DoE-It-Yourself: A Case Study for Implementing Design of Experiments into Nanoparticle Synthesis

Niamh Mac Fhionnlaoich, Ye Yang, Federico Galvanin, and Stefan Guldin*

Department of Chemical Engineering, UCL, London

E-mail: s.guldin@ucl.ac.uk

Abstract

Predictable and repeatable outcome is a major issue in nanoparticle synthesis. Traditionally, a one-factor-at-a-time (OFAT) method is relied upon to investigate and optimise synthetic processes; however, this method is inefficient and often misleading. Design of experiments (DoE), in contrast, can provide a greater amount of information in fewer experiments and lends itself to more reproducible results. Nevertheless, DoE techniques are only used by a relatively low number of practitioners in nanoparticle research. Here, we provide a step-by-step tutorial for the synthesis of oleylamine-capped gold nanoparticles (AuNPs). Through the use of DoE, we are able to achieve a marked reduction in dispersity and develop a model for detailed control over the mean diameter of the nanoparticle populations. Principles of the case study presented herein are applicable and should serve for facile implementation of DoE to other synthetic routes.

Introduction

For many applications, monodisperse nanoparticles of a specific size and morphology must be synthesised in a manner that is repeatable.[1] The synthetic procedures however, are typically rather complex with numerous experimental variables and their interactions affecting the resulting particle outcome. This issue is compounded by the fact that most experimental approaches rely on one factor at a time method (OFAT) variation, which seriously limits the understanding of exactly how the experimental conditions affect the outcome of the synthesis.[2] Since OFAT methods neglect any variable interactions, process optimisation may often lead to false conclusions and suboptimal results. Furthermore, a lack of understanding on the global experimental domain delays scientific exchange and overall progress.

DoE can be implemented to overcome these limitations. In essence, DoE is simply strategic planning of experiments and the application of statistics. Even so, it provides a robust framework designed to maximise the amount of information obtained for a given number of experiments. DoE provides more accurate results as averages are compared with averages which reduces bias due to random variance. Furthermore, it can detect and define interactions between experimental conditions. These attributes make it significantly more powerful than the OFAT method.[3] We refer to an introduction to experimental design and optimization by Lundstedt *et al.*.[4] The wider context of data-driven approaches to materials synthesis, in particular the context between OFAT, DoE, machine learning and sequential learning was recently discussed by Braham *et al.*.[5] Tao *et al.* highlighted viable routes for the accelerated development of efficient protocols for nanoparticle synthesis by the implementation of machine learning algorithms.[6]

DoE has been applied to nanoparticle synthesis with favourable outcome. Hao *et al.* used a DoE-based approach in optimising the synthesis of solid lipid nanoparticles for drug delivery.[7] The results of their experimental campaign revealed strong interactions between the experimental variables, which would not have been discovered through an OFAT approach. Similarly, non-linear and interaction effects evidenced by a response surface design

enabled Barglik-Chory *et al.* to achieve control over the band-gap energies of bio-stabilized CdS nanoparticles using DoE.[8] Burrows *et al.* investigated the effect of eight experimental variables in a fractional factorial series, revealing novel insights on the seed-mediated silver-assisted synthesis of gold nanorods.[9] Indeed, there are numerous examples of DoE being used to varying degrees in the optimisation of various characteristics of nanoparticles, such as size, phase, stoichiometry, yield or drug loading.[10–20]

The synthesis of olelyamine-capped gold nanoparticles (AuNPs) represents an ideal case study for a tutorial view on DoE due to its versatility as well as the conflicting literature describing the optimal approach to their synthesis. Well-defined AuNPs in a tunable size range from 6 to 21 nm have been reported by the use of only two reactants ($HAuCl_4$ and oleylamine) in the presence of a solvent (here toluene), highlighting the dual role of oleylamine as both reducing agent and capping agent.[21] An alternative route involves the use of gold(I) halides, including AuCl and AuBr.[22] The use of borane *tert*-butylamine (tBAB) as an additional reducing agent for $HAuCl_4$ provides access to a size range of 2-10 nm, where the size can be tuned due to its dependence on the reaction temperature.[23] Even within this simple experimental procedure, there has been conflicting results on what affects the outcome of the experiment. Peng *et al.* used tetralin as a solvent in their experimental campaign and achieved low degrees of dispersity and a size tunable by temperature.[23] In contrast, Wu *et al.* found that using tetralin drastically increased polydispersity when compared to linear hydrocarbons such as octane. Furthermore, they noted that while the mean size of AuNPs synthesized in tetralin was highly dependent on the reaction temperature, the size of the AuNPs synthesized in octane was much more affected by the amount of reducing agent in the system.[24] Conversely, more recent studies by Yang *et al.* have found that the size of the AuNPs can be tuned via temperature in Octane producing monodisperse AuNPs in the range of 2-6 nm.[25] While oleylamine effectively stabilizes the AuNPs, this work also showed that it can easily be replaced with prescribed mixtures of thiol-terminated ligands to provide AuNPs target functionalities.

Here, DoE was applied for two purposes. First, to determine the sub-set of experimental conditions that reliably produce monodisperse AuNPs. Second, within this sub-set, to understand the relationship between these experimental conditions and the mean particle diameter. Despite the limited number of reagents and steps involved in this synthesis, the interplay between experimental conditions, or factors, to produce a given AuNP population can be very complex. A standard, OFAT method seriously limits the understanding of how these factors interact to determine the outcome. Neglecting interactions can lead to false conclusions and substandard results during process optimisation. This synthesis is therefore an ideal case study to demonstrate the capabilities of DoE in resolving the relationships between the experimental conditions and the outcome of the experiment with the aim to produce monodisperse particles of a given size in a repeatable fashion.

Methods

Synthesis

The general protocol to synthesis oleylamine-capped AuNPs required $HAuCl_4 \cdot 3H_2O$ to be dissolved in 20 of an octane oleylamine solution, the ratio of which was set according to the experimental design. A jacketed flask was connected to a Grant GD120-R2 thermostatic bath which maintained a set temperature with a resolution of 0.1 °C. The flask was flushed with argon to provide an inert atmosphere then sealed. The gold salt solution was stirred vigorously for 10 minutes to ensure full dissolution and to equilibrate the temperature in the flask. A second solution was prepared containing tert-butylamine borane (tBAB) in octane and oleylamine at a concentration of 0.125 M. Once fully dissolved, this solution was quickly injected into the jacketed flask containing the gold salt. After a set reaction time (30 minutes to 2 hours), the reaction was quenched with ethanol. The quantities of $HAuCl_4 \cdot 3H_2O$, molar equivalents of tBAB, temperature, and composition of the the octane/oleylamine solution were determined by the experimental design. The ranges for the experimental conditions are

summarised in Table 1 & 2.

To wash the particles, the reaction solution was divided between two 50 ml effendorf tubes and precipitated using ethanol. A Thermo Scientific Multifuge X1R was used to centrifuge the samples at 5,000 rpm and 10 °C for 10 mins. After decanting, approximately 2 ml dichloromethane was used to resuspend the particles before the washing protocol was repeated. The AuNPs were left to dry overnight under vacuum at room temperature. TEM images were obtained using a JEOL JEM-2100 (200 kV). The images were analysed by imageJ to determine the area of the AuNPs and, assuming spherical particles, the diameter of each NP was obtained. The resolution of the measurement for the image acquisition and analysis was estimated to be 0.1 nm. This was used as the bin width to calculate the nanoparticle entropy.[26]

Design of Experiments

Two designs were used in the study of these oleylamine-capped AuNPs. The first experimental campaign was used to determine a set of conditions which minimized the dispersity of the resulting nanoparticle population. The aim of the second design was to investigate the effect of experimental conditions on the mean particle size and develop a model. These two designs are detailed below.

Case Study 1: Minimizing Nanoparticle Dispersity

A three level, two factor full factorial design was used to study the effects of the reaction time and ratio of the reducing agent to the gold salt on the nanoparticle dispersity. Previous studies on this and similar systems had highlighted these two as key variables in the dispersity of the resulting population and this design would provide a response surface model to reveal any non-linear effects or interactions between the two factors. The levels investigated are presented in Table 1. For these experiments, a solution of 12.5 mM of gold salt in solution of 6.67 mL of oleylamine and 13.33 mL of octane was prepared. The reaction temperature

was set at 15 °C. The full experimental design can be found in SI Table S1.

Table 1: **Experimental factors for nanoparticle dispersity.** The experimental factors investigated in the DoE for nanoparticle dispersity and the values corresponding to the low, medium, and high levels.

Factor	**-1**	**0**	**+1**
Reducing Agent Stoichiometric Ratio	0.5	1.25	2
Reaction Time (minutes)	30	75	120

Case Study 2: Modelling Nanoparticle Diameter

Prior work on the synthesis of oleylamine-capped AuNPs have identified the concentration of the gold salt and capping agent as well as the reaction temperature as experimental variables by which mean particle diameter could be controlled. Here, a central composite inscribed design was used to explore the full experimental domain and develop a response surface model for mean nanoparticle diameter. This experimental design is shown in SI Table S2. The values corresponding to the low, medium, and high levels are summarised in Table 2. A stoichiometric ratio of 1.6 moles of tBAB to gold salt and a reaction time of 120 minutes was used for this design.

Table 2: **Experimental factors for mean nanoparticle diameter.** The experimental factors investigated in the DoE for mean nanoparticle diameter and the values corresponding to the low, medium, and high levels.

Factor	**-1**	**0**	**+1**
Gold Salt Concentration (mM)	3.125	12.5	21.875
Ratio of Oleylamine to Reaction Solvent	0.5	1.25	2
Reaction Temperature (°C)	5	15	25

Results and Discussion

Case Study 1: Dispersity

The three level, full factorial design allowed for the estimation of the second order effects and interactions between the reaction time and reducing agent ratio. The results are shown

Table 3: **Experimental factors that determine dispersity.** The experimental factors and scaled estimates that determine the dispersity as measured by entropy.

Factor	**Scaled Estimate**	**p-value**
Intercept	1.6119	0.0002
Reducing Agent Stoichiometric Ratio	-0.2381	0.1192
Reaction Duration (minutes)	-0.5653	0.0066
[Reducing Agent Stoichiometric Ratio]^2	0.5709	0.0328
[Reducing Agent Stoichiometric Ratio]* [Reaction Duration (minutes)]	-0.3503	0.0737
[Reaction Duration (minutes)]^2	0.4493	0.0695

in Table 3.

The variance associated with this model was minimal; the RMSE and R^2 were found to be 0.31 and 0.90 respectively. From this model, the optimal conditions to minimise the nanoparticle entropy could be identified. Based on the values presented in Table 3, a reducing agent ratio of 1.6 and reaction time of 111 minutes should result in minimal dispersity. These results must be tempered by a physical understanding of the system. The model attempts to fit the effect of reaction time on entropy as a second order polynomial; however, for nanoparticle synthesis, a singular optimal reaction time is unlikely. Most probably, once sufficient time has passed for the synthesis to complete, and the in presence of an effective capping agent, additional time has little to no impact on the entropy. Therefore, it was determined that the optimal conditions for minimal dispersity were a reducing agent ratio of 1.6 and a reaction time of 120 minutes. The effects of reducing agent ratio and reaction time on the entropy are shown in Figure 1.

The dispersity of the AuNP populations as measured by the normalised nanoparticle entropy was found to be predominately determined by the reaction duration and the molar ratio of the reducing agent ratio to the gold salt. Studies on the mechanism of AuNP synthesis have noted a slow growth stage after nucleation where the core increases in diameter either through coalescence or Oswald ripening and a notable decrease in dispersity occurs.[27–29] These studies suggest an interruption in the slow growth process results in a broader particle size distribution. Furthermore, Liu *et al.* suggested the relationship between particle size

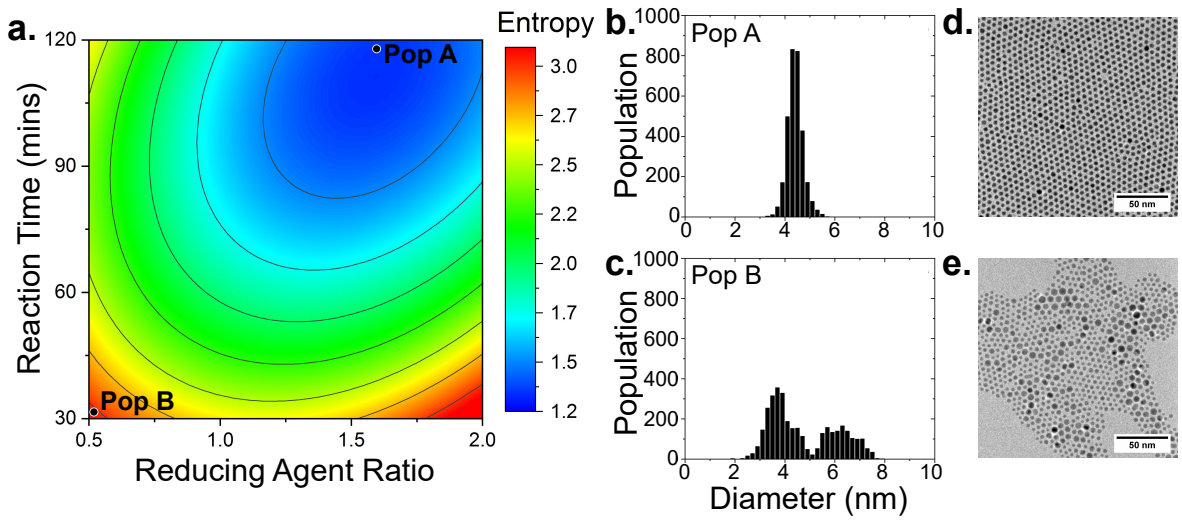

Figure 1: **Entropy model. a.** Response surface diagram depicting the effect of reducing agent ratio and reaction time on entropy. **b & c.** The AuNP diameter distribution for points A & B. **d & e.** TEM images for the samples A & B.

and time followed a logistic trend.[29] This is in good agreement with the observations from this study. Initially, increasing the reaction time substantially improved monodispersity. However, at longer reaction times, little to no reduction in dispersity was observed.

Zheng *et al.* investigated the strength and relative concentration of the reducing agent on the dispersity of the synthesised AuNP population.[30] Strong reducing agents rapidly reduced the gold salt and produced polydisperse samples. Meanwhile, weaker reducing agents enabled a slow, controlled nucleation and growth process resulting in a low dispersity. The concentration of reducing agent acted similarly. High concentrations promoted rapid reduction and increased polydispersity, while the inverse was observed for low concentrations. However, insufficient reducing agent may also generate highly disperse populations.[31,32] To achieve optimal monodispersity, the nucleation and growth phases would ideally be temporally separated, which is facilitated by a greater presence of reducing agent. The results from the DoE demonstrated a non-linear relationship between the stoichiometric ratio of tBAB to the gold salt and the normalised nanoparticle entropy. This behaviour illustrated the delicate balance between too much and too little reducing agent.

Case Study 2: Mean Diameter

To accurately determine the factors that affect the mean particle diameter, monodisperse, or near-monodisperse, populations are required. Therefore, the optimised conditions to minimise dispersity were applied to all runs in the experimental design to investigate mean particle size. The central composite design used allowed the estimation of all main effects as well as interactions and non-linear effects involving the gold salt concentration, the ratio of the capping agent to the reaction solvent, and the reaction temperature. The results from the analysis of variance is presented in Table 4.

Table 4: **Experimental factors that determine mean gold nanoparticle diameter.** The experimental factors and scaled estimates that determine the mean AuNP diameter.

Factor	**Scaled Estimate**	**p-value**
Intercept	4.8706	<0.0001
Gold Salt Concentration (mM)	-0.4043	0.0175
Ratio of Oleylamine to Reaction Solvent	0.3643	0.0291
Reaction Temperature (°C)	-0.8543	<0.0001

All three factors, gold salt concentration, reaction temperature, and composition of the solvent solution, were important in governing the mean particle size. The relationship between these parameters is shown in Figure 2.

Statistical evaluation of the model fit demonstrated it satisfactorily represented the data. The R^2 value was calculated at 0.76 with an RMSE of 0.51; these values reflect good agreement between the predicted and experimental results. The analysis of variance tests the model to determine if it sufficiently explains the variance in the data, i.e. the dependence on the experimental conditions; the presented model passed this test with a p-value below 0.0005. Additionally, the lack of fit test determined the model described the non-random variance in the data without over-fitting ($p = 0.2068$). Figure 3 plots the actual data against the predicted values.

Using the model from Table 4, a number of experimental conditions were identified to synthesize AuNPs in the range 4-6 nm. The resulting particles were analysed and plotted

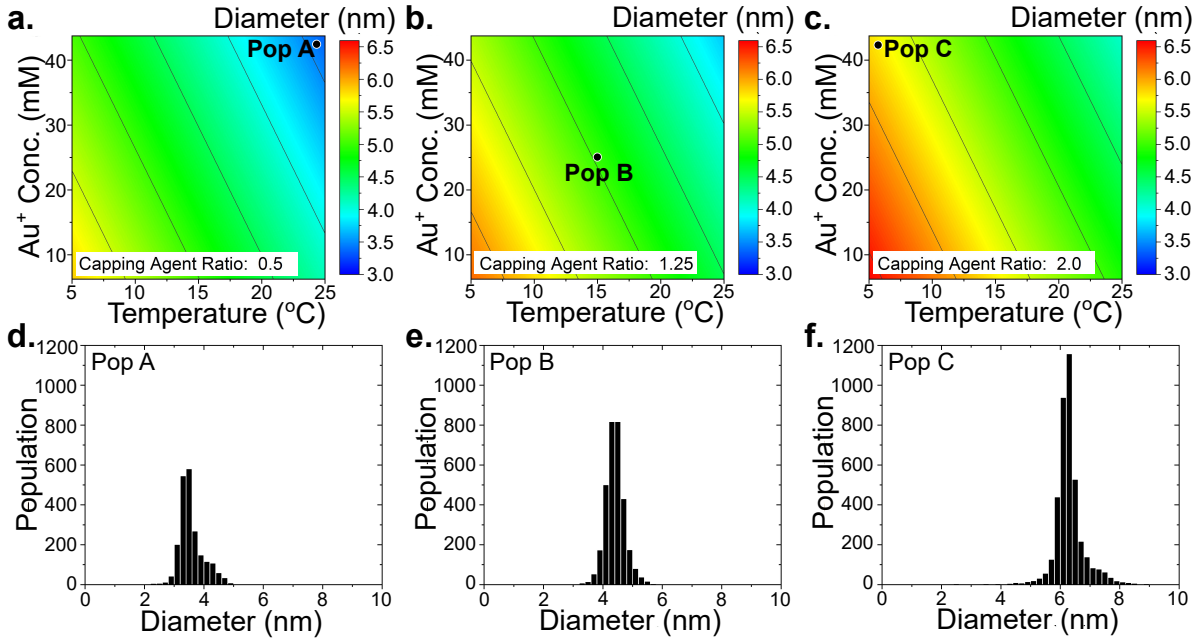

Figure 2: **Effect of experimental conditions on mean gold nanoparticle diameter.** Effect of gold salt concentration and reaction temperature on the mean AuNP diameter for ratios of oleylamine to octane equal to **a.** 0.5 (low level), **b.** 1.25 (centre point), and **c.** 2.0 (high level). Sample AuNP populations are plotted in **d-f.** with reference to the corresponding experimental conditions.

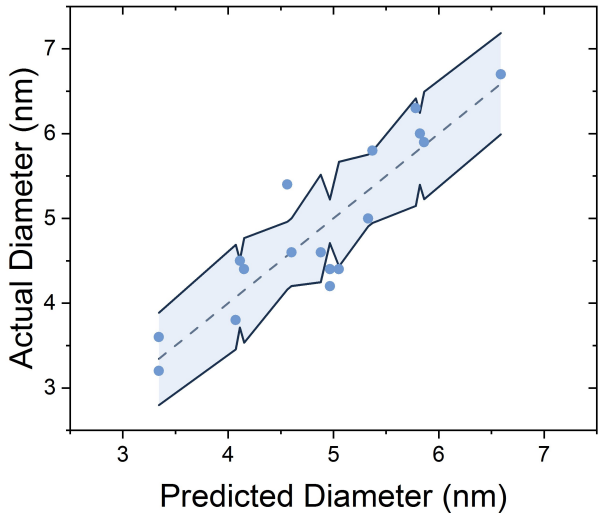

Figure 3: **Actual vs. predicted mean particle diameter.** Actual data plotted against the predicted values for the mean particle diameter model.

against the predicted values in Figure 4. The R^2 and RMSE were found to be 0.52 and 0.33, respectively, demonstrating a reasonable fit.

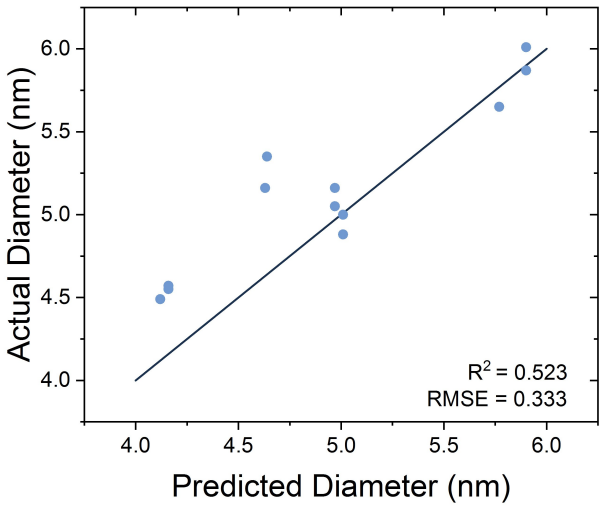

Figure 4: **Gold nanoparticle mean diameter model validation.** Actual data plotted against the predicted values for the mean particle diameter model.

The effect of reaction temperature on mean particle size has been well established by previous studies and was reflected by this DoE.[23,25,33] Furthermore, the interaction between capping agent ratio and reaction temperature partly explains the insensitivity to temperature reported by Wu *et al.* which contrasted with the resulted from other studies.[24] The model presented here shows reaction temperature is the dominant factor in determining mean particle size; however, for the capping agent ratio and temperature range investigated in the study by Wu *et al.*, only a small change was observed. The interaction between oleylamine content and reaction temperature has also been demonstrated in literature. The efficacy of oleylamine in forming a stabilising layer on the surface of the nanoparticle depends on the temperature and relative concentration of oleylamine.[29,34] In agreement with the work done by Aslam *et al.*, a decrease in the concentration of the oleylamine corresponds to an increase in particle size. Overall, the combination of these two parameters have a sizeable impact on the mean AuNP diameter.

It was found that the particle diameter varied proportionally with the concentration of the gold salt in the range investigated. This dependence has been noted in the synthesis of citrate and thiol stabilised AuNPs.[31,35,36] It is difficult to make direct comparisons with

such different systems; however, these lend credence to the impact of gold salt on the mean particle size. Furthermore, the profoundly non-linear behaviour observed by Zabetakis *et al.* on the effect of the ratio between the citrate and the gold salt and the mean particle size cautions against extrapolation of this effect to other concentrations.

Lastly, it is important to note that the non-negligible experimental variance revealed in this study. During an OFAT study, this increased variation can inhibit detection of significant effects or impact the accuracy when attempting to produce a model. This highlights the need for DoE to overcome these sources of variance to uncover the fundamental relationships.

Conclusion

The synthesis of oleylamine-capped AuNPs is a complex process, which cannot be easily understood by a traditional OFAT process. In contrast, the DoE-based approach highlighted interactions between factors and mitigated experimental variance, allowing the development of models for nanoparticle dispersity and diameter. Key factors for nanoparticle dispersity were identified and optimised to reliable produce monodisperse populations. Subsequently, experimental conditions relating to the mean particle size were analysed. A functional model of the system was produced enabling synthesis of low dispersity AuNPs in the range of 3-7 nm. The principles of experimental design and material optimization presented herein are applicable to a broad range of synthetic protocols and should serve for facile implementation to other nanoparticle platforms.

Supporting Information

The following files are available free of charge.

- Supplementary Information: Table outlining the full experimental design for the synthesis of oleylamine-capped gold nanoparticle.

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