

A MICROBIAL PROTEIN-ASSISTED SILICA BALL COMPRISING OF SILICA-NANOPARTICLES WITH PLAUSIBLE OPTICAL PROPERTIES FOR MULTIPLE APPLICATIONS

Research

AUTHOR: Brajadulal Chattopadhyay

August 2017

Shilpi Show¹, Krishna Chattopadhyay², Chetana Ghosal¹, Brajadulal Chattopadhyay^{1*}

¹Department of Physics, Jadavpur University, Kolkata 700032, India

²Department of Chemistry, Jadavpur University, Kolkata 700032, India

CORRESPONDENCE AUTHOR

Brajadulal Chattopadhyay
Department of Physics, Jadavpur University
Raja S.C. Mallick Road, Kolkata-700032
India
Email: bdc_physics@yahoo.co.in
Phone: +91-9433343917
Fax: +91-33-24138917

CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

Received Date: 30th June 2017

Accepted Date: 09th July 2017

Published Date: 08th Aug 2017

Copy rights: © This is an Open access article distributed under the terms of Creative Commons Attribution 4.0 International License.

ABSTRACT

Background: Production of mesoporous silica nanoparticles and its conceivable applications in the fields of chromatography, surface polishing, catalysis and drug delivery etc. has gained momentum recently. We demonstrate here an efficient methodology for the amicable synthesis of silica balls consisting of mesoporous silica nanoparticles (SiO₂-NPs) by using a secretory protein (bioremediase). The protein was isolated from a thermophilic non-pathogenic bacterium BKH1 (GenBank Accession No. FJ177512).

Methods: Silica ball was formed at ambient temperature by mixing the dissolved bacterial protein dropwise to an organic precursor tetra-ethyl-orthosilicate (TEOS) solution at neutral pH environment. Surface morphology and compositional studies of prepared silica ball were carried out by using High-Resolution Transmission Electron Microscope (HRTEM) and Field Emission Scanning Electron Microscope equipped with Energy Dispersive X-ray Analyzer (FESEM-EDX). Fourier Transform Infrared Spectroscopy (FTIR) and X-ray diffraction (XRD) studies were further carried out for auxiliary characterization to determine the nature of SiO₂-NPs. Stability of the as prepared SiO₂-NPs was determined by noting the Zeta potential (ζ). The dye degradation activity of the silica balls was noted against different dyes.

Results: Silica ball thus formed consisted of silica nanoparticles whose average dimensions were 20 ± 10 nm ($n = 100$). The size of the silica ball and also the sizes of the constituents' nanoparticles depend on the protein concentration in the reaction mixture. The result of zeta potential implied the moderate stability of SiO₂-NPs at neutral pH environment. The SiO₂-NPs showed green fluorescence emission which might have feasibly applications in the field of biomedical imaging. The decolourizing effect of silica ball on various dyes is a cost effective phenomenon as it can be used repeatedly.

Conclusion: The protein-assisted silica balls preparation has a special consequence as it is an environmentally benign, lucrative and one pot synthesis approach which could be used repeatedly for various biomedical and chromatographic packing purposes.

KEYWORDS: Bioremediase protein, Nanoparticles, Silica ball, Tetraethyl orthosilicate,

INTRODUCTION

There is an immense compact of interest in the field of nanoscale particles like mesoporous silica and its conceivable applications in the different fields like chromatographic, surface polishing, catalysis, medical implants, and drug delivery etc. [1-3]. Silica nanoparticles (SiO_2 -NPs) have fascinated considerable attention from researchers due to their enormous potential applications. First, the band gap of α - SiO_2 is very large which means that vacuum ultraviolet excitation is needed for optical experiments. Second, the fact that the material is amorphous allows for a wide variety, and greater number, of atomic-scale defects than would be accommodated by the ordered structure of a crystal. As a result, many of the optical and electronic properties of α - SiO_2 are dominated by these defects which have, therefore, been the focus of much of the research on this material. The defects may be extremely important, by the way, in both electronic and optical applications of α - SiO_2 . Defects in thermal oxides on Si devices, especially if charged, can influence carrier mobility near the surface as well as the threshold of field-effect transistors. Defects in optical fibres may limit the ultimate optical attenuation either by absorption or scattering.

Properties like biocompatibility, low toxicity, and scalable accessibility have fuelled studies that extent its reasonable applications from cell differentiation to drug or Si-RNA delivery systems and imagenology [4-7]. However, conventional methods for the production of SiO_2 -NPs follow a self assembly mechanism where physical, chemical as well as structural properties of the nanoparticle (NPs) are controlled by both reactant ratios and investigational conditions [8-10]. Anderson et al. (1998) and others synthesized NPs using both charged and neutral templates and showed that the addition of co-solvent produced more spherical particles [11, 12].

Present efforts are focused on the search for new biological synthetic routes for SiO_2 -NPs formation involving lower costs and a less ecological impact while also maintaining both reproducibility and biocompatibility. This work recommends a single step, rapid, convenient green technique for the formation of amorphous, along with thermally and chemically stable silica balls consisting of SiO_2 -NPs with controlled size using a microbial secretary bacterial protein (bioremediase), which can be used in cost effective manner in various purposes. Moreover, we have explored the characteristics of the final product with a view of the possible nanobiotechnological and optoelectronics applications, wherein properties such as size, dispersion, surface charge of the SiO_2 -NPs are essential.

MATERIALS AND METHOD

The bacterial strain BKH1 (Gen Bank accession number FJ177512) was obtained from the Biophysics Laboratory, Department of Physics, Jadavpur University [13]. The analytical grade TEOS was purchased from the Merck, USA. All other fine chemicals were purchased from Spectro Chem. Pvt. Ltd. India.

Isolation and purification of the bioremediase Protein

Bioremediase protein (UniProt Knowledgebase Accession Number P86277) is secreted by the bacterium BKH1 in the growth medium while growing in the medium. The protein was purified from 6 to 7 days old bacterial culture medium similarly as described by Biswas et al. [13]. Double step purification through Sephadex G-100 column chromatographic technique was employed to purify the enzyme. Biosilicification assay was done to ensure the silica leaching activity of the purified protein as described earlier [13].

Bio-production of SiO_2 -NPs

For the biosynthesis of silica ball consisting of SiO_2 -NPs, 100 μg purified bioremediase bacterial protein (1 $\mu\text{g}/10 \mu\text{l}$ deionized water) was added drop wise in a 1 ml organic silica rich substrate (TEOS, 0.1 mol/L) solution in a 5 ml plastic vial and kept at ambient temperature for 24 h at pH neutral condition. Same experiment was performed by taking different concentrations of the protein (25, 50 and 150 μg respectively) separately. After adding the protein, spherical ball like structure was formed within the reaction mixture. The bio-transformed reaction commodities were collected using a long for-shape. Afterwards, the product was subsequently washed twice with ethanol-deionized (DI) water solution and dried at 65 $^\circ\text{C}$ temperature in vacuum desiccators for 24 h. Finally, some of the dried balls were crushed by mortar pestle to get fine powder for further auxiliary characterizations.

Optical and Electron Microscopic Characterizations

The morphology of silica ball was studied by FESEM. The as-prepared silica ball was crushed into fine powder by using mortar-pestle. The SiO_2 -NPs powder sample was dispersed in DI-water and the optical characterizations were performed with UV-Vis spectrophotometer (UV-3101PC, Shimadzu) and fluorescence spectrophotometer (LS 50B, Perkin Elmer). The 280 nm was used as excitation wavelength (λ_{exc}). A pinch of SiO_2 -NPs

powder sample was taken, coated with gold for the surface morphology images and compositional studies of the SiO₂-NPs were examined further in Field Emission Scanning Electron Microscopy (FE-SEM, FEI INSPECT F50, The Netherlands) equipped with Energy dispersive spectrometer (EDX, Bruker System) using QUANTAX ESPIRIT 1.9 software. The morphological study of as-prepared SiO₂-NPs was also performed by using a High Resolution Transmission Electron Microscopy (HR-TEM, JEOL JEM 2100, Japan). Samples for the TEM were prepared by drop casting the isolated and re-suspended solution on carbon coated copper grids.

Zeta Potential

The synthesized SiO₂-NPs powder was dispersed in Milli-Q water and the particles size as well as zeta potential experiment was characterized through DLS (Zeta Sizer, Nano ZS 90, Malvern) experiment.

X-ray Diffraction

XRD measurements of as-prepared powder SiO₂-NPs sample was carried out on a Bruker, D8 Advance, X-ray diffractometer instrument operated at a voltage of 40 kV and a current of 40 mA with Cu-K_α radiation.

FTIR and Raman Spectra Analysis

FTIR was used to identify the chemical and functional groups involves in SiO₂-NPs. The prepared SiO₂-NPs powder was dried and crushed with KBr (1% wt), pelleted and the FTIR spectra were recorded on a FTIR-8700, Shimadzu one instrument at a resolution of 4 cm⁻¹. The Raman spectroscopy for the as-prepared SiO₂-NPs was carried out using Laser Raman spectrometer (alpha 300, Witec, Germany) with the excitation wavelength of 532 nm and 20 mW output power for the irradiation time of 5 seconds.

Thermo-gravimetric Weight-loss Analysis (TGA)

The thermal stability of SiO₂-NPs was observed by determining the weight loss of the sample against elevated temperature in TGA/SDTA 851 °Mettler Toledo thermal analyzer system.

Photo-catalytic Activity of SiO₂-NPs for Decolourization of Dyes

The photo-catalytic of the as prepared silica balls containing SiO₂-NPs was tested for decomposing various dyes (Methylene blue, Methyl orange and Bromophenol blue). The silica ball was immersed in a 100 ml solution of a dye (10 μM) and incubated for several minutes at room temperature. The optical density of the dye solution was checked at different intervals (5 min) at respective absorption maximum of the corresponding dye. Percentage of decolourization of the dye was determined and plotted in the graph. The same silica balls were repeatedly used for decolourization of different dyes. After each degradation reaction, the silica balls were washed repeatedly with deionized water: acetone (1:1 v/v) and then air dried for next experimental purposes.

Statistical analysis

For each experiment prepared sample was tested repeatedly to confirm the obtained results.

RESULTS AND DISCUSSION

Morphological and Compositional Characterization of SiO₂-NPs

Formation of silica balls by using bioremediase protein is shown in the Fig. 1. The size of the ball depends on the protein concentration as observed visually. The FESEM image of the silica ball surface showed that the ball consisted of mostly silica nanoparticles along with some silica nano-ball of bigger sizes (Fig. 2).

The formation of silica ball consisting of silica nanoparticles was observed by the FE-SEM image (Fig. 3A), TEM image (Fig. 3B) and confirmed by EDX analysis (inset of Fig. 3A). The results suggested that SiO₂-NPs assembled to form silica ball due to the interaction of bioremediase protein and TEOS solution. It similarly confirmed the successful synthesis of Si-NPs using bioremediase protein from TEOS as an organic precursor [14]. The synthesized Si-NPs were quite regular in shape and consistently dispersed as observed in both FE-SEM and TEM images. The average size of synthesized SiO₂-NPs was measured as 20 ± 10 nm (n = 100) when analyzed from both FE-SEM and TEM images. EDX analysis (Inset of Fig. 3) of the specimen indicated the two strong evidence peaks of Silicon (Si) and Oxygen (O₂) that confirmed that powder form of sample consisted of Silicon (Si) and Oxygen (O₂) with atomic wt. 33.33% and 66.67% respectively which corroborated to the previous result [15]. TEM image also suggested the amorphous nature of the as prepared SiO₂-NPs.

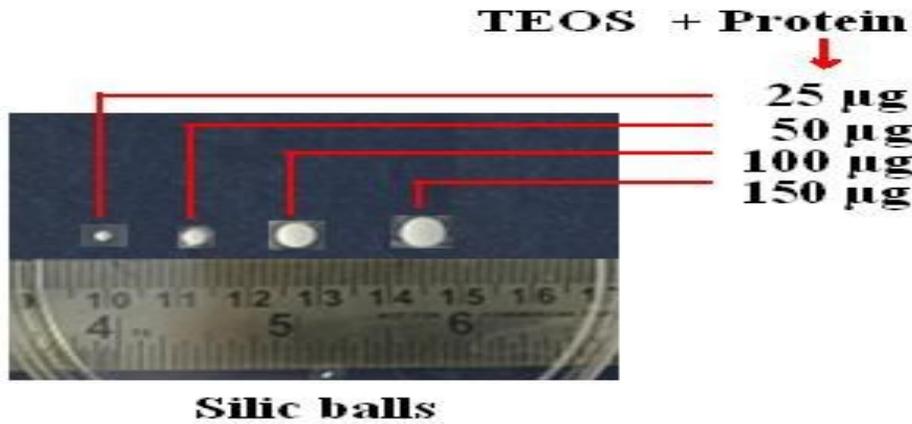


Fig. 1: Photographic images of silica balls formed by the interaction of different concentrations of bacterial protein with TEOS at neutral pH.

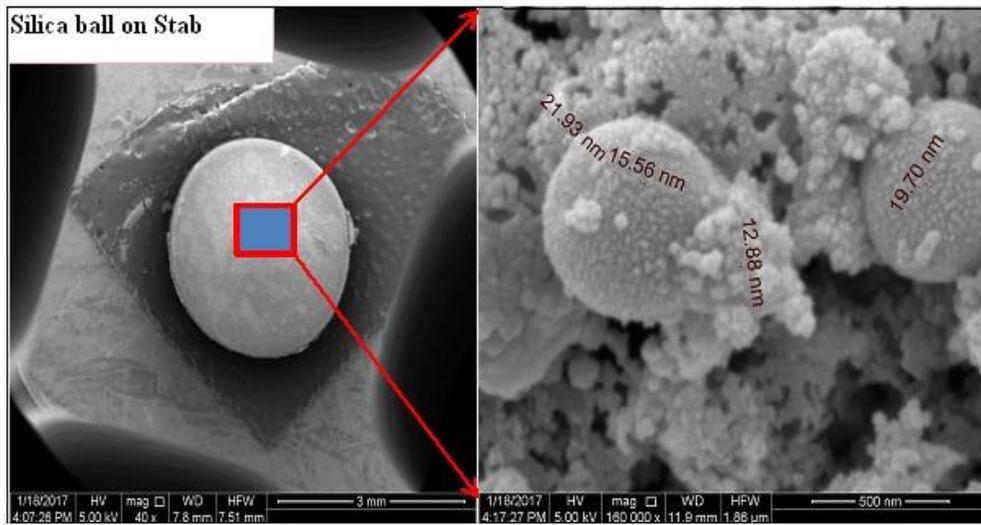


Fig. 2: FE-SEM image of silica ball and its constituents SiO₂-NPs formed by the Interaction of bacterial protein with TEOS at neutral pH.

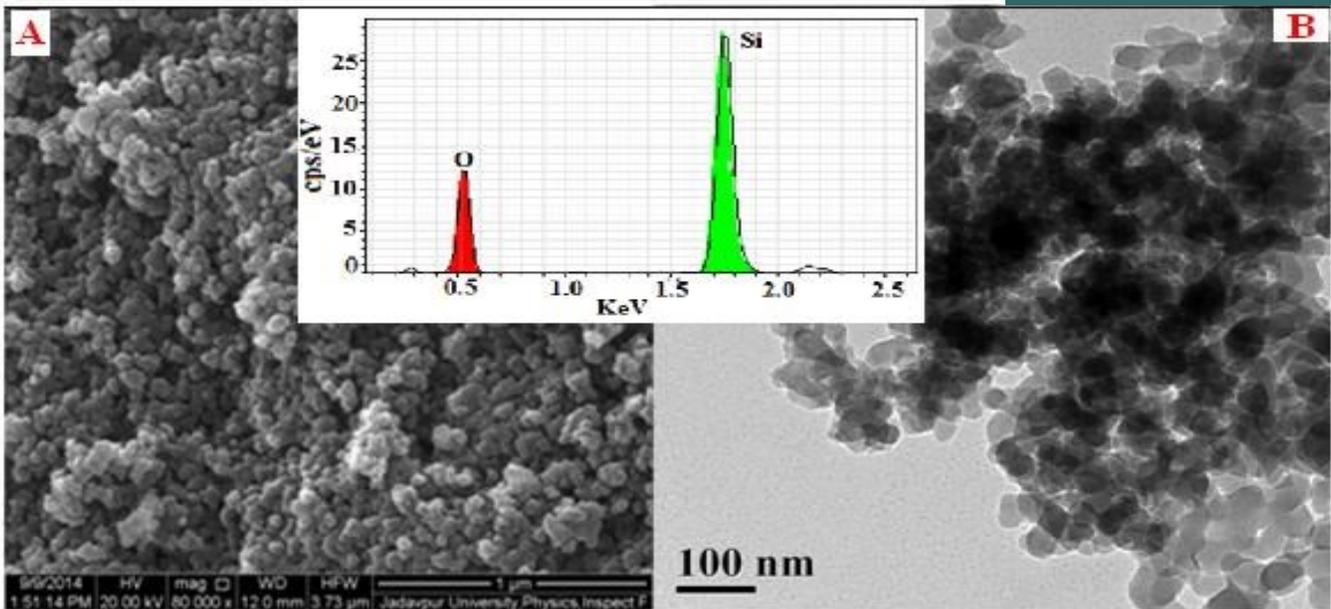


Fig. 3: (A) FE-SEM image of constituents SiO₂-NPs obtained from silica ball. (B) TEM image of constituents SiO₂-NPs obtained from silica ball. Inset of the Fig. 3 shows the composition analysis by EDX of constituents SiO₂-NPs obtained from silica ball.

The possible vibrational bands shown at 1097 cm^{-1} (ν_{as} : Si-O) and 790 cm^{-1} (ν_{s} : Si-O) were the two strong evidence of the formation of SiO_2 -NPs. The absorption bands appeared between 800 and 1269 cm^{-1} had been ascribed as a superimposition of assorted SiO_2 peaks, Si-OH bonding and residual organic groups [14]. The amorphous nature of prepared SiO_2 -NPs was confirmed from the XRD observation (Fig. 4B), as there is no sharp crystalline diffraction peak [15, 16]. The broadness of XRD graph might be due to the small size as well as infectivity of inner configuration of the particles [16, 17].

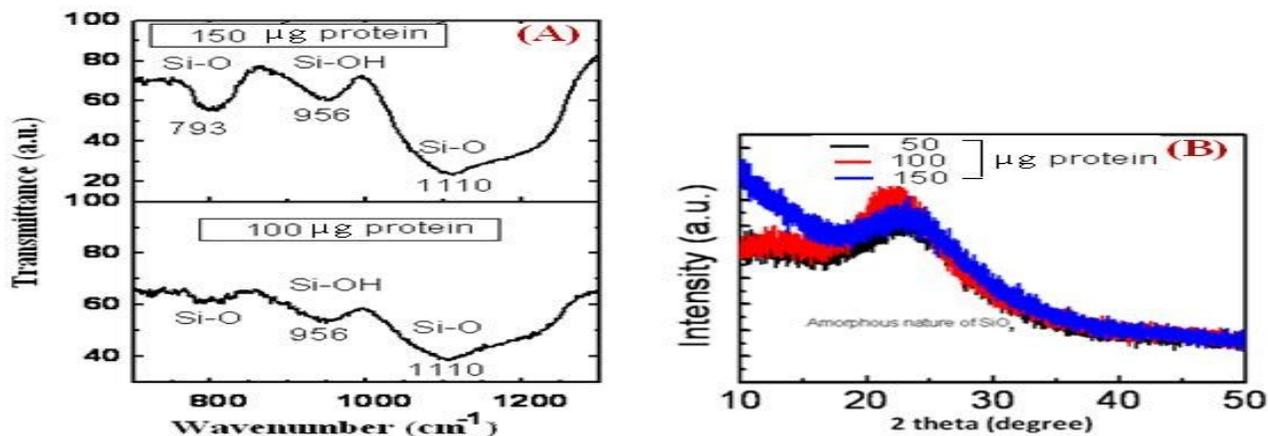


Fig. 4: (A) FT-IR spectra of constituents SiO_2 -NPs obtained from silica ball when reaction mixture contained $150\text{ }\mu\text{g}$ proteins (upper panel) and $100\text{ }\mu\text{g}$ proteins (lower panel) respectively. (B) XRD pattern of the as prepared SiO_2 -NPs obtained from silica balls at different concentration of proteins used.

Stability of SiO_2 -NPs

The Zeta potential (ζ) of the SiO_2 -NPs was found to be $(-)$ 30.9 mV (Fig. 5A) in balanced pH surroundings. SiO_2 -NPs were stable in nature and bounded with negative type of surface charges as it is the typical characteristic of SiO_2 -NPs. The nature of Zeta potential was negative which further suggested the good dispersion stability property as well as preventing the sample from further agglomeration. Fig. 5B indicated conventional thermal properties of biosynthesized SiO_2 -NPs measured from room temperature ($30\text{ }^\circ\text{C}$) to a very high temperature ($800\text{ }^\circ\text{C}$) by using TGA method. TGA is mainly used for characterizing the structural properties as well as for confirmation of the thermal stability of the materials. A ceramic (Al_2O_3) crucible was used for heating and measurements were carried out in N_2 atmosphere at the heating rate of $10\text{ }^\circ\text{C}/\text{min}$. It is known that all the water molecules of the surfactant are removed when temperature is raised nearly equal to $130\text{ }^\circ\text{C}$ [18]. There was practically no change in mass of the SiO_2 -NPs within the temperature range of 500 to $800\text{ }^\circ\text{C}$ which implied that the as-prepared SiO_2 -NPs could be used in chromatographic packing for its stability at higher temperature.

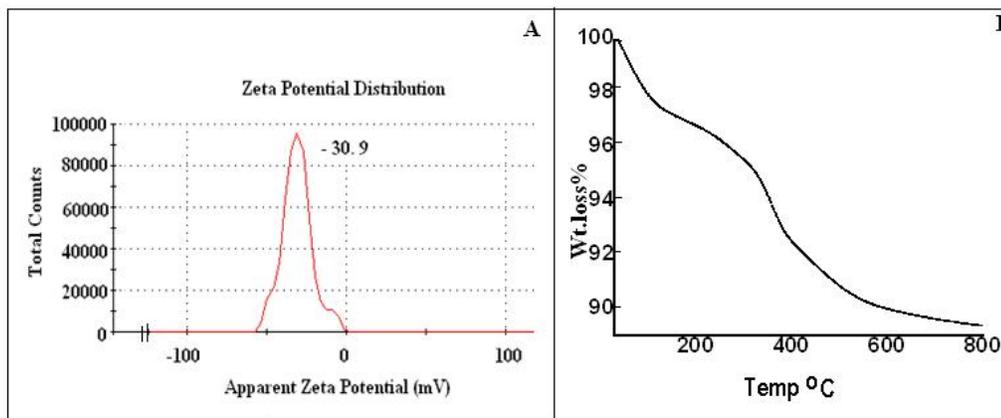


Fig. 5:(A) Zeta Potential (ζ) curve of synthesized SiO_2 -NPs in neutral environment.

(B) Thermogravimetric analysis of weight-loss curves for SiO_2 -NPs.

Fig. 5A represents the UV-Vis absorption spectra of SiO₂-NPs when well dispersed in DI-water. It observes a small absorption peak centered at about 360 nm. The estimated band gap (E_g) of SiO₂-NPs was found to be 3.4 eV which was similar to the previous data [15, 16]. The room temperature fluorescence spectra of as-prepared SiO₂-NPs are shown in Fig. 5B. A broad emission observed at UV-region, which is composed of a sharp peak centered at 440 nm. The sharp visible (blue) emission peak may be due to the electron-hole recombination of the self-trapped exciton (STE). This peak is slightly blue shifted because of the smaller-sized SiO₂-NPs as evident from the TEM image (Fig. 3B). The mechanism involved in blue band is an oxide associated process either from the oxide itself or from infectivity within structure of the oxide. However, sometimes the blue band could be resultant of a non-stoichiometric suboxide, SiO_x ($x < 2$), having value of band gap smaller than that of SiO₂. The appropriate justification for blue band formation from deficiency of non-bridging oxygen hole centre, or from infectivity of the oxide itself is now more eye-catching. SiO₂-NPs can be utilized in a range of biomedical applications like killing of unwanted bacteria with UV irradiation and use as an optical probe as well as optoelectronic devices for various medical diagnoses by using the visible emission.

Raman Spectra Analysis

Fig. 7 illustrates room temperature Raman spectra of synthesized SiO₂-NPs in a wavelength range of 4050–50 cm⁻¹. The Raman spectra of the prepared materials similarly confirmed the typical nature of SiO₂-NPs. In details, the major peak at 410 cm⁻¹ was the main characteristic peak of SiO₂-NPs. It is due to mainly coming from the bending mode of oxygen in n-membered rings ($n > 4$) and it was well known as R line. Peak appears at 818 cm⁻¹ was the involvement of Silica network optical mode as well; vibrational mode due to (OH) - group with admiration to Si contributed the peaks at 980 cm⁻¹. The typical symmetric stretching peak at ~490 (D1) have been attributed to the bulk infectivity in fourfold and threefold ring like structure of SiO₂ tetrahedral atom [19 - 22]. Subsequently, Raman studies of SiO₂-NPs have been established the surface-related optical properties as well. However, Raman peak amplitude of SiO₂-NPs scientifically depends on the specific surface areas as similar to previous literature [23].

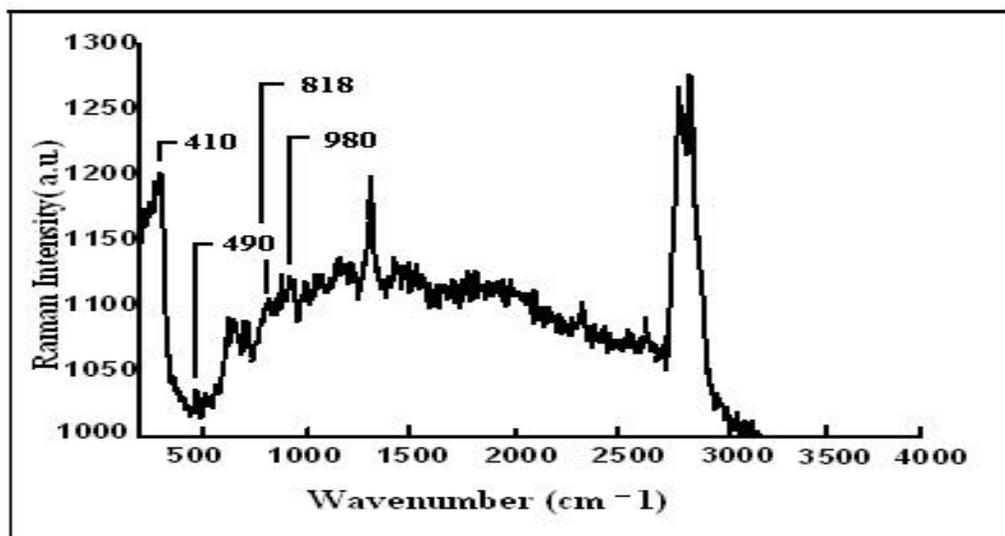


Fig. 7: Raman spectra analysis for synthesized SiO₂-NPs obtained from silica ball when 150 μg proteins used in the reaction mixture.

Decolourizing Effect of Silica ball

The photo-catalytic activity of the prepared silica ball consisting of SiO₂-NPs was successfully demonstrated (Fig. 8). The degradation of Methyl orange (Fig. 8A), Bromophenol blue (Fig. 8B) and Methylene blue (data not supplied) dyes was carried out in the presence of silica ball at different time intervals in the visible region. It was observed that the absorption maxima of Methyl orange (460 nm), Bromophenol blue (492) and Methylene blue (663 nm) were decreased regularly with the incubation time indicating the photo-catalytic degradation those dyes due to the action of the silica balls. The percentage of degradation efficiency of biosynthesized silica balls was determined and plotted in the Fig. 8. Almost 100% degradation of Bromophenol blue and 70 to 80% degradation of Methylene blue and Methyl orange dyes were noted in presence of silica ball. The lesser degradation of the last two dyes might be due to deposition of the Bromophenol blue dye on the surface of silica ball which was used first and thus less exposed surface of SiO₂-NPs was available for those two dyes. The repeated use of the biosynthesized silica balls suggested that it could

be an effective photo-catalyst for water purification system and dye effluent treatment.

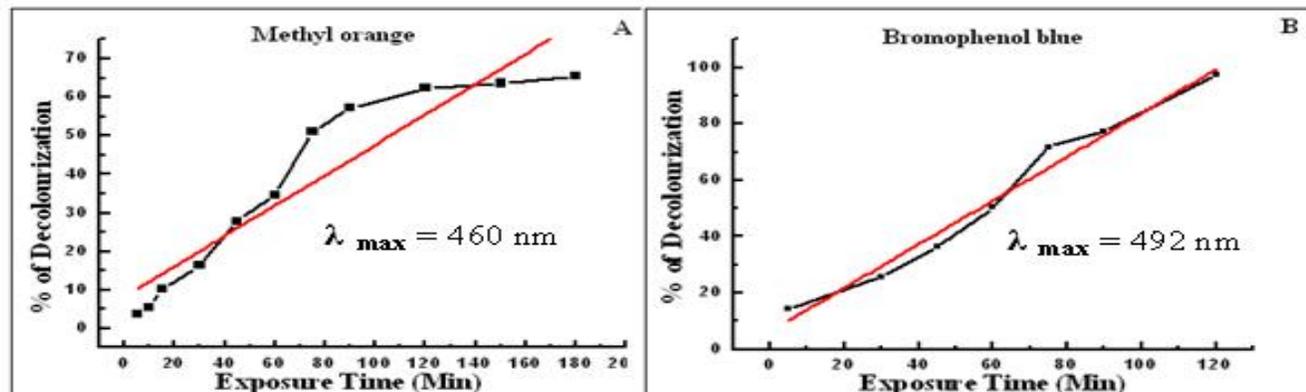


Fig. 8: Dye degradation activities of silica ball where (A) Methyl orange and (B) Bromophenol blue dyes were used as substrate.

CONCLUSIONS

A microbial protein assisted silica ball consisting SiO_2 -NPs using an Organic (TEOS) silica precursor is effectively established. The constituent particles of the as-prepared samples are systematically characterized using different tools like UV-Vis, Fluorescence, HR-TEM, FE-SEM, XRD, TGA and FTIR spectra. Fluorescence data shows a broad emission in visible region. This result suggests its further utilization in various biomedical applications as an optical probe for medical diagnosis. The value of Zeta potential (ζ) of the SiO_2 -NPs indicates the immovability of SiO_2 -NPs in dispersed neutral medium, preventing it from the further agglomeration. The formation of silica balls via an effortless biocompatible protein assisted methodology shows its potential and notable optical properties for direct use in various fields.

Author's contributions

Shilpi Show: She did the all experimental work and substantially contributed to conception, acquisition of data and analysis of data.

Krishna Chattopadhyay: She involved in drafting the manuscript and revising it critically for important intellectual content.

Chetana Ghosal: She took part in dyes degradation work and substantially contributed to acquisition and analysis of data

Brajadulal Chattopadhyay: He made the substantial contributions to conception and designs the experimental procedures for acquisition of data and interpretation of data. He was also involved in drafting the manuscript and revising it critically for important intellectual content.

Acknowledgments

The experimental facilities obtained from Biophysical Laboratory of Physics Department of Jadavpur University are gratefully acknowledged.

Funding/Financial Disclosure

The work was performed under the supervision of Professor Brajadulal Chattopadhyay and there was no financial support obtained for the work. Only support from Biophysics Laboratory from its own research facilities involved in the work.

REFERENCES

- [1] Huo, Q., Feng, J., Schuth, F., Stucky, G. D. (1997). Preparation of Hard Mesoporous Silica Spheres. *Chemistry of Material*, 9(1): 14 – 17.
- [2] Ozin, G. A. (1992). Nanochemistry: Synthesis in diminishing dimensions. *Advance Materials*, 4: 612 – 649.
- [3] Gunter. B., Grün, M., Unger K. K., Matsumoto, A., Kazuo, T. (1998). Tailored syntheses of nanostructured silicas: Control of particle morphology, particle size and pore size. *Supramolecular Science*, 5: 253– 259.
- [4] Bao, X., Wei, X., Wang, Y., Jiang, H., Yu, D., Hu, M. (2014). Effect of Silica-Based Nanomaterials and Their Derivate with PEGylation on Cementoblasts. *Annals of Biomedical Engineering*, 42 (8): 1781 – 1789.

- [5] Kell, A. J., Barnes, M. L., Slater, K., Lavigne, C. (2012). Functionalised silica nanoparticles stable in serum-containing medium efficiently deliver siRNA targeting HIV-1 co-receptor CXCR4 in mammalian cells. *International Journal of Nano and Biomaterials* 2, 4(3-4): 223 – 242.
- [6] Liu, X., Qian, H., Ji, Y., Li, Z., Shao, Y., Hu, Y., Tong, G. X., Li, L., Guo, W., Guo, H. (2012). Mesoporous silica-coated NaYF₄ nanocrystals: facile synthesis, in vitro bioimaging and photodynamic therapy of cancer cells. *RSC Advances*, 2(32): 12263 – 12268.
- [7] Natarajan, S. K., Selvaraj, S. (2014). Mesoporous silica nanoparticles: Importance of surface modifications and its role in drug delivery. *RSC Advances*, 4: 14328 – 14334.
- [8] Monnier, A., Schuth, F., Huo, Q., Kumar, D., Margolese, D., Maxwell, R. S., Stucky, G. D., Krishnamurthy, M., Petroff, P., Firouzi, A., Janicke, M., Chmelka, B. F. (1993). Cooperative formation of inorganic-organic interfaces in the synthesis of silicate mesostructures. *Science*, 261: 1299– 1303.
- [9] Firouzi, A., Kumar, D., Bull, L. M., Besier, T., Sieger, P., Huo, Q., Walker, S. A., Zasadzinski, J. A., Glinka, C., Nicol, J., Margolese, D., Stucky, G. D., Chmelka, B. F. (1995). Cooperative organization of inorganic-surfactant and biomimetic assemblies. *Science*, 267: 1138 – 1143. .
- [10] Huo, Q., Margolese, D. I., Ciesla, U., Demuth, D. G., Feng, P., Gier, T. E., Sieger, P., Firouzi, A., Chmelka, B. F.; Schuth, F. Stucky, G. D. (1994). Organization of Organic Molecules with Inorganic Molecular Species into Nanocomposite Biphase Arrays. *Chemistry of Materials*, 6: 1176 – 1191.
- [11] Anderson, M. T., Martin, J. E., Odinek, J. G., Newcomer, P. P. (1998). Surfactant-Templated Silica Mesophases Formed in Water: Cosolvent Mixture. *Chemistry of Materials*, 10: 311.
- [12] Gruⁿ, M., Unger, K. K., Matsumoto A., Tsutsumi, K. (1999). Novel pathways for the preparation of mesoporous MCM-41 materials: control of porosity and morphology. *Microporous and Mesoporous Materials*, 27: 207 – 216.
- [13] Biswas, M., Majumdar, S., Chowdhury, T., Chattopadhyay, B.D., Mandal, S., Halder, U., Yamasaki, S. (2010). Bioremediase a unique protein from a novel bacterium BKH1, ushering a new hope in concrete technology. *Enzyme and Microbial Technology*, 46: 581 – 587.
- [14] Chowdhury, T., Sarkar, M., Chaudhuri, B., Chattopadhyay, B.D., Halder, U.C. (2015). Participatory role of zinc in structural and functional characterization of bioremediase: a unique thermostable microbial silica leaching protein. *Journal of Biological Inorganic Chemistry*, 20(5): 791 – 803.
- [15] Show, S., Tamang, A., Chowdhury, T., Mandal, D., Chattopadhyay, B.D. (2015). Bacterial (BKH1) Assisted Silica Nanoparticles from Silica Rich Substrates: A Facile and Green approach for biotechnological applications. *Colloids and Surfaces B: Biointerfaces*, 126: 245 – 250.
- [16] Show, S., Chattopadhyay, B.D. (2016). Protein Mediated Silica Particles with pH Controlled Porosity and Morphology. *Advances in Microbiology*, 6: 986 – 998.
- [17] Wei, Z., Yan, P., Feng, W., Dai, J., Wang, Q., Xia, T. (2006). Microstructural characterization of Ni nanoparticles prepared by anodic arc plasma. *Materials Characterization*, 57(3): 176 – 181.
- [18] Unger, K.K. (1979). Porous Silica. *Journal of Chromatography Library*, 16: 237-242.
- [19] Umari, P., Pasquarello, A. (2003). First-principles analysis of the Raman spectrum of vitreous silica: comparison with the vibrational density of states, *Journal of Physics: Condensed Matter*, 15(16): S1547 – S1552.
- [20] Anedda, A., Carbonaro, C.M., Clemente, F., Corpino, R., Ricci, P. C. (2003). Raman Investigation of Surface OH-Species in Porous Silica. *Journal of Physical Chemistry B*, 107 (49): 13661 – 13664.
- [21] Anedda, A., Carbonaro, C.M., Clemente, F., Corda, L., Corpino, R., Ricci, P.C. (2003). Surface hydroxyls in porous silica: a Raman spectroscopy study, *Materials Science and Engineering: C*, 23 (6-8): 1069 – 1072.
- [22] Galeener, F.L. (1982). Planar rings in vitreous silica, *Journal of Non-Crystalline Solids*, 49(1-3): 53 – 62.
- [23] Alessi, A., Agnello, S., Buscarino, G., Gelardi, F.M. (2013). Structural properties of core and surface of silica nanoparticles investigated by Raman spectroscopy. *Journal of Raman Spectroscopy*, 44(6): 810 – 816.

Contact Us:

SIFT DESK

Deerpark Dr, #75, Fullerton, CA, 92831, United States.

E-mail: helpdesk@siftdesk.org

Visit us on the web at: www.siftdesk.org