
**Implants for surgery — Hydroxyapatite —
Part 3:
Chemical analysis and characterization
of crystallinity and phase purity**

Implants chirurgicaux — Hydroxyapatite —

*Partie 3: Analyse chimique et caractérisation de la cristallinité
et de la pureté de phase*

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ISO 13779-3:2008

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 13779-3 was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 1, *Materials*.

ISO 13779 consists of the following parts, under the general title *Implants for surgery — Hydroxyapatite*:

- *Part 1: Ceramic hydroxyapatite*
- *Part 2: Coatings of hydroxyapatite* [ISO 13779-3:2008](https://standards.iteh.ai/catalog/standards/sist/2482838f-4a6a-43da-a7ae-759fafdd7e5/iso-13779-3-2008)
- *Part 3: Chemical analysis and characterization of crystallinity and phase purity*
- *Part 4: Determination of coating adhesion strength*

Introduction

No known surgical implant material has ever been shown to cause absolutely no adverse reactions in the human body. However, long term clinical experience of the use of the material referred to in this part of ISO 13779 has shown that an applicable level of biological response can be expected, if the material is used in appropriate applications.

The biological response to coating of hydroxyapatite ceramic has been demonstrated by a history of its clinical use and by laboratory studies.

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Implants for surgery — Hydroxyapatite —

Part 3: Chemical analysis and characterization of crystallinity and phase purity

1 Scope

This part of ISO 13779 specifies methods of test for the chemical analysis and assessment of crystallinity and phase composition of hydroxyapatite-based materials such as coatings and sintered products.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 565, *Test sieves — Metal wire cloth, perforated metal plate and electroformed sheet — Nominal sizes of openings*

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ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 10993-14, *Biological evaluation of medical devices — Part 14: Identification and quantification of degradation products from ceramics*

ISO 13779-2, *Implants for surgery — Hydroxyapatite — Part 2: Coatings of hydroxyapatite*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

Sheets JCPDS 09-0169; JCPDS 9-348; JCPDS 9-432; JCPDS 72-1243; JCPDS 25-1137; JCPDS 70-1379; JCPDS 4-0777; JCPDS 82-1690 *Elements of X-ray Diffraction*, B. D. CULLINITY, 2nd ed., Addison-Wesley, Reading, MA, 1978 (JCPDS = Joint Committee on Powder Diffraction Standards)

3 Terms, definitions and symbols

3.1 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1.1

calibration curve

calculating plot translating the integrated intensity measured on the X-ray diffraction pattern into the foreign phases content or calcium:phosphorus (Ca:P) ratio

3.1.2

detection limit

ten times the standard deviation of a blank test

3.1.3

height

distance between the peak summit and the base line of the X-ray diffraction pattern from which the background has been subtracted

3.1.4

integrated intensity

surface area between the plot of the peak and the base line of the X-ray diffraction pattern from which the background has been withdrawn

3.1.5

scraping

removal of the coating from the base material without removing any of the base material itself

3.1.6

signal:noise ratio

height of a peak of the X-ray diffraction pattern divided by the maximum deviation of the base line oscillation

3.2 Symbols

p density

d inter-reticular distance

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4 Analytical methods

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The methods listed below have been tried and tested. The list is not restrictive:
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- a) atomic absorption spectroscopy, hydride method with “background noise” correction;
- b) atomic absorption spectroscopy with electro-thermal atomization using matrix modifiers;

EXAMPLE Palladium-magnesium nitrate.

- c) flame atomic absorption spectroscopy after complexion and extraction;
- d) inductive by coupled plasma (ICP) hydride;
- e) inductive by coupled plasma mass spectrography (ICP-MS).

The following spectroscopic methods are generally used for the analysis of trace elements:

- f) atomic absorption spectroscopy (AAS);
- g) inductively coupled plasma mass spectroscopy (ICP-MS).

5 Apparatus, reagents and calibration specimens

5.1 Apparatus for chemical analysis

The test sample shall be kept in devices with at least the following characteristics:

- a) class A glassware carefully washed with acid then rinsed with grade 2 water in accordance with ISO 3696:1987;

or

b) PTFE flask (or similar).

An appropriate quantitative analysis apparatus, having a detection limit which is at most equal to the required limit value as given in ISO 13779-2.

5.2 Reagents for chemical analysis

All reagents shall be of analytical quality:

5.2.1 grade 2 water, according to ISO 3696:1987.

5.2.2 minimum 52,5 % nitric acid, $p = 1,33$.

5.2.3 30 % hydrogen peroxide (by mass), $p = 1,11$.

5.2.4 standard solutions of the elements to be determined, prepared either by weighing or from commercially available standard solutions.

5.3 Apparatus and calibration specimens for X-ray diffraction analysis

5.3.1 General

The X-ray diffractometer shall have a resolution and reproducibility of at least $0,02^\circ$ on a 2θ angle scale and allow the recording of the diffraction peak positions and intensities. A stabilized power supply is necessary in the case of goniometric recording. The conditions of the X-ray diffraction pattern collection will allow the contribution of the apparatus to the half-intensity width to be minimized; these conditions shall be identical for the test sample and for the mixture used to prepare the calibration curves.

5.3.2 Apparatus

The apparatus shall consist of the following:

5.3.2.1 mortar, in alumina, agate or other suitable matter;

5.3.2.2 sieve, complying with ISO 565;

5.3.2.3 oven, capable of maintaining a temperature of $1\,000^\circ\text{C} \pm 25^\circ\text{C}$;

5.3.2.4 desiccator, with a CO_2 trap.

5.3.3 Calibration specimens

The calibration specimens listed below shall be used.

a) Pure β -tricalcium phosphate having an X-ray diffraction pattern as described in JCPDS 09-0169. It shall conform to the requirements described in Annex B.

NOTE 1 Pure β -tricalcium phosphate can either be prepared as described in Annex E, or purchased as commercially available standard powder.

b) Pure α -tricalcium phosphate having an X-ray diffraction pattern as described in JCPDS 9-348.

NOTE 2 Pure α -tricalcium phosphate can either be prepared as described in Annex E, or purchased as commercially available standard powder.

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- c) Pure apatite having an X-ray diffraction pattern as described in JCPDS 9-432 or JCPDS 72-1243. It shall conform to the requirements described in Annex B.

NOTE 3 Pure apatite can either be prepared as described in Annex E, or purchased as commercially available standard powder.

- d) Pure tetracalcium phosphate having an X-ray diffraction pattern as described in JCPDS 25-1137 or JCPDS 70-1379.

NOTE 4 Tetracalcium phosphate can either be prepared as described in Annex E or purchased as commercially available standard powder.

- e) Pure calcium oxide having an X-ray diffraction pattern as described in JCPDS 4-0777 or JCPDS 82-1690. It shall conform to the requirements described in Annex B.

NOTE 5 Pure calcium oxide can be obtained commercially.

5.4 Infrared apparatus

Fourier transform infrared (FTIR) spectroscopy can be used to identify chemicals that are either organic or inorganic. The wavelength of light absorbed is characteristic of the chemical bond. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

If an FTIR apparatus is used, it should allow at least a resolution of 4 cm^{-1} and analyse a region between 400 cm^{-1} and $4\,000\text{ cm}^{-1}$.

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6 X-Ray diffraction pattern collection (standards.iteh.ai)

6.1 General

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The diffractometer settings shall allow a resolution of 0.02° on a 2θ angle scale and a signal:noise ratio greater than 20 for peak 211 of the apatite (in the majority of cases, a signal:noise ratio greater than 50 is recommended).

The integrated intensities of the peaks taken into consideration shall be able to be measured without having recourse to deconvolution or peak decomposition software.

The peak integrated intensities of all phases shall be determined to an accuracy greater than 5 %, either using a planimeter or suitable software in the case of computerized installations. The integrated intensity corresponds to the surface area between the base line of the recording, from which the background has been removed, and the plot of the line.

Integration time will allow quantification of at least 5 % of any foreign phase.

6.2 Identification of the crystallized phases

The isolated crystallized phases shall be identifiable according to their characteristic lines:

- β -tricalcium phosphate shall be identifiable according to its lines given in sheet JCPDS 09-0169;
- α -tricalcium phosphate shall be identifiable according to its lines given in sheet JCPDS 9-348;
- apatite shall be identifiable according to its lines given in sheets JCPDS 9-432 and JCPDS 72-1243;
- tetracalcium phosphate shall be identifiable according to its lines given in sheets JCPDS 25-1137 and JCPDS 70-1379;
- calcium oxide shall be identifiable according to its lines given in sheets JCPDS 4-0777 and JCPDS 82-1690.

The selected lines could be:

- the line 0.2.10 ($d = 2,88 \times 10^{-10}$ m) of the β -tricalcium phosphate;
- the line 441,170 ($d = 2,905 \times 10^{-10}$ m) of the α -tricalcium phosphate;
- the line 040 ($d = 2,995 \times 10^{-10}$ m) of the tetracalcium phosphate;
- the line 200 ($d = 2,405 \times 10^{-10}$ m) of the calcium oxide;
- the line 210 or 211 ($d = 3,08 \times 10^{-10}$ m or $d = 2,81 \times 10^{-10}$ m) of the apatite.

Other lines may be chosen provided that they do not affect the sensitivity of the determination. When foreign phase contents are higher and in particular, if the five crystallized phases are present (apatite, tetracalcium, α - and β -tricalcium, calcium oxide) intensity corrections shall be made using suitable computer software in order to take into account the line spectral interference.

NOTE The quantification of the foreign phases is often delicate to carry out on account of, on the one hand, spectral interference and of broadening of the lines due to the foreign phases and, on the other hand, of the modifications of the line intensity of the projected apatite due to the existence of oxyhydroxyapatite. The low tetracalcium phosphate, α -tricalcium phosphate, β -tricalcium phosphate and calcium oxide contents are determined in relation to the reference line of the apatitic phase which does not interfere with the intense lines of these phases.

Further information is given in Annex C.

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7 Preparation of the test sample

7.1 Coatings

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In the case of coatings, a prior separation from the substrate is necessary and can be carried out using any method (scraping, tearing, etc.) resulting in negligible contamination of the sample. For thin coatings, it is important that the coating is detached from its substrate.

NOTE A solution might be coated on to a thin, lightly grit-blasted substrate that could be bent afterwards to collect the coating.

For thermally sprayed coatings, it is common that the layers near to the coating/substrate interface contain more amorphous phase than those areas far from the interface. Therefore, the sample shall be taken from a mixture of the whole coating layer to obtain a representative sample of the coating.

7.2 Bulk sample

Bulk samples shall be reduced to powder form.

7.3 X-ray analysis

For X-ray analysis, all the samples (deposit, powder and bulk) shall be crushed and the particle size distribution shall be checked. The maximum grain size shall be 40 μm . The particle size is an important parameter in the measurement. The same method of crushing shall therefore be applied for the preparation of the test sample and the mixtures for the calibration curves. It is necessary to avoid any contamination. Keep all the test samples in the desiccator.