DONG THAP UNIVERSITY JOURNAL OF SCIENCE
Tạp chí Khoa học Đại học Đồng Tháp
Natural Sciences Issue
ISSN 0866-7675 | e-ISSN 2815-567X

DOI: https://doi.org/10.52714/dthu.14.5.2025.1530

FACTORS AFFECTING THE MORPHOLOGY AND SIZE OF SILICA NANOPARTICLES AS DRUG DELIVERY FOR CANCER TREATMENT

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Article history

Received: 11/3/2025; Received in revised form: 31/3/2025; Accepted: 01/4/2025

Abstract

Currently, the use of nanoparticles as "carriers" to deliver drugs to the correct location has attracted the attention of scientists. Nano silica (SNs) is widely studied as material for biomedical applications. In this study, SNs particles were synthesized using the Stober method. The study investigated two factors affecting the morphology and particle size: reaction time and NH_3 solution addition rate. Determining the characteristic properties of the material, methods used include transmission electron microscopy (TEM), X-ray diffraction (XRD), dynamic light scattering (DLS), and the N_2 adsorption-desorption. SNs particles are spherical and highly homogeneous, with a 105.06 ± 0.74 nm size. The efficiency and loading capacity of Dox of SNs particles were 17.70 ± 0.52 % and 4.11 ± 0.11 %, respectively. The SNs/Dox drug carrier system has good potential for controlled drug release.

Keywords: *Doxorubicin, drug loading efficiency, drug loading content, nano silica, particle size.*

Cite: Nguyen, T. N. T., & Nguyen, T. N. H. (2025). Factors affecting the morphology and size of silica nanoparticles as drug delivery for cancer treatment. *Dong Thap University Journal of Science, 14*(5), 88-97. https://doi.org/10.52714/dthu.14.5.2025.1530

KHẢO SÁT YẾU TỐ ẢNH HƯỞNG ĐẾN HÌNH THÁI VÀ KÍCH THƯỚC HẠT NANO SILICA, ỨNG DỤNG MANG THUỐC ĐIỀU TRỊ BỆNH UNG THƯ

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Lịch sử bài báo

Ngày nhận: 11/3/2025; Ngày nhận chỉnh sửa: 31/3/2025; Ngày duyệt đăng: 01/4/2025

Tóm tắt

Hiện nay, việc sử dụng các hạt nano làm "vật tải" để đưa thuốc đến đúng vị trí đã thu hút sự chú ý của các nhà khoa học. Nano silica là vật liệu được nghiên cứu rộng rãi cho các ứng dụng y sinh. Trong nghiên cứu này, các hạt nano silica (SNs) được tổng hợp theo phương pháp Stober. Nghiên cứu tiến hành khảo sát hai yếu tố ảnh hưởng đến hình thái, kích thước hạt bao gồm thời gian phản ứng và tốc độ thêm dung dịch NH_3. Các phương pháp xác định tính chất đặc trưng của vật liệu bao gồm kính hiển vi điện tử truyền qua (TEM), phổ nhiễu xạ tia X (XRD), phương pháp tán xạ ánh sáng động học (DLS) và phương pháp hấp phụ - khử hấp phụ khí N_2. Các hạt SNs có dạng hình cầu, độ đồng nhất cao với kích thước 105,06 ± 0,74 nm. Hiệu suất và khả năng mang tải thuốc Dox của các hạt SNs tương ứng là 17,70 ± 0,52 % và 4,11 ± 0,11 %. Hệ chất mang thuốc SNs/Dox có tiềm năng kiểm soát phóng thích thuốc tốt.

Từ khóa: *Doxorubicin, hiệu suất mang thuốc, khả năng mang thuốc, kích thước hạt, nano silica.*

1. Introduction

Chemotherapy is a relatively common form of traditional cancer treatment, using drugs to treat rapidly growing and proliferating cells as essential characteristics of cancer cells (Ardizzoni et al., 2007). Doxorubicin (Dox) is a chemotherapeutic drug for many types of cancer, metabolized from the actinomycete Streptomyces peucetius (Octavia et al., 2012). Dox is water-soluble, so after intravenous injection, the drug molecules diffuse and distribute freely throughout the body, leading to common unwanted side effects such as nausea, gastrointestinal problems, and nervous system disorders (often causing hallucinations and dizziness). Therefore, the development of chemotherapeutic methods that can passively or actively target cancer cells to increase treatment efficacy and minimize drug side effects has attracted the attention of scientists. For example, passive targeting is based on the unique biological properties of tumors, which allows nanocarriers to accumulate in tumors by the enhanced permeation and retention (EPR) effect (Peer et al., 2020).

Nanotechnology as a new field of science has developed rapidly in the 21st century and widely applied in many fields, such as industry, agriculture, medicine, and cosmetics. Compared with polymer nanoparticles, micelles, and liposomes, silica nanoparticles are known as promising nanocarriers due to their high surface area, large pore volume, tunability of pore size, high biocompatibility, and ease of surface modification (Cotí et al., 2009; Nguyen et al., 2020; Wu et al., 2015). In 1968, Stober reported a pioneering method for synthesizing spherical silica nanoparticles, the reactants including alkyl silicate, alcohol, water, and ammonia. For alkyl silicates (alky groups including methyl, ethyl, n-propyl, n-butyl, and n-pentyl), the fastest reaction and the smallest particle size (less than 0.2 µm) were formed with tetramethyl ester. In contrast, with tetrapentyl ester, the reaction was slow (it took 24 hours for the condensation reaction, and the resulting particle size was considerable); the particle size distribution was wider. However, because it is difficult to control the rapid hydrolysis rate of TMOS (Tetramethyl orthosilicate), which often leads to forming free silica nanoparticles with an internal porous structure, TEOS is a commonly used silica-containing precursor (Stöber et al., 1968).

Many studies on the influence of reaction parameters in the synthesis of silica nanoparticles have been published to control particle size (Peer et al., 2020; Tacar et al., 2013). In this study, the effects of reaction time and ammonia solution drop rate on the size of the resulting silica nanoparticles were studied to create silica materials with the appropriate size as drug carriers. Then, as previously indicated, Dox was encapsulated inside silica nanoparticles to provide a tailored drug delivery system that improved therapeutic efficacy and lessened side effects.

2. Experiment

2.1. Materials

The chemicals used in the study were all analytical grade with high purity, including Doxorubicin (Sigma), Tetraethyl orthosilicate (TEOS) from Sigma, Ethanol (Prolabo), NH_3 (aq) 28% (Merck), and deionized water. Cellulose membrane MWCO 12–14 kDa, 6–8KDa (Spectrum Laboratories, Inc., Rancho Dominguez, CA 90220, USA).

2.2. Characterization

- Transmission electron microscopy (TEM) was used to examined the material's morphology and surface.

- The particle size and zeta potential were assessed with a high degree of precision using dynamic Light Scattering with a Horiba Nano SZ100 particle size measuring equipment.

- The X-ray Diffraction (XRD) was instrumental in our research, analyzing the structure and space group characteristics of the material.

- The N_2 adsorption-desorption method, performed on a Trista Micromeritics 3000 device, was a key step in our research, evaluating the surface area of the material.

- The solution was quantified by ultraviolet-visible absorption spectroscopy on a UV-Vis 1800 device (Shimadzu, Japan).

2.3. Synthesis of solid silica nanoparticles (SSN)

The synthesis process was carried out under the Stober method in Figure 1 (Stöber et al., 1968); all concentrations of substances were calculated based on the total volume of the initial substances participating in the reaction. A mixture of ethanol 13.48 mol/L and water (with fixed volume) was placed into a 250 mL round-bottom flask and cover. The flask was placed on a stove and stirred at 50^{o}C for 10 minutes. Then, NH_3 solution 0.38 mol/L and TEOS 0.22 mo/L were added to the mixture and stirring was on for T hours to end the reaction - the time T was investigated respectively: 0.5, 2, 3, 4, and 5 hours. The amount of NH_3 was added to the mixture under investigation: 200 µL/minute and total volume/5 seconds. The solution was put into a 12-14 KDa membrane and dialyzed with distilled water. The dialysis time was 4 days, and the dialysis water was changed 4 times/day, ensuring the purity of the product. Then, the obtained product was freeze-dried to form silica nanoparticles (denoted as SNs). The optimal nanoparticles (particles with spherical shape and high homogeneity, size about 100 nm, suitable as drug carriers) were selected for drug loading and release evaluation.

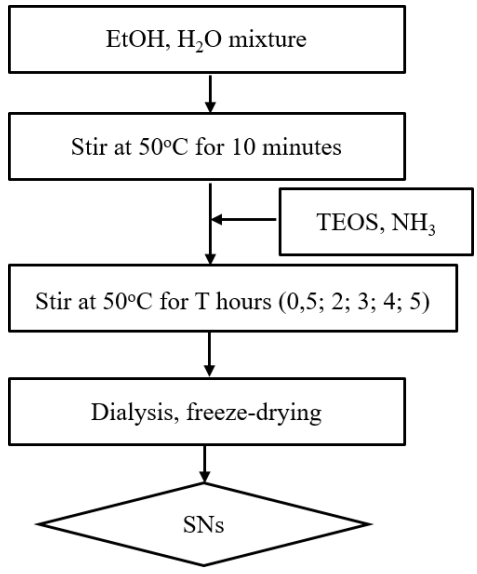

Figure 1. Synthesis of SNs

2.4. Encapsulation of cancer treatment drug Doxorubicin (denoted as SNs/Dox)

Disperse 20 mg of each carrier in deionized water, sonicate for 15 minutes, and place in a brown glass bottle. Next, add 1000 ppm Dox solution. The mixture was stirred for 24 hours at room temperature in the dark (Mas et al., 2014). Then, divide into three parts, corresponding to three treatments. Put each part into a 6-8 KDa cellulose membrane and dialyze in 10 ml deionized water for 6 hours. Change the water outside the membrane 3 times; the distilled water outside the membrane corresponding to each change determines the free Dox content by UV-Vis.

The evaluation of the drug loading capacity of the material is a crucial step in our process. It is assessed through the parameters: Drug loading efficiency - DLE and drug loading content – DLC (Chen et al., 2010; Liu et al., 2016):

$$\text{DLE (\%)} = \frac{\text{Amount of encapsulated drug}}{\text{Initial amount of drug for loading}} \text{ x } 100\% \quad (1)$$

$$\text{DLC (\%)} = \frac{\text{Amount of encapsulated drug}}{\text{Total amount of drug and carriers}} \text{ x } 100\% \quad (2)$$

2.5. Investigation of the release rate of Doxorubicin of the SNs/Dox system

Disperse a certain amount of the SNs/Dox drug-carrier system into 1 ml of distilled water. Transfer the samples to separate 6-8 KDa cellulose membranes with meticulous attention to detail. Place the dialysis membranes in 20 ml of phosphate buffer (PBS) with pH 5.5. Stir the solution at a physiological temperature of 37^{o}C at a speed of 100 rpm. At predetermined times, including 1, 3, 6, 9, 12, 24, 36, 48, 72, and 96 hours (Corrêa et al., 2012), every 2 ml of the solution outside the membrane is taken out, and 2 ml of buffer is added. Measure the absorbance of the solutions outside the membrane by UV-Vis spectrophotometry, then plot the drug release curve of the corresponding samples.

3. Results and Discussion

3.1. Effect of synthesis time

The effect of reaction time from 0.5 h to 5 h was carried out at the concentrations of reactants TEOS 0.22 mol/l, NH_3 0.39 mol/l, and ethanol 13.48 mol/L. During the reaction, the color of the solution in the flask turned slightly cloudy after 30 minutes of adding TEOS solution. This may be due to the hydrolysis reaction of TEOS to form silicic acid, followed by the condensation of silicic acid in a supersaturated state, so the solution became more cloudy than before (Stöber et al., 1968). After more than 1 hour, the solution turned opaque white, and no color change was observed until the end of the reaction. This is consistent with the result of increased particle size with increased reaction time shown in Figure 2 and consistent with the published study by Li et al. (2015).

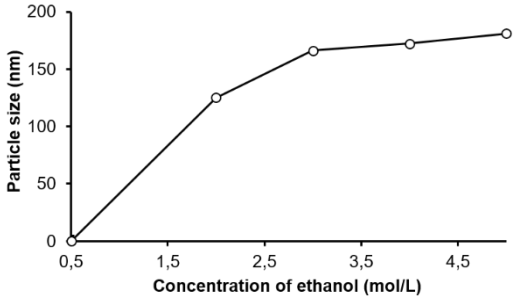

Figure 2. Effect of reaction time on particle size of SNs

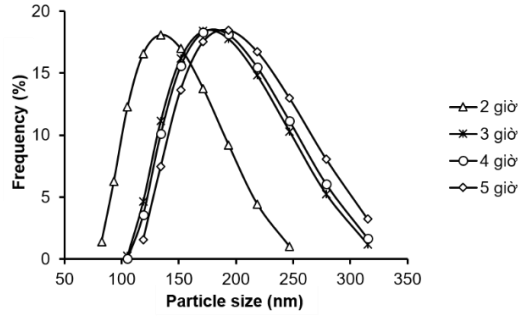

Figure 3. Effect of reaction time on size distribution of SNs

Figure 3 shows that the particle size distribution obtained when reacting for 3 - 5 hours is relatively similar, with a reasonably broad distribution line compared to the particles obtained for 2 hours. However, the TEM image in Figure 4 provides compelling evidence that supports this data. It can be seen that at a reaction time of 0.5 hours (Figure A), after two hydrolysis and condensation reactions, the primary particles lacked time to condense and form spherical nanoparticles as at a reaction time of 5 hours (Figure B).

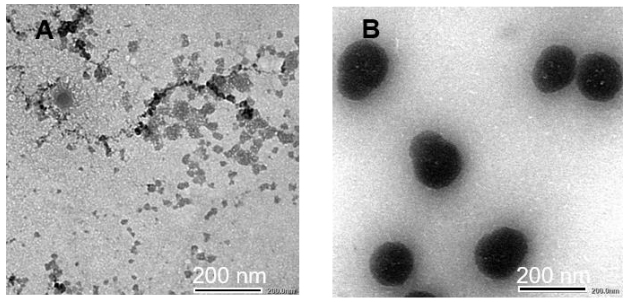

Figure 4. TEM images of silica particles synthesized at 0,5 (A) and 5 hours (B)

3.2. Effect of ammonia solution feed rate

To control the particle size, most publications studied the rate of adding TEOS solution to the mixture of H_2O, NH_3, and ethanol during the synthesis of nanosilica (Park et al., 2002). However, the rate of adding NH_3 solution to the reaction mixture also significantly affects the particle size. Based on Figure 5, it can be seen that when adding the total volume of NH_3 solution/5 seconds to the mixture, the hydrolysis reaction rate increases rapidly, resulting in a rapid increase in the concentration of primary particles. At the same time, the reaction rate also increases rapidly, so the primary particles condense rapidly, increasing the particle size rapidly (Rahman et al., 2007).

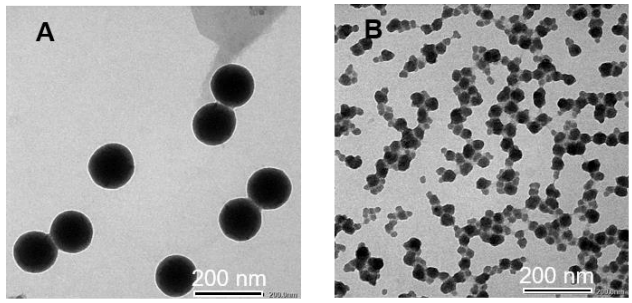

Figure 5. TEM images of SNs at NH_3 feed rate: (a) total volume/5 seconds (A) and 200µl/minutes (B)

3.3. The characterization of SNs at total volume/5 seconds

Based on the TEM images under two experimental conditions shown in Figures 4 and 5, SNs particles with rapid NH_3 addition rate were selected to evaluate the ability to carry and release the Dox drug (denoted as SNs-A). SNs particles were evaluated for size and structure using various techniques.

3.3.1. Size distribution of SNs-A

Particle morphology and size were observed by transmission electron microscope (TEM), Figures 5A and 6. The results showed that the formed nanoparticles were spherical in shape, had high uniformity, were 105,06 ± 0.74 nm in size, and had a narrow particle size dispersion.

3.3.2. Zeta potential

The zeta potential appears due to the charge existing on the surface of the particles and shows the stability of the colloidal system formed (Vazquez et al., 2017). When there is a significant zeta potential, whether positive or negative, the electrostatic repulsion between the particles increases, resulting in reduced aggregation between the particles and improved

stability and dispersion of the particles in water. The surface charge of all the formed nanoparticles is shown by the zeta potential in Figure 7. The zeta potential of SNs particles with hydroxyl groups on the surface should carry a negative charge of -44,53 ± 0,23 mV.

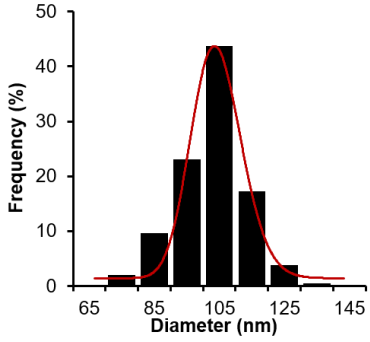

Figure 6. Size distribution of Sns-A calculated from TEM imaging

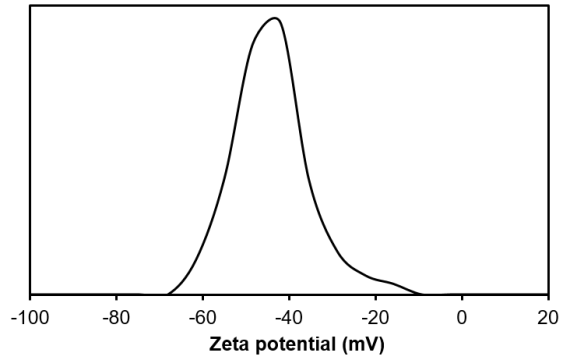

Figure 7. Zeta potential of SNs-A

3.3.3. XRD pattern

As observed in Figure 8, the resulting nanoparticles have a broad XRD diffraction peak ($2\theta = 23^o$), showing that SNs-A nanoparticles have an amorphous structure. This is consistent with the published theory that the resulting nanoparticles will have an amorphous structure when synthesized by the Stober method (Penkova et al., 2009).

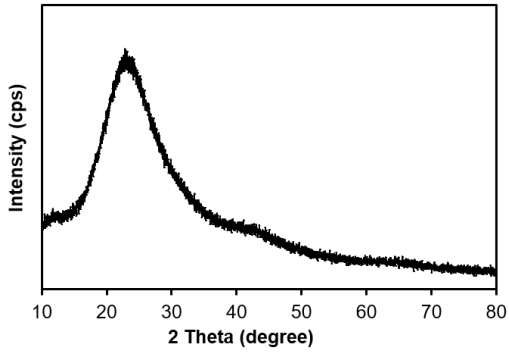

Figure 8. XRD patterns of SNs

3.3.4. Surface area via BET method

Nitrogen adsorption-desorption and the BET method (Barrett-Emmet-Taller) evaluated the material's surface area to be 75 m^2/g. Based on the synthesis process, the adsorption-desorption isotherm shape can be either type I or II (Li et al., 2015; Szekeres et al., 2002). As can be seen from Figure 9, the nitrogen adsorption-desorption isotherms of SNs-A belong to type II, according to the IUPAC classification. Furthermore, the graph displays a broad hysteresis loop in the desorption branch, possibly due to the gaps formed at the surface between spherical nanoparticles.

3.3.5. Drug loading efficiency and content

The Dox content in the nanocarrier system was determined by UV-Vis spectroscopy with the maximum absorption wavelength at 570 nm. Pure Dox was dissolved in deionized

water at concentrations ranging from 0 - 5 µg/ml and 5 to 50 µg/ml to construct a standard curve. The graph representing the ratio between Dox concentration and absorbance gives a straight-line equation of y = ax + b, where y is absorbance and x is concentration. Drug loading efficiency and content (DLE and DLC) are two important factors in any drug delivery system, as they directly affect the therapeutic activity (Colilla et al., 2013). The results of drug loading efficiency and capacity of SNs-A particles were DLE (17,70 ± 0,52 %) and DLC (4,11 ± 0,11 %), respectively.

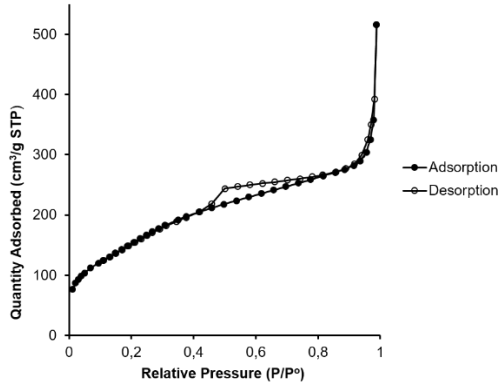

Figure 9. Nitrogen adsorption-desorption isotherms of SNs-A

3.3.6. The doxorubicin release rate of SNs-A/Dox

The drug release capacity of the carrier/drug system was investigated overtime at 1 to 96 hours at an acidic pH of 5.5 (to simulate the tumor environment), Figure 10. The graph shows that after 20 hours, the amount of Dox released into the environment is less than 40%. This shows that Dox is released slowly from the carrier system compared to free Dox, confirming the control potential of SNs-A carrier systems.

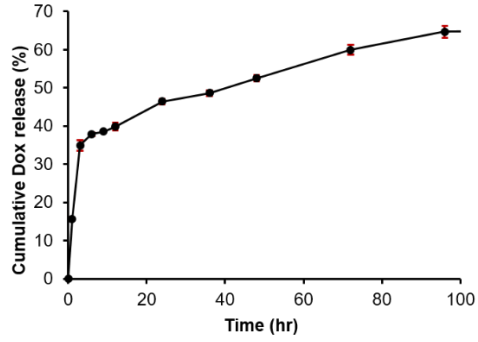

Figure 10. *In vitro* release profile of SNs/Dox at pH 5,5. The marked points correspond to 0,1,3,6,9,12, 24,36, 48, 72 and 96h, respectively

4. Conclusion

Based on the findings of the analysis of two variables influencing the size and shape of silica nanoparticles, the SN particles synthesized with the NH_3 solution addition rate (total volume/5 seconds) have a spherical shape, uniform with a size of 105.06 ± 0.74 nm, with a surface area of 75 m^2/g. These particles were selected as Dox drug carriers. The results of the efficiency and ability to carry the anticancer drug Dox of SN particles are 17.70 ± 0.52 % and 4.11 ± 0.11 %, respectively. More importantly, the SNs/Dox drug carrier system demonstrates a high potential for controlled drug release.

References

Ardizzoni, A., Boni, L., Tiseo, M., Fossella, F. V., Schiller, J. H., Paesmans, M., Radosavljevic, D., Paccagnella, A., Zatloukal, P., & Mazzanti, P. (2007). Cisplatin-versus carboplatin-based chemotherapy in first-line treatment of advanced non–small-cell lung cancer: an individual patient data meta-analysis. *Journal of the National Cancer Institute, 99*(11), 847-857. https://doi.org/10.1093/jnci/djk196.

Chen, Y., Chen, H., Guo, L., He, Q., Chen, F., Zhou, J., Feng, J., & Shi, J. (2010). Hollow/rattle-type mesoporous nanostructures by a structural difference-based selective etching strategy. *ACS Nano, 4*(1), 529-539. https://doi.org/10.1021/nn901398j.

Colilla, M., González, B., & Vallet-Regí, M. (2013). Mesoporous silica nanoparticles for the design of smart delivery nanodevices. *Biomaterials Science, 1*(2), 114-134. https://doi.org/10.1039/C2BM00085G.

Corrêa, D. S., Magalhães, R. T. d., & Siqueira, D. C. B. d. (2012). In situ dry matter and fiber fraction degradability of the Mineirão stylos. *Acta Scientiarum. Animal Sciences, 34*, 203-207. https://doi.org/10.4025/actascianimsci.v34i2.13138

Cotí, K. K., Belowich, M. E., Liong, M., Ambrogio, M. W., Lau, Y. A., Khatib, H. A., Zink, J. I., Khashab, N. M., & Stoddart, J. F. (2009). Mechanised nanoparticles for drug delivery. *Nanoscale, 1*(1), 16-39. https://doi.org/10.1039/B9NR00162J.

Li, S., Wan, Q., Qin, Z., Fu, Y., & Gu, Y. (2015). Understanding Stöber silica's pore characteristics measured by gas adsorption. *Langmuir, 31*(2), 824-832. https://doi.org/10.1021/la5042103.

Liu, J., Luo, Z., Zhang, J., Luo, T., Zhou, J., Zhao, X., & Cai, K. (2016). Hollow mesoporous silica nanoparticles facilitated drug delivery via cascade pH stimuli in tumor microenvironment for tumor therapy. *Biomaterials, 83*, 51-65. https://doi.org/10.1016/j.biomaterials.2016.01.008.

Mas, N., Galiana, I., Hurtado, S., Mondragón, L., Bernardos, A., Sancenón, F., Marcos, M. D., Amorós, P., Abril-Utrillas, N., & Martínez-Máñez, R. (2014). Enhanced antifungal efficacy of tebuconazole using gated pH-driven mesoporous nanoparticles. *International Journal of Nanomedicine*, 2597-2606. https://doi.org/10.2147/IJN.S59654.

Nguyen, T. N. T., Le, N. T. T., Nguyen, N. H., Ly, B. T. K., Nguyen, T. D., & Nguyen, D. H. (2020). Aminated hollow mesoporous silica nanoparticles as an enhanced loading and sustained releasing carrier for doxorubicin delivery. *Microporous and Mesoporous Materials, 309*, 110543. https://doi.org/10.1016/j.micromeso.2020.110543.

Octavia, Y., Tocchetti, C. G., Gabrielson, K. L., Janssens, S., Crijns, H. J., & Moens, A. L. (2012). Doxorubicin-induced cardiomyopathy: from molecular

mechanisms to therapeutic strategies. *Journal of molecular and cellular cardiology, 52*(6), 1213-1225. https://doi.org/10.1016/j.yjmcc.2012.03.006.

Park, S. K., Do Kim, K., & Kim, H. T. (2002). Preparation of silica nanoparticles: determination of the optimal synthesis conditions for small and uniform particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 197*(1-3), 7-17. https://doi.org/10.1016/S0927-7757(01)00683-5.

Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R., & Langer, R. (2020). Nanocarriers as an emerging platform for cancer therapy. *Nano-enabled medical applications*, 61-91. https://doi.org/10.1038/nnano.2007.387.

Penkova, A., Blanes, J. M., Cruz, S. A., Centeno, M., Hadjiivanov, K., & Odriozola, J. A. (2009). Gold nanoparticles on silica monospheres modified by amino groups. *Microporous and Mesoporous Materials, 117*(3), 530-534. https://doi.org/10.1016/j.micromeso.2008.07.041.

Rahman, I., Vejayakumaran, P., Sipaut, C., Ismail, J., Bakar, M. A., Adnan, R., & Chee, C. (2007). An optimized sol–gel synthesis of stable primary equivalent silica particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 294*(1-3), 102-110. https://doi.org/10.1016/j.colsurfa.2006.08.001.

Stöber, W., Fink, A., & Bohn, E. (1968). Controlled growth of monodisperse silica spheres in the micron size range. *Journal of Colloid and Interface Science, 26*(1), 62-69. https://doi.org/10.1016/0021-9797(68)90272-5.

Szekeres, M., Tóth, J., & Dékány, I. (2002). Specific surface area of stoeber silica determined by various experimental methods. *Langmuir, 18*(7), 2678-2685. https://doi.org/10.1021/la011370j.

Tacar, O., Sriamornsak, P., & Dass, C. R. (2013). Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *Journal of Pharmacy and Pharmacology, 65*(2), 157-170. https://doi.org/10.1111/j.2042-7158.2012.01567.x.

Vazquez, N. I., Gonzalez, Z., Ferrari, B., & Castro, Y. (2017). Synthesis of mesoporous silica nanoparticles by sol–gel as nanocontainer for future drug delivery applications. *Boletín de la Sociedad Española de Cerámica y Vidrio, 56*(3), 139-145. https://doi.org/10.1016/j.bsecv.2017.03.002.

Wu, S., Huang, X., & Du, X. (2015). pH-and redox-triggered synergistic controlled release of a ZnO-gated hollow mesoporous silica drug delivery system. *Journal of Materials Chemistry B, 3*(7), 1426-1432. https://doi.org/10.1039/C4TB01794C.