



BSI Standards Publication

Nanotechnologies — Assessment of protein secondary structure during an interaction with nanomaterials using ultraviolet circular dichroism

National foreword

This Published Document is the UK implementation of ISO/TS 23459:2021.

The UK participation in its preparation was entrusted to Technical Committee NTI/1, Nanotechnologies.

A list of organizations represented on this committee can be obtained on request to its committee manager.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

© The British Standards Institution 2021
Published by BSI Standards Limited 2021

ISBN 978 0 539 01802 8

ICS 07.120

Compliance with a British Standard cannot confer immunity from legal obligations.

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 28 February 2021.

Amendments/corrigenda issued since publication

Date	Text affected
------	---------------

**Nanotechnologies — Assessment of
protein secondary structure during an
interaction with nanomaterials using
ultraviolet circular dichroism**

*Nanotechnologies — Évaluation de la structure secondaire des
protéines durant une interaction avec des nanomatériaux à l'aide du
dichroïsme circulaire ultraviolet*





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

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Abbreviated terms	2
5 Nanomaterial protein interactions	2
6 Sample preparation	3
6.1 General.....	3
6.2 Desired properties of the UV-CD quartz cell.....	3
6.3 Preparation of protein solution.....	3
6.4 Instrumental setting condition.....	4
6.5 Recording UV-CD spectra procedure.....	4
6.5.1 General.....	4
6.5.2 Buffer.....	4
6.5.3 Protein sample.....	4
6.5.4 Stability of NP suspension in the protein solution.....	5
6.6 Preparation of protein-NPs conjugated suspension.....	5
6.7 UV-CD spectra measurement.....	5
6.8 Calculation of molar ellipticity.....	6
6.9 Data analysis.....	6
7 Test report	7
Annex A (informative) Typical UV-CD spectra of proteins	8
Annex B (informative) Literature survey on structural changes of NOAA and proteins	9
Annex C (informative) Description of buffers that can be used for protein solubility	15
Annex D (informative) Unit conversions in CD measurements	19
Annex E (informative) Calculating the concentration range of the sample	20
Annex F (informative) Methods for estimation of secondary structures of protein	21
Annex G (informative) Typical data of UV-CD used for estimation of secondary structures of protein	22
Bibliography	23

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 229, *Nanotechnologies*.

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

Nano-objects and their aggregates and agglomerates (NOAA) are currently produced in large mass quantities globally and used in a variety of applications. However, there is concern about their interaction with biological systems, including proteins, which could lead to reversible or irreversible alterations in their secondary structure. The latter could affect the functionality and conformation of protein, which in turn might affect the overall bio-reactivity of the proteins. The monitoring of the occurrence of such alterations could thus provide important information on the interaction of NOAA with biological systems.

The process of folding of polypeptides in biological media produces the secondary structure of proteins which determines their bioactivity. The important features of this structure include hydrogen bonds between the amine hydrogen and carbonyl oxygen atoms in the peptide backbone and disulfide bonds between two cysteine residues.

The protein secondary structure could be affected by exposing it to certain metal ions and bioactive compounds. Furthermore, it is also influenced by different buffer ionic strength, pH values, and temperature^[1]. Alterations in the functionality and conformation of proteins can be attributed to reorganization (so-called misfolding) and changes of the overall molecular dimension that accompany the folding process. Some diseases, such as amyotrophic lateral sclerosis (ALS), Alzheimer's and Parkinson's, are a consequence of misfolded proteins^[2].

There are several standard techniques for determining the molecular structures/conformations and folding process of proteins and upon their interaction with NOAA. These include high-field nuclear magnetic resonance (NMR), Fourier-transform infrared (FT-IR), Raman spectroscopy and ultraviolet circular dichroism (UV-CD) spectroscopies^{[3][4][5][6]}. In addition, a novel technique synchrotron radiation circular dichroism (SRCD) spectroscopy is a sensitive method to provide information on protein secondary structures and folding^[7].

Nanotechnologies — Assessment of protein secondary structure during an interaction with nanomaterials using ultraviolet circular dichroism

1 Scope

This document specifies measurement protocols and test conditions to determine alterations to protein secondary structure induced by their interaction with nanomaterials using ultraviolet circular dichroism (UV-CD) spectroscopy.

This document does not apply to the characterization of conformational changes of dissolved proteins.

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/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

ISO/TS 80004-4, *Nanotechnologies — Vocabulary — Part 4: Nanostructured materials*

ISO/TS 80004-6, *Nanotechnologies — Vocabulary — Part 6: Nano-object characterization*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-1, ISO/TS 80004-2, ISO/TS 80004-4, ISO/TS 80004-6 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

nanoparticle

NP

nano-object with all external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nano-object do not differ significantly

Note to entry: If the dimensions differ significantly (typically by more than three times), terms such as “nanofibre” or “nanoplate” may be preferred to the term “nanoparticle”.

[SOURCE: ISO/TS 80004-2:2015, 4.4]

3.2

nanomaterial

material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale

Note 1 to entry: This generic term is inclusive of nano-object and nanostructured material.