Read across in nanosafety research: Dissolution behaviour of a library of 37 nanomaterials in simplified physiological media

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Overview

- Scope
- Dissolution study and statistical analysis
- Classification modelling
- Conclusions

Scope

The aims of this study were:

- To **monitor dissolution** of a library of **37 ENMs** used by the EU FP7 project NanoMILE, using the **ECETOC tier 1 test**, to **identify patterns and descriptors (particulate, atomic/ionic)** correlated with dissolution
- The **potential to group ENMs** based on their **dissolution behaviour assuming** that dissolution is **driven by the same physicohemical or atomic/ionic descriptors**
- To **develop a classification model to predict ENM dissolution** based on the most significant physicochemical and/or atomic descriptors.

Overview

- Scope
- **Dissolution study and statistical analysis**
- Classification modelling

Materials and methods

- The **ENMs assessed** comprised of **metal** (Ag), **oxides** of Ti, Ce, Zr, Co, Zn, Fe(II) and Fe(III), Ca, Ba and Al, **chemically doped bimetal oxides** (Zr doped Ce ENMs with different dopant ratios), and **physical mixtures** of CeO_2 with ZnO or CoO
- **pH** values of **1.5 and 7.0** simulating **simplified physiologically significant environments** of the **gastrointestinal tract and lungs** and which present the **main routes of ENM exposure**, via ingestion and inhalation, respectively
- **Dispersion concentration: 0.5 mg / mL**, when possible. Respective scaling was performed when pristine dispersions were more dilute than required
- **Sampling** took place for **5 timepoints**: **2, 4, 8, 24 and 48 hours**.
- **Analysis** took place for short-term (**2 hours timepoint**) and long-term (**48 hours timepoint**) **dissolution**

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Materials and methods

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- Due to the **small sample size** (< 50 data points / descriptor) the **Kruskal-Wallis H test with the Dunn-Bonferroni post-hoc test** was used to identify significant differences **between ENM and their bulk analogues**.
- **Categorical Principal Component Analysis (CatPCA)** was used to **statistically identify the descriptors** that **contributed the most to the variance** of the produced **dataset**.
- **Data imputation** was used to **fill the gaps**, as this helps **reduce bias originating from smaller datasets** and consequent increased difficulty in data handling and analysis

NanoMILE

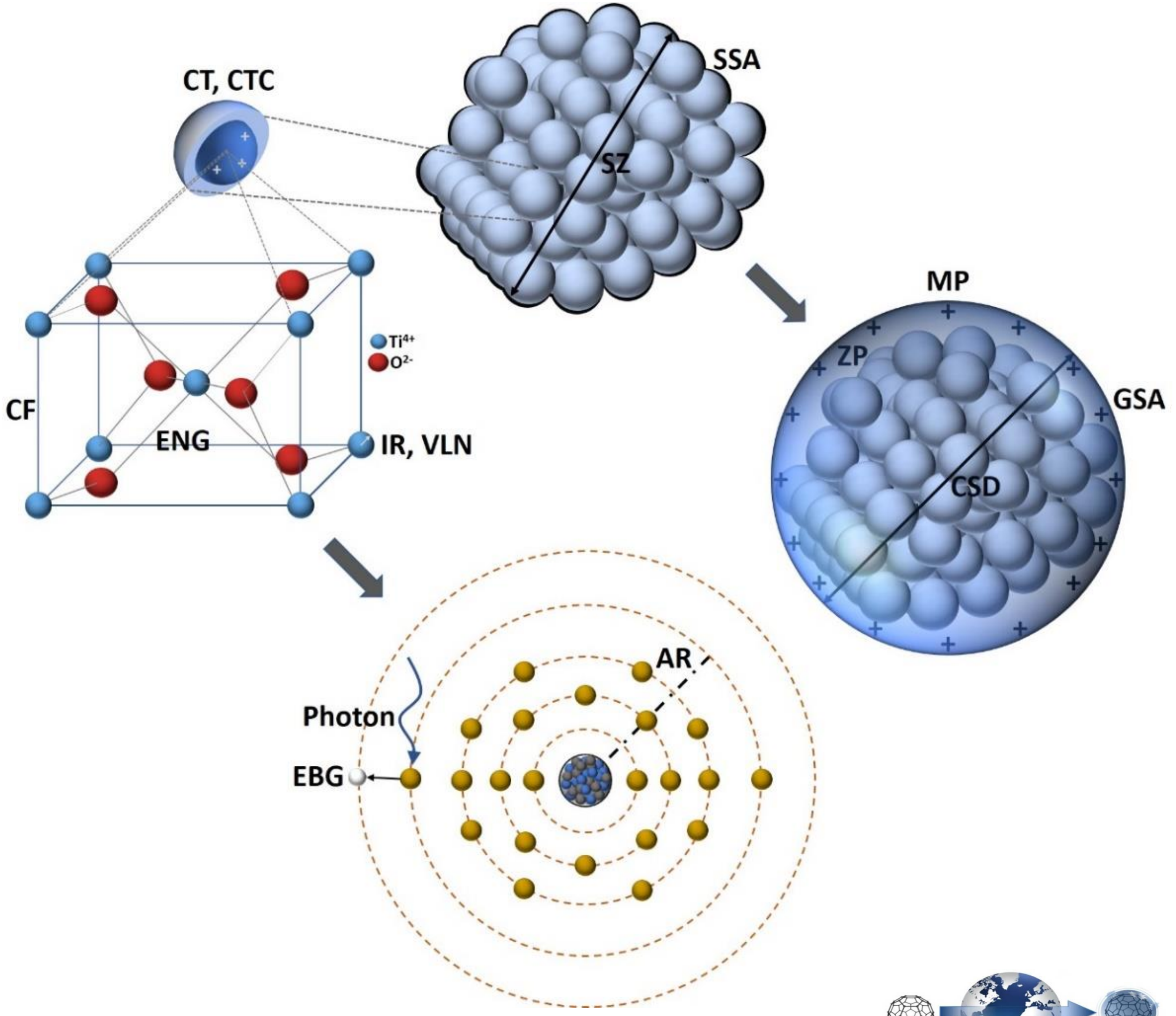
Descriptors studied

Particle descriptors

- Morphology (MP)
- Coating (CT)
- Coating charge (CTC)
- Size (SZ, including hydrodynamic diameter)
- Geometric surface area* (GSA)
- Corresponding sphere diameter* (CSD)
- Specific surface area (SSA, BET)
- ZP – zeta potential

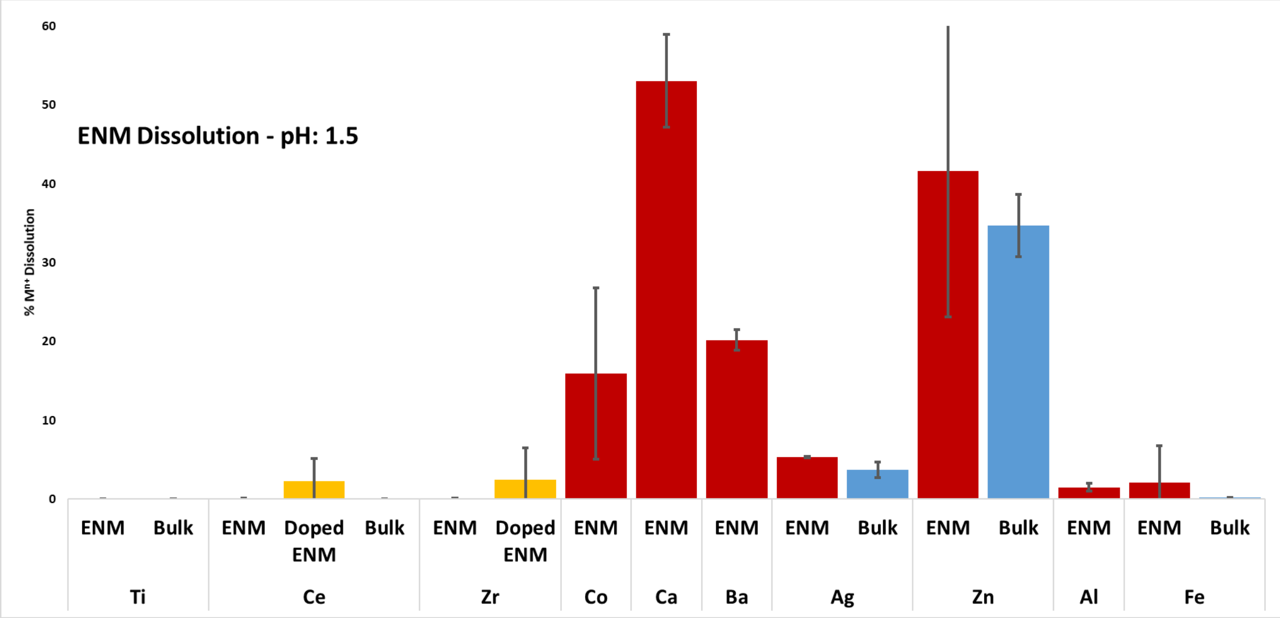
Atomic descriptors

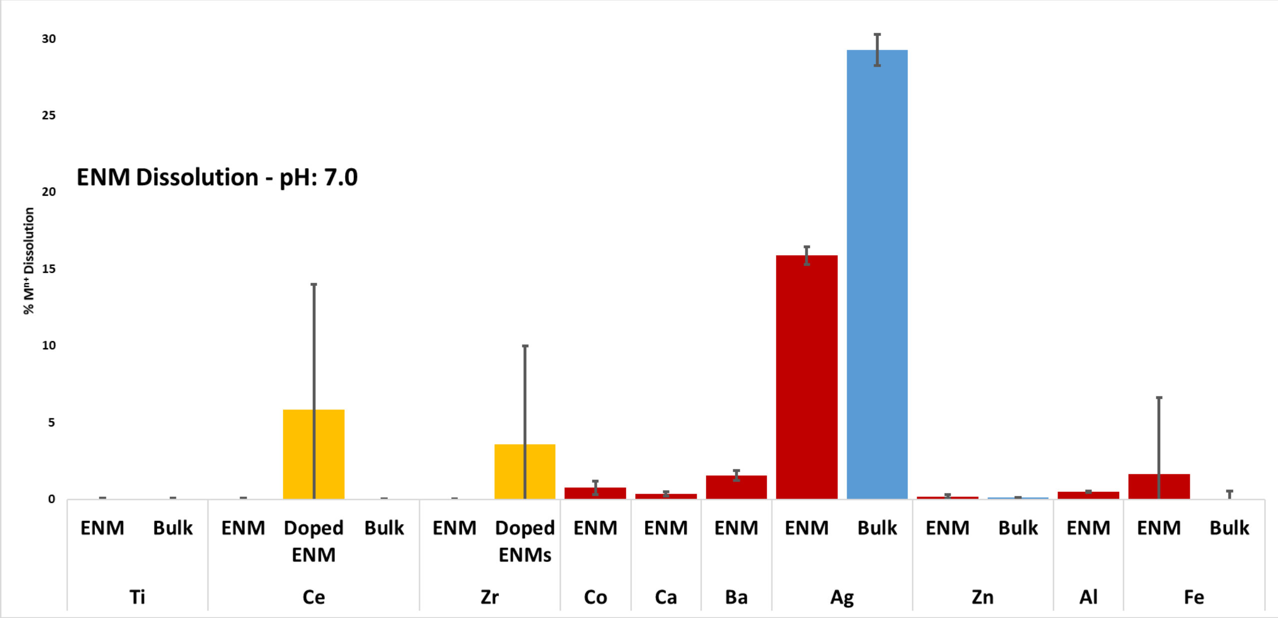
- Chemical formula (CF)
- Atomic radius (AR)
- Electronegativity (ENG)
- Energy bang gap* (EBG)
- Ionic radius (IR)
- Valency (VLN)



* Corresponds to calculated descriptors

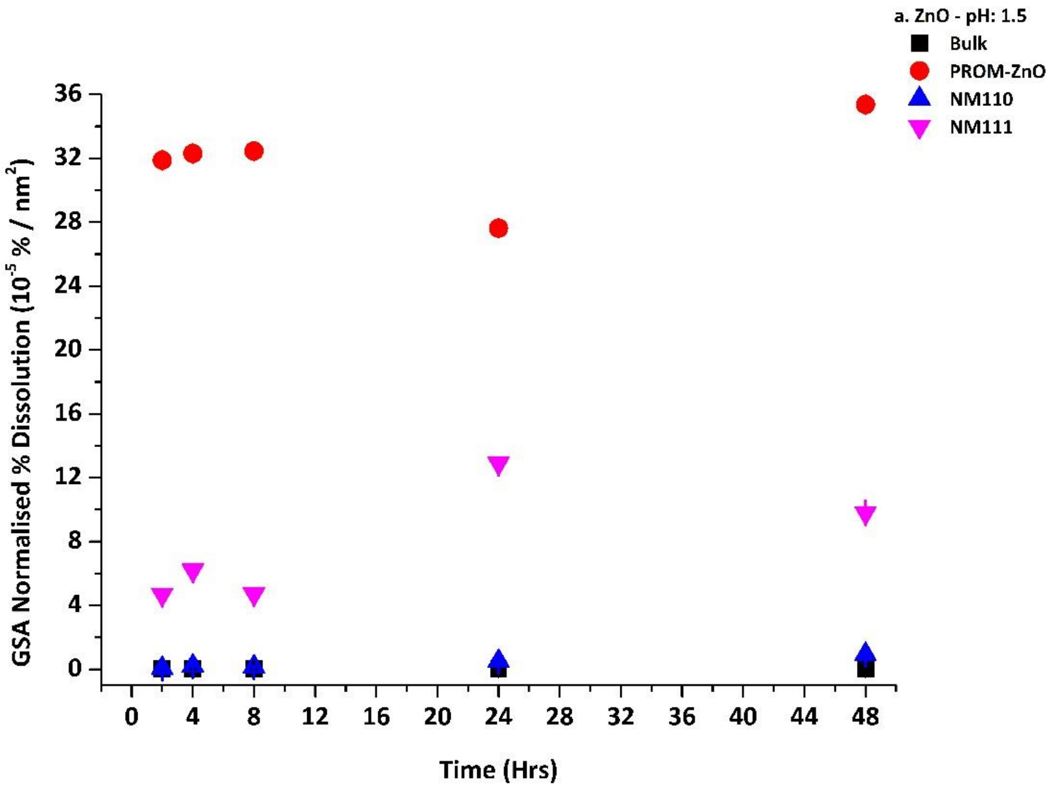
Results – 2 hours timepoint

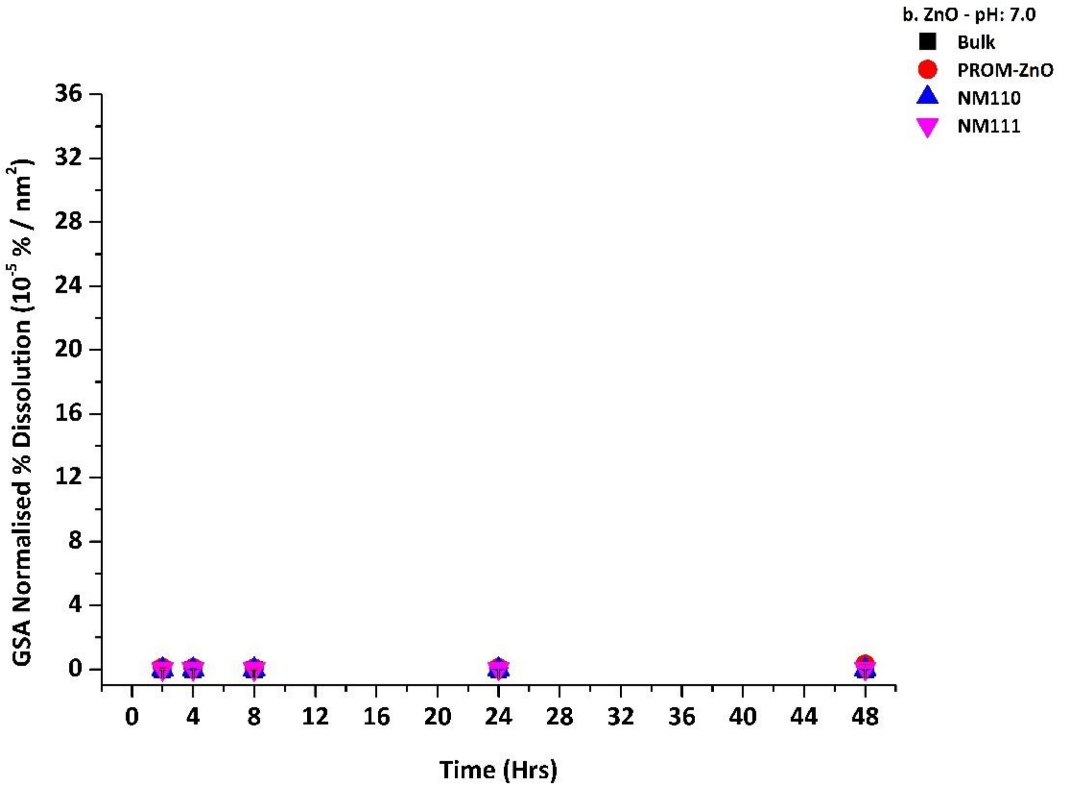




- ENMs and their bulk analogues demonstrate **higher dissolution under low pH conditions** than at neutral pH
- **Low pH: Ca-bearing ENM are the most soluble, followed by Zn-, Co-, Ba-bearing and Ag ENMs**
- **Neutral pH, Ag ENMs are most soluble followed by Zr-doped Ce-, Ba- and Co-bearing ENMs**
- **No statistically significant differences** were observed **between the ENM and the bulk** (ZnO: $p = 0.392$, TiO_2: $p = 0.433$, CeO_2: $p = 0.406$, Ag: $p = 0.416$) for both pH values

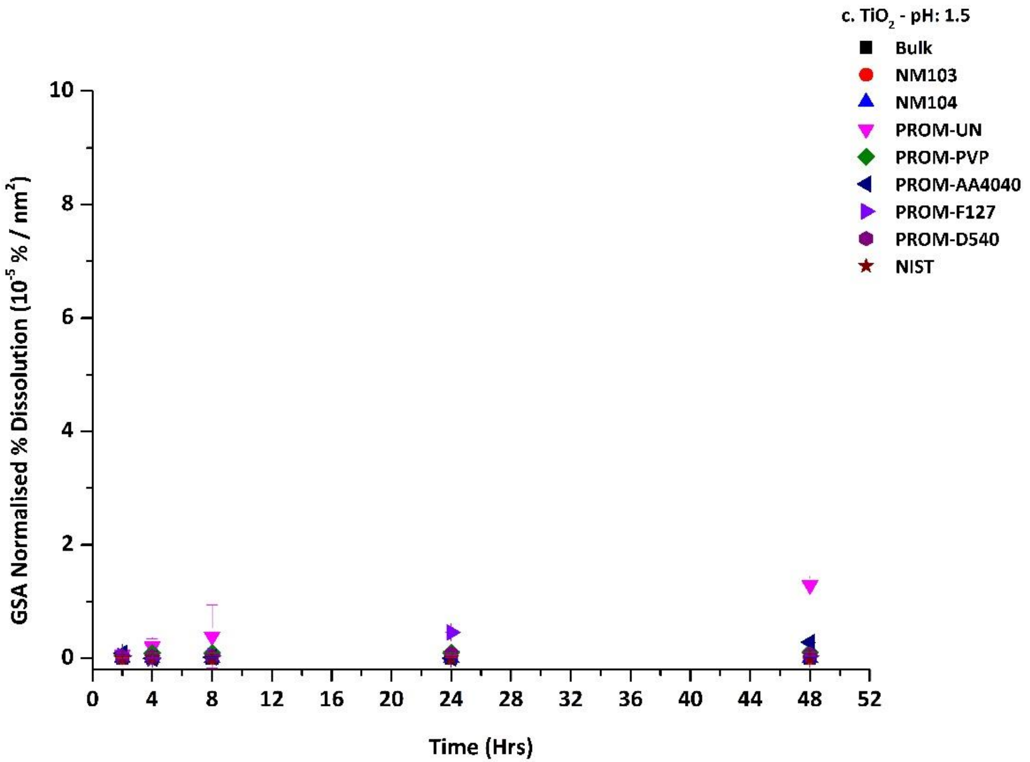
Results – 48 hours timepoint ZnO

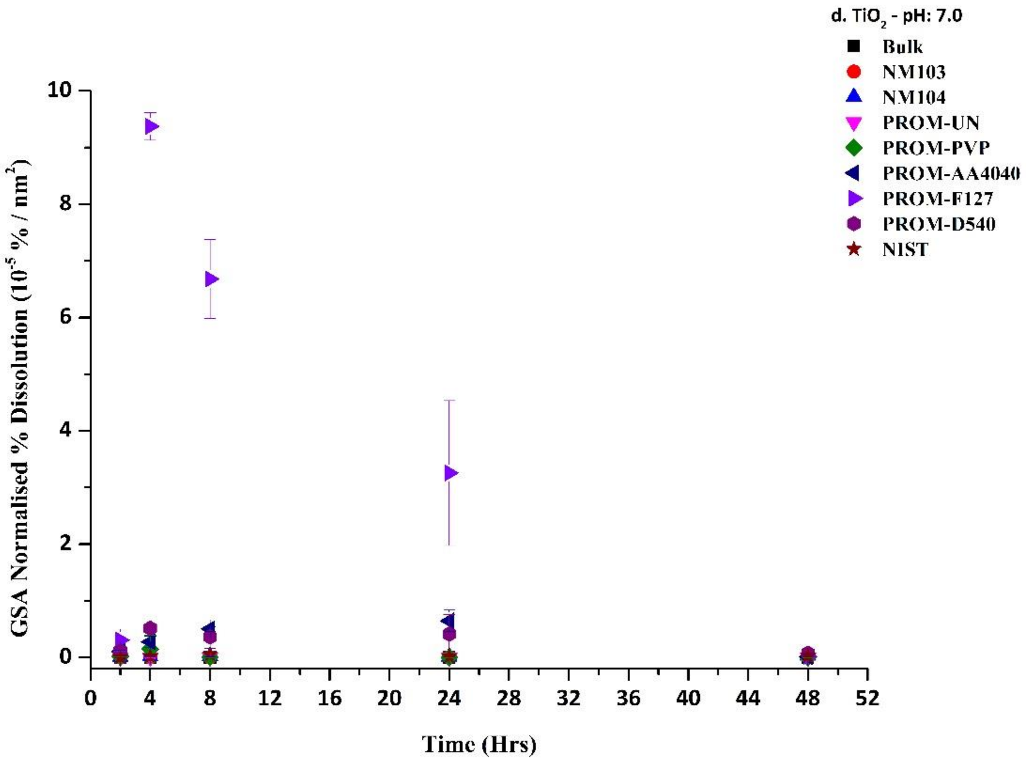




- **Higher dissolution at low vs neutral pH**
- **Significant difference between ENM and bulk** dissolution for **both pH values** (KWH: $p << 0.001$)
- Dunn Bonferroni post hoc test: **Only** the **uncoated ZnO ENM** (PROM-ZnO, p=0.001 for both pH values) was **significantly different than the bulk**

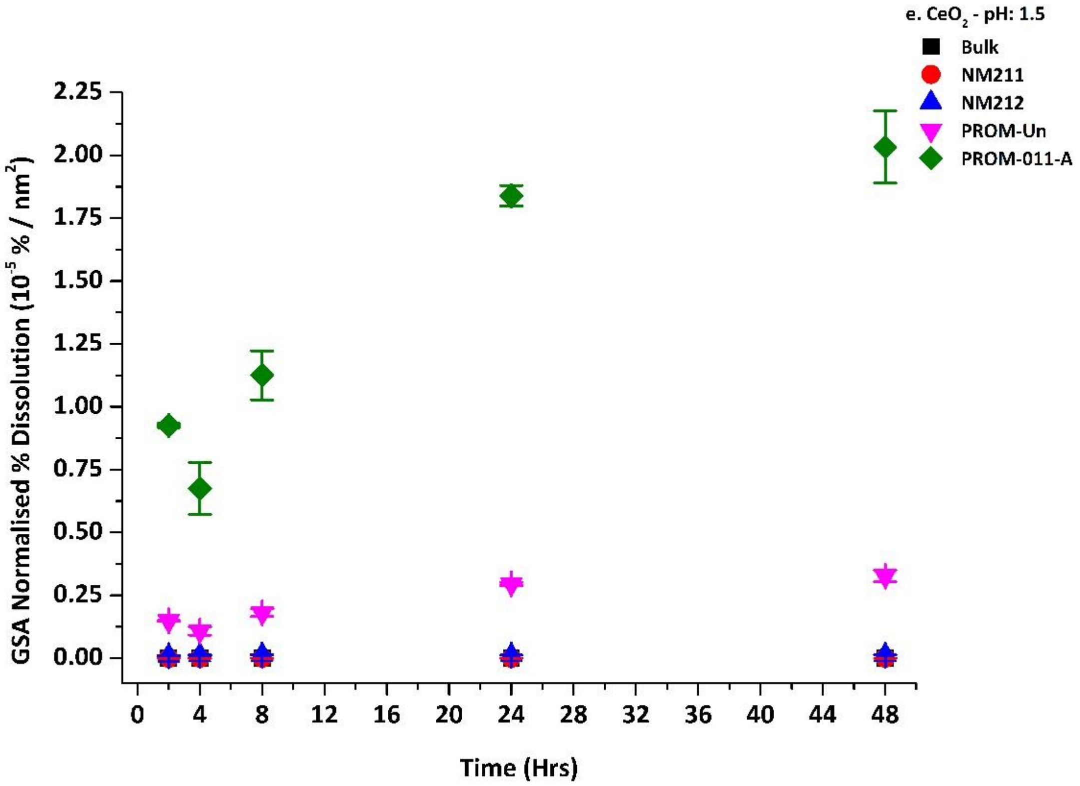
Results – 48 hours timepoint TiO_2

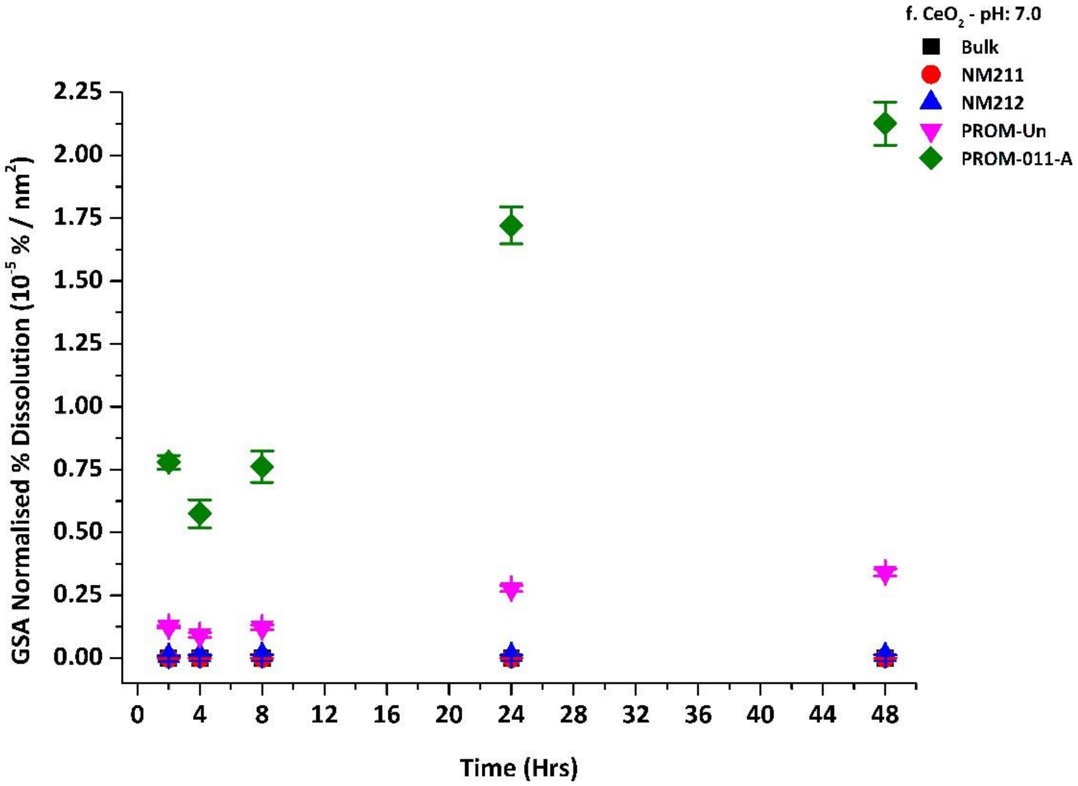




- **Higher dissolution at low vs neutral pH, with exceptions** (JRC NM-104, PROM-AA4040, PROM-F127, TiO_2-NIST)
- **Significant difference between ENM and bulk dissolution** for both pH values (Low pH: $p=0.003$; neutral pH: $p << 0.001$)
- Statistically significant difference:
  - **Low pH: Uncoated (PROM-UN, p=0.003) and PVP coated (PROM-PVP, p=0.010)**
  - **Neutral pH: PROM-D540 (p=0.004), AA4040 (PROM-AA4040) (p=0.011) and F127 (PROM-F127) (p=0.001) coated**

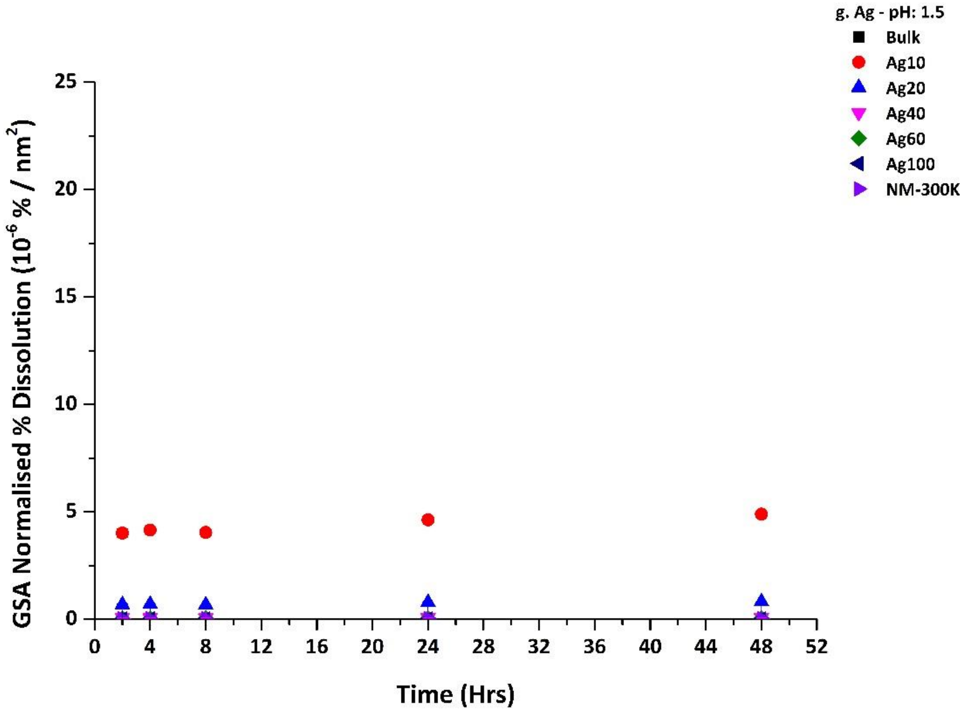
Results – 48 hours timepoint CeO_2

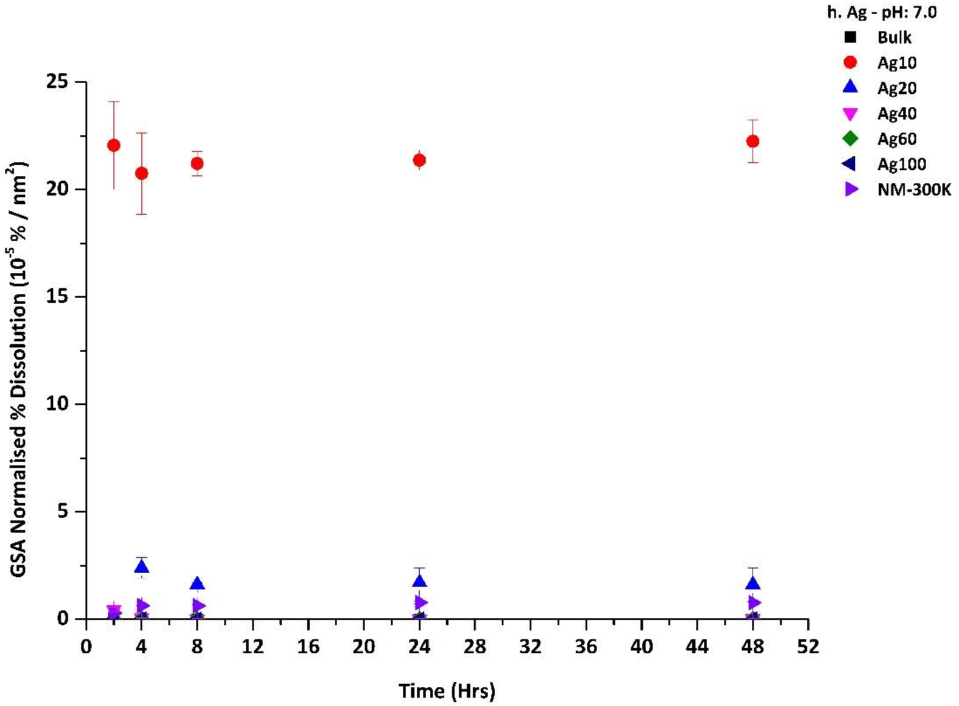




- **Higher dissolution at low vs neutral pH**
- **Significant difference between ENM and bulk dissolution** for both pH values (KWH: $p << 0.001$)
- Statistically significant difference: **uncoated CeO_2 ENMs** (PROM-Un, $p=0.019$ and PROM-011-A, $p<<0.001$)

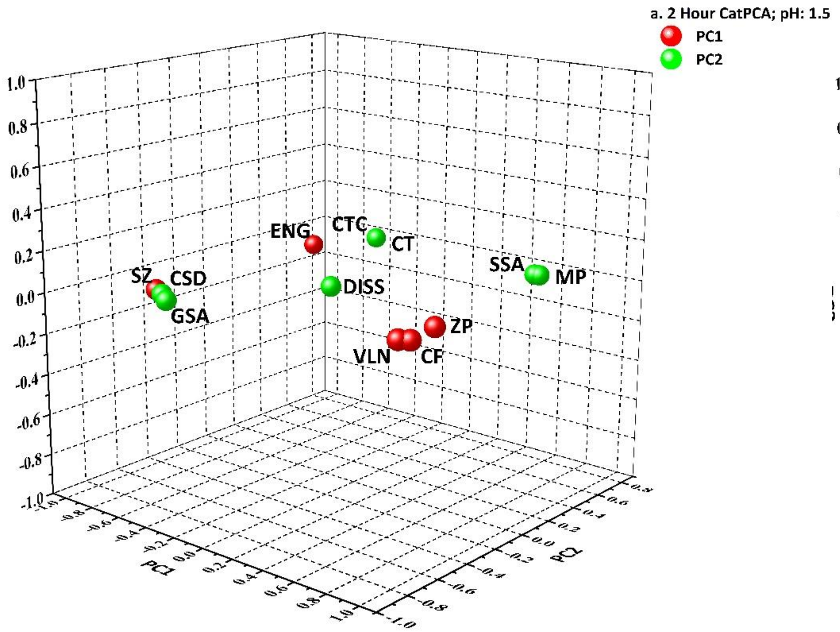
Results – 48 hours timepoint Ag

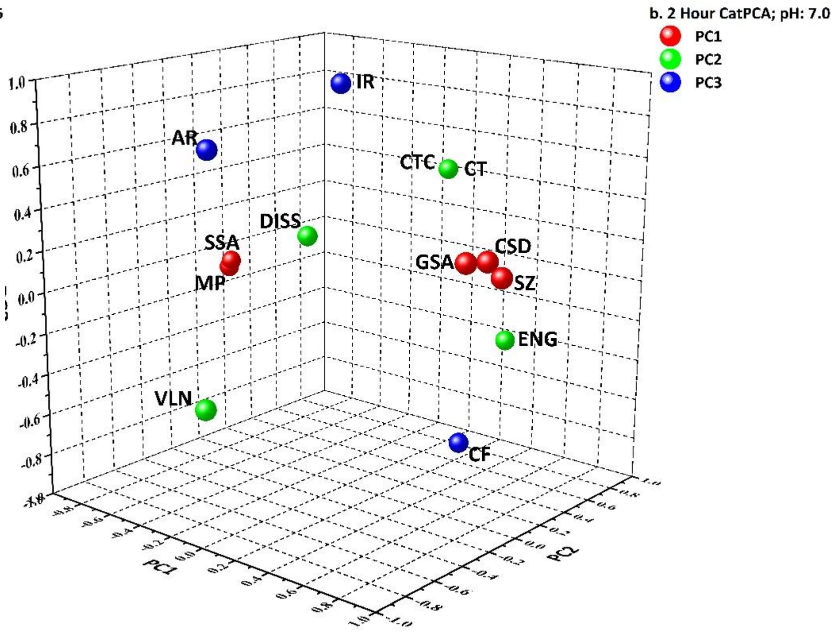




- **Higher dissolution at low vs neutral pH with exceptions** (Ag10, Ag20 and NM-300K)
- **Significant difference between ENM and bulk dissolution** for both pH values (KWH: $p << 0.001$)
- Statistically significant difference from bulk:
  - **Low pH, Ag10 ($p=0.003$) and Ag20 ($p<<0.001$)**
  - **Neutral pH, Ag10 ($p<<0.001$), Ag20 ($p=0.007$) and NM-300K ($p=0.037$)**

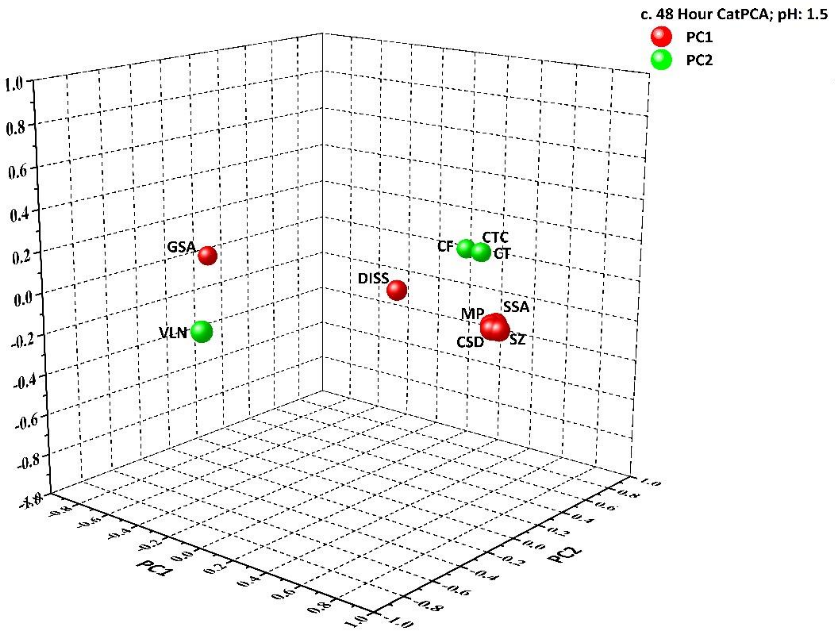
Results – 2 hours timepoint CatPCA

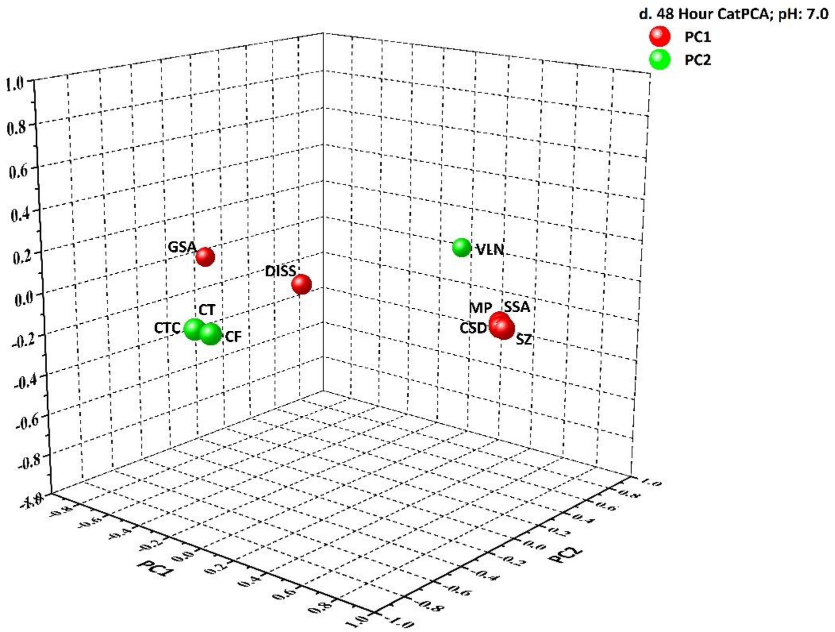




- **Two principle components** (PC_i, i = 1,2,…) at **low pH (75.3% of variance)** and **three at neutral pH (84.3% of variance)**
- **ENM dissolution** is in PC_2 in both cases along with:
  - **Low pH: coating, coating charge, morphology, surface area and corresponding sphere diameter**
  - **Neutral pH: coating, coating charge, valency and electronegativity**
- PC_1 includes:
  - Low pH: chemical formula, valency, size, ζ-potential and electronegativity
  - Neutral pH: morphology, size and surface area

Results – 48 hours timepoint CatPCA





- **Same two principle components** in both cases with **variance of 78.5% and 78.4%** for low and neutral pH respectively
- **ENM dissolution** is in PC_1 in both cases along with:
  - Both pH values: **morphology, size and surface area**
- **PC_2** includes:
  - Both pH values: **chemical formula, coating and coating charge and valency**
- **In all cases** (both pH values and time points), **Cronbach's α values** (0.70 < α < 0.87) and **total percentage of variance** (75.3-84.3%) suggest **high internal component consistency**

Conclusions

- ENM dissolution is not always statistically significantly different from the respective bulk analogues
- Surface characteristics and size seem to affect ENM dissolution
- In the short-term (2 hours) dissolution results suggest that dissolution is driven by both particle and atomic ENM characteristics
- In the longer-term (48 hours) particle characteristics dominate the process, with the exception of core metal valency
- Results suggest that an underlying mechanism of dissolution/reprecipitation (chemical transformation, Ostwald ripening) exists affecting the measured results
- ENM dissolution, especially in the short-term, should be studied taking also into account the atomic ENM characteristics

Overview

- Scope
- Dissolution study and statistical analysis
- **Classification modelling**

Materials and methods

- **ENM clustering** was attempted, in the entire dataset, using the categories defined during the **OECD case study on Ag ENM**s:
  - **High solubility (> 70%, 6 data rows)**
  - **Moderate solubility (10 – 70%, 35 data rows)**
  - **Low solubility (1 – 10%, 79 data rows)**
  - **Negligible solubility (< 1%, 270 data rows)**
- Due to the **resulting unbalanced clustering, two clusters were defined**:
  - **Soluble ENM (> 1%, 120 data rows)**
  - **Negligible solubility (< 1%, 270 data rows)**
- **Descriptors with data gaps were removed** from analysis to **increase model robustness and reliability**.

Materials and methods

- **Fourteen descriptors used: pH, time, chemical formula, coating, coating charge, ζ-potential, size, morphology, atomic radius, ionic radius, electronegativity, valency, geometric surface area, corresponding sphere diameter**
- **Gaussian normalisation was applied to all data.**
- The **CFS (Correlation based Feature Selection) algorithm** with **BestFirst evaluator** was used to identify the **most significant predictive descriptors**.
- **Prediction** was performed using the **J48 algorithm and the EnaloskNN algorithm for 3 neighbours with a random 75% : 25% ratio of training to test sets**.

Materials and methods

- **Read across testing** was performed using the **EnaloskNN algorithm** (Enalos Chem/Nanoinformatics tools) to study the selected training neighbours for each test ENM.
- The. **Applicability Domain (area of reliable predictions) was tested using Euclidian distance of the used descriptors.**
- **Model validation and robustness was tested based on the OECD criteria for model validation and Y-randomisation** (10 randomised calculations).

Significant parameters selection

- The **most significant parameters** used for prediction are:
  - pH,
  - chemical formula,
  - ζ-potential
  - coating,
  - size,
  - geometric surface area,
  - Corresponding sphere diameter
  - atomic radius and
  - electronegativity.

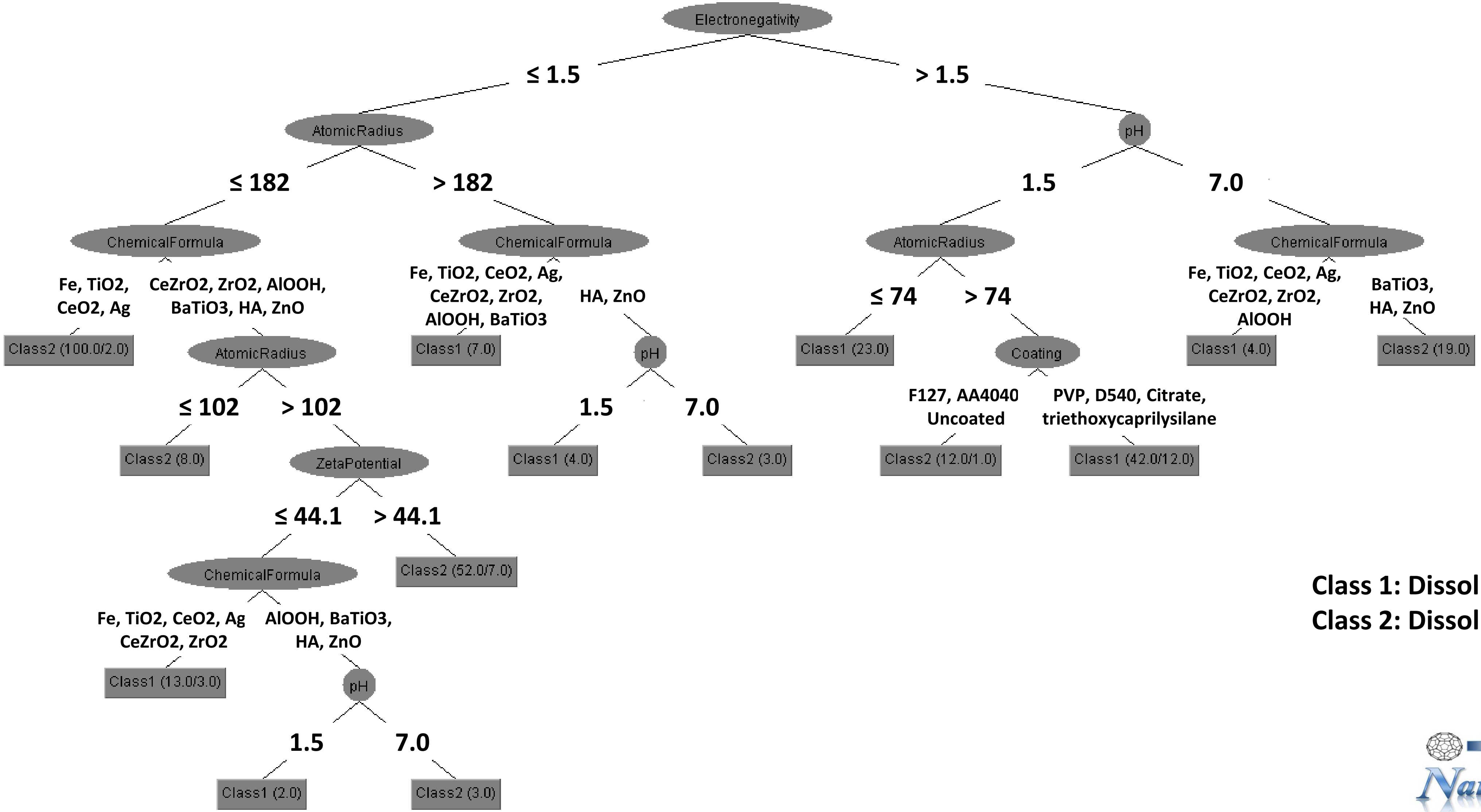
J48 confusion matrix and statistics

- Statistics:
  - Total test classes: 98
  - Correct classification: 90 (91.837%)
  - Wrong classification: 8 (8.163%)
  - Cohen's κ: 0.808
  - Accuracy: 0.918
  - Sensitivity (< 1%): 0.941
  - Specificity (< 1%): 0.867

Initial classification / classification	> 1%	< 1%
> 1%	26	4
< 1%	4	64

- Y-randomisation yielded in all cases statistically significant lower predictive power
- APD: 100% of predictions were reliable (APD limit value: 3.921)

J48 decision tree



Class 1: Dissolution > 1%
Class 2: Dissolution < 1%

EnaloskNN confusion matrix and statistics

- Statistics:
  - Total test classes: 98
  - Correct classification: 91 (92.857%)
  - Wrong classification: 7 (7.143%)
  - Cohen's κ: 0.836
  - Accuracy: 0.929
  - Sensitivity (< 1%): 0.926
  - Specificity (< 1%): 0.933

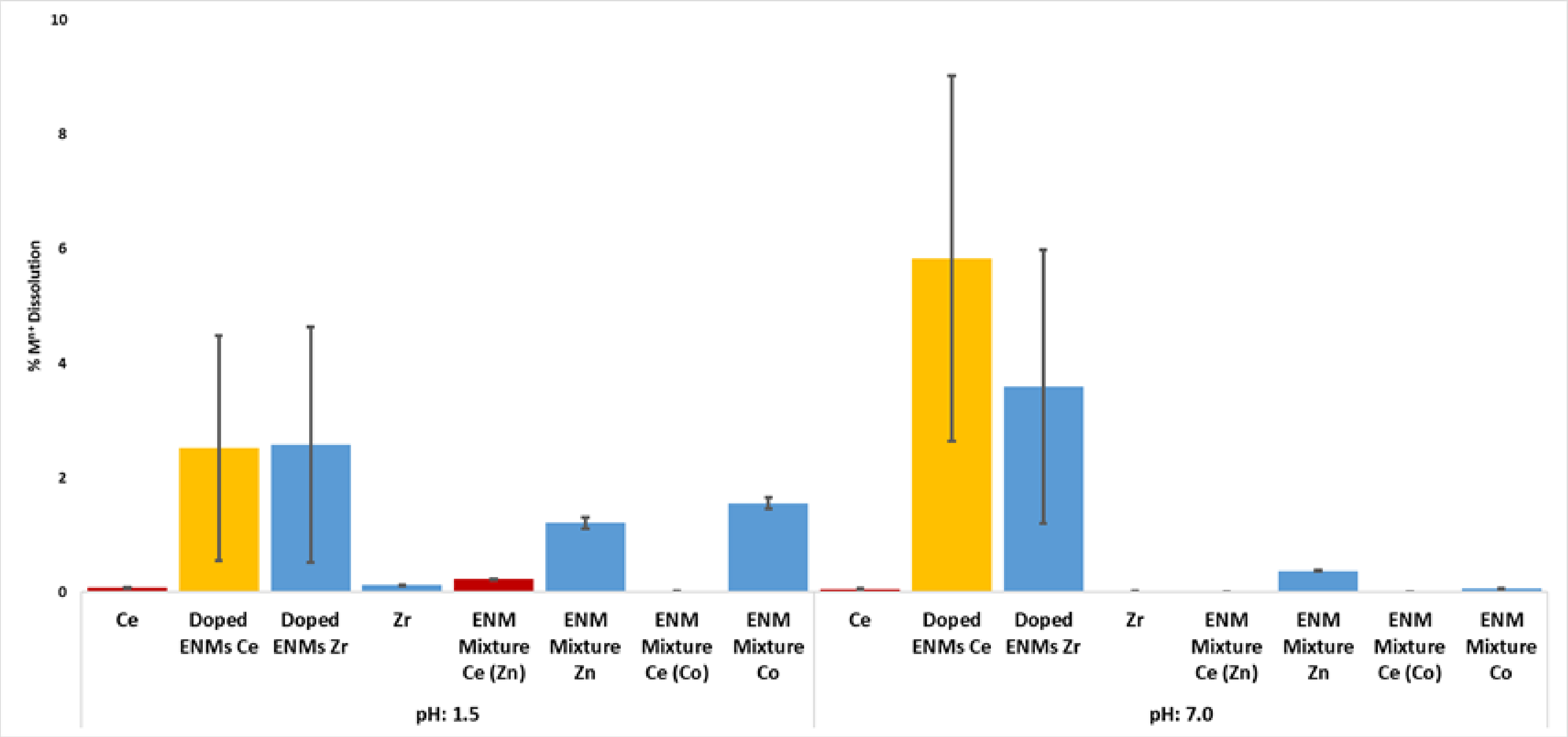
Initial classification / classification	> 1%	< 1%
> 1%	28	2
< 1%	5	63

- Y-randomisation yielded in all cases statistically significant lower predictive power
- APD: 100% of predictions were reliable (APD limit value: 2.955)

Read across examples

ENM / Neighbours	Neighbour 1	Neighbour 2	Neighbour 3
TiO_2 – F127 (8 H)	TiO_2 – F127 (24 H)	TiO_2 – F127 (48 H)	TiO_2 – AA4040 (24 H)
JRC TiO_2 NM104 (48 H)	JRC TiO_2 NM104 (4 H)	JRC TiO_2 NM104 (24 H)	JRC TiO_2 NM103 (24 H)
PROM-CeO_2-11A (48 H)	PROM-CeO_2-11A (24 H)	PROM-CeO_2-11A (4 H)	CeO_2 Uncoated (24 H)
$Ce_{0.08}Zr_{0.92}O_2$ (48 H)	$Ce_{0.08}Zr_{0.92}O_2$ (24 H)	$Ce_{0.08}Zr_{0.92}O_2$ (8 H)	$Ce_{0.22}Zr_{0.78}O_2$ (24 H)
AlOOH (24 H)	AlOOH (48 H)	AlOOH (8 H)	ZrO_2 (2 H)

Complex ENM systems dissolution



- **Hume-Rothery rules**: metal alloys present **high solubility** if the **difference in atomic radii of the high (solvent) and low (solute) concentrated metals is < 15%, and if the metals present similar crystal structures and small differences in valency and electronegativity.**

Conclusions

- Modelling results are in good agreement with statistical analysis
- The statistically significant parameters identified for the entire dataset were: pH, chemical formula, size, geometric surface area, ζ-potential, coating, electronegativity, atomic radius
- Both the J48 and EnaloskNN provide similar classification results
- Both J48 and EnaloskNN provided robust and validated models
- J48 further refined the significant parameters to: pH, chemical formula, ζ-potential, coating, electronegativity, atomic radius
- EnaloskNN provided meaningful results for the read across of ENM
- Complex ENM systems dissolution could potentially be explained by the Hume-Rothery rules for alloy and solid solutions dissolution.

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