
**Wear of implant materials — Polymer and
metal wear particles — Isolation,
characterization and quantification**

*Usure des matériaux d'implant — Particules d'usure des polymères et
des métaux — Isolation, caractérisation et quantification*



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Foreword

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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.

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Introduction

The biological responses to wear debris contribute to the failure of joint prostheses through bone resorption and consequent implant loosening. A standardized method of particle retrieval from the tissue, followed by debris characterization and quantification, is required for a uniform approach to debris response investigations. The examination of the debris generated from implants in joint simulators also provides valuable information on the wear properties and performance of the implant being studied.

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Wear of implant materials — Polymer and metal wear particles — Isolation, characterization and quantification

1 Scope

This International Standard specifies methods for sampling wear debris generated by total joint prostheses in humans and in joint simulators. It specifies the apparatus, reagents and test methods to isolate, characterize and quantify both polymer and metal wear debris from samples of tissue excised from around the joint prosthesis, obtained at revision surgery or post mortem, and from samples of joint-simulator test fluids.

The method given in this International Standard does not quantify the level of wear the implant produces, nor does it determine the amount of wear from any particular surface. This International Standard does not cover the biological effect of wear debris or provide a method for evaluation of biological safety.

The method given in this International Standard is not applicable to the measurement of poly(methyl methacrylate) (PMMA) debris.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1

polymer wear debris

particles of ultra-high molecular weight polyethylene (UHMWPE) generated from the wear of polymeric components of a joint prosthesis

2.2

metal wear debris

particles and particulate corrosion products generated from the wear of metal components of a joint prosthesis

3 Methods of sampling and analysis of polymer and metal wear debris from tissue samples

3.1 Principle

Particles of polymeric and metal wear debris are released from tissue samples by digestion (see 3.5.1 and 3.6.1). The yield of each particle species is then purified by eliminating any remaining organic debris.

NOTE The methods involved in polymer and metal particle isolation are slightly different and are described in 3.5 and 3.6, respectively.

The particles are collected, and are characterized and counted using scanning electron microscopy (SEM) or transmission electron microscopy (TEM). The concentration of particles in the original tissue sample is then calculated.

3.2 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled water or water of equivalent purity.

All reagent solutions shall be filtered through a filter of 0,2 μm pore size or less (3.3.4) prior to use, to avoid contamination of the sample by extraneous particles.

3.2.1 Sodium hydroxide solutions (NaOH), $c = 0,1 \text{ mol/l}$ and 5 mol/l .

3.2.2 Hydrochloric acid solution (HCl), $c = 0,01 \text{ mol/l}$.

3.2.3 Sucrose solutions, $\rho = 1,35 \text{ g/cm}^3$, $1,17 \text{ g/cm}^3$, $1,08 \text{ g/cm}^3$, $1,04 \text{ g/cm}^3$ and $1,02 \text{ g/cm}^3$.

3.2.4 Isopropanol-water mixtures, $\rho = 0,96 \text{ g/cm}^3$ and $\rho = 0,90 \text{ g/cm}^3$.

3.2.5 Distilled water.

3.2.6 Papain solution.

Mix 100 μm of pure papain, 3,26 mg of *N*-acetylcysteine, 9 ml of distilled water and 1 ml of phosphate buffer (3.2.7).

3.2.7 Phosphate buffer.

Dissolve 3,55 g Na_2HPO_4 and 3,45 g of NaH_2PO_4 in 100 ml of distilled water to obtain a solution of pH 6,5; add 0,744 g EDTA added after pH titration.

3.2.8 Ethylenediaminetetraacetic acid solution (EDTA).

Dissolve 250 g of EDTA salt in 1,75 l of distilled water.

3.2.9 Fixative, e.g. formalin.

3.2.10 High density polyethylene (HDPE) powder (particle length $< 10 \mu\text{m}$).

3.2.11 Titanium powder (particle length $< 10 \mu\text{m}$).

3.3 Apparatus

All apparatus shall be cleaned and triple-rinsed with distilled water which has been filtered through a filter of pore size 0,2 μm or less (3.3.4) before use, to remove any contaminant particles.

3.3.1 Lint-free cloth.

3.3.2 Ultracentrifuge.

3.3.3 Ultracentrifuge tubes.

3.3.4 Filters, with pore size of 0,2 μm or less for filtering reagents and distilled water.

3.3.5 Polycarbonate membrane filters, of pore sizes 0,1 μm and 0,02 μm , for collecting particles.

3.3.6 Agitating temperature-controlled water bath.

3.3.7 Pipettes, at least one of which is fine-tipped.

3.3.8 Petri dishes, with lids.

3.3.9 Ultrasonicator.

3.3.10 Polarizing light microscope.

3.3.11 Scanning electron microscope (SEM).

3.3.12 Transmission electron microscope (TEM).

3.3.13 Aluminium stub.

3.3.14 Carbon sticker.

3.3.15 Fourier transform infra-red (FTIR) spectroscope.

3.3.16 Syringe, with wide-bore needle.

3.3.17 Filtration unit.

3.3.18 Balance, with an accuracy of at least 0,1 mg.

3.4 Storage and preparation of samples

Store the tissue frozen in a freezer at -70°C or in a fixative such as formalin. Thaw the tissue, if applicable, and rinse it thoroughly in distilled water before continuing with the extraction method. Remove excess water from the rinsed tissue by blotting with a lint-free cloth. Accurately measure the wet mass of the tissue and record the volume of the subsequently applied solutions to enable the calculation of the number of wear particles per gram of wet tissue (see 3.10).

Unfixed tissue should be handled under universal conditions.

Dry tissue may also be used, and the number of wear particles per gram of dry tissue calculated.

The nature of surgical instruments used for sample retrieval should be recorded in case of contamination.

NOTE Sampling variability due to specimen origin may occur.

3.5 Procedure for polymer particle isolation

3.5.1 Tissue digestion

Cut the tissue into smaller pieces using a scalpel and blade before digestion, to speed up the digestion times. Extract the lipids from the minced tissue by placing into a 2:1 (volume ratio) chloroform: methanol mixture for 24 h or until the tissue sinks to the bottom of container. Remove and rinse the tissue.

Add 5 mol/l NaOH to the tissue in a ratio 10 ml of NaOH to 5 g tissue, and leave to digest for 1 h to 3 h in an agitating water bath at 65°C . Digestion can be judged to be complete when no visible solid pieces of tissue remain in the suspension.

3.5.2 Purification of the polymer particle yield

Place sucrose solution ($\rho = 1,02 \text{ g/cm}^3$) into ultracentrifuge tubes so that the tubes are roughly three-quarters full, and apply measured aliquots of the digested tissue suspension to the surface of the sucrose solution in each tube. Ultracentrifuge at $100\,000\,g$ for 3 h at 2°C . Carefully collect the top layer into a sterile tube and dilute with distilled water at 65°C to help dilute the residual sucrose. Ultrasonicate for 10 min to break up the agglomerated particles and then heat for 1 h at 80°C to dissolve the sucrose.

Apply measured volumes of the suspension to two layers of isopropanol-water mixture of densities $0,90 \text{ g/cm}^3$ and $0,96 \text{ g/cm}^3$ formed in the ultracentrifuge tubes. Ultracentrifuge these at $100\,000\,g$ for 1 h at 20°C . After removing the tubes from the ultracentrifuge rotor, a layer of white particles should be visible at the interface of the two layers. Remove this layer, containing the UHMWPE particles, and place into a sterile tube using a fine-tipped glass pipette inserted through the top isopropanol layer. Ultrasonicate for 10 min to break up any aggregates.

Different ultracentrifugation times and speeds may be used, provided that they have been demonstrated to give the same degree of separation and the results of the verification procedure have been documented.

NOTE The first ultracentrifugation step serves to separate the lighter UHMWPE wear debris from the heavier fractions. The second ultracentrifugation step purifies the UHMWPE particle yield by putting it through a finer density gradient.

3.6 Procedure for metal particle isolation

3.6.1 Tissue digestion

Cut tissue into smaller pieces using a scalpel and blade before digestion, to speed up the digestion times. Add 5 mol/l NaOH to the minced tissue in a ratio of 10 ml of NaOH to 5 g tissue in ultracentrifuge tubes and leave for a maximum of 1 h in an agitating water bath at 65 °C.

3.6.2 Purification of the metal particle yield

Remove the ultracentrifuge tubes containing the digested tissue suspension from the water bath and dilute with distilled water to the total volume of the ultracentrifuge tubes. Ultracentrifuge for 1 h at 100 000 *g* at 15 °C. Collect the pellet and resuspend the particles by addition of distilled water. Ultrasonicate the suspension for 10 min.

Prepare sucrose density gradients with sucrose layers $\rho = 1,04 \text{ g/cm}^3$, $1,08 \text{ g/cm}^3$, $1,17 \text{ g/cm}^3$ and $1,35 \text{ g/cm}^3$ in ultracentrifuge tubes. Apply measured aliquots of the resuspended pellet to each of the sucrose density gradient tubes. Ultracentrifuge at 100 000 *g* for 3 h at 2 °C. After ultracentrifugation, discard the supernatant and retain the pellet containing the heavier particles. Wash the pellet in distilled water by resuspending the pellet in distilled water at 65 °C, followed by centrifugation at ambient temperature to reform the pellet, and discarding the supernatant. Repeat this procedure at least three times to ensure that all of the viscous sucrose solution is rinsed away. After the final wash with distilled water, discard the supernatant and transfer the pellet to a sealable tube.

To eliminate bone particles from this pellet, add EDTA solution and leave for 3 h in an agitating water bath at 65 °C. Following treatment with EDTA, reform the pellet by centrifuging the suspension at ambient temperature and discard the supernatant. Add papain solution to the pellet to eliminate any remaining organic debris. Readjust the pH of the solution to 6,5 after addition to the pellet by using small amounts of 0,01 mol/l HCl and 0,1 mol/l NaOH solutions. Leave this suspension in the sealed tube in an agitating water bath at 65 °C for 18 h to 24 h. After papain treatment, ultracentrifuge the suspension over a sucrose density gradient as described previously. Discard the supernatant and wash the pellet with distilled water three times. After the final wash, resuspend the particles in 10 ml of distilled water, in sealed tubes, and ultrasonicate for 10 min to break up particle aggregates.

Different ultracentrifugation times and speeds may be used, provided that they have been demonstrated to give the same degree of separation and the results of the verification procedure have been documented.

3.7 Collection of particles

Collect particles onto filters of pore size 0,1 μm . Use either 10 μm to 300 μm aliquots of the particle suspension or larger volumes of diluted suspension (see Note). If diluting, record the exact dilution for each sample. For filtration, take up a known volume of the particle suspension into a syringe through a wide-bore needle. Discard the needle, having first ensured that it is free of liquid, and attach the end of the syringe to the filtration unit. Apply gentle pressure to the syringe to push the water through the filter and out at the other end of the filtration unit at a rate of about 1 drop (0,045 ml) per second, depositing the particles on the surface of the filter. Change the filter if it becomes blocked. Carry out the following procedure on each filter used. Flush the filter and syringe through with distilled water. Finally, flush the filter with air in the same direction as the filtration before removing the filter from the unit with tweezers and leaving it to dry in a clean, covered Petri dish.

If available, filters with a pore size smaller than 0,1 μm may be used. In this case, sequential filtering may be required to avoid pore clogging.

NOTE The appropriate dilution is achieved when the suspension appears almost clear, usually at dilutions between 1:10 and 1:100. The aim is to produce a concentration of particles on the filter which is not so dense as to make visualization of discrete particles difficult in the SEM, while ensuring a reasonable number of particles is available for analysis (at least 100 particles).

3.8 Particle size and shape characterization

For SEM imaging of particles larger than $0,1\text{ }\mu\text{m}$, attach the filter with particles on it to an SEM mount using a carbon sticker. For polymeric particles, coat the filter with gold to make the particles conductive. For metal particles, coat the filter with carbon or gold. Image the polymer particles at an accelerating voltage of not greater than 10 keV and metal particles at an accelerating voltage not greater than 15 keV.

To characterize particles larger than $0,1\text{ }\mu\text{m}$, select random non-overlapping fields on the filter carrying the particles at a magnification of $\times 5\,000$, until a total of 100 particles have been imaged. For particles larger than $10\text{ }\mu\text{m}$, use a lower magnification such as $\times 1\,000$.

Characterise the size, shape and area of the particles using a series of pre-defined descriptions such as length, breadth, equivalent circle diameter (diameter of a circle with the same area as particle), area, perimeter, aspect ratio (length/breadth) and roundness ($\text{perimeter}^2/4\pi \times \text{area}$). The magnification at which size and shape analysis was performed shall be stated in the test report.

For characterization of metal particles smaller than $0,1\text{ }\mu\text{m}$, use TEM. Place aliquots of the metal particle suspension onto copper TEM grids. Use magnifications of $\times 20\,000$ to $\times 25\,000$ at an accelerating voltage of 80 keV.

NOTE 1 All particles can be imaged using scanning electron microscopy (SEM), and metal particles smaller than $0,1\text{ }\mu\text{m}$ can be imaged using transmission electron microscopy (TEM).

NOTE 2 For metal particles, it might not be possible to image as many as 100 particles.

NOTE 3 Differentiation of fibrillar and rounded polymer particles may also be useful.

NOTE 4 Computerized or manual image-analysis software can be used to determine particle sizes and shapes.

NOTE 5 Particle analysers may also be used if their limit of resolution is $0,1\text{ }\mu\text{m}$ or less, however, there is a risk of size overestimation due to particle agglomeration.

3.9 Control study

Add accurately measured quantities of commercially available high density polyethylene (HDPE) powder (particle length $< 10\text{ }\mu\text{m}$) (3.2.10) and titanium powder (particle length $< 10\text{ }\mu\text{m}$) (3.2.11) to an accurately measured quantity of capsule tissue from primary total joint replacement surgery. Isolate the control powders from the tissue in accordance with 3.5 and 3.6, but wash the particles in alcohol rather than water after the final purification step, then leave until the alcohol has evaporated. Obtain the mass of the HDPE and titanium residues by differential weighing (tube with residue minus empty tube) using a balance with minimum accuracy of $0,1\text{ mg}$. Repeat this procedure three times and calculate a mean retrieval ratio (see Note). In addition to the retrieval ratio, also calculate the number of control particles before and after the control study according to 3.10. For evaluation of experimental reproducibility, perform the control study three times and calculate a mean and a standard deviation.

NOTE The retrieval ratio is the mass of retrieved particles divided by the mass of actual starting particles.

3.10 Calculation of the number of particles

The number of retrieved particles per gram of wet tissue shall be calculated by counting the number of particles in a known area of SE micrograph and correcting this according to the dilution ratio used in the extraction method and the retrieval ratio obtained from the control study. Fields without particles shall be included in the calculations.

Calculations of numbers of particles larger than 10 µm and smaller than 0,1 µm can be performed by repeating the calculations on images of the particles at magnifications of ×1 000 and ×20 000 to ×25 000, respectively. The exact magnifications at which the numbers of particles were calculated shall be reported in the test report.

The following example illustrates the calculation procedure:

a) **Data:** Metal particles are extracted from 3,01 g of wet tissue. The total dilution throughout the extraction procedure is 1/3250. After filtration, 141 particles are counted on 7 SE micrographs at a magnification of ×5 000.

b) **Calculate** the total number of particles on the whole filter:

$$\text{Area of 1 SE micrograph} = 3,35 \times 10^{-10} \text{ m}^2$$

$$\text{Therefore area of 7 SE micrographs} = 2,35 \times 10^{-9} \text{ m}^2$$

$$\text{Total area of filter} = \pi \times (0,0045)^2 = 6,35 \times 10^{-5} \text{ m}^2$$

$$\text{Therefore total number of particles on whole filter} = (141 \times 6,36 \times 10^{-5}) / 2,35 \times 10^{-9} = 3,82 \times 10^6$$

c) **Correct** this number according to the dilutions made throughout the procedure:

$$\text{Total dilution ratio through extraction method} = 1/3250$$

$$\text{Therefore corrected number of particles in 3,01 g of wet tissue} = 1,24 \times 10^{10}$$

d) **Correct** this number according to the retrieval ratio obtained from the control study:

$$\text{Retrieval ratio} = 37,4 \%$$

$$\text{Therefore corrected number of particles in 3,01 g of wet tissue} = 1,24 \times 10^{10} \times 1,626 = 2,02 \times 10^{10}$$

e) **Result:** The number of particles per gram of wet tissue = $6,70 \times 10^9$.

NOTE This number is an estimate of the particles present in the tissue studied, because the retrieval ratio is an estimate.

3.11 UHMWPE particle verification

Verification of the identity of the retrieved particles as UHMWPE shall be carried out using FTIR spectroscopy. Particles shall be prepared for FTIR spectroscopy by drying and pressing into potassium bromide (KBr) discs or using a microscope attachment.

Particles shall be considered to be UHMWPE if the dominant peaks in the FTIR spectra are comparable to those of a reference UHMWPE spectrum, such as the spectrum obtained from medical grade UHMWPE powder.

Particle morphology may be used as an additional basis for identifying UHMWPE particles by reference to published images of UHMWPE particles (see e.g. ASTM F1877-98).

NOTE Particles from different samples may need to be pooled to provide a sufficient volume of material for FTIR spectroscopic analysis.

3.12 Metal particle verification

The identity of the metal particles shall be determined using SEM-EDS (energy-dispersive spectroscopy) or TEM-EDS.