The Effect of Silica Nanoparticles Stability in Biological Media

A. Ahmad¹, N.D Zakaria², Z. Lockman¹ and K.A.Razak¹,²,*

¹ School of Materials and Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, 14300 Nibong Tebal, Penang, Malaysia.
² NanoBiotechnology Research and Innovation (NanoBRI), Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 11800 Penang, Malaysia.

Email: kairunisak@usm.my

Abstract. The stability and level of aggregation of nanoparticles (NPs) in physiological conditions or different media are important for biomedical applications. The interaction of NPs in different media could affect the physicochemical properties of NPs. In this study, two different sizes of amorphous silica nanoparticles (SiNPs) encapsulated dye were synthesised using the micelle entrapment method. The SiNPs encapsulated dyes suspension was mixed with different concentration of salt solution, NaCl and mouse serum and incubated at 37°C to mimic human body environment to study the interaction of SiNPs encapsulated dyes in physiological conditions. Particles agglomeration or aggregation of SiNPs encapsulated dyes in NaCl solution and mouse serum were investigated and analysed. The absorbance spectra and the stability efficacy were recorded and calculated using UV-Vis spectrometer, while the particle size was measured using Zetasizer particle analysis and transmission electron microscope (TEM). The results obtained showed that 53 nm of SiNPs was more stable compared to 30 nm both in NaCl solution and mouse serum.

1. Introduction

Amorphous SiNPs encapsulated dyes have been widely explored especially for bio-imaging and bio-labeling applications. Amorphous SiNPs becomes popular due to the desired properties of high hydrophilic property that could give high solubility in water [1]. The solubility of amorphous SiNPs in water at body pH is normally around 130–150 ppm (μg/mL) [2]. Apart from that, SiNPs is optically transparent hence does not affect the fluorescence properties of encapsulated dyes. SiNPs also provides protection for dye molecules from the buffer. For biomedical application, it is important to determine the toxicological properties cause by SiNPs. However, very few and limited initial study on the stability effect of SiNPs in biological media can be found and is still not fully understood.

The stability and level of aggregation of NPs in physiological conditions or different media are important parameters prior to toxicological studies [3]. The interaction between the NPs in biological environment...
could affect the physicochemical properties of NPs such as particle size distribution, aggregation/agglomeration, surface charge and surface chemistry. Hence, the stability of NPs is influenced by the local environment conditions such as temperature, pH and electrolytes concentration [4,5]. In most studies, NPs for biomedical applications are usually suspended in highly salted solutions or serum-containing media, conditions likely to have a large influence on the hydrodynamic diameter hence affect the stability of nanoparticles [6]. Thus, it is important to understand the effect of size of SiNPs in biological media.

In this study, two different sizes of amorphous silica nanoparticles encapsulated 1,1'-Diocadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) dye (SiDiI) were synthesised using the micelle formation method. The particle agglomeration and stability of different sizes SiDiI in biological media that mimic the realistic biological conditions were determined and analysed.

2. Experimental

The silica nanoparticles were synthesised using the micelle entrapment method. Tween 80 (Polysorbate 80, Sigma Aldrich) as surfactant was dissolved in distilled water. 200 μl of ammonium hydroxide (Sigma Aldrich) was added as the pH adjuster to basic condition (pH 9-10). Then, different volume (2 and 6 ml) of co-solvent, 2-butanol (Merck) as the synthesis parameter was added into solution and stirred continuously to obtain different size of SiNPs. The solution was transferred into a preheated bioreactor at 50°C with 320 rpm stirring speed for 1 hour. After that, 2 ml solution of DiI fluorescence dye (Sigma Aldrich) was added into the micelle suspension and followed by organosilica precursor, Vinyltrimethoxysilane (VTMS) (98%, Sigma Aldrich). The mixture was continuously stirred overnight. The excess surfactant and solvent were removed by dialysis using 12 kDa dialysis tube (Fisher Scientific) in 4°C water for 4-5 days. The dialysis water was changed twice daily. After dialysis process, the silica encapsulated Dil dye (SiDiI) was re-concentrated using 30 kDa cellulose membrane (Fisher Scientific). The concentrated of SiDiI suspension were surface modified with D-glucose (Sigma Aldrich) and trimetaphosphate (TSTMP, Sigma Aldrich). The hydrodynamic particle size of SiDiI suspension was analysed using Zetasizer particle analysis. The morphology and size distribution were observed using transmission electron microscope, TEM.

SiDiI suspension was further tested in biological media and serum to study its stability. The SiDiI suspension (2%) was mixed with 0.1, 0.5 and 1.0 M NaCl (Sigma Aldrich) solution or 5, 10, and 25 % of mouse serum (Sigma Aldrich). The stability of SiDiI suspension in biological medium was further analysed by incubation in a water bath at body temperature, 37 ± 0.5 °C for 5 hours. The absorbance value before and after incubation of the filtered samples was analysed using the UV-Vis spectrophotometer. The stability efficacy of SiDiI suspension was calculated using the Equation 1:

\[
\text{Stability efficacy \%} = \frac{\text{Concentration SiNPs encapsulated fluorescence dye} \text{tn (after filtration)}}{\text{Concentration of SiNPs encapsulated fluorescence dye} \text{ t0}} \times 100 \tag{1}
\]

3. Results and discussion

Two different sizes of amorphous silica nanoparticles encapsulated DiI (SiDiI) were synthesised using the micelle entrapment method. In this study, micelle entrapment method was chosen because of the ability to produce good monodispersed spherical particles of amorphous SiNPs. Micelles act as a temporary template to define the morphology of silica nanoparticles (SiNPs). Micelle structure formed from the interaction of
nonionic surfactant, Tween 80 with aqueous solvent containing water and co-solvent at the critical micelle concentration (CMC). The addition of organosilica precursor, VTMS, produced the silanol groups and deposited around the micelle structure by hydrolysis and condensation reactions. The hydrolysis reaction was enhanced with the addition of ammonia by producing more hydroxyl ions (OH⁻) to react with organosilanes. The condensation of alcohol and water caused interaction of silanols with each other and formed crosslinking between Si–O–Si chains until all Si precursors reacted.

In this study, 2ml and 6ml of 2-butanol that acted as co-solvent were used as the synthesis parameter to produce different size of SiDiI. The effect of volume of co-solvent during the synthesis method on the particle formation was determined using Zetasizer particles analyser and TEM. Figure 1 shows the hydrodynamic particle size distribution of SiDiI using 2 and 6 ml co-solvent measured using Zetasizer. SiDiI produced using 2 and 6 ml 2-butanol have hydrodynamic particle size of 32.6 and 59.0 nm with PDI values of 0.27 and 0.09, respectively. Particle size of SiDiI increases with increasing volume of 2-butanol resulted from the reducing hydrolysis rate of silica precursors during the silica formation. In this method, hydrolysis rate was affected by the nature of the solvent involving steric effect and hydrogen bonding between silica precursor and solvent. Hydrolysis rate of silica precursor is faster in water with low amount of alcohol as the co-solvent. Hydrolysis rate reduces with increasing amount of alcohol. In this work, high volume of alcohol such as 2-butanol (6ml), the effective availability of water molecules are limited causes slower hydrolysis rate of silica precursor [6]. As a consequence, less nucleation of particles took place during the hydrolysis which lead to the formation larger particle size [7].

![Figure 1. Hydrodynamic size distribution of SiDiI produced using different volumes of 2-butanol (2 and 6 ml).](image)

The morphology of SiDiI produced using different 2-butanol volume is shown in

![Figure 2. Spherical shape and well dispersed of SiDiI were observed from TEM images of both samples.](image)

100 particles were measured by using the ImageJ software and the average particle size of SiDiI was calculated and tabulated in Table 1. The average particle size of SiDiI synthesised using 2 and 6 ml of 2-butanol is 30 and 53 nm, respectively. The particle size of SiDiI observed using TEM is smaller than hydrodynamic particle size measured by Zetasizer due to the Brownian motion that influences the hydrodynamic particles in colloidal form [6].

**Table 1.** Hydrodynamic size, polydispersity index (PDI) and TEM size of SiDiI produced using different volume of 2-butanol (2, 4 and 6 ml)

| Volume of 2-butanol (ml) | Hydrodynamic size (nm) | Polydispersity index | TEM size (nm) |
|--------------------------|------------------------|---------------------|--------------|
| 2                        | 33                     | 0.27                | 30           |
| 6                        | 59                     | 0.09                | 53           |
Figure 2. TEM images and histograms of particle size distribution of SiDiI produced using different volume of 2-butanol: (a) 2 ml and (c) 6 ml.

Two different sizes of SiDiI suspension of 30 and 53 nm were added into different concentration of sodium chloride salt solution, NaCl. NaCl was chosen since the ionic strength in the cell culture media study mainly arises from this salt with only 0.15 M [8]. In this study, 20% of SiDiI with sizes 30 and 53 nm were mixed with different concentrations of NaCl medium: 0, 0.1, 0.5 and 1.0 M and incubated in water bath for 24 hours at 37°C to study the stability effect on particle agglomeration. The absorbance value of SiDiI suspension in NaCl solution was measured by using UV-Vis spectroscopy to identify the presence of particle agglomeration. The stability efficacy was calculated from the measured absorbance value by the ratio between the maximum absorbance value of SiDiI after 24 hour incubation after filtration and maximum absorbance value of SiDiI before incubation. After 24 hours of incubation, the stability efficacy of SiDiI at NaCl concentration at 0.0, 0.1, 0.5 and 1.0 M for size 53 nm decreasing from 71, 68, 54 and 51 %, respectively. Similar trend was observed for SiDiI with size 30 nm with stability efficacy of 67, 56, 44 and 38 % with the increasing of NaCl concentration, respectively, as shown in Figure 3(a).

The results revealed that the increase of NaCl concentration caused colloidal suspension became unstable and started to flocculate [8]. In nanoparticle suspensions, particles have high tendency to aggregate more quickly at higher ionic strengths. This behavior can be explained by Derjaguin, Landau, Verwey, and Overbeek, DLVO theory where the agglomeration and stability of particle dispersions are influenced by the attractive and repulsive forces between individual particles [9]. In this case, the increase of NaCl concentration led to increasing of ionic strength in the suspension medium. At high NaCl concentration, ions exist in the dispersion covers the silica nanoparticles and neutralize the charge of their surface. In this condition, the thickness of double layer of SiDiI was reduced and the repulsive force between the nanoparticles was reduced. Hence, the total potential energy of interaction became more attractive and van der Waals forces became dominant resulted to particles agglomeration [10]. In addition, the result shows that SiDiI with size 53 nm has higher stability in NaCl solution than and SiDiI with size 30 nm revealed that SiDiI with size 30 nm has high particle agglomeration (less stable). The particle agglomeration of SiDiI was proven by the effective hydrodynamic particle measured by Zetasizer as shown in Figure 3(b). This is due to the large surface area of smaller particle size, which has high tendency of agglomeration between each particle [5]. On the other hand, small particles have less surface charge per particles leading to weaker electric double layer repulsion forces hence subsequently have greater aggregation tendency [11]. Thus, it can be concluded that at the same ionic strength, smaller particles have faster rate of aggregation than larger particles.
The particles agglomeration of SiDiI was further studied in different concentration of mouse serum. 5, 10 and 25 % of mouse serum were mixed with 30 and 53 nm of SiDiI and incubated for 5 hours at 37°C. The absorbance properties were measured before and after incubation and after filtration to investigate stability of SiDiI. The filtration efficacy of SiDiI was derived from the maximum absorbance value of SiDiI before incubation and after filtration after 5 hours incubation. From the result showed in Figure 6, increasing of mouse serum concentration from 5, 10 and 25 % decreased the SiDiI stability after incubation from 89, 81 and 70 %, respectively, for 53 nm SiDiI. While the stability efficacy of 30 nm SiDiI decreased from 75, 52 and 64 %, respectively. The decrease of stability efficacy with increasing of mouse serum concentration revealed the formation of particles agglomeration in mouse serum at higher concentration. This could be due to the formation of protein corona on the surface of NPs resulted from the interaction of the NPs with the proteins presence in the biological media. Protein corona formed on the surface of NPs lead to modification on properties of NPs, hence, de-stabilised the colloidal systems of NPs favouring the formation of agglomerates [5,12]. Thus, more protein molecules adsorbed on the NPs surface would form more particle agglomeration of NPs [4].

The different agglomeration behaviour of different size of SiDiI was observed from the stability efficacy as shown in Figure 4. SiDiI with size 53 nm had high stability efficacy of 70 % when interacted with high concentration of mouse serum. With the same concentration of mouse serum, the lower filtration efficacy of SiDiI in size 30 nm was observed at 64 %. This result revealed that 53 nm SiDiI formed less agglomeration compared with 30 nm SiDiI. This result is in agreement with Halamoda-Kenzaoui et al., (2015), where smaller NPs has high tendency to agglomerate than larger NPs. In addition, Clemments et al. (2015) also demonstrated that smaller particles adsorbed large amount of protein due to their larger external surface area. Large amount of adsorbed protein lead to the greater accumulation of protein on the surface of NPs, hence, cause agglomeration [13, 14]. Although all the SiDiI samples have same surface chemistry and only differed in size, they behaved differently in cellular media containing the proteins as also observed by other authors [5].
4. Conclusion

Spherical shaped and monodispersed SiDiI were successfully produced using the micelle formation method. 30 and 53 nm of SiDiI were produced by varying the volume of co-solvent. The particle size of SiDiI increased with increasing volume of co-solvent. Further study on the stability of 30 and 53 nm SiDiI in NaCl solution and mouse serum showed similar trend. The stability of SiDiI for both sizes decreased as the concentration of NaCl solution and mouse serum increased. Furthermore, 53 nm SiDiI size had higher stability in both NaCl solution and mouse serum compared to 30 nm SiDiI.

Acknowledgment

The authors appreciate technical support from School of Materials & Mineral Resources Engineering, and Institute for Research in Molecular Medicine, USM. This research was supported by Research University (RU) grant 1001/Pbahan/870028. One of the author appreciates scholarship from Ministry of Higher Education Malaysia (MyBrain15).

References

[1] Iler K R 1979 Colloid and Surface Properties and Biochemistry of Silica.
[2] Vittal R, Areva S, Jokinen M & Koskinen M 2008 Sol-Gel Methods for Materials Processing.
[3] Pavlin M & Bregar V B 2012 D. J. Nanomaterials & Biostructures (DJNB), 7.
[4] Pfeiffer C, Rehbock C, Hühn D, Carrillo-Carrion C, De Aberasturi D J, Merk V, Barcikowski S & Parak W J 2014 J. of The Royal Society Interface 11 20130931
[5] Halamoda-Kenzaoui B, Ceridono M, Colpo P, Valsesia A, Urbán P, Ojea-Jiménez I, Gioria S, Gilliland D, Rossi F & Kinsner-Ovaskainen A 2015 PloS one 10 e0141593
[6] Dos Santos Neves C S 2014 Development of fluorescent silica nanoparticles encapsulating organic and inorganic fluorophores; synthesis and characterization. PhD, de Ciências e Tecnologia da Universidade Nova de Lisboa.
[7] Song A, Zhang J, Zhang M, Shen T & Tang J A 2000 Colloids and Surfaces A: Physicochemical &Eng. Aspects, 167, 253
[8] Orts-Gil G, Natte K, Drescher D, Bresch H, Mantion, Kneipp J & Österle W 2011 Journal of Nanoparticle Research, 13, 1593
[9] Jiang J, Oberdörster G & Biswas P 2009 Journal of Nanoparticle Research 11 77
[10] Jenkins S, Kirk S R, Persson M, Carlen and J Abbas Z 2008 The Journal of Chemical Physics 128 16471
[11] Hotze E M, Phenrat T & Lowry G V 2010 Journal of environmental quality 39 1909
[12] Moore T L, Rodriguez-Lorenzo L, Hirsch V, Balog S, Urban D, Jud C, Rothen-Rutishauser B, Lattuada M & Petri-Fink A 2015 Chemical Society Reviews 44 6287
[13] Clemments A M, Botella P & Landry C C 2015 ACS Applied Materials & Interfaces 7 21682
[14] Albanese A, Walkey C D, Olsen J B, Guo H, Emili A & Chan W C 2014 Secreted biomolecules alter the biological identity and cellular interactions of nanoparticles