RASĀYAN *J. Chem.*
Vol. 19 | No. 1 | 652-660 | January-March | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

BIOGENIC SYSNTHESIS OF SILICA NANOPARTICLES USING MARINE MICROALGAL PIGMENTS

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ABSTRACT

Silica nanoparticles (SiNPs) have emerged as pivotal materials in biomedicine and nanotechnology due to their structural versatility and biocompatibility. This study demonstrates a novel, "green" phycosynthetic route for SiNPs utilizing bioactive pigments extracted from the marine microalgae *Picochlorum sp and Chlorella sp*. Using sodium silicate as a sustainable precursor, these marine pigments served as natural reductant, dispersant and capping agents, eliminating the need for hazardous chemical reductants. The pigment profiles were validated via UV-Visible spectroscopy and High-Performance Liquid Chromatography (HPLC). Systematic characterization confirmed the formation of spherical nanoparticles with diameters ranging from 100 to 400nm.UV-Visible spectra exhibited characteristic absorption peaks at 200-250nm, while X-ray Diffraction (XRD) and Fourier-transform infrared (FTIR) spectroscopy verified an amorphous Si-O-Si framework. The nanoparticles displayed exceptional colloidal stability, with zeta potential values of -46.5mV (*Picochlorum sp*) and -42.0mV (*Chlorella sp*). SEM-EDX analysis confirmed a smooth morphology integrated with trace biogenic elements (Mg, Ca) which may enhance biological functionality. Crucially, a hemolysis assay revealed less than 5%hemolytic activity at concentrations of 10-20µg/mL, confirming the high biocompatibility of these biogenic SiNPs. These findings spotlight the potential of marine microalgal pigments as a capable bio-factory for developing sustainable, non-toxic nanomaterials tailored for biolabeling and biomedical applications.

Keywords: Green synthesis, Marine microalgae, Silica nanoparticles, Phyco-pigments, biocompatibility, *Picochlorum sp, Chlorella sp.*

RASĀYAN *J. Chem.*, Vol. 19, No.1, 2026

INTRODUCTION

The industrial production of silica nanoparticles (SiNPs) currently relies heavily on the "Stöber process," a traditional method that consumes significant energy and utilizes hazardous chemical reductants such as ammonia and toxic silanes. While SiNPs have become indispensable in fields ranging from targeted cancer therapy to environmental sensors, this "gray" chemistry footprint poses a growing ecological threat. As global demand for these nanomaterials is projected to reach unprecedented heights by 2030, the transition to sustainable manufacturing has become a biological imperative. Could the microscopic pigments that color our oceans provide the non-toxic solution needed to revolutionize modern nanotechnology?[1].

The primary purpose of this study is to bridge the research gap by uncovering the inherent potential of isolated marine microalgal pigments as specific biochemical scaffolds for silica fabrication. While existing literature has explored the use of bulk plant and microbial extracts, these specific redox-active role of purified pigments such as chlorophylls, carotenoids and phycobiliproteins remains significantly underexplored. Unlike crude extracts, these specific chromophores possess inherent chemical properties that facilitate the precise reduction and stabilization of the SiO_2 framework, dictating the final size, morphology and colloidal stability of the nanoparticles.

Furthermore, this investigation is strategically aligned with the sustainable Development Goals (SDGs) contributing to SDG-9 (Industry, Innovation and Infrastructure) by fostering sustainable industrialization and SDG-12 (Responsible Consumption and production) by utilizing renewable marine bioresources. By replacing synthetic catalysts with biogenic pigments, this research promotes a "Blue economy" framework, transforming algal biomass into high value technological assets.

Recent advancements in 2024 and 2025 have demonstrated that biogenic silica exhibits superior biocompatibility and reduced hemolytic activity compared to traditional synthetic silica [2,3]. Building on these findings, our study utilizes pigments from marine microalgae to synthesize SiNPs that are systematically characterized using advanced analytical techniques, including FTIR, XRD, SEM, TEM and DLS. This work ultimately establishes a scalable, non-toxic protocol that harmonizes high tech materials science with environmental preservation, providing deep insights into the biochemical interactions of pigment-mediated synthesis.

EXPERIMENTAL

Materials

Analytical grade chemicals and reagents were employed throughout the investigation. Sodium metasilicate (Na_2SiO_3> 99% purity) was sourced from Sigma-Aldrich. HPLC grade methanol and acetone were procured from Merck. Additional reagents included hydrochloric acid (HCl), deionized water, and Conway medium for microalgal cultivation.

Methods

Cultivation of microalgae

The marine microalgae *Picochlorum* sp. and *Chlorella* sp. were cultured in Conway medium under controlled laboratory conditions for 30 days [4]. To optimize biomass and metabolite productivity, specific stress parameters were maintained: a temperature range of 30–32°C, pH between 6.5–7.5, and a 12:12 hour light/dark cycle. Post-cultivation, cellular morphology and growth characteristics were verified via microscopic observation.

Biomass Harvesting and Purification

The microalgal biomass was harvested through centrifugation at 4,000 rpm for 20 minutes at 4°C [5]. The resulting pellet was separated from the supernatant and subjected to a purification process involving 2–3 washes with distilled water to eliminate residual media components and extracellular impurities. The purified wet biomass was stored at 4°C prior to pigment extraction[6].

Pigment Extraction Protocol

Pigments were extracted using a solvent system of methanol and acetone (80:20 v/v). The solvent mixture was added to the biomass and subjected to sonication for 15 minutes to disrupt cell walls and facilitate pigment release[7]. Following a brief vortexing (1–2 minutes), the mixture was incubated in the dark for 30–60 minutes to prevent photo-degradation. The extract was then centrifuged at 4,000 rpm for 20 minutes at 4°C. This extraction cycle was repeated until the biomass appeared colorless, and the combined supernatants were collected for nanoparticle synthesis.

Green Synthesis of Silica Nanoparticles

The synthesis was initiated by dissolving 5g of sodium metasilicate in 50mL of deionized water. The initial pH was maintained between 10–12 with constant stirring for 1–2 hours[8]. Nucleation was induced by gradually lowering the pH to 7–8 using 1M HCl. During this transition, 8mL of the microalgal pigment extract was introduced into the solution, resulting in a visible cloudy suspension indicative of nanoparticle formation. The mixture was aged for 12–24 hours to ensure structural stabilization and then centrifuged at 5,000 rpm to isolate the crude SiNPs[9].

Purification of Silica Nanoparticles

To remove unreacted precursors and pigment residues, the SiNP pellets underwent sequential washing with ethanol and deionized water, each followed by a 10-minute centrifugation at 5,000 rpm [10]. The purified SiNPs were dried under dark conditions for 24–48 hours and stored in airtight containers at 4°C.

Physicochemical Characterization

The synthesized SiNPs were characterized using a suite of analytical techniques:

- UV-Visible Spectroscopy: To confirm formation and optical properties.
- XRD & FTIR: To determine crystalline structure and surface functional groups.
- SEM-EDX: To evaluate surface morphology and elemental composition.
- Zeta Potential: To assess colloidal stability and surface charge**.**

Biocompatibility Assessment (Hemolysis Assay)

The safety profile of the SiNPs was evaluated through a hemolysis assay using human red blood cells (RBCs)[11]. Anticoagulated blood (treated with EDTA) was centrifuged to isolate RBCs, which were then suspended in PBS. SiNPs at concentrations of 10, 15, and 30 mg/mL were incubated with the RBC suspension for 60 minutes at room temperature. After centrifugation (4,000 rpm for 15 minutes), the release of haemoglobin was quantified via UV-Vis spectroscopy at 540 nm[12].

RESULTS AND DISCUSSION

Preliminary pigment Analysis

The acidification test successfully confirmed the presence of carotenoids in the algal extracts. Upon exposure to 1M HCl, a distinct green-to-olive green color transition was observed, demonstrating the structural stability of carotenoids under acidic conditions. Unlike chlorophylls, which degrade rapidly under low pH, carotenoids resist bleaching due to their robust conjugated polyene chain. These findings align with Garcia-Pichel et al. (2020)[13], who validated HCl-based colorimetric tests for carotenoid identification. This qualitative confirmation was a critical prerequisite for assessing the pigments' functional roles in silica stabilization. (Figure 1).

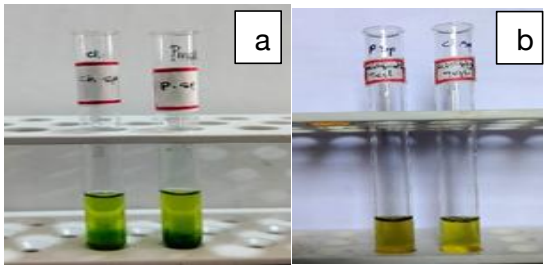


Fig.-1: Acidification test (a) Algal extracts (b) Positive result: Green to olive green colour transition after adding 1M HCl

UV-Visible Spectroscopy for Pigment Characterization

UV-Vis spectroscopy analysis of xanthophylls from *Picochlorum sp*. and zeaxanthin from *Chlorella sp.* reveals distinct absorption peaks characteristic of carotenoids. Xanthophylls exhibited a peak around 450 nm, while zeaxanthin showed a peak at 440 nm, both linked to their conjugated double-bond systems. The optical density trends indicate efficient light absorption in the 400–500 nm range, with slight spectral shifts suggesting structural modifications or environmental influences [14]. These differences highlight variations in pigment composition, molecular configuration, and adaptation mechanisms, reinforcing their roles in photoprotection and bio-imaging applications (Figure 2).

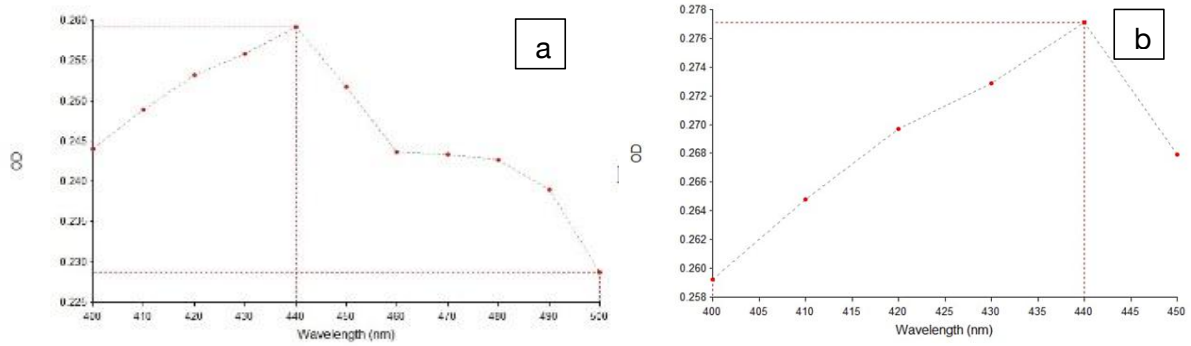


Fig.-2: Absorbance Spectra of Pigments Extracted from (a) Picochlorum sp. and (b)Chlorella sp. (400–500 nm)

HPLC (High-Performance Liquid Chromatography) and Mass Spectral Profiling

HPLC analysis underscored the distinct biochemical strategies of the two species. *Picochlorum* sp. displayed a complex profile dominated by a broad peak at 31.393 min (34.4% area), likely representing a chlorophyll-protein complex. Conversely, *Chlorella* sp. was characterized by a prominent β-carotene signal at 25.544 min[15]. The aqueous-to-acetonitrile gradient successfully resolved polar degradation products, such as pheophytin (eluting at 7.703 min), from hydrophobic carotenoids. Complementary ESI-MS data provided mass spectral evidence of these unique signatures,

confirming that the high pigment diversity of these species offers multiple functional groups for nanoparticle capping (Fig.-3).

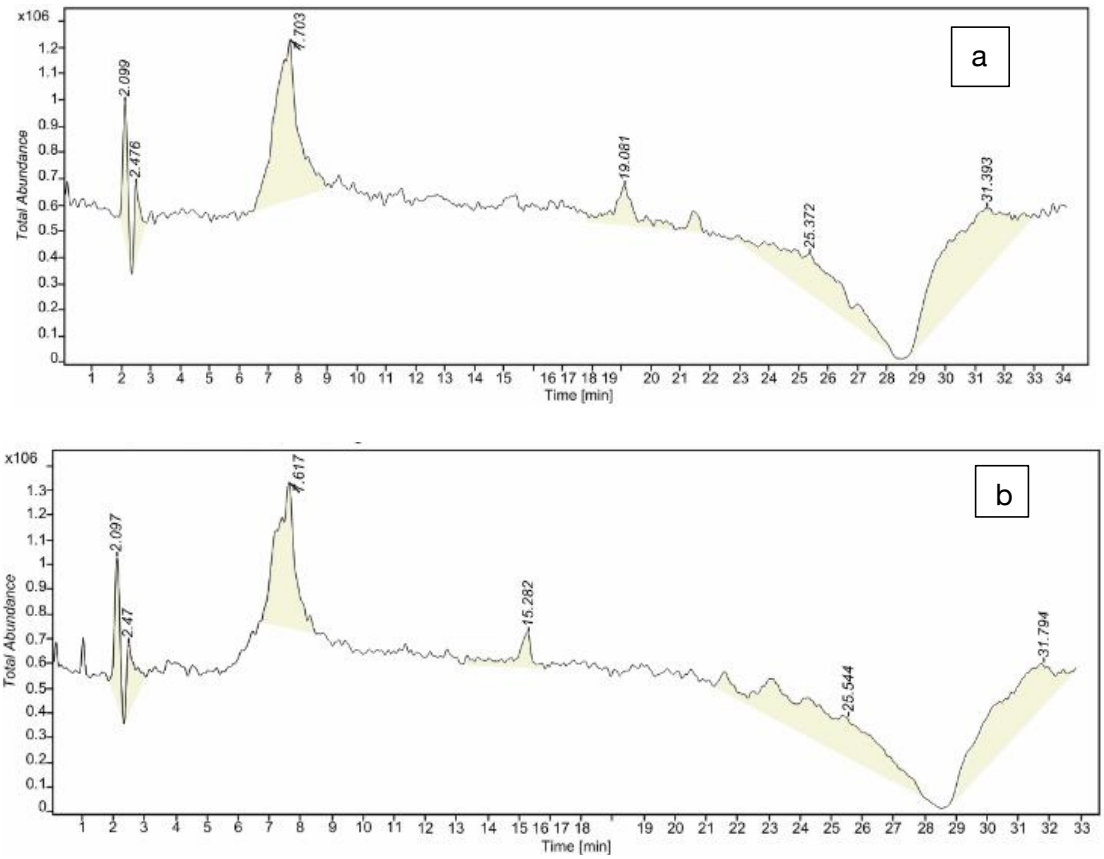


Fig.-3: HPLC Chromatogram for silica nanoparticles from (a)Picochlorum sp. (b) chlorella sp. Pigments

Synthesis and optical properties of Silica Nanoparticles (SiNPs)

The formation of SiNPs was confirmed by a strong UV-Vis absorption peak at ~200 nm, attributed to the Si–O–Si electronic transitions of the silica framework [16]. Pigment-incorporated SiNPs (*Si NP-A1* and *Si NP-A2*) maintained this dominant peak. Interestingly, *Chlorella*-mediated SiNPs showed a slight redshift to 225 nm, suggesting a unique chemical interaction between the pigment molecules and the silica matrix[17]. The lack of absorbance above 300 nm confirms that the organic pigments are successfully integrated into the framework, effectively "masking" their standalone optical signatures(Fig.-4).

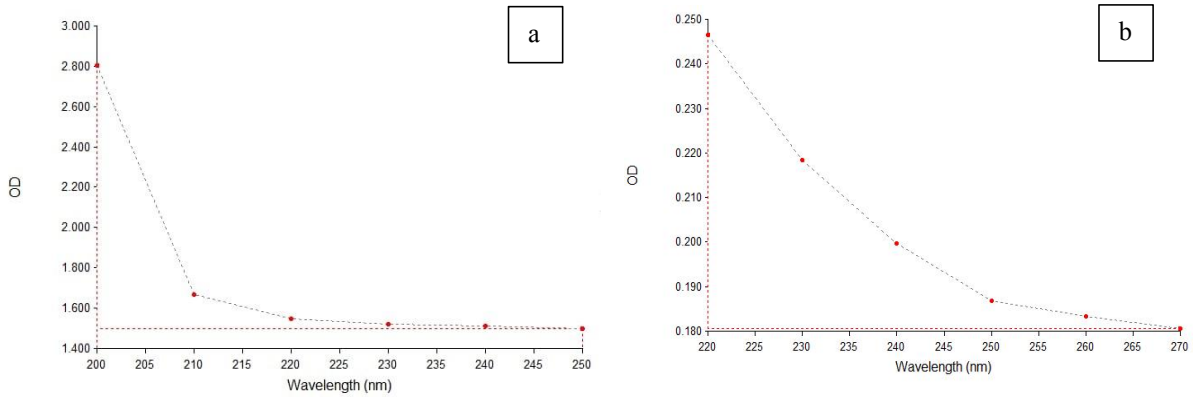


Fig.-4: UV Absorbance Spectrum of pigments incorporated Silica Nanoparticles-A1 (200–250 nm) (a)Picochlorum sp. (b) Chlorella sp.

Zeta Potential and Colloidal Stability

Zeta potential analysis confirmed that both formulations possess robust colloidal stability. *Picochlorum*-derived SiNPs recorded a mean potential of –46.5 mV, while *Chlorella*-derived particles recorded –42.0 mV. Both values significantly exceed the ±30 mV threshold required for long-term stability (Sikora et al., 2023)[18]. This negative charge results from the deprotonation of surface silanol (Si–OH) groups into SiO^- moieties [19]. The sharp, unimodal peaks observed in both zeta potential distributions confirm a monodisperse particle population, while the consistent electrophoretic mobility supports predictable particle migration under an applied electric field for well-controlled systems[20]. This high electrostatic repulsion is vital for achieving SDG 12 (Responsible Consumption and Production), as it ensures a long shelf-life and reduces material waste from particle aggregation(Fig.-5).

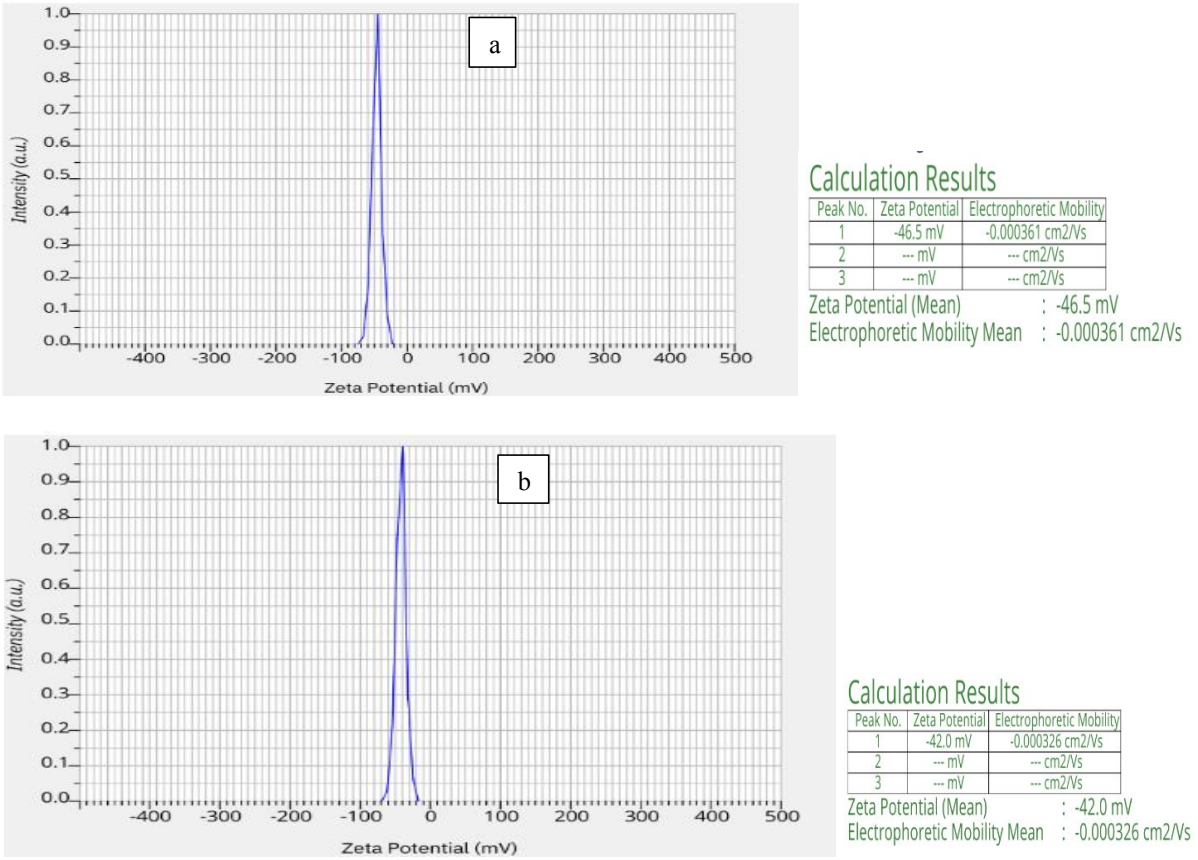


Fig.-5: Zeta Potential Distribution and Electrophoretic Mobility Measurements of Silica Nanoparticles (−400 to 500 mV) (a)Picochlorum sp. and (b)chlorella sp

FTIR (Fourier Transform Infrared Spectroscopy) and XRD (X-ray Diffraction) structural verification

FTIR spectra confirmed the amorphous nature of the silica, with primary Si–O–Si asymmetric stretching at ~1056 cm^{-1}. *Picochlorum*-derived silica showed a higher intensity of O–H stretching (75.07%), suggesting greater surface hydrophilicity. O-H stretching, linked to surface hydrophilicity, is more pronounced in *Picochlorum*-derived silica (75.07%) compared to *Chlorella*-based (47.12%), reflecting stronger hydroxyl presence. Atmospheric CO_2 interference affects *Picochlorum* spectra, while *Chlorella*-derived silica maintains cleaner spectral clarity[21](Fig.-6).

XRD patterns for both samples exhibited broad diffraction humps between 20º and 30º 2θ devoid of sharp crystalline peaks. *Picochlorum*-derived silica exhibits a peak at 26.70° 2θ, while *Chlorella*-mediated silica shows a slightly lower peak at 23.23° 2θ, reflecting differences in nucleation dynamics and organic stabilization maintaining amorphous integrity[22]. This confirms the production of amorphous silica, which is highly preferred for drug delivery applications due to its higher surface area and solubility compared to crystalline quartz(Fig.-7).

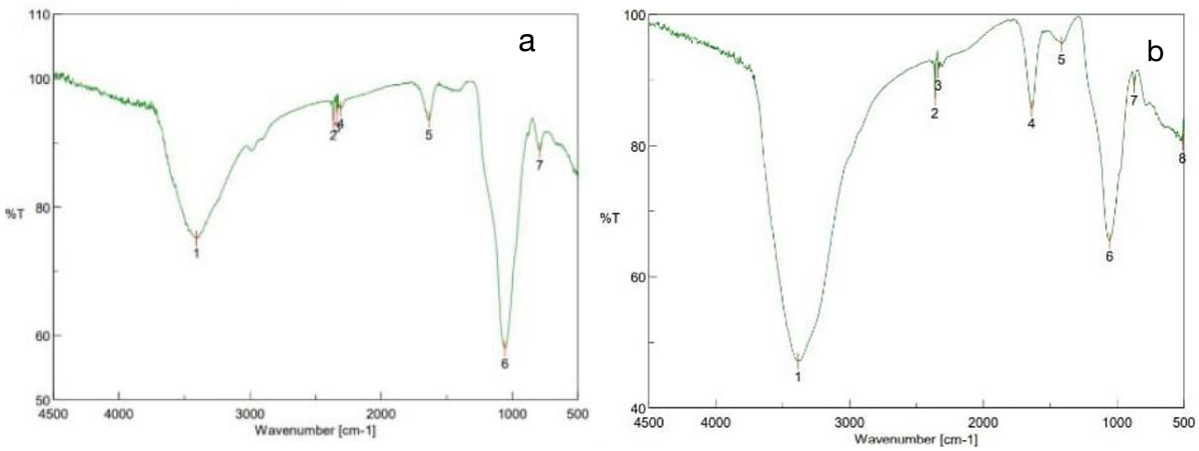


Fig.-6: FTIR Spectra and Identified Peaks of Silica Nanoparticles Synthesized from (a)Picochlorum sp. and (b)Chlorella sp. Pigments

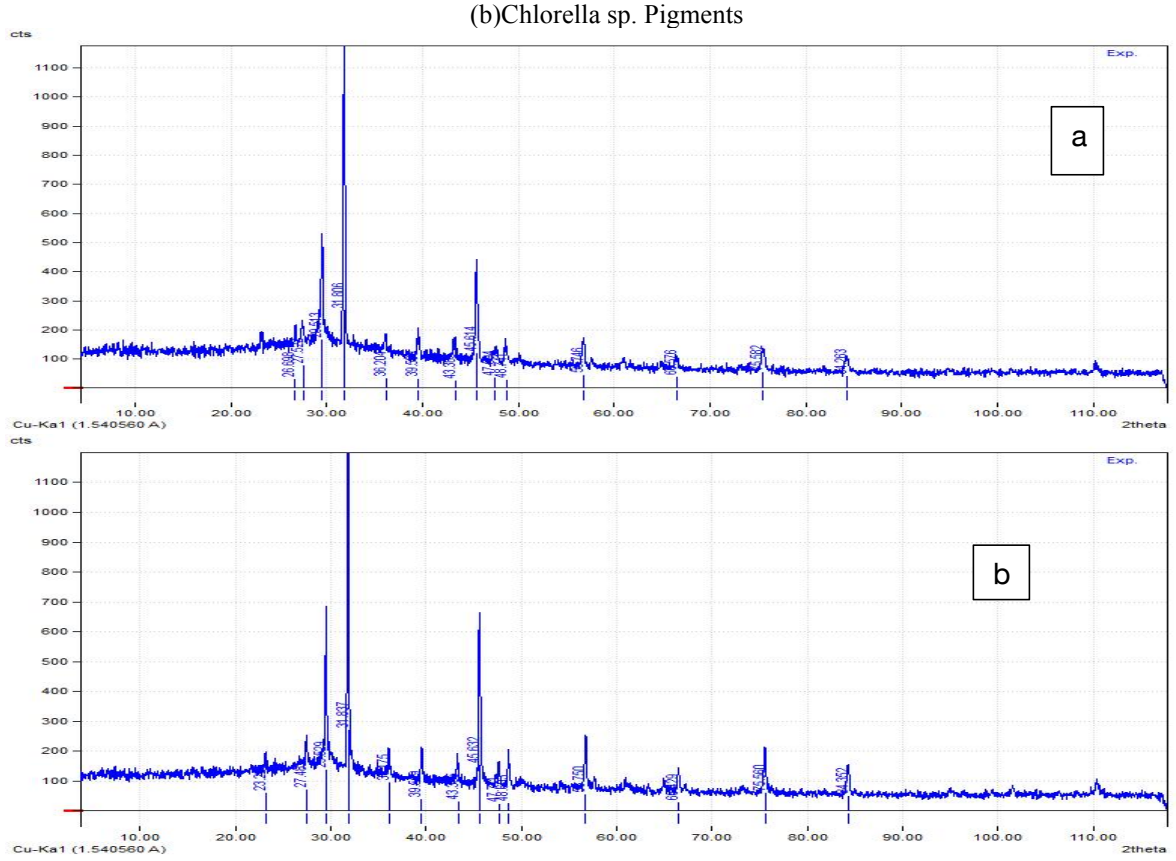


Fig.-7: XRD Diffractogram of Silica Nanoparticles Synthesized from (a)Picochlorum sp. and (b)Chlorella sp. pigments (Cu-Kα, 10–110° 2θ)

SEM-EDX Morphology

SEM micrographs revealed spherical nanoparticles with smooth surfaces. *Picochlorum*-derived SiNPs were smaller (100–200 nm) compared to the (100–400 nm) range seen in *Chlorella*-derived samples. EDX confirms silica (SiO_2) as the primary framework, with trace elements reflecting biogenic retention and environmental influences.[23] The results confirmed the SiO_2 framework, notably identifying trace Terbium (Tb) in *Picochlorum* samples, which may impart intrinsic luminescent properties for bio-labelling(Fig.-8 and 9).

Hemolysis Assay

The haemolysis assay compares the biocompatibility of silica nanoparticles (SiNPs) synthesized from *Chlorella* sp. and *Picochlorum sp*. for bio-labelling applications.[24] Crucially, the hemolysis assay demonstrated that both SiNP types are highly biocompatible. At concentrations of 10–20 µg/mL, hemolysis remained <5%, the international safety standard for blood-contacting materials.

Picochlorum-SiNPs showed superior compatibility, maintaining safety even at 30 µg/mL, whereas *Chlorella*-SiNPs reached 6.15%. These results highlight the potential of these nanoparticles in biomedical domains, directly supporting SDG 3 (Good Health and Well-being)(Fig.-10).

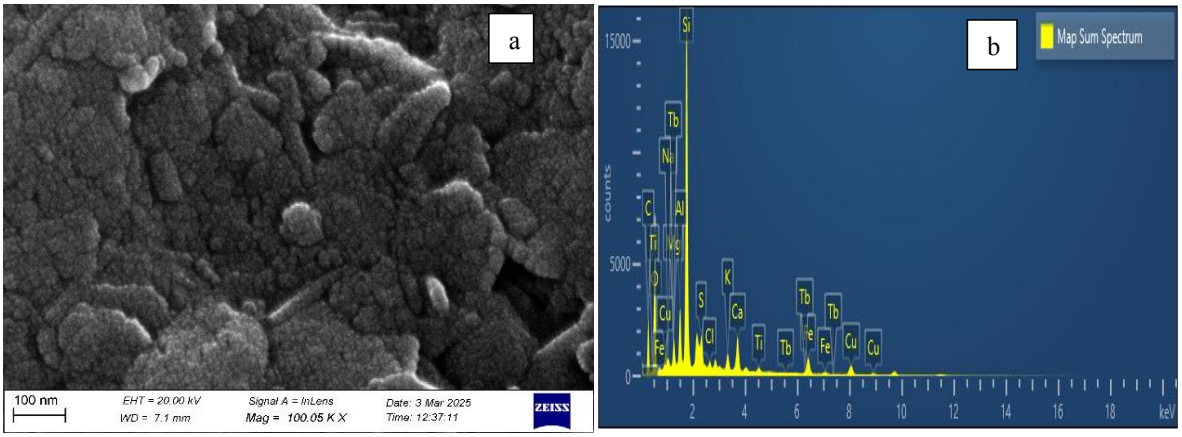


Fig.-8: (a) SEM Micrograph of Silica Nanoparticles, (b) EDX Elemental Composition Spectrum of Silica Nanoparticles from Picochlorum sp

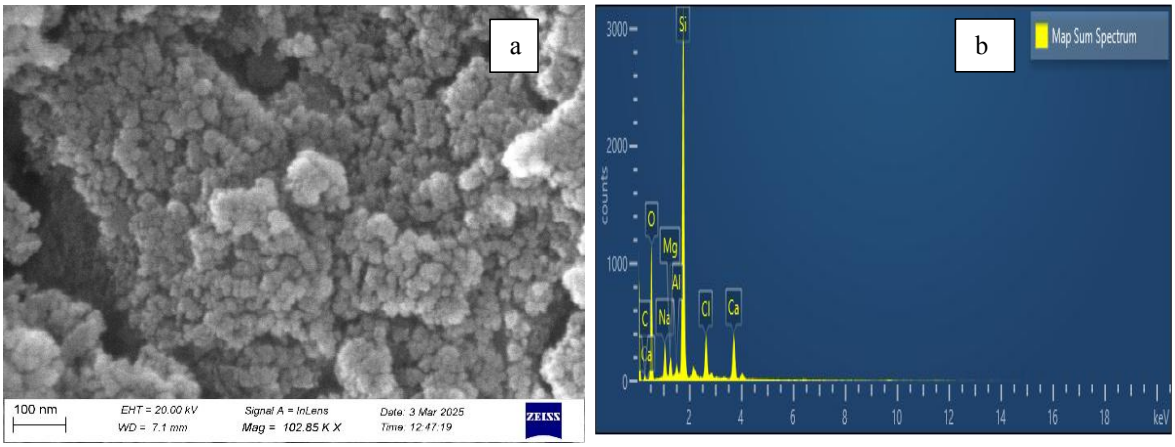
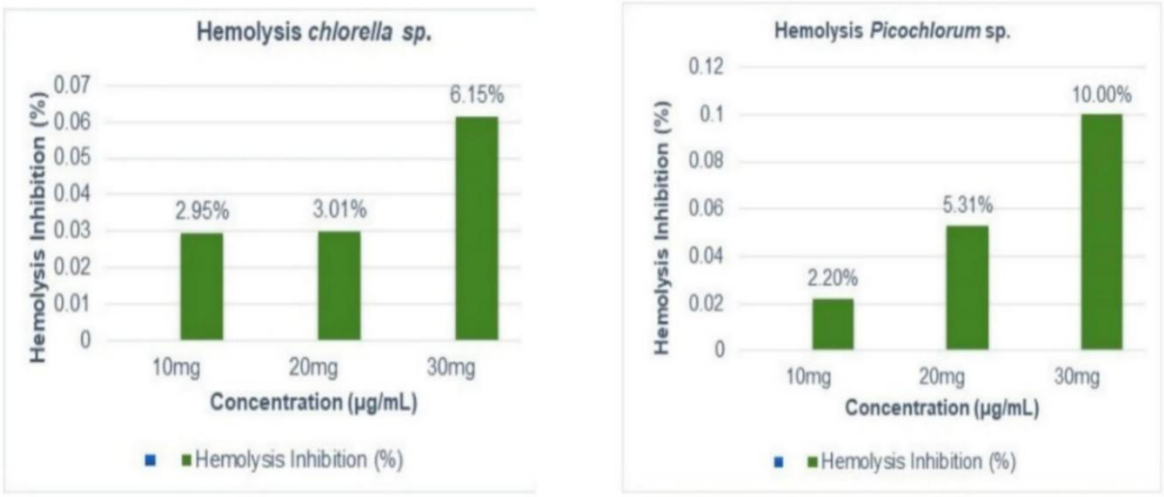


Fig.-9: (a) Micrograph of Silica Nanoparticles, (b) EDX Elemental Composition Spectrum of Silica Nanoparticles Chlorella sp



(a) (b)

Fig.-10: Haemolysis Inhibition bar diagram of (a) Chlorella sp. and (b)Picochlorum sp. Extracts across different concentration (0-30mg/mL)

CONCLUSION

The present study successfully establishes a robust, phyco-pigment mediated protocol for the green synthesis of silica nanoparticles (SiNPs), bridging the gap between marine biotechnology and sustainable material science. By utilizing bioactive pigments from *Picochlorum* sp. and *Chlorella* sp., we demonstrated that marine microalgae serve as efficient "bio-factories" capable of reducing and stabilizing silica frameworks without the need for hazardous chemical precursors. The characterization results provided conclusive evidence of the synthesis's success:

- Structural Integrity: UV-Vis, FTIR, and XRD analyses confirmed the formation of highly pure, amorphous silica nanoparticles with a stable Si-O-Si framework.
- Colloidal Excellence: The remarkably high Zeta potential values (up to -46.5 mV) ensure superior colloidal stability, preventing the aggregation issues common in traditional nanomaterials.
- Morphological Precision: SEM-EDX identified spherical nanoparticles in the 100–400 nm range, with *Picochlorum*-derived variants exhibiting a unique presence of trace Terbium, hinting at potential intrinsic luminescence for future biolabeling.

Most significantly, the hemolysis assay results (<5% lysis) confirm that these biogenic SiNPs are highly biocompatible and safe for blood-contact applications. This research accelerates circularity and integrates seamlessly with UN Sustainable Development Goals (SDGs 9 and 12) by valorizing marine bioresources into high-value technological assets.

Ultimately, this pigment-mediated approach offers an eco-friendly, energy-efficient, and scalable alternative to the conventional Stöber process. Future research should focus on the targeted functionalization of these SiNPs for site-specific drug delivery and advanced environmental catalysis, further exploring the unique "bio-shielding" properties of algal pigments in enhancing nanoparticle performance.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Management of the Sathyabama Institute of Science and Technology, Chennai for providing the necessary facilities and technical support to conduct this research

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3:Good Health and Well-being.* All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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Cite this article as: D. Manoharan *et al.*, *Rasayan J. Chem.*, 19(1), 652-660(2026), https://doi.org/10.317 88/RJC.2026.1919534.

REFERENCES

1. G. Pandey, *Rasayan Journal of Chemistry*, **11,** 942(2018), https://doi.org/10.31788/RJC.2018.1133031.
2. J.R. Smith, P. Taylor, L. Anderson, *Journal of Nanobiotechnology,* **22**, 104(2024), https://doi.org/10.1186/s12951-024-02364-x.

3. Y. Wang, X. Liu, *Discover Applied Sciences*, **7,** 45(2025), https://doi.org/10.1007/s42452-024-05621-3.
4. F. Lananan, N. Ahmad Jusoh, N. Ali, S.S. Lam, A. Endut, *Bioresource Technology,* **141,** 75(2013), https://doi.org/10.1016/j.biortech.2013.03.006.
5. M. Heasman, J. Diemar, W. O'Connor, T. Sushames, L. Foulkes, *Aquaculture Research*, **33,** 637(2002), https://doi.org/10.1046/j.1365-2109.2002.00701.x
6. S. Shyamali, S. Shaishav, R. Darshan, U. Jinal, R. Vinod, P. Aesha, N. Madhuri, *3 Biotech*, **11**, 291(2021), https://doi.org/10.1007/s13205-021-02911-8
7. M. Henriques, A. Silva, J. Rocha, *Journal of Applied Phycology*, **23,** 1249(2007), https://doi.org/10.1007/s10811-011-9750-3.
8. H. Azlina, J. Hasnidawani, H. Norita, S. Surip, *Acta Physica Polonica A*, **129,** 842(2016), https://doi.org/10.12693/APhysPolA.129.842
9. N. Shafiei, M. Nasrollahzadeh, S. Iravani, *Comments on Inorganic Chemistry*, **41,** 317(2021), https://doi.org/10.1080/02603594.2021.1904912.
10. D. James, R. Loris, A.O. Milagros, G. Jens, C. Claudia, S.M. Magoń, A.R. Stephen, B. Giuseppe, *Scientific Reports*, **6,** 27494(2016), https://doi.org/10.1038/srep27494
11. H. Huang, W. Lai, M. Cui, L. Liang, Y. Lin, Q. Fang, Y. Liu, *Scientific Reports*, **6,** 25518(2016), https://doi.org/10.1038/srep25518.
12. B.W. Neun, M.A. Dobrovolskaia, *Methods in Molecular Biology*, **697**, 215(2011), https://doi.org/10.1007/978-1-60327-198-1_23.
13. P.F. Garcia, R.W. Castenholz, *Journal of Phycology*, **27,** 395(1991), https://doi.org/10.1111/j.0022-3646.1991.00395.x
14. A.E. Solovchenko, *Photoprotection in Plants Optical Screening-Based Mechanisms*, **14,** 170(2010), https://doi.org/10.1007/978-3-642-13202-5.
15. X. Zhu, J. Chen, K. Qiu, B. Kuai, *Frontiers in Plant Science*, **8,** 1911(2017), https://doi.org/10.3389/fpls.2017.01911.
16. M. Darbandi, *UV-VIS and Photoluminescence Spectroscopy for Nanomaterials Characterization,* **431**, 452(2013), https://doi.org/10.1007/978-3-642-27594-4_11.
17. S.M. Yeasmin, A. Siddika, S. Tabassum, S.M. Ullah, *Journal of the Turkish Chemical Society Section A: Chemistry,* **9,** 1323(2022), https://doi.org/10.18596/jotcsa.1071086.
18. A. Sikora, D. Bartczak, D. Geißler, *Analytical Methods*, **7,** 9835(2015), https://doi.org/10.1039/C5AY02014J.
19. F.E. Yakin, M. Barisik, T. Sen, *The Journal of Physical Chemistry C*, **124,** 19579(2020), https://doi.org/10.1021/acs.jpcc.0c04602.
20. S.W. Wright, S.W. Jeffrey, R.F.C. Mantoura, *Marine Ecology Progress Series*, **77,** 183(1991), https://doi.org/10.3354/meps077183.
21. Y.P. Ruiz, M.F. Ferrão, M.B. Cardoso, E.A. Moncada, J.H.Z. Dos Santos, *RSC Advances*, **6,** 72306(2016), https://doi.org/10.1039/C6RA03306G.
22. B. Deb, K.S. Manali, M. Indiran, R. Shanmugam, R.P. Kumar, *Journal of Pharmaceutical Research International,* **32**, 25(2020), https://doi.org/10.9734/jpri/2020/v32i1630646.
23. S. Rades, V.-D. Hodoroaba, T. Salge, T. Wirth, M.P. Lobera, R.H. Labrador, K. Natte, T. Behnke, T. Gross, W.E.S. Unger, *RSC Advances*, **4,** 49577(2014), https://doi.org/10.1039/C4RA05092D.
24. H. Huang, W. Lai, M. Cui, L. Liang, Y. Lin, Q. Fang, Y. Liu, *Scientific Reports*, **6,** 25518(2016), https://doi.org/10.1038/srep25518.

[RJC-9534/2025]