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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, butanol and TMVS were changed during synthesis process and their effects on particle size were investigated. Various sizes of silica nanoparticles in the range 28 – 168 nm were synthesized by changing the synthesis parameters. The size of silica nanoparticles increases by increasing the synthesis temperature and amount of butanol. The influence of the changing the reaction temperature from 30 °C to 70 °C, the particles size increase from 28.91 nm to 113.22 nm. Similar trends observed by addition the amount of butanol, the particle size increases from 44.43 nm to 103.50 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 26.39 nm, 24.60 - 56.78 nm and 51.48 - 167.79 nm, respectively. The particle size of silica nanoparticles were analysed using Malvern Zetasizer Nano ZS 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). Silica has been widely studied for drug delivery system based on its properties like non-toxic, biocompatible, 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 and release. The body distribution of colloidal drug delivery system is mainly influenced by two physicochemical properties, particle size and surface characteristics (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

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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 (Wab et al., 2012). In this study, reaction temperature, amount of butanol and amount of trimethoxyvinylsilane was changed in a wide range during synthesis process to investigate their effects on the particle size of silica nanoparticles.

Particles sizes of nanoparticles are measured by two methods, that is, Zetasizer Nano ZS and TEM observation in this study. When we used both methods for the same sample and found that the particle size measured by Zetasizer was always larger than the particle size from TEM observation. In addition, there are several measurements from Zetasizer for the same sample which gives at least two peaks appear in the figure of the particle size distribution which was due to the agglomeration of silica nanoparticles in the solution. Therefore, to determine the true particle size of silica nanoparticles, we chose to use the measurement from TEM observation. To avoid such an error argument, since TEM only looks at a discrete portion (not the whole part) of the sample, we took at least five portions for one sample in TEM measurement. For comparison in determining the accuracy of particles size, the results from Zetasizer and TEM was presented in Table 1.

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 Aldrich. Butanol (99% pure), and ammonium hydroxide solution 5 M (31.5% NH_3 pure) were obtained from Fischer Scientific. All these chemicals were of analytical grade and used without any purification. De-ionized water (18.2 Ω 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, 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 was then subject to dialysis process for five days to remove the excess substances. Finally, the sample will then be collected in the bottle and stored in the refrigerator until further test.

2.3 Characterization of silica nanoparticles

The characterization of particle sizes were carried out using a particle size analysizer, Zetasizer Nano ZS (Malvern Instrument) while the images of the samples were also checked optically using Philips CM12, 120 kV, transmission electron microscopy (TEM) system. To prepare samples for TEM analysis, a drop of silica nanoparticles was placed on a copper grid coated with carbon and left it for three minutes at room temperature. Then, the grid is inserted to the slot of TEM ready for analysis.

3. Result and Discussion

In the present study, a systematic study was carried out by changing a wide range of reaction temperature, amount of 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)	BuOH (mL)	TMVS (mL)	Average size Zetasizer (nm)	Mean particle size TEM (nm)
Temperature	30	6	2	31.41	28.91
	50			60.25	53.68
	60			76.42	61.54
	70			140.60	113.22
Amount of Butanol	50	4	2	47.64	44.43
		8		66.75	67.67
		12		109.30	103.50
Amount of TMVS	50	6	1	28.66	26.39
			3	57.05	24.60-56.78
			5	156.9	51.48 - 167.79

The term particle sizes in this paper refer to the average diameter of the silica particles. The average diameter of the silica particles 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. The amount of 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.

3.1 Effect of temperature on size of nanoparticles

The reaction temperature changed between 30 °C and 70 °C and the results were given in Table 1. While the amount of butanol and the amount of TMVS was kept constant as 6 mL and 2 mL, respectively. 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 noticed in Figure 1, the monodisperse and uniform-sized silica nanoparticles were obtained when temperature increased.

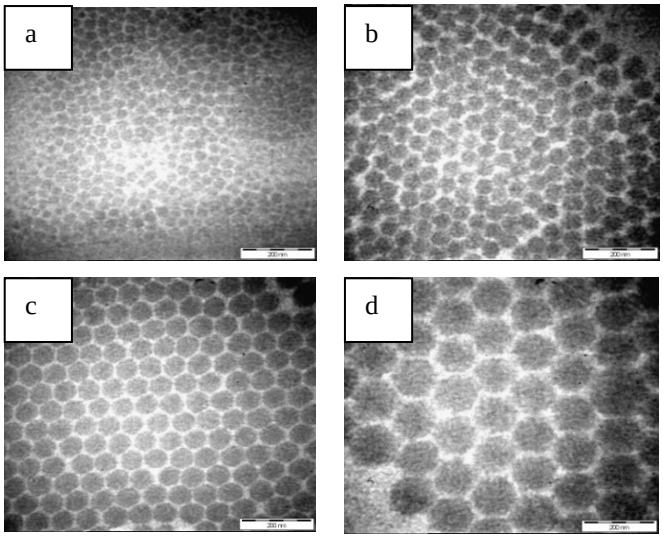

Figure 1: TEM images of silica nanoparticles prepared at (a) 30 °C, (b) 50 °C, (c) 60 °C and (d) 70 °C at fixed experimental conditions

From Figure 1 (a), silica particles of minimum average size of 28.91 nm under TEM measurements were obtained at 30 °C. The silica nanoparticles with maximum mean particle size of 113.22 nm were obtained at 70 °C as shown in Figure 1 (d). The high temperature promotes the hydrolysis and rate of condensation reaction (Zawrah et al. 2009). At high temperatures, the ammonia gets evaporated easily in the reaction mixture causes an increase of particle size (Rao et al., 2005). Hence, the high reaction temperature achieves less agglomeration compared to at low temperature as illustrated in Figure 1. There was significant change in the mean particle size was observed at higher temperature.

3.2 Effect of butanol on size of nanoparticles

In order to investigate the effect of butanol amount on the particle size of silica nanoparticles, the amount of butanol was changed during synthesis process. When the amount of butanol was changed at 4 mL, 8 mL and 12 mL, the silica nanoparticle with average size of 44.43 nm, 67.67 nm and 103.50 nm was produced, respectively. As can be seen from Figure 2, increasing the butanol amount presented the uniform and larger size of silica nanoparticles. Figures 2 (d) shown silica particles of maximum average size of 103.50 nm were obtained at 12 mL butanol while maintaining other parameters. The spherical and agglomerated silica nanoparticles were obtained using a lower amount of solvent as seen in Figure 2 (a). This may indicate that the solvent have interactions with silanol groups (Si-O-H) in the formation of silica nanoparticles. The particle size increases with increasing the chain length of the alcohol. Therefore, the butanol serves an important role as solvent in affecting the size of silica nanoparticles. However, different solvents have different effect on the size of the particles (Tabatabaei et al., 2006).

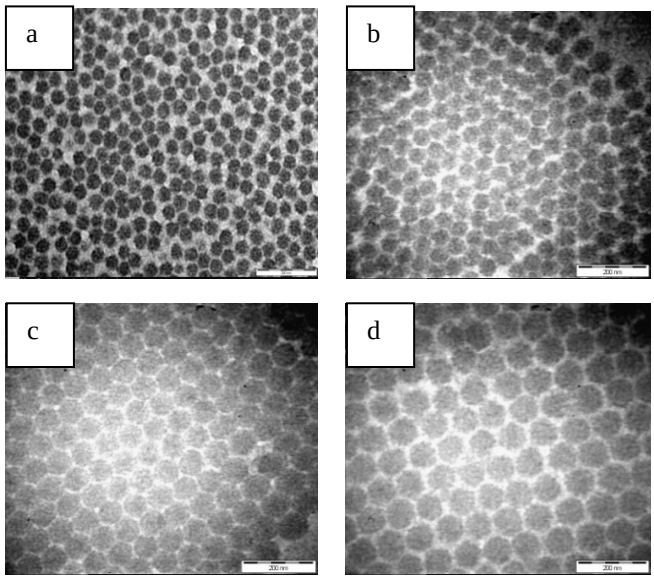

Figure 2: TEM images of silica nanoparticles with varying BuOH amount; (a) 4 ml, (b) 6 ml, (c) 8 ml and (d) 12 ml at fixed experimental conditions.

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 in literature (Wab et al., 2012). When the amount of TMVS increased, 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. However, the particle size distribution difficult to measure at the lowest amount of TMVS since the structure of monodispersed silica was not spherical formed. 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. Therefore, because of the excess of silica precursor, condensation reactions produced the new nuclei among the grown silica particles as called heterogeneous or bimodal distribution (Yuan et al., 2010). From

Table 1, zetasizer and TEM indicates the size of silica nanoparticles increased from 26 to 168 nm. In the part of study, the amount of TMVS of 2 mL was taken as it gives the best nanoparticles formation while other parameters were kept constant as indicated before.

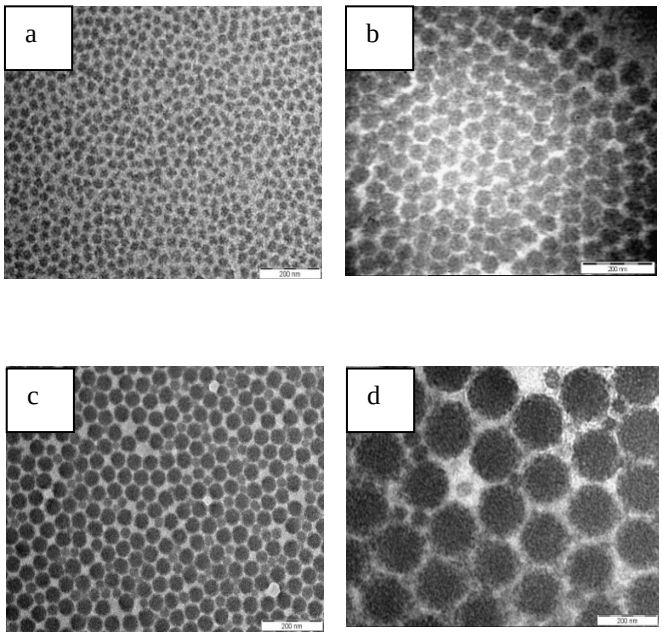

Figure 3: TEM images of silica nanoparticles with varying TEVS amount; (a) 1 mL, (b) 2 mL, (c) 3 mL and (d) 5 mL at fixed experimental conditions.

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 agitating, pH 9 - 11, 200 mL water and 5.5 mL tween 80) of this method, all of 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. By adjusting the reaction temperature, the silica nanoparticles with average size of 28.91 nm – 113.22 nm were obtained. Butanol also has much influence on the size of silica nanoparticles. The silica nanoparticles become larger and uniform with average size of 44.43 nm – 103.50 nm when the amount of butanol changed from 4 mL, 8 mL, and 12 mL. Meanwhile, the increase of silica precursor leads to produce bimodal structures. An increase the amount of TMVS in the value of 1 mL, 3 mL and 5 mL, the average size of silica nanoparticles were obtained as 26.39 nm, 24.60 – 56.78 nm, and 51.48 - 167.79 nm, respectively. As a result, varying their parameters during the synthesis process give the different sizes of silica nanoparticles entrapped rifampicin.

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