FINAL
DRAFT

INTERNATIONAL
STANDARD

**ISO/FDIS
20579-3**

ISO/TC **201**/SC **2**

Secretariat: ANSI

Voting begins on:
2020-11-03

Voting terminates on:
2020-12-29

Surface chemical analysis — Sample handling, preparation and mounting —

Part 3: Biomaterials

Analyse chimique des surfaces — Lignes directrices pour la manipulation, préparation et montage des échantillons — Partie 3: Biomatériaux

Reference number
ISO/FDIS 20579-3:2020(E)

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

Contents

Page

Foreword ..... **iv**
Introduction ..... **v**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 Symbols and abbreviated terms **2**
5 Explanation of the structure of this documen **2**
6 General requirements and classes of specimens **3**
6.1 General information 3
6.2 Handling 3
6.3 Packaging 3
6.4 Toxins and other hazardous materials 4
7 Specimen considerations **4**
7.1 History of the specimen 4
7.2 Information sought 4
7.3 Categories of specimen 4
8 Sources of specimen contamination **5**
8.1 Sample preparation 5
8.2 Tools 5
8.3 Sample handling 5
8.3.1 General 5
8.3.2 Exposure to gases 5
8.3.3 Minimize contamination of the analysis area 5
8.4 Separation between neighbouring areas 6
9 Specimen storage and transfer of biomaterials **6**
9.1 Storage 6
9.1.1 Storage time 6
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 **6**
11 Specimen mounting procedures of biomaterials **6**
12 Methods for reducing specimen charging **6**
13 Specimen preparation techniques of biomaterials **7**
13.1 Mechanical separation 7
13.2 Sectioning techniques 7
13.3 Solvents for biomaterials 7
13.4 Chemical etching 7
13.5 Ion sputtering 7
14 Fracturing, cleaving and scribing **7**
15 Specimen-handling techniques **7**
Bibliography **8**

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 201, *Surface chemical analysis*, Subcommittee SC 2, *General procedures*.

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 www.iso.org/members.html.

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 being general requirements for (ISO 20579-1) sample handling and storage, and (ISO 20579-2) mounting and treatment of samples. 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 energy-loss 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.

0.2 ISO 20579-3 introduction

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. Surface-analysis 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[4-7] 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 is 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.

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:

— attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR);

— total reflection X-ray fluorescence (TXRF);

— ultraviolet photoelectron spectroscopy (UPS).

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, will be 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 standard include: preparation of specimens for electron or light microscopy.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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 Symbols and abbreviated terms

AES	Auger electron spectroscopy
ATR-FTIR	-FTIR aAttenuated Ttotal rReflection - Fourier tTransform iInfrared Sspectroscopy
ESCA	electron spectroscopy for chemical analysis
SIMSSEM	secondary-ion mass spectrometry scanning electron microscopy
TXRFSIMS	total reflection X-ray fluorescence spectroscopy secondary-ion mass spectrometry
UPSTXRF	ultra-violet photoelectron spectroscopy total reflection X-ray fluorescence spectroscopy
XPSUPS	X-ray photoelectron spectroscopy ultra-violet photoelectron spectroscopy
XPS	X-ray photoelectron spectroscopy

5 Explanation of the structure of this documen

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.

— Clause 6 provides specific recommendations on specimen handling procedures necessary to minimize contamination of the biomaterial specimen surface. Moreover, Clause 6 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.

— Clause 7 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.

— Clause 8 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.

— Clause 9 discusses specimen storage with respect to time, humidity and temperature. Clause 10 also describes different specimen containers for biomaterials that may be used in different conditions.

— Clause 10 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.

— Clause 11 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.

— Clause 12 provides methods for reducing specimen charging, and in particular for using electron and ion beams for the analysis of biomaterials.

— Clause 13 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.

— Clause 14 discusses fracturing, cleaving and scribing of samples.

— Finally, Clause 15 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

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

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 steel[4]. 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 Clause 15. 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 Clause 8 below.

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