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# Implants for surgery — Hydroxyapatite —

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

*Implants chirurgicaux — Hydroxyapatite —*

*Partie 3: Analyse chimique et caractérisation du rapport de cristallinité et de la puréte de phase*

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# Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 150, *Implants for surgery*, Subcommittee SC 1, *Materials*.

This second edition cancels and replaces the first edition (ISO 13779-3:2008), which has been technically revised.

A list of all parts in the ISO 13779 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

# 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 hydroxyapatite has shown that an applicable level of biological response can be expected, if the material is used in appropriate applications.

Biocompatibility and resorption rate of hydroxyapatite material for surgical application may depend of the presence of trace elements, foreign crystalline phases and crystallinity ratio. Amorphous calcium phosphate, tetracalcium phosphate, $\alpha$-tricalcium phosphate and $\beta$-tricalcium phosphate have demonstrated to have a higher solubility and may resorb more rapidly than hydroxyapatite in the body. CaO and heavy metals may impair the biocompatibility of the material. As a consequence, it is important to assess the composition of the material.

In this field, the assessment of the different crystalline and amorphous phase components has been under continuing development (of both equipment and processing software). In this document a new method for measuring the crystallinity ratio of hydroxyapatite is introduced and the Rietveld method is introduced as an alternative method for measuring the foreign phase content.

# Implants for surgery — Hydroxyapatite —

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

## 1 Scope

This document specifies methods of test for the chemical analysis, assessment of crystallinity ratio and phase composition of hydroxyapatite-based materials such as powders, coating or bulk products.

NOTE These tests are intended to describe properties of the material and to communicate these between organizations. These tests are not written with the objective of replacing a company's internal operational and assessment tests although they could be used as such.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 3310-1, *Test sieves — Technical requirements and testing — Part 1: Test sieves of metal wire cloth*

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

## 3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at http://www.electropedia.org/

**3.1**
**calibration curve**
calculating plot translating the ratio of integrated intensity of foreign phases, measured on the X-ray diffraction pattern into the mass fraction of foreign phases compared to crystalline hydroxyapatite

**3.2**
**detection limit**
**DL**
lowest quantity of the foreign phase or trace element that can be distinguished from the absence of that foreign phase or trace element

Note 1 to entry: Requirements and procedure for estimating the detection limit of foreign phases is established in 5.6.3.

### 3.3
**quantification limit**
**QL**
lowest quantity of the foreign phase or trace element that can be quantified

Note 1 to entry: Requirements and procedure for estimating the quantification limit of foreign phases is established in 5.6.3.

### 3.4
**height**
distance between the peak summit and the base line of the X-ray diffraction pattern from which the background has been subtracted

### 3.5
**integrated intensity**
area between the plot of the peak and the base line of the X-ray diffraction pattern from which the background has been subtracted

### 3.6
**scraping**
removal of the coating from the base material minimising contamination from of the base material itself

### 3.7
**signal:noise ratio**
height of a peak of the x-ray diffraction pattern divided by the maximum deviation of the base line oscillation

Note 1 to entry: The height of each peak of the X-ray diffraction pattern divided by the maximum deviation of the base line oscillation near to the peak location, in an area not likely to present a crystalline phase peak.

### 3.8
***α*-tricalcium phosphate**
***α*-TCP**
chemical compound with a crystallographic structure characterized by IC DD PDF 09-0348

Note 1 to entry: The chemical formula is $Ca_3(PO_4)_2$.

### 3.9
***β*-tricalcium phosphate**
***β*-TCP**
chemical compound with a crystallographic structure characterized by IC DD PDF 09-0169

Note 1 to entry: The chemical formula is $Ca_3(PO_4)_2$.

### 3.10
**hydroxyapatite**
**HA**
chemical compound with a crystallographic structure characterized by IC DD PDF 09-0432 or IC DD PDF 72-1243

Note 1 to entry: The chemical formula is $Ca_5(OH)(PO_4)_3$.

### 3.11
**tetracalcium phosphate**
**TTCP**
chemical compound with a crystallographic structure characterized by IC DD PDF 25-1137 or IC DD PDF 70-1379

Note 1 to entry: The chemical formula is $Ca_4(PO_4)_2O$.

**3.12**

**calcium oxide**

**CaO**

chemical compound with a crystallographic structure characterized by ICDD PDF 4-0777 or IC DD PDF 82-1690

**3.13**

**uncertainty**

95 % confidence interval of the measurement, taking into account reproducibility of the measurement

**3.14**

**crystallinity ratio**

ratio between the sum of integrated intensities of a selection of peaks of HA in the sample and the sum of integrated intensities of the same peaks of HA after calcination of the sample at 1 000 °C for 15 h

Note 1 to entry: Sometimes crystallinity is defined as the ratio between the mass fraction of crystalline HA and the total mass fraction of HA (crystalline and amorphous). However, in this standard there is no method given to measure the total mass fraction of HA. For this reason, the term crystallinity ratio, as defined above, instead of crystallinity is used.

**3.15**

**background**

signal produced by the non-diffracted X-ray beam

Note 1 to entry: This is illustrated in Figure 1.

**3.16**

**noise**

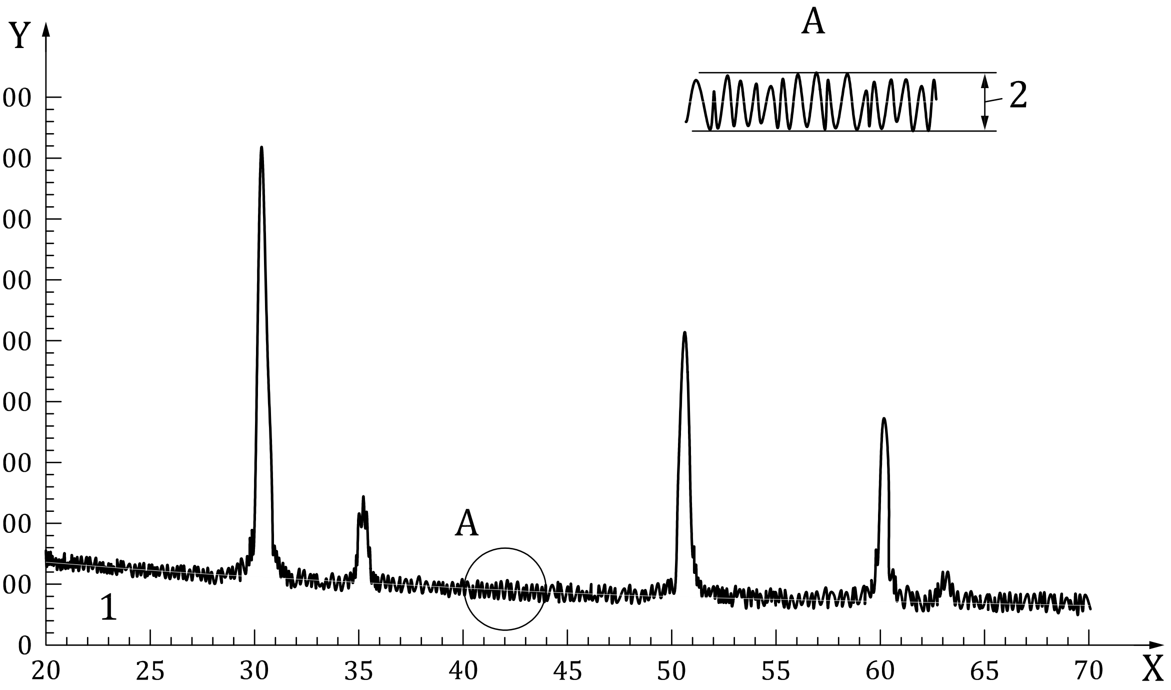
variation of the signal intensity produced by the X-ray apparatus

Note 1 to entry: This is illustrated in Figure 1.

**3.17**

**foreign crystalline phases**

crystalline phases other than hydroxyapatite, being detectable by X-ray diffraction analysis and consisting of tetracalcium phosphate, $\alpha$-tricalcium phosphate, $\beta$-tricalcium phosphate, and calcium oxide (CaO)



**Key**

1 background

2 noise

X = 2 theta

Y = counts

Figure 1 — Illustration of background and noise

## 4 Chemical analysis

### 4.1 General

This clause specifies the methods for determining arsenic, cadmium, lead, mercury, and heavy metals content in hydroxyapatite-based materials for surgical implants.

Heavy metals content is considered as the sum of lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper and molybdenum.

NOTE This list of elements is contained in ISO 13779-6. Comparison of trace elements in an HA coating with the same trace elements in an HA powder indicates any detriment to the HA by coating process.

This method can also be used to measure calcium and phosphorous to calculate the Ca:P ratio as an alternative to the X-ray diffraction method detailed in 5.8. The equivalence of such results obtained from the chemical analysis with the results from the X-ray method should be demonstrated.

There can be a need to analyse other elements (see Annex A).

### 4.2 Analytical methods

The following methods can be used after validation and determination of detection limit, quantification limit and measurement tolerances. Each analytical method has its own DL and QL relevant to that method. An appropriate quantitative analysis apparatus, having a quantification limit which is not less than the required limit value in the analysed sample, shall be used.

The list is not restrictive:

a) atomic absorption spectroscopy, hydride method;

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) inductively coupled plasma / mass spectroscopy (ICP-MS);

e) inductively coupled plasma / atomic emission spectroscopy (ICP-AES); or

f) atomic absorption spectroscopy (AAS).

Calibration can be external or based on the standard addition method. If external, the calibration solution shall contain HA with known content in elemental impurities to take into account the effect of the HA matrix. The HA concentration used for calibration shall be the same as the HA concentration in the tested solution.

## 4.3 Apparatus for chemical analysis

Vessels used shall be either of the following:

### 4.3.1 Class A glassware, or

### 4.3.2 PTFE flask (or similar).

The vessels used for dissolution of the sample shall not contaminate the solution.

NOTE For analysis of Mercury, PTFE vessel is suitable to avoid potential contamination of the solution.

Before use they shall be carefully washed with acid and then rinsed with grade 2 water (4.4.1).

## 4.4 Reagents for chemical analysis

All reagents shall be of analytical quality:

### 4.4.1 Grade 2 water, according to ISO 3696.

### 4.4.2 Analytical grade nitric or hydrochloric acid.

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

## 4.5 Procedure

Bulk samples shall be crushed. In the case of coatings, the HA needs to be removed by scraping. Scraping of coatings and crushing of bulk samples shall be performed to minimize contamination of the sample.

The HA sample shall be dissolved in acid. The mass of the HA sample and acid type and concentration might need to be adjusted depending on the analytical method used as well as the quantity of the element to analyse, present in the HA matrix. The mass of the HA sample as well as the type, volume and concentration of the acid used shall be recorded.

Place ground sample into PTFE or glass flask (4.3), carefully add the acid solution consisting of grade 2 water (4.4.1) and of nitric or hydrochloric acid [4.4.2].

NOTE As a starting point using (1,000 ± 0,001) g of ground sample, (30 ± 0,05) ml of grade 2 water and (1 ± 0,05) ml of 52,5 w% nitric or hydrochloric acid can be appropriate.

Dilute (e.g. to 50 ml volume) using grade 2 water (4.4.1), seal and shake thoroughly. Ensure that the sample is totally dissolved. A blank test shall be conducted at the same time.

The solution shall be analysis by a suitable analytical method (4.2).

### 4.6 Expression of results

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

X < DL,

where X is the analysed chemical element and DL the detection limit expressed in mg/kg.

Values of impurities which are between the detection limit and the quantitation limit shall be expressed as follows:

DL < X < QL, where X is the analysed chemical element, DL the detection limit expressed in mg/kg, and QL is the quantitation limit expressed in mg/kg.

For heavy metals content determination, the content of chemical elements below the detection limit shall be considered as equal to DL. If the value is between DL and QL the QL shall be used.

Values of heavy metal content and impurities which are greater than the detection limit shall be rounded to the nearest 0,1 mg/kg. The values for each heavy metal impurity shall be reported.

## 5 X-ray diffraction analysis

### 5.1 General

The X-ray diffraction (XRD) method described in Clause 5 is based on the comparison of the integrated intensities of the XRD pattern of a sample with reference materials.

An alternative method is the Rietveld method (see Annex G) for the calculation of the foreign phases content. The Rietveld method may be used instead of the method described in Clause 5 once correlated with the integrated intensity method. The QL, DL and accuracy of the Rietveld method shall be calculated and the difference of the results of the two methods quantified.

### 5.2 Apparatus

The apparatus shall consist of the following:

**5.2.1 Mortar and pestle**, in alumina, agate or other suitable material (minimising contamination of the sample).

**5.2.2 38 or 40 micrometre sieve**, complying with ISO 3310-1.

**5.2.3 Oven**, capable of maintaining a temperature of (1 000 ± 25) °C.

**5.2.4 Desiccator.**

**5.2.5 Intensity reference material**, to determine instrument intensity, suitable materials include bulk gold or alumina reference (not in the form of powder, for example NIST SRM 1976).

**5.2.6 X-ray diffraction apparatus** having a resolution and reproducibility of at least 0,02° on a 2 $\theta$ angle scale and allowing the recording of the diffraction peak positions and intensities. A stabilized power supply is necessary. Software of the diffraction apparatus shall allow adjusting the peaks position, subtracting the background and measuring the integrated intensities of the peaks over a determined angular range.

## 5.3 Preparation of test samples

### 5.3.1 General

The test samples shall be crushed and sieved so that the particle size does not exceed 40 µm. Care should be taken not to crush the samples too much as the particle size does have an influence on the width of the diffraction peaks. It is necessary to minimize any contamination or transformation in contact with humidity. Keep all the test samples in the desiccator.

### 5.3.2 Coatings

In the case of coatings, the HA needs to be removed by scraping. Scraping shall be performed to minimise contamination of the sample.

For thermally sprayed coatings, it is common that the layers near to the coating/substrate interface contain more amorphous material 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.

### 5.3.3 Bulk sample

Bulk samples shall be reduced to powder form according to 5.3.1.

## 5.4 Calibration specimens

The calibration specimens listed below shall be used:

a) Pure $\beta$-tricalcium phosphate having an X-ray diffraction pattern as described in ICDD PDF 09-0169.

b) Pure $\alpha$-tricalcium phosphate having an X-ray diffraction pattern as described in ICDD PDF 9-348.

c) Pure hydroxyapatite having an X-ray diffraction pattern as described in ICDD PDF 9-432 or ICDD PDF 72-1243.

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

e) Pure calcium oxide having an X-ray diffraction pattern as described in ICDD PDF 4-0777 or ICDD PDF 82-1690.

Calibration specimens shall be prepared by the methods described in Annex E. They shall comply with the requirements described in Annex B.

## 5.5 X-ray diffraction pattern collection

### 5.5.1 General

The conditions of the X-ray diffraction pattern collection will allow the contribution of the apparatus to the full width half maximum, detection limit and tolerances to be minimized; these conditions shall be identical for the test sample and for the mixtures used to prepare the calibration curves. The signal: noise ratio shall be greater than 20 for peak 211 of HA. If an amorphous hump is visible in the pattern, it should be subtracted with the background before measuring the noise.