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Fast Nucleation for Silica Nanoparticle Synthesis in Sol-Gel Method

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Note 1: Kinetics of silica nucleation

Silica particle formation in base medium via TEOS hydrolysis in pure alcoholic solutions has been extensively studied.[1] Fairly recently, attempts were made to understand nucleation behaviour in pure water medium, although TEOS is immiscible in water. These studies provided valuable insight into the growth of silica particles. Previously, it has been established that base-catalyzed TEOS follow a first order relation with the concentrations of TEOS and hydroxyl ions (OH^-) but an overall second order kinetics.[2–4] Our findings were concomitant with these studies. Our findings follow a probabilistic model of nucleation which can be depicted with

$$d[TEOS]/dt \; \alpha \; [TEOS][OH^-] \text{----------(1)}$$

In order to approximate the kinetics of TEOS consumption we have based it on a simple assumption that the recovered dry weight of the particles equates to the number of TEOS molecules as per the following calculations

1M or 1000mM TEOS = 208 g/L TEOS

90mM TEOS (employed in this study) = 18.7 g/L TEOS = 187 mg/10 mL TEOS

If, dry weight of the obtained sample from 10 mL reaction medium = 2 mg/10 mL

Then, it will have ~0.96 mM TEOS.

Now, based on these calculations, we have recovered different TEOS amounts as summarized in the Supplementary Table 1.

Supplementary Table 1: TEOS consumption and recovery with time

Time (min)	Dry particle weight (mg)	Recovered TEOS as particles (mM)	$[TEOS]_R/[TEOS]_0$
0	0	0	0
20	25±2.0	12.03	0.13
30	55±1.1	26.5	0.29
45	90±1.2	43.3	0.48
60	135±1.0	64.9	0.72
75	150±1.3	72.2	0.80

TEOS added initially $[TEOS]_0$ is 90mM equivalent to 187mg/10mL; Reaction volume for each sample was 10mL and the recoveries are reported from 10mL reaction medium

In order to obtain hydroxyl ion concentration, we have measured pH over the whole reaction time and calculated the number of [OH-] in the sample as summarized in Supplementary Table 2.

Supplementary Table 2. Hydroxyl ion concentration with time

Time (min)	pH	Hydroxyl ion concentration (μM)
0 (at NaOH addition)	10.2	158.5
20	8.3	1.99
30	8.6	3.98
45	8.6	3.98
60	8.6	3.98
75	8.7	5.02

From Supplementary Tables 1 & 2, we can calculate the rate of TEOS consumption as described below:

The TEOS consumption rate can be written as

$$[TEOS]_t/[TEOS]_0 = e^{(-kt)} \text{----------(2)}$$

or,

$$\log ([TEOS]_t/[TEOS]_0) = - kt \log e = - 0.43\ (kt) \text{ ----------(3)}$$

where k is rate constant, t is the reaction time. The calculation based on eq (3) for TEOS consumption and particle formation rates are summarized in Supplementary Table 3.

Supplementary Table 3. Rate of TEOS consumption

Time (min)	$[TEOS]_R/[TEOS]_0$	Log $([TEOS]_R/[TEOS]_0)$	kt	k (min^{-1})
0	-	-	-	-
20	0.13	– 0.886	2.060	0.103
30	0.29	– 0.537	1.248	0.042
45	0.48	– 0.318	0.739	0.016
60	0.72	– 0.142	0.332	0.006
75	0.80	– 0.097	0.225	0.003

$[TEOS]_R$ is the amount of silica recovered as nanoparticles and represents the amount of TEOS corresponding to a specific reaction time and is obtained from Supplementary Table 1.

Note 2: Phasing behaviour of TEOS in water

TEOS is immiscible in water. In order to understand the dependence of the extent of miscibility, we have experimentally confirmed the hydrolysis behaviour of TEOS in various water ratios against ethanol which serves as an excellent solvent for dissolving TEOS.

Supplementary Table 4. TEOS recovery as emulsion

Time (min)	Unreacted TEOS (mM)	$[TEOS]_t/[TEOS]_0$
0	90	1
20	77.97	0.86
30	63.5	0.71
45	46.7	0.51
60	23.1	0.25
75	17.8	0.20

Supplementary Table 5: TEOS reactivity relation with water and ethanol concentrations (TEOS: 90mM, NaOH: 18mM)

Volumetric Ratio (Water : Ethanol)	Result
10:90, 20:80	Very fast gelation
30:70	Gelation and particle nucleation
40:60	Particles with high precipitation
50:50	Particles as clear suspension
60:40	Hydrolysis without nucleation and growth
70:30	TEOS slowly phased out
80:20, 90:10, 100:0	TEOS phased out very fast

Note 3: Nanoparticle growth prediction

By the mass conservation law,

Mass of total TEOS = mass of unreacted TOES + mass of nanoparticle (recovered TEOS) ------(4)

or,

$M_{Total} = M_{Unreacted} + M_{NP}$

$V_{Total} \cdot \rho_{TEOS} = V_{Unreacted} \cdot \rho_{TEOS} + N(V_{NP} \cdot \rho_{SiO2})$

$\rho_{TEOS} (V_{Total} - V_{Unreacted}) = N \cdot 4/3\Pi R^3 \cdot \rho_{SiO2}$

$\rho_{TEOS} (V_{Total} - V_{Unreacted})/\rho_{SiO2} = N \cdot 4/3\Pi R^3$

$\rho_{TEOS}/\rho_{SiO2} \cdot \Delta V = N \cdot 4/3\Pi R^3$

$(\rho_{TEOS}/\rho_{SiO2}) \cdot (\Delta V/\Delta t) = N \cdot 4/3\Pi \cdot (R^3/\Delta t)$ ---------- (5)

For ease, derivative equation for rate or change of analysis, this can be rewritten as

$(\rho_{TEOS}/\rho_{SiO2}) \cdot V_{Consumed} = N \cdot 4/3\Pi \cdot R^3$ ---------- (6)

Considering ρ_{TEOS}/ρ_{SiO2} and $4/3\Pi$ as constant values then rate of change of TEOS volume in the reaction medium has a direct relation with the rate of change of radius. Therefore, by carefully predicting number of particles in sample we can predict the particle size.

TEOS density = 0.940 g/cm3

SiO2 density = 2.65 g/cm3

$\rho_{TEOS}/\rho_{SiO2} = 0.35$

$4/3\Pi = 4.18$

Supplementary Table 6. Predicted nanoparticle radius using eq. 6 supplementary note 2

For 20 min duration	**For 30 min duration**	**For 45 min duration**
$V_{Consumed} = 12$ mM	$V_{Consumed} = 26.5$ mM	$V_{Consumed} = 43.3$ mM
$N = 5.70 * 10^{19}$	$N = 5.66 * 10^{19}$	$N = 8.9 * 10^{18}$
$R = 0.21 * 10^{-7} = 26 * 10^{-9}$	$R = 0.34 * 10^{-7} = 34 * 10^{-9}$	$R = 0.74 * 10^{-7} = 74 * 10^{-9}$
$D = 42 * 10^{-9}$ m	$D = 68 * 10^{-9}$	$D = 148 * 10^{-9}$
SEM size: 50 nm	SEM size: 80 nm	SEM size: 170 nm
For 60 min duration	**For 75 min duration**	
$V_{Consumed} = 64.9$ mM	$V_{Consumed} = 72.2$ mM	
$N = 1.57 * 10^{18}$	$N = 5.5 * 10^{17}$	
$R = 1.51 * 10^{-7} = 151 * 10^{-9}$	$R = 2.23 * 10^{-7} = 223 * 10^{-9}$	
$D = 302 * 10^{-9}$	$D = 446 * 10^{-9}$	
SEM size: 300 nm	SEM size: 400 nm	

References:

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Experimental

Chemicals: Tetraethylorthosilicate (TEOS, ≥99%), ethanol (200 proof), sodium hydroxide were from Sigma–Aldrich. The silica wafer for electron microscopy (SEM) was from Ted Pella, Inc. All solutions were prepared in 18 MΩ cm^{-1} deionized water (DIW). All the prepared solutions were filtered using 0.2 μm filters (Fisher) prior to use.

Nanoparticle synthesis: NaOH at 18mM was added from a stock of 2M solution to make a 10 mL reaction volume with 1:1 ethanol-water solution and was homogenized with mixing at 400 rpm. TEOS was then added to the NaOH mixture at a final concentration of 90mM. Reaction was then allowed to take place for 20 min with continuous mixing. Cloudiness was observed at 15 min as a result of TEOS condensation.

Nanoparticles were allowed to develop for duration of 20-75 min as different sets, such that each set was processed at the designated time, *viz.* 20, 30, 45, 60, 75 min. After designated time the reaction was allowed for, DIW was added at 15-folds excess by volume relative to ethanol volume. This will phase out TEOS from the ethanol:water system due to its immiscibility in water leaving the already formed nanoparticles in the solution. Immediately after, the top layer consisting of TEOS phase was pipetted out and collected separately. The process of phasing out was repeated to collect any left-out TEOS species to restrict any undesired growth of silica nanoparticles or developing irregular shaped products. The SiNPs from the suspension were collected after centrifugation and were washed twice each with ethanol and DIW. Finally, the nanoparticles were stored in DIW at room temperature for further studies.

For mechanistic studies, 50nm seed particles synthesized via previously described approach was prepared under three different sets where TEOS was reacted on the seeds in (i) ethanol, (ii) 1:1 ethanol:water, and (iii) 1:1 ethanol:water solution prespiked with 18mM NaOH. Each reaction was allowed to continue for 3 h and 12 h.

Luminol entrapment: Briefly, luminol was prepared at a concentration of 50mM in 20mM NaOH. A solution of luminol was added to the reaction mixture saturated with seed particles of ~150nm in size in two different sets with 5 and 20 mM final concnetrations. The mixture with a final NaOH concentration of approximately 8mM was allowed to stand for one hour followed by addition of TEOS to a final concentration of 25mM. The reaction with TEOS was allowed to take place for 1h and particles were retrieved out by centrifugation at 5000 rpm for 10 min. Particles were thoroughly washed 3 times each with 10mM NaOH, 100mM PBS, and DIW. These were stored in DIW for further analysis.

Luminol characterization: Luminol at 0, 5, and 20mM were individually reacted with 1mM hydrogen peroxide solution followed by addition of Horseradish Peroxidase (HRP). Under similar conditions, sets of 5 and 20mM luminol-nanoparticles were subjected to the reaction with peroxide in presence of HRP enzyme. Images of all the reactions were captured using a CCD camera for a duration of 10 min and were analyzed with ImageJ ROC analysis.

High Resolution-Low Vacuum SEM Analysis: High Resolution Scanning Electron Microscopy (SEM) was performed with the Teneo HR-LVSEM. Nanoparticles were re-suspended in ethanol and drop coated on a silica wafer. The samples were dried overnight at room temperature. The dried samples were gold coated to a thickness of ~10 nm layer using chemical vapor deposition system. The prepared samples were loaded into the microscope. Imaging was performed at 10KV potential while internal pressure was maintained at 2.6 μTorr.

Particle size analysis: Dynamic Light Scattering Analysis was performed to analyze hydrodynamic size of nanoparticles using ALV/CGS-3 instrument. Size was recorded as scattering of light at 90^o light incidence. The G2(t) intensity correlation function representing particle motion was fitted against the delay time (τ) to obtain decay rates of particle motion as per the Seigert's relationship between G2(t), τ, $g_1(\tau)$

$$G_2(\tau) = B[1 + \beta|g_1(\tau)|^2]$$

According to this, larger particles will decay slower attributed to their poor diffusion while contrary is true for smaller particles.

Particle stability analysis: It was performed by measuring Zeta Potential of nanoparticle colloid using ZetaPlus analyzer (Brookhaven Instruments Corp.).

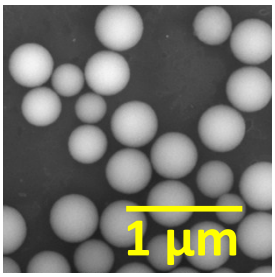
Monitoring hydroxyl content in the reaction: It was measured as a function of pH. The measured pH was employed to calculate pOH using the following relation

$$pH + pOH = 14.$$

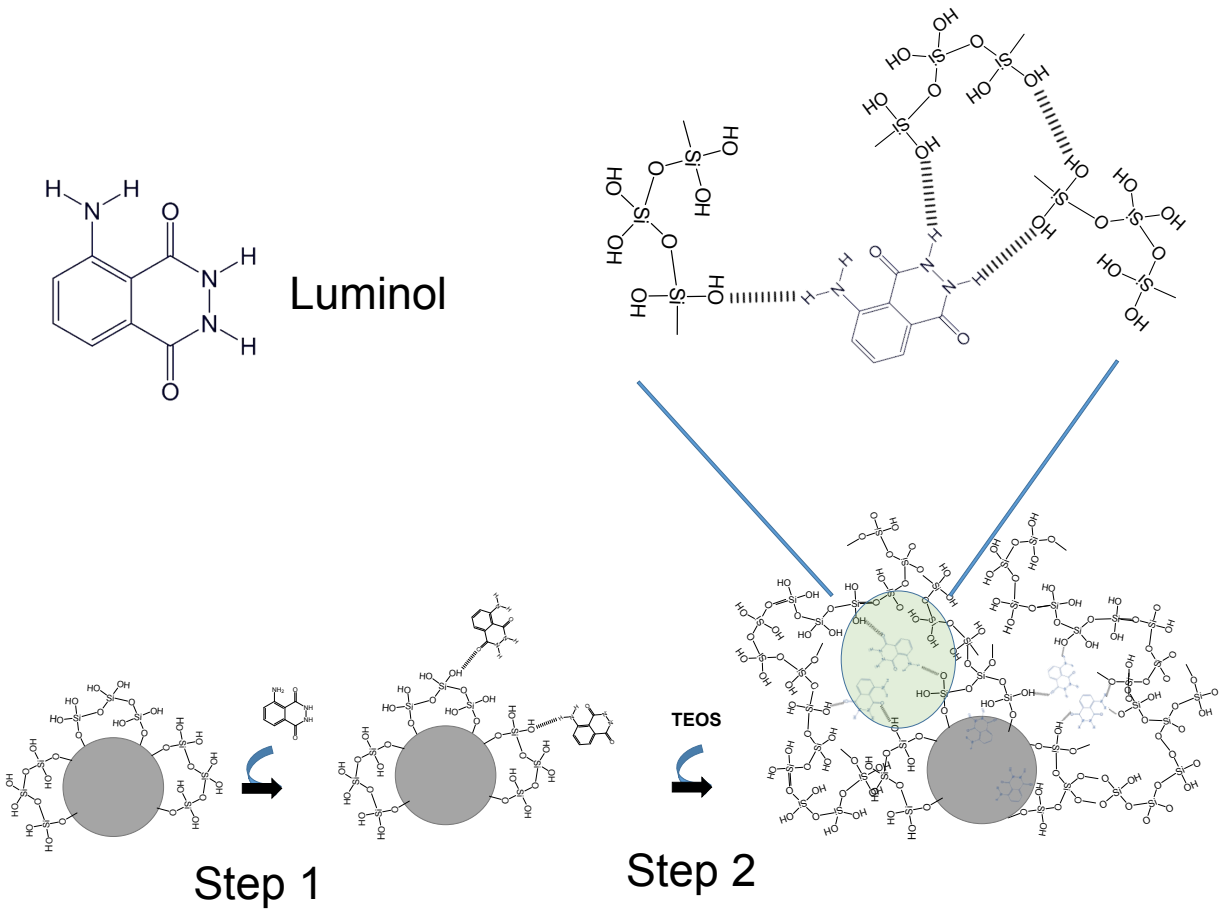
Later, pOH will be used to calculate hydroxyl as

$$pOH = -\log [OH^-]$$

Supplementary Figures



Supplementary Fig 1: Highly polydispersed nanoparticles synthesized after addition of 18mM NaOH to the reaction mixture 1:1 ethanol:water with 90mM TEOS and reaction was allowed to take place for 3 h. This is supplementary to Figure 2D.



Supplementary Figure 2: Illustration for entrapping luminol on the seed silica nanoparticles. In step 1, luminol is allowed to interact with the nanoparticle surface for an hour. In step 2, TEOS is added to the reaction mixture and a coat is grafted on the surface entrapping luminol within the nanoparticles. It is a simplified diagram to propose a probable mechanism; however, further studies are required to understand the complete entrapment procedure for achieving higher luminol entrapment.