

Temperature Variation on Doxorubicin Adsorption by Mesoporous Silica Nanoparticles and its Effect towards Release Rate

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ABSTRACT – Mesoporous silica nanoparticles (MSN) were reported to have many advantages to be used as an ideal drug carrier. In this study, MSN was prepared using the sol gel method with the addition of a pore expander namely triethoxysilyl propylamine (APTES). The prepared MSNs were characterized by using X-ray diffraction (XRD), and Transmission electron microscopy (TEM) to study the crystallinity and topology of the MSN. Adsorption of doxorubicin (DOX) which is a type of anticancer drug onto the MSN was carried out at different temperatures ranging from 50°C to 80°C to obtain different drug loading capacities. The mechanism for the adsorption of DOX onto MSN has also been explained in this study. The drug release profile of DOX from MSN-DOX was then evaluated after the adsorption process to know the effect of different drug loading values on the release level and rate. Based on the findings, drug loading values obtained at adsorption temperatures of 50°C to 80°C were 77.58%, 80.27%, 88.86% and 84.69%, respectively. The highest adsorption percentage was obtained at 70°C. As for the drug release study, high drug loading resulted in faster drug release. The drug loading value that released drug at the slowest rate was 77.58% at the rate of 0.038% min⁻¹ with a percentage of release 22.82% after 24 hours.

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INTRODUCTION

Nanoparticles are used in the drug delivery system, and they provide a lot of advantages as compared to conventional treatment. These includes enhancing the therapeutic activity and solubility of hydrophobic drugs, increasing drug half-life, reducing potential immunogenicity and side effects of drugs, and releasing drugs sustainably [1,2]. Cancer is a very serious health issue, and researchers are continuously conducting studies to find the best solutions to cure the disease effectively. Chemotherapy which is the current treatment to combat cancer cells, has brought many bad outcomes to patients as they also affect healthy cells and tissues. Nanotechnology is a promising solution as an effective drug delivery vehicle in which the main function is to carry anticancer drugs and deliver them to the targeted organs. Mesoporous silica nanoparticles (MSN) can be used as a nano carrier for targeted drug delivery; besides, it prevents drug resistance by cancer cells [3]. Malfanti et al. [4] has stated that MSN has various advantages as non-organic nano material such as heat and chemical stability, easy chemical alteration on the surface of silanol group, stable mesoporous structures, large surface area, high pore volume, and tunable mesoporous diameter.

MSN has potentially proven to adsorb hydrophilic drugs, but its potential to adsorb hydrophobic drugs like doxorubicin (DOX) is not yet known. Previous research works have focused on the adsorption of DOX on different carriers such as chitosan, nano polymeric system, and polymeric micellar. In cancer treatment using DOX, the maximum adsorption percentage of the drug onto the carrier will be considered economical, and at the same time, a slow and continuous release rate of the drug is also required. Therefore, it is important to study the optimization of the adsorption process and control the release rate effectively. According to research done by Ganesh et al. [5], adsorption of DOX on MSN at room temperature resulted in an adsorption percentage of 33% after 3 days. Adsorption percentage from previous research is measured as little for a quite long adsorption time. Wu et al. [6], in their research on the adsorption of DOX on graphene oxide, explained that an increase in temperature caused an increase in adsorption capacity. Therefore, it is important to study the effect of temperature variation on drug release. The adsorbed drug will be released at 37°C. The difference in adsorption temperature is hypothesized to cause variation in drug loading value. The effect of different drug loading values on the release rate of the drug also will be studied. Ganesh et al. [5] stated that high drug loading caused the percentage of drug release to increase. Manipulation of drug loading values through temperature variation during the adsorption process and its effect on the release process is not yet known. In previous works, the loading/adsorption process was normally standardised and only the release behaviour was studied.

Drug adsorption and release profiles are important to determine the loading capacity of drug carriers and controlled release. There are various factors that influence the adsorption and release of the drug. Wu et al. [6] stated that pH, the concentration of adsorbents, contact time, and temperature control the adsorption of the drug. The structure of DOX consists of an amine group which causes the physicochemical properties to depend on pH value. According to Roik et al. [7], DOX is dominantly not ionized at neutral or alkali pH and will undergo protonation in an acidic medium. However, in acidic conditions, the silanol group of MSN is not ionized. This has caused the adsorption of DOX on MSN to be ineffective. Interaction between ionized surfaces of silica group with positively charged DOX is possible at a neutral pH value. Almost 50% of silica groups are ionized in this pH thus, limiting the study for adsorption of DOX on MSN on pH variation. The release of drugs is controlled by the solubility of drugs, desorption of adsorbed drugs, drugs diffusion through the matrix of nanoparticles, degradation of nanoparticles matrix, and the combination between erosion and diffusion.

DOX has widely been used for chemotherapy treatment. It is under the anthracycline antibiotic class and antineoplastic agent that is useful for the treatment of various types of malignant such as breast cancer, leukaemia, and ovary [8]. However, according to Knezevic [9], DOX will also kill healthy tissues, thus preventing them from being used effectively. Besides, DOX was reported to bring many bad effects to human health, including heart damage, cancer cells resistance to the drug, blood circulation becoming less stable, heterogeneous tumour tissue, and others [8]. Ghanbari [8] later stated that the combination between DOX and drug carriers such as liposomes could reduce the dosage, relying on effect and cardiac toxicity. Therefore, this study will focus on the effect of different DOX loading values on the DOX release rate from MSN.

MATERIALS AND METHODS

Material synthesis and characterization

Cetyl trimethylammonium bromide (CTAB), distilled water, ethylene glycol, and ammonia solution were added into a conical flask and stirred continuously for 30 minutes at 50°C. Tetraethyl orthosilicate (TEOS) and triethoxysilyl propylamine (APTES) were added into the flask, and the temperature was increased to 80°C. The solution was kept stirred for two hours and kept in the refrigerator for complete separation between the solvent and the product. The centrifugation process using ethanol was carried out three times to wash away the ammonia. The gel product obtained was dried at 110°C overnight. The sample was then crushed to fine particles before calcination at 550°C for three hours. The synthesized MSN was analysed for X-ray diffraction (XRD) and transmission electron microscopy (TEM) analysis to determine the physical and chemical properties. The crystallinity of MSN was measured with a Bruker Advance D8 X-ray powder diffractometer with Cu K α ($\lambda = 1.5418 \text{ \AA}$) radiation as the diffracted at 40 kV and 40 mA. TEM was performed using a Philip TEMCM12 microscope. The samples were ultrasonically dispersed in acetone and deposited on an amorphous, porous carbon grid.

DOX adsorption

MSN samples were loaded with DOX in a ratio of 1:3 by soaking them in diluted NaOH solution and followed by continuous stirring for 24 h at 50°C to 80°C. The concentration of DOX was fixed to 80 ppm for the loading which 110 mg of DOX was dissolved in 1 L of dilute NaOH, and 100 mg of dry MSN were added into the solution. The wasted DOX has been separated by filtration process by washing in ethanol and dried in an oven overnight at 105°C. The samples of aliquots 2 ml were taken at the pre-defined time interval and centrifuged. DOX was detected by using UV-Vis spectrophotometer at an adsorption wavelength of 380 nm.

DOX release

The release profile of DOX from MSN was obtained by mixing MSN-DOX produced into 100ml of Ultrapure water. The sample was heated in an incubator shaker at a temperature of 37°C at 180 rpm for 24 hours. 2ml of the sample were taken at 1-hour interval for 24 hours for UV-vis analysis at the same wavelength. This is to determine the release percentage and release rate after certain times.

RESULTS AND DISCUSSION

Materials characterization

X-ray diffraction (XRD) is used to analyze a single crystal and fine crystalline powder. Powder X-ray diffraction can be used to determine the crystallization of the amorphous material. In mesopores of silica, amorphous there in nature, where the powder pattern shows the arrangement of mesopores in the framework of its own. The results of the XRD analysis show the mesostructure. Mesoporous silica nanoparticles have shaped of hexagonal. Figure 1 shows the X-Ray diffraction of the hexagonal MSN. Small-angle XRD patterns exhibit a strong diffraction peak at coordinates (100) and two small peaks indicate the coordinates on (110) and (200). Sample diffraction occurs at 2θ of 2.5, 4.4, and 5.0, which shows a hexagonal array of mesopores. The patterns reflect reflections such as MCM-41, which consists of various regular hexagonal silica tubes and can be indexed in line with the assumption of a hexagonal unit cell (100), (110), and (200).

Transmission electron microscopy (TEM) imaging was also done on the MSN sample (Figure 1B). From the results, it can be concluded that the diameter size of MSN produced is approximately 273nm.

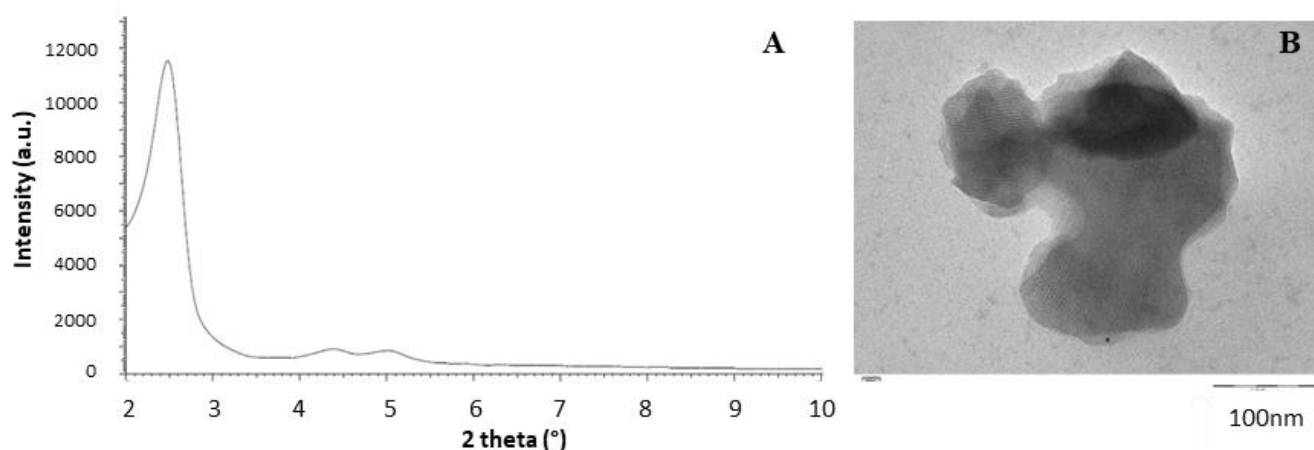


Figure 1. XRD diffraction (A) and TEM image (B) of MSN

DOX adsorption

Adsorption of DOX by MSN was carried out at 50 °C, 60 °C, 70 °C, and 80 °C for 24 hours. The highest adsorption percentage was obtained at 70 °C. As shown in Figure 2, the percentage of adsorption has shown an increasing trend as the temperature increased but started to decrease at 80 °C. The uptake increases with increasing temperature can be explained by the availability of more interactable DOX towards MSN. In this case, as the temperature increases, the favourable intermolecular forces between DOX and MSN are much stronger than those between DOX and the solvent. A previous study by Wu et al. [6] for the adsorption of DOX using graphene oxide (GO) also shows a similar pattern but due to the activation of more GO surfaces as the temperature increase [6]. Table 1 shows the summary of DOX adsorption percentage after 24 hours.

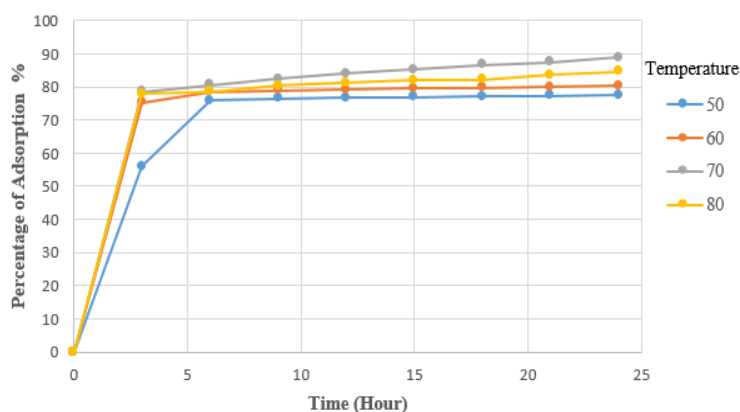


Figure 2. Adsorption of DOX against time at different temperature

Table 1. Percentage of adsorption of DOX on MSN after 24 hours

Adsorption temperature (°C)	Percentage of adsorption (%) after 24 hours
50	77.58
60	80.27
70	88.86
80	84.69

The percentage of adsorption also shows a rapid increment at the beginning and started to increase slowly with time. Rapid adsorption is due to the driving force that causes DOX to move towards the active sites of MSN. However, active sites begin to decrease with time. Therefore, more time will be needed for the adsorption process to achieve an equilibrium level. The study by Ganesh et al. [5] stated that the adsorption of Doxorubicin by mesoporous silica nanoparticles at room temperature resulted in an adsorption percentage of 33% after a time period of three days. Thus, the value in this study is acceptable because the increase in temperature facilitates the adsorption process in a shorter time.

Figure 3 shows the mechanism for adsorption of DOX on MSN. According to Prokopowicz et al. [10], DOX exists in positive charge when the pH value is 6. In this experiment, adsorption of DOX was done at pH 7. Prokopowicz also stated that in this pH range, the silanol group of MSN will react as acid and undergo partial protonation. Roik et al. [7] suggest that 50% of silanol groups were ionized while 93% of amine groups were protonated at neutral pH. The ionized silanol group formed electrostatic force with a positively charged amine group of DOX. Hydrogen bond also formed between neutral charged DOX and nonionized silanol group, which contributed to the adsorption process. Hydrogen bonds that may be formed were $-OH$ from MSN with $-NH_3^+$ from DOX.

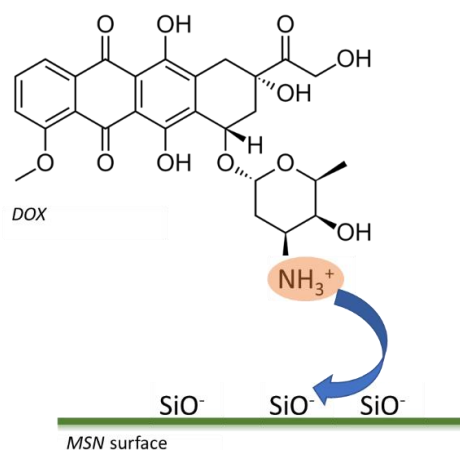


Figure 3. Mechanism for adsorption of DOX on MSN

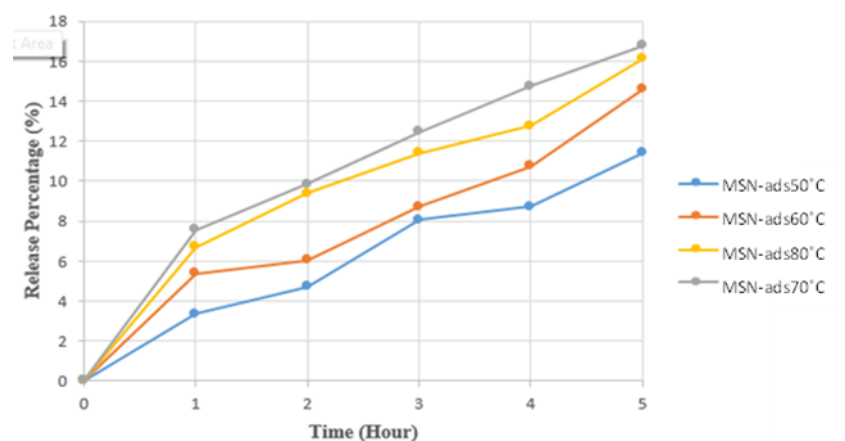


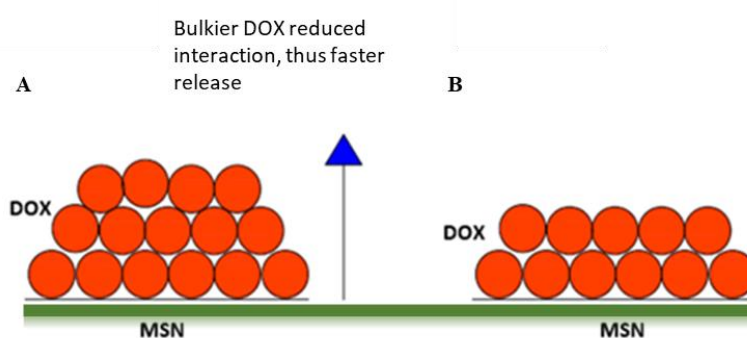
Figure 4. Percentage of release of DOX from MSN against time

Figure 4 shows the release progress of DOX from MSN in 5 hours. From Table 2, the percentage of release obtained after 5 hours for MSN-ads50°C, MSN-ads60°C, MSN-ads70°C, and MSN-ads80°C were 11.41%, 14.57%, 16.78%, and 16.11%, respectively. The highest percentage of drug release after 5 hours was recorded at 16.78% of loading capacity 84.69%, from MSN-ads70°C. More drugs were released as drug loading capacity was increased. The same goes for the release rate. An increase in drug loading values will cause the release rate of DOX to increase.

Table 2. Release and release rate of DOX from MSN after 5 hours

Sample	Percentage of release (%)	Release rate (% min ⁻¹)
MSN-ads50°C	11.41	0.038
MSN-ads60°C	14.57	0.049
MSN-ads80°C	16.11	0.054
MSN-ads70°C	16.78	0.056

Figure 5 shows the comparison between highly loaded and less loaded MSN. Different release rates obtained due to different drug loading values can be explained by the mechanism of drug release. The bond between DOX and MSN is controlled by electrostatic interaction within it. Positively charged DOX will get attracted to negatively charged MSN. MSN that carries a high drug load will release drugs faster compared to MSN that carries less drug load due to the lesser interaction among the lower amount of DOX. Thus, it is speculated as to the reason for the faster release of MSN-ads70°C.

**Figure 5.** Speculated mechanism on the difference between adsorption amount that led to different release behavior.

Release time was continued to 24 hours for the slowest initial drug release at a percentage of 11.41% from MSN-ads50°C. The release percentage obtained after 24 hours was 22.82%, whereas, for the fastest drug release of MSN-ads70°C, the release percentage obtained after 24 hours was 29.53%. In conclusion, an increase in drug loading will lead to a faster drug release rate.

CONCLUSION

MSN was prepared using the sol gel method. XRD pattern exhibited two peaks at 100 and 110 hkl miller indices which indicated the formation of mesoporous silica structures. Adsorption of DOX by MSN at temperature 50°C, 60°C, 70°C, and 80°C for 24 hours resulted in 77.58%, 80.27%, 88.86%, and 84.69% adsorption percentage, respectively. DOX adsorption percentage on MSN increased as the temperature increased up until 70°C. Release rate for drug loading following the previous order was 0.038% min⁻¹, 0.049% min⁻¹, 0.054% min⁻¹ and 0.056% min⁻¹. Slow-release of the drug is required for the application as an anticancer drug. Therefore, a lower drug loading value is more suitable to be used as it provides slow drug release. However, for pain relievers such as paracetamol, a fast release is required. In this case, a high drug loading value is more suitable to be used. In conclusion, the release rate of the drug can be controlled by manipulating the temperature during drug adsorption, which leads to differences in drug loading value.

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