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Nanotechnologies — Method to estimate cellular uptake of carbon nanomaterials using optical absorption

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Nanotechnologies — Method to estimate cellular uptake of carbon nanomaterials using optical absorption

Nanotechnologies — Méthode d'estimation de la captation cellulaire des nanomatériaux carbonés par mesure d'absorption optique

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Foreword

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This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

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Introduction

Owing to their unusual physical and chemical properties, carbon nanomaterials (CNMs), such as carbon nanotubes, carbon black, graphene, and carbon nanohorns, have been considered for various applications such as in the fields of electronics, energy, nanotechnology, and biology. With the increase of CNM-based products on the market, the public concern regarding possible toxicities has also increased. Estimation of the amount of CNM associated with the targeted cells is useful for an initial toxicological screening of CNMs and for developing applications in medicine[1][2][3][4].

Fluorescent dyes and/or radioactive isotopes have been routinely used to measure cellular uptake. Because CNMs absorb light in near infrared (NIR) region, where the bio-components such as protein and water in cells or tissues have relatively low light absorption, the cellular uptake of CNMs can be estimated from the absorbance of cell-lysate[5][6][7][8].

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Nanotechnologies — Method to estimate cellular uptake of carbon nanomaterials using optical absorption

1 Scope

This document describes a near-infrared optical absorption method to estimate the in vitro cellular uptake of carbon nanomaterials including both internalized and/or tightly adhered to the cell membrane from liquid dispersions. This is a simple method to screen carbon nanomaterials uptake; additional analysis using a different technique can be required if quantification is desired.

2 Normative references

The following document is referenced 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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO/TS 80004-3, *Nanotechnologies — Vocabulary — Part 3: Carbon nano-objects*

3 Terms, definitions and abbreviated terms

For the purposes of this document, the following terms, definitions and abbreviations as well as the terms and definitions given in ISO/TS 80004-3 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 Terms and definitions

3.1.1
cellular uptake
internalization or association of a substance by a living cell

3.1.2
cell lysis
destruction or dissolution of cells with release of contents

3.1.3
absorbance
measure of the capacity of a substance to absorb light at a specified wavelength

3.2 Abbreviated terms

CNH	carbon nanohorn
CNM	carbon nanomaterial
CNT	carbon nanotube

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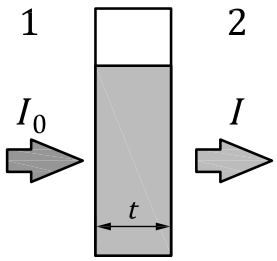
SWCNT	single-wall carbon nanotube
MWCNT	multiwall carbon nanotube
PBS	phosphate-buffered saline
SDBS	sodium dodecylbenzene sulfonate
NIR	near infrared
UV	ultraviolet
Vis	visible

4 Method overview

4.1 General

When an optical beam goes through a solution sample, some of the beam is attenuated by the solution, and the rest is transmitted (see Figure 1). The amount of optical beam attenuated by the solution is related to the property of solution itself and the thickness of the solution sample.



Key

1 incident light

2 transmitted light

Figure 1 — Optical attenuation by a solution sample

The optical absorbance is directly proportional to the concentration of the dissolved substance in a solution. When the concentration of solution is expressed as $g \cdot l^{-1}$, the relationship between absorbance and concentration can be written as follows.

$$A = \log_{10} (I_0/I) = k_m tc \quad (1)$$

where

A is the optical absorbance of the solution sample;

I_0 is the radiant fluxes of incident;

I is the radiant fluxes of transmitted beams;

k_m is the wavelength-dependent mass absorptivity coefficient with units of $g^{-1} \cdot cm^{-1}$;

t is the thickness of the solution sample;

c is the concentration of a substance dissolved in the solution sample expressed in units of $g \cdot l^{-1}$.

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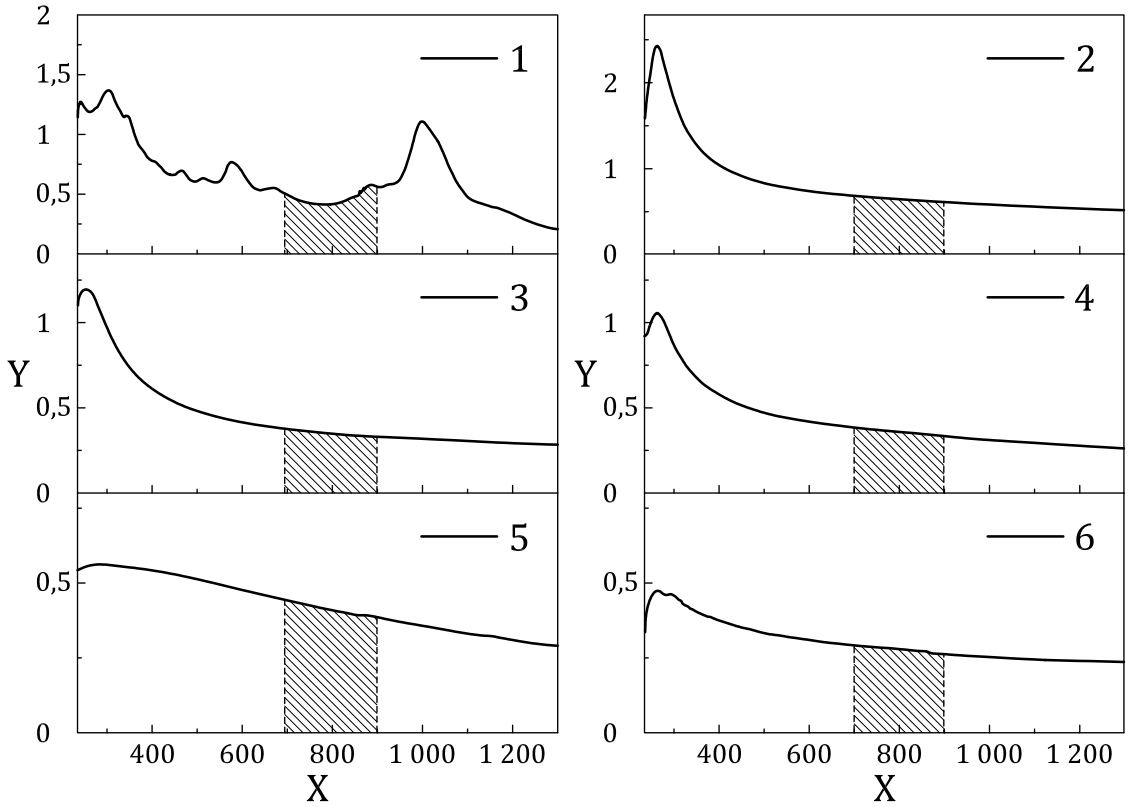
If A, k_m and t are known, then the concentration of a substance dissolved in the solution sample can be obtained. A is obtained by measurement, k_m is obtained by calibration using the known amount of the substance dissolved in the solution sample, and t is a fixed value determined by a cuvette optical pathlength for absorbance measurement.

4.2 Optical absorption of carbon nanomaterials

The optical absorption spectra of carbon nanomaterials in dispersion show a strong absorption peak in 300 nm to 200 nm (4 eV to 6 eV) that is attributed to the collective excitations of π electron systems (π-plasmons).[9] This π-plasmon absorption peak can be also observed in most graphitic compounds.[10] The peak is superimposed on the featureless background extending to the vis-NIR and IR regions. The typical spectra of SWCNTs, MWCNTs, CNHs, carbon blacks and graphene are shown in Figure 2. The broad non-resonant absorbance of MWCNTs, CNHs, carbon black, graphene and/or SWCNTs in vis-NIR region (e.g. 700 nm to 900 nm) is typical for most carbonaceous nanomaterials in samples.



Key

X	wavelength (nm)	3	MWCNTs
Y	absorbance (arbitrary units)	4	CNHs
1	individual SWCNTs	5	carbon black
2	SWCNTs bundles	6	graphene

NOTE The oblique regions show the absorbance of each carbon nanomaterial in 700 nm to 900 nm.

Figure 2 — Typical absorption spectra of individual SWCNTs, SWCNT-bundles, MWCNTs, CNHs, carbon black and graphene nanoplates in dispersions

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