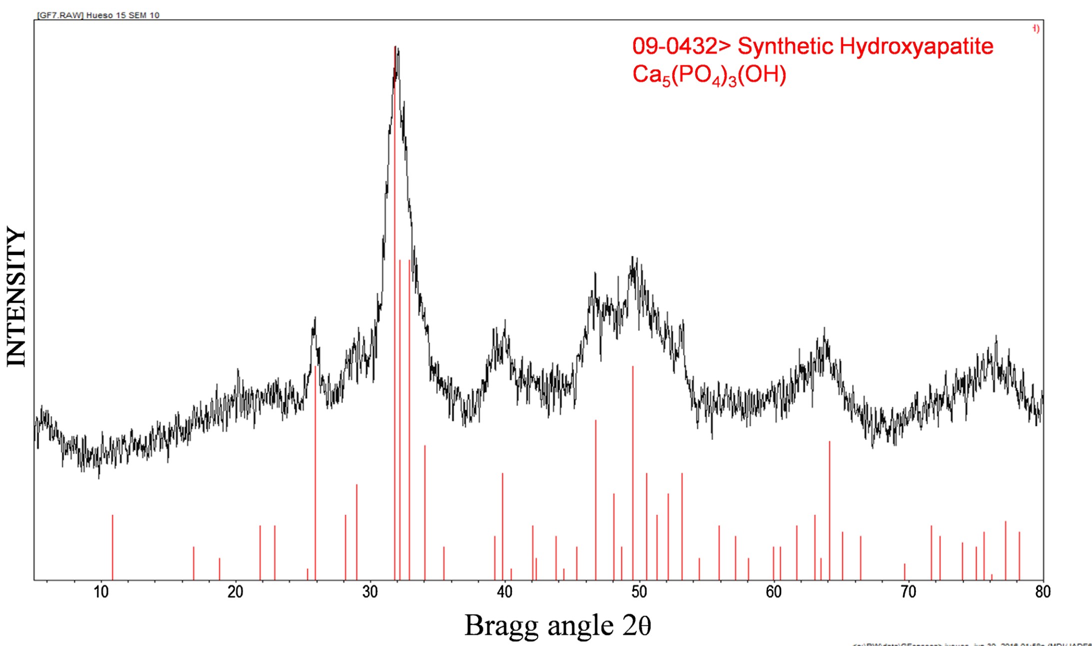

Figure 2. Wistar rat bone diffractogram at 15 weeks of age (black line), comparison with synthetic hydroxyapatite.

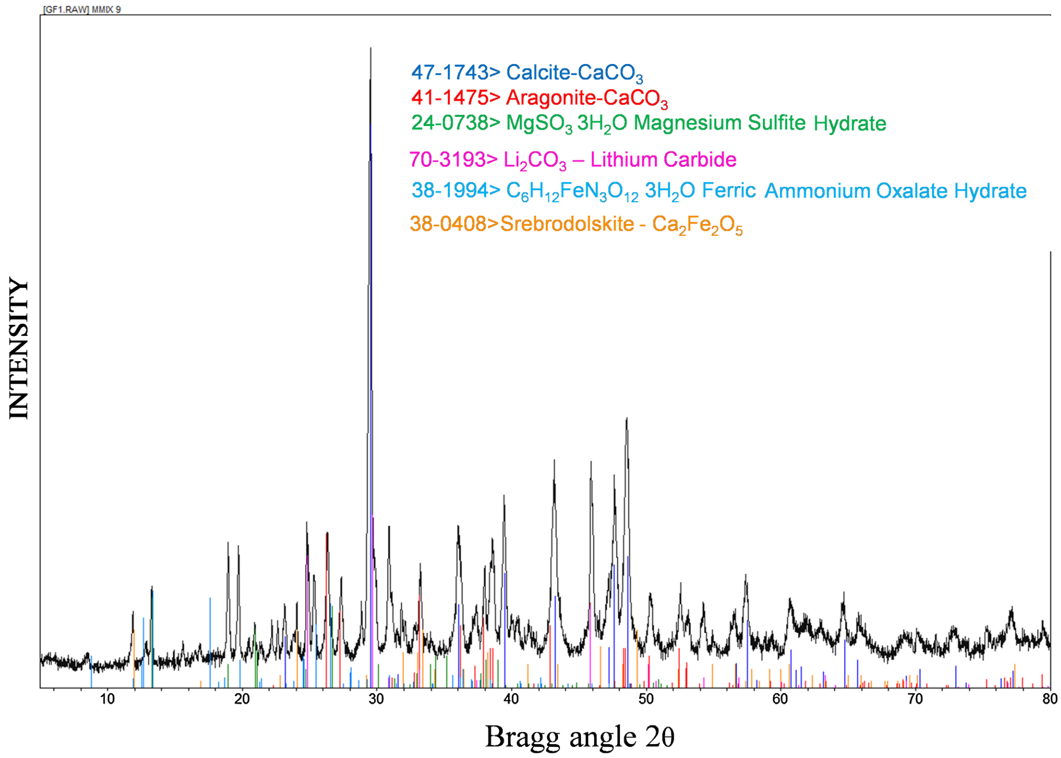

Figure 3. X-ray diffractogram for the mixture of food grade minerals mix.

4. Discussion

Using the X-ray technique test, we found that the samples have a similar pattern to that generated by hydroxyapatite (reference material), which is a material noted for its use for bone implants. The characteristics of the samples that are registered trademarks for commercial use of biomedical applications, so it is intended that in the future, the mix of samples can be used inside the human or animal body. Within the long-term perspective, the aim is to replace the PMMA used with another polymer of natural origin, the new material could achieve greater biocompatibility. Because hydroxyapatite is a used material for restorative medical applications due to its biocompatibility, we suggest the use of experimental new resembled the bone organic (proteins and PMMA) and inorganic (food grade mineral mix) phases (**Figure 1**, **Figure 3**). Principally, for their biostability similar to the hydroxyapatite (**Figure 2**) and bone rat crystallinity properties can maintain the physiological conditions and integration of live tissues.

The fabrication of new materials derives numerous benefits from X-ray diffraction techniques, during the processing of samples, composition analysis can be performed accurately to evaluate the percentage of impurities found within the sample. Mainly due to a mixture of protein, polymer and food minerals mix (**Figure 3**) by experimental ways can perform the organic and inorganic phases of bone. Additionally, the possible synthetized mixtures can also be thoroughly analyzed before giving them any use [18].

As a perspective of application of the tested samples, when comparing the hardness of used samples might have varying porosities, the block with less pores is found to possess hardness, while the block with more pores has less hardness, but the presence of pores will accommodate their integration for use in temporal dental or orthopedic applications [19] [20]. An *in vitro* biostability/corrosion test showed that porous hydroxyapatite is stable under human physiological conditions as it is not absorbed in the simulated body fluid and also allows the integration of body tissues, which is in line with the present findings.

5. Conclusion

After analyzing the bulk of results, we concluded that the mixtures of the evaluated samples are likely to be used for biomedical applications, such as replacement or implants. However, it is suggested that X-ray test be carried out to determine the quality of substances that present the adequate crystallinity quality properties to guarantee their uses for bone recovering applications. The samples showed similar structure pattern to that generated by hydroxyapatite, which is a material applied for its use for bone implants, due to the similarity of organic and inorganic bone phases. The use of proteins and polymers for possible bone replacement is also reinforced because of the similar pattern of Wistar bone rat and hydroxyapatite. We suggest realizing similar tests of mixtures of collagen/casein polymer and mineral mix (**Figures 1-3**) for their similar uses of hydroxyapatite, which could achieve greater biocompatibility.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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