

Rough Hollow Mesoporous Silica Nanoparticles as Carrier for Agarwood Oil to Treat Cancer Cells

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ABSTRACT

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Natural products such as agarwood oil (AO) are found to exhibit potential anticancer properties. They provide safer and better alternative to the conventional drugs used in chemotherapy treatment. However, due to the limitation of AO in terms of volatility, stability and solubility in water, a suitable carrier is needed to enhance the performance of the essential oil. Mesoporous silica nanoparticles are found to be promising carrier for AO as it is biocompatible, hydrophilic, possesses high stability, high total surface area and high pore volume properties which can reduce the limitations possessed by AO. Rough mesoporous hollow silica (RMHS) nanoparticles were used in this study as the particles known to have high loading capacity due to its pores and rough surface. By using ethanol as solvent, the ratio of AO to RMHS 20:1 with loading time of 24 hour has successfully achieved high loading efficiency as much as 62.79%. Successful loading of AO in RMHS was confirmed by Fourier transform infrared (FTIR). Sustained release of AO from RMHS has been observed, with release up to 63.8% after 72 hours. Cell viability assay (CVA) by using MTT assay showed that RMHS loaded with AO (RMHS-AO) was able to reduce the viability of MCF-7 cells up to 73% reduction after 48 hours of treatment.

Keywords:

Agarwood oil, mesoporous silica, nanoparticles, MCF-7, cell viability assay

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1. Introduction

American Cancer Society estimated that there will be 1,735,350 new cancer cases in 2018 with 609,640 deaths. By type, American Cancer Society estimated that the highest number of new case will be breast cancer for both sexes with 268,670 cases, while most deaths are estimated from lung and bronchus cancer with 154,050 deaths. Chemotherapy is one of the conventional ways to treat cancer with the use of one or more anti-cancer drugs. There were studies conducted to study the use of nanoparticles such as mesoporous silica as drug delivery vehicle of the drugs [1]. However, the use of anti-cancer drugs in chemotherapy or radiotherapy gives negative side effects towards the patients such as hair loss, fatigue and weight changes. Studies are going on to find alternative treatment to replace the use of toxic drug and chemotherapy for treating cancer. Previously, studies

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have been conducted to investigate anti-cancer properties of various essential oils [2-5]. Agarwood essential oils have proved to have anti-cancer property as one of its pharmacological activities [6]. However, as essential oil is highly volatile and not soluble in water, it needs a carrier to improve its release in the body fluid [7]. Previous studies have proved that essential oils loaded onto nanoparticles could improve volatility problems and have better release control [8].

Hollow mesoporous silica nanoparticles (HMSNs) were found to be promising nanocontainer as it is biocompatible, hydrophilic, high stability, high total surface area and high pore volume properties which make them suitable candidates as nano-vehicles for essential oil [9,10]. Recently, it is reported that nanoparticles with rough surface structure possess additional advantages as delivery system such as large storage capacity, control release of payload and increase interaction with cell surface [11]. Hence, in this study, rough hollow mesoporous silica (RMHS) nanoparticles have been applied for the first time as the carrier for agarwood oil (AO) and their anti-cancer properties were investigated.

2. Materials & Methods

2.1 Materials

Agarwood oil was obtained from IIUM OUD Research Group. MCF-7 cell lines were obtained from Cell and Tissue Engineering Laboratory IIUM. The cells were maintained in Dulbecco's Modified Eagle Media, DMEM with 10% fetal bovine serum (v/v) at 37 °C with 5% CO₂. DMEM was purchased from Sigma Aldrich, fetal bovine serum was purchased from Gibco and accutase was purchased from Innovative Cell Technologies Inc. Other chemicals were purchased from Sigma Aldrich.

2.2 Preparation of Rough Mesoporous Hollow Silica (RMHS) Nanoparticles

RMHS nanoparticles were reproduced by using method described by Song *et al.*, [12].

2.3 Loading of Agarwood Oil

AO was loaded onto RMHS with varied weight of AO (mg) to RMHS nanoparticles weight (mg) at ratios of 10:1, 20:1 and 30:1 respectively. Absolute ethanol was used as the solvent. For every variation, the mixture of ethanol and AO was added to the total volume of one (1.0) mL. The mixture of ethanol, agarwood oil and RMHS was shaken at three different durations; 12, 24 and 36 hours. The variation of parameters was generated by Design Expert software using response surface method (RSM). Each run was done in duplicate. As shown in Table 1, there are total 13 suggested combinations for the loading. Next, the mixture was centrifuged at 15,000 rpm for 5 minutes. The supernatant was collected and the concentration was measured by UV-Vis spectrophotometer at the absorbance reading of 260 nm. After that, RMHS nanoparticles loaded with agarwood oil (RMHS-AO) was dried at room temperature to remove ethanol residue. The loading efficiency was calculated as in Equation (1), where M_0 and M is the concentration of agarwood oil before and after loading respectively

$$\text{Loading efficiency} = [(M_0 - M)/M_0] \times 100\% \quad (1)$$

Table 1
Variation of parameters generated by Design Expert software

Run	Ratio of oil to nanoparticles (mg:mg)	Loading time (hour)	Loading efficiency (%)
1	10:1	12	16.84
2	30:1	36	22.82
3	20:1	24	51.24
4	30:1	12	24.25
5	20:1	24	43.19
6	20:1	24	62.79
7	20:1	24	46.13
8	30:1	24	12.56
9	20:1	24	44.52
10	20:1	36	41.02
11	10:1	24	58.91
12	20:1	12	13.41
13	10:1	36	61.10

2.4 Sample Characterization

RMHS and RMHS-AO were characterized by using scanning electron microscopy (SEM) and Fourier Transform Infrared (FTIR). SEM imaging was from JEOL InTouchScope, JSM-IT500HR operated at 10kV and 15kV. The FTIR spectra of the sample was recorded using Thermo Scientific Nicolet iS50 (Thermo Fisher Scientific, USA) in mid-IR mode, equipped with a Universal ATR (attenuated total reflectance) sampling device containing diamond.

2.5 Release and Volatility Behavior Study

For release study, one milligram (1.0 mg) RMHS loaded with 5 mg of AO was suspended in 1 mL PBS (pH 5.6) and shaken at 200 rpm at 37 °C. At intervals of 8, 24, 48 and 72 hours, the mixture was centrifuged at 5000 rpm for 5 minutes using Eppendorf Minispin plus. The absorbance of the supernatant was measured by using a UV-vis spectrophotometer at wavelength of 260 nm. For volatility test, the remaining AO loaded inside the RMHS was measured by thermogravimetric analysis (TGA) before and after 72 hours of exposure to air.

2.6 In Vitro Anti-Cancer Test

80% confluent MCF-7 cells were seeded at the concentration of 1×10^5 cell/mL in 100 μ L of fresh culture medium in each of 96-well plate. The cells were allowed to attach overnight before treated with RMHS, free AO and RMHS loaded with AO (RMHS-AO) separately. Two plates were prepared for treatment at 24 and 48 hours. All cell culture activities were performed under Safeflow 1.2 biosafety cabinet. Cells were incubated in LabServ CO₂ incubator.

Two-time serial dilution was performed to prepare the sample for treatment. Sample was dispersed in DMEM. For RMHS, the concentrations used were 0.25 mg of RMHS dispersed in 1 mL of DMEM (1mg/mL) until 0.0625 mg/mL. For free AO, the concentrations used were 1.25 mg of AO in 1 mL of DMEM (5 mg/mL) until 0.3125 mg/mL. Finally, for 1 mg of RMHS-AO, the concentrations used were from 0.25 mg RMHS-AO in 1 mL of DMEM (1 mg/mL) until 0.0625 mg/mL. After treatment at the corresponding period, absorbance at 570 nm was measured. Untreated cells were used as the control. The % cell viability was calculated as following

$$\% \text{ Cell viability} = (\text{Absorbance of Sample} / \text{Absorbance of Control}) \times 100\% \quad (2)$$

3. Results

3.1 Agarwood Oil (AO) Loading Efficiency

The highest loading efficiency (62.79%) as seen in Table 1 and Figure 1 was obtained with the AO:RMHS in the ratio of 20:1 with loading time of 24 hours. The runs that have the mentioned parameters were Run 3, 5, 6, 7 and 9. All of these experimental runs showed loading efficiency of more than 40% with small deviation. Higher AO to RMHS ratio (20:1) exhibited significantly higher loading efficiency, but further increasing the ratio reduced the loading efficiency. Similarly, longer loading time increased the loading efficiency, however when the time is further prolonged, the efficiency decreased as there will be some leakage of the payload afterwards [13]. Results interpreted using analysis of variance (ANOVA) from Design Expert software reported that the model and model term are significant with Model F-value of 5.58 and Values of "Prob > F" less than 0.05 respectively (Table 2).

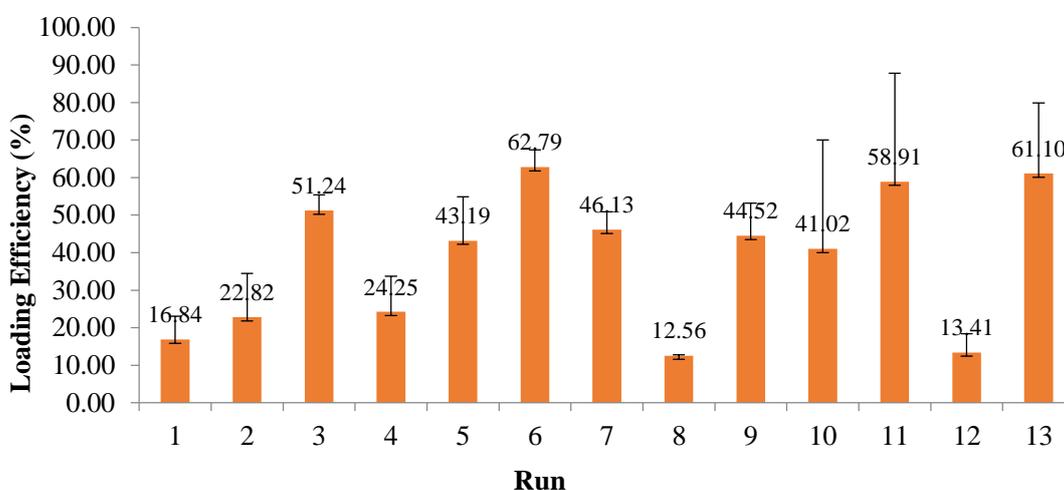


Fig. 1. Average loading efficiency for each run

Table 2

Analysis of variance table (partial sum of square)

Source	Sum of Squares	F-Value	Prob > F	
Model	3215.33	5.58	0.0218	significant
A: ratio of AO to RMHS	993.82	8.62	0.0219	
B: loading time	826.97	7.17	0.0316	
A ²	74.94	0.65	0.4467	
B ²	520.58	4.51	0.0712	
AB	521.89	4.52	0.0710	
Residual	807.36			
Lack of Fit	551.76	2.88	0.1667	not significant
Pure Error	255.60			

3.2 Characterization of the Nanoparticles

The SEM images of RMHS and RMHS-AO are shown in Figure 2 (a) and (b). It is observed that RMHS maintained its spherical morphology with slight aggregation after AO adsorption. RMHS size was measured to be 237 ± 31 nm. It is expected that the AO is encapsulated inside the hollow core of the nanoparticles as well as between the nanospike between rough structure.

To confirm the presence of AO on RMHS, FTIR analysis was performed for the sample. From Figure 3, the spectral features of AO are similar to a previous study by Dahham *et al.*, [6]. The spectra of AO, RMHS-AO (direct mixing) and RMHS-AO (with ethanol as solvent) exhibit all the features, indicating successful loading of AO into RMHS. Meanwhile, the spectral features of RMHS, RMHS-AO (direct mixing) and RMHS-AO (with ethanol as solvent) exhibit the presence of ether bond at 1150 cm^{-1} and 1030 cm^{-1} , indicating their prevalence in the RMHS.

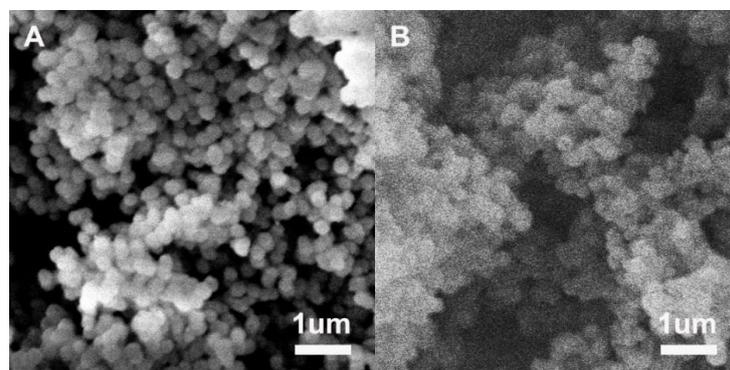


Fig. 2. SEM images of (A) RMHS and (B) RMHS-AO at magnification of 10k

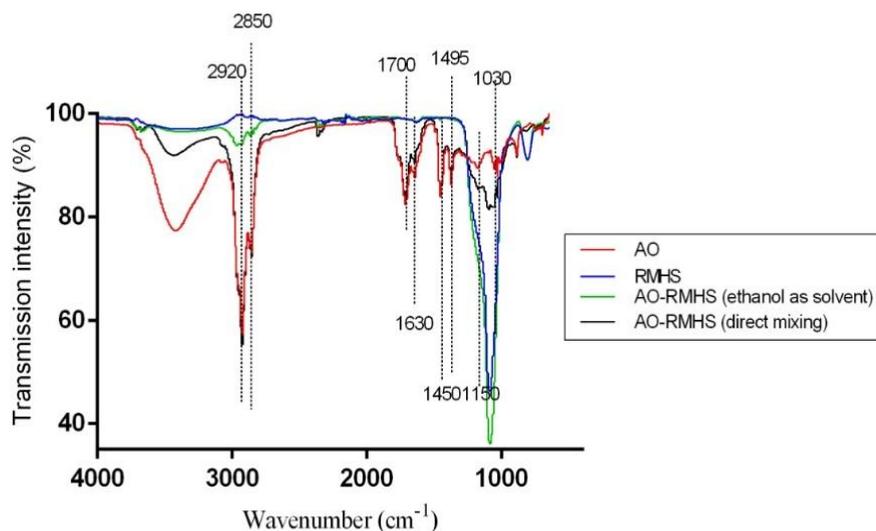


Fig. 3. FTIR spectroscopy of AO, RMHS and RMHS-AO

3.3 Release Study of Agarwood Oil from RMHS-AO

Controlled released is the aimed key feature in AO loading. PBS solution of pH 5.6 was used for the release study to mimic the local condition of cancer cell which gradually becomes acidic after incubation. The acidic condition of the medium can be observed when the colour of DMEM changes from red to orange. Prior to the release, it was observed that RMHS-AO in PBS pH 7.4 exhibited undesirable cloudy mixture once mixed with the solution (figure not shown), compared to that of pH 5.6 which is having a clearer solution. It is expected that the cloudy mixture occurred due to strong hydrophobic nature of the AO which repel water at neutral pH. Based on Figure 4, within 24 hours, there was almost 20% AO released from RMHS-AO. After 72 hours, 63.8% of AO was released. It is expected that more AO will be released after a longer period. Thermogravimetric analysis was done to study the volatility of the loaded AO in RMHS. Figure 5 shows that when the RMHS-AO was exposed to air for 72 hour, there is only about 8% weight loss of AO were observed which indicate the protective property by the RMHS to reduce AO volatility.

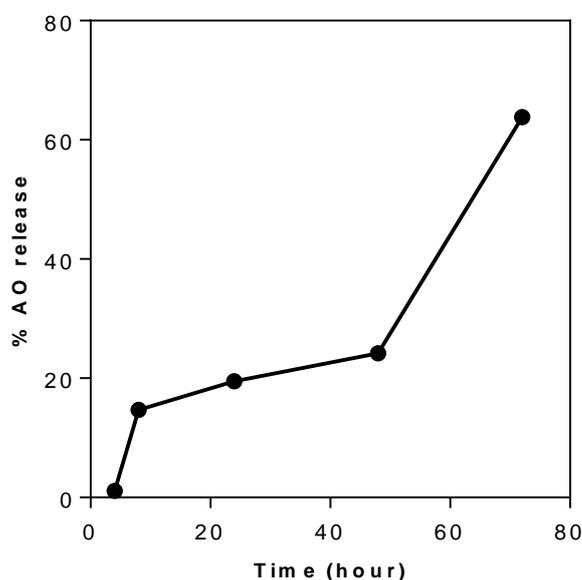


Fig. 4. Release profile of AO from RMHS-AO at pH 5.6

3.3 In Vitro Anti-Cancer Test

After 24 hours of treatment, as shown in Figure 5A, cells treated with RMHS-AO have lesser percentage of viability (~30%) than cells treated with free (>30%) AO at the same dosage of AO. It is noted that at 24 hours, only less than 20% of the total payload is released from RMHS carrier which shows improved efficiency of the nanoformulation. Meanwhile, after 48 hours of treatment, cell viability was further reduced for RMHS-AO compared to free AO as shown in Figure 5B. At the concentration of AO of 1.25, 0.625 and 0.3125 mg/mL, the percentage viability was 28%, 43% and 60% respectively. On the other hand, after treatment using RMHS-AO, the cell percentage viability was 25%, 26% and 27% respectively. At low concentration of RMHS-AO (0.0625 mg/ml) huge difference of 33% compared to AO alone on cell viability was observed, which could be explained by the sustained release of AO from the RMHS container. From the result, it was also observed that RMHS alone are not toxic to the cells.

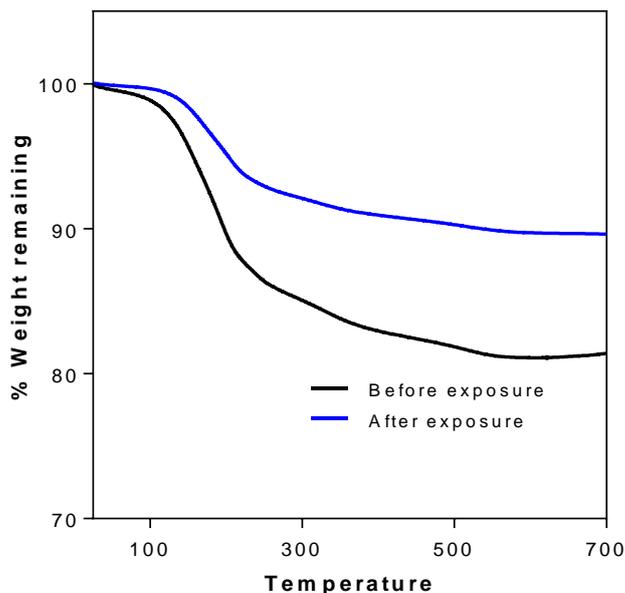


Fig. 5. TGA curve of RMHS-AO before and after 72 hours exposure to air

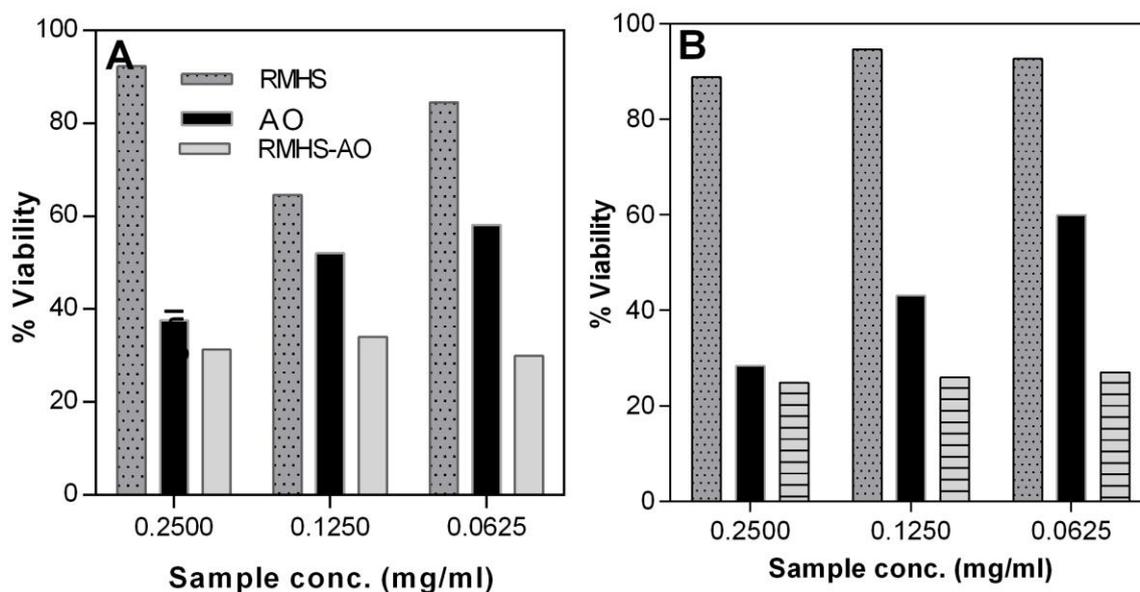


Fig. 6. Cell viability after 24 hours (A) and 48 hours (B). Sample concentration is labeled with respect to the weight of the RMHS-AO

4. Conclusions

Biocompatible mesoporous silica nanoparticles with rough and hollow morphology used in this study were found to improve agarwood oil physiochemical and anticancer properties. Based on this study, optimum loading was successfully achieved with agarwood oil to nanoparticles ratio of 20:1 with 24 hour of loading time. Agarwood oil loaded onto RMHSs show better anti-cancer properties

compared to free AO with 33% difference in killing efficiency at concentration AO of 0.3125 mg/ml at 48 hours. RMHSs have provided sustained release for the loaded agarwood oil with 60% release up to 72 hours. The sustained release was applicable in the anti-cancer test where 73% of the cells were treated at 48 hours of treatment with loaded agarwood oil compared to 70% of cells were killed at 24 hours of treatment with the same sample.

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