FINAL DRAFT
Technical Report

ISO/DTR 23652

Nanotechnologies — Considerations for radioisotope labelling methods of nanomaterials for performance evaluation

Nanotechnologies — Considérations relatives aux méthodes de marquage radio-isotopique des nanomatériaux pour l'évaluation des performances

ISO/TC **229**

Secretariat: **BSI**

Voting begins on:
2024-02-20

Voting terminates on:
2024-04-16

Reference number
ISO/DTR 23652:2024(en)

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Published in Switzerland

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Foreword

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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

Prior to the clinical trials of nanomaterials intended for use in human medicine, their in vivo behaviour has been evaluated in animal experiments. Several quantitative methods for assessing the biodistribution of nanomaterials have been developed. Among these methods, the biodistribution of radioisotope-labelled nanomaterials provides quantitative information on their distribution throughout the entire body.

The use of radioisotope-labelled nanomaterials for biodistribution studies is a well-established method for understanding the pharmacokinetics or toxicokinetics of nanomaterials in vivo. These methods assume that the distribution pattern of nanomaterials and radioisotope-labelled nanomaterials will be similar or nearly identical in vivo.

Radioisotope labelling of nanomaterials can be accomplished using a wide variety of radionuclides and associated labelling methods. However, for nanomaterials used for medicinal purposes, there are only a few matching pairs of nanomaterial and radioisotope labelling method that ensure the in vivo integrity of the radioisotope-labelled nanomaterial. Failure to identify and apply matching pairs of nanomaterial and radioisotope labelling method in studies preceding the clinical trial phase can lead to experimental data on biodistribution in which the nanomaterial and radio-label separate during the experiment. This in turn can result in a large number of nanomaterials or nano-drugs failing in the clinical trial phase.

Nanotechnologies — Considerations for radioisotope labelling methods of nanomaterials for performance evaluation

1 Scope

This document provides:

a) a review of radioisotope labelling methods that can be used for nanomaterials;

b) the pros and cons of each radioisotope labelling method;

c) information on the selection of a matched pair of nanomaterial and radioisotope labelling method to ensure the in vivo integrity of radioisotope-labelled nanomaterials[1] or the stability of their performance.

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 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core vocabulary*

ISO/TS 80004-8, *Nanotechnologies — Vocabulary — Part 8: Nanomanufacturing processes*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 80004-1, ISO/TS 80004-8 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
nanoscale
length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from a larger size are predominantly exhibited in this length range.

[SOURCE: ISO 80004-1:2015, 2.1]

3.2
nanomaterial
material with any external dimension in the *nanoscale* (3.1) 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.

[SOURCE: ISO 80004-1:2015, 2.4]

3.3
nanoparticle
nano-object with all three external dimensions in the *nanoscale* (3.1),

Note 1 to entry: If the lengths of the longest to the shortest axes of the nano-object differ significantly (typically by more than three times), the terms nanofibre or nanoplate are intended to be used instead of the term nanoparticle.

[SOURCE: ISO/TS 27687:2008, 4.1]

3.4
radioisotope
unstable isotope of an element that decays or disintegrates spontaneously, emitting ionizing radiation that can be alpha particles, beta particles and/or gamma rays

Note 1 to entry: Approximately 5 000 natural and artificial radioisotopes have been identified.

[SOURCE: ISO 19461-1:2018, 3.9, modified — "that can be alpha particles, beta particles and/or gamma rays" has been added to the definition.]

3.5
biodistribution
technique used to monitor the movement and distribution of specific radiolabelled *nanomaterials* (3.2) within an experimental animal or human subject

3.6
chelating agent
substance having a molecular structure embodying several electron-donor groups which render it capable of combining with metallic ions by chelation

[SOURCE: ISO 862:1984, 81]

3.7
specific activity
total radioactivity of the sample divided by its mass

Note 1 to entry: Specific activity is expressed in Bq/g.

[SOURCE: ISO 3925:2014, 3.4, modified — In the term, "activity" has been changed to "radioactivity"; Note 1 to entry has been added.]

4 Abbreviated terms

BFC	bifunctional chelating agent
NP	nanoparticle
TATE	1,4,8,11- tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
CB-TE2A	4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane
NOTA	1,4,7-triazacyclononane-1,4,7-triacetic acid
DOTA	1,4,7,10-tetraazacyclododecane- 1,4,7,10-tetraaceticacid
DFO	desferrioxamine
PET	positron emission tomography
SPECT	single photon emission computed tomography

5 Biodistribution study and radioisotopes

5.1 Biodistribution study

Biodistribution studies involve tracing the movement of materials of interest in an experimental animal or human subject.[1][2] For any medicinal product intended for human administration, whether in experimental conditions during clinical trials or as an established treatment after approval by regulatory authorities, extensive information is generated in anticipation of human administration to understand the potential benefits and risks, as well as to anticipate and estimate the potential benefit-risk ratio profile.[3] The biodistribution of radiolabelled nanomaterials can be assessed using various methods, including image quantification, tissue radioactivity measurement, or autoradiography. The injection route of nanomaterials, such as intravenous, oral, or intraperitoneal administration, can also be considered based on the intended clinical application.

Image-based biodistribution can be done by tracking the distribution of radiolabelled nanomaterials of interest in an experimental animal or human subject using imaging techniques such as positron emission tomography (PET), single-photon emission computed tomography (SPECT). This approach allows researchers to visualize and quantify the spatial and temporal distribution of the nanomaterials of interest in vivo, enabling them to better understand their pharmacokinetics and pharmacodynamics[4]. Tissue-based biodistribution is a preclinical research technique that involves the analysis of the distribution and accumulation of radiolabelled nanomaterials in different tissues and organs of an animal or animal model. This technique provides information on the pharmacokinetics and pharmacodynamics of nanomaterials, which includes its absorption, distribution, metabolism and elimination (ADME) in different organs and tissues.[5] To perform a tissue-based biodistribution study, animals are typically treated with radiolabelled nanomaterials. After a predetermined time, animals are sacrificed, and different tissues and organs are collected and analysed for the presence and concentration of the drug. Whole-body slices can be made to visualize the distribution of radioactivity in different organs and tissues using autoradiography. This technique involves exposing the sliced tissue sections to a photographic film or imaging plate, which detects the radioactive emissions and generates an image that can be analysed to determine the distribution of the radiolabelled nanomaterial. Autoradiography is a widely used technique in preclinical research for evaluating the biodistribution of radiolabelled nanomaterials, and it can provide valuable insights into how the drug or nanomaterial is distributed throughout the body[6].

The injection route of nanomaterials, such as intravenous, oral, or intraperitoneal administration, can also be considered based on the intended clinical application. The injection route is an important consideration when assessing the biodistribution of nanomaterials since different routes can lead to distinct distribution patterns and pharmacokinetic profiles. Therefore, researchers carefully select the appropriate method and injection route to obtain accurate and reliable results.

5.2 Radioisotopes

Radioisotopes are isotopes that emit radiation and are commonly used for the diagnosis and treatment of various human diseases. Diagnostic purposes make up about 90 % of radioisotope usage, while therapeutic treatment makes up the remaining 10 %. Radioisotopes are labelled onto disease-targeting molecules, which are then administered and targeted to specific organs or tissues through specific mechanisms. The information from the radioisotopes is then collected and reconstructed by an imaging instrument to provide information about disease localization and specific biological processes. Typically, diagnostic radioisotopes are preferred for biodistribution studies because they have longer penetration depth and lower toxicity than therapeutic radioisotopes. γ or β^+ emitters are commonly used for this purpose[7].

The ability of γ rays to penetrate through the body depends on their energy, with higher energy leading to higher penetration ratios. However, excessively high energy can decrease the detector's sensitivity and resolution. As such, moderate-energy γ rays (between 30 keV and 300 keV) are optimal for γ camera or SPECT imaging.[8][9] β^+ particles can create two γ photons (each with an energy of 511 keV) via an annihilation reaction, making them suitable for PET imaging. The range of a positron is influenced by its kinetic energy, with lower kinetic energy leading to better imaging quality.[10] When labelling nanomaterials with radioisotopes, the physical half-life is also a critical factor to be considered because specific nanomaterials have varying biological half-lives.

6 Radioisotope labelling methods for nanomaterials

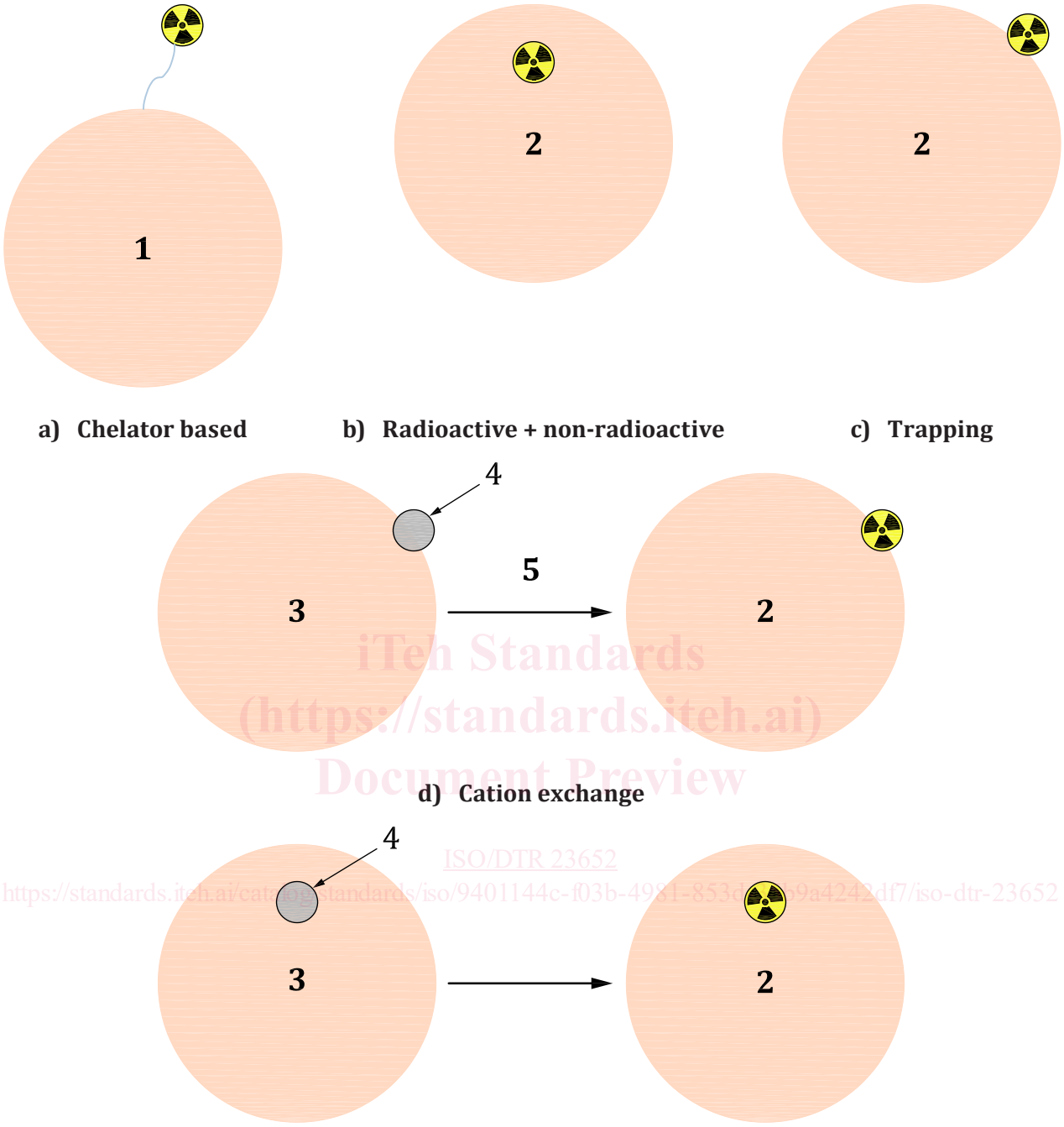
6.1 General

The methods for radioisotope labelling and imaging of nanomaterials are crucial for various biomedical applications. Two common approaches for radioisotope labelling are chelating agent-based (Figure 1, a) and chelating agent-free (Figure 1, b to e) methods, as shown in the figure below.

Chelating agent-based methods involve the attachment of a chelating agent to the surface of the nanomaterial, which then binds to a radioisotope. This approach provides stable binding and high labelling efficiency. However, it can also result in the modification of the nanomaterial's properties and potential toxicity.

Chelating agent-free methods involve the direct binding of the radioisotope to the nanomaterial surface without the use of a chelating agent. This approach preserves the nanomaterial's properties and reduces potential toxicity. However, it can have lower labelling efficiency and stability.

Overall, the choice of labelling method depends on the specific application and desired properties of the labelled nanomaterial[11].



a) Chelator based b) Radioactive + non-radioactive c) Trapping

d) Cation exchange

e) Activated with high energy particle

Key

1 nanomaterial
2 radioactive nanomaterial
3 non-radioactive nanomaterial
4 high energy particle
5 radiometal

Figure 1 — Radioisotope labelling methods for nanomaterials