

Stöber reaction, the so-called seeded polymerization [16], but in this process, the effect of reactant addition rate and the number or size of seeds highly influence the product properties, which factors make it more difficult to control the reactions, with this the properties of SNPs.

We aimed to develop a simple, well-size-controlled synthesis method with high reproducibility and product yield for SNPs under 200 nm particle size. We want to focus on only the synthesis procedure, not on the reaction mechanism; it may provide practical help to researchers who deal with the application of SNPs. We assumed that with the proper choice of tetraethyl orthosilicate (TEOS), ammonium hydroxide, or water concentration and reaction temperature, we could find relationships between the SNPs' size and reaction conditions that may be suitable to calculate the reaction conditions for synthesizing SNPs with the desired size.

We chose the reaction conditions and reagents based on literature data and our previous observations and experiences. We worked with TEOS as a silanol precursor and ethanol as a solvent. Green and co-workers described before that if the alcohol chain length increases, the first nucleation step will be slower, and the size of the nuclei will be bigger, so the final particle size will also be bigger [17]. Therefore, in our experiments, we worked with ethanol instead of methanol because if we would like to synthesize SNPs in a large amount, it is an important practical aspect that ethanol is cheaper and more eco-friendly. The choice of TEOS and ammonium hydroxide concentration is based on our previous experiments. We worked with 0.26 M TEOS concentration in all cases because the product yields were unsatisfactory at lower values. If the TEOS concentration is higher than 0.3 M, the SNP size is higher than 500 nm, and here, we focus on the 5–200 nm particle size range. We have also examined previously if the ammonium hydroxide concentration is lower than 0.0192 M, then at 0.26 M TEOS concentration particles do not form, presumably under this ammonium hydroxide concentration value the hydrolysis rate is very slow, only partially hydrolyzed monomers are formed. On the other hand, the particle size increases significantly if the ammonium hydroxide concentration is higher than 0.5 M. Based on these, we worked with a constant 0.26 M TEOS concentration and in the 0.29–0.097 M ammonium hydroxide concentration range.

Plumeré et al. [18] and J. Yang et al. [19] established that if the reaction temperature is higher, the particle size and the polydispersity index will be smaller; if the reaction temperature increases, the hydrolysis and condensation rates also increase, and at higher temperature, more secondary particles form, which leads to faster nucleation kinetics. Two research teams independently studied the formation of SNPs, and they found that because of the faster nucleation process, more nuclei are produced, so smaller and more uniform particles can be synthesized at higher reaction temperatures [14,20]. Other than this, Park et al. [21] and Kim et al. [22] determined that if the temperature is higher than 55 °C, the aggregation of nuclei dramatically increases, which will control the final particle size; hence, the particle size increases again. We considered these observations and experiences and varied the reaction temperature between 25 and 55 °C.

The water concentration also influences the hydrolysis and nucleation rates; it enhances the aggregation of nuclei, which controls the primary particle number and size. Lindberg et al. [23] and Zukoski et al. [24] observed the following trend in particle size between 0.5–17 M water concentrations: the particle size decreases if the water concentration decreases under 9 M, but over this value, the particle size increases again because of the enhanced aggregation of primary particles. High water concentration is rarely used in the Stöber synthesis because its high value limits the TEOS solubility [21], which decreases the reaction yield and lengthens the reaction time. Based on these observations, we worked in the 2–5 M water concentration range.

We characterized the SNPs using dynamic light scattering (DLS) and transmission electron microscopy (TEM), as our main focus was to tailor the size and morphology of SNPs.

2. Materials and Methods

2.1. Materials

This study used tetraethoxysilane [TEOS], (Alfa Aesar [USA], purity 98%), absolute ethanol AnalR Normapur, purity $\geq 99.8\%$ (VWR Chemicals, Debrecen, Hungary), 28 w/w% ammonium solution AnalR Normapur, analytical reagent (VWR Chemicals, Debrecen, Hungary), and ultrapure water (membraPure Astacus Analytical with UV, VWR Chemicals, Debrecen, Hungary). TEOS was freshly distilled before each reaction.

2.2. Silica Nanoparticle Synthesis

The silica nanoparticles were prepared using hydrolysis of TEOS in ethanol in the presence of ammonium hydroxide as a catalyst. The concentration of TEOS was constant in each reaction, $c = 0.26 \text{ mol}/\text{dm}^3$. We systematically changed the concentration of ammonia, water, or temperature as follows:

- varied the ammonia concentration between $0.29 \text{ mol}/\text{dm}^3$ and $0.097 \text{ mol}/\text{dm}^3$, at a constant water concentration ($5 \text{ mol}/\text{dm}^3$) and a constant temperature (25°C)
- varied the water concentration between $2 \text{ mol}/\text{dm}^3$ and $5 \text{ mol}/\text{dm}^3$ at a constant ammonia concentration ($0.29/0.194/0.097 \text{ mol}/\text{dm}^3$) and a constant temperature (25°C)
- varied the temperature between 25°C and 50°C at a constant ammonia concentration ($0.29/0.194/0.097 \text{ mol}/\text{dm}^3$) and a constant water concentration ($5 \text{ mol}/\text{dm}^3$)

The total volume for the systematic study was 8 cm^3 . The water, ethanol, and TEOS mixture were stirred at constant temperature for 15 min. Then, the reaction mixtures were sonicated for 15 min (Bandelin Sonorex, RK 52 H, Berlin, Germany), followed by adding 10 m/m% ammonium hydroxide solution under stirring. The reaction mixtures were stirred for 24 h at a constant temperature. For all experiments, we used vials of the same size 8 or 100 cm^3 , magnetic stirring bars of the same size and shape, and a constant stirring rate (1000 min^{-1}). For each synthesis, a new factory-cleaned vial and cap with PTFE membrane were used to avoid contamination with silica seeds, which can be the main cause of size heterogeneity. The synthesized silica nanoparticles were stored and found to be stable without aggregation and any change in size or morphology for several months in the residual reaction solution.

2.3. Characterization of Silica Nanoparticles

The size distribution, hydrodynamic diameter (d), and polydispersity index (PDI) were determined by DLS using a Malvern Zetasizer Nano S instrument. The size distribution was confirmed, and the morphology of silica nanoparticles was studied with TEM (JEM 1200 EX II and JEOL-1400 TEM [JEOL Ltd., Tokyo, Japan]). Samples for TEM experiments were prepared using drop casting “as is” samples on 400 mesh copper grids with carbon coating (Micro to Nano Ltd., Haarlem, The Netherlands). ImageJ 1.53 was used for size distribution analysis from TEM images, and the equivalent diameter for 300–400 particles was determined.

2.4. Statistical Analysis

Statistical analyses were conducted using Microsoft Excel[®] 2016. Correlations between the studied parameters and the diameter of silica nanoparticles were assessed using linear regression. The coefficient of determination (R^2) was determined to establish the fit of the models. Finally, confidence intervals for each model were examined to determine the accuracy of the prediction made by the models. All experimental results were performed in triplicates and are presented as mean \pm SD.

3. Results

Firstly, we investigated the influence of the ammonium hydroxide concentration on the particle size if the temperature is constant, $t = 25^\circ\text{C}$. We determined that in the $0.29\text{--}0.097 \text{ M}$ ammonium hydroxide concentration range, SNPs between 27.1 and 190.8 nm could be

We determined that by increasing the reaction temperature, the SNP particle size decreases at constant TEOS (0.26 M), water (5 M), and ammonium hydroxide (0.29/0.194/0.097 M) concentration. At different ammonium hydroxide concentrations, the SNP size decreased at different rates. At a 0.29 M ammonia concentration, the particle size is between 190.8 nm and 69.3 nm in the 25–55 °C temperature range (see Table 1, A1 and T30A-T55A samples). At lower an ammonia concentration, 0.194 M, the particle sizes are in the 30.2–99.1 nm interval (see Table 1, A2 and T30B-T55B samples), and at 0.097 M, these values are in the 6.1–27.1 nm size range (see Table 1, A3 and T30C-T55C samples). We performed all syntheses three times, and we found that the reproducibility of SNP synthesis is excellent; the standard deviations are very low. At each temperature and ammonium hydroxide concentration, the SNPs are highly monodisperse (PDI: 0.002–0.091) and spherical (see Figure 2). Higher PDI values (0.016–0.091) were measured at higher temperatures; we assumed this phenomenon might indicate the temperature heterogeneity in the reaction vessels. We found a linear correlation between particle size and reaction temperature, and we used it to calculate the reaction temperature for tailored particle size by regulating the temperature at constant TEOS and ammonia concentration (see Figure 3). Our results show that precise temperature control makes it possible to synthesize SNPs with a desired particle size in three different size ranges at each ammonia concentration (Figure 3 and Table 2). Statistical analysis has confirmed the model's quality (Table 3). These calculations in the different SNP size intervals allow for SNP size tailoring or fine-tuning. At a 0.29 M ammonia concentration, we can precisely control the particle size to be between 65 and 200 nm. If we want to synthesize SNPs in the 30 and 100 nm particle size range, we should work with a constant 0.194 M ammonia concentration. At a 0.097 M ammonia concentration, SNPs under 30 nm size can be produced, and we can fine-tune the particle size from 6 nm to 23 nm.

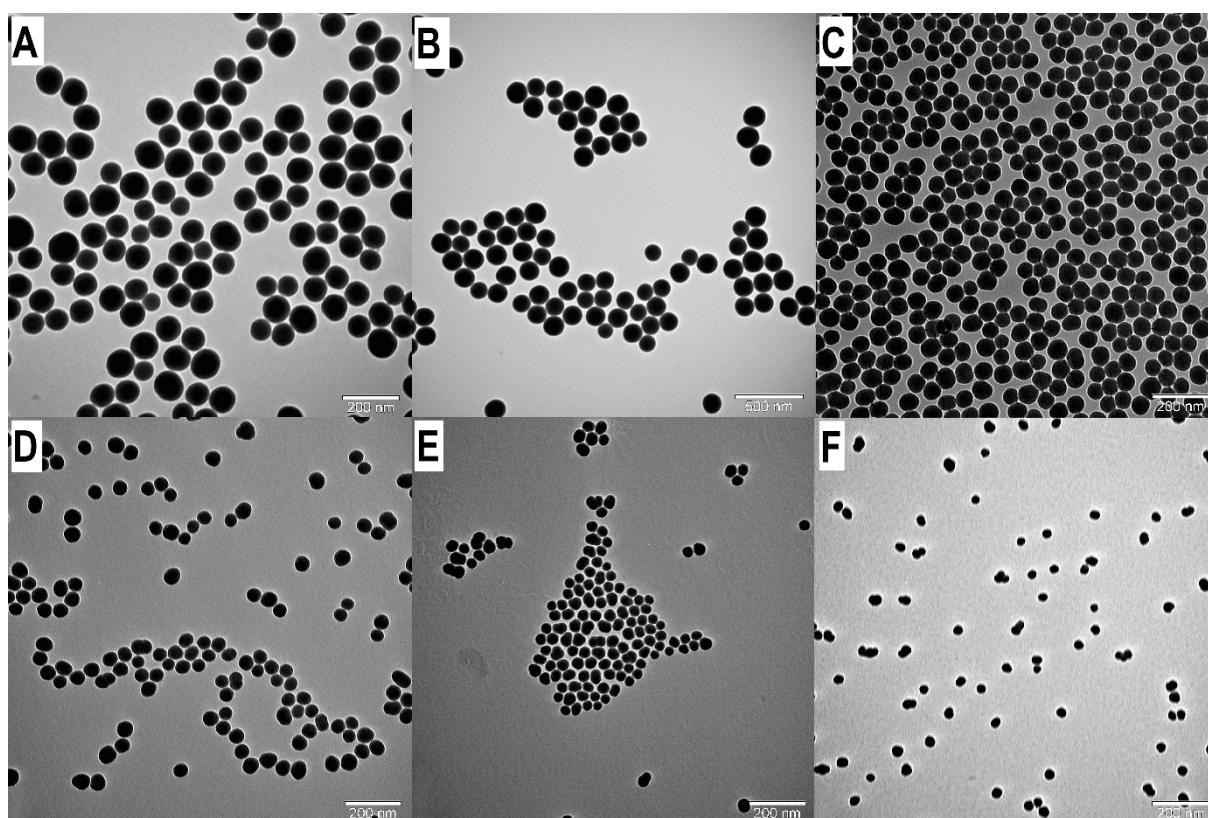


Figure 2. TEM images of SNPs: Examination of the influence of reaction temperature on the SNP size. (A): T25B $d = 91.72$ nm. (B): T30B $d = 81.85$ nm. (C): T35B $d = 65.43$ nm. (D): T40B $d = 55.42$ nm. (E): T45B $d = 42.14$ nm. (F): T50B $d = 31.96$ nm.

Table 3. Statistical analysis of temperature–size dependence on SNP synthesis.

cNH ₃ (mol/dm ³)	Linear Regression	CI	R2
0.291 M	y = -4.906x + 316.720	93%	0.9909
0.194 M	y = -2.819x + 168.630	95%	0.9846
0.0197 M	y = -0.814x + 44.412	92%	0.9326

Similarly to the size dependence of temperature, at different ammonium hydroxide concentrations, the SNP particle sizes decrease in different degrees: at a 0.29 M ammonia concentration, the particle size is between 52.18 and 190.80 nm, and there is an increase in the 2–5 M water concentration range (see Table 1 A1 and W4A-W2A samples). At a lower ammonia concentration, 0.194 M, the particle sizes are in the 27.81–99.09 nm interval (see Table 1 A2 and W4B-W2B samples), and at 0.097 M, these values are in the 6.92–27.1 nm size range (see Table 1 A3 and W4C-W2C samples, Figure 4). Here, we also found a correlation between water concentration and SNP size. By decreasing the water concentration, the SNP particle size decreased at a constant TEOS (0.26 M) and ammonia concentration (0.29/0.194/0.097 M) at 25 °C; the relation between water concentration and SNP size can be fitted with quadratic equation (Figures 5 and 6). These relations also make it possible to calculate and tailor the SNP size. It is important to note that if the water concentration is lower than 3 M, the sphericity of SNPs decreases while polydispersity increases. Statistical analysis has verified the quality of the model, as shown in Tables 4 and 5.

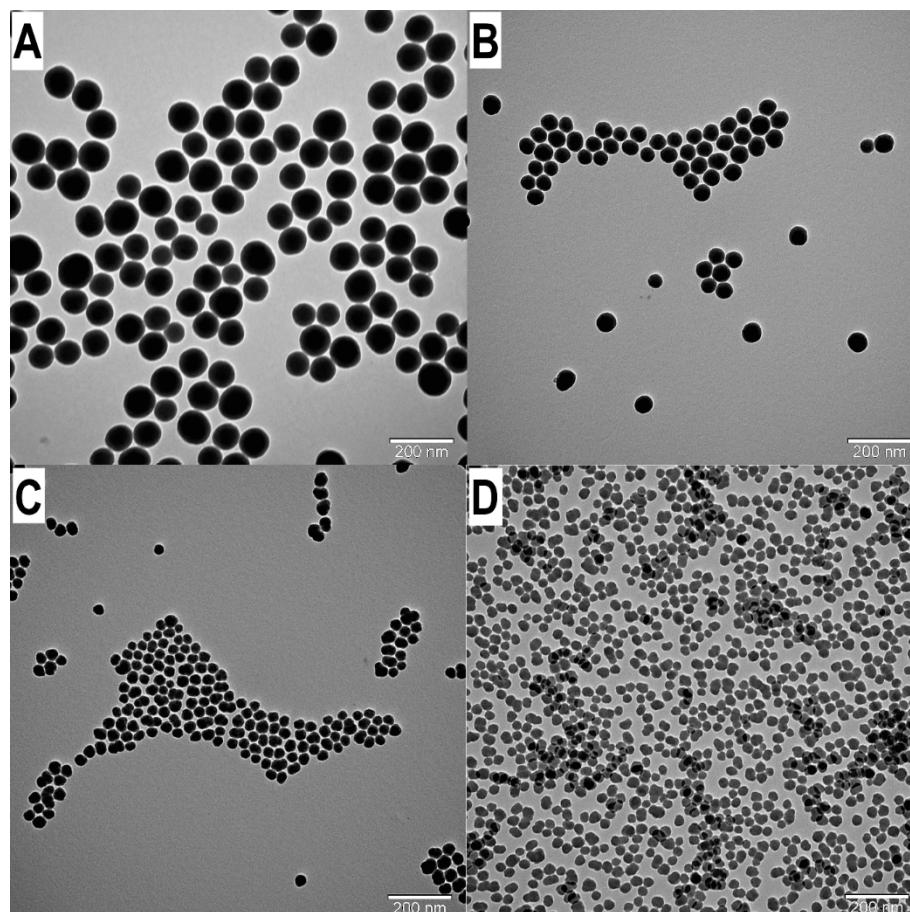


Figure 4. TEM images of SNPs: Examination of the influence of water concentration on the SNP size. (A): W5B d = 91.73 nm. (B): W4B d = 60.29 nm. (C): W3B d = 33.60 nm. (D): W2B d = 28.20 nm.

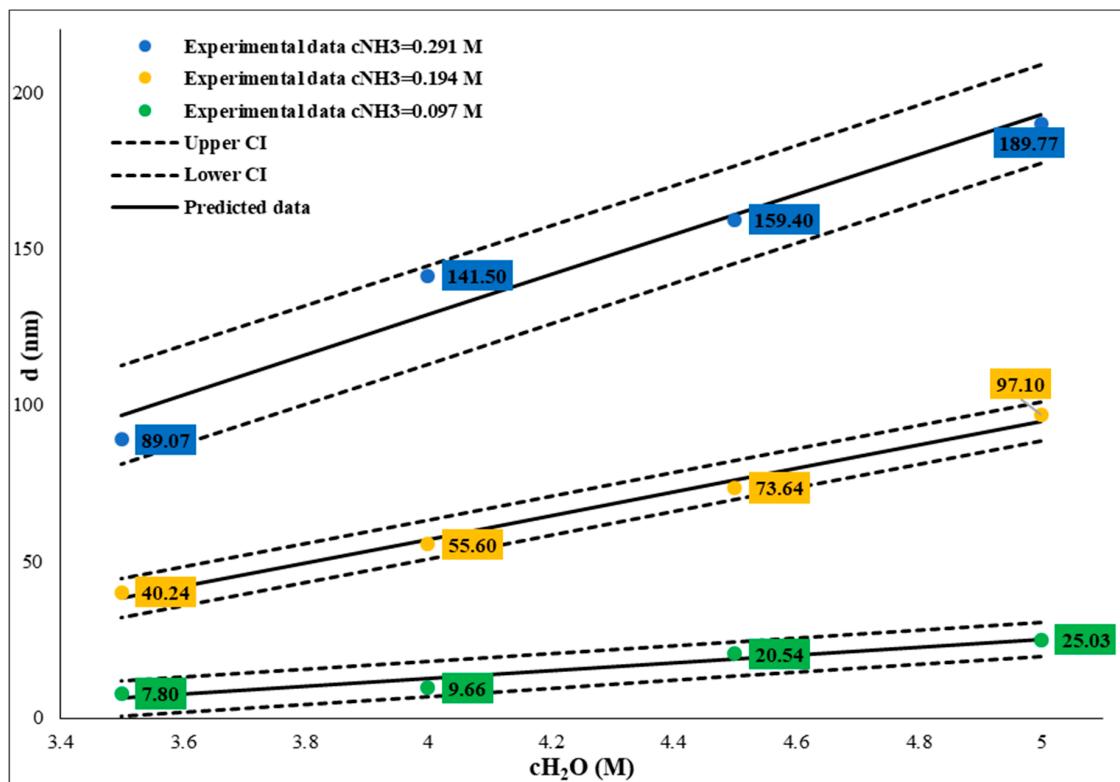


Figure 5. Influence of water concentration between 3.5 and 5 mol/dm³ on size of SNPs at different ammonium hydroxide concentrations. The TEOS concentration was constant, $c = 0.26\text{ M}$.

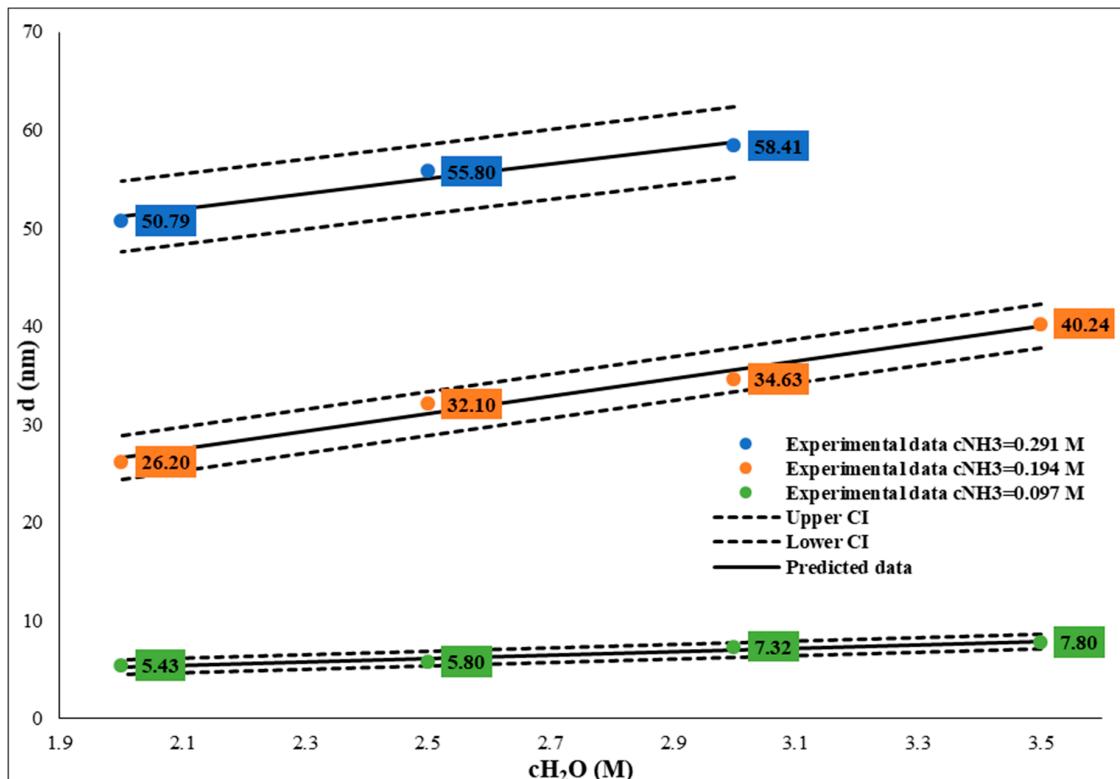


Figure 6. Influence of water concentration between 2 and 3.5 mol/dm³ on size of SNPs at different ammonium hydroxide concentrations. The TEOS concentration was constant, $c = 0.26\text{ M}$.

Table 4. Statistical analysis of a water concentration of 3.5–5 mol/dm³ and size dependence during SNP synthesis.

cNH ₃ (mol/dm ³)	Linear Regression	CI	R ²
0.291 M	y = 63.998x + 127.057	99%	0.9566
0.194 M	y = 37.222x + 96.647	95%	0.9906
0.097 M	y = 12.514x + 34.427	95%	0.9350

Table 5. Statistical analysis of a water concentration of 2–3.5 mol/dm³ and size dependence during SNP synthesis.

cNH ₃ (mol/dm ³)	Linear Regression	CI	R ²
0.291 M	y = 7.623x + 35.940	99%	0.9679
0.194 M	y = 8.931x + 8.730	95%	0.9793
0.097 M	y = 1.726x + 1.840	95%	0.9385

4. Discussion

The study focused on the controlled growth of various sizes of silica nanoparticles (SNPs) by systematically adjusting the ammonium hydroxide concentration, water concentration, and temperature. SNP synthesis of particles sized less than 200 nm was targeted using common approaches while ensuring high yields, narrow size distributions, and reproducibility in the synthesis of the nanoparticles. In this discussion, the consequences that the results may have will be appreciated; a comparison of the results obtained with what is already known from past work will be made, and finally, how the findings can be applied will be considered.

General rules for successfully synthesizing silica nanoparticles with the desired size are: always use freshly distilled TEOS, which tends to hydrolyze and form oligomers and silica seeds at room temperature without catalysts, even with atmospheric water. Always use a new factory-cleaned vial and screw cap with a PTFE sealing membrane, as the complete removal of adsorbed silica particles from the used vial wall is only plausible by HF-containing cleaning mixtures. Magnetic stirring bars have to be cleaned with great care. Silica contaminants are major reasons for polydispersity and formation of particles with undesired size.

4.1. Influence of Ammonium Hydroxide Concentration

The study confirmed a strong correlation between ammonium hydroxide concentration and the size of the SNPs. Higher concentrations of ammonium hydroxide yielded larger particles, with sizes ranging from 27.1 nm to 190.8 nm within the 0.29–0.097 M concentration range at 25 °C. This trend aligns with previous findings in the literature, where the hydrolysis and condensation rates are affected by the catalyst concentration, influencing the nucleation and growth stages of particle formation. The reproducibility of the results with low standard deviations demonstrates the method's robustness, which is crucial for scaling up the production of SNPs for industrial applications. The results also suggest that fine-tuning the ammonium hydroxide concentration can effectively control particle sizes within a targeted range, offering flexibility for different use cases, such as drug delivery systems or biosensing applications requiring specific nanoparticle sizes.

4.2. Influence of Temperature

The study also established that increasing the reaction temperature results in smaller SNPs across different ammonium hydroxide concentrations. The linear correlation observed between temperature and particle size provides a predictable means to tailor SNP sizes through temperature control, allowing researchers to adjust reaction conditions to achieve desired particle dimensions. This finding corroborates earlier studies, which noted that higher temperatures accelerate nucleation rates, leading to smaller and more

uniform particles. However, the study also found higher temperatures increased polydispersity, especially at temperatures above 50 °C. We can assume that the temperature heterogeneity within the reaction vessel may cause uneven particle formation. Hence, while temperature control is effective for size tuning, there is a need for careful monitoring to maintain uniformity.

4.3. Influence of Water Concentration

Water concentration had a significant but complex influence on SNP size. The study demonstrated that decreasing water concentration generally resulted in smaller particles. However, at concentrations below 3 M, the particles tended to be less spherical and more polydisperse. This observation is consistent with existing research indicating that water concentration affects the aggregation of primary particles during the growth phase. The quadratic relationship between water concentration and particle size offers a more nuanced approach to size control than the linear temperature relationship. While this provides additional flexibility, it also necessitates careful optimization to avoid undesirable particle morphologies and polydispersity.

4.4. Practical Implications

The ability to precisely control SNP sizes through systematic variation of reaction conditions holds significant promise for various applications, particularly in biomedical fields. For instance, particle size can critically influence the biodistribution, cellular uptake, and clearance of nanoparticles in drug delivery. The study's findings offer a clear framework for researchers to tailor SNPs for such applications with high reproducibility and efficiency (Stöber article). Additionally, the study's emphasis on using eco-friendly solvents like ethanol enhances the practical utility of the method by making it more sustainable and cost-effective, especially for small and mid-scale production; this aligns with current trends in green chemistry, where minimizing environmental impact is a priority.

5. Conclusions

A reproducible and scalable method was successfully developed for synthesizing silica nanoparticles of sizes less than 200 nm by adjusting the ammonium hydroxide concentration, water concentration, and temperature.

The research findings indicated that ammonium hydroxide concentration possesses a linear relationship with particle size distribution, whereby increasing the concentration will lead to the formation of bigger particles. Temperature is an effective way of controlling and predicting particle size alteration linearity in which polydispersity must also be controlled at higher temperatures. Water concentration is another factor that can be used in controlling particle size, giving a parabolic dependence of particle size on the water concentration. However, the particles might not be in regular spherical shape at particularly low concentrations.

Author Contributions: Conceptualization, B.K., A.S. (Aleksandar Széchenyi) and B.V.-H.; methodology and investigation, A.S. (Aleksandar Széchenyi) and B.V.-H.; Software, A.S. (Ala' Salem) and S.P.; data curation, A.S. (Ala' Salem), S.P., A.S. (Aleksandar Széchenyi) and B.V.-H.; writing—original draft preparation, A.S. (Aleksandar Széchenyi) and B.V.-H.; writing—review and editing, B.K., A.S. (Aleksandar Széchenyi), S.P. and B.V.-H.; visualization, A.S. (Aleksandar Széchenyi) and B.V.-H.; supervision, A.S. (Aleksandar Széchenyi). All authors have read and agreed to the published version of the manuscript.

Funding: Some TEM studies were performed using the JEOL-1400 TEM electron microscope funded by the grant GINOP-2.3.3-15-2016-0002 (New generation electron microscope: 3D ultrastructure). The project has been supported by the NKFI, grant 2021_1.2.6_TET_IPARI_MA_2022_00015.

23. Lindberg, R.; Sjöblom, J.; Sundholm, G. Preparation of silica particles utilizing the sol-gel and the emulsion-gel processes. *Colloids Surf. A Physicochem. Eng. Asp.* **1995**, *99*, 79–88. [[CrossRef](#)]
24. Bogush, G.H.; Tracy, M.A.; Zukoski, C.F. Preparation of monodisperse silica particles: Control of size and mass fraction. *J. Non-Cryst. Solids* **1988**, *104*, 95–106. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.