materials

MDPI

Supplementary Materials

Applicability and limitations in the characterization of poly-dispersed engineered nanomaterials in cell media by Dynamic Light Scattering (DLS).

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Table S1. Z-average and PDI measured on the stock suspensions of the NMs in the media following the traditional and standardized protocol.

Sample	Z-Average (nm) Traditional Protocol	PDI Traditional Protocol	Z-Average (nm) Standardized Protocol	PDI Standardized Protocol
NM-200	467.4 ± 26.6	0.772 ± 0.074	238.4 ± 3.9	0.185 ± 0.019
NM-203	172.1 ± 2.3	0.144 ± 0.015	147.7 ± 3.7	0.181 ± 0.022
NM-100	252.8 ± 10.2	0.200 ± 0.014	222.7 ± 14.7	0.383 ± 0.066
NM-101	573.3 ± 44.2	0.326 ± 0.042	473.5 ± 27.7	0.325 ± 0.039
NM-212	248.3 ± 11.1	0.376 ± 0.035	349.8 ± 72.2	0.420 ± 0.100

Table S2. Composition of the cell media DMEM and RPMI as declared by the provider.

	DMEM	RPMI
Osmolality	260–310 mOsm/kg	300–340 mOsm/kg
pH	7.0–7.4	7.0–7.2
Amino Acids (mM)		
Glycine	0.4	0.13333334
L-Alanyl-Glutamine	3.9723501	2.0552995
L-Arginine hydrochloride	0.39810428	1.1494253
L-Asparagine	-	0.37878788
L-Aspartic acid	-	0.15037593
L-Cystine	0.15335463	0.20833333
L-Glutamic Acid	-	0.13605443
L-Histidine hydrochloride-H_2O	0.2	0.09677419
L-Hydroxyproline	-	0.15267175
L-Isoleucine	0.8015267	0.3816794
L-Leucine	0.8015267	0.3816794
L-Lysine hydrochloride	0.7978142	0.21857923
L-Methionine	0.20134228	0.10067114
L-Phenylalanine	0.4	0.09090909
L-Proline	-	0.17391305
L-Serine	0.4	0.2857143
L-Threonine	0.79831934	0.16806723
L-Tryptophan	0.078431375	0.024509804
L-Tyrosine	0.39779004	0.110497236
L-Valine	0.8034188	0.17094018
Vitamins (mM)		
Biotin	-	8.1967213×10^{-4}
Choline chloride	0.028571429	0.021428572
D-Calcium pantothenate	0.008385744	5.24109×10^{-4}
Folic Acid	0.009070295	0.0022675737
Niacinamide	0.032786883	0.008196721
Para-Aminobenzoic Acid	-	0.00729927
Pyridoxine hydrochloride	0.019417476	0.004854369
Riboflavin	0.0010638298	5.319149×10^{-4}
Thiamine hydrochloride	0.011869436	0.002967359
Vitamin B12	-	3.690037×10^{-6}
i-Inositol	0.04	0.19444445
Inorganic Salts (mM)		
Calcium nitrate	-	0.42372882
Calcium Chloride	1.7959183	-
Ferric Nitrate	2.4752476E-4	-
Magnesium Sulfate	0.8130081	0.40650406
Potassium Chloride	5.3333335	5.3333335
Sodium Bicarbonate	44.04762	23.809525
Sodium Chloride	110.344826	103.44827
Sodium Phosphate monobasic	0.90384614	5.633803
Other Components (mM)		
D-Glucose (Dextrose)	5.5555553	11.111111
Phenol Red	0.039851222	0.0032573289
Sodium Pyruvate	1.0	0.013283741

Table S3. ζ -potential measured on the suspensions of the NMs in the media following the traditional and standardized protocol. (NM concentration 100μg/mL).

Media	Sample	ζ-Potential (mV) Traditional Protocol	ζ-Potential (mV) Standardized Protocol
DMEM	**NM-200**	−10.5 ± 0.5	−6.65 ± 1.05
	NM-203	−10.9 ± 1.1	−7.33 ± 5.21
	NM-100	−12.1 ± 0.5	−5.50 ± 1.18
	NM-101	−13.8 ± 1.6	−6.83 ± 0.66
	NM-212	−11.3 ± 0.1	−11.7 ± 0.4
RPMI	**NM-200**	−10.7 ± 0.5	−16.7 ± 2.0
	NM-203	−11.2 ± 0.3	−21.4 ± 1.4
	NM-100	−11.4 ± 0.7	−0.154 ± 1.830
	NM-101	−11.8 ± 0.2	−0.189 ± 1.740
	NM-212	−11.2 ± 0.2	−11.7 ± 0.8

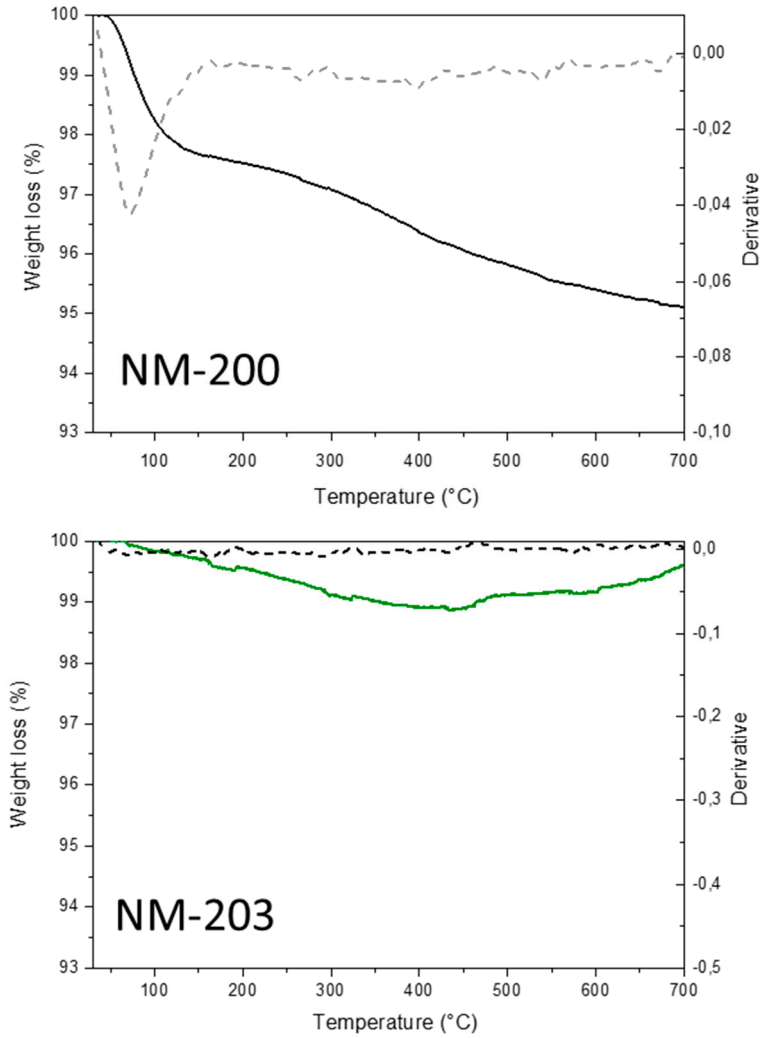

Figure S1. TGA weight loss (full line) and derivative (dotted line) curves for NM-200 and NM-203, under N_2 flow (TGA heating ramp = 15 °C/min, N_2 flow rate = 35 mL/min).

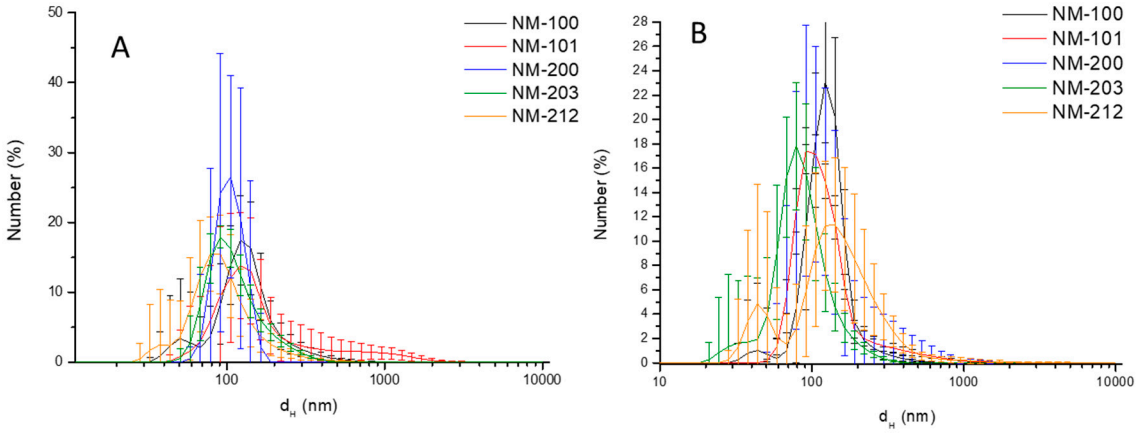

Figure S2. d_H distribution (number)of stock suspensions. (**A**) traditional protocol (**B**) standardized protocol.

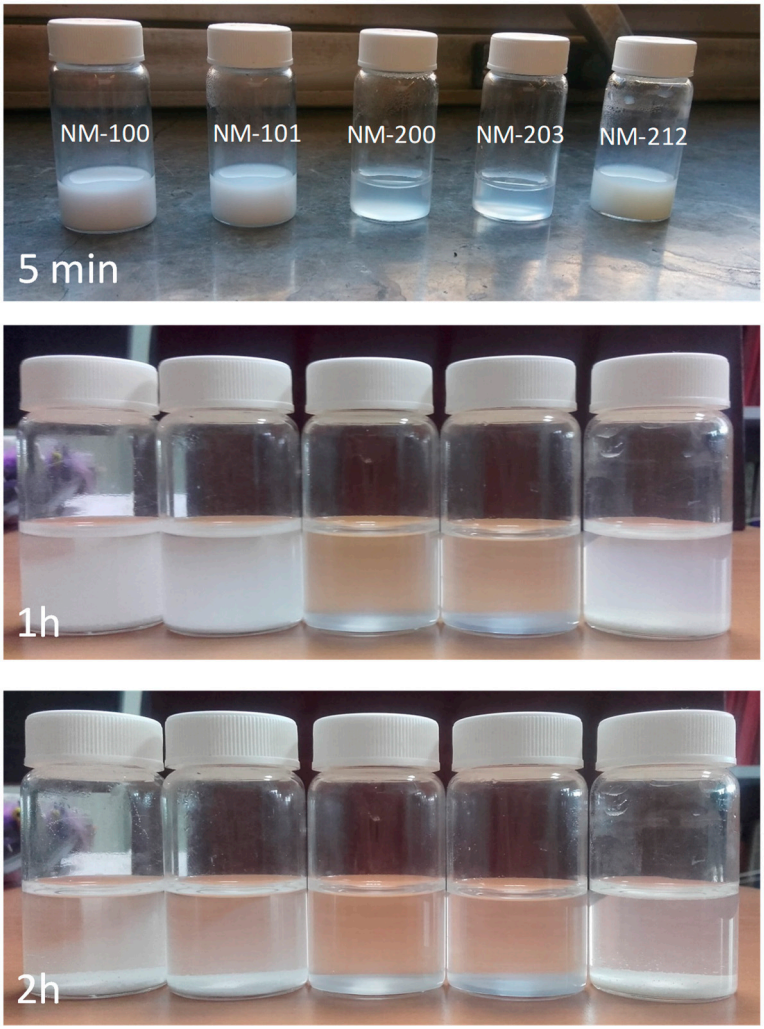

Figure S3. Images of the stock suspension prepared by the traditional protocol just after sonication, after 1 h and after 2 h.

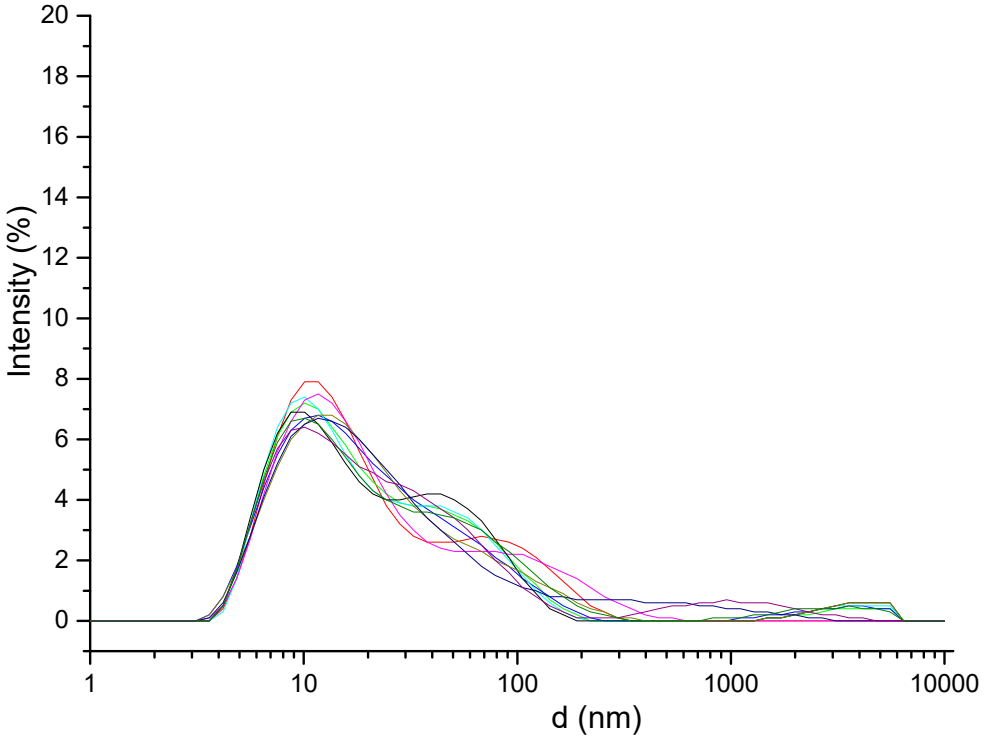

Figure S4. DLS analysis of the RPMI media.

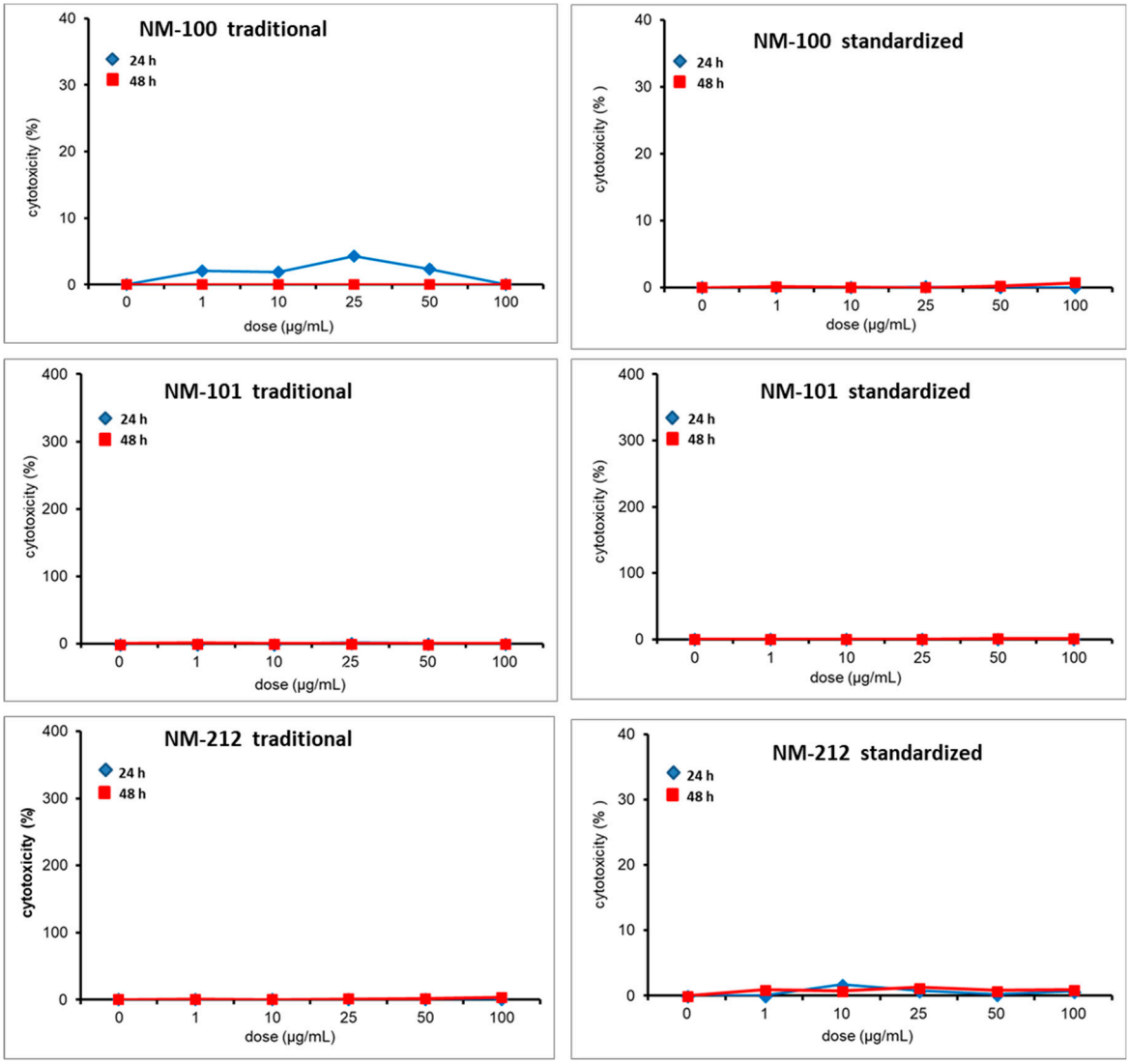

Figure S5. Effect of the dispersion protocols on the cytotoxicity of NM-100, 101 and 212 toward THP-1 cells measured as LDH leakage.

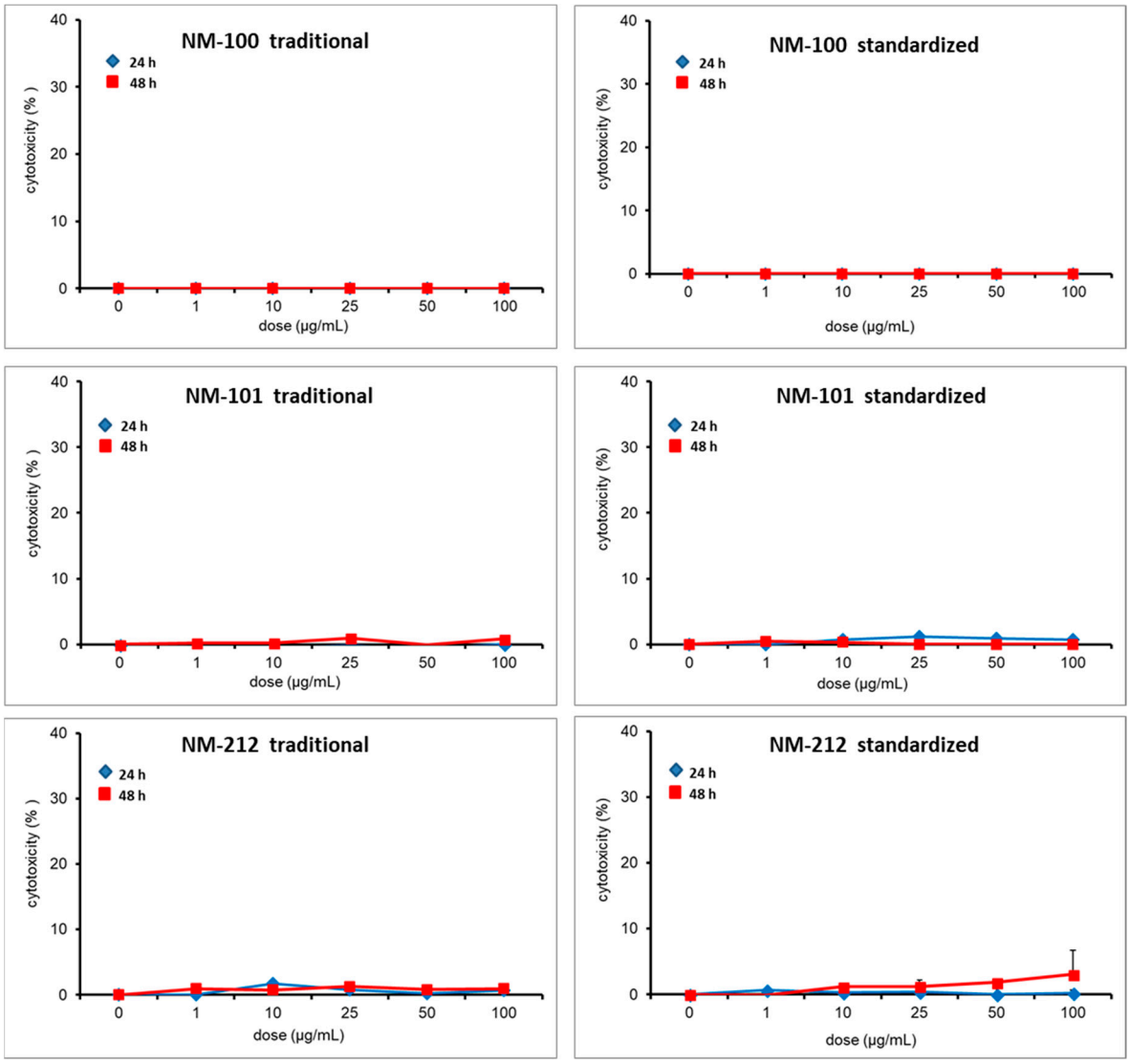

Figure S6. Effect of the dispersion protocols on the cytotoxicity of NM-100, 101 and 212 toward RAW 264.7 cells murine macrophages measured as LDH leakage.

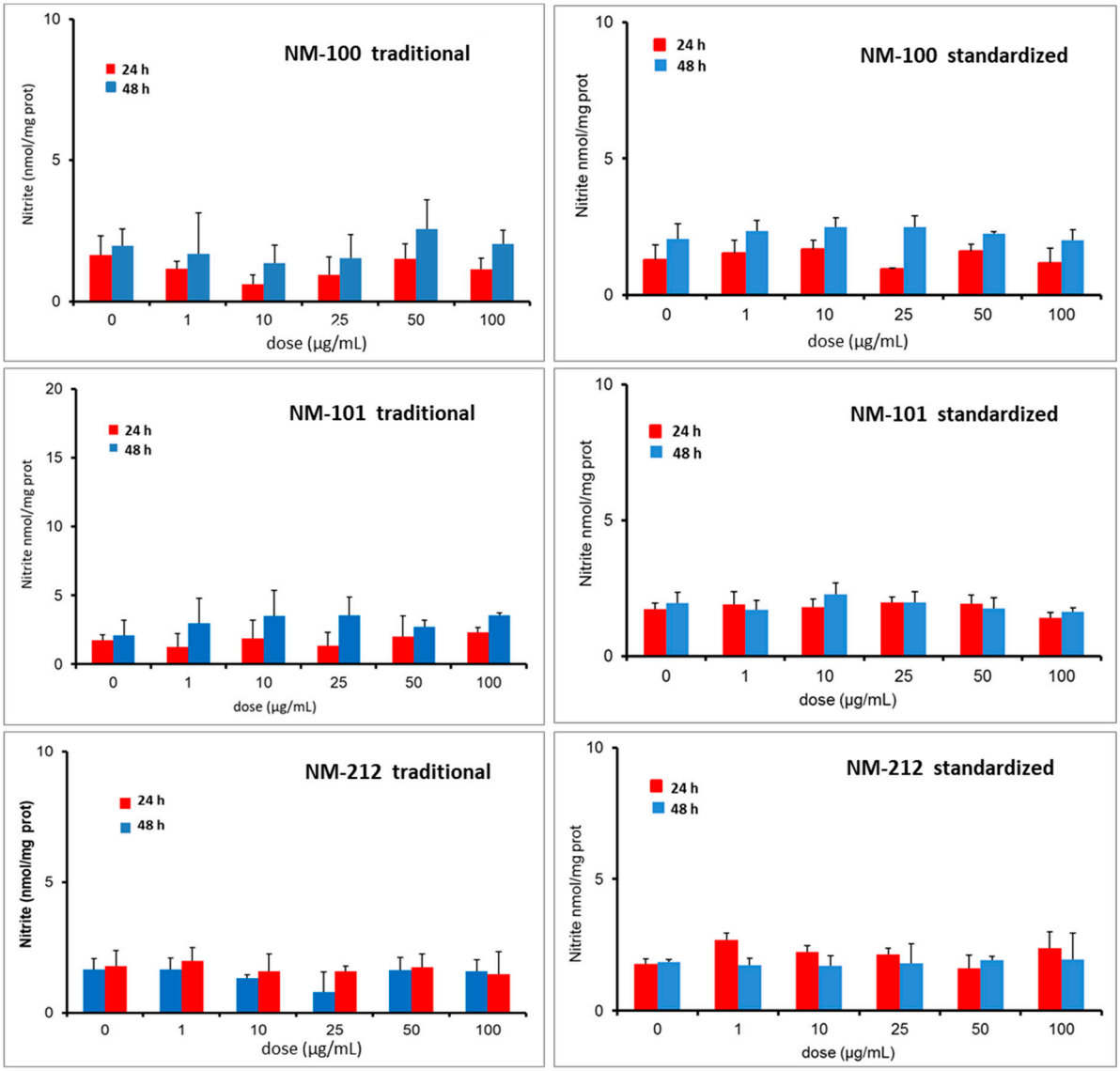

Figure S7. Effect of the dispersion protocols on the induction of NO release by RAW 264.7 murine macrophages by NM-100, 101 and 212.