SRI LANKA STANDARD 12004:2013
ISO 10801:2010

NANOTECHNOLOGIES - GENERATION OF METAL NANOPARTICLES FOR INHALATION TOXICITY TESTING USING THE EVAPORATION/CONDENSATION METHOD

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard

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SLS 12004:2013
ISO 10801:2010

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SRI LANKA STANDARDS INSTITUTION
17, Victoria Place,
Elvitigala Mawatha,
Colombo 8,
Sri Lanka.

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This standard does not purport to include all the necessary provisions of a contract.

Sri Lanka Standard

NANOTECHNOLOGIES - GENERATION OF METAL NANOPARTICLES FOR INHALATION TOXICITY TESTING USING THE EVAPORATION/CONDENSATION METHOD

NATIONAL FOREWORD

This standard was approved by the National Mirror Committee on Nanotechnology and authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2013.11.27.

This Sri Lanka Standard is identical with **ISO 10801:2010**, Nanotechnologies - Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method, published by the International Organization for Standardization (ISO).

TERMINOLOGY AND CONVENTIONS

The text of the International Standard has been accepted as suitable for publication, without any deviation as a Sri Lanka Standard. However, certain terminology and conventions are not identical with those used in Sri Lanka Standards. Attention is therefore drawn to the following:

a) Wherever the words “International Standard” appear referring to this standard they should be interpreted as “Sri Lanka Standard”.

b) The comma has been used throughout as a decimal marker. In Sri Lanka Standards, it is the current practice to use a full point on the baseline as the decimal marker.

Wherever page numbers are quoted, they are “ISO” page numbers.

CROSS REFERENCES

Corresponding Sri Lanka standards for International Standards listed under references in **ISO 10801:2010** are not available.

SLS 12004:2013

INTERNATIONAL STANDARD

ISO 10801

First edition
2010-12-15

Nanotechnologies — Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method

Nanotechnologies — Génération de nanoparticules de métal pour essais de toxicité par inhalation en utilisant la méthode de condensation/évaporation

Reference number
ISO 10801:2010(E)

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 10801 was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Introduction

The number of nanotechnology-based consumer products containing silver, gold, carbon, zinc oxide, titanium dioxide and silica nanoparticles is growing very rapidly. The population at risk of exposure to nanoparticles continues to increase as the applications expand. In particular, workers in nanotechnology-based industries are at risk of being exposed to manufactured nanoparticles. If nanoparticles are liberated from products, the public could be exposed as well.

There is currently limited, but growing, knowledge about the toxicity of nano-sized particles. The processes of nanoparticle production include gas-phase, vapour-phase, colloidal and attrition processes. Potential paths of exposure include inhalation, dermal and ingestion. Inhalation may arise from direct leakage from gas-phase and vapour-phase processes, airborne contamination of the workplace from deposition or product recovery and handling of product, or post-recovery processing and packing[7]. Exposure to manufactured nano-sized particles might occur during production, use and disposal in the ambient air or workplace and is of concern for public and occupational health.

There are currently neither generally accepted methods of inhalation toxicology testing for nano-sized particles nor specific nanoparticle generation methods for such testing. The ability to disperse respirable nano-sized particles from powders has been an obstacle to evaluating the effects of inhalation of nano-sized particles on the respiratory system. Although it is possible to disperse nanoparticles in air from powders, the size of the particles so generated may be larger than desired due to aggregation and agglomeration. In order to gain vital information for evaluating the health effects of nanoparticles by inhalation, nano-sized particles need to be generated and transported to a test environment containing experimental animals for testing short- or long-term inhalation toxicity. The nanoparticle generation method based on evaporation of metal (silver in this example) and subsequent condensation is capable of providing a consistent particle size distribution and stable number concentrations, suitable for short- or long-term inhalation toxicity study.

This International Standard provides a method for stable silver nanoparticle generation with particle sizes up to 100 nm. A detailed method is described in Annex A. The generation method provided here has sufficient stability for continuous inhalation toxicity testing up to 90 days. The generated nanoparticles can be used in various experimental systems, including high-throughput human cell-based labs-on-a-chip, a variety of additional *in-vitro* methods [8][9][10][11], as well as the animal experiments that may still be performed at this time, which include, but are not limited to, whole-body, head-only and nose-only. The method is not limited to the silver nanoparticles used in this example and may be used to generate other metallic nanoparticles with a similar melting temperature and evaporation rate, such as gold. However, this method is not applicable to the generation of nanoparticles of all metals.

INTERNATIONAL STANDARD **ISO 10801:2010(E)**

Nanotechnologies — Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method

1 Scope

This International Standard gives requirements and recommendations for generating metal nanoparticles as aerosols suitable for inhalation toxicity testing by the evaporation/condensation method. Its application is limited to metals such as gold and silver which have been proven to generate nanoparticles suitable for inhalation toxicity testing using the technique it specifies (see Annex A).

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 amendments) applies.

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

ISO 15900, *Determination of particle size distribution — Differential electrical mobility analysis for aerosol particles*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

OECD Test Guideline (TG) 403, *Acute Inhalation Toxicity*[1)]

OECD Test Guideline 412 (TG) 412, *Subacute Inhalation Toxicity: 28-Day Study*[1)]

OECD Test Guideline 413 (TG) 413, *Subchronic Inhalation Toxicity: 90-day Study*[1)]

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 27687 and ISO 15900 and the following apply.

3.1
differential mobility analysing system
DMAS
system used to measure the size distribution of submicrometre aerosol particles consisting of a DEMC, a particle charge conditioner, flow meters, a particle detector, interconnecting plumbing, a computer and suitable software

NOTE Adapted from ISO 15900:2009, definition 2.8.

1) Organization for Economic Cooperation and Development (OECD) publication.

3.2
differential electrical mobility classifier
DEMC
differential electrical mobility spectrometer
DEMS
classifier that is able to select aerosol particle sizes from a distribution that enters it and pass only selected sizes to the exit

NOTE 1 A DEMC classifies aerosol particle sizes by balancing the electrical force on each particle in an electrical field with its aerodynamic drag force. Classified particles have different sizes due to their number of electrical charges and a narrow range of electrical mobility determined by the operating conditions and physical dimensions of the DEMC.

NOTE 2 Adapted from ISO 15900:2009, definition 2.7.

3.3
condensation particle counter
CPC
instrument that detects particles and that can be used to calculate particle number concentration given the known flow rates into the detector

NOTE 1 The range of particles detected are usually smaller than several hundred nanometers and larger than a few nanometers. A CPC is one possible detector for use with a DEMC.

NOTE 2 In some cases, a condensation particle counter may be called a condensation nucleus counter (CNC).

NOTE 3 This definition is different from the one given in ISO 15900.

3.4
inhalation exposure chamber
inhalation chamber
exposure chamber
system prepared to expose experimental animals to an inhaled test substance of predetermined duration and dose by either the nose-only or whole-body method

NOTE 1 The term “nose-only” is synonymous with “head-only” or “snout-only”.

NOTE 2 Adapted from OECD TG 403, OECD TG 412, OECD TG 413.

3.5
evaporation/condensation nanoparticle generator system
device used to make a nanoparticle aerosol by the evaporation/condensation method, which can be connected to an inhalation chamber or other toxicity testing device

3.6
geometric mean diameter
GMD
measure of the central tendency of particle size distribution using the logarithm of particle diameters, computed for the DMAS by

$$\ln(\mathrm{GMD}) = \frac{\sum_{i=m}^{n} \Delta N_i \ln\left(d_i\right)}{N}$$

where

d_i is the midpoint diameter for size channel i;

N is the total concentration;

ΔN_i is the concentration within size channel i;

m is the first channel;

n is the last channel.

NOTE The GMD is normally computed from particle counts and, when noted, may be based on surface area or particle volume with appropriate weighting.

3.7
geometric standard deviation
GSD
measure of width or spread of particle sizes, computed for the DMAS by

$$\ln(\mathrm{GSD}) = \sqrt{\frac{\sum_{i=m}^{n} N_i \left[\ln d_i - \ln(\mathrm{GMD})\right]^2}{N-1}}$$

3.8
count median diameter
CMD
diameter equal to GMD for particle counts assuming a logarithmic normal distribution

NOTE The general form of the relationship as described in ISO 9276-5 is

$$\mathrm{CMD} = x_{50,r} = x_{50,p} \mathrm{e}^{(r-p)s^2}$$

where

e is the base of natural logarithms, e = 2,718 28;

p is the dimensionality (type of quantity) of a distribution, where

- p = 0 is the number,
- p = 1 is the length,
- p = 2 is the area, and
- p = 3 is the volume or mass;

r is the dimensionality (type of quantity) of a distribution, where

- r = 0 is the number,
- r = 1 is the length,
- r = 2 is the area, and
- r = 3 is the volume or mass;

s is the standard deviation of the density distribution;

$x_{50,r}$ is the median particle size of a cumulative distribution of dimensionality r.

4 Principle

4.1 Generation

The test airborne nanoparticles are generated by heating solid silver to evaporate silver from the solid silver precursor. The entrained silver vapour is then cooled to nucleate and the vapour condensed to form a silver nanoparticle aerosol. One experimental method that describes the generation of silver nanoparticles with the evaporation/condensation method is described in Annex A.

4.2 Preparation of system

4.2.1 Prior to interfacing the nanoparticle generating system with the exposure chamber or chambers, nanoparticle size analysis should be performed to establish the number concentrations and size distribution of nanoparticles and to determine the stability of the generated aerosol. For this process, parameters selected to generate the silver nanoparticle aerosol include flow rate, evaporation temperature, quench-zone length and temperature gradients, among others. During exposure tests, analysis should be conducted continuously and/or intermittently, depending on the method of analysis, so as to determine the consistency of particle size distribution without disrupting the inhalation exposure.

4.2.2 Inhalation chambers and supporting equipment shall be prepared in accordance with OECD TG 403, OECD TG 412 and OECD TG 413.

4.2.3 Inhalation chambers and supporting equipment shall be prepared for nanoparticle exposure studies.

NOTE 1 Aerosolized nanoparticles can be deposited to walls by Brownian diffusion and particle size change due to aggregation/agglomeration. This deposition process depends on the particle size, electrostatic charge, particle number concentration and residence time. See standard texts on aerosol science, viz. Reference [12].

NOTE 2 Charge neutralization might be required, depending on the purpose of the study.

If charge distribution is considered a characterization requirement, this shall be specified and measured in the study.

NOTE 3 To reduce deposition losses, conductive tubing of minimum length and diameter consistent with instrument tube diameters is selected to interface with instrumentation and thereby avoid expansions and restrictions.

4.2.4 An inhalation chamber or chambers and supporting equipment, such as sampling probes and manifolds, shall be characterized to ensure compliance with OECD TG 403, OECD TG 412 and OECD TG 413 or US EPA Guidelines[31], for determining any sampling bias.

NOTE The sampling manifold consisting of conductive tubing, solenoid valves and/or other elements required for routing samples from each inhalation chamber to on-line monitoring equipment may increase particle losses and alter downstream particle size distributions if losses are dependent upon particle size.

4.2.5 Measurement instruments used in inhalation testing shall be calibrated and/or tested in accordance with ISO/IEC 17025.

The differential mobility analysing system (DMAS) is usually calibrated at the factory and this should be documented in the report.

5 Requirements

5.1 Capacity and control

Output, reliability and control of the generator shall be adequate for the planned study, as follows:

a) metal evaporation rate (μg/h);

b) air flow rate (m^3/h);

c) continuous operation of generator at target evaporation and air flow rates for study duration to be considered.

5.2 Nanoparticle properties

5.2.1 The geometric mean diameter (GMD) of nanoparticles shall be less than 100 nm. This is accomplished primarily by controlling the metal evaporation and condensation rates and the residence time in each of the reactor zones. If, despite all reasonable effort, this requirement is unable to be met, expert judgement will need to be provided.

5.2.2 The geometric standard deviation (GSD) shall be less than 2 (as proposed in OECD TG 403, OECD TG 412 and OECD TG 413).

5.2.3 Test article purity, including particle purity and particle surface purity, shall be established to meet the objective of the study. Preferably prior to the start of the study, there should be a characterization of the test article that includes its purity and, if technically feasible, the name and quantities of unknown contaminants and impurities (OECD GD 39).

NOTE Determination of the chemical purity may require characterization of the surface chemistry of the generated particles in addition to bulk chemical purity.

5.3 Exposure chamber atmosphere

5.3.1 Air delivered to test animals shall be breathable, with an adequate oxygen content of at least 19 % (OECD TG 403, OECD TG 412 and OECD TG 413; US EPA Guidelines[31]).

This may be accomplished by supplying appropriate dilution air to the generator.

5.3.2 Care shall be taken that contaminants are not generated by evaporation of volatile compounds in binders, lubricants, finishes and sealants used in the aerosol generator. This can be accomplished by selection of appropriate materials and adequate bake-out of the system.

5.3.3 The temperature of the air delivered to the test inhalation chamber shall be within the limits for inhalation studies (OECD TG 403, OECD TG 412 and OECD TG 413; US EPA Guidelines[31]).

5.3.4 Supply air to both the generator and inhalation chambers shall be free of oil, volatile compounds and other contaminants, and shall be HEPA-filtered to remove aerosols, including nanoparticles, dust and microorganisms.

5.4 System operational safety

5.4.1 All local safety requirements shall be respected.

5.4.2 Contact with hot surfaces and electrical conductors associated with the electrical heater or other components shall be prevented.

5.4.3 Gas discharged to the atmosphere from the system shall be HEPA-filtered.

5.4.4 There shall be no measurable leaks to the atmosphere from the aerosol generator.

5.4.5 Exposure chambers should be maintained at negative pressure ($\leqslant$ 5 mm water) with respect to ambient conditions in order to avoid worker exposure in case of leakage. This pressure differential should be monitored on a continuous basis and arranged to be kept within alarm limits. An alternative approach is to maintain the apparatus at positive pressure with respect to ambient conditions to ensure that aerosols or airborne contaminants cannot enter the exposure chamber. The apparatus at positive pressure should be enclosed within ventilated secondary containment in order to minimize worker exposure.

For nose-only exposure, pressure should be slightly positive so as to ensure that animals will be properly exposed. Due to potential leakage from this positive pressure, nose-only experiments should be conducted inside the boundaries of an adequately designed fume hood (OECD GD 39).

NOTE Frequent leak checks, e.g. by the soap bubble method, or the installation of permanent leak detectors may be necessary when there is a risk of nanomaterial leakage. In nose-only exposure systems, the test atmosphere could leak around the animal where it meets the exposure apparatus. Leaks can be prevented by using a restraint system that seals the tube, although heat and moisture buildup in the tube is a concern[29].

6 Characterization of generator performance

6.1 Requirements for particle size distribution and mass concentration

Measurement of particle size distribution and total particle mass concentration are essential for the characterization of nanoparticles for inhalation toxicity testing. In the case of particle size distribution, this is because the knowledge of particle size influences dose and dose distribution while mass concentration is the dosimetric parameter used routinely in inhalation toxicity testing. In evaluating the nanoparticle aerosol generator used for inhalation toxicity testing, these particle size distribution and total particle mass-concentration measurements shall always be made.

6.2 Particle size distribution measurement

The method shall include near-continuous monitoring based upon the scan speed of the classification and detection instruments, with a time resolution appropriate for verifying the stability of the nanoparticle generator in terms of particle size distribution and concentration. The measurement method used shall be comprehensive for nanoparticle aerosols produced by the generator. The accuracy of particle size and concentration measurements shall be sufficient for nanoparticle toxicity testing, and may be validated by methods such as calibration against appropriate reference standards. The particle diameter range of particle sizing shall be sufficiently broad so that all relevant data are recorded to reduce errors in conversion from number-weighted distribution to surface-area-weighted or volume-weighted distribution.

NOTE For number-based particle size distribution, measurement using DMAS is the only currently available method that meets all the above requirements in the size range below 100 nm.

6.2.1 Sampling with DMAS

Nanoparticles should be measured following manufacturer's recommendations and in accordance with ISO 15900.

6.2.2 Sampling for microscopy

The filters on which the particles are sampled shall be coated with carbon (to reduce charging during analysis), mounted on an electron microscope grid (200 mesh), and observed under a transmission electron microscope (TEM). Diameters of randomly selected particles should be measured at 100 000× magnification, and analysed using an energy-dispersive x-ray analyser (EDXA) at an appropriate accelerating voltage for the target. ISO 10312[3] can be adapted for the sampling and analysis to determine nanoparticle morphology. Additional details on sampling may be obtained from other sources [6][13][15][17][18][27].

6.3 Mass concentration measured by filter sampling

Gravimetric filter analysis is the method used for measuring total nanoparticle concentrations, in which the test atmosphere is sampled from the animal's breathing zone. The mass concentration is calculated by dividing the mass of the nanoparticles collected on the filter by the volume of air passed through the filter.

NOTE Beta attenuation monitor (BAM), tapered element oscillating microbalance (TEOM), piezoelectric microbalance, gravimetric filter and other methods based on the chemical analysis of particles collected on filter media may meet requirements for nanoparticle mass-concentration measurement.

Obtaining adequate mass loading to generate data above lower detection limits should be considered [5].

Mass concentration can be derived from number-based size distribution measurement data by making an assumption regarding particle density, particularly for spherical particles, which may match bulk material density [19]. However, significant errors in calculated mass concentration may result if particle density is inaccurate or unknown. Therefore, derived mass concentration from number-weighted size distribution data shall be accepted only when no other acceptable methods meet the measuring requirements.

6.3.1 Filter sampling for aerosol mass concentration

Nanoparticle aerosol shall be sampled with a probe onto a membrane filter at an appropriate flow rate. Sampling times will be selected based on the ability to obtain weighable samples with a microbalance. The actual concentration is commonly expressed in mass units per unit volume of air (mg/l, mg/m^3).

6.3.2 Frequency of sampling

6.3.2.1 Frequency of sampling forms an important part of the study plan. Sampling shall be conducted as often as necessary to determine consistency of nanoparticle size distribution, number and mass dose. Individual exposure chamber concentration samples should not deviate from the mean chamber concentration by more than $\pm$ 20 % for nanoparticles (as proposed in OECD GD 39 and US EPA Guidelines[31]).

6.3.2.2 Process parameters for generating silver nanoparticle aerosol, such as the flow rate through the evaporation/condensation reactor zones and temperatures of the evaporation and condensation zones, shall be monitored continuously.

7 Nanoparticle generation specifications

7.1 Test particle purity/impurities

Test particle purity/impurities shall be established to meet the objective of the study.

7.2 Size range

The GMD of nanoparticles shall be less than 100 nm, measured at the breathing zone according to OECD TG 403, OECD TG 412 and OECD TG 413.

7.3 Number concentration

Number concentration shall be measured in the unit of particles, per unit volume of air, cm^{-3}, measured at the breathing zone according to OECD TG 403, OECD TG 412 and OECD TG 413.

7.4 Nanoparticle shape

7.4.1 Primary nanoparticles should be nearly spherical. The desired shape of the primary particle, however, may be different, based on the purpose of the study. Particle shape shall be documented as transmission electron microscopy (TEM) or scanning electron microscopy (SEM) images, or other methods; the degree of dispersion or aggregation/agglomeration should be evaluated quantitatively, if possible, using available methods.

7.4.2 Consideration should be given to the fraction of nonagglomerated nanoparticles.

7.5 Stability

Nanoparticles should remain in a dispersed non-agglomerated or agglomerated morphology, depending on the study objective. Stability of nanoparticle morphology shall be documented by TEM or SEM, or other methods; the degree of dispersed non-agglomerated or agglomerated morphology should be evaluated quantitatively, if possible, using available methods.

7.6 Animal exposure

7.6.1 The nanoparticle generator shall conform to the requirements of the inhalation studies.

7.6.2 The duration for use in the inhalation studies shall be established for the generation system.

7.6.3 There shall be ten to 15 air exchanges (continuous flow) per hour in whole body exposure, adequate oxygen content of at least 19 % and uniform conditions throughout the exposure chamber (OECD TG 403, OECD TG 412 and OECD TG 413; US EPA Guidelines[31]). For animals exposed in flow-past inhalation equipment (such as nose-only) designed to sustain a dynamic air flow, an adequate air exchange of at least two to three times the respiratory minute volume of animals exposed (i.e. at least 0,5 $l \cdot min^{-1}$ per exposure port for rats) shall be ensured (see OECD GD 39). Each exposure port should have similar exposure conditions, with an oxygen concentration of at least 19 % (see OECD GD 39).

7.6.4 Estimate the mass dose by DMAS and the filter-sampled dose.

8 Assessment of results

The generation shall demonstrate that stable and continuous operation can be achieved for use in short- and long-term inhalation toxicity testing. In order to comply with the current relevant guidelines for inhalation toxicity (such as OECD TG 403, OECD TG 412 and OECD TG 413), generation should be stable during the period of exposure in terms of number and diameter of nanoparticles (GMD and total number concentration). In addition, the concentration of nanoparticles in the exposure chamber should remain within 20 % of the mean.

The results shall be assessed with respect to conformity to study and quality plans.

9 Test report

9.1 The test report shall be in accordance with the test procedures used.

9.2 In addition, the test report shall include the following:

a) complete identification of the source material of nanoparticles generated (manufacturer's code, catalogue or formulation number, batch number or date of manufacture, trade name, etc.);

b) procedures for the preparation of test samples;

c) quantification of nanoparticle size distribution and number by DMAS.

Annex A
(informative)

Example method for evaporation/condensation generation of silver nanoparticles

A.1 General

Silver nanoparticles are often synthesized by evaporation/condensation, which is normally carried out in a tube furnace at atmospheric pressure. The source material within a boat centred at the furnace is vaporized into a carrier gas and then cooled at the exhaust of the furnace to form nanoparticles. Nanoparticles of various materials, such as Ag, Au, Pb and fullerene, have previously been produced using the evaporation/condensation method [11][18][21][23][24][25].

However, the generation of nanoparticles using a tube furnace has several drawbacks in terms of aerosol generator applications, because a tube furnace occupies a large space, consumes a great deal of energy and requires long periods to achieve thermal stability. A typical tube requires more than 3 kW, and 30 min or more, to attain stable operating temperature. Most importantly, for inhalation toxicology studies, the tube furnace raises the environmental temperature around the source material, which can negatively affect nanoparticle condensation as well as make aerosol temperatures unacceptably high for delivery to test animals.

This annex describes a new approach, where material is heated on the surface of a small, flat ceramic electrical heater.

A.2 Mechanisms

Source metal on the surface of the small (50 mm × 5 mm × 1,5 mm), flat, ceramic electric heater is vaporized and converted to nanoparticles in a carrier gas stream [16]. Because of the relatively small heating surface and short residence time during which carrier gas is exposed to the heater, the aerosol generated cools at a faster rate than with a tube furnace. As particles nucleate at the thin area near the heater surface and flow out with the carrier air, coagulation decreases rapidly due to the quenching and dilution effect of the low temperature carrier gas. Additionally, the thermophoretic force, a positively unipolar electric force, and diffusion mixing contribute to the formation of non-agglomerated spherical nanoparticles [17].

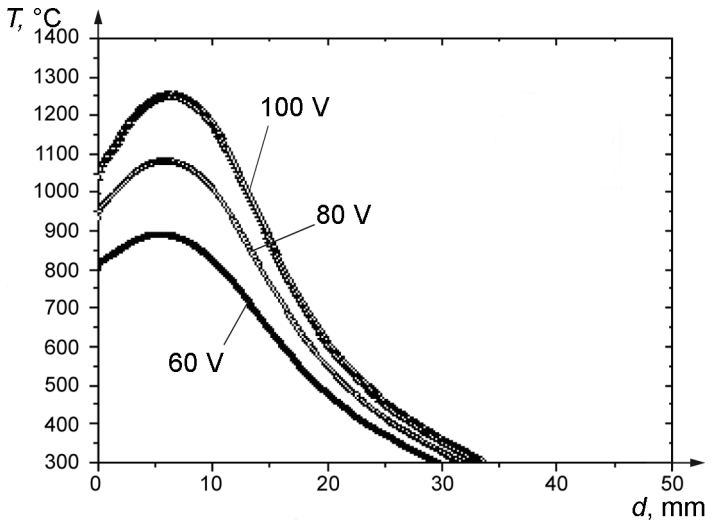
A.3 Temperature characteristics of small ceramic heater and exposure chamber

The properties of a heater with respect to temperature are important for nanoparticle generation. In the evaporation/condensation method in particular, a high temperature near the melting point of the source material is required. In order to obtain synthesized nanoparticles with stable characteristics, stable thermodynamic conditions are needed during their generation. The temperature distribution of the heater surface is depicted in Figure A.1, measured at various applied voltages using a thermocouple and infrared ray thermometer. Comparing surface temperatures of the heater, the respective emissivity at various surface temperatures is evaluated. Emissivity changes incrementally with temperature and is 0,9 for a temperature range of 894 °C to 1 417 °C, which is representative of the maximum temperature required (see Figure A.2).

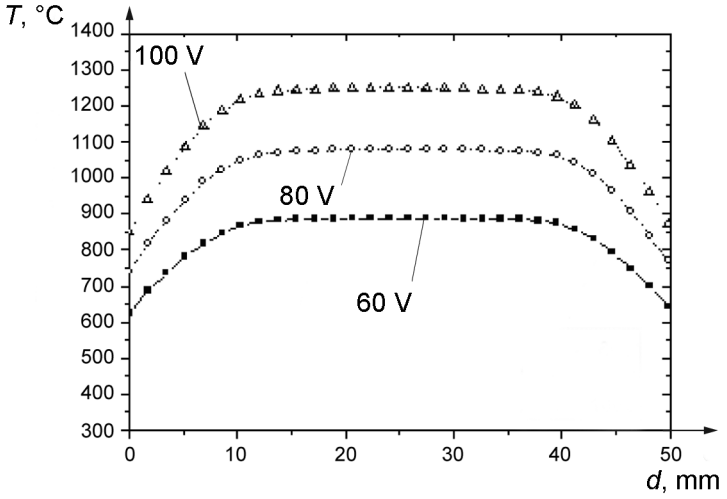
Using this value, the heater surface temperature distribution is measured. The high-temperature surface on the ceramic heater, required for this experiment, is a uniform 3 mm × 3 mm. Temperature characteristics of the exposure chamber are monitored and recorded.

As shown in Figure A.3, the time to reach maximum temperature is about 10 s for each target temperature. Compared with the tube furnace method, the small heater quickly attains requisite thermal conditions for

nanoparticle generation. After approximately 10 s, the surface temperature of the heater, which directly impacts evaporation rate of the source material, is maintained at a constant level. Hence, the small ceramic electric heater surface is appropriate for stable generation of nanoparticles.



a) X axis



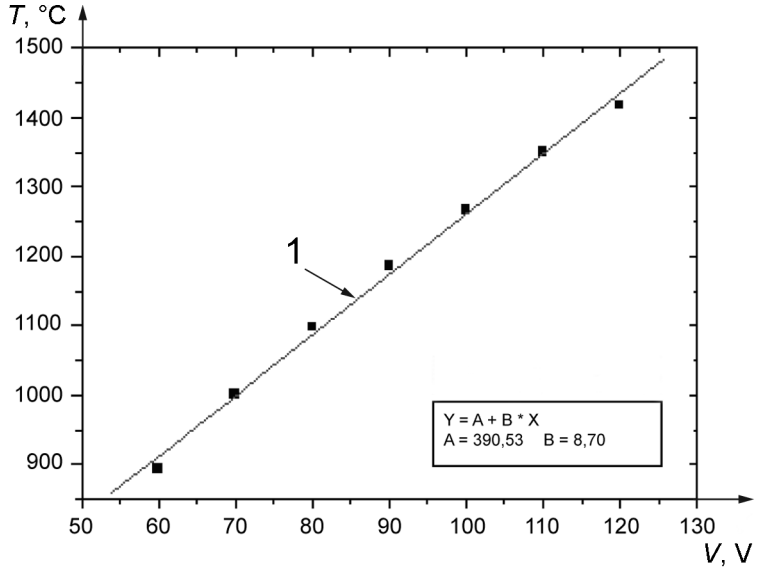
b) Y axis

Key

T temperature

d distance

Figure A.1 — Temperature distributions about X and Y axes for heating surface of small ceramic heater using various applied voltages [13]

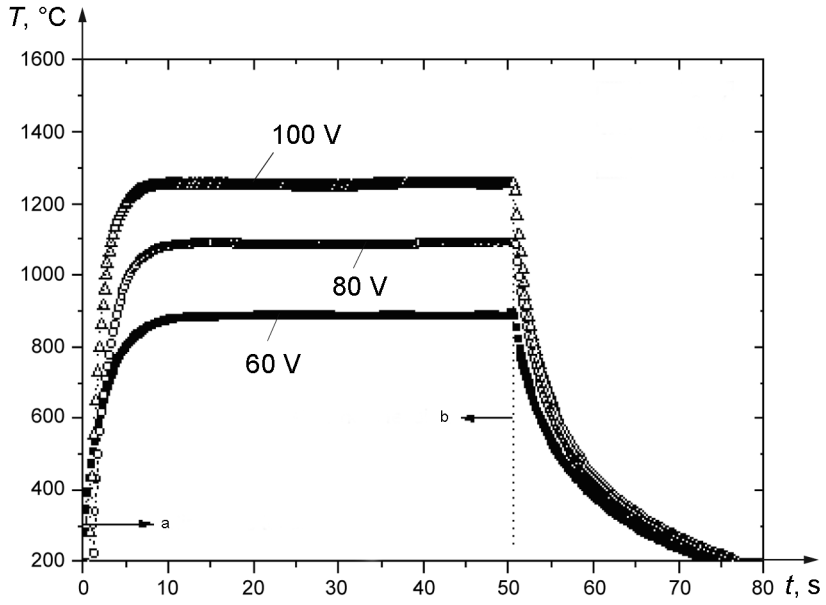


Temperature increases almost linearly with the applied voltage.

Key

T temperature
V applied voltage
1 linear fitting

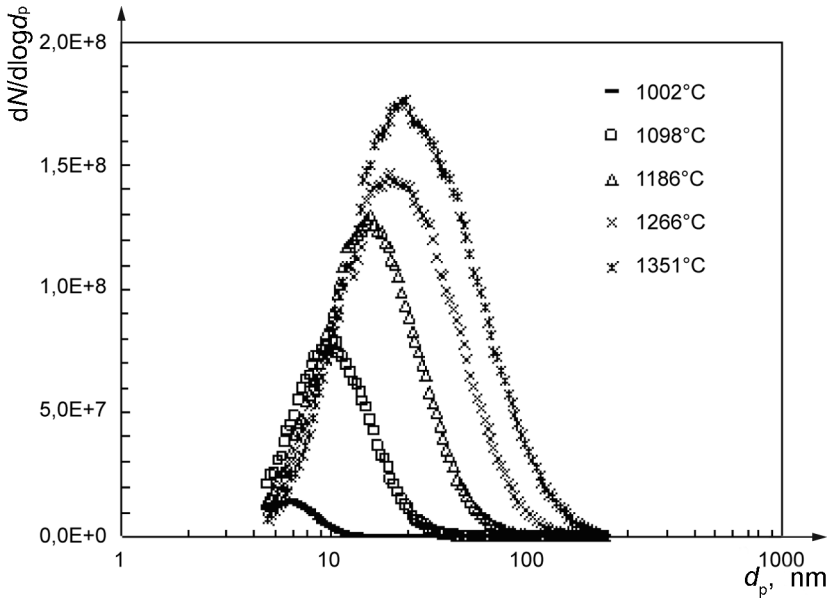
Figure A.2 — Maximum temperature of heater as function of applied voltage [13]



Key

T temperature
t time
a Power on.
b Power off.

Figure A.3 — Temperature as function of time for various applied voltages [13]



Carrier gas flow rate is constant at 4 l·min^{-1}.

Generator case diameter: 34 mm; initial silver loading mass: 14,76 mg.

Key

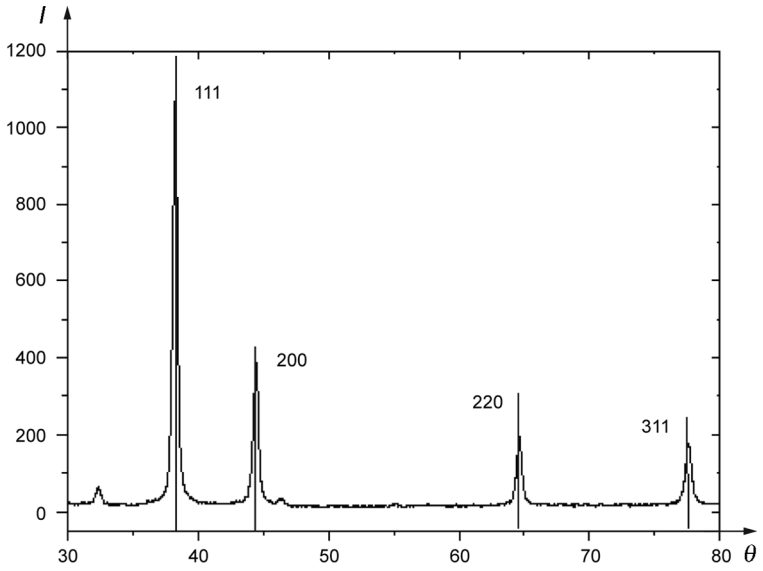
dN/dlogd_p	particles per cm^3
d_p	mobility diameter

Figure A.4 — Silver nanoparticle size distributions generated using different heater surface temperatures [13]

A.4 TEM and X-ray diffraction (XRD) analysis of silver nanoparticles under air carrier gas

Particle size distribution is affected by the heater temperature as described in Figure A.4. TEM images of silver nanoparticles created at various heater temperatures indicate that nanoparticles are spherical and non-agglomerated [13]. In the initial stage of particle formation, those growing via coagulation are sintered into spherical particles because of the high temperature near the source material [20]. Because the small electric ceramic heater system has a high-temperature surface, generated silver nanoparticles may grow spherically via the same process. However, as nucleated particles at the small heater surface flow out with the carrier air, coagulation between particles decreases rapidly due to quenching and the dilution effect. Additionally, the thermophoretic force near the source material, a positive unipolar electric force, and diffusion mixing by the local high-temperature gradient, contribute to the formation of non-agglomerated spherical nanoparticles.

Figure A.5 shows the results of XRD analysis of Ag nanoparticles performed using an X-ray diffractometer and CuK radiation. As shown in the crystal structure peaks, the synthesized silver nanoparticles are not silver oxides, but pure silver, even when air is used as a carrier gas. However, it is possible to form some oxidized silver nanoparticles using this generation method.



Key

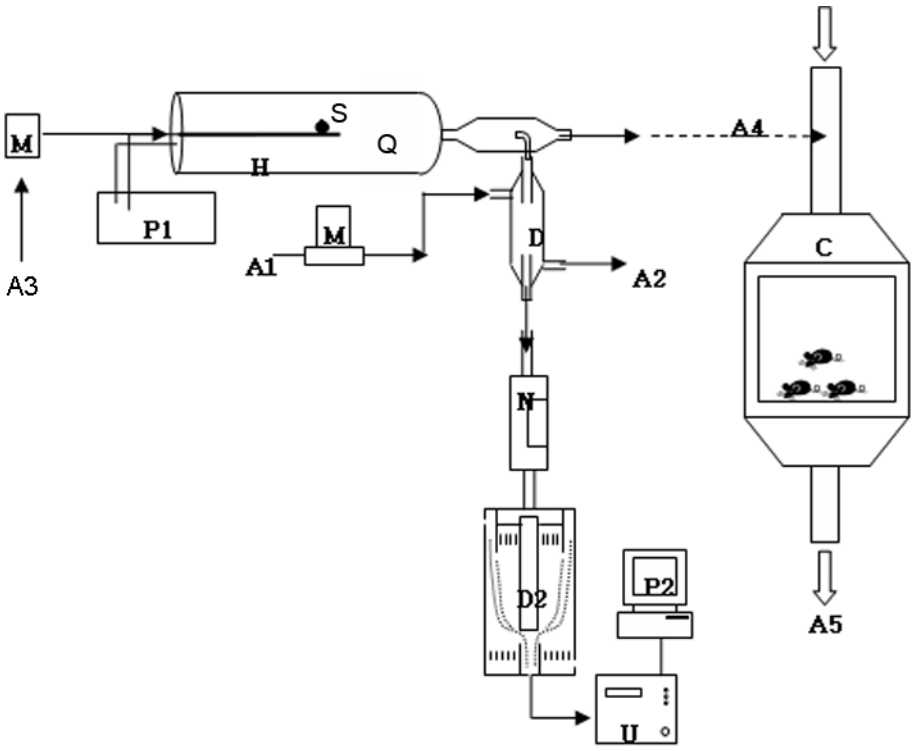
I intensity (arbitrary unit)

θ theta angle (degrees)

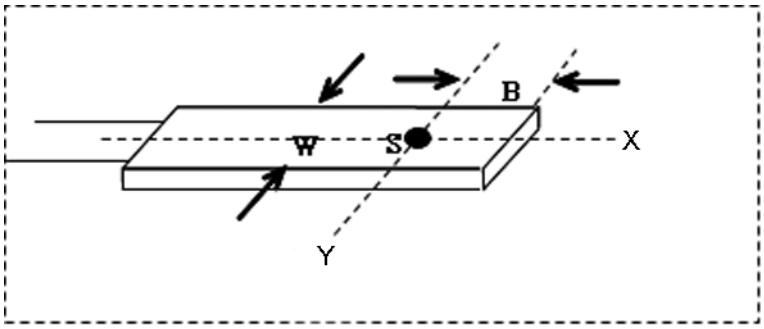
Figure A.5 — XRD analyses of Ag nanoparticles [13]

A.5 Long-term stability characteristics for inhalation toxicity studies

The experimental set-up for the long-term experiment consists of two main parts: one for the synthesis of silver nanoparticles by aerosol generation and the other for dilution and measurement (see Figure A.6.) [14]. For aerosol generation, a small, flat-plate ceramic heater is connected to an AC power supply and housed within a quartz case. The case is 70 mm in diameter and 140 mm in length. An overall heater element dimension of 50 mm × 5 mm × 1,5 mm is capable of generating a surface temperature of approximately 1 500 °C with a local heating area of 5 mm × 10 mm. When 85 V is applied, the highest temperature on the local heating area, T_{max}, is 1 140 °C. Silver source material (99,99 % pure) is located at the position of highest temperature. Dry, filtered air is used as the carrier gas, with laminar flow maintained at 22 l·min^{-1} (Reynolds Number of approx. 420) using a mass flow controller (MFC). The size distribution of silver nanoparticles is measured using a DMAS and a condensation particle counter (CPC).



a) Arrangement of components



b) Ceramic heater detail

Key

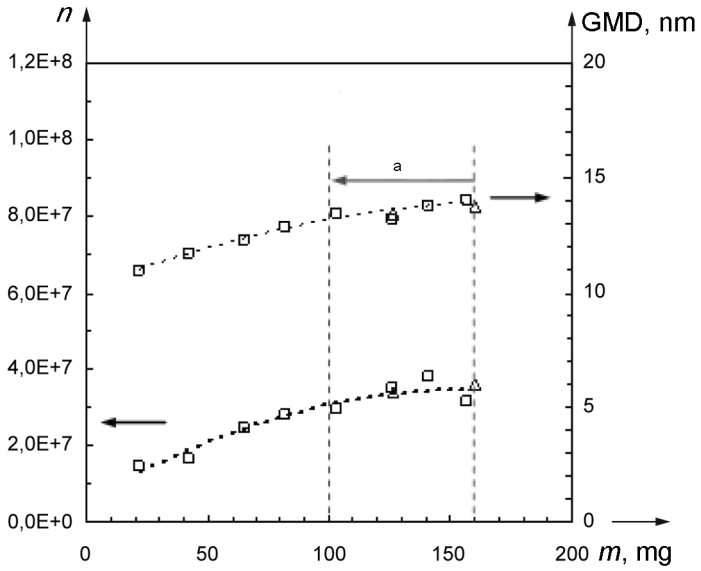
A1	HEPA-filtered air at 200 l·min^{-1}	D	dilutor
A2	excess air	N	neutralizer (^{210}Po)
A3	dried filtered air	D2	differential electrical mobility classifier (DEMC)
A4	nanoparticle containing air at 22 l·min^{-1}	U	ultrafine condensation particle counter (UCPC)
A5	exhaust air at 222 l·min^{-1}	P2	personal computer
M	mass flow controller (MFC)	C	inhalation chamber
P1	AC power supply	W	heater width, 5 mm
Q	quartz tube	B	source material to edge distance, 6,2 mm
H	ceramic heater		
S	source material		

Figure A.6 — Schematic diagram of experimental set-up [14]

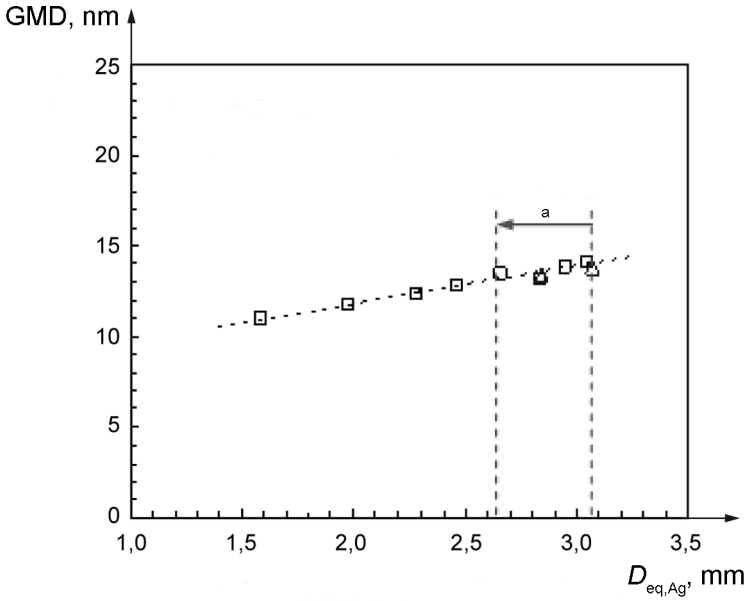
A.6 Estimation of long-term size distribution by changing the loaded mass of silver

For the evaluation of the health effects of nanoparticle inhalation, nano-sized particles are delivered to the test environment containing experimental animals, which are subsequently tested for effects of inhalation toxicity. These studies are performed for short- or long-term exposure. The silver-nanoparticle generation method provides a consistent particle-diameter distribution and stable number concentrations, suitable for both short- and long-term inhalation toxicity studies.

Figure A.7 a) shows the size distribution of silver nanoparticles as measured in the stability test, corresponding to data for the change in loaded mass of evaporating silver [14]. Figure A.7 b) shows the change in GMD, decreasing slowly as the mass of loaded silver evaporates from 160 mg to 100 mg, calculated to be volumetric equivalents of diameters 3,08 mm and 2,63 mm, respectively. For a silver mass of less than 100 mg, GMD decreases relatively quickly as the silver evaporates. GMD is affected by the diameter and surface area of the loaded silver lump. If the initial loading mass is 160 mg, it is possible to generate consistent-size silver nanoparticles until 60 mg of the loaded mass is evaporated and 100 mg of the lump remains.



a) Variation of number concentration and GMD of particles with silver mass on heater



b) Change in GMD with size of bulk source material

Generator case diameter: 70 mm.

Key

n	number concentration, particles per cm^3
m	mass of silver on heater surface
GMD	geometric mean diameter
$D_{eq,Ag}$	equivalent diameter of silver
□	mass change data
△	stability data

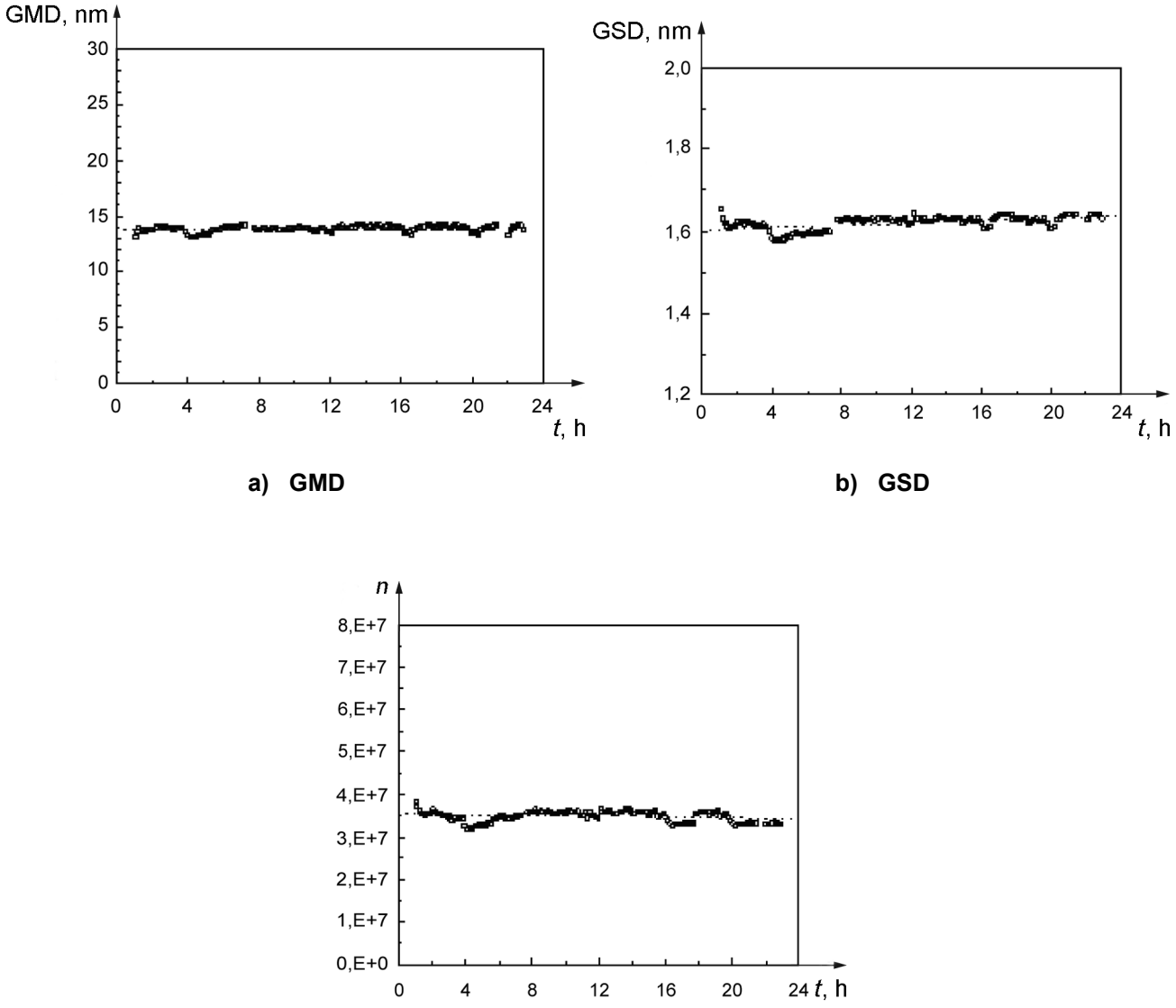
[a] 36 hours.

Figure A.7 — Variation of number concentration and GMD of particles with silver mass and variation of GMD with equivalent diameter of silver on heater — Comparison between 24 h long-term stability test data and predicted data from initial mass vs. mass reduce rate using short-term test [14]

A.7 Long-term stability test and distribution of nanoparticles

A 160 mg initial bulk silver load is selected for the long-term test. A 160 mg silver sphere has a diameter of 3,08 mm. The GMD and GSD of nanoparticles generated from this load are 14 nm and 1,6 nm, respectively [see Figures A.8 a) and b)]. The total number concentration is $3,5 \times 10^7$ particles/cm^3 [14] (see Figure A.8 c)].

Figure A.9 shows the size distribution of silver nanoparticles over time up to 24 h for a long-term inhalation toxicology test. Figures A.9 a), b) and c) show the number distribution, surface-area distribution and mass distribution, respectively. DMAS software is used to calculate surface area and mass on the basis of particle diameter, assuming the nanoparticles are spherical. The approximate surface-area concentration is obtained from the particle-number size distribution as silver nanoparticles are nearly spherical and non-aggregate. During operation of the heater as described in Reference [14], the silver maintained its spherical shape for the duration of the operation due to the surface tension of the liquid silver. A solid silver sphere formed on the heater surface when the applied voltage was initially turned on; however, this shape eventually collapsed due to the pull of gravity. The bulk silver source maintained its size and shape throughout the 24 h of operation [14].



a) GMD

b) GSD

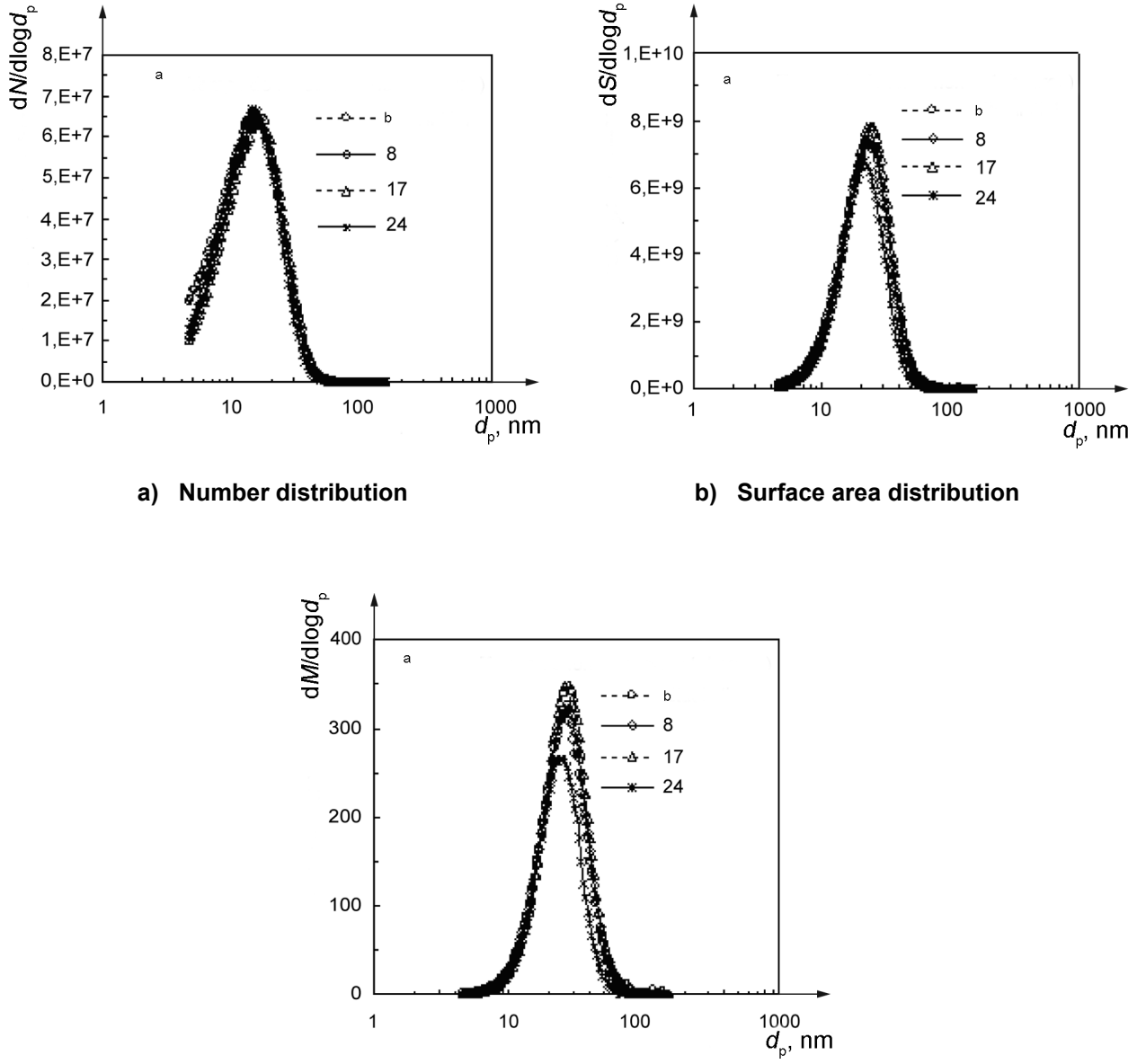
c) Total number concentration

Initial silver loading mass: 160 mg
Air flow rate: 22 l·min^{-1}
Applied voltage: 85 V (T_{max} = 1 140 °C)
Generator case diameter: 70 mm

Key

GMD	geometric mean diameter
GSD	geometric standard deviation
t	operation time
n	number concentration, particles per cm^3

Figure A.8 — Variations of GMD, GSD and total number concentration of Ag nanoparticles with time for long-term stability testing[14]



a) Number distribution

b) Surface area distribution

c) Mass distribution

Generator case diameter: 70 mm.
Initial loading silver mass: 160 mg.

Key

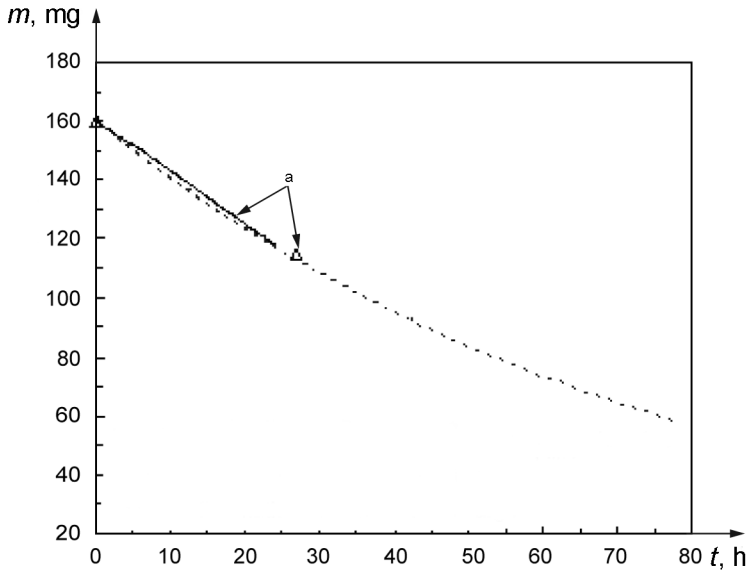
dN/dlogd_p	particles per cm^3
dS/dlogd_p	cm^{3}per cm^3
dM/dlogd_p	µg/m^3
d_p	mobility diameter
n	number concentration, particles per cm^3

a Operation time (hours).

b Initial state.

Figure A.9 — Size distributions of silver nanoparticles with time for long-term inhalation toxicology test [14]

Silver mass decreases with operation time as shown in Figure A.10. The initial loaded silver mass is 160 mg. Silver loading mass is weighed by microbalance after 24 h of continuous operation. This gravimetric data is similar to the expected mass data calculated from DMAS data collected from 24 h continuous monitoring. The number concentration calculated from DMAS data is converted to mass concentration for the toxicity study.



Key

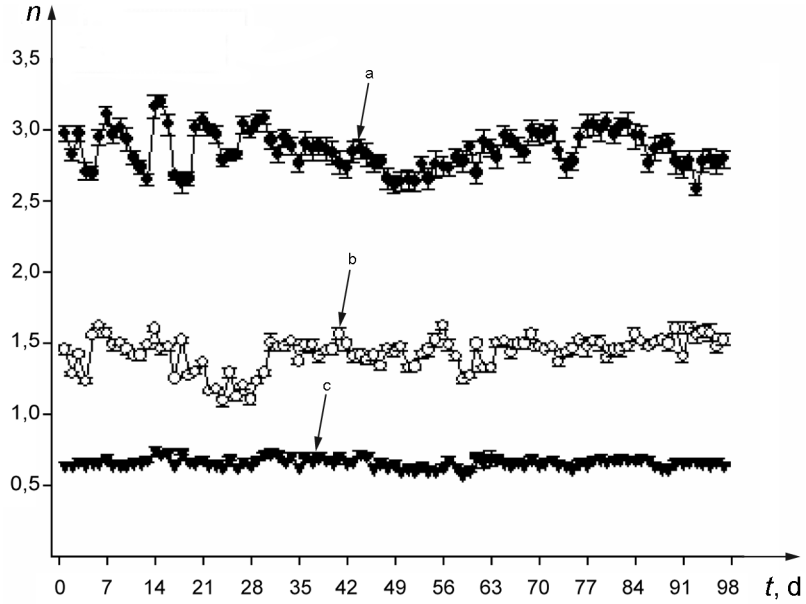
m	loading silver mass
t	time
———	SMPS estimation
△	microbalance measure
- - - - - - - -	loading change estimation

a From stability data.

Figure A.10 — Comparison between long-term 24 h stability test data and the predicted data from initial vs. mass reduction rate using the short-term test [14]

A.8 Stability of silver-nanoparticle generation and maintenance concentrations during 90-day inhalation toxicity study

As shown in Figure A.11, concentration of silver nanoparticles is maintained consistently during the 90-day inhalation toxicity study [26]. The generator is stable enough and suitable for subacute inhalation exposure study.



Key

n number (# $\times$ 10^6 particles/cm^3)

t time, days

a High.

b Middle.

c Low.

Figure A.11 — Maintenance of concentrations during 90-day silver nanoparticle generation and inhalation exposure [26]

A.9 Generation of other metal nanoparticles (e.g. gold)

The method described in this annex is not limited to silver nanoparticles, but may be used to generate gold or other metallic nanoparticles having a similar melting temperature and vaporization rate. Although the evaporation rate of gold is low and generation is not easy [22], a useful concentration of gold nanoparticles can be generated with this device [28].

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