Suspending and diluting Nanomaterials

Metal Oxides and NM purchased as monodisperse suspensions

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1 Introduction

Cytotoxicity assays are performed with cells growing in cell culture medium. Therefore nanomaterials (NM) have to be suspended in an aqueous medium that allows their application to cells (Schulze et al., 2008). NMs in general and carbon based NMs in particular tend to form agglomerates when put in suspension. Agglomeration behavior depends largely on NM physico-chemical properties and the surrounding liquid. The goal is to find a biocompatible solvent and combine this with ultrasonication to reduce NM agglomeration to a minimum.

2 Principle of the Method

Double-distilled water (ddH_2O) is used as the solvent of choice to suspend metal oxide NMs. A 1 mg/ml stock suspension of metal oxide NMs is prepared in ddH_2O by 10 min ultrasonication in an ultrasound bath. NMs that are purchased as monodisperse suspensions are further diluted in the solvent recommended by the manufacturer. If no such solvent is available ddH_2O is used.

3 Applicability and Limitations

Typically metal oxide NMs suspend easily in ddH_2O at a stock concentration of 1 mg/ml. Some metal oxide NM (e.g. ZnO) dissolute in aqueous suspension and release metal ions (e.g. Zn^{2+}). Therefore suspensions have to be prepared and diluted right before application to cells. Storage of suspensions is not recommended. A special dilution procedure has to be followed to assure homogenous distribution of NMs (adapted from Zook et al., 2011).

4 Related Documents

Table 1: Documents needed to proceed according to this SOP and additional NM-related interference control protocols.

Document ID	Document Title

5 Equipment and Reagents

5.1 Nanomaterial type

Metal oxide NM (e.g. ZnO, Cr_2O_3, TiO_2), Polystyrene beads and all NM delivered as monodisperse suspensions by the supplier.

5.2 Equipment

- Glass tubes
- Ultrasound bath
- Vortex®
- Pipettes
- Micro scales

5.3 Reagents

- Double-distilled water (ddH_2O)

6 Procedure

6.1 Preparation of NM stock suspension

- Weigh 2-4 mg of metal oxide NM into a sterile glass tube.
- Add appropriate volume of ddH_2O to reach a final concentration of **1 mg/ml** NM. Avoid raising dust from NM powder.
- Sonicate for 10 min in an ultrasound bath.
- Use directly for further dilution and application. Do not store.

6.2 Dilution of NM

6.2.1 General remarks

Important: "Mixing" in the context of diluting NMs means, the solvent containing tube is put on a continuously shaking Vortex® and the previous sub-dilution (or stock suspension, respectively) is put drop wise into the shaking solvent. The resulting suspension stays on the Vortex® for additional 3 seconds before proceeding with the next sub-dilution (Procedure according to Zook et al., 2011).

The solvent concentration has to be exactly the same in each cellular sample and in the solvent control. Therefore NMs are serially pre-diluted in solvent (ddH_2O) before final dilution in complete cell culture medium (or any other adequate buffer used in the respective assay). The exact procedure is illustrated in the example (6.2.2) below.

6.2.2 Example

The following six different concentrations of a metal oxide NM and the solvent control (7.) shall be finally tested on cells:

1. 100 µg/ml
2. 50 µg/ml

3. 25 µg/ml
4. 12.5 µg/ml
5. 6.25 µg/ml
6. 3.13 µg/ml
7. Solvent control: ddH_2O

A] In the first step the following sub-dilutions from the 1 mg/ml stock suspension (6.1) are prepared in ddH_2O:

2. 300 µl of 1 mg/ml stock suspension (1) are mixed with 300 µl of ddH_2O
   → 500 µg/ml [2]
3. 300 µl of 500 µg/ml [2] are mixed with 300 µl ddH_2O → 250 µg/ml [3]
4. 300 µl of 250 µg/ml [3] are mixed with 300 µl ddH_2O → 125 µg/ml [4]
5. 300 µl of 125 µg/ml [4] are mixed with 300 µl ddH_2O → 62.5 µg/ml [5]
6. 300 µl of 62.5 µg/ml [5] are mixed with 300 µl ddH_2O → 31.25 µg/ml [6]

B] The same volume from each of these sub-dilutions [2-6], from the stock suspension [1] as well as from the solvent [7] is then used to prepare the final concentrations in complete cell culture medium or any adequate buffer that is used in the respective assay. See Figure 1.

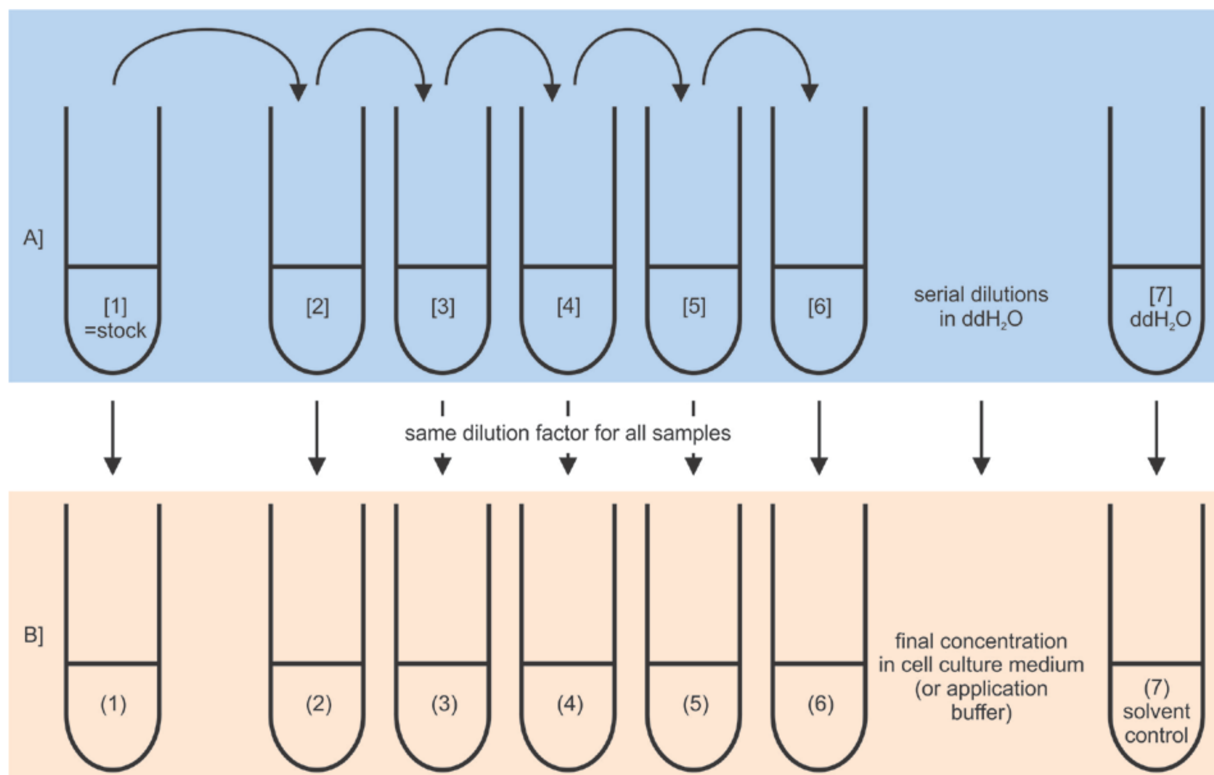

Figure 1: Pipetting scheme for NMs. First serial dilutions in ddH_2O are prepared from the stock suspension [1]. Each of these as well as the stock suspension and the solvent is then further diluted in complete cell culture medium (or any other kind of application buffer). This procedure assures the same amount of solvent in each sample.

7 Quality Control, Quality Assurance, Acceptance Criteria

8 Health and Safety Warnings, Cautions and Waste Treatment

Raising dust from NM powders has to be avoided. Operators should wear masks, gloves and laboratory coat for all handling steps.

Discard NMs separately and according to the institution's rules.

9 Abbreviations

ddH_2O	double-distilled water
NM	nanomaterial

10 References

Schulze C, Kroll A, Lehr CM, Schäfer UF, Becker K, Schnekenburger J, Schulze-Isfort C, Landsiedel R, Wohlleben W; 2008: Not ready to use - overcoming pitfalls when dispersing nanoparticles in physiological media. *Nanotoxicology* 2(2), 51-61

Zook JM, MacCuspie RI; Locascio LE, Halter, MD; Elliott JT; 2011: Stable nanoparticle aggregates/agglomerates of different sizes and the effect of their size on hemolytic cytotoxicity. *Nanotoxicology* 5(4): 517-530