INTERNATIONAL STANDARD



First edition 2021-04

Surface chemical analysis — Sample handling, preparation and mounting —

Part 3: **Biomaterials**

iTeh STAnalyse chimique des surfaces — Manipulation, préparation et montage des échantillons — (stancards iten.ai) Partie 3: Biomatériaux

<u>ISO 20579-3:2021</u> https://standards.iteh.ai/catalog/standards/sist/47cb4d8f-051a-4d41-b788-68f7d558e462/iso-20579-3-2021



Reference number ISO 20579-3:2021(E)

iTeh STANDARD PREVIEW (standards.iteh.ai)

<u>ISO 20579-3:2021</u> https://standards.iteh.ai/catalog/standards/sist/47cb4d8f-051a-4d41-b788-68f7d558e462/iso-20579-3-2021



COPYRIGHT PROTECTED DOCUMENT

© ISO 2021

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office CP 401 • Ch. de Blandonnet 8 CH-1214 Vernier, Geneva Phone: +41 22 749 01 11 Email: copyright@iso.org Website: www.iso.org

Published in Switzerland

Page

Contents

Fore	eword	iv	
Intro	oduction	v	
1	Scope		
2	Normative references		
3	Terms and definitions		
4	Abbreviated terms	2	
5	Explanation of the structure of this document	2	
6	General requirements and classes of specimens	3	
	6.1 General information	3	
	6.2 Handling	3	
	6.4 Toxins and other hazardous materials		
7	Specimon considerations		
/	7.1 History of the specimen	4 4	
	7.2 Information sought	4	
	7.3 Categories of specimen	4	
8	Sources of specimen contamination		
	8.1 Sample preparation ANDARD PREVE	5	
	8.2 10015 8.3 Sample handling (standards itch ai)	ວ 5	
	8.3.1 General		
	8.3.2 Exposure to gases $150 \cdot 20579 = 3 \cdot 2021$	5	
	8.3.3 https://www.animatice.com/animation.of.the.analysis-area.4d41-b788-	6	
	8.4 Separation between neighbouring areas -3=2021		
9	Specimen storage and transfer of biomaterials	6	
	9.1 Storage time		
	9.1.2 Descriptive list of containers for biomaterials	6	
	9.2 Temperature and humidity	6	
10	Education of specimen owner on appropriate specimen handling procedures	7	
11	Specimen mounting procedures of biomaterials	7	
12	Methods for reducing specimen charging	7	
13	Specimen preparation techniques of biomaterials	7	
	13.1 Mechanical separation	7	
	13.2 Sectioning techniques		
	13.5 Solvents for biomaterials		
	13.5 Ion sputtering	7	
14	Fracturing, cleaving and scribing	7	
15	Specimen-handling techniques	8	
Bibli	iography	9	

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.

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 201, *Surface chemical analysis*, Subcommittee SC 2, *General procedures*. https://standards.iteh.ai/catalog/standards/sist/47cb4d8f-051a-4d41-b788-

A list of all parts in the ISO 20579 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 <u>www.iso.org/members.html</u>.

Introduction

0.1 Common introduction

The ISO 20579 series is intended to assist analysts and those seeking surface chemical analysis in the handling, storage, mounting and treatment of specimens. This is a multipart series, with the first two parts specifying general requirements for reporting of sample handling and storage (ISO $20579-1^{1}$), and reporting of mounting and treatment of samples (ISO 20579-2¹)). The ensuing parts combine any new requirements of sample handling/storage and/or sample mounting/preparation for classes of new materials. This document focuses on biomaterials analysis and handling, and ISO 20579-4 focuses on reporting and handling needs for nano-objects. Each part of this series can be used independently of the other parts, although the general procedures described in ISO 20579-1 and ISO 20579-2 are applicable to a wide range of materials and are not reproduced in detail in material-specific sections.

Although primarily prepared for the surface-analysis techniques of Auger electron spectroscopy (AES), X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS), the methods described in this series will also be applicable to many other surface-sensitive analytical techniques such as ion-scattering spectrometry, low-energy electron diffraction and electron energyloss spectroscopy, where specimen handling can influence surface-sensitive measurements. AES, XPS and SIMS are sensitive to surface layers that are typically a few nanometers in thickness. Such thin layers may be subject to severe perturbations caused by specimen handling or surface treatments that may be necessary prior to introduction into the analytical chamber. Proper handling and preparation of specimens is particularly critical for dependable analysis. Improper handling of specimens can result in alteration of the surface composition and unreliable data.

11en SIANDARD

0.2 ISO 20579-3 introduction

standards.iteh.ai) This document is specifically intended to assist analysts in the handling, preparation and mounting of specimens submitted for surface chemical analysis of biomaterials. Applications of synthetic materials in a body includes metals, ceramics, polymers, glasses, carbons and composite materials. Surfaceanalysis techniques such as AES, XPS and SIMS were originally developed for the analysis of inorganic materials, but the methods described in this document may also be applicable to biomaterials. Many other surface-sensitive analytical techniques such as ion-scattering spectrometry, low-energy electron diffraction and electron energy-loss spectroscopy can be applied for specimen analysis. A few examples of biomaterial applications are artificial hip and knee joints, bone plates for fracture fixation, dental implants, optical devices (intraocular lenses), heart valves and stents for cardiovascular systems, and membrane materials for guided tissue regeneration. More examples are discussed elsewhere^{[1],[2]}.

Specimen handling can influence surface-sensitive measurements. Surface methods for chemical analysis are sensitive to surface layers that are typically only a few nanometers in thickness. Such thin layers may be subject to severe perturbations caused by improper specimen handling^{[4],[Z]} or surface treatments that may be necessary prior to introduction into the analytical chamber. Proper handling and preparation of specimens is particularly critical for biomaterial analysis. Improper handling of specimens can result in alteration of the surface composition and unreliable data.

Proper preparation and mounting of specimens are particularly critical for surface chemical analysis of biomaterials. Improper preparation may result in the alteration of the surface composition and in unreliable analyses. Specimens are handled carefully so that the introduction of spurious contaminants is avoided or minimized. The goal prior to analysis is to preserve the state of the surface during preparation and mounting so that the analysis remains representative of the original specimen. This document describes methods that the surface analyst may need to use in order to minimize the effects of specimen preparation when using any analytical method.

In addition, the change of composition of the surface of a biomaterial before and after implantation may be an issue related to contamination. It is intended to highlight general ideas about surface chemical analysis, in particular solid surfaces but also soft surfaces, such as self-assembled monolayers (SAMs), hydrogels, scaffolds and some polymers.

¹⁾ To be developed.

iTeh STANDARD PREVIEW (standards.iteh.ai)

<u>ISO 20579-3:2021</u> https://standards.iteh.ai/catalog/standards/sist/47cb4d8f-051a-4d41-b788-68f7d558e462/iso-20579-3-2021

Surface chemical analysis — Sample handling, preparation and mounting —

Part 3: **Biomaterials**

1 Scope

This document gives guidance on methods of handling, mounting and surface treatment for a biomaterial specimen prior to surface chemical analysis. It is intended for the analyst as an aid in understanding the specialized specimen-handling conditions required for analyses by the following techniques:

- X-ray photoelectron spectroscopy (XPS or ESCA);
- secondary ion mass spectrometry (SIMS);
- Auger electron spectroscopy (AES).

The protocols presented are also applicable to other analytical techniques that are sensitive to surface composition, such as: **i i eh Si ANDARD PREVIEW**

- attenuated total reflectance -Fourier transform infrared spectroscopy (ATR-FTIR);
- total reflection X-ray fluorescence (TXRF); 79-3:<u>2021</u>
- ultraviolet photoelectron spectroscopy (UPS) s/sist/47cb4d8f-051a-4d41-b788-

68f7d558e462/iso-20579-3-2021 The influence of vacuum conditions applied and the issue of contamination before and after analysis and implantation, as well as issues related to contamination during analysis, are addressed. Biomaterials covered here are hard and soft specimens such as metals, ceramics, scaffolds and polymers.

This document does not cover such viable biological materials as cells, tissues and living organisms. Other related topics not covered in this document include: preparation of specimens for electron or light microscopy.

Normative references 2

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 18115-1, Surface chemical analysis — Vocabulary — Part 1: General terms and terms used in spectroscopy

ISO 18115-2, Surface chemical analysis — Vocabulary — Part 2: Terms used in scanning-probe microscopy

Terms and definitions 3

For the purposes of this document, the terms and definitions given in ISO 18115-1 and ISO 18115-2 and the following 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

biomaterial

material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body

3.2

biological material

organic and/or mineral substances produced by living organisms (e.g. bone, skin, seashell, woods, and cultured cells)

4 Abbreviated terms

AES	Auger electron spectroscopy
ATR-FTIR	attenuated total reflection - Fourier transform infrared spectroscopy
ESCA	electron spectroscopy for chemical analysis
HDPE	high density polyethylene
LDPE	low density polyethylene
рНЕМА	poly (2-hydroxyethyl methacrylate) DARD PREVIEW
PTFE	polytetrafluoroethylene (standards.iteh.ai)
SIMS	secondary-ion mass spectrometry
SEM	scanning electron/smidro/scopy/catalog/standards/sist/47cb4d8f-051a-4d41-b788-
TXRF	total reflection X-ray fluorescence spectroscopy
UHV	ultra-high vacuum
UPS	ultra-violet photoelectron spectroscopy
XPS	X-ray photoelectron spectroscopy

5 Explanation of the structure of this document

ASTM E1829 - 14 and ASTM E1078 – 14 provide specific information on specimen preparation techniques prior to surface analysis of materials, covering general considerations, mechanical separation, thinning versus removal of layers, removal from the substrate, and sectioning techniques^{[1],[2]}. The growth of overlayers, solvents, chemical etching as well as ion sputtering, and plasma etching followed by sample heating and ultraviolet radiation is emphasized.

- <u>Clause 6</u> provides specific recommendations on specimen handling procedures necessary to minimize contamination of the biomaterial specimen surface. Moreover, <u>Clause 6</u> gives a series of alternative specimen handling procedures based on maintaining increasing degrees of specimen cleanliness during handling and transfer of the specimen to storage containers.
- <u>Clause 7</u> discusses additional considerations, such as specimen history and previous analyses of the specimen that affect the composition of the surface. Documentation of these influences should accompany the carefully handled and packaged specimen when submitted for analysis.
- <u>Clause 8</u> provides recommendations for sample handling and preparation with emphasis on tools, gloves and wiping materials. Exposure to gases, instrumental vacuum, electrons, ions and X-rays as well as the contamination of the analytical chamber is discussed.

- <u>Clause 9</u> discusses specimen storage with respect to time, humidity and temperature. <u>Clause 10</u> also
 describes different specimen containers for biomaterials that may be used in different conditions.
- <u>Clause 10</u> emphasizes that specimen handling has an effect on the information derived from surface analytical measurements, and that specimen owners as well as analysts will benefit from improved analyses when prescribed specimen handling protocols are followed.
- <u>Clause 11</u> discusses specimen mounting procedures for various types of solid samples, i.e. powders, wires and filaments. It provides information on pedestal mounting and reduction of thermal heating during analysis.
- <u>Clause 12</u> provides methods for reducing specimen charging, and in particular for using electron and ion beams for the analysis of biomaterials.
- <u>Clause 13</u> highlights specimen preparation techniques of biomaterials, such as sectioning techniques, using solvents and chemical etching. Ion sputtering is discussed in particular as well as the influence of UV radiation.
- <u>Clause 14</u> discusses fracturing, cleaving and scribing of samples.
- Finally, <u>Clause 15</u> on specimen handling techniques refers to ASTM E1078 14 where pre-pumping
 of gassy specimens, viscous liquids and solute residues is discussed.

6 General requirements and classes of specimens

6.1 General information STANDARD PREVIEW

creams, skin oils and other skin materials are not suitable for high vacuum.

General information on specimen handling of solid biomaterials is available in References [4] to [14]. Biomaterials analysis requires special methods to control many of the biological reactions occurring in response to a biomaterial. Functionality, biocompatibility and durability are of concern. Some information for solid surfaces is found in Reference [5]. Contamination is a key concern. The degree of cleanliness in particular for biomaterials required by surface-sensitive analytical techniques is much higher than for many other forms of analysis^[4]. Specimens and mounts must never be in contact with the bare hand. Handling of the surface to be analysed should be eliminated or minimized whenever possible. Fingerprints contain mobile species that may contaminate the surface of interest. Hand

6.2 Handling

Care should be taken in the handling of biomaterials to ensure that nothing, apart from air or clean inert gases, comes in contact with the surface to be investigated. In particular, avoid contacting the specimen surface with solvents or cleaning solutions, gases such as compressed air or solvent vapours, metals, tissue or other wrapping materials, tape, cloth, tools, packing materials, or the walls of containers. In response to hydrophobic environment compatible components may migrate to the surface of the specimen, thus reducing interfacial energy. Responding to an aqueous environment, the surface may reverse its structure and point polar (hydrophilic) groups outward to interact with the polar water molecules. Many materials can undergo a reversal of surface structure when transferred from air into a water environment. A hydroxylated polymer, such as a pHEMA contact lens, exhibits a surface rich in methyl groups in air, and a surface rich in hydroxyl groups under water. Energy minimization drives this process. For metal alloys, one component tends to dominate the surface, as chromium in stainless steel⁴. In particular, the use of low-density poly(ethylene) (LDPE) bags, which are known to be contaminated with slip agents, should be avoided. In cases where these precautions are not feasible due to the size of the specimen, some alternative specimen storage and transporting methods are presented in <u>Clause 15</u>. Some approaches to sample handling will apply to all biomaterials and some only to subsets, i.e. liquid specimen.

6.3 Packaging

If a sample is placed in a package for shipping or storage prior to surface analysis it is critical to know whether the packaging material can induce surface contamination. Plain paper in contact with most biomaterials will transfer atoms or molecules to the surface. Many plastics are processed with silicone oils or other additives that can be transferred to the specimen surface^[4].

Ideally, specimens should be transported to the analyst in a container that does not come into direct contact with the surface of interest. A small vacuum desiccator is preferred. When this is not possible, clean packaging materials should be used. Examples of clean containers include Piranha-cleaned glass vials, UHV foil or tissue and specific polystyrene Petri dishes. The surface cleanliness of the container should be verified prior to use. Compare <u>Clause 8</u>.

In some cases, it may be necessary to take a representative sample from the specimen. Selection of a smaller sample from a larger specimen should be done after considering the information being sought because in homogeneities are often present. It is recommended that this choice be made in consultation with an experienced analyst. Specific care should be taken to avoid contaminating the surface of interest during the cutting procedure (for more details see ASTM E1829 - 14 and ASTM E1078 - 14).

These general principles are applicable to all materials. There are properties that are specific to specific classes of materials. Compared with metals, ceramics, glasses and carbons, organic and polymeric materials are more easily damaged by surface analysis methods. Polymeric systems also exhibit greater surface molecular mobility than inorganic systems. Surfaces of inorganic materials are contaminated more rapidly than polymeric materials because of their higher surface energy. Electrically conductive metals and carbons are often easier to characterise than insulators using electron, X-ray or ion interactive methods^[1].

6.4 Toxins and other hazardous materials

Special caution shall be exercised with speci**mens** containing potential toxins or other hazardous materials. Whenever possibles material safety data sheets (MSDS) should be supplied with the specimen. The severity of the requirement for careful handling varies dramatically with the condition of the surface, the depth from the surface of the information being sought, and the detection level required for the material being analysed. Triggered release of bound ligands by smart polymer-engineered protein bioconjugates could be used to release therapeutics. Triggered release could also be used to remove inhibitors, toxins or fouling agents from the recognition sites of immobilised or free enzymes and affinity molecules, such as those used in biosensors, diagnostic assays or affinity separations^[4].

7 Specimen considerations

7.1 History of the specimen

In the case of samples that are surface treated with a plasma process or similar treatment intended to increase the surface polarity of remove surface contaminants, it is important to also keep track of the time between treatment and analysis. Surfaces of polymeric materials with molecular mobility are known to undergo molecular rearrangement to minimize the surface free energy. A polymer surface can change rapidly after a plasma treatment.

7.2 Information sought

The information sought is described in ASTM E1078 - 14, 7.1

7.3 Categories of specimen

The categories of specimen are explained in ASTM E1829 - 14, Clause 6.

Surface chemical analysis can be performed on a wide range of specimens and can be used to obtain very different types of information about surfaces or interfaces. The degree of care that shall be