Single Particle ICP-MS Characterization of Magnetoliposomes: Toward a Measurement of Number Distribution of Encapsulated Magnetic Nanoparticles

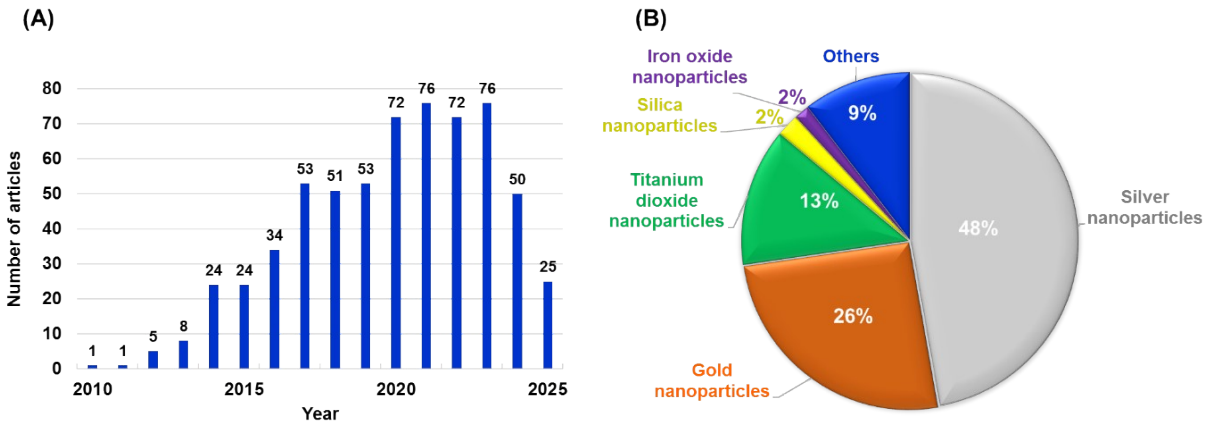
Linda Ayouni-Derouiche,[a,*] Jules Mistral,[b] Ece Ates,[b] Marie Boutry,[a] Nadia Baskali-Bouregaa,[a] Laurent David,[b] Sabri Derouiche,[a] Catherine Ladavière,[b,*]

[a]. Université Claude Bernard Lyon 1, CNRS, ISA, UMR5280, 5 rue de la Doua, F-69100 Cedex, Villeurbanne, France.

[b]. Université Claude Bernard Lyon 1, INSA Lyon, Université Jean Monnet, CNRS UMR 5223, Ingénierie des Matériaux Polymères, F-69622 Cedex, Villeurbanne, France.

* To whom correspondence should be addressed. linda.ayouni-derouiche@isa-lyon.fr ; catherine.ladaviere@univ-lyon1.fr

SI1. Evolution of the article number about the spICP-MS technique since 2010, and main types of NPs studied by this technique.



(A) Evolution of the article number about the SP ICP-MS technique since 2010 (used keywords: “"single particle ICP MS" or "single particle ICP-MS" or "SP ICP MS" or "SP ICP-MS", source: Web of Science).

(B) Main types of nanoparticles (NPs) studied by this technique (used keywords: “"single particle ICP MS" or "single particle ICP-MS" or "SP ICP MS" or "SP ICP-MS" and “silver nanoparticles” or “gold nanoparticles” or “titanium dioxide nanoparticles” or “silica nanoparticles” or “iron oxide nanoparticles” or “nanoparticles”, source: Web of Science).

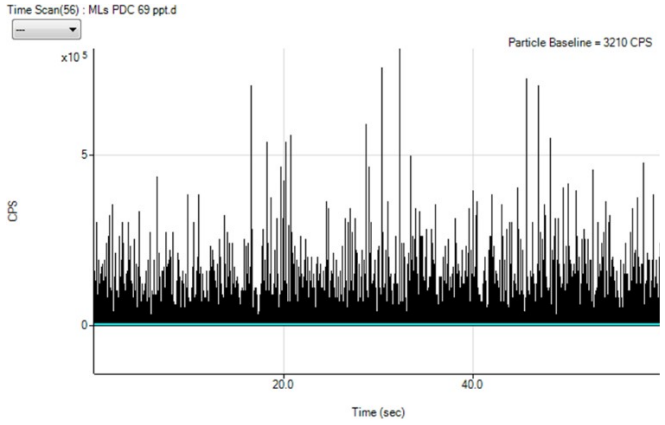
SI2. Equation of log-normal distribution.

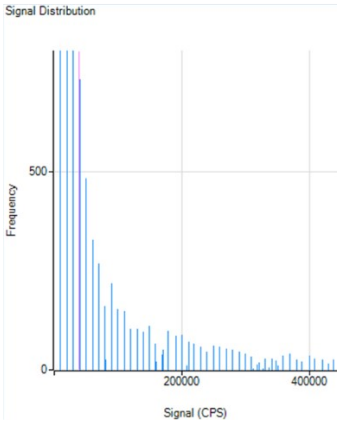
$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

x: the magnetic nanoparticle number per magnetoliposome, μ: the mean (or expectation) of the distribution, and σ: the standard deviation of the distribution.

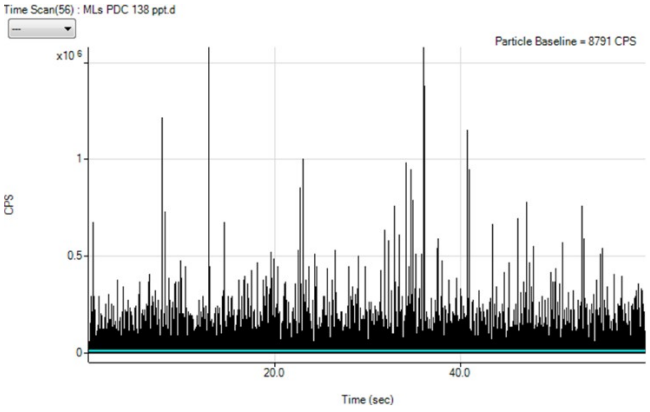
SI3. Representation of spICP-MS raw data of MLs for four iron concentrations in ppt.

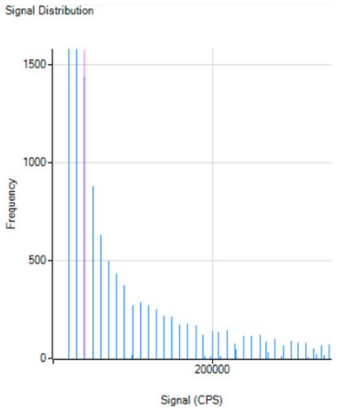
69 ppt



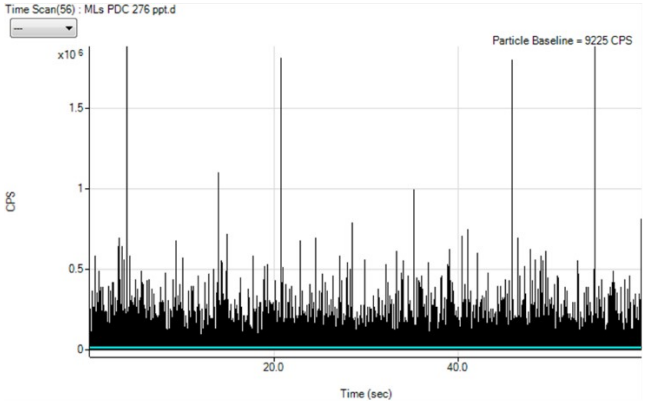


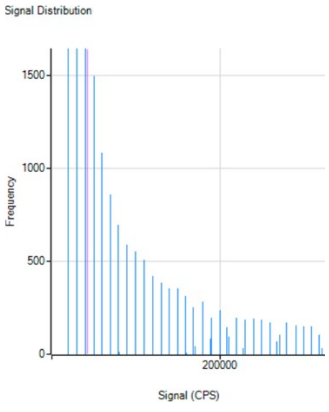
138 ppt



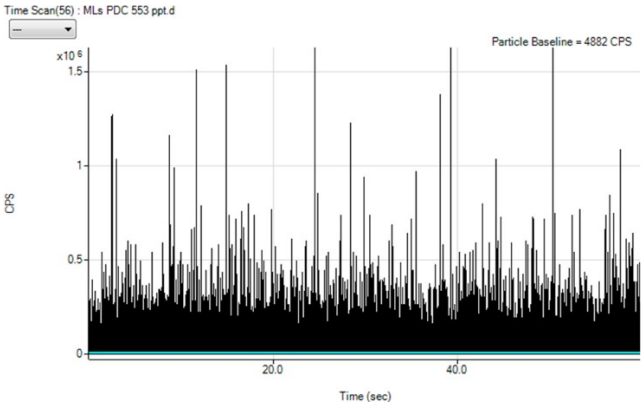


276 ppt





553 ppt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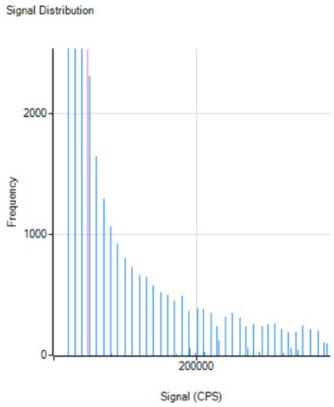
Particle Baseline = 4882 CPS
x10 6
1.5
1
0.5
0
CPS
20.0
40.0
Time (sec)


Signal Distribution
2000
1000
0
Frequency
200000
Signal (CPS)

SI4. Detailed calculations of the average number of magnetic NPs per ML measured by ICP-MS.

1) Determination of the number of magnetic nanoparticles in the final purified ML suspension

The mass of 1 magnetite nanoparticle can be expressed by:

$$m_{magnetic\ nanoparticle} = \rho_{magnetite} \times V_{SPION}$$

Where $\rho_{magnetite}$ is the volumic mass for magnetite (5.17g/cm^3) and $V_{magnetic\ nanoparticle}$ is the volume of 1 magnetic nanoparticle with a diameter (d_{TEM}) of 10 nm.

The experimental concentration of iron (Fe) in the final ML suspension is given by the ICP-MS experiments. Thus, the concentration of magnetite (Fe_3O_4) in the ML suspension is obtained by considering the mass ratio (R), which is the inverse of "f" described in the manuscript, between iron and oxygen in magnetite:

$$R = \frac{3 \times M_{Fe}}{3 \times M_{Fe} + 4 \times M_O}$$

Where M_{Fe} and M_O are the molecular mass of iron and oxygen, respectively.

Thus, the experimental concentration of magnetite (Fe_3O_4) in the purified ML suspension is expressed by:

$$C_{Fe_3O_4} = \frac{C_{Fe}}{R}$$

Finally, the average number per liter of magnetic nanoparticles in the final purified ML suspension is given by the following formula:

$$n_{magnetic\ nanoparticles} = \frac{C_{Fe_3O_4}}{m_{magnetic\ nanoparticles}}$$

2) Determination of the number of MLs in the final purified ML suspension

First, the surface of 1 ML (S_{ML}) was calculated considering a ML diameter of 120 nm, according to DLS measurements.

The surface of 1 lipid head (S_{lipid}) was considered to be 0.7 nm^2, based on literature data for a DPPC head [1].

Thus, the number of lipids in 1 ML is given by the following formula:

$$n_{lipid/ML} = 2 \times \frac{S_{ML}}{S_{lipid\ corrected}} \times C$$

Where:
- $S_{lipid\ corrected}$ is the part of the ML surface which is occupied by 1 lipid head whose surface is S_{lipid}.
- The factor 2 stands for the lipid bilayers (2 different monolayers). Each one was considered to have the same amount of lipids.
- C is the compacity of a packing of an infinite number of circles on spheres. Based on the literature data [2,3], C was considered equal to 0.9069.

Finally, the experimental concentration of phosphorous (C_{Phosp}) in the final MLs suspension is given by the ICP-MS experiments.

Thus, the number of lipids per liter in the suspension is expressed by:

$$n_{lipid} = \frac{N_A \times C_{Phosphorus}}{0.95}$$

Where:
- N_A is the Avagadro number
- C_{Phosp} is the experimental phosphorous concentration (mol/L)
- The factor 0.95 comes from the fact that the 5% of DPTAP in the lipid formulation do not have phosphorous atoms.

Thus, the average number of ML in the final purified ML suspension is given by

$$n_{MLs} = \frac{n_{lipids}}{n_{lipids/ML}}$$

3) Determination of the number of magnetic nanoparticles per ML

Based on previous calculations in sub-sections (1) and (2), the number of magnetic nanoparticles per ML is expressed by the following formula:

$$n_{magnetic\ nanoparticles/ML} = \frac{n_{magnetic\ nanoparticles}}{n_{MLs}}$$

[1] Lis L. J., McAlister M., Fuller N., Rand R. P., Parsegian V. A., *Interactions between neutral phospholipid bilayer membranes*, *Biophysical Journal* , **37**, 657-65 (1982)

[2] Clare B. W., Kepert D. L., *The optimal packing of circles on a sphere*, *Journal of Mathematical Chemistry,* **6**, 325-49 (1991)

[3] Clare B. W., Kepert D. L., *The closest packing of equal circles on a sphere, Proceedings of the Royal Society A,* **405**, 329-44 (1986)