"Just Add Water" – "Instant" singly-dispersed suspensions of silver nanoparticles on demand.

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ABSTRACT

The intrinsic instability of silver nanoparticles (AgNPs) is a substantial challenge to achieving stable long-term storage for on-demand use of AgNPs. Here, a "Just Add Water" approach is presented for achieving suspensions of principally singly dispersed AgNPs. By lyophilizing (freeze-drying) the formulated AgNPs into a solid powder, or cake, water is removed thereby eliminating solution-based chemical changes such as oxidative dissolution. Storing under inert gas should further reduce surface reactions. An example of an optimized lyophilization formulation is presented for 75 nm AgNPs. This "Just Add Water" approach enables ease of use for the researcher desiring on-demand singly dispersed AgNP suspensions from a single master batch. Implementation of this methodology will enable studies to be performed over long periods of time and across different laboratories using particles that are identical chemically and physically and available on-demand, and serve as prototypes for future nanoscale reference materials.

Keywords: silver nanoparticles, lyophilization, freeze drying, reference materials, nanoparticle dispersions

1 INTRODUCTION

The rapid pace of development of new nanomaterials and new applications has also attracted much attention from the environmental health and safety (EHS) risk assessment community. Subsequently, calls for well-characterized common test materials from a single batch for use in nanoEHS biological and ecological toxicity studies continue to be made to facilitate the intercomparison of these results. However, solution-based decomposition of silver nanoparticles (AgNPs) prevents using a single batch over years or sometimes even months of study. To overcome this limitation, lyophilization of AgNPs is proposed to recover reproducible singly-dispersed AgNP suspensions. This work will show how formulation of the initial suspension of AgNPs to be lyophilized is critical to recovering singly-dispersed suspensions. Additionally, a "Just Add Water" redispersion protocol that requires only adding deionized water and shaking by hand will be shown to effectively and efficiently reconstitute the lyophilized powders of AgNPs. The "Just Add Water" approach minimizes the variability in producing uniform dispersions that can be introduced by either sonication or user error in complex procedures. For example, it has been shown that formulations greatly impact the reproducibility of dispersion protocols, with factors such as the potential capping agents found in biological media [1], synthetic lung fluid [2], environmental waters [3,4] all providing various degrees of colloidal stabilization. User procedure details, even apparently trivial details such as the timing and order of addition of AgNPs and serum proteins to cell culture media play a role, and can even be exploited to fabricate controlled agglomerate dispersions [5]. Lyophilization of AgNPs and subsequent bottling under inert atmosphere will enable assessment of whether long-term stability can be increased over solution-based storage approaches. Additionally, this lyophilization approach will be used by the National Institute of Standards and Technology for the development of upcoming silver nanoparticle reference materials.

2 MATERIALS AND METHODS[1]

2.1 Lyophilzation

Suspensions containing 1 mg mL^{-1} Ag of nominally 75 nm AgNPs with 10 mg mL^{-1} polyvinylpyrrolidone (PVP), were purchased from nanoComposix, Inc. (San Diego, CA), stored in the dark at 4 ºC, and used as received within 3 weeks of receipt. Temperature loggers were used during shipment to ensure the temperature did not vary more than 2 ºC from the targeted 4 ºC. Working in a class 2 biological safety cabinet, suspensions were dispensed in 2.000 mL aliquots into 10 mL serum bottles for lyophilization. The samples were frozen for 6 h in a stoppering tray drier (Model 7948020, LabConco, Kansas City, MO) with a shelf temperature of –40 ºC, then lyophilized for 30 h. Figure 1 shows an example record of the system and sample temperatures and system pressure over the course of one lyophilization run. The "shelf" temperature is the temperature of the refrigerant flowing through the shelves the bottles were sitting on and thus the amount of cooling. After the initial freezing step, the sample, or "cake", temperature is lower than the shelf temperature. At this point, heat is flowing into the cake to provide the energy required for the water molecules to sublimate. When the

[1] The identification of any commercial product or trade name does not imply endorsement or recommendation by the National Institute of Standards and Technology.

sublimation has completed, the temperature of the samples will rise to an equilibrium temperature greater than the shelf temperature.

The stoppering tray drier was backfilled with argon gas to approximately 400 Pa before the serum bottles were stoppered inside the freeze drier. Bottles were crimp-sealed and stored in the dark at room temperature until reconstitution. A photograph of the lyohpilized solids, or "cakes", in the crimp-sealed bottles is shown in Figure 2.

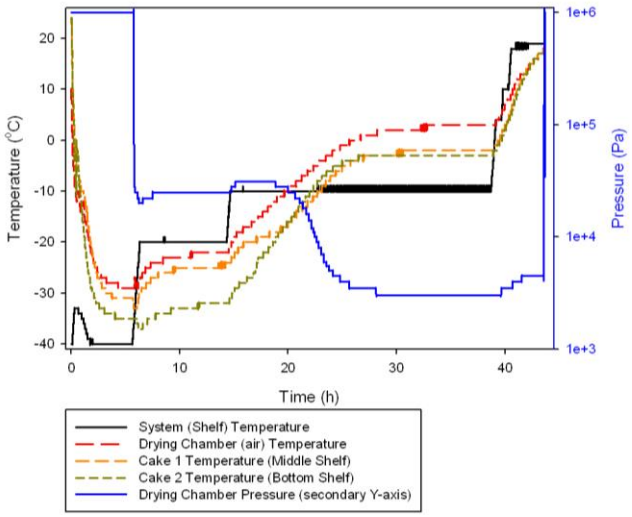

Figure 1. Example record of system pressure, shelf temperature (system temperature), air temperature of a thermocouple probe sitting on a shelf, and temperature of two sample cakes.

Figure 2. Photograph of lyophilized 75 nm AgNPs with 1 mg mL^{-1} PVP.

2.2 Reconstitution of lyophilized cakes

Reconstitution of lyophilized PVP-AgNP cakes involved addition of 2.00 mL of DI water, gentle shaking of the bottle by hand for approximately one minute, and allowing the capped suspension to stand for one hour under ambient laboratory light and temperature conditions before measurements.

2.3 Instrumentation

Dynamic light scattering (DLS) was performed using a Malvern Zetasizer Nano (Westborough, MA), and using disposable semi-micro UV-transparent plastic cuvettes (BrandTech, Inc., Essex, CT). Samples were diluted 1:100 with biological grade deionized (DI) water before measurement. DLS measurements were analyzed with both the refractive index and viscosity values of DI water and the refractive index and viscosity values of a PVP solution in water; however, at these Ag and PVP concentrations, < 1 % difference in reported z-average size was found. All reported z-average diameter values are the mean of five consecutive measurements, made under repeatability conditions, with uncertainty of one standard deviation about the mean. Note, this uncertainty relates to the precision of the measurement, and is not reflective of the width of the size distribution. DLS intensity-based size distributions were obtained with Zetasizer Software v6.20 (Malvern, Westborough, MA) using the general purpose analysis model.

Immediately upon completion of DLS measurements, cuvettes were transferred to a Lambda 750 Spectrophotometer (Perkin Elmer, Waltham, MA, USA) for ultraviolet-visible (UV-vis) absorbance spectroscopy.

3 RESULTS AND DISCUSSION

For the nominally 75 nm AgNPs, a 10 mg mL^{-1} PVP formulation was selected. This formulation is a smaller PVP amount compared to what is required for smaller diameter AgNPs [6], due to the decrease in number concentration of larger nanoparticles when the silver mass concentration remains constant.

To determine both the degree of recovery of silver nanoparticles and look for signs of agglomeration, UV-vis spectroscopy was employed. Figure 3 compares the optical properties of the 75 nm AgNPs before and after one lyophilization-reconstitution cycle. A good recovery of the silver mass was observed, based on the intensity of the absorbance spectra for the reconstituted AgNPs being almost as strong as the intensity of the stock AgNPs. Additionally, the shape of both absorbance spectra was essentially identical (see Figure 3). No increases at longer wavelengths were observed, which indicates no signs of agglomeration. Additionally, there was no shifting of the peak wavelength to shorter wavelengths, which indicates no dissolution into smaller sizes of AgNPs, such as has been seen with UV-induced oxidation and dissolution of AgNPs [7].

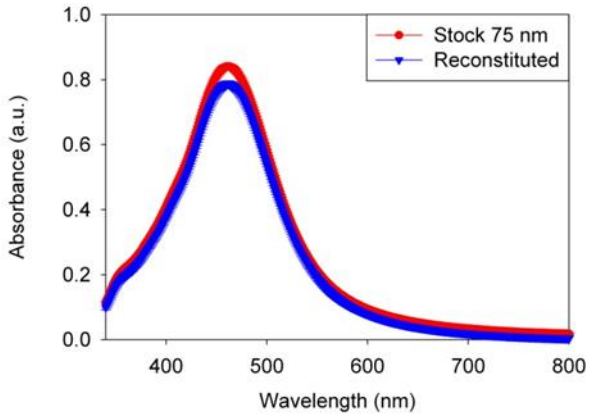

Figure 3. Absorbance spectra before (red) and after (blue) lyophilization and "Just Add Water" resuspension of 75 nm AgNPs. Data are the mean of three measurements, with uncertainty of one standard deviation smaller than the size of the symbols.

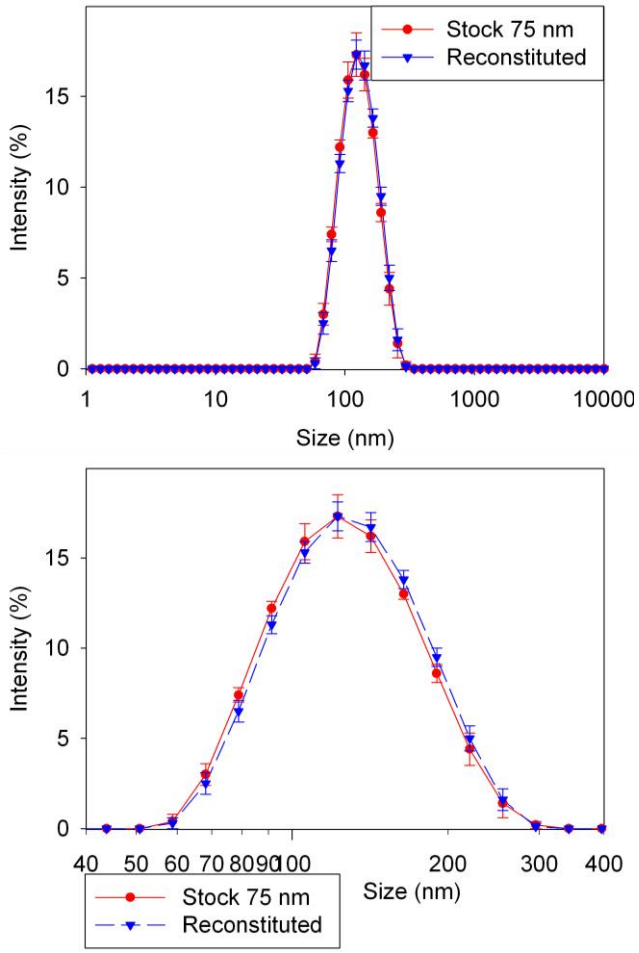

Figure 4. DLS size distributions before (red) and after (blue) lyophilization and "Just Add Water" resuspension of 75 nm AgNPs. Data are mean of 5 consecutive measurements on one suspension, with uncertainty of one standard deviation. Top panel is full data range collected, bottom panel is same data set magnified.

To further investigate whether signs of agglomeration were present in the optimized formulation, DLS was employed. DLS is the natural choice for this, as the intensity-based size distributions obtained by DLS are volume-squared weighted and thus especially sensitive to larger particle sizes in heterogenous mixtures. Therefore, one of the strengths of DLS is detecting the earliest signs of aggregation or agglomeration.

The DLS intensity-based size distributions for the pre- and post-lyophilization suspensions were the same, within the uncertainty of the measurements. The upper and lower panels of Figure 4 show the same dataset (the average of 5 consecutive measurements) of stock (pre-lyophilization) and reconstituted (post-lyophilization) AgNP suspensions, with the size axis scaling changed to highlight both size distributions being within the uncertainty of the measurements.

Examining the DLS and UV-vis data together confirm that no aggregation was occurring due to the lyophilization process. Using routine screening approaches to monitor for signs of aggregation has been demonstrated to be an efficient and reasonable approach elsewhere [8].

4 CONCLUSION

This work demonstrates a proof of concept for a "Just Add Water" methodology for generating on-demand suspensions of singly dispersed AgNPs from a lyophilized cake of AgNPs using PVP as the lyophilizing agent. An optimized formulation for nominally 75 nm AgNPs is presented that reproduces the pre-lyophilization size distribution within the uncertainty of the measurements used. The lyophilized cakes were easily reconstituted by just the addition of DI water, gentle shaking by hand for one minute, and allowing the suspensions to stand on the benchtop for one hour. This formulation serves as a prototype for the forthcoming NIST Reference Materials RM8017, nominally 75 nm AgNPs. This "Just Add Water" approach of resuspension after lyophilization and packaging under inert gas could potentially enable new classes of moderately stable nanoparticles to become candidates for future reference material development, or benchmarks or common test materials for long-term studies.

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