Designation: F 2024 – 00

Standard Practice for X-ray Diffraction Determination of Phase Content of Plasma-Sprayed Hydroxyapatite Coatings[1]

This standard is issued under the fixed designation F 2024; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice is for the determination, by the Reference Intensity Ratio External Standard Method, of the percent by weight of the crystalline phases, hydroxyapatite (HA), beta-(whitlockite) tricalcium phosphate (β-TCP), and calcium oxide (CaO) in coatings deposited upon metallic substrates by plasma-spraying hydroxyapatite.

1.2 A major component in plasma-sprayed HA coatings other than HA is expected to be amorphous calcium phosphate (ACP). Crystalline components other than HA that may be present include alpha- and beta- (whitlockite) tricalcium phosphates, tetracalcium phosphate (TTCP), calcium oxide, and calcium pyrophosphates. Quantification of the minor crystalline components has proven to be very unreliable due to extreme overlap and confounding of X-ray diffraction peaks. Therefore, this practice addresses the quantification of only HA, β-TCP, and CaO.

1.3 This practice was developed for plasma-sprayed HA coatings with HA contents of at least 50 % of the total coating. It is recognized that the analysis of the crystalline components uses diffraction from regions of the pattern that also includes a small contribution from the amorphous component. However, within the limits of applicability of this practice, the effect of such interference is believed to be negligible.

1.4 The coating analyzed shall be produced and processed under equivalent manufacturing conditions to that on the device of interest.

1.5 This practice requires the use of monochromated copper Kα radiation and flat samples.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

F 1185 Specification for Composition of Ceramic Hydroxylapatite for Surgical Implants[2]

F 1609 Specification for Calcium Phosphate Coatings for Implantable Materials[2]

3. Terminology

3.1 *Definitions*:

3.1.1 *crystalline phases*:

Chemical and Mineral Names	Formula	PDF Card No.[3]
whitlockite beta-tricalcium phosphate	β-$Ca_3(PO_4)_2$	9-169
calcium phosphate alpha-tricalcium phosphate	α-$Ca_3(PO_4)_2$	9-348
lime calcium oxide	CaO	37-1497
hydroxyapatite (hydroxylapatite)	$Ca_5(PO_4)_3OH$	9-432

3.2 *plasma-sprayed hydroxyapatite coating*—a coating, consisting of at least 50 % hydroxyapatite by weight, prepared by plasma-spraying hydroxyapatite on a substrate.

4. Significance and Use

4.1 Calcium phosphate coatings have been shown in animal and clinical studies to be biocompatible and to enhance the early attachment of bone to implant surfaces (see Refs. **1-5)**[4]

4.2 It is believed that the form of calcium phosphate ceramic and its purity with respect to secondary crystalline phases and amorphous material have an effect on its physical, mechanical, and biological properties. However, no definitive studies of effects on biological properties have been completed. To achieve reproducible clinical results and to permit the determination of the effects of properties of the coating on biological performance, it is essential that the properties of both clinical and experimental materials be well-characterized and consistent.

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[1] This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.13 on Ceramic Materials.

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[2] *Annual Book of ASTM Standards*, Vol 13.01.

[3] Joint Committee on Powder Diffraction Standards, Swarthmore, PA.

[4] The boldface numbers in parentheses refer to the list of references at the end of this standard.

4.3 This practice provides procedures for determination of the percentage by weight of the crystalline phases identified as hydroxyapatite, β-TCP and CaO in plasma-sprayed hydroxyapatite coatings.

5. Quantitative Phase Analysis by the External Standard Technique

5.1 The external standard technique allows the determination of weight fractions of individual phases in a mixture containing an amorphous fraction by comparison of the integrated intensity of one or more peaks from the phase(s) of interest to the external standard under identical instrumental conditions **(6)**. The sample analyzed may be a solid such as a plasma-sprayed coating or may be a powder. The mass absorption coefficients of the sample and standard must be known.

5.2 The weight fraction of the analyte phase in the mixture is given by Equation 11 of Ref **(6)**, as follows:

$$W_i = \left(\frac{I_i^{hkl}}{I_i^{REL}}\right) \cdot \left(\frac{\chi_m}{\chi_s}\right) \cdot \left(\frac{1}{I_s^{Pure} \cdot RIR_i}\right) \quad (1)$$

where:

I_i^{hkl} = integrated intensity of the analyte phase (hkl) peak or sum of peaks,
I_i^{REL} = relative intensity of the analyte phase (hkl) peak or sum of peaks,
χ_m = mass absorption coefficient of the mixture,
χ_s = mass absorption coefficient of the standard,
I_s^{Pure} = integrated intensity of the most intense peak of the pure standard measured under identical conditions, and
RIR_i = reference intensity ratio of the analyte phase to the standard.

Values of the relative intensities, mass absorption coefficients, and reference intensity ratios which have been measured for HA, β-TCP, and CaO are given in Appendix X1.

6. Procedure

6.1 *Sample Preparation*:

6.1.1 Plasma sprayed coating samples in the form of flat coupons of nominal dimensions 2.5 by 2.5 by 0.6 cm (1 by 1 by 0.125 in.) may be analyzed directly on the coated surface. The coating must be at least 44 µm thick to provide a sample opaque to the X-ray beam. Thinner samples must be removed from the substrate and either deposited in a layer of at least 44-µm thickness and area sufficient to exceed the dimensions of the irradiated area.

6.1.2 Reliable quantitative analysis cannot be performed by X-ray diffraction on curved surfaces because of errors caused by absorption and defocusing.

6.1.3 Microabsorption caused by variations in either particle size or surface roughness will produce errors in the measured diffracted intensity. The effective particle size and variation in surface roughness of the alpha-corundum external standard must be less than 5 µm.

6.2 *X-ray Equipment*:

6.2.1 A standard Bragg-Brentano focusing diffractometer equipped with a pyrolytic graphite monochromator is recommended. Because of the need to resolve closely spaced and overlapping peaks, a diffracted beam monochromator is required unless a solid-state detector is used. Linearity of the instrument and associated electronics must be verified daily prior to utilizing this method. Use of NIST silicon powder standard, SRM 640 is suggested.[5]

6.2.2 An X-ray source with a copper target is required. Characteristic copper radiation provides the needed X-ray diffraction peak resolution and allows for separation of peaks from contaminant phases at a suitable range of diffraction angles from nominally 20 to 60° 2θ. A 1.0° incident beam divergence, a 0.2° receiving slit, and soller slits in either incident or diffracted beam, or both, are suitable.

6.3 *X-ray Method and Data Reduction Strategy*:

6.3.1 Collect a diffraction pattern from 20 to 60 ° 2θ at 0.02° increments for a minimum of 1s/point.

6.3.2 X-ray diffraction peaks (or peak groups) from the crystalline phases must be separated in order to quantify the HA content. The following outline provides a data reduction strategy in order to provide the integrated intensities necessary to determine the HA, β-TCP, and CaO content of mixtures of amorphous calcium phosphate, α-TCP, β-TCP, CaO, β-$Ca_2P_2O_7$, tetracalcium phosphate, and hydroxyapatite. Accomplish the determination of integrated intensities using computer techniques, with least-squares fitting of the selected peak shape to the experimental data. Manual fitting of peak and background is not permitted under this standard practice.

6.3.2.1 Obtain the β-TCP content by integration from 30.5 to 31.5° 2θ. The β-TCP peak being used for quantification is the (0 2 10) peak. This region is integrated by assuming a linear background and a Pearson VII functional form of the peaks surrounding the region.

6.3.2.2 Determine the calcium oxide content by integration from 37.0 to 38.5° 2θ and correct for the β-TCP (1 2 11) and (315) peaks. This region contains the 100 % (200) calcium oxide peak, and is integrated by assuming a linear form to the background.

6.3.2.3 Finally, determine by integration the region from 38.5 to 59.0° 2θ HA and correct for interference by β-TCP and calcium oxide. A large angular range is used in order to use as many peaks as possible and to reduce the effects of preferred orientation. Again, this region is integrated assuming a linear form of the background.

6.3.3 Perform the analysis as an external standard technique with reference to an alpha-corundum standard, using the relative intensities, mass absorption coefficients, and reference intensity ratios shown in Appendix X1. Reference intensity ratios determined experimentally using the equipment and conditions used for analysis of unknown samples may be substituted for those shown, provided that their validity under the experimental conditions used for analysis has been verified using known standards. An example calculation is shown as Appendix X2.

6.3.4 Verify the validity of the analytical procedures applied using known mixtures of powders ranging from nominally 50 to 95 % hydroxyapatite. Conduct periodic revalidation (at least

[5] Available from National Institute of Standards and Technology (NIST), 100 Bureau Dr., Stop 3460, Gaithersburg, MD 20899-3460.

annually) of instrument conditions and analytical technique using retained plasma-sprayed hydroxyapatite samples.

7. Report

7.1 Report following information:

7.1.1 Sample identification,

7.1.2 Condition of analyzed sample, as-sprayed coating on coupon or powder spalled from sample,

7.1.3 Analytical results expressed as percent hydroxyapatite, percent β-TCP, and percent CaO relative to the entire sample,

7.1.4 Balance expressed as balance-amorphous calcium phosphate (ACP) and other minor phases, and

7.1.5 Statistical variability of the results based on the variability in the RIR values (shown in Table X1.1) and instrumental conditions.

7.2 Further reporting by the device manufacturer shall include any treatment applied to the coating after plasma-spraying.

8. Precision and Bias

8.1 The precision and bias of this practice are currently being determined in an interlaboratory test program. Individual experience indicates that reproducibility is on the order of ±3 % for determination of the HA content of plasma-sprayed coatings.

9. Keywords

9.1 amorphous calcium phosphate (ACP); hydroxyapatite coatings; hydroxyapatite (HAP); hydroxylapatite coatings; phase analysis; tricalcium phosphate; whitlockite

APPENDIXES

(Nonmandatory Information)

X1. REFERENCE VALUES FOR ANALYSIS

TABLE X1.1 Experimentally Determined Reference Intensity Ratios (RIR) Relative to Alpha-Corundum, αAl_2O_3

Phase	RIR
Hydroxyapatite (commercial powder)	1.276 ± 0.001
β-TCP	1.146 ± 0.004
CaO	3.375 ± 0.004

TABLE X1.2 Combined Relative Intensities for the Integration Regions Indicated (6)

Phase	Range 2θ,°	I^{Rel}
Hydroxyapatite	38.5 - 59.0	2.16 ± 0.02
β-TCP	30.5 - 31.6	1.00 ± 0.00
	37.0 - 38.5	0.145 ± 0.001
	38.5 - 59.0	2.35 ± 0.01
CaO	37.0 - 38.5	1.00 ± 0.00
	38.5 - 59.0	1.32 ± 0.007

TABLE X1.3 Mass Absorption Coefficients

Phases(s)	Mass Absorption Coefficient
Ca-P from hydroxyapatite (all phases)	χ_m= 87.23
Alpha-corundum, αAl_2O_3	χ_s= 31.78

X2. EXAMPLE CALCULATION

X2.1

Phase	Net Intensity (after correction), counts · s/°
β-TCP	23.9
CaO	12.4
HA	603.2
Standard	989.0

X2.2 The weight percentages are determined from Equation 11 of Ref **(6)** with the RIR, I_{rel}, and mass absorption coefficients from Appendix X1.

$$W_{\beta-TCP} = \left(\frac{23.9}{1}\right)\cdot\left(\frac{87.23}{31.78}\right)\cdot\left(\frac{1}{989.0\cdot 1.146}\right) = 0.058 = 5.8\ \% \quad \text{(X2.1)}$$

$$W_{CaO} = \left(\frac{12.4}{1}\right)\cdot\left(\frac{87.23}{31.78}\right)\cdot\left(\frac{1}{989.0\cdot 3.375}\right) = 0.010 = 1.0\ \% \quad \text{(X2.2)}$$

$$W_{HA} = \left(\frac{603.2}{2.16}\right)\cdot\left(\frac{87.23}{31.78}\right)\cdot\left(\frac{1}{989.0\cdot 1.276}\right) = 0.607 = 60.7\ \% \quad \text{(X2.3)}$$

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(**5**) Thomas, K.A., "Hydroxyapatite Coatings," *Orthopedics*, Vol 17, 1994, p. 267.

(**6**) Prevey, P.S., and Rothwell, R.J., "X-ray Diffraction Characterization of Percent Crystallinity and Contaminants in Plasma-Sprayed Hydroxylapatite Coatings," *Characterization and Performance of Calcium Phosphate Coatings for Implants*, *ASTM STP 1196*, Emanuel Horowitz and Jack E. Parr, eds., American Society for Testing and Materials, Philadelphia, PA, 1994, p. 63.