pH-Sensitive Biogenic Silica-chitosan modified for Targeted Folic Acid delivery

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ABSTRACT

The drug loading onto silica derived from organic compounds satisfies the need for effective biocompatible carriers for sustained and targeted delivery. Silica is a naturally occurring material that is biocompatible and biodegradable. However, studies reveal silica nanoparticles' drug loading and release capabilities are uncontrolled and erratic. In this paper, biogenic silica (BS) was modified with chitosan to improve the loading and release capabilities in vitro. The BS was prepared by calcining for 4 hours at 600 ℃, and the modification was accomplished by immersing the BS in a chitosan solution overnight. The modified BS (BS-C) was characterised using Fourier transform infrared spectroscopy (FTIR) and X-ray diffractometry (XRD). Folic acid loading and release studies were performed for the BS alone and the BS-C using UV-Vis spectrophotometry analysis at a wavelength of 285 nm. The folic acid loading was done at a pH of 9, and release studies were done at a pH of 7.24 and 10.40. Results from the comparative analysis of the BS and the BS-C showed improvements in drug adsorption efficiency of 29.79 and 73.96%, respectively, in a 2-hour period. Thus, the findings show the potential application of folic acid delivery in the small intestine.

Keywords: Drug delivery, Folic acid, Biogenic silica

1.0 INTRODUCTION

Silica is a material that has become of keen interest in the area of nanomaterials. They have provided a different avenue for biomedical applications in areas such as bio-sensing, cellular uptake, and drug delivery (Devi *et al.,* 2016). Following advancements in research, the use of silica particles as drug delivery systems for sustained drug release is quickly becoming a sought-after application for scientists and engineers due to significant research focused on the design and implementation of biomaterials, which provide a sustained release of therapeutics and

can be modified for the bioavailability of drugs at specific locations in the body (Fenton et al., 2018). The sustained drug release reduces the high consumption of drugs and reduces toxicity in patients (Žid et al., 2020), which has inspired material scientists and engineers to study materials that have high stability and flexibility for the administration of drugs through various pathways (Menon & Pillai, 2022). One of the many materials that have been studied for use as a drug delivery agent is silica particles (Isa et al., 2021; Trzeciak et al., 2021). These particles can be acquired by organic or inorganic means. The use of chemicals such as tetraethyl orthosilicate (TEOS) and cetyl trimethyl ammonium bromide (CTAB), (Song *et al.*, 2019) constitutes the inorganic means, whereas the contrary makes use of natural materials composed of biogenic silica (BS) (de Cordoba et al., 2019).

Rice husk has been found to have the highest amount of silica among all plant-based resources (Chun et al., 2020), with a study reporting that about 95% by weight of amorphous silica can be obtained from rice husk after acid leaching treatment and calcination at 600° (Dorairaj et al., 2022; Suyanta & Kuncaka, 2011). Its use in obtaining BS is rapidly catching the attention of material scientists due to the low cost of production and eco-friendliness of these materials (Prabha et al., 2021). Approximately 148.2 million tonnes of rice husk are produced worldwide each year (Park et al., 2021), which speaks to their availability for utilisation. This allowed the use of rice husks in the production of magnetic Mobil Composition of Matter 41 (magMCM-41) for use as adsorbents and sensor bases (Kamari & Ghorbani, 2021). However, their use in designing drug delivery systems reveals a major challenge: insufficient drug delivery profiles. This was evident when BS was used for targeted release studies at pH above 7 (Salazar Hernández et al., 2014). Subsequent research observed an issue of uncontrolled drug delivery with BS, using Folic acid (FA) as the model drug (Carmen et al., 2017). Folic acid (FA) is an essential vitamin used in the regulation of homocysteine, to mediate the formation of cardiovascular diseases when metabolised (Ganguly & Alam, 2015; Ratajczak et al., 2021). A targeted delivery of FA prevents having an excess amount in the bloodstream and any possible development of cancer (Khan & Jialal, 2022; Tam et al., 2012).

Thus, we propose the use of chitosan for surface modification of BS for controlled folic acid studies at a selected pH of 7 and 10, since FA is mostly absorbed in an alkaline medium in the small intestine. Chitosan, a natural polymer with minimal toxicity, biodegradability, and biocompatibility (Ibrahim et al., 2015), has the presence of hydroxyl and amine functional groups that can interact with organic and inorganic compounds (Lizardi-Mendoza et al., 2016; Zhang et al., 2010). The modified material was characterized using X-ray diffraction spectroscopy and Fourier infrared spectroscopy, while drug loading and release studies were done using an ultraviolet (UV) visible spectrophotometer

2.0 MATERIALS

The rice husk used in this study was obtained from rice fields in the northern region of Ghana. Hydrochloric acid (HCl), nitric acid (HNO_3), and acetic acid (AcOH) were purchased from Sigma Aldrich. Industrial chitosan as well as pharmaceutical folic acid from Sundown® were used. Phosphate buffer saline was also used in the dissolution of the pharmaceutical folic acid.

3.0 METHODS

3.1 Extraction of SiO2 from Rice

A mass of 125g of rice husk was measured and washed with water to remove all organic impurities. Concentrations of 2 M of both hydrochloric acid (HCl) and nitric acid (HNO_3) in volumes of 700 and 150 mL, respectively, were mixed in 150 mL of distilled water to form a solvent. The rice husk was added to the solvent, stirred, and placed in a water bath at 75 °C for 1 h, after which it was dried at room temperature for 48 h. The dried rice husk was then calcined in a muffle furnace at 600 °C for 4 h.

3.2 Surface Modification of Biogenic Silica

A mass of 0.15 g of chitosan was completely dissolved in 25 mL of 10% acetic acid to form a homogenous solution. 1g of biogenic silica (BS) was added to this solution in a beaker and stirred with a magnetic stirrer for 24 h. The solution was centrifuged and dried for 72 h to form a biogenic silica-chitosan composite (BS-C).

3.3 Preparation of Folic Acid (FA)

A mass of 50 mg of pharmaceutical folic acid was dissolved in 100 mL of 1% phosphate buffered saline (PBS) with a pH of 9.131 in a beaker. The solution was stirred for 20 min and filtered to obtain an aliquot with a concentration of 0.5 mg/mL.

3.4 Drug Loading and Release

Folic acid with a concentration of 0.5 mg/mL was added to 0.5 mg of both the BS and the BS-C in separate Eppendorf tubes. A 360° mechanical rotator with a speed of 10 rpm was used to aid in the drug loading process in triplicate for 2 h at intervals of 30 min. The solution was then centrifuged for 5 min at a speed of 1000 rpm. The supernatants from the preparation were pipetted, and absorbance was read using a UV-Vis spectrophotometer at a wavelength of 285 nm. The materials were then dried. Drug release was carried out for both BS and BS-C in PBS at a pH of 7.24 and 10.10. The drug release was studied in triplicate for 2 hours with time intervals of 30 minutes with a 360 mechanical rotator at a speed of 10 rpm. The absorbance was recorded at each time interval using UV-Vis spectrophotometer at a wavelength of 285 nm after centrifugation.

3.5 Characterization Techniques

The BS and BS-C were characterised with an X-Ray Diffractometer (XRD) equipped with Cu-Kα radiation of wavelength 1.54Å. Also, a Fourier transform infrared spectrometer (FTIR) was used to determine functional groups over a wavelength of 400 to 4000 cm^{1}.

4.0 RESULTS AND DISCUSSION

4.1 XRD of BS, chitosan and BS-C

Figure 1 compares the X-ray diffractometry (XRD) patterns of biogenic silica (BS), chitosan, and BS modified with chitosan (BS-C). The BS is seen to have a major peak at the 50 2θ/degrees position, whereas the chitosan shows more peaks at 8.5, 20, 28.9, 38.1, 42.8, 44.4, 47.1, 48.3, 64.8, and 78 2θ/degrees positions (Ghorbani et al., 2015). The profile in Fig. 1 reveals that BS is amorphous while chitosan is semicrystalline. However, when the BS is modified with chitosan, the BS-C forms an amorphous material. The amorphous nature of the BS-C is due to the silica and chitosan polymeric chains being completely mixed at a molecular level (Rajiv Gandhi & Meenakshi, 2012) and masking the minor crystalline peaks in chemical reactions that result in an amorphous structure (Kamari & Ghorbani, 2021).

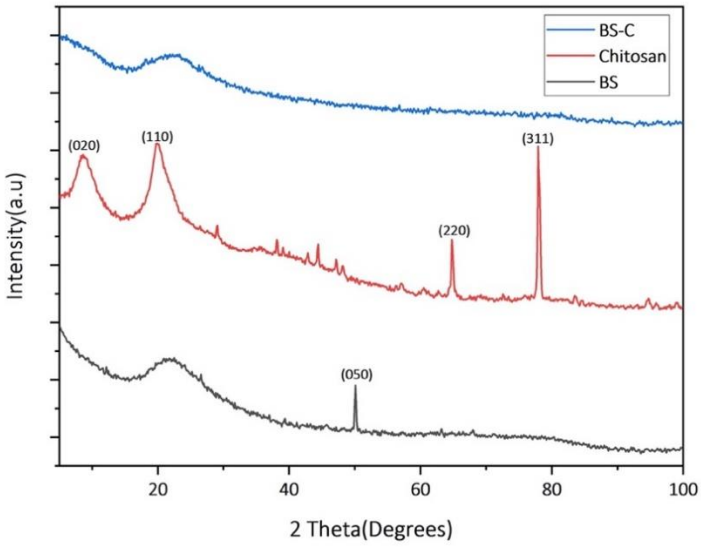

Figure 1: XRD patterns of BS, Chitosan and BS modified with chitosan (BS-C).

4.2 FTIR of BS, chitosan and BS-C Composite

Figure 2 represents the FTIR spectra of BS, chitosan, and BS-C. The broad band between 3610 and 3000 cm^{-1} in BS, chitosan, and BS-C is assigned to the stretching vibration of surface hydroxyl. The stretching vibration of Si-OH is seen in the band between 3610 and 3000 cm^{-1} in the BS. The bands at 1042, 788, and 439 cm^{-1} in both the BS and the BS-C are assigned to the Si-O group and Si-O-Si and

and Si-O-Si, respectively (Yuan et al., 2006). The bending, symmetric, and asymmetric stretching of the Si-O-Si and Si-O at 1042, 788, and 439 cm^{-1} are consistent with the fingerprint region seen for silica particles in literature (Chen et al., 2014). Chitosan reveals a vibrating NH band, which is spotted at 1640 cm^{-1}. Other vibrating C-H groups in the chitosan can be found at 952 and 800 cm^{-1}. From the BS-C, new bands occurred at 1640, 1557, 1408, and 952 cm^{-1}. The new bands are attributed to the N-H bending vibration of the amine group and the C-H stretching bond of the CH_2 and CH_3 groups, respectively, which are characteristic functional groups present in the chitosan structure.

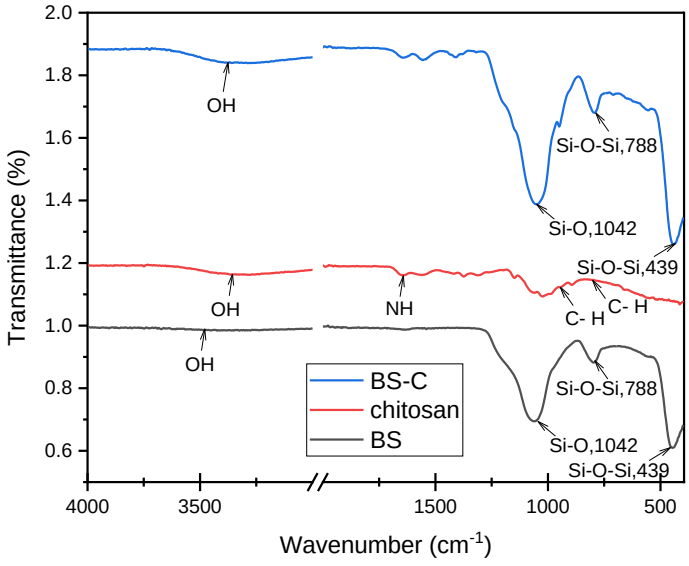

Figure 2: FTIR of BS, chitosan and BS modified with chitosan (BS-C).

4.3 FA Loading of BS And BS-C

FA loading studies were conducted on BS and BS-C at room temperature. The drug adsorption efficiency (DAE), which quantifies the amount of drug loaded onto the material, of BS and BS-C was then calculated using the concentration after 2 hours obtained from the UV-VIS spectrophotometer:

$$DAE = \frac{(C_i - C_f)}{C_i} \times 100\% \quad (1)$$

C_i is the initial concentration and C_f is the final concentration.

The DAE of BS after 2 h was calculated as 29.79%, while that of the BS-C composite was 73.96%, which confirms that the BS-C has a higher drug entrapment efficiency when compared to BS. Figure 3 compares the absorbance readings of the BS and BS-C. It shows irregular drug adsorption by the BS, confirming the inefficient and uncontrolled drug loading characteristic of the BS (Salazar Hernández et al., 2014). On the contrary, there was a steady decrease in drug adsorption by the BS-C. This indicates efficient drug loading onto the material, which could be attributed to the chemical interactions taking place between the FA and the composite.

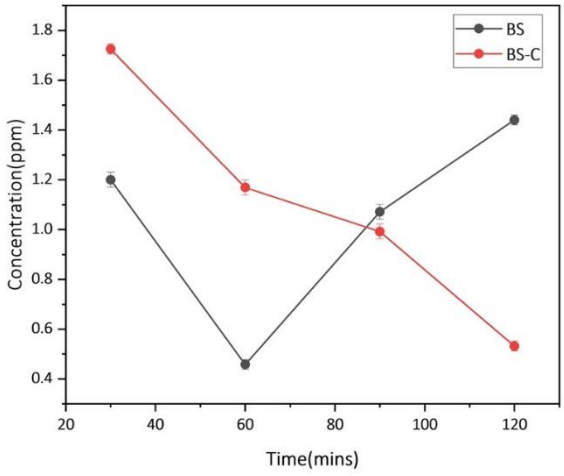

Figure 3. Drug loading of biogenic silica (BS) and BS modified with chitosan (BS-C).

4.4 FA Release and Kinetic model of BS and BS-C

FA release studies were carried out for both the BS and the BS-C composite to analyse their release behaviour at different pH (7 and 10). At a pH of 7.24, FA was released into solution by the BS in the first 30 minutes, and progressively adsorbed again after 90 minutes (at 30 minutes interval), which confirms the poor release character of BS. This could be due to the high surface energy of silica (Bauer et al., 2021; Seied Reza Saeid Jalali et al., 2018), causing minimal release from the BS surface. On the contrary, there was a sustained release of the FA by BS-C composite as seen in *Figure 4.* This is partially due to the character of chitosan reducing the surface energy of BS and the interactions between the FA and chitosan rather than the BS. At a pH of 10.1, FA continued to be released from the BS-C with increased affinity of FA to the OH ions in solution as well as the character of chitosan. In the BS sample, it was suspected that FA released into solution with time as a result of FA affinity for OH ions and solubility of FA at high pH causing it to release from the BS sample.

Based on dependence of the substance amount released with time *Figure 4*, the data was fitted to the Korsemeyer-Peppas kinetic model. Korsmeyer-Peppas equation, also called power law, is a semi-empirical model based on the diffusion phenom that is used to describe in a general way the main transport phenomena involved in release procedures, which occurs by either diffusion or swelling (Salah Eldeen et al., 2019). Although a good fit has been achieved with this model, several authors have shown that Korsmeyer-Peppas is applicable only to the first 60% of the release profile (Heredia et al., 2022; Korsmeyer et al., 1983). The diversity of this process can be summarised by the equation :

$$F = K_m \qquad (2)$$

t^n where K is kinetic constant, t is time, n is diffusion exponent. The diffusion exponent(n) of the Korsmeyer–Peppas model preparation that releases the FA as a result of both diffusion and erosion is 0.384, with an R^2 value of 0.9812. Reference values

value of 0.9812. Reference values for non-fickian transport are between 0.5< n <= 0.89 (Ballantine et al., 1997). It has been clearly shown in *Figure 5* that the process is consistent with this model and was observed only in a period of time between 30 and 120 min.

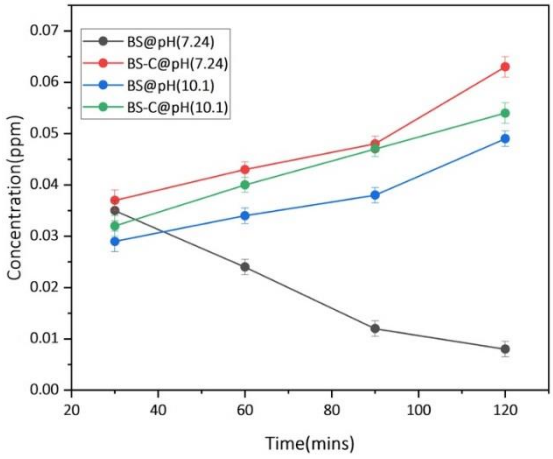

Figure 4. Drug release of biogenic silica (BS) and BS modified with chitosan (BS-C) at pH of 7.24 and 10.10.

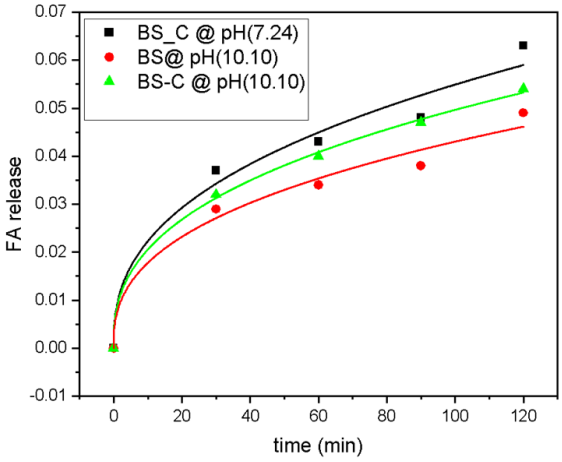

Figure 5. Kosmeyer-Pepper model of biogenic silica (BS) and BS modified with chitosan (BS-C) at pH of 7.24 and 10.10.

5.0 CONCLUSION

The potential of biogenic silica (BS) modified with chitosan (C) (BS-C) was evaluated for the adsorption and release of folic acid (FA) over a period of 120 min. The BS-C displayed better drug loading and release capability for FA as compared to the BS. The release of FA from the BS-C was influenced by the pH of the medium. The pH range was chosen to mimic the pH in the small intestine, where folic acid is absorbed. The release profile of the BS-C agrees with the Kosmeyer-Pepper model. The mechanism of reaction between BS-C and FA, as well as BS and FA, was mainly governed by the adsorption of the functional groups. The study shows the potency of biogenic silica from rice husk modified with chitosan as an effective system for the delivery of FA.

REFERENCES

Ballantine, D. S., Martin, S. J., Ricco, A. J., Frye, G. C., Wohltjen, H., White, R. M., & Zellers, E. T. (1997). Materials Characterization. In D. S. Ballantine, S. J. Martin, A. J. Ricco, G. C. Frye, H. Wohltjen, R. M. White, & E. T. Zellers (Eds.), *Acoustic Wave Sensors* (pp. 150–221). Academic Press. https://doi.org/10.1016/B978-012077460-9/50004-6

Bauer, F., Meyer, R., Bertmer, M., Naumov, S., Al-Naji, M., Wissel, J., Steinhart, M., & Enke, D. (2021). Silanization of siliceous materials, part 3: Modification of surface energy and acid-base properties of silica nanoparticles determined by inverse gas chromatography (IGC). Colloids and Surfaces A: Physicochemical and Engineering Aspects, 618, 126472. https://doi.org/10.1016/J.COLSURFA.2021.126472

Carmen, S.-H., Mercedes, S.-H., Rocío, L.-R., Enrique, E.-R., Agustín Hilario, R.-R., Carmen, S.-H., Mercedes, S.-H., Rocío, L.-R., Enrique, E.-R., & Agustín Hilario, R.-R. (2017). Silica from Rice as New Drug Delivery Systems. Rice - Technology and Production. https://doi.org/10.5772/66723

Chen, X., Jiang, J., Yan, F., Tian, S., & Li, K. (2014). A novel low temperature vapor phase hydrolysis method for the production of nano-structured silica materials using silicon tetrachloride. RSC Advances, 4(17), 8703–8710. https://doi.org/10.1039/C3RA47018K

Chun, J., Mo Gu, Y., Hwang, J., Oh, K. K., & Lee, J. H. (2020). Synthesis of ordered mesoporous silica with various pore structures using high-purity silica extracted from rice husk. Journal of Industrial and Engineering Chemistry, 81, 135–143. https://doi.org/10.1016/J.JIEC.2019.08.064

de Cordoba, M. C. F., Matos, J., Montaña, R., Poon, P. S., Lanfredi, S., Praxedes, F. R., Hernández-Garrido, J. C., Calvino, J. J., Rodríguez-Aguado, E., Rodríguez-Castellón, E., & Ania, C. O. (2019). Sunlight photoactivity of rice husks-derived biogenic silica. Catalysis Today, 328, 125–135. https://doi.org/10.1016/J.CATTOD.2018.12.008

Dorairaj, D., Govender, N., Zakaria, S., & Wickneswari, R. (2022). Green synthesis and characterization of UKMRC-8 rice husk-derived mesoporous silica nanoparticle for agricultural application. Scientific Reports 2022 12:1, 12(1), 1–11. https://doi.org/10.1038/s41598-022-24484-z

Fenton, O. S., Olafson, K. N., Pillai, P. S., Mitchell, M. J., & Langer, R. (2018). Advances in Biomaterials for Drug Delivery. Advanced Materials, 30(29), 1705328. https://doi.org/10.1002/ADMA.201705328

Ganguly, P., & Alam, S. F. (2015). Role of homocysteine in the development of cardiovascular disease. Nutrition Journal, 14(1). https://doi.org/10.1186/1475-2891-14-6

Ghorbani, F., Sanati, A. M., & Maleki, M. (2015). Production of Silica Nanoparticles from Rice Husk as Agricultural Waste by Environmental Friendly Technique. 2(1), 56–65.

Heredia, N. S., Vizuete, K., Flores-Calero, M., Katherine Pazmiño, V., Pilaquinga, F., Kumar, B., & Debut, A. (2022). Comparative statistical analysis of the release kinetics models for nanoprecipitated drug delivery systems based on poly(lactic-co-glycolic acid). PLoS ONE, 17(3 March). https://doi.org/10.1371/journal.pone.0264825

Ibrahim, H. M., Zairy, E. M. R. E.-, Ibrahim, H. M., & Zairy, E. M. R. E.-. (2015). Chitosan as a Biomaterial — Structure, Properties, and Electrospun Nanofibers. Concepts, Compounds and the Alternatives of Antibacterials. https://doi.org/10.5772/61300

Isa, E. D. M., Ahmad, H., Rahman, M. B. A., & Gill, M. R. (2021). Progress in Mesoporous Silica Nanoparticles as Drug Delivery Agents for Cancer Treatment. Pharmaceutics 2021, Vol. 13, Page 152, 13(2), 152. https://doi.org/10.3390/PHARMACEUTICS13020152

Kamari, S., & Ghorbani, F. (2021). Extraction of highly pure silica from rice husk as an agricultural by-product and its application in the production of magnetic mesoporous silica MCM–41. Biomass Conversion and Biorefinery, 11(6), 3001–3009. https://doi.org/10.1007/S13399-020-00637-W/METRICS

Khan, K. M., & Jialal, I. (2022). Folic Acid Deficiency. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK535377/

Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., & Peppas, N. A. (1983). Mechanisms of solute release from porous hydrophilic polymers. In international Journal of Pharmaceutics (Vol. 15).

Lizardi-Mendoza, J., Argüelles Monal, W. M., & Goycoolea Valencia, F. M. (2016). Chemical Characteristics and Functional Properties of Chitosan. Chitosan in the Preservation of Agricultural Commodities, 3–31. https://doi.org/10.1016/B978-0-12-802735-6.00001-X

Menon, A. A., & Pillai, R. G. (2022). Source optimization, characterization, assessing biocompatibility and drug loading efficiency of biogenic silica particles from agro wastes. Chemical Biology Letters, 9(1), 311–311. https://www.pubs.thesciencein.org/journal/index.php/cbl/article/view/311

Park, S. J., Son, S. H., Kook, J. W., Ra, H. W., Yoon, S. J., Mun, T. Y., Moon, J. H., Yoon, S. M., Kim, J. H., Kim, Y. K., Lee, J. G., Lee, D. Y., & Seo, M. W. (2021). Gasification operational characteristics of 20-tons-Per-Day rice husk fluidized-bed reactor. Renewable Energy, 169, 788–798. https://doi.org/10.1016/J.RENENE.2021.01.045

Prabha, S., Durgalakshmi, D., Rajendran, S., & Lichtfouse, E. (2021). Plant-derived silica nanoparticles and composites for biosensors, bioimaging, drug delivery and supercapacitors: a review. In Environmental Chemistry Letters (Vol. 19, Issue 2, pp. 1667–1691). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s10311-020-01123-5

Rajiv Gandhi, M., & Meenakshi, S. (2012). Preparation and characterization of silica gel/chitosan composite for the removal of Cu(II) and Pb(II). International Journal of Biological Macromolecules, 50(3), 650–657. https://doi.org/10.1016/J.IJBIOMAC.2012.01.012

Ratajczak, A. E., Szymczak-Tomczak, A., Rychter, A. M., Zawada, A., Dobrowolska, A., & Krela-Kaźmierczak, I. (2021). Does Folic Acid Protect Patients with Inflammatory Bowel Disease from Complications? Nutrients 2021, Vol. 13, Page 4036, 13(11), 4036. https://doi.org/10.3390/NU13114036

Salah Eldeen, T., Ahmed, L., Atif, R., Yahya, I., Omara, A., & Eltayeb, M. (2019). Study the Using of Nanoparticles as Drug Delivery System Based on Mathematical Models for Controlled Release. In International Journal of Latest Technology in Engineering: Vol. VIII. www.ijltemas.in

Salazar Hernández, M., Salazar Hernández, C., Gutiérrez Fuentes, A., Elorza, E., Carrera-Rodríguez, M., & Puy Alquiza, M. J. (2014). Silica from rice husks employed as drug delivery for folic acid. Journal of Sol-Gel Science and Technology, 71(3), 514–521. https://doi.org/10.1007/S10971-014-3378-5/METRICS

Seied Reza Saeid Jalali, Sobat, S., & Farzi, G. (2018). Surface modification of silica nanoparticle using dichlorodimethylsilane for preparation of self-cleaning coating based on polyurethane and polydimethylsiloxane. Materials Research Express, 5(9), 095311. https://doi.org/10.1088/2053-1591/AAD607

Suyanta, S., & Kuncaka, A. (2011). UTILIZATION OF RICE HUSK AS RAW MATERIAL IN SYNTHESIS OF MESOPOROUS SILICATES MCM-41. Indonesian Journal of Chemistry, 11(3), 279–284. https://doi.org/10.22146/IJC.21393

Tam, C., O'Connor, D., & Koren, G. (2012). Circulating Unmetabolized Folic Acid: Relationship to Folate Status and Effect of Supplementation. Obstetrics and Gynecology International, 2012, 1–17. https://doi.org/10.1155/2012/485179

Trzeciak, K., Chotera-ouda, A., Bak-sypien, I. I., & Potrzebowski, M. J. (2021). Mesoporous Silica Particles as Drug Delivery Systems—The State of the Art in Loading Methods and the Recent Progress in Analytical Techniques for Monitoring These Processes. Pharmaceutics 2021, Vol. 13, Page 950, 13(7), 950. https://doi.org/10.3390/PHARMACEUTICS13070950

Yuan, W. Z., Peng, M., Yu, Q. M., Tang, B. Z., & Zheng, Q. (2006). Synthesis and Characterization of Polystyrene/Nanosilica Organic-Inorganic Hybrid. Chemical Research in Chinese Universities, 22(6), 797–802. https://doi.org/10.1016/S1005-9040(06)60215-8

Zhang, J., Xia, W., Liu, P., Cheng, Q., Tahirou, T., Gu, W., & Li, B. (2010). Chitosan Modification and Pharmaceutical/Biomedical Applications. Marine Drugs, 8(7), 1962. https://doi.org/10.3390/MD8071962

Žid, L., Zeleňák, V., Almáši, M., Zeleňáková, A., Szücsová, J., Bednarčík, J., Šuleková, M., Hudák, A., & Váhovská, L. (2020). Mesoporous Silica as a Drug Delivery System for Naproxen: Influence of Surface Functionalization. Molecules 2020, Vol. 25, Page 4722, 25(20), 4722. https://doi.org/10.3390/MOLECULES25204722