Three strategies to stabilise nearly monodisperse silver nanoparticles in aqueous solution

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Supplementary Data

Bootstrapping is a very useful tool in statistics in cases when there is doubt that distributional assumptions and asymptotic results are valid and accurate [S1]. The fundamental concept in bootstrapping is in building a sampling distribution for a particular statistic by resampling from the data at hand. Based on the two independent samples obtained by TEM and AFM we modelled nanoparticle sphericity by constructing an ellipse with diameter from TEM and height from AFM, and calculate the corresponding statistic eccentricity ($\varepsilon$, eq (1)):

$$\varepsilon = \sqrt{\frac{a^2 - b^2}{a^2}} = \sqrt{1 - \left(\frac{b}{a}\right)^2} \quad 0 < \varepsilon < 1$$

(1)

where $a$ and $b$ are an ellipse's half major and minor axes (diameter and height) respectively.

The eccentricity of an ellipse is a measure of how circular the ellipse is. As the eccentricity value ranges from 0 to 1, the ellipse ranges from circular to highly elongated. According to eq (1) and non-parametric bootstrap techniques ($B = 1000$ resampling) we plot the distribution of our bootstrapped
statistic as a histogram (Fig. S1A) and we summarise the results by using the percentile bootstrap confidence intervals (95% confidence level) (Table S1). In addition, we address the question of interest “How spherical are the nanoparticles synthesised in this study?” according to the the Kruskal-Wallis hypothesis test. The Kruskal-Wallis test is the non-parametric analog to One-way Analysis of Variance. This test is used to determine if there are “significant” differences among the population medians (instead of the population means). The Kruskal-Wallis H statistic is an overall test statistic that enables one to test the general hypothesis that all population medians are equal. However, the investigator is not extremely interested in this general hypothesis but is interested in comparisons amongst the individual groups. According to that we compare the nanoparticle types to establish a ranking of eccentricity, and hence sphericity (where a low eccentricity corresponds to a high sphericity). We find (Fig. S1B, Table S2) that the silver-chromium alloy nanoparticles are the most spherical, followed by the silver-gold nanoshells, PEG-functionalised silver and finally pure silver nanoparticles.

Fig. S1. Statistical analysis of nanoparticle shape from combining TEM (diameter) and AFM (height) data distributions. (A) Eccentricity bootstrap distributions obtained for each nanoparticle type. High frequencies to the left x-axes (low eccentricity values) indicate a spherical nanoparticle. (B) Results of non-parametric Kruskal-Wallis hypothesis testing on particle eccentricity (a low eccentricity corresponds to a high sphericity).

Table S1
Bootstrap eccentricity percentile confidence intervals (95% confidence level).

| Nanoparticle | Percentile CI (95%) |
|--------------|---------------------|
| Ag           | [0.25, 0.99]        |
| AgPEG        | [0.30, 0.99]        |
| Ag-Au        | [0.21, 0.98]        |
Table S2
Kruskal Wallis hypothesis test and Multiple comparisons (significance level= 0.05).

**Eccentricity (e) test:**

### Kruskal-Wallis: Multiple Comparisons

Kruskal-Wallis Test on the data

| Group  | N  | Median | Ave Rank | Z     |
|--------|----|--------|----------|-------|
| AgCr   | 1000 | 0.7741 | 1706.8   | -9.29 |
| Ag     | 1000 | 0.8584 | 2249.0   | 7.86  |
| AgPEG  | 1000 | 0.8522 | 2263.4   | 8.31  |
| AgAu   | 1000 | 0.7805 | 1782.9   | -6.88 |
| Overall| 4000 |        | 2000.5   |       |

\[ H = 198.31 \quad DF = 3 \quad P = 0.000 \]

\[ H = 198.31 \quad DF = 3 \quad P = 0.000 \quad (adjusted \ for \ ties) \]

### Kruskal-Wallis: All Pairwise Comparisons

Comparisons: 6
Ties: 73
Family Alpha: 0.2
Bonferroni Individual Alpha: 0.033
Bonferroni Z-value (2-sided): 2.128

Sign confidence interval for median

|               | Achieved | Interval | Confidence | Lower | Upper | Position |
|---------------|----------|----------|------------|-------|-------|----------|
|               | N  | Median |            |       |       |          |
| AgCr          | 1000 | 0.7741 | 0.8628     | 0.7551| 0.7830| 477      |
|               |     |        | 0.8676     | 0.7551| 0.7832| NLI      |
|               |     |        | 0.8787     | 0.7550| 0.7838| 476      |
| Ag            | 1000 | 0.8584 | 0.8628     | 0.8479| 0.8689| 477      |
|               |     |        | 0.8676     | 0.8478| 0.8691| NLI      |
|               |     |        | 0.8787     | 0.8476| 0.8695| 476      |
| AgPEG         | 1000 | 0.8522 | 0.8628     | 0.8451| 0.8638| 477      |
|               |     |        | 0.8676     | 0.8451| 0.8639| NLI      |
Kruskal-Wallis: Conclusions

The following groups showed significant differences (adjusted for ties):

| Groups            | Z vs. Critical value | P-value |
|-------------------|----------------------|---------|
| AgCr vs. AgPEG    | 10.7775 >= 2.128     | 0.000   |
| AgCr vs. Ag       | 10.4985 >= 2.128     | 0.000   |
| AgPEG vs. AgAu    | 9.3035 >= 2.128      | 0.000   |
| Ag vs. AgAu       | 9.0245 >= 2.128      | 0.000   |

The graph displays boxplots of the groups with their sign confidence intervals for the medians. This graph is extremely useful because you can visually see the eccentricity values of the nanoparticles with respect to the others (a low eccentricity corresponds to a high sphericity)
Due to the presence of extreme values in our dataset we performed bootstrapping by weighting frequency values. In other words, once the value becomes extreme enough, the weight for that observation becomes very small in the resampling procedure so the influence of the observation is minimal. These weights are the relative frequencies of particle diameters from TEM and particle heights from AFM.

**References**

S1. Efron B, Tibshirani RJ: *An introduction to the Bootstrap*. London: Chapmann & Hall; 1993.