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Nanoteknologi – Ytkaraktärisering av guldnanopartiklar för kontroll av giftighet – FT-IR-metod (ISO/TS 14101:2012, IDT)

Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening – FT-IR method (ISO/TS 14101:2012, IDT)

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Foreword

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ISO/TS 14101 was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Introduction

Gold nanoparticles (AuNPs) can be controlled with regard to size, shape and surface ligands, making them ideal for the study of relationships between their physicochemical properties and cytotoxicity on living bodies[1][2][3]. Among the various properties of AuNPs, surface ligand characteristics, such as the chemical composition, molecular structure and quantity of bound molecules, were found to play an important role in determining the behaviour of AuNPs, e.g. the degree of aggregation or agglomeration in solution, binding with biomolecules in cell culture media and cytotoxicity to living cells[4][5][6][7][8][9][10][11][12]. On the other hand, surface ligand modification is not always successful in the synthesis step, and the degree of ligand exchange should be identified prior to the property specific cytotoxicity test of AuNPs in order to obtain reliable and consistent results.

FT-IR (Fourier transform infrared) absorption spectroscopy is one of the most useful tools of NP surface ligand identification and quantification. By using the FT-IR method, the structures and relative quantities of ligand molecules bound to NP surfaces can be analysed[13][14][15][16][17][18][19][20]. However, the low concentrations and aqueous environment of synthesized AuNPs will complicate the interpretation of measurement results. Low concentrations of AuNPs result in small absorbance values, which can easily be influenced by background noise or the absorbance of trace impurities. Since cytotoxicity tests are performed in aqueous environments, we should analyse what is on the surface of AuNP in aqueous solutions if we want to study the effect of the surface characteristics on cytotoxicity of AuNPs. However, water molecules strongly absorb IR light over a wide frequency range, disabling IR absorption analysis on the solutes in very low concentrations. It is necessary to develop measurement guidelines by which the above issues can be minimized. In this project, we seek to develop a Technical Specification (TS) for the observation of chemical moieties bound to the synthetic AuNP in the form of dehydrated films, which can deliver the information about the molecular species bound to AuNPs when they were in aqueous solutions. Although the standardization of FT-IR measurement procedures will be the basis for this Technical Specification, a great deal of weight will also be given to the sample preparation procedure for correct FT-IR analysis.

Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: FT-IR method

1 Scope

This Technical Specification provides guidelines for the identification of the surface bound molecules using FT-IR of dehydrated gold nanoparticle (AuNPs) films both before and after nanomaterial (NM) cytotoxicity testing.

NOTE 1 AuNPs may have surface bound ligands prior to testing and may be additionally covered (or coated) with organic- or bio-molecules during the cytotoxicity test.

NOTE 2 Nucleic acids, amino acids, lipids or membrane components binding to AuNPs can be observed by FT-IR spectroscopy by detection of absorption bands corresponding to phosphodiester, amine or lipid, respectively, although the type of nucleic acids, proteins or lipid cannot be identified in detail based on IR spectra.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendment) applies.

ISO/TS 27687, *Nanotechnologies — Terminology and definitions for nano-objects — Nanoparticle, nanofibre and nanoplate*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 27687 and the following apply.

3.1
attenuated total reflection mode
ATR Mode
instrumental mode of operation in which the incident angle of IR light on the crystal is adjusted to be higher than the critical angle

NOTE The light is completely reflected by the upper surface of the crystal, and the intensity of the light is attenuated through absorption by materials covering the upper surface of the crystal. The frequency of IR light absorbed is used to identify the absorbed chemical moiety, and the fraction of light that is absorbed is used to quantitate the amount of that moiety present.

3.2
dialysis
process by which small molecules or ions diffuse through a membrane, thus causing their separation from larger molecules in solution and from suspended matter

[ISO 6107-2:2006, definition 38]

3.3
Fourier transform infrared spectroscopy
FT-IR
analytical chemical technique based on absorption of infrared radiation by chemical moieties in the specimen, used to identify and quantitate the absorbing chemical moieties

3.4
limit of detection
LOD
measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence

NOTE 1 Adapted from ISO/IEC Guide 99:2007, definition 4.18.

NOTE 2 LOD may be determined as 2,776 times the standard deviation of the measurements of 5 replicate blanks under conditions of repeatability with IUPAC recommended values of 0,05 for both α and β.

NOTE 3 See also ISO 17191.

3.5
limit of quantification
LOQ
lowest value of an analyte that can be determined with an acceptable level of accuracy and precision

NOTE 1 LOQ may be determined as 10 times the standard deviation of the photometric noise, which will give relative precision $\sigma_A/A \leq 10$ % for the minimum signal level A.

NOTE 2 See Reference [24].

3.6
molecular weight cut-off value
MWCO
molecular weight of solute that is retained by more than 90 % after 16 h dialysis

NOTE See References [25] and [26].

3.7
nano-object
material with one, two or three external dimensions in the nanoscale

[ISO/TS 27687:2008, definition 2.2]

NOTE Generic term for all discrete nanoscale objects.

3.8
nanoparticle
NP
nano-object with all three external dimensions in the nanoscale

[ISO/TS 27687:2008, definition 4.1]

NOTE If the lengths of the longest to the shortest axes of the nano-object differ significantly (typically by more than three times), the terms nanorods or nanoplate are intended to be used instead of the term nanoparticle.

3.9
nanoscale
size range from approximately 1 nm to 100 nm

[ISO/TS 27687:2008, definition 2.1]

NOTE 1 Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate.

NOTE 2 The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as nano-objects or elements of nanostructures, which might be implied by the absence of a lower limit.

3.10
pre-tested distilled water
DW
distilled water validated to be free from IR absorbing impurities by FT-IR measurement

3.11
relative centrifugal force
RCF
acceleration force relative to the Earth's gravity

3.12
surface plasmon resonance band
SPR
range of frequencies of absorbed light, where the absorption is the result of the collective oscillation of electrons within the near-surface region of a solid

NOTE SPR occurs in thin metal films or metallic NPs.

4 Symbols and abbreviated terms

AuNP	gold nanoparticle
IR	infrared
MW	molecular weight
SCM	serum containing media
UV/Vis	ultraviolet/visible
× *g*	Earth's gravimetric acceleration as a reference unit for the relative centrifugal force

5 Sample preparation mode

5.1 Removal of unbound molecules

5.1.1 General

Since FT-IR absorption spectroscopy measures total molecular species in the sample film, all unbound molecules that are active IR absorbing species except solvents shall be removed from the solution before preparing the sample film in order to correctly identify the molecules bound to the surface of AuNPs.

5.1.2 Dialysis

Dialysis is an efficient method for separating unbound molecules from AuNPs when membranes with adequate MWCOs are available. If used adequately, dialysis membranes reduce the concentration of unbound molecules according to the volume ratio of the sample and dialysis solutions, generally retaining more than 90 % of NPs. It is recommended that MWCO is lower than half the MW of the species to be retained, and higher than three times the MW of the species intended to pass through. Because the efficiency of dialysis membrane depends on the charge and shape of molecules, the unbound molecular removal efficiency of a dialysis membrane shall be verified before the separation of AuNPs from unbound molecules. Prior to the efficiency test, the membrane shall be tested if it is free from IR absorbing impurities. The procedure for impurity test is as follows:

a) fill the dialysis bag with 0,5 ml to 3 ml of pretested DW;