AIDIC CONFERENCE SERIES

VOL. 11, 2013
Chief Editor: Sauro Pierucci

ISBN 978-88-95608-55-6; **ISSN** 2036-5969

A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/acos

DOI: 10.3303/ACOS1311044

Study on the Effect of Synthesis Parameters of Silica Nanoparticles Entrapped with Rifampicin

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Silica nanoparticles were synthesized using trimethoxyvinylsilane (TMVS) as silica precursor and butanol as solvent by micelles entrapment approach. Addition of Tween 80 as anionic surfactant and NH_3 as base catalyst controlled the size of silica nanoparticles. The aim of this study is to investigate the effect of synthesis parameters on the size of silica nanoparticles. For this purpose, reaction temperature, 2-butanol and TMVS were varied during synthesis process and their effects on particle size were investigated. Various sizes of silica nanoparticles in the range 28 – 182 nm were synthesized by changing the synthesis parameters. The size of silica nanoparticles increases with increasing synthesis temperature and amount of 2-butanol. The influence of varying the reaction temperature from 30 °C to 70 °C resulting in increase of particle size from 28.91 nm to 113.22 nm. Similar trends were observed by changing the amount of 2-butanol from 4 ml to 12 ml, the particle size increases from 40.21 nm to 102.18 nm. From TEM observations, increasing the amount of TMVS in the range 1 mL, 3 mL and 5 mL produced bimodal structures with mean average size of 25.10 nm, 21.71 – 54.30 nm and 32.26 – 182.75 nm, respectively. The particle size of silica nanoparticles were analysed using dynamic light scattering (DLS) while the shape and diameter of the silica nanoparticles verified using transmission electron microscope (TEM). TEM images showed the almost spherical in shape of silica nanoparticles were produced.

1. Introduction

Nanoparticles have been intensively researched during the last few decades due to their unique properties and potential application in medicinal and therapeutic world such as drug delivery system. Nanoparticles provide immense technological advantages to be used as drug carriers as nanoparticles have high carrier capacity, high drugs stability, high surface to volume ratio, tunable size for targeted delivery and controllable release of molecules (Gelperina et al., 2005).

Nanoparticles-based drug delivery system is hundreds and thousands times smaller than human cells but is similar to large biomolecules size such as enzymes and receptors. It improves drug bioavailability, has minimal side effects, reducing toxicity to the organ and lower cost production (Chiang et al., 2011). Nanoparticles are normally made of biodegradable and biocompatible materials such as polymer and ceramic. Silica is a member of ceramic family that has potential to be used as carrier for drug delivery system. Silica has been widely studied for drug delivery system based on its properties like non-toxic, biodegradable, highly stable and porous in structure (Slowing et al., 2008).

The controllability of the silica particle size for drug delivery system is paramount because the particle size strongly affects the efficiency of endocytosis, sensing as well as drug release. For instance, body distribution studies have shown that larger nanoparticles are more rapidly removed by the liver and spleen than smaller particles due to the size of the capillaries in the organ. Besides that, different *in vitro* studies show that the particle size also affects the cellular uptake of nanoparticles. The body distribution of colloidal drug delivery system is mainly influenced by two physicochemical properties, particle size and surface characteristics

Please cite this article as: Zainal N.A., Abdul Shukor S.R., Wab H.A.A., Abdul Razak K., 2013, Study on the effect of synthesis parameters of silica nanoparticles entrapped with rifampicin, AIDIC Conference Series, 11, 431-440 DOI: 10.3303/ACOS1311044

(Jahanshahi et al., 2008). Hence, it is important to develop a research method that able to tune the size of the nanoparticles towards the targeted drug delivery system. The size of nanoparticles can be tuned by changing synthesis variables (i.e. temperature, silica precursor and butanol concentration). According to (Chou and Chen, 2003), the effect of various parameters plays an important role on the particle size.

In general, silica can be synthesized by different methods such as sol-gel process, micro-emulsion, reverse micelles and surfactant templates. In this present work, micelles entrapment approach was used which has advantages of enhance drug solubility, prolong circulation blood half-life, selective accumulation at tumor sites and possesses lower toxicity (Ab-Wab et al., 2012). In this study, reaction temperature, amount of 2-butanol and amount of trimethoxyvinylsilane (TMVS) as silica precursor was varied over in a wide range during synthesis process to investigate their effects on the size of silica nanoparticles.

In this study, the size of nanoparticles is measured by two methods; particle size determination via DLS and via TEM. When both methods were used for the same sample, it was found that the particle size measured by DLS was always larger than the particle size from TEM observation. In addition, there are several measurements from DLS for the same sample which gives at least two peaks appear in the figure of the particle size distribution. This could be due to the agglomeration of silica nanoparticles in the solution. Therefore, to determine the true particle size of silica nanoparticles, the measurement from TEM observation was chosen for this study. TEM observation is important to determine the morphology and distribution of the nanoparticles and also to evaluate the results obtained from DLS techniques (Rahman et al., 2007). To avoid such error argument, since TEM only looks at a discrete portion (not the whole part) of the sample, we took at least five random portions for one sample in TEM measurement. For comparison in determining the accuracy of particles size, the results from DLS and TEM was presented in Table 1.

In this paper, micelles entrapment approach is used to synthesis silica nanoparticles as carriers for drug delivery system and the essential parameters are considered. The objective of the present study is to investigate the effects of synthesis parameters on the resulting particles size and distribution of silica nanoparticles. In addition, TEM characterizes the shape and morphology of all the samples. This study is intended to establish a rational basis for develop a formulation in tuning size of silica nanoparticles as drug carrier system.

2. Experiment

2.1 Materials

The materials required for the synthesis of silica nanoparticles are described as follows. Tween 80 - viscous liquid, trimethoxyvinylsilane (TMVS 98% pure), and rifampicin were obtained from Sigma-Aldrich Co. (MO, USA). 2-butanol (99% pure), and ammonium hydroxide solution 10 M (31.5% NH_3 pure) were obtained from Fischer Scientific (Fairlawn, NJ). All these chemicals were of analytical grade and used without any purification. De-ionized water from a Millipore filtration system (operating at 18.2 MΩ cm) was used in this study.

2.2 Preparation of Silica Nanoparticles

Silica nanoparticles were prepared by micelles formation approach. First, 5.5 mL Tween 80 was dissolved in 200 mL of de-ionized water. The mixture was stirred for 15 minutes before 200 μL of prepared NH_3 (1 mL NH_3 solution was dissolved in 1 mL de-ionized water) was added to ensure the pH is maintain in the range 9 - 11. Then, 2-butanol was poured into the mixture and continuously stirred for five minutes. The mixture was then transferred into a preheated reactor at the set temperature and continuously stirred at 320 rpm for one hour. After that, the prepared rifampicin drug solution (0.0839 g of rifampicin dissolved in 1.5 mL methanol) was added into the above mixture and continuously stirs under the same condition. After an hour, trimethoxyvinylsilane (TMVS) as silica precursor was added. The mixture was left overnight with continuously stirred at 320 rpm and maintain with the set temperature, which yield a total volume of 250 mL. The effect of synthesis temperature, butanol and TMVS on the particle size of silica was investigated. The produced silica nanocolloids were then subjected to dialysis process for five days to remove the excess surfactant. Finally, the sample will then be collected in the bottle and stored in the refrigerator until further test.

2.3 Characterization of silica nanoparticles

Silica nanoparticles were synthesized in a reactor with total volume of 500 mL. Various types of experiments were conducted in which the amount of one reagent was varied and the amount of other reagents and operating conditions were fixed. The hydrodynamic diameter was determined by dynamic light scattering (DLS) technique using a Zetasizer Nano ZS from Malvern Instrument, UK. The particle size was analysed using a dilute suspension of nanoparticles in deionized water. The particles size measurements were performed with a disposable cuvette and setup measurement using refractive index of colloidal silica (n= 3.5)

with material absorption at 0.01 and water as dispersant material (n=1.330) at 25 °C. Analysis was done in triplicate measurements for each sample. The particle size refers to the average size of silica nanoparticles.
The images of the samples were also checked optically using transmission electron microscopy (TEM; Philips, Model CM12, Eindhoven, Netherlands), operating at 120 kV. To prepare samples for TEM observation, a clean dropper was used to transfer a droplet of each sample of silica nanocolloids on a carbon-coated copper grid. The samples were allowed to dry-air about three minutes at room temperature. Then, the grid is examined with TEM without being stained. The micrographs were taken at a number of random locations on the grid. Diameter of nanoparticles was measured using the *ImageJ Version 1.43* software. The size of the nanoparticles was calculated from the TEM micrographs using an average of 100 particles for all samples.

3. Result and Discussion

A systematic study was carried out by varying over a wide range of the reaction temperature, amount of 2-butanol and amount of TMVS during synthesis process and the results are discussed. Varying synthesis parameters will give the different sizes of silica nanoparticles. The main parameters and the particle size distribution are summarized in Table 1.

Table 1: The synthesis parameters affecting particle size

Parameters	T (˚C)	2-Butanol (mL)	TMVS (mL)	Z-Average DLS (nm)	Mean particle size TEM (nm)
Temperature	30	6	2	31.41	28.91
	50			60.25	56.99
	60			76.42	61.54
	70			140.60	113.22
Amount of 2-Butanol	50	4	2	52.05	40.21
		8		69.13	66.63
		10		88.27	86.27
		12		109.30	102.18
Amount of TMVS	50	6	1	27.28	25.10
			2	60.25	56.99
			3	57.46	21.71-54.30
			5	156.80	32.26 – 182.75

The term particle sizes in this paper refer to the average diameter of the silica nanoparticles. The average diameter of the silica nanoparticles which are almost spherical were determined based on the diameter of about a hundred particles from the TEM micrographs for each sample as illustrated in Figure 1.
Figure 1 represents the effect of temperature on size of silica nanoparticles as mentioned in Table 1. Increasing the temperature presented the uniform and larger size of silica nanoparticles as illustrated in Figure 1. The amount of 2-butanol was varied between 4 mL and 12 mL under the experimental conditions 50 ˚C temperature and 2 mL TMVS. The particles sizes increased with increasing butanol as observed in Figure 2. Varying the amount of TMVS between 1 mL to 5 mL under the experimental conditions 50 ˚C temperature and 6 mL butanol leads to produce bimodal size distribution as stated in Table 1. Figure 3 represents the effect of TMVS on size of silica nanoparticles. In the present study, two main types of reactions are involved in the synthesis of silica nanoparticles: (i) silanol groups are formed by hydrolysis and (ii) silaxone bridges are formed by a condensation polymerization reaction:

Hydrolysis: $Si(OR)_4 + nH_2O \rightarrow Si(OR)_{4-x}(OH)_x + nROH$ (1)

Alcohol Condensation: $Si\text{-}OR + HO\text{-}Si \rightarrow Si\text{–}O\text{–}Si + ROH$ (2)

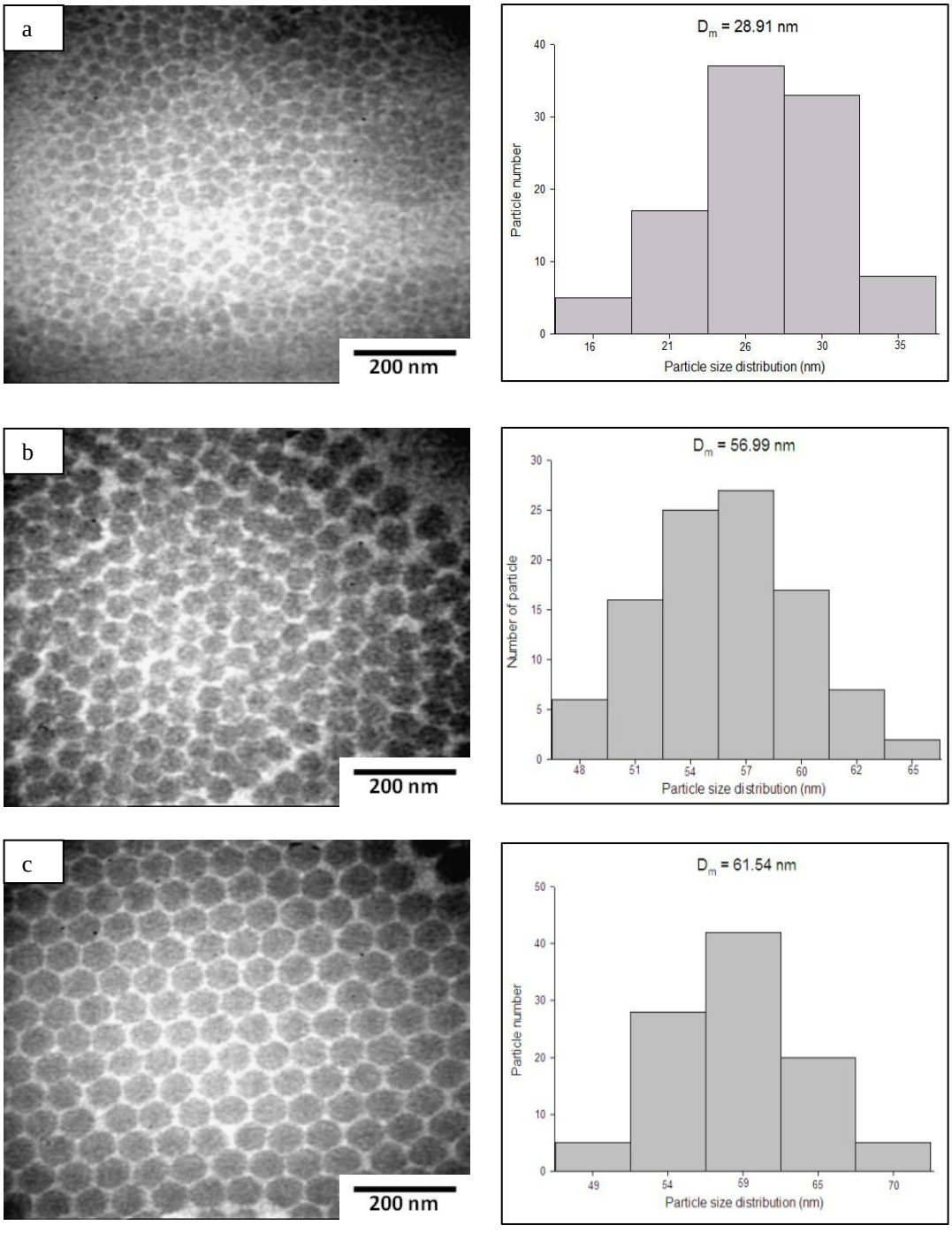
Water Condensation: $Si\text{-}OH + HO\text{-}Si \rightarrow Si\text{–}O\text{–}Si + H_2O$ (3)

Silica nanoparticles were prepared by micelles entrapment approach, which consist of non-ionic surfactant, butanol and water, and conducted the process under the basic conditions. The synthesis process involved the hydrolysis and condensation of silica precursor in the mixture containing Tween 80 in water with ammonia as catalyst and butanol to form micelles (Davies et al., 2009). Therefore, the resulting particle size and distribution can be affected by several synthesis parameters, including operating conditions such as

temperature and agitation rate during the silica nanoparticles preparation. In this study, the reaction temperature, amount of 2-butanol and amount of silica precursor were varied to allow verifying the influence of these experimental parameters on the final average size of silica nanoparticles that were measured by DLS and TEM, as shown in Table 1.

3.1 Effect of temperature on size of nanoparticles

The effect of temperature on size of silica nanoparticles was explored. The reaction temperature was varied between 30 °C and 70 °C under the fixed experimental conditions; 5.5 mL of Tween 80, 200 mL of water and 320 rpm agitating rate. While the amount of butanol and the amount of TMVS was kept constant as 6 mL and 2 mL, respectively. The results of these studies were given in Table 1.



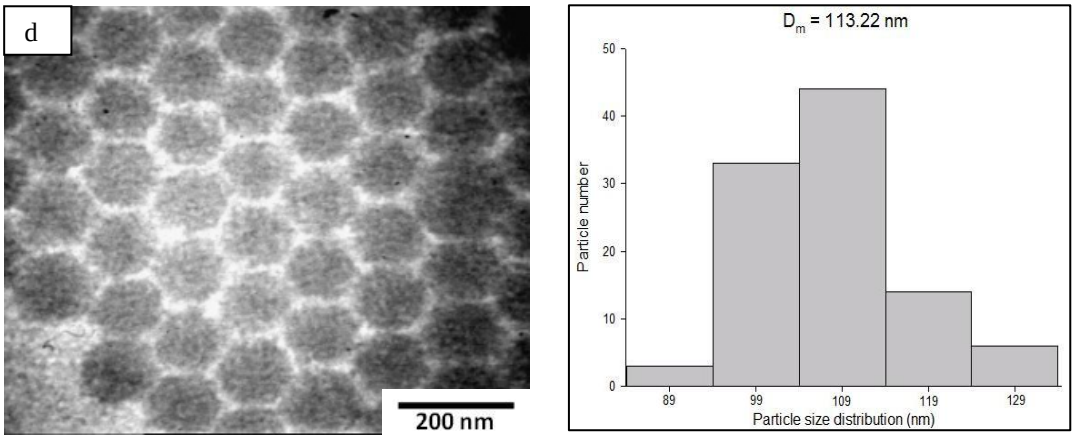
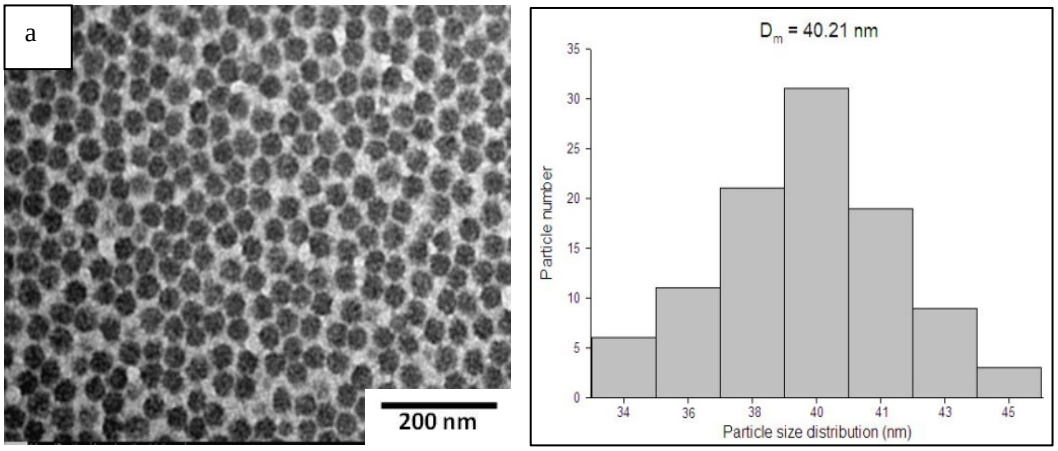


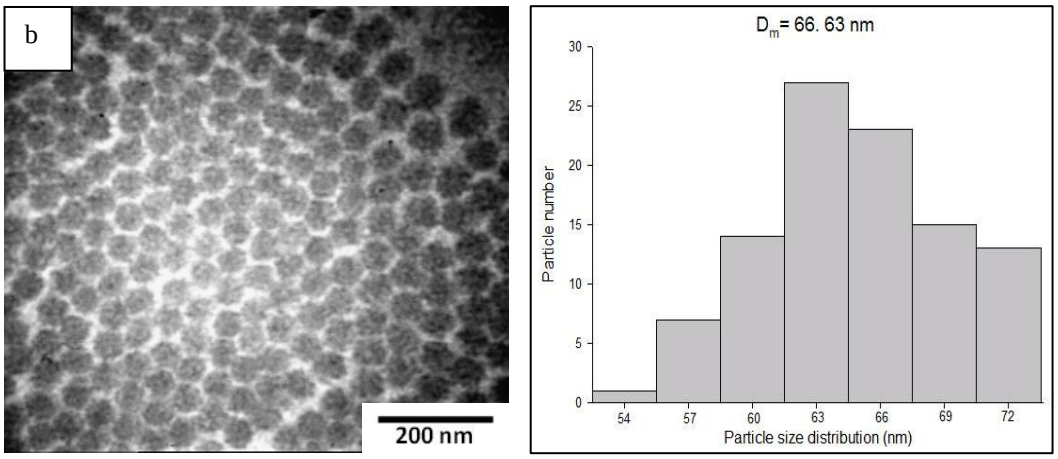
Figure 1: TEM images of silica nanoparticles prepared at (a) 30 °C, (b) 50 °C, (c) 60 °C and (d) 70 °C at fixed experimental conditions

In these conditions, the silica nanoparticles with mean particle size in the range 28.91 – 113.22 nm were obtained according to transmission electron microscopy results. Particle size generally increased with an increase in the reaction temperature. As can be seen in Figure 1, the monodisperse and uniform-sized silica nanoparticles were obtained at higher temperature. As observed from TEM images, the size of silica nanoparticles also becomes bigger with increasing temperature from 30 °C to 70 °C. From Figure 1 (a), silica particles of minimum average size of 28.91 nm under TEM measurements were obtained at temperature 30 °C. At low reaction temperature, narrow particle size distribution was produced compared to higher reaction temperature. At temperature 60 °C, it was clear that the most of the produced silica nanoparticles were smooth and spherical as can seen in Figure 3 (c).The silica nanoparticles with maximum mean particle size of 113.22 nm were obtained at temperature of 70 °C as shown in Figure 1 (d). High temperature promotes the hydrolysis and rate of condensation reaction (Zawrah et al. 2009). Raising the reaction temperature in the synthesis process of silica nanoparticles also promotes the polycondensation reaction among the siliceous micelles coated on the silica surfaces, resulting in the formation of silica shells with a dense structure and giving a larger size (Ge et al., 2009). Therefore, the size of silica nanoparticles becomes larger at the higher temperature. According to (Rao et al., 2005), the ammonia gets evaporated easily in the reaction mixture causes an increase of particle size at high temperatures. Hence, higher reaction temperature influences high agglomeration as compared to at low temperature as illustrated in Figure 1. There was significant change in the mean particle size observed at higher temperature. From Table 1, the silica nanoparticles size of DLS analysis are slightly larger compared to TEM analysis because of the hydrodynamic size usually is affected by surface charge of particles. Varying temperature from 30 °C to 70 °C, the particle size increases from 31.41 nm to 140.60 nm refer to the average size of DLS results. Therefore, temperature at 60 °C was chosen as the best conditions for the fabrication of the amorphous silica nanoparticles since it gives higher yield of nanoparticles with uniform size.

3.2 Effect of butanol on size of nanoparticles

Alcohols were the most common solvents in many of these studies since many of the condensation products of simple alkoxysilanes were immiscible in the reaction mixture (Shi et al., 2012). In order to investigate the effect of alcohol on the particle size of silica nanoparticles, the amount of 2-butanol was varied during synthesis process. These studies were conducted between 4 mL and 12 mL of 2-butanol under fixed experimental conditions; 200 mL of water, temperature 50 °C and 320 rpm agitating rate. While the amount of Tween 80 and the amount of TMVS was kept constant as 5.5 mL and 2 mL, respectively. 2-butanol was dissolved in the mixture under basic condition, using NH_3 as catalyst. In the present study, the nanoparticle size was generally increased with increasing amount of 2-butanol. When the amount of 2-butanol were varied at 4 mL, 8 mL, 10 mL and 12 mL, the silica nanoparticles with mean size of 40.21 nm, 66.63 nm, 86.27 and 102.18 nm were produced, respectively. As can be seen from Figure 2, increasing the 2-butanol amount produced a more uniform and larger size of silica nanoparticles. Figures 2 (d) shown silica particles of maximum average size of 102.18 nm were obtained at 12 mL of 2-butanol while maintaining other parameters.


a
200 nm
D_m = 40.21 nm
Particle number
Particle size distribution (nm)
0
5
10
15
20
25
30
35
34
36
38
40
41
43
45


b
200 nm
D_m= 66. 63 nm
Particle number
Particle size distribution (nm)
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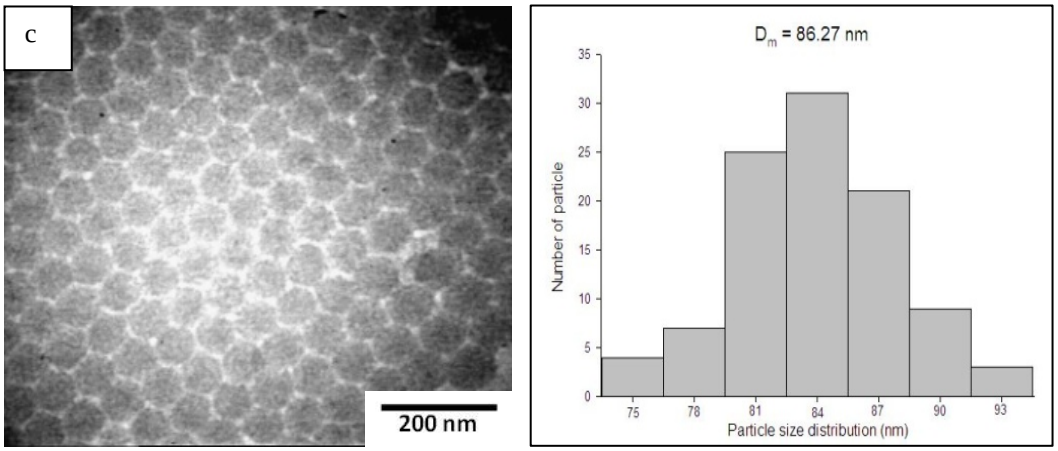
5
10
15
20
25
30
54
57
60
63
66
69
72


c
200 nm
D_m = 86.27 nm
Number of particle
Particle size distribution (nm)
0
5
10
15
20
25
30
35
75
78
81
84
87
90
93

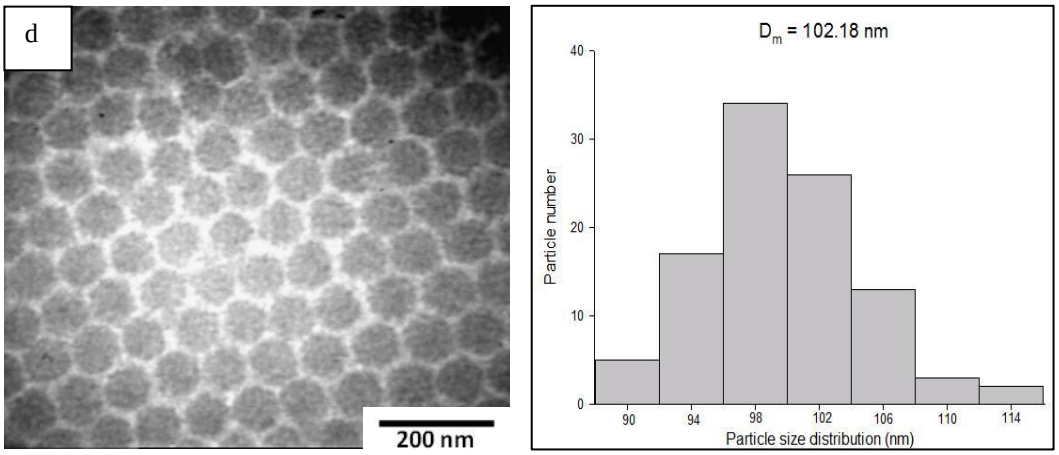
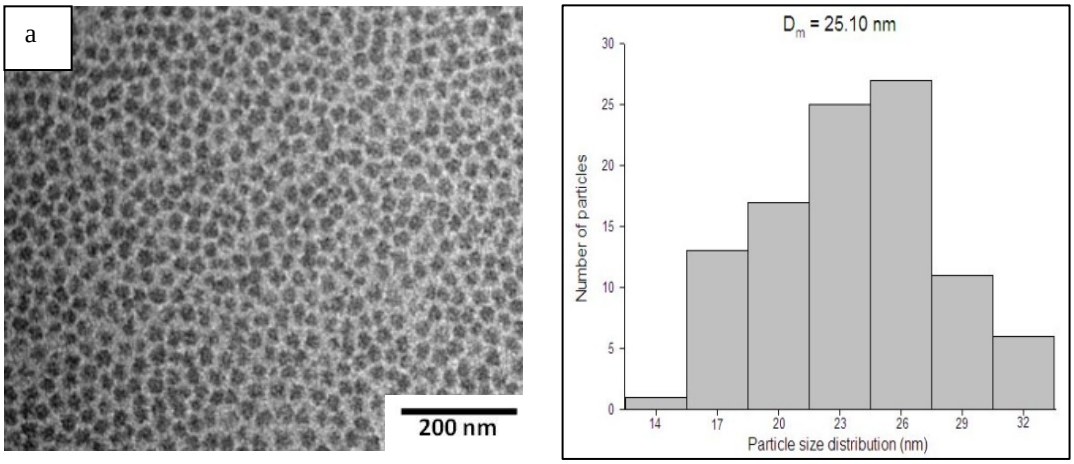


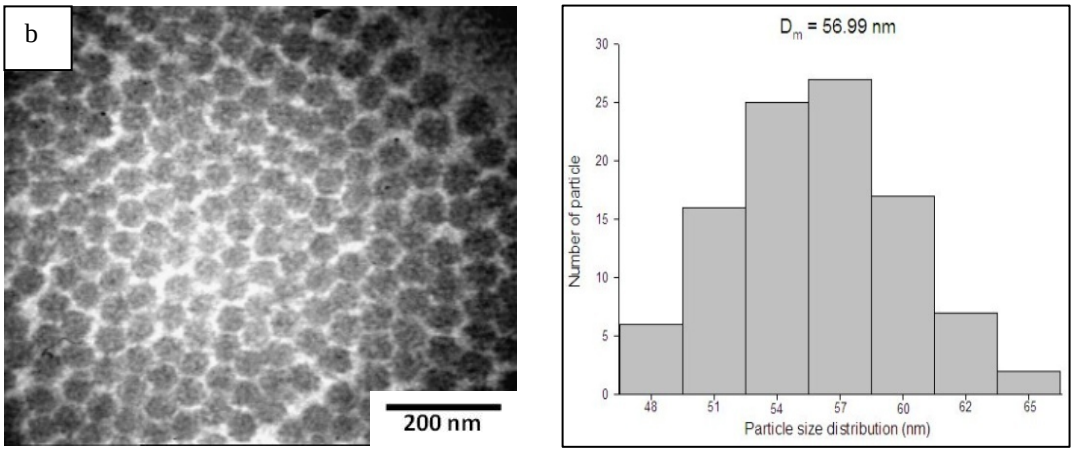
Figure 2: TEM images of silica nanoparticles with varying 2-butanol amount; (a) 4 ml, (b) 8 ml, (c) 10 ml and (d) 12 ml at fixed experimental conditions.

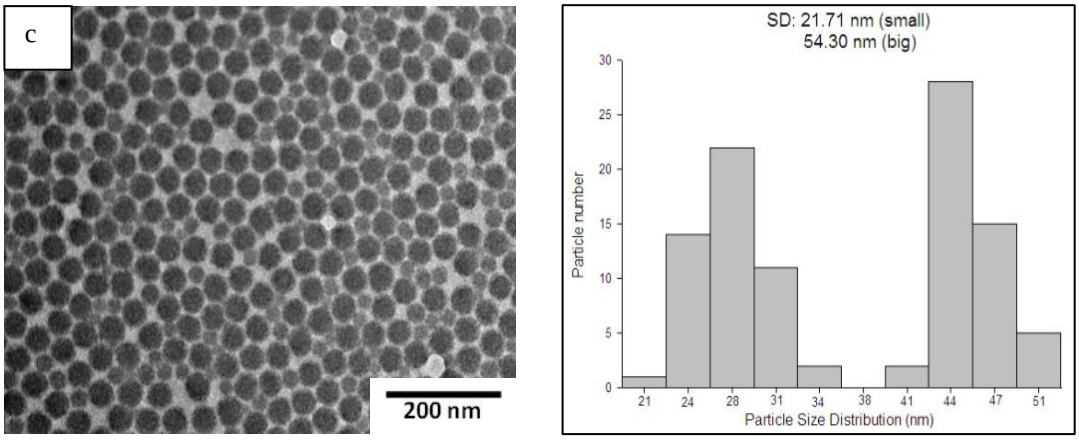
The increase of 2-butanol amount leads to the formation of fewer and bigger sites available for the reaction of silane-surfactant micelles and the silica nucleation formation (Guo et al., 2008). Hence, the total number of nuclei can be formed will be less in numbers and the final size of silica nanoparticles will be relatively larger as illustrated in Figure 2 (d). Meanwhile, the smaller size of spherical silica nanoparticles was obtained using a lower amount of solvent as seen in Figure 2 (a). This may indicate that the solvent has interactions with silanol groups (Si-O-H) in the formation of silica nanoparticles. Equation 2 show the hydroxyl group of intermediate $[Si(OR)_{4-x}(OH)_x]$ reacts with either the ethoxy group of other TMVS (alcohol condensation) or the hydroxyl group of another hydrolysis intermediate (water condensation) to form Si-O-Si bridges until all TMVS have reacted. During the condensation process, the silanol groups reacted with TMVS to form trimethylsilyl groups and the particles become hydrophobic (Ibrahim et al., 2010). With lower 2-butanol amount, the number of nuclei is increased and therefore smaller silica nanoparticles were obtained. However, different solvents have different effect on the size of the particles (Tabatabaei et al., 2006). The particle size also increases with increasing the chain length of the alcohol and also becomes broader when longer-chain alcohols are used as solvents (Rao et al., 2005). Therefore, the butanol plays an important role as solvent in affecting the size of silica nanoparticles.

3.3 Effect of silica precursor on size of nanoparticles

Trimethoxyvinylsilane (TMVS) was used in this study to investigate the effect of silica precursor on the size silica nanoparticles. The TMVS amount was changed between 1 mL and 5 mL and amount of butanol was used as 6 mL at the set temperature, 50 °C based on the previous study by (Ab-Wab et al., 2012). When the amount of TMVS was increased to 2 mL, the particle size distribution changed from monodispersed to bimodal distributions as shown in Figure 3. It can be seen clearly in Figure 3 (c) and (d) that small secondary particles were formed with increasing TMVS amount beyond 2 mL. From TEM measurements, increasing the amount of TMVS at 3 mL and 5 mL produced bimodal structures with mean average size of 21.71 – 54.30 nm and 32.26 – 182.75 nm, respectively. A plausible explanation is that when silica precursor reaches a certain size at the induction period, TMVS become excess in the limiting reactant leads to rapid hydrolysis of silica precursor. The increase in particle size is attributed to the increase in concentration of primary nuclei particles at the nucleation stage. However, ammonia and 2-butanol became the limiting reactants and resulting in an inefficient hydrolysis and condensation reactions. Therefore, because of the excess of silica precursor, condensation reactions produced new nuclei among the grown silica particles promoting heterogeneous or bimodal distribution (Yuan et al., 2010). Table 1, DLS indicates the size of silica nanoparticles increased from 27 to 156 nm and unimodal distributions different from the results obtained via TEM. This could happen because of the small particles tend to stick around the larger particles and make the size measured by DLS bigger than the real size.


a
200 nm
D_m = 25.10 nm
Number of particles
Particle size distribution (nm)


b
200 nm
D_m = 56.99 nm
Number of particle
Particle size distribution (nm)


c
200 nm
SD: 21.71 nm (small)
54.30 nm (big)
Particle number
Particle Size Distribution (nm)

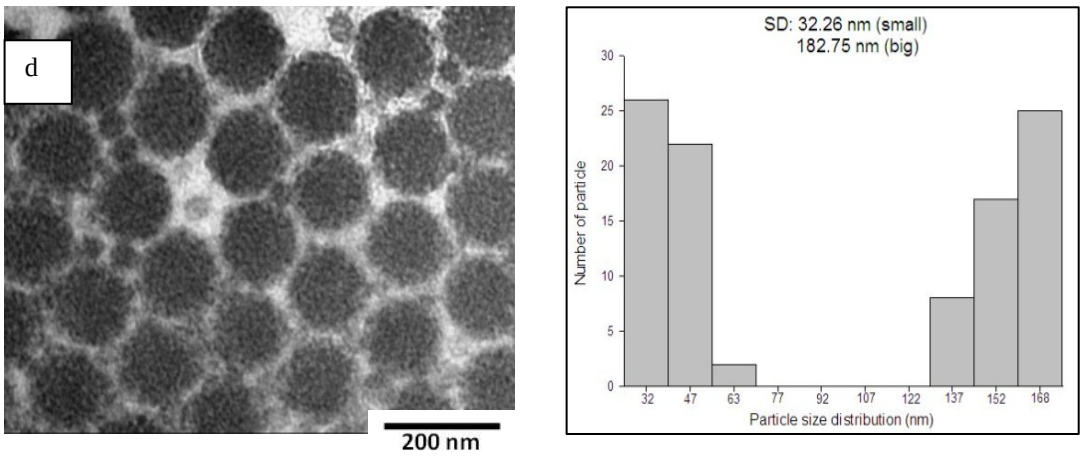


Figure 3: TEM images of silica nanoparticles with varying TMVS amount; (a) 1 mL, (b) 2 mL, (c) 3 mL and (d) 5 mL at fixed experimental conditions.

Besides, DLS is determined by intensity, the bigger particles size give higher intensity compared to the smaller sizes. Thus, the results show measurement for big particles only. In part of this study, the amount of TMVS of 2 mL was taken since it gives the best nanoparticles formation while other parameters were kept constant as indicated before. It also sufficient to consume the primary particles nucleated after the induction period and produces the monodispersity of silica nanoparticles size.

4. Conclusions

Spherical silica nanoparticles with various sizes have been synthesized by micelles entrapment approach. This research investigated the effect of synthesis parameters on particle size of silica nanoparticles. At fixed experimental conditions (320 rpm, pH 9 - 11, 200 mL water and 5.5 mL Tween 80) of this method, all of the three synthesis parameters showed their influential upon the average size of silica nanoparticles. It was found that the average size of silica particles depend on the proportion of the reactants and temperature. By adjusting the reaction temperature, the silica nanoparticles with average size of 28.91 nm –113.22 nm were obtained. 2-butanol as a solvent in the preparation method also has much influence on the size of silica nanoparticles. The silica nanoparticles become larger and uniform with average size of 40.21 nm – 102.18 nm when the amount of 2-butanol changed from 4 mL, 8 mL, 10 mL and 12 mL. Meanwhile, the increase of silica precursor leads to produce bimodal structures beyond 2 mL. An increase the amount of TMVS in the value of 1 mL, 2 mL, 3 mL and 5 mL, the average size of silica nanoparticles were obtained as 25.10 nm, 56.99 nm, 21.71-54.30 nm, and 32.26 – 182.75 nm, respectively. As a result, varying their parameters during the synthesis process give the different sizes of silica nanoparticles entrapped rifampicin. The amount of the alcohol and silica precursor, and also temperature were proportional to the nanoparticle size as a response.

Acknowledgement

The authors gratefully acknowledge for technical support from School of Chemical Engineering and Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia (USM). We are also thankful to School of Biological Sciences, USM for providing TEM measurement for all the samples in this study. This research was financially supported by Research University Cluster Grant (1001/PSF/861001).

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