RELEASE OF 5-FLUOROURACIL IN ACIDIC MEDIUM BY 2-VINYLPYRIDINE-CAPPED MESOPOROUS SILICA NANOPARTICLES

[1]NUR HIDAYATUL NAZIRAH KAMARUDIN, [2]SHARIFAH NAJIHATIMMIATI, [3]GEE SHIUH CHAU, [4]ROHAYU JUSOH

[1,2]Chemical Engineering Programme, Faculty of Engineering and Built Environment, UniversitiKebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.
[3]Research Centre for Sustainable Process Technology (CESPRO), Faculty of Engineering and Built Environment, UniversitiKebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.
[1]Fuel Cell Institute (SELFUEL), UniversitiKebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.
[4]Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, LebuhrayaTunRazak, 26300 Gambang, Kuantan, Pahang, Malaysia.
E-mail: [1]nhnazirah@ukm.edu.my, [2]najiha@ukm.edu.my, [3]shiuhchau.gee@gmail.com, [4]rohayu@ump.edu.my

Abstract - Ever since the encounter of ordered mesoporous silica materials in early 90's, synthesis and applications of mesoporous solids have received increasing attention due to their high surface area, highly ordered structures, and larger pore sizes. Over the past few decades, mesoporous silica nanoparticles (MSN) have been advancing as pharmaceutical drugs delivery system by adsorption and release test of various anti-cancer drugs. Although MSN possessed great possibilities as drug delivery vehicle, its cell targeting specificity is still a question to be answered. Thus, the purpose of this study is to explore the potential of MSN to be functionalized based on stimuli such as pH by using 2-vinylpyridine (2-VP) as the acidic pH responsive material, as the cancerous cell is more acidic than the normal cell, it will assist the material to release drugs to more specific target. MSN was synthesized by sol gel method and further used to adsorb the 5-fluorouracil (5-FU) as the model drug. The addition of 2-VP was conducted after the adsorption of 5-FU. It was found that in pH 5, the initial release rate of 5-FU from MSN was significant, which was 0.37 mg/min, compared to almost no release of 5-FU in pH 7, which indicated that 2-VP partially exist in protonated form in acidic condition, which promote the breaking of 5-FU bond with MSN, which subsequently formed the release path for 5-FU form MSN channel.

Keywords - Mesoporous silica nanoparticles; Drug delivery; Adsorption; Fluorouracil; 2-vinylpyridine

I. INTRODUCTION

Over the past few decades, mesoporous silica nanoparticle (MSN) has been receiving great attention and is advancing in pharmaceutical drugs delivery for various diseases, especially chemotherapeutic drugs. Its potential was uncovered since the discovery of mesoporous silica materials or called M41S in early 1900s by researchers from Mobil Research and Development Corporation and others[1]. According to recommendation adopted by IUPAC, mesopores are particles with size range within 2nm to 50nm [2]. There is large variety of differently structured mesoporous silica known, ranging from firms, to micrometer-sized particles, rod-like silica structures, and nanometer-sized mesoporous materials which is the M41S and its derivatives while out of so many structures, MSN stands out as MCM-41 due to its unique physical and chemical properties which includes its biocompatibility[3]. MCM-41 is in a uniformly organized hexagonal pore phase and can be synthesized through a counter-ion initiated, self-assembled liquid crystal mechanism involving, which form a liquid equivalent to a liquid crystal-micelle structure. Other M41S discrete members includes the cubic (MCM-48) and the lamellar (MCM-50) forms [4]. MSN possesses large number of mesoporous channels which endow them with unique structural features including uniform and tunable pore size (2 - 15 nm), high surface area (over 700m^2/g), large pore volume (ranges from 0.6 to 1 cm^3/g) and readily functionalized surface [5]. Among the large variety of inorganic structures, MSN has been intensively studied as potential candidate for catalytic, biological, and biomedical system, etc, while the main criteria for its suitability as drug delivery vehicle are having good biocompatibility, high loading capacity, and targeted cell and controlled release rate as described by [6-10].

Due to the acidic nature of cancer cell, the pH at the cancer cells is usually slightly lower than neutral. 2-vinylpyridine is a chemical that tend to protonates in the acidic condition. This property can be seen as an advantage to increase the cell specificity of MSN to release the anticancer drugs towards the more acidic cells, compared to the normal neutral cell. Thus, this research will focus on the applicability of 2-VP that protonates in the acidic condition, to assist the release of 5-Fluorouracil, an anti-cancer drug as the model drug in this study.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Tetraethyl orthosilicate (TEOS), 3-Aminopropyl triethoxysilane (APTES), Ammonium hydroxide and were purchased from Merck, Malaysia.

Cetyltrimethylammonium bromide (CTAB) and Ethylene glycol (EG) was purchased from R&M Chemicals, Malaysia. Ethanol (EtOH) was bought from HmbG Chemicals, Malaysia. 5-Fluorouracil (5-FU) was purchased from NacalaiTesque, Japan. 2-Vinylpyridine (2-VP), was purchased from Sigma Aldrich, USA). (MERCK, Germany). All the chemicals were used as received without further treatment.

2.1. Synthesis of mesoporous silica nanoparticles

In the synthesis of MSN, the procedure is based on our previous study [11]. TEOS was used as the precursor or in other words, the provider of silica component for synthesis process. Before starting the synthesis process of MSN, the surfactant solution was prepared. CTAB was weighted and added into a conical flask together with EG, NH_4OH, and distilled water. The surfactant solution was stirred at 50°C before adding the TEOS and APTES was added drop wise using pipette and micropipette respectively into the surfactant solution.

The solution is ensured to be warm at 50°C before the addition of TEOS and APTES. The solution then heated up to 80°C with continuous stirring for 2 hours. After centrifugation process, the white gel was dried in oven for overnight at 110°C, prior to calcination at 550°C for 5 hours. MSN formed as white solid powder upon the completion of calcination.

2.2. Loading of 5-FU

5-FU solution was prepared and mixed with MSN. The adsorption process was monitored by UV-Vis Spectrophotometer at wavelength 265 nm. After the adsorption process shows plateau, the sample was dried in 110°C and further immersed with 2-VP for 24 hours to cap the surface of MSN-5-FU. The resulted mixture then was dried in oven at 110°C overnight, and denoted as MSN-5-FU-2-VP.

2.3 Material Characterization

The MSN produced was analyzed by using X-ray diffraction (XRD), and Fourier transforms infrared spectrometer (FTIR).

The purpose of XRD analysis is to verify on successful synthesis of MSN through the identification of crystalline structure in the sample, while FTIR analysis was conducted to determine the functional groups existed in the sample. All the analysis results were compared with the reference data obtained.

2.4 Release of drugs

The release of 5-FU from MSN-5-FU-2VPwas tested at pH 5 and pH 7 to compare the performance in acidic and normal cell pH.

The pH of water was adjusted by adding hydrochloric acid. Sample was taken from the mixture for every 5 minutes for the first 30 minutes and every 10 minutes for the next 30 minutes and the desorption of 5-FU was monitoredby UV-Vis Spectrophotometer in order to identify the release rate and percentage of 5-FU from MSN-VP.

III. RESULTS AND DISCUSSION

3.1. Characterization

MSN had been successfully synthesized using the Sol-Gel method and the crystallinity of MSN was characterized by XRD. Figure 1 shows the XRD spectra of MSN.

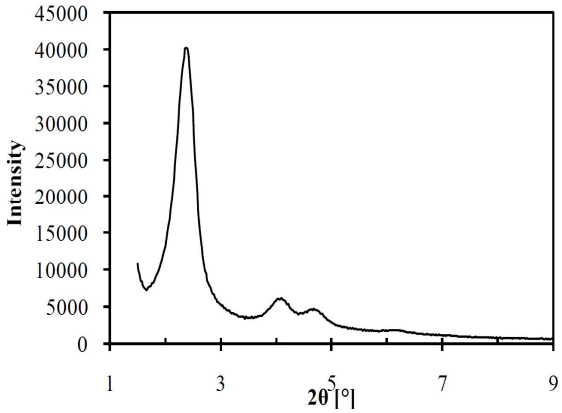


Fig.1.X-Ray diffraction of MSN

XRD analysis was carried out through low angle scanning, which is between 0.5 to 10. Theoretically, 3 peaks associated withhexagonal MSN structure angle was observed at 100°, 110° and 200°. Compared to the study found by He et al. (2013), this peak was identical to typical mesoporous silicas thus the MSN was successfully synthesized[12].

3.2. Adsorption of Drugs

During drugs loading, small amount of solution sample was taken for every stated time interval and analyzed by UV-Vis to identify the adsorption rate. The result showed that the adsorption of 5-FU anticancer drugs was rapid, where the adsorption rate is 0.53mg/min at the beginning 5 minutes.

The adsorption rate sharply dropped after 5 minutes and one of the possible reasons is that the capacity of MSN is approaching maximum. At time 40 minutes, the adsorption rate became constant, indicating the achievement of total capacity.

The total amount of 5-FU anticancer drugs in the solution adsorbed by MSN is approximately 65%, which is 3.25mg. Thus, we can conclude that for every liter of 100ppm 5-FU solution, 65% of 5-FU anticancer drugs existing in the solution is adsorbed by 15mg of MSN. Also, for every 1g of MSN, 0.217g of 5-FU in 1 liter solution is being adsorbed.Fig.2 illustrates the amount of 5-FU adsorbed by MSN against time.

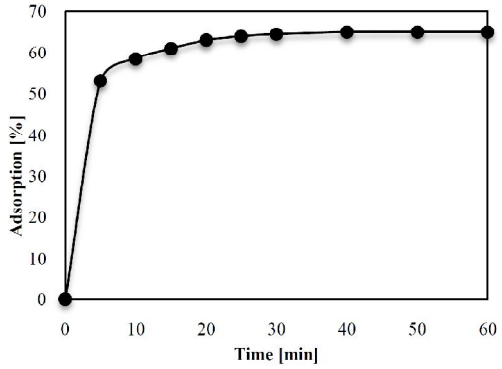


Fig.2. Adsorption of 5-FU onto MSN

To verify the existence of 5-FU in MSN, FTIR analysis was carried to identify the functional groups in the sample. From the FTIR spectra of MSN-5-FU (Figure 3), a combination of wave number peaks of MSN and 5-FU were identified. Most of the peak wave numbers were shifted due to the arrangement of chemical bonding as well as the amount of existed component in the sample, which is indicated by the changes in transmittance. Some identical peak wave numbers of 5-FU are identified, for example at 3131.7cm^{-1} and 3067.0cm^{-1} which are attributed to hetero-aromatic N-H stretch, 1722.0cm^{-1} and 1660.2cm^{-1} attributed to C=O stretch, and 1245.0cm^{-1} attributed to C-F stretch.

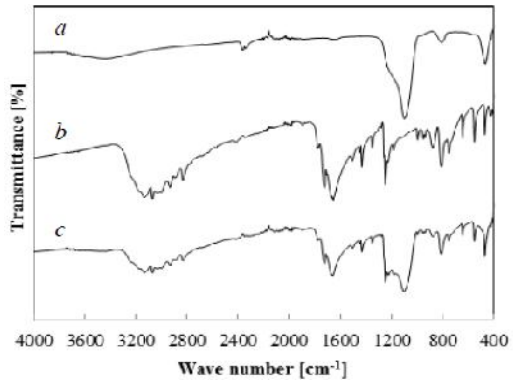


Fig.3. FITR spectra of (a) MSN, (b) 5-FU, (c) MSN-5-FU

At 1101.5cm^{-1}, a peak wave number is identified which is attributed to Si-O stretch of MSN. Some shifted transmittance value within the wave number range from 800cm^{-1} to 1000cm^{-1} are identified. The peak wave numbers within this range are attributed to Si-O stretch, Si-F stretch and Si-H bending vibrations. After drugs loading, 2-VP was added into the mixture to enclose the pores of MSN which contained 5-FU shows the comparison among the FTIR spectra of MSN-5-FU, 2-VP and MSN-5-FU-2-VP.

3.3. Release of Drugs

The release of 5-FU was controlled at pH 7, which is for neutral cell and pH 5, which simulated the acidic condition of cancer cell [13]. Small amount of sample was taken at every stated time interval and was analyzed by UV-Vis to obtain the desorption rate of 5-FU from MSN-5-FU-2-VP. Figure 4shows the percentage of 5-FU release in 2 different pH.

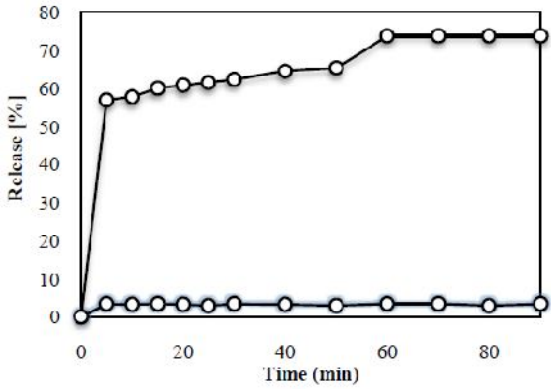


Fig.4.Percentage of 5-FU release from MSN-5-FU-2-VP$_{st}$

The result showed that the release of 5-FU anticancer drugs was rapid in pH 7, where the desorption rate is 0.37mg/min at the first 5 minutes. The desorption rate sharply dropped after 5 minutes and remained almost constant. In neutral pH, very little release of 5-FU was observed due to the entrapment of 5-FU molecules by 2-VP that not protonated in neutral condition. This is most probably caused by the strong interaction between MSN and 5-FUOne of the reasons is that entrapment of 5-FU anticancer drugs in MSN might happen[14].

CONCLUSION

In conclusion, the results of this research showed that 2-VP has the potential to be used as pH-responsive material to functionalize MSN so that the fundamental pre-requisites of good anticancer drugs vehicles can be achieved.2-VP was successfully added onto the 5-FU loaded MSN to enclose the drugs in the pores of MSN. FTIR was done to characterize the existence of 2-VP in MSN-5-FU-2-VP and result showed that 2-VP was successfully mixed onto the MSN matrix in the post-mixing method. On the other hand, co-mixing of 2-VP onto MSN showed negative result and the possible reason of causing this scenario is due to the inappropriateness of solvent used in the mixing process which failed to modify the MSN to provide active site for 2-VP to bind with.

ACKNOWLEDGMENTS

The author were very grateful to the financial support ofGeranGalakanPenyelidik Muda GGPM-2016-027 from UniversitiKebangsaan Malaysia.

REFERENCES

[1] Sazegar, M.R., Mahmoudian, S., Mahmoudi, A., Triwahyono, S., Jalil, A.A., Mukti, R.R., Kamarudin, N.H.N.,

Ghoreishi. M.K. 2015. Catalyzed Claisen–Schmidt reaction by protonated aluminate mesoporous silica nanomaterial focused on the (E)-chalcone synthesis as a biologically active compound. RSC Advances 6 (13): 11023-11031.

[2] Rouquerolt, J., Avnir, D., Fairbridge, C. W., Everett, D. H., Haynes, J. H., Pernicone, N., Ramsay, J. D. F., et al. 1994. Recommendations for the characterization of porous solids. Pure & Appl. Chem. 66(8): 1739–1758.

[3] A H Karim, A AJalil, S Triwahyono, S M Sidik, N H N Kamarudin, R Jusoh, N W C Jusoh, B H Hameed. Amino modified mesostructured silica nanoparticles for efficient adsorption of methylene blue. Journal of Colloid and Interface Science 386(1) (2012) 307-14.

[4] Kresge, C. T., Vartuli, J. C., Roth, W. J. &Leonowicz, M. E. 2004. Mesoporous Crystals and Related Nano-Structured Materials, Proceedings of the Meeting on Mesoporous Crystals and Related Nano-Structured Materials. Studies in Surface Science and Catalysis 148: 53–72. doi:10.1016/S0167-2991(04)80193-9

[5] Rosenholm, J. M., Sahlgren, C. & Lindén, M. 2010. Towards multifunctional, targeted drug delivery systems using mesoporous silica nanoparticles – opportunities & challenges. Nanoscale 2(10): 1870-1883.

[6] 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.

[7] N H N Kamarudin, AAJalil, S Triwahyono, N F M Salleh, A H Karim, R RMukti, B H Hameed, A Ahmad. Role of 3-aminopropyltriethoxysilane in the preparation of mesoporous silica nanoparticles for ibuprofen delivery: Effect on physicochemical properties, Microporous and Mesoporous Materials. 180 (2013) 235-241

[8] N H N Kamarudin, AAJalil, S Triwahyono, V Artika, N F M Salleh, A H Karim, N F Jaafar, M R Sazegar, R RMukti, B H Hameed, A Johari. Variation of the crystal growth of mesoporous silica nanoparticles and the evaluation to ibuprofen loading and release. Journal of Colloid and Interface Science, 421 (2014) 6-13.

[9] N H N Kamarudin, AAJalil, S Triwahyono, M R Sazegar, S Baba, S Hamdan, A Ahmad. Elucidation of acid strength effect on ibuprofen adsorption and release by aluminated mesoporous silica nanoparticles. RSC Advances, 5 (2015) 30023-30031.

[10] Kamarudin, N.H.N., Jalil, A.A., Triwahyono, S.T., Timmiati, S.N. (2016). Microwave-assisted Synthesis of Mesoporous Silica Nanoparticles as A Drug Delivery Vehicle. Malaysian Journal of Analytical Science 20 (6): 1382-1389.

[11] A H Karim, A AJalil, S Triwahyono, N H N Kamarudin, A Ripin. Influence of multi-walled carbon nanotubes on textural and adsorption characteristics of in situ synthesized mesostructured silica. Journal of Colloid and Interface Science. 421 (2014) 93–102.

[12] R Jusoh, AAJalil, S Triwahyono, N H N Kamarudin. Synthesis of dual type Fe species supported mesostructured silica nanoparticles: Synergistical effects in photocatalytic activity RSC Advances, 5 (13) (2015) 9727-9736.

[13] Boyer, M. J., Barnard, M., Hedley, D. W. & Tannock, I. F. 1993. Regulation of intracellular pH in subpopulations of cells derived from spheroids and solid tumours 890–897.

[14] Olukman, M., Oya, Ş. & Solak, E. K. 2012. Release of Anticancer Drug 5-Fluorouracil from Different Ionically Crosslinked Alginate Beads 2012(October): 469–479.

★★★