Supplementary Information

Rapid and highly sensitive protein extraction via cobalt oxide nanoparticle based liquid-liquid microextraction coupled with MALDI mass spectrometry

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Calculation of concentration of Co_3O_4/CTA^+ NP

Number of surface atoms of Co_3O_4/CTA^+ NP and concentration were calculated according to reported methods [1–3]. The average size of the Co_3O_4/CTA^+ NP was about 50 nm measured by SEM. We can assume NP as a large spherical metal clusters with the following conditions (R. L. Johnston, "Atomic and Molecular Clusters" Taylor & Francis, 2002):

$V_{cluster} = NV_{atom}$ ----- (1)

$4/3\pi (R_{cluster})^3 = N\ 4/3\ \pi (R_{atom})^3$ ------- (2)

Where $V_{cluster}$ = cluster or atomic volume; R_{atom} = cluster or atomic radius; N = total number of atoms within the cluster.

Further,

$R_{cluster} = N^{1/3} R_{atom}$ ------- (3)

Then, we can calculate the cluster radius by calculating

Calculation of Concentration of Co_3O_4/CTA^+ NP

From SEM result, we know that $R_{cluster}$ = 50 nm and and R_{Co3O4}=141 pm. The number of Co_3O_4 atoms per nanoparticle is estimated by Eq. 3.

$N = (R_{cluster} / R_{Co3O4/CTA^{+}})$

$=(50 \times 10^{-9}\ m / 141 \times 10^{-12}\ m)^3$

44591588 Co and O atoms per Co_3O_4 /CTA^+ NP

1 mg of Co_3O_4/CTA^+ NP prepared was dissolved in 100 mL of toluene, so the molar (M) concentration was 0.041M and the number of the Co_3O_4 particles in the solutions:

N_{atom}=100 x 0.041 x NA (N_A is the Avagadro's number)

= 100 x 0.041 x 6.022 x 10^{23}

=2.47 x 10^{24}

$N_{NP} = N_{atom}/N$

N_{NP} = (2.47 x 10^{24}) / (44591588)

$N_{NP} = 5.5 \times 10^{16}$

Six different dilute concentrations of Co_3O_4/CTA^+ particles were prepared in 100 μL toluene for the separation of biomolecules from the sample solution and in 1 μL (used for MALD-MS analysis) the particle/ μL would be:

1). 0.7 x 10^{12}/ μL

2). 1.4 x 10^{12}/ μL

3). 4.0 x 10^{12}/ μL

4). 6.7. x 10^{12}/ μL

5) 8.0 x 10^{12}/ μL

5). 9.4 x 10^{12}/ μL

This values of concentration of Co_3O_4/CTA^+ particles/μL used in the experiments throughout the manuscript were calculated based on the following references:

1. J. D. Lewis, M. T. Day, V. J. MacPherson, Z. Pikeramenou, *Chem. Comm.* 2006, **13**, 1433 - 1435.

2. A. Murugadoss, A. Chattopadhyay, 2008, **9**, Nanotechnology (9 pages).

3. I.Yu, T. Isobe, M. Senna, *J. Phys. Solids*, 1996, **57**, 373 – 379.