
**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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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*
- *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.

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*

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

4 Analytical methods

The methods listed below have been tried and tested. The list is not restrictive:

- 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.

- 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 $4\,000\text{ cm}^{-1}$.

6 X-Ray diffraction pattern collection

6.1 General

The diffractometer settings shall allow a resolution of $0,02^\circ$ 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.

7 Preparation of the test sample

7.1 Coatings

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.

8 Plotting the calibration curves

8.1 General

Use the calibration specimens described in 5.3.3 to plot the calibration curve. Crush all the calibration specimens, avoid contamination and check the particle size distribution. The maximum grain size shall be 40 µm. Keep all the test sample in a desiccator. The particle size is an important parameter in the measurement; therefore, the same method of crushing shall be applied for the preparation of the test sample and the mixtures for the calibration curves.

At least three X-ray diffraction patterns shall be collected for each of the mixtures below, as described in Clause 6.

8.2 Plotting the calibration curves for the foreign phases

8.2.1 Plotting the hydroxyapatite/ β -tricalcium phosphate calibration curve

Produce, by weighing and crushing, pure calibration specimen mixtures (see 5.3.3) of hydroxyapatite and of β -tricalcium phosphate containing increasing quantities of β -tricalcium phosphate. Produce the X-ray diffraction pattern in the range corresponding to the peaks selected (peak 210 or peak 211 of the apatite and peak 0.2.10 of the β -tricalcium phosphate).

Measure the integrated intensities of the peaks selected for the apatite and β -tricalcium phosphate and calculate the ratio:

$$R_1 = \frac{\text{peak 210 or peak 211 integrated intensity of the apatite}}{\text{peak 0.2.10 integrated intensity of the } \beta\text{-tricalcium phosphate}}$$

where R_1 is a function of the percentage mass fraction of β -tricalcium phosphate.

8.2.2 Plotting the hydroxyapatite/calcium oxide calibration curve

Use calcium oxide that has been freshly calcined and stored in a desiccator.

Produce by weighing and crushing pure calibration specimen mixtures (see 5.3.3) of hydroxyapatite and of calcium oxide containing increasing quantities of calcium oxide. Produce the diffractogram in the range corresponding to the peaks selected (peak 210 or peak 211 of the apatite and peak 200 of the calcium oxide).

Measure the integrated intensities of the peaks selected for the apatite and calcium oxide and calculate the ratio:

$$R_2 = \frac{\text{peak 210 or peak 211 integrated intensity of the apatite}}{\text{peak 200 integrated intensity of the calcium oxide}}$$

where R_2 is a function of the percentage mass fraction of calcium oxide.

8.2.3 Plotting the hydroxyapatite/ α -tricalcium phosphate calibration curve

Produce by weighing and crushing pure calibration specimen mixtures (see 5.3.3) of hydroxyapatite and of α -tricalcium phosphate containing increasing quantities of α -tricalcium phosphate. Produce the X-ray diffraction pattern in the range corresponding to the peaks selected (peak 210 or peak 211 of the apatite and peak 441,170 of the α -tricalcium phosphate).

Measure the integrated intensities of the peaks selected for the apatite and α -tricalcium phosphate and calculate the ratio:

$$R_3 = \frac{\text{peak 210 or peak 211 integrated intensity of the apatite}}{\text{peak 44 1,170 integrated intensity of the } \alpha\text{-tricalcium phosphate}}$$

where R_3 is a function of the percentage mass fraction of α -tricalcium phosphate

8.2.4 Plotting the hydroxyapatite/tetracalcium phosphate calibration curve

Produce by weighing and crushing pure calibration specimen mixtures (see 5.3.3) of hydroxyapatite and tetracalcium phosphate containing increasing quantities of tetracalcium phosphate. Produce the X-ray diffraction pattern in the range corresponding to the peaks selected (peak 210 or peak 211 of the apatite and peak 040 of the tetracalcium phosphate).

Measure the integrated intensities of the peaks selected for the apatite and tetracalcium phosphate and calculate the ratio:

$$R_4 = \frac{\text{peak 210 or peak 211 integrated intensity of the apatite}}{\text{peak 040 integrated intensity of the tetracalcium phosphate}}$$

where R_4 is a function of the percentage mass fraction of tetracalcium phosphate.

8.3 Plotting the calibration curves for the calculation of the calcium:phosphorus (Ca:P) ratio

8.3.1 Plotting the hydroxyapatite/ β -tricalcium phosphate calibration curve

Produce by weighing and crushing pure calibration specimen mixtures (see 5.3.3) of hydroxyapatite and of β -tricalcium phosphate containing increasing quantities of β -tricalcium phosphate. Produce the X-ray diffraction pattern in the range corresponding to the peaks selected (peak 210 or peak 211 of the apatite and peak 0.2.10 of the β -tricalcium phosphate).

Measure the integrated intensities of the peaks selected for the apatite and β -tricalcium phosphate and calculate the ratio:

$$R_5 = \frac{\text{peak 210 or peak 211 integrated intensity of the apatite}}{\text{peak 0.2.10 integrated intensity of the } \beta\text{-tricalcium phosphate}}$$

where R_5 is a function of the percentage mass fraction of β -tricalcium phosphate or of the Ca:P ratio of the mixture calculated according to the weighed-in quantities.

8.3.2 Plotting the hydroxyapatite/calcium oxide calibration curve

Use calcium oxide, which has been freshly calcined and stored in a desiccator.

Produce by weighing and crushing pure calibration specimen mixtures (see 5.3.3) of hydroxyapatite and calcium oxide containing increasing quantities of calcium oxide. Produce the X-ray diffraction pattern in the range corresponding to the peaks selected (peak 202 of the apatite and peak 200 of the calcium oxide).

Measure the integrated intensities of the peaks selected for the apatite and calcium oxide and calculate the ratio:

$$R_6 = \frac{\text{peak 210 or peak 211 integrated intensity of the apatite}}{\text{peak 200 integrated intensity of the calcium oxide}}$$

where R_6 is a function of the percentage mass fraction of calcium oxide or of the Ca:P ratio of the mixture calculated according to the weighed-in quantities.

9 Chemical analysis

9.1 General

This clause specifies the methods for determining arsenic, mercury, cadmium and lead in calcium phosphate based coatings for surgical implants. There can be a need to analyse other elements (see Annex A).

The test consists of forming a solution in a water/acid/hydrogen peroxide mixture and the determination of desired elements using a spectrometric method.

Take a test sample (as described in Clause 7), which is compatible with the determination method used. If necessary, treat the test samples in a PTFE flask (or similar) (due to the fact that Hg is difficult to determine) with 30 ml of grade 2 water +1 ml of nitric acid and 2 ml of hydrogen peroxide; put this in an oven for at least 4 h at $110^{\circ}\text{C} \pm 10^{\circ}\text{C}$. Leave to cool and pour into a 50 ml graduated flask adding the water used for rinsing. Add grade 2 water to 50 ml, cork and homogenise. A blank test shall be conducted at the same time.

Perform the measurement on a least three test samples. Calculate the mean value and the standard deviation and record these in the test report.

9.2 Expression of results

Values of impurities which are less than the detection limit shall be expressed as follows:

As < 1 mg/kg

Cd < 1,5 mg/kg

Hg < 1,5 mg/kg

Pb < 10 mg/kg

Values of impurities which are greater than the detection limit shall be rounded to the nearest 0,1 mg/kg for As, Hg and Cd contents and to the nearest 1 mg/kg for Pb contents.

10 Ca:P ratio

10.1 General

After homogenizing and calcination at 1 000 °C, the calcium phosphates having a Ca:P atomic ratio between 1,50 and 2,0 inclusive comprise, at the most, two phases:

- α - and/or β -tricalcium phosphate and hydroxyapatite if the Ca:P ratio is below 1,667;
- hydroxyapatite and calcium oxide if the Ca:P ratio is above 1,667.

The method consists of comparing the intensity of an X-ray diffraction peak characteristic of hydroxyapatite and of a β -tricalcium phosphate peak for the samples having a Ca:P atomic ratio between 1,50 and 1,667 inclusive or of a calcium oxide peak for the samples having a Ca:P atomic ratio between 1,667 and 2,0 inclusive.

10.2 Procedure

Calcine the test sample in air at $1\,000\text{ °C} \pm 25\text{ °C}$ for at least 15 h in a platinum or alumina crucible. Withdraw the sample from the oven (still at $1\,000\text{ °C}$) and put it immediately into the desiccator. The sample should be free of oxyapatite. To verify this, use infrared spectroscopy according to the following procedure:

- prepare a 400 mg pellet of KBr containing 2 mg of the test sample;
- record the infrared spectrum; the absence of a band at 434 cm^{-1} indicates the absence of oxyapatite.

If oxyapatite is identified, either:

- heat treat the sample once again at $1\,000\text{ °C} \pm 25\text{ °C}$ for at least 15 h in a platinum or alumina crucible in a water vapour atmosphere of 10^5 Pa ,

or

- carry out a second heat treatment at $840\text{ °C} \pm 10\text{ °C}$ for 15 h in ambient air.

If necessary, recrush and carry out a granulometric analysis. 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 mixture for the calibration curves. Keep the test portion in the desiccator.

NOTE At temperatures of $1\,000\text{ °C}$ in air, partial dehydroxyation of hydroxyapatite (HA), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, to oxyhydroxyapatite (OHA), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-x}\text{O}_{0,5x}$, can occur.

10.3 Measurements on the sample

Produce the X-ray diffraction pattern on the basis of the test sample. If more than one foreign phase is observed or if the presence of a foreign phase other than β - and/or α -tricalcium phosphate or calcium oxide are detected (tetracalcium phosphate, calcium pyrophosphate, etc.), recrush the sample and recalcine it (see Clause 7).

Measure the intensities of the peaks selected, calculate the R_5 or R_6 ratio and refer to the calibration curves in order to determine the corresponding Ca:P ratio. When neither calcium oxide nor β - and/or α -tricalcium phosphate are detected, the apatite is considered to be stoichiometric. Perform the measurement on at least three test samples. Calculate the mean value and the standard deviation and record these in the test report.

10.4 Choice of the diffraction peaks

Ca:P < 1,667:

- peak 210 or peak 211 of the hydroxyapatite ($d = 3,08 \times 10^{-10}\text{ m}$);
- peak 0.2.10 of the tricalcium phosphate ($d = 2,880 \times 10^{-10}\text{ m}$).

Ca:P > 1,667:

- peak 200 of the calcium oxide ($d = 2,405 \times 10^{-10}\text{ m}$);
- peak 202 of the hydroxyapatite ($d = 2,631 \times 10^{-10}\text{ m}$).

10.5 Expression of results

The results shall be given by a dimensionless value representing the Ca:P atomic ratio expressed to the nearest two significant figures.

11 Qualitative and quantitative determination of the foreign phases

11.1 Procedure

Produce the X-ray diffraction pattern of the test sample. Measure the intensities of the peaks selected as described in Clause 6, calculate the R_1 to R_4 ratios and refer to the calibration curves in order to determine the corresponding content of the foreign phases. Perform the measurement on at least three test samples. Calculate the mean value and the standard deviation and record these in the test report.

11.2 Expression of results

The results shall be expressed as a percentage of foreign phases in relation to the apatitic phase.

NOTE 1 The apatitic phase, which constitutes a large number of plasma deposits, is essentially formed of oxyhydroxyapatite, the diffraction line intensities of which differ noticeably from those of stoichiometric hydroxyapatite.

NOTE 2 The apatitic phase defects (composition difference, lattices, stacking faults, etc.) lead to an X-ray diffraction pattern of which the line integrated intensities (line surfaces) are less than those of a 100 % crystalline apatite. The values found therefore cannot rigorously represent the true composition of the deposit. Furthermore, all the foreign phases are determined in relation to the hydroxyapatite. The sum of percentages found will not necessarily be 100 %.

12 Determination of the crystallinity ratio

12.1 General

The determination of the proportion of properly crystallized apatitic phase is based on the measurement of the integrated intensity of ten lines, suitably chosen from the sample's X-ray diffraction pattern, and of the same ten lines of a fully crystallized reference compound. The positions of the intensity lines are reported in Annex D.

NOTE 1 The calculated crystallinity ratio is an estimation of the crystallinity of the apatitic phase without taking into account the foreign phases.

NOTE 2 The apatitic phase, which constitutes a large number of plasma deposits, is essentially formed of oxyhydroxyapatite, the diffraction line intensities of which differ considerably from those of stoichiometric hydroxyapatite.

12.2 Preparation of the sample

The preparation of the sample is described in Clause 7. The sample shall not be heated or calcined.

12.3 Procedure

Produce the X-ray diffraction pattern of the test sample and of the standard as described in Clause 6. The integrated intensities of the apatitic phase ten lines, of the sample and of the standard, shall be determined to an accuracy greater than 5 %, either using a planimeter, or with the aid of appropriate software in the case of computerized installations. Perform the measurement on at least three test samples. Calculate the mean value and the standard deviation and record these in the test report.

12.4 Expression of results

The hydroxyapatite crystallinity ratio: (integrated intensity of ten lines of the sample) \times 100/(integrated intensity of ten lines of the standard) represents the crystallinity content. The results shall be expressed in percent.

13 Degradation of ceramics

The identification and quantification of degradation products from ceramics shall be carried out in accordance with ISO 10993-14.

14 Test report

The presentation of test reports shall meet the relevant requirements in ISO/IEC 17025, and shall include the visual inspection carried out on receipt (inclusion check).

Furthermore, the test report shall contain the following information:

- a) reference to this International Standard, i.e. ISO 13779-3:2008;
- b) location of the laboratory and date of the test;
- c) number and identification of specimens tested;
- d) nature of the apparatus used for chemical analysis;
- e) nature of the apparatus used for XRD (generator, goniometer, etc.), the recording conditions, (wavelength, filters, apertures monochromators, assembly types, etc.);
- f) X-ray diffraction patterns of the sample produced;
- g) diagram illustrating the sample and the position of the test portion;
- h) the average and the standard deviation for the following characteristics:
 - 1) the foreign phase, if any: the percentages of foreign phases in relation to the apatitic phase; when these phases are present in quantities lower than the detection limit of the instrumentation, the detection limits shall be indicated;
 - 2) the determined Ca:P ratio;
 - 3) the crystallinity ratio;
 - 4) the amount of trace elements.

Annex A
(informative)

Contamination of calcium phosphate

Due to manufacturing techniques currently in use, it is possible that undesirable chemicals, other than those cited in this part of ISO 13779, contaminate the calcium phosphate. Manufacturers are therefore advised to carry out objective analysis of the risks of contamination due to the various manufacturing processes used within their company or by sub-contractors and, if necessary, to qualify, quantify and set the limits of acceptability for each chemical liable to be a contaminant.

Particular attention should be paid to the limits of the following metals: copper, iron, tungsten (arising during use of the plasma torch) and other types of selective or random contamination.

Annex B (normative)

Testing of the purity of the phases used in the production of the calibration curves

B.1 Hydroxyapatite

The hydroxyapatite shall be considered as being stoichiometric with regard to the Ca:P ratio and purity if, after calcination at 1 000 °C for 15 h, it meets the following three conditions.

- a) The absence of β - and α -tricalcium phosphate is confirmed. For testing, confirm absence of the line 0.2.10 at $d = 2,88 \times 10^{-10}$ m for the β -form and 441,170 at $d = 2,905 \times 10^{-10}$ m for the α -form on an X-ray diffraction pattern having a resolution $\geq 0,02^\circ$ and a signal (line 211 of the apatite)/noise ratio ≥ 50 .
- b) The absence of calcium oxide is confirmed. For testing, prepare an aqueous solution of phenolphthalein by mixing 0,1 ml of 0,2 % phenolphthalein in ethanol in 50 ml of water. Calcine 100 mg of apatite powder at 1 000 °C for 15 h and introduce all of this still-hot powder into the solution. The absence of colouring indicates the absence of calcium oxide. This test is very sensitive.
- c) The absence of oxyapatite is confirmed. For testing, use infrared spectroscopy. Prepare a 400 mg pellet of KBr containing 2 mg of the hydroxyapatite test sample. Record the infrared spectrum; the absence of a band at 434 cm^{-1} indicates the absence of oxyapatite.

If oxyapatite is identified, either:

- heat treat the sample once again at $1\,000 \text{ °C} \pm 25 \text{ °C}$ for at least 15 h in a platinum or alumina crucible in a water vapour atmosphere of 10^5 Pa ,

or

- carry out a second heat treatment at $840 \text{ °C} \pm 10 \text{ °C}$ for 15 h in ambient air.

B.2 β -tricalcium phosphate

The β -tricalcium phosphate shall be considered pure if, after calcination at 1 000 °C for 15 h, it meets the following two conditions.

- a) The absence of hydroxyapatite is tested using X-ray diffraction. The absence of the line 211 of the apatite at $d = 2,81 \times 10^{-10}$ m on an X-ray diffraction pattern having a resolution $\geq 0,02^\circ$ and a signal (line 0.2.10 of the tricalcium phosphate)/noise ratio ≥ 30 indicates the absence of hydroxyapatite.
- b) The absence of calcium pyrophosphate is confirmed, tested using infrared spectrometry. For this purpose prepare a pellet of KBr having a ratio of 2 mg of tricalcium phosphate to 300 mg of KBr. Record the infrared spectrum; the absence of bands at 757 cm^{-1} and 434 cm^{-1} indicates the absence of the α -form of calcium pyrophosphate and the absence of bands at $1\,210 \text{ cm}^{-1}$, $1\,185 \text{ cm}^{-1}$, 723 cm^{-1} and 454 cm^{-1} indicates the absence of the β -form of calcium pyrophosphate.

B.3 Calcium oxide

Calcium oxide shall be considered pure if it is prepared by calcining calcium carbonate, containing less than 1 % of impurities by mass, at 1 000 °C for 15 h and has been stored in a desiccator immediately after calcining.

Annex C (informative)

Examples of X-ray diffraction patterns collected from various mixtures used to plot the calibration curves

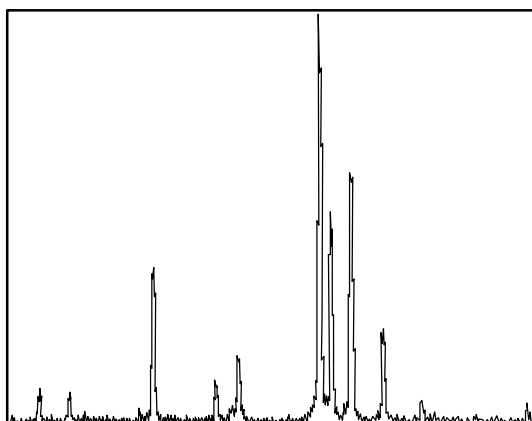


Figure C.1 — Pure HA

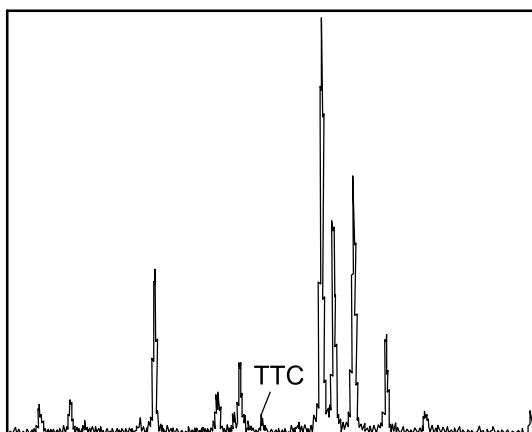


Figure C.2 — Mixture of hydroxyapatite and 5 % TTC

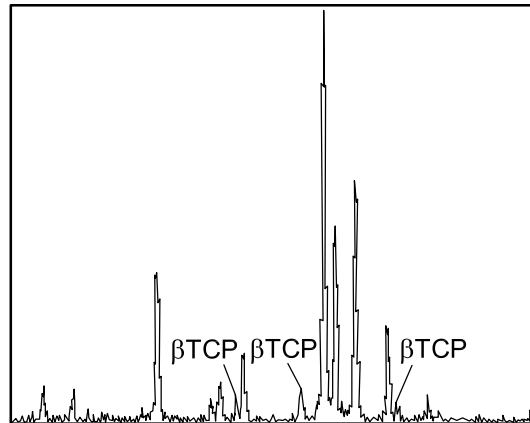


Figure C.3 — Mixture of hydroxyapatite and 5 % TCP

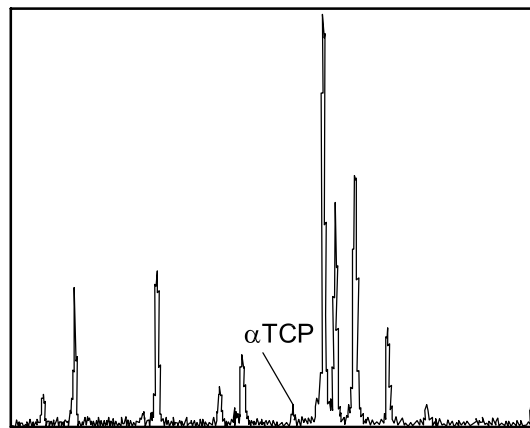


Figure C.4 — Mixture of hydroxyapatite and α -tricalcium phosphate (α -TCP) at 5 % of mass

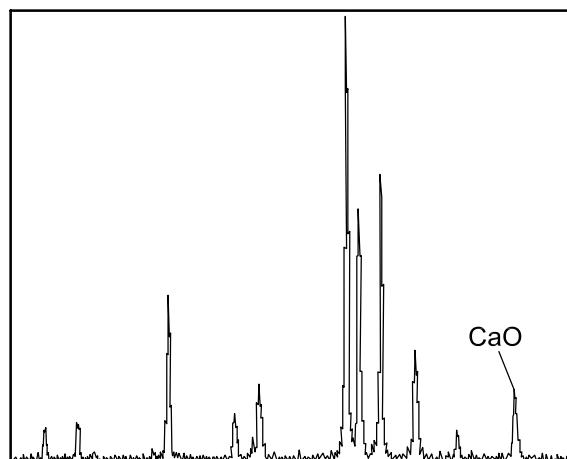


Figure C.5 — Mixture of hydroxyapatite and 5 % CaO

Annex D (normative)

Positions of lines used to measure the crystallinity ratio

The apatite selected to produce the standard mixtures shall only contain calcium, phosphate ions and hydroxide. The mixture shall not contain more than 100 µg/g of any other impurities. The hydroxyapatite shall be considered as being stoichiometric with regard to the Ca:P ratio if, after calcination at 1 000 °C for 15 h, it meets the following two conditions.

- a) The absence of α - and β -tricalcium phosphate is tested. For testing, confirm absence of the line 441,170 at $d = 2,905 \times 10^{-10}$ m for the α -form and 0.2.10 at $d = 2,88 \times 10^{-10}$ m for the β -form on an X-ray diffraction pattern having a resolution $\geq 0,02^\circ$ and a signal (line 211 of the apatite)/noise ratio ≥ 50 .
- b) The absence of calcium oxide is tested. For testing, prepare an aqueous solution of phenolphthalein by mixing 0,1 cm³ of 0,2 % phenolphthalein in ethanol in 50 cm³ of water. Calcine 100 mg of apatite powder at 1 000 °C for 15 h and introduce all of this still-hot powder into the solution. The absence of colouring indicates the absence of calcium oxide. This test is very sensitive.

Table D.1 — Position of the 10 lines used to measure the crystallinity ratio

Lines
$d_1 = 3,44 \times 10^{-10}$ m
$d_2 = 3,17 \times 10^{-10}$ m
$d_3 = 3,08 \times 10^{-10}$ m
$d_4 = 2,81 \times 10^{-10}$ m
$d_5 = 2,78 \times 10^{-10}$ m
$d_6 = 2,72 \times 10^{-10}$ m
$d_7 = 2,63 \times 10^{-10}$ m
$d_8 = 2,26 \times 10^{-10}$ m
$d_9 = 1,94 \times 10^{-10}$ m
$d_{10} = 1,84 \times 10^{-10}$ m

Annex E (informative)

Examples of methods for the preparation of reference materials

E.1 Example of a method of preparation for the β -TCP

Pure β -TCP can be obtained from a commercial powder of β -TCP. It shall meet the following two conditions as received or after calcination at 1 000 °C for 15 h.

- a) The absence of hydroxyapatite is tested, using X-ray diffraction. The absence of the line 211 of the apatite at $d = 2,81 \times 10^{-10}$ m on an X-ray diffraction pattern having a resolution $\geq 0,02$ degrees and a signal (line 0.2.10 of the tricalcium phosphate)/noise ratio ≥ 30 confirms the absence of hydroxyapatite.
- b) The absence of calcium pyrophosphate is tested, using infrared spectrometry. For this purpose prepare a pellet of KBr having a ratio of 2 mg of tricalcium phosphate to 300 mg of KBr. Record the infrared spectrum; the absence of bands at 757 cm^{-1} and 434 cm^{-1} indicates the absence of the α -form of calcium pyrophosphate and the absence of bands at $1\,210 \text{ cm}^{-1}$, $1\,185 \text{ cm}^{-1}$, 723 cm^{-1} and 454 cm^{-1} indicates the absence of the β -form of calcium pyrophosphate.

E.2 Example of a method of preparation for the tetra-calcium phosphate (TTCP)

E.2.1 Reagents

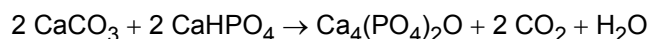
- E.2.1.1 **Dicalcium phosphate**, (dihydrated: DCPD or anhydrous: DCPA).
- E.2.1.2 **Calcium carbonate**.
- E.2.1.3 **Liquid nitrogen**.

E.2.2 Apparatus

- E.2.2.1 **Wide platinum crucible**.
- E.2.2.2 **Blender**.
- E.2.2.3 **Oven**.

E.2.3 Reaction

The solid state reaction is:



E.2.4 Procedure

Thoroughly mix equal quantities of calcium carbonate and DCPD (or DCPA) powders in the blender (E.2.2.2). Heat the mixture to 1 400 °C and maintain it at that temperature for 6 h, preferably under nitrogen atmosphere. Take the crucible (E.2.2.1) out of the oven and quench it in liquid nitrogen (E.2.1.3).

Check the sample by X-ray diffraction for the presence of foreign phases.

E.3 Example of a method of preparation for the α -tricalcium phosphate (α -TCP)

E.3.1 Reagents

E.3.1.1 β -tricalcium phosphate, (β -TCP) free of Mg^{2+} , Fe^{2+} and any other bivalent ions impurities susceptible to stabilize the β -TCP.

E.3.1.2 Liquid nitrogen.

E.3.2 Apparatus

E.3.2.1 Wide platinum crucible.

E.3.2.2 Oven.

E.3.3 Reaction

The reaction is a phase transition occurring at 1 125°C.

E.3.4 Procedure

Heat β -tricalcium phosphate in the platinum crucible (E.3.2.1) in the oven (E.3.2.2) at 1 350 °C and maintain it at this temperature for 1 h. Take the crucible out of the oven and quench it with liquid nitrogen (E.3.1.2).

Check the sample by X-ray diffraction for the presence of foreign phases.

E.4 Standard reference materials

Standard reference materials exist and can be used (to plot the calibration curve and as a reference material for the crystallinity ratio) if they meet the requirements described in Annexes B and D.

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- [3] ASTM F 1185-03, *Standard Specification for Composition of Hydroxyapatite for Surgical Implants*
- [4] NF S 94-065, *Materials for surgical implants — Determination of arsenic, mercury, cadmium and lead on coatings based on phosphate of calcium*
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- [6] *Octacalcium Phosphate*, Eds CHOW, L.C. and EANES, E.D. *Monographs in Oral Science*, **18**, Karger Press, p. 152, 2001
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