

Solvent-volume effects on the structural and textural properties of biosurfactant-assisted mesoporous silica nanoparticles

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ABSTRACT

This study investigated the use of the biosurfactant P34, a low-molecular-weight lipopeptide produced by *Bacillus velezensis* P34, as a green structure-directing agent for the synthesis of mesoporous silica nanoparticles (MSNs). A modified sol-gel method was employed, in which the water content was varied while keeping constant amounts of ethanol, tetraethyl orthosilicate (TEOS), ammonia, and biosurfactant. In the presence of P34, spherical nanoparticles were obtained under all conditions, whereas control syntheses without the biosurfactant yielded particles only at lower solvent volumes. Solvent volume played a decisive role in particle morphology and porosity: compact non-porous nanoparticles were formed at lower water contents, while mesoporous structures with pore diameters between 4.7 and 12.2 nm appeared at higher dilutions. Small-angle X-ray scattering confirmed quasi-spherical morphologies; however, it also revealed increased heterogeneity and rougher interfaces with higher solvent volumes. Fractal analysis of N₂ adsorption isotherms showed that P34 strongly influenced particle texture, enhancing surface roughness at low pressures while reducing the volumetric fractal dimension in mesoporous domains. In addition, zeta potential measurements indicated that all P34-assisted particles were electrostatically stable in suspension (−41 to −59 mV), with the highest stability observed for the non-porous sample. These findings demonstrate that P34 functions as an effective and sustainable bio-template, enabling control over morphology, porosity, and colloidal stability. The resulting nanomaterials are well suited for applications in catalysis, drug delivery, and gas separation, highlighting the potential of biosurfactants as environmentally friendly alternatives to synthetic surfactants in nanotechnology.

1. Introduction

Mesoporous silica nanoparticles (MSNs) have become a cornerstone in nanotechnology due to their high surface area, tunable pore size, and wide applicability in areas such as medicine, corrosion, catalysis, fire suppressors and drug delivery, just to mention a few [1], [2], [3], [4], [5], [6]. Traditional methods for MSN synthesis, such as the Stöber process, rely on the hydrolysis and condensation of alkoxy silanes (e.g., tetraethyl orthosilicate, TEOS) in alcoholic solutions, with ammonia acting as the catalyst [7], [8]. It is worth remarking that these methods

often require surfactants like cetyltrimethylammonium bromide (CTAB) to create mesoporosity, increasing the surface area and pore volume of the particles [9]. However, the widespread use of such chemical surfactants has raised environmental and health concerns due to their toxicity and persistence in the environment [10], [11], [12], [13].

In response to these concerns, alternative surfactant-free methods have been explored. For example, Chen et al. [14] reported a simple and effective approach in which mesoporous silica particles were synthesized through the precise pre-mixing of TEOS, water, and ethanol in controlled molar ratios. This initial step was followed by post-synthetic

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treatment with ammonium hydroxide solutions, which induced mesoporosity. The resulting particles exhibited remarkable textural properties, including a specific surface area of approximately 500 m²/g, accessible mesopores with diameters around 3.0 nm, and a high pore volume of 0.87 cm³/g [14]. However, this proposed method also generated large amounts of ammonia-containing waste, presenting a significant environmental challenge.

To address these limitations, recent research has turned to environmentally friendly molecules, such as biosurfactants, as sustainable templating agents [15], [16], [17]. Biosurfactants are biodegradable, low-cost, and renewable biomolecules produced by microorganisms from different carbon sources, including carbohydrates, industrial or domestic wastewater, starch-based substrates, and vegetable oils [18], [19], [20]. Their applications span multiple industries, including detergents, food, personal care, and agriculture. Significant production is concentrated in Europe (e.g., Biotensidon, Evonik), India, China, Japan, Brazil, and the USA (e.g., Jeneil Biotech), highlighting their global economic importance [21].

Given the potential of biosurfactants in MSN syntheses and the need to explore new sustainable molecules, recent research has focused on innovative alternatives to optimize the formation and textural properties of these nanoparticles. Bacterial lipopeptides have emerged as particularly promising due to their amphiphilic characteristics, enabling excellent emulsification and stabilization. The lipopeptide biosurfactant produced by *Bacillus subtilis* BBK006 was used to synthesize MSNs via a modified Stöber method [22]. After calcination at 550 °C, the resulting particles exhibited uniform spherical shapes (61–300 nm), a specific surface area of 8.3 m²/g, and mesopores with diameters of approximately 15 nm [22]. In this context, bacterial lipopeptides have gained attention due to their ability to modulate the morphology and porosity of silica particles.

Inspired by these advancements, we investigated the application of the P34 lipopeptide, a biosurfactant derived from a *Bacillus velezensis* P34 isolated from the gut of an Amazonian fish, as a green templating agent for MSN synthesis. In addition to its biosurfactant properties, P34 exhibits strong antimicrobial activity, functioning as a bacteriocin-like substance (BLS) with a membrane-active mode of action. It has demonstrated bactericidal effects against *Listeria monocytogenes* at 200 AU/mL and inhibitory effects against *Escherichia coli* and *Salmonella Enteritidis* and *Salmonella enterica* sv. *Enteritidis* in the presence of EDTA [23]. Fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) analyses have revealed that P34 disrupts bacterial cell membranes, leading to structural damage and loss of intracellular material [23]. Furthermore, P34 possesses antiviral properties, inhibiting the replication of equine arteritis virus (EAV) and feline herpesvirus type 1 (FHV-1) by 99.9% and 94.4%, respectively, with a virucidal effect detected against EAV [24].

While the amphiphilic nature of P34 drives the self-assembly mechanism required to template and modulate the morphology and porosity of the silica matrix, its intrinsic broad-spectrum antimicrobial and antiviral activities make it a uniquely functional structure-directing agent. By using P34, the resulting silica nanoparticles may form mesopores with both well-defined structural characteristics and intrinsic biological functionalities. This approach eliminates the necessity for additional post-synthesis modification with external biocidal agents.

In the present study, we compare the textural and morphological properties of silica particles synthesized using P34 with those obtained under identical conditions without the lipopeptide. This investigation provides a comprehensive evaluation of the interaction of P34 with silica precursors and demonstrates its potential as a sustainable alternative for MSN syntheses. By contributing to the development of environmentally friendly nanomaterials, this work advances the field of green nanotechnology with potential applications in biomedicine, catalysis, and other industrial sectors.

2. Methods

2.1. Chemicals and biosurfactant P34

Silica particles were synthesized using aqueous ammonia solution (NH₄OH 28–30%, Sigma-Aldrich), ethyl alcohol (EtOH 99.5% assay, Merck), tetraethyl orthosilicate (TEOS 99.0% purity, Fluka). Distilled water was further purified through the Simplicity185 apparatus (Millipore, Molsheim, France).

The biosurfactant was produced by *Bacillus velezensis* P34, isolated from the intestinal contents of Piau-com-pinta (*Leporinus* sp.) in the Amazon basin, Brazil [23]. Brain heart infusion (BHI) medium (Oxoid, Basingstoke, UK) was used for strain maintenance with 20% (v/v) glycerol at –20 °C.

For biosurfactant production, *Bacillus velezensis* P34 was grown in 200 mL BHI medium at 30 °C in a rotary shaker at 180 rpm for 24 h. Cells were harvested by centrifugation at 10,000 × g for 15 min at 4 °C, and the culture supernatant was subjected to a butanol extraction protocol as described elsewhere [25]. The resulting extract was sterilized by filtration using 0.22 µm membranes (Millipore, Bedford, USA).

2.2. Silica synthesis via basic catalysis using lipopeptide P34 as the biosurfactant

The synthesis of silica nanoparticles using lipopeptide P34 as a biosurfactant was carried out following a procedure described in the literature for another biosurfactant [14], with some modifications. Three solvent volumes (water) were tested to evaluate their effect on the morphological and textural properties of the obtained particles. The volumes used were 50 mL, 75 mL, and 100 mL of water, each containing 1 mL of P34. In parallel, blank syntheses were conducted under identical conditions in the absence of P34 (i.e., without any surfactant/biosurfactant additive), enabling a direct comparison with the P34-containing systems and assessment of the effect of solvent volume. Under magnetic stirring at 400 rpm, 76 mL of ethanol, 10 mL of ammonia (28–30%), and 10 mL of TEOS (added dropwise at a rate of 7.4 mL/h) were incorporated into the initial solution. The reaction mixture was stirred for 1 h 45 min, followed by 24 h of resting, after which the solution was centrifuged at 5000 rpm for 20 min (or 40 min for smaller particles). The resulting sediment was collected, washed with pure water, and dried at 40 °C for 24 h. The samples were labeled as P34-SiNP_{vol} when the lipopeptide was used and SiNP_{vol} for the correspondent blank, with the subscript annotation of the water volume, namely 50, 75 and 100 (mL), respectively. The molar ratios between the reagents for each synthesis are shown in Table 1.

2.3. Characterization of the nanoparticles

2.3.1. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

The morphology of the micro- and nanoparticles of silica was investigated using Scanning Electron Microscopy (SEM) at the Interdepartmental Laboratory of Electron Microscopy (LIME), Roma Tre University. Calcined nanoparticles were examined by Transmission Electron Microscopy (TEM) at the Centro Grandi Attrezzature (CGA), University of Tuscia. SEM images were obtained using a Zeiss Sigma 300 FE-SEM with an ETSE detector, operating at voltages of 3–15 kV and a working

Table 1

Molar proportion between reagents for each silica synthesis with (P34-SiNP_{vol}) and without P34 (SiNP_{vol}).

Sample	TEOS	H ₂ O	EtOH	NH ₄ OH
P34-SiNP ₅₀ & SiNP ₅₀	1.0	61.3	28.8	5.8
P34-SiNP ₇₅ & SiNP ₇₅	1.0	92.0	28.8	5.8
P34-SiNP ₁₀₀ & SiNP ₁₀₀	1.0	122.7	28.8	5.8

distance of 3–8 cm. For TEM, the samples were suspended in 10 μL of distilled water, agitated for 1 min, sonicated for 10 min, then adsorbed onto copper grids coated with Formvar carbon and air-dried. TEM micrographs were taken using a JEOL 1200 EXII electron microscope, operating at an acceleration voltage of 100 kV. Particle size and distribution were analyzed using ImageJ software.

2.3.2. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was performed using a TGA Q50 V6.4 system (TA Instruments) under a continuous flow of high-purity nitrogen at 50 mL/min. Approximately 11 mg of dried sample was heated from 25 to 600 $^{\circ}\text{C}$ at a constant heating rate of 10 $^{\circ}\text{C}/\text{min}$. Prior to TGA, the nanoparticles were recovered by centrifugation and washed to remove non-incorporated or weakly adsorbed biosurfactant from the supernatant. No calcination or solvent-extraction step was applied, since the objective was to evaluate the hybrid silica particles after synthesis while preserving the organic fraction retained in the material.

The mass-loss profiles of P34-mediated silica nanoparticles were compared with those of the corresponding bare silica nanoparticles synthesized under the same solvent-volume conditions. This comparative approach was used to distinguish thermal events associated with the silica matrix, such as water loss and silanol condensation/dehydroxylation, from additional mass losses related to thermally detectable organic material retained in the hybrid particles.

2.3.3. Fourier transform infrared spectroscopy (FTIR)

FTIR analyses were performed using a Shimadzu FTIR-8300 spectrophotometer in the range of 4000–400 cm^{-1} , with 32 scans and a spectral resolution of 4 cm^{-1} . The spectra were used to identify the main functional groups and to compare the structural organization of bare silica nanoparticles and P34-mediated silica nanoparticles synthesized under the same solvent-volume conditions.

For a more detailed evaluation of the silica network, the spectral region between 1350 and 700 cm^{-1} was selected for deconvolution, since this region contains the main Si–O–Si stretching contributions, including transverse optical (TO) and longitudinal optical (LO) modes, as well as silanol-related bands. Peak deconvolution was performed using the Peak Analyzer tool in OriginPro® software. Before fitting, the spectra were baseline-corrected and normalized in the selected region. The deconvolution was performed using Gaussian components assigned to $\text{LO}(\text{SiO})_6$, $\text{LO}(\text{SiO})_4$, $\text{TO}(\text{SiO})_4$, $\text{TO}(\text{SiO})_6$, Si–OH, Si–O $^{\cdot}$, and symmetric Si–O–Si vibrations. Initial peak positions were selected based on literature assignments and on the experimental maxima and shoulders observed in the spectra. During fitting, peak centers were allowed to vary within a restricted interval around the initial positions, while peak widths and intensities were optimized to minimize residuals.

The relative area of each component was calculated as the integrated area of the fitted peak divided by the total integrated area of all fitted components in the 1350–700 cm^{-1} region. The goodness of fit was evaluated using the coefficient of determination, R^2 .

The relative contribution of LO-associated siloxane modes was calculated by Equation (1).

$$\% \text{LO} = [A_{\text{LO}(\text{SiO})_6} + A_{\text{LO}(\text{SiO})_4}] / \Sigma A_i \times 100 \quad (\text{Equation 1})$$

whereas the relative contribution of six-membered siloxane ring components was calculated by Equation (2).

$$\% (\text{SiO})_6 = [A_{\text{LO}(\text{SiO})_6} + A_{\text{TO}(\text{SiO})_6}] / \Sigma A_i \times 100 \quad (\text{Equation 2})$$

where ΣA_i corresponds to the total integrated area of the fitted components in the selected spectral region.

2.3.4. Nitrogen adsorption Porosimetry (N_2) and fractal dimension analysis

The structure and texture of the calcined samples were investigated by N_2 physisorption after thermal treatment at 200 $^{\circ}\text{C}$ for 2 h under

helium flow. Adsorption/desorption isotherms were obtained at -196.15°C using a Micromeritics Gemini V instrument. The specific surface area was calculated using the Brunauer–Emmett–Teller (BET) method [26]. The pore size distribution (PSD) and the total pore volume were determined using the Barrett–Joyner–Halenda (BJH) method [27] applied to the adsorption branch of the isotherms, utilizing the Halsey thickness equation and the Faas correction for the thinning of the adsorbed film.

The textural characterization of the samples was also carried out by calculating their fractal dimension (D) from the N_2 adsorption data, using the Frenkel–Halsey–Hill (FHH) theory of multilayer gas adsorption expressed by Equation (3). The relationship between the parameters D and α , which depends on the interaction of the solid–liquid–gas interface, was calculated using Equations (4) and (5). The equations reflect the dominance of van der Waals forces or liquid–gas surface tension forces, which influence the interface's behavior at different surface coverages.

$$\ln (V/V_m) = \text{const} + \alpha \ln [\ln (P_0/P)] \quad (\text{Equation 3})$$

where V is the volume of adsorbed gas, V_m is the monolayer volume (from BET), α is the slope (power-law exponent), P_0 is the saturation pressure, and P is the equilibrium pressure. The relationship between α and D depends on the dominant interfacial forces. When van der Waals forces prevail, the gas/film interface mimics the surface roughness, and D is calculated using Equation (4).

$$\alpha = (D - 3)/3 \quad (\text{Equation 4})$$

At higher adsorbate coverage, surface tension becomes dominant and capillary condensation occurs, requiring the use of Equation (5):

$$\alpha = (D - 3) \quad (\text{Equation 5})$$

These analyses enabled a detailed assessment of mesopore roughness, complementing the morphological and structural characterization of the synthesized silica nanoparticles.

2.3.5. Small-angle X-ray scattering (SAXS)

A Small-angle X-ray scattering (SAXS) measurements were carried out using a Nano-InXider instrument (Xenocs®, France) at the Nanoscience and Nanotechnology Center of the Federal University of Rio Grande do Sul (CNANO/UFRGS, Brazil). The instrumental apparatus is equipped with a Dectris Pilatus 3 detector and a $\text{CuK}\alpha$ X-ray source ($\lambda = 0.154 \text{ nm}$), calibrated using a silver behenate standard. In this technique, the scattering contribution at each structural level is modeled as the sum of a Guinier exponential term and a structurally limited power-law tail [28]. The Porod region was analyzed to determine the exponent ρ from the power-law decay $I(q) \propto q^{-\rho}$, which provides information on particle surface smoothness and structural heterogeneity. Linear regressions of $\log I(q)$ versus $\log q$ were performed using OriginPro® (OriginLab, USA) to extract the Porod exponents and corresponding statistical parameters (R^2 , adjusted R^2 , Pearson's r , and RSS).

2.3.6. Zeta potential

The zeta potential of the silica particles was measured using a dynamic light scattering technique on a Zeta Sizer Nano (Malvern Instruments, Worcestershire, UK). The dispersions of the P34-SiNP₅₀, P34-SiNP₇₅, and P34-SiNP₁₀₀ samples were diluted with deionized water (approximately 50 mg L^{-1}) to ensure that the light scattering intensity was within the sensitivity range of the instrument and introduced into a closed capillary cell (DTS 1060) for measurement. The zeta potential (mV), electrophoretic mobility ($\mu\text{m cm/V s}$), and conductivity (mS/cm) values were recorded directly from the instrument software.

3. Results and discussion

The results of our multi-technique approach, for the morphological and structural characterization of the synthesized nanoparticles, are

reported and discussed below.

3.1. SEM and TEM observations

The particles obtained from each synthesis were analyzed using scanning electron microscopy (FE-SEM). The images for the syntheses without P34 are shown in Fig. 1(a–d,g), while those with P34 are presented in Fig. 1(b–e,h). Syntheses without the lipopeptide served as controls, facilitating an assessment of the effects of adding this biomolecule.

FE-SEM images at $100,000\times$ magnification, performed for syntheses validation, revealed spherical nanoparticles in the case of the most concentrated reaction medium (SiNP₅₀). As the medium was diluted, clusters without nanoparticles can be visualized at this magnification in the absence of the biosurfactant (Fig. 1d–g). Conversely, the addition of P34 under the same conditions resulted in monodispersed nanospheres observable in all three dilutions, with different size (Fig. 1c–f,i).

The influence of P34 on nanoparticle dimensions can be observed in Fig. 2, which presents the comparison of particle size histograms for silica synthesized in 50 mL of aqueous solution, both with and without the biosurfactant. Fig. 2 also shows the inverse relationship between particle sizes and the solvent volume at constant P34 amount. The control synthesis (SiNP₅₀, Fig. 2a) yielded particles with unimodal size distribution with an average diameter of approximately 136 nm. In contrast, the P34-SiNP₅₀ sample (Fig. 2b) exhibited particles primarily in the 268 nm and 343 nm ranges, suggesting the successful templating

action of the biosurfactant. As the dilution increased, the particle diameters decreased. The P34-SiNP₇₅ sample shows a unimodal distribution centered around 85 nm. Notably, P34-SiNP₁₀₀ exhibits multimodal distribution with smaller primary particles (~ 30 nm, Fig. 2c), and biggest rearranged hollow spheres, as observed by TEM analysis, with diameters centered at ~ 183 nm and at ~ 617 nm (Fig. 2d). SEM images (Fig. 1) further support these findings, showing the formation of spherical particles in all dilutions, with particle size decreasing as the dilution increased.

These results suggest that reaction medium dilution and consequent pH variation modulate TEOS–P34 interactions, affecting the extent of biosurfactant incorporation into the silica framework, in agreement with the mechanism proposed by Sharma et al. [22]. Moreover, the increase in particle size at higher surfactant concentrations is consistent with radially arranged micellar aggregates composed of longer individual micelles, as demonstrated by Privitera et al. (2024) [4].

3.2. Thermogravimetric analysis (TGA)

Fig. 3 presents the thermograms of the synthesized particles, used to determine thermal stability and behavior using thermogravimetric analysis (TGA), in a temperature range of 25 °C to 600 °C. The TGA curve revealed multiple stages of weight loss. All samples showed an initial weight loss of $\sim 10\%$ up to ~ 130 °C, attributed to evaporation of physisorbed water [29]. Above this temperature, control silica nanoparticles (SiNPs) exhibit an additional $\sim 3.5\%$ loss, associated with silica

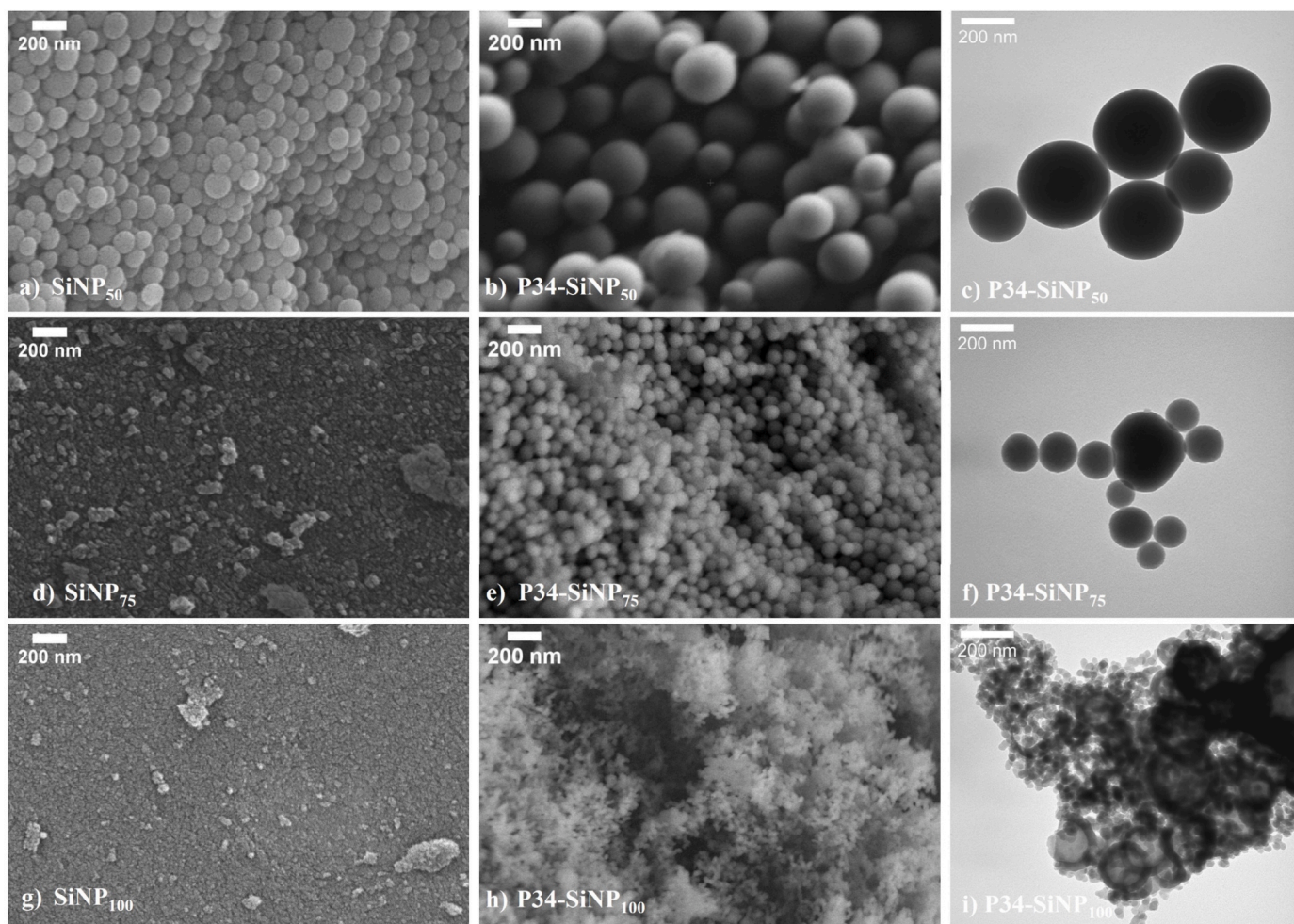


Fig. 1. FE-SEM images of silica particles synthesized with different solvent volumes (water) at magnifications of $100,000\times$. Silica nanoparticles prepared without the biosurfactant are shown in (a, d, g), while those synthesized with the biosurfactant are shown in (b, e, h). TEM micrographs at $100,000\times$ depict silica particles produced in the presence of the biosurfactant (c, f, i).

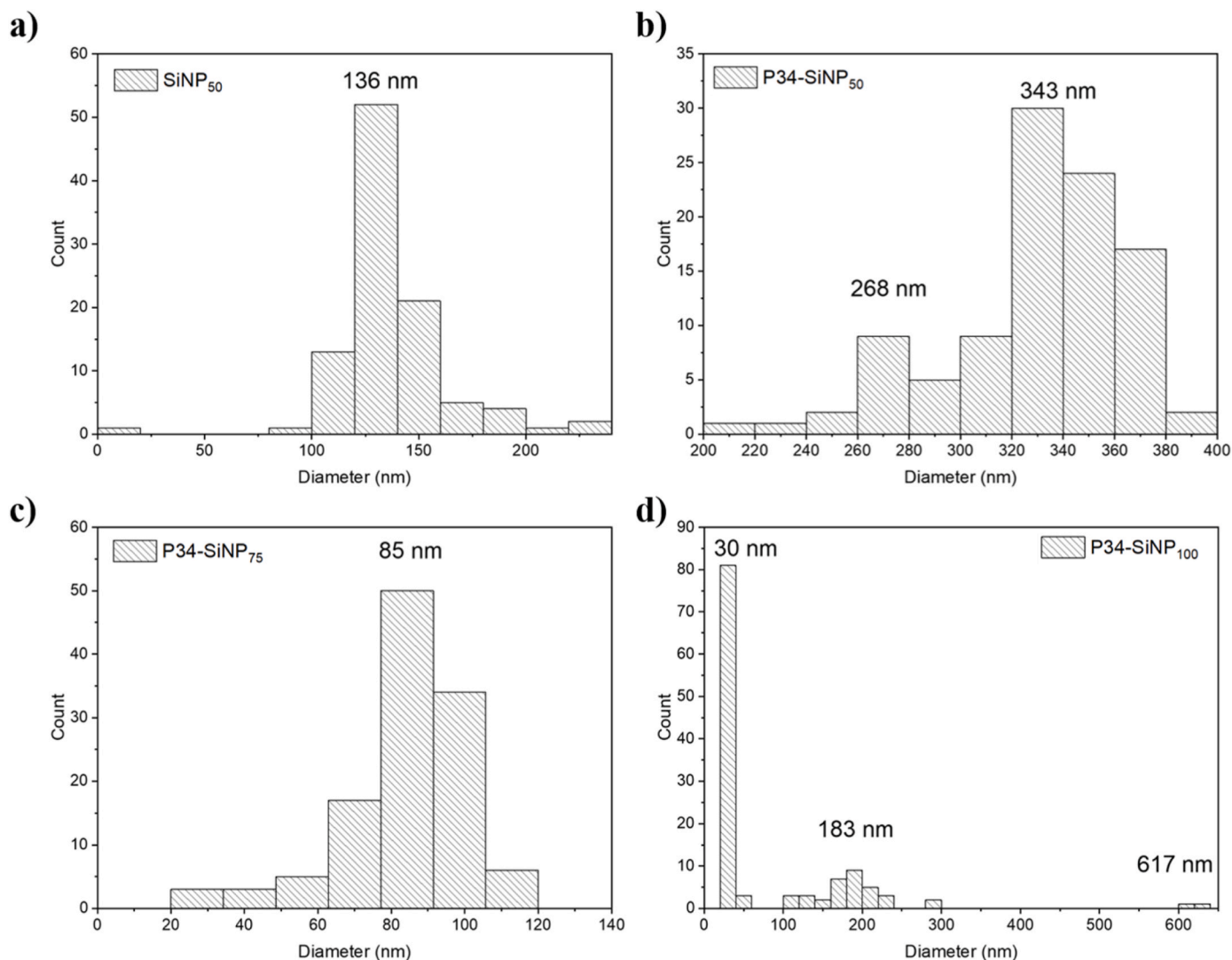


Fig. 2. Histograms of the particle size distribution of synthesized silica: (a) SiNP₅₀, (b) P34-SiNP₅₀, (c) P34-SiNP₇₅ and P34-SiNP₁₀₀ (N = 100).

dihydroxylation [29].

Bare silica nanoparticles showed an additional mass loss (~3.5%) at higher temperatures, associated with condensation of silanol groups and partial dehydroxylation of the silica surface [29]. These events were considered as the thermal contribution of the silica matrix.

Among the P34-mediated samples, P34-SiNP₅₀ showed the most pronounced additional mass loss (~6.7%) above 350 °C, with a degradation event centered around 480–490 °C. This thermal event is consistent with the decomposition of organic material [22], [30] retained in the hybrid silica particles after the washing steps, suggesting the incorporation of P34 into silica channels. In contrast, P34 P34-SiNP₇₅ and P34-SiNP₁₀₀ showed weaker additional mass losses compared with their corresponding bare silica controls, suggesting lower retention of thermally detectable organic material and/or partial overlap between organic degradation and silica-related thermal events. Such limitations are intrinsic to the method and are influenced by the purge gas composition and temperature rather than by the sample mass or balance sensitivity [22], [31].

These results indicate that, although P34 was used during the synthesis of all hybrid samples, the final amount retained in the particles was dependent on the synthesis condition. The higher relative concentration of P34 in the P34-SiNP₅₀ synthesis medium likely favored stronger retention of the biosurfactant within or at the silica network, whereas higher solvent volumes reduced this effect.

3.3. Small-angle X-ray scattering (SAXS)

Complementarily, we employed SAXS measurements to investigate the morphology of the synthesized nanoparticles using different solvent volumes. Fig. 4 displays the scattering intensity $I(q)$ as a function of the scattering vector q for samples synthesized with and without the biosurfactant, red circles and gray squares respectively. In the absence of the lipopeptide, all three samples (SiNPs) exhibit a shoulder feature, whose intensity gradually decreases and shifts toward higher q values with increasing solvent volume, as highlighted by the dashed blue line (Fig. 4a–c). This finding is consistent with the reduction in particle size due to the greater dilution of the reaction medium, which may also explain the absence of detectable particles in the SEM micrographs for SiNP₇₅ and SiNP₁₀₀ (at least at this magnification). A similar behavior is observed in the biosurfactant-embedding systems, where TEM observations confirm that more diluted syntheses yield smaller nanoparticles.

For the P34-template syntheses, the scattering of P34-SiNP₇₅ and P34-SiNP₁₀₀ (Fig. 4b and c) display distinct oscillation. Such behavior is indicative of increased polydispersity and may point to a more heterogeneous distribution of silica inner structures [32]. This observation suggests that the biosurfactant influences both average particle size and the overall organization and uniformity of the nanostructures.

In addition, orange arrows in Fig. 4 highlight the q^{-4} slope expected for smooth spherical particles according to Porod's law. The fitted

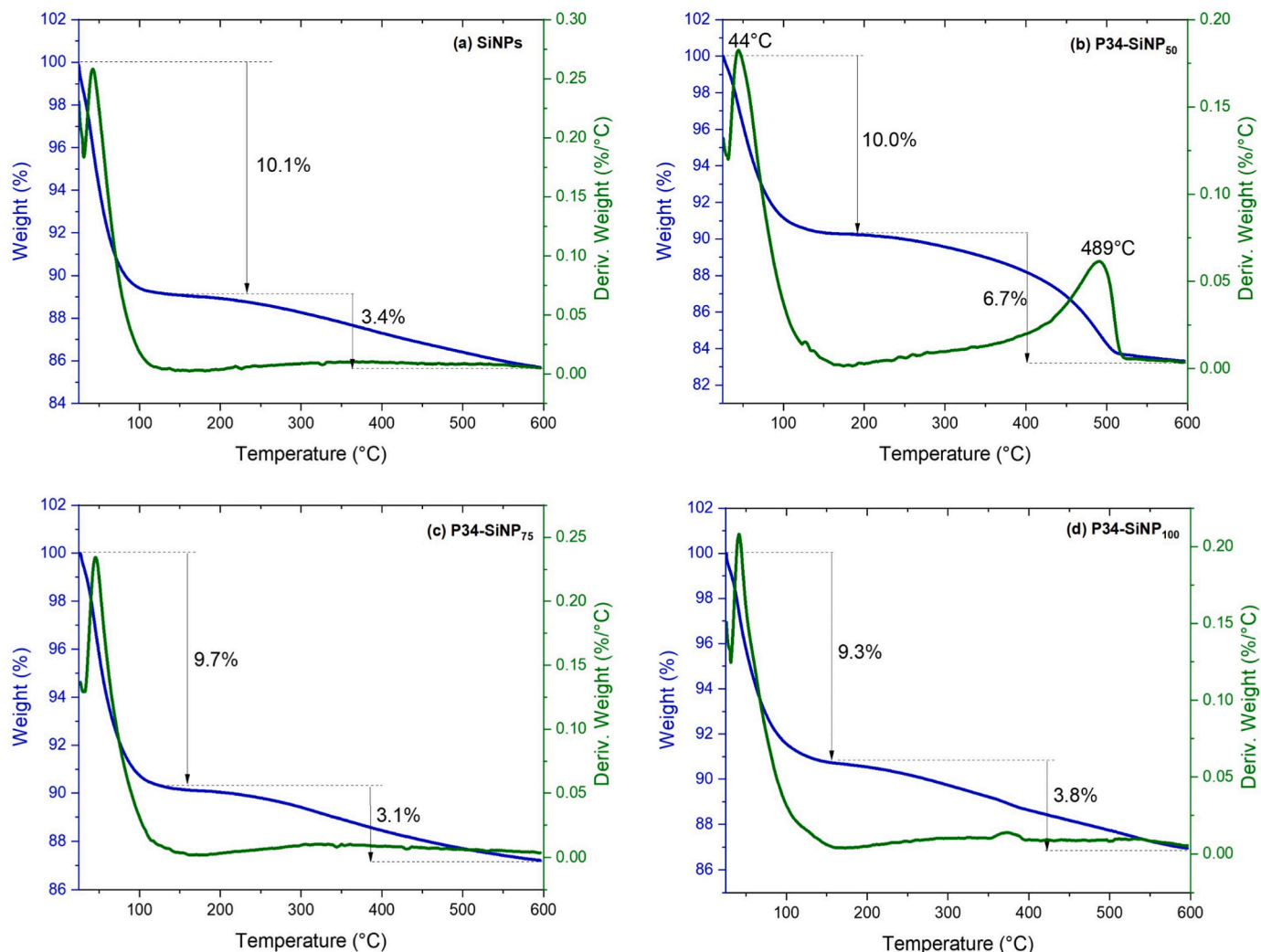


Fig. 3. Thermogravimetric analysis patterns of silica nanoparticles without addition of P34, (a) SiNP_s, and with addition varying the solvent volume: (b) P34-SiNP₅₀, (c) P34-SiNP₇₅ and (d) P34-SiNP₁₀₀.

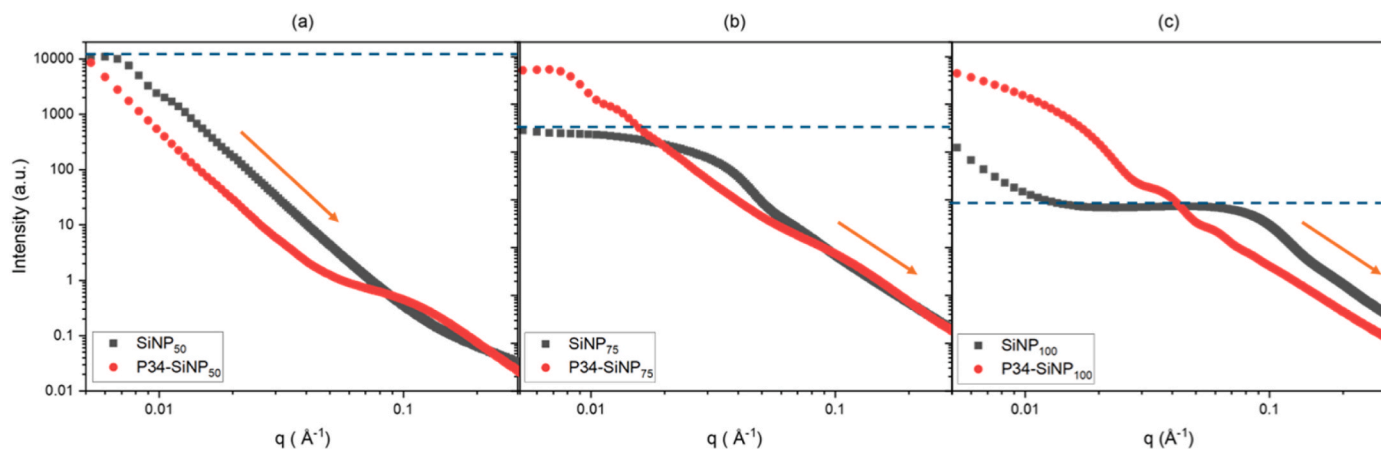


Fig. 4. SAXS intensity profiles $I(q)$ as a function of the scattering vector q for silica nanoparticles synthesized with different solvent volumes: (a) 50 mL, (b) 75 mL, and (c) 100 mL. Gray squares correspond to SiNPs, and red circles correspond to P34-SiNPs. The horizontal dashed line highlights the shoulder feature observed in SiNPs, while the orange arrows indicate the q^{-4} slope expected for smooth spherical particles according to Porod's law. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

exponents, however, deviate slightly from the ideal value (Table 2). For the 50 mL systems, the slopes are close to -3.99 , consistent with quasi-

spherical particles. In contrast, the more diluted samples (75 and 100 mL) show fewer steep decays, with exponents between -3.16 and

Table 2

Fitting parameters of the Porod decay for SiNPs and P34-SiNPs samples.

Sample	SiNP ₅₀	P34-SiNP ₅₀	SiNP ₇₅	P34-SiNP ₇₅	SiNP ₁₀₀	P34-SiNP ₁₀₀
Intercept	-4.548 ± 0.008	-5.275 ± 0.042	-3.509 ± 0.004	-4.312 ± 0.023	-3.263 ± 0.003	-3.545 ± 0.002
Slope (ρ)	-3.991 ± 0.006	-3.985 ± 0.025	-3.368 ± 0.005	-3.779 ± 0.015	-3.563 ± 0.005	-3.162 ± 0.003
Residual Sum of Squares (RSS)	0.0102	0.0982	0.0633	0.0095	0.0033	0.0112
Pearson's r	-0.9999	-0.9990	-0.9997	-0.9997	-0.9999	-0.9999
R^2 (COD)	0.9998	0.9980	0.9994	0.9993	0.9997	0.9998
Adj. R^2	0.9998	0.9980	0.9994	0.9993	0.9997	0.9998

–3.78. Similar deviations have been reported in the SAXS/SANS studies of polymeric microgels, where they were attributed to internal density fluctuations or surface roughness of the collapsed particles [33]. These findings suggest that, while nanoparticles retain an overall spherical morphology, higher dilution induces structural heterogeneity and rougher interfaces. Far from indicating a lack of synthetic control, this structural shift reflects the dynamic micellar polymorphism intrinsic to the multi-component P34 lipopeptide matrix. Because the extract naturally contains a mixture of fengycin and iturin families with distinct hydrophobic chain lengths, variations in the solvent volume directly modulate their self-assembly boundaries and interfacial curvature. Therefore, this structural heterogeneity represents the physical-chemical fingerprint of a flexible, bio-templated directing process, which actively drives the expanded pore tunability observed in the subsequent textural analyses.

Table 2 shows the statistical parameters and confirms the robustness of the Porod fits. Coefficients of determination (R^2 and adjusted- $R^2 > 0.998$) and Pearson's r values close to -1 indicate good correlations between the theoretical model ($I(q) = q^{-\rho}$) and the experimental data. Moreover, the low uncertainties associated with the fitted slopes (± 0.006 – 0.025) strengthen the reliability of the observed deviations from the ideal Porod exponent ($\rho = 4$). Thus, the statistical quality of the fits reflects intrinsic structural heterogeneities of the nanoparticles.

3.4. Fourier-transform infrared spectroscopy (FTIR)

FTIR analysis revealed relevant differences between the silica systems synthesized with and without the P34. The symmetric and asymmetric vibrations of siloxane were identified by deconvolution in the spectral range of 1350–700 cm^{-1} , along with contributions associated with silanol and Si–O[−] groups Fig. 5. This spectral region provides valuable insights into the structural characteristics of these materials, particularly through the band corresponding to Si–O–Si stretching vibrations, including transverse optical (TO) and longitudinal optical (LO) components. These bands correspond to primary siloxane ring structures, such as four-membered (SiO)₄ and six-membered (SiO)₆ rings, as detailed in Table 3.

Deconvolution of FTIR spectra enabled the accurate assignment of vibrations characteristic of the silica structure, providing deeper insight into how reaction parameters affect the resulting particles, especially the organization of siloxane arrangements. The fitting was performed using Gaussian components in OriginPro®, after baseline correction of the selected spectral region. The quality of the fittings was evaluated by the coefficient of determination (R^2) and adjusted R^2 , which are presented in Table 3. Variations in the percentages of these components (TO and LO modes) have been reported in the literature upon the incorporation of organic groups or molecules into the silica network [22]. Generally, a higher concentration of organic groups or molecules favors the formation of six-membered rings, which are energetically less strained and more capable of accommodating non-reactive groups within the silica framework [34]. Using the areas under the peaks corresponding to these components, the relative area contribution of each vibrational mode was determined, as shown in Table 3.

Additionally, two structural descriptors calculated from the deconvoluted areas were compared with the solvent volume, as illustrated in

Fig. 6. The first descriptor corresponds to the relative contribution of LO-associated siloxane modes, calculated as the sum of the areas assigned to LO(SiO)₆ and LO(SiO)₄. The results indicate that the LO component ranged between approximately 25% and 30% for control particles, while for particles synthesized with the biosurfactant, this value increased to approximately 30%–40%, decreasing with the dilution of the synthesis medium (Fig. 6a). The second descriptor corresponds to the relative contribution of six-membered siloxane ring components, calculated as the sum of the areas assigned to LO(SiO)₆ and TO(SiO)₆. The percentage of (SiO)₆ increased from approximately 17% at the lowest dilution to 21% at the highest dilution when P34 was added compared with the control. At the intermediate dilution, however, the values were comparable to those of the control, showing no evident variation under this condition.

Notably, these variations in the silica network suggest that P34 influenced the condensation and local organization of the silica framework during synthesis. This effect is likely associated with the surfactant behavior of P34 and its possible interactions with hydrolyzed silica species, rather than with the simple physical presence of an inert molecule.

3.5. Nitrogen adsorption Porosimetry (BET and BJH methods)

In addition to the changes in particle size and morphology, textural modifications, such as the formation of mesopores, were observed via nitrogen adsorption analyses, as shown in Fig. 7.

In Fig. 7 (a) and Fig. 8 (a) the synthesis performed with the lowest solvent volume (SiNP₅₀) exhibits no evidence of porosity in the whole pore size range (type III isotherms without hysteresis loop). In contrast, the samples carried out with higher solvent volumes (SiNP₇₅ and SiNP₁₀₀) display pores with diameters of around 3.8 nm and type IV isotherms with a hysteresis loop, typical of mesoporous materials. However, for these samples, the available data only demonstrates the presence of a mesoporous environment and does not provide direct evidence that porosity is associated with discrete mesoporous nanoparticles. The observed textural properties could arise from a porous continuous phase rather than individual particles, although the possibility that the material consists of mesoporous nanoparticles cannot be excluded at this stage.

Replicating these same experimental conditions in the presence of P34, also lead to non-porous particles at the lowest dilution (P34-SiNP₅₀). For the two higher dilutions (P34-SiNP₇₅ and P34-SiNP₁₀₀), mesopores were observed as shown in Fig. 7 (b) and Fig. 8 (b), suggesting that the synthesis conditions strongly influenced pore formation. Table 4 summarizes the textural analysis results, obtained via BET and BJH methods, and indicates an increase in specific surface area and pore volume with increasing solvent volume, both in the absence (from 26 to 392 m^2/g and 0.068 to 0.420 cm^3/g) and in the presence of P34 (from 22 to 131 m^2/g and 0.035 to 0.365 cm^3/g). Additionally, in the presence of the biosurfactant, an increase in pore diameter was also observed (from 0 to 12.2 nm).

Table 4 also shows that the textural properties achieved in this work surpass those reported by Sharma et al. for biosurfactant-mediated mesoporous silica synthesized at 550 °C [22]. Although both studies employ TEOS, ammonia, and ethanol within sol–gel routes, the

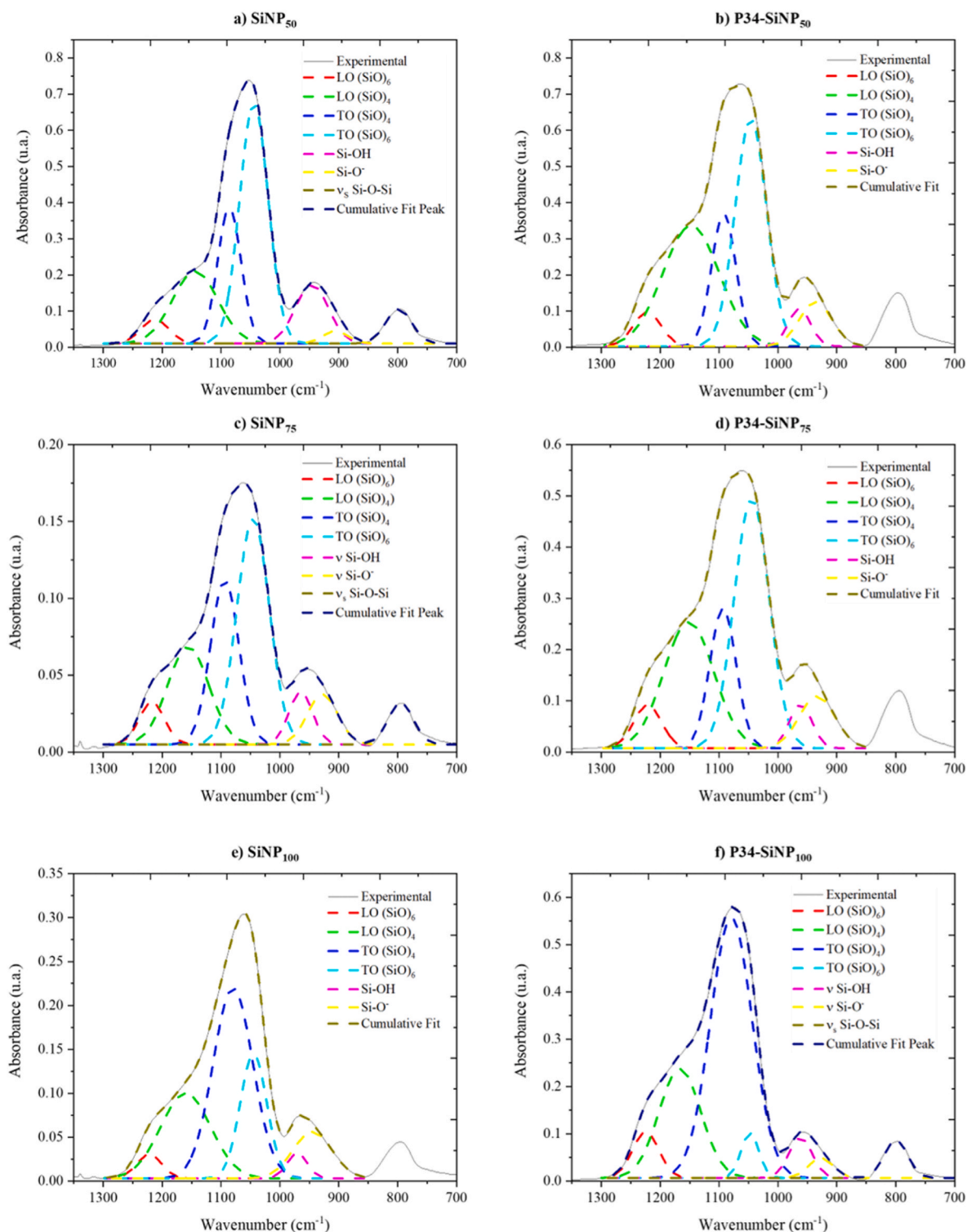


Fig. 5. Deconvolution of the spectral region obtained by FTIR between 1350 and 700 cm^{-1} for nanoparticles produced with different volumes of solvent, with and without P34.

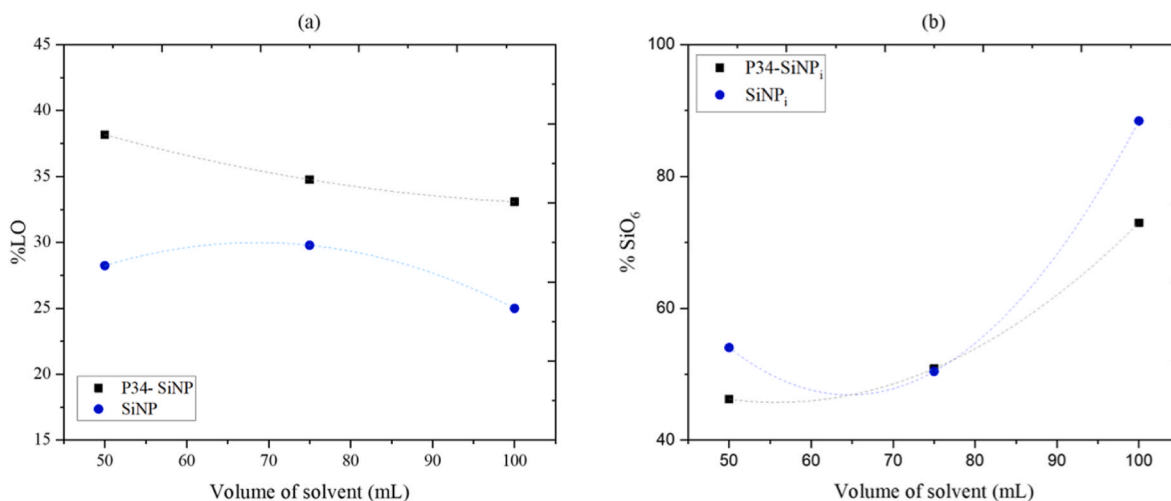
experimental conditions are not directly comparable: Sharma et al. varied only calcination temperature, whereas the present work systematically altered water content, which strongly affects hydrolysis, condensation kinetics, and ultimately the development of porosity. It is worth noting that the occurrence of capillary condensation at higher relative pressures ($P/P_0 > 0.9$) for the P34-SiNP₁₀₀ sample - while resulting in an average BJH pore size of 12.2 nm - is attributed to the hybrid organic-inorganic nature of the uncalcined pore walls. Residual

lipopeptide matrices change the local surface energy and nitrogen adsorption film dynamics, shifting the condensation boundary without implying an interstitial or interparticle origin. This intraparticle nature is further validated by the strict structural differences against the template-free control samples, where the pore size remained locked at 3.8 nm, proving the active structure-directing role of the biosurfactant.

A more fundamental difference concerns the biosurfactant source and the level of chemical characterization available for each system.

Table 3Main results obtained from the deconvolution of the characteristic silica region ($1350\text{--}700\text{ cm}^{-1}$) into Gaussian components for the synthesized nanoparticles.^a

Sample		$\nu_{\text{as}}\text{Si-O-Si}$				$\nu\text{Si-OH}$	$\nu\text{Si-O}^-$	$\nu_{\text{s}}\text{Si-O-Si}$
		LO (SiO) ₆	LO (SiO) ₄	TO (SiO) ₄	TO (SiO) ₆			
SiNP ₅₀	ν	1212.2	1144.0	1085.9	1044.1	945.8	905.8	798.3
	% A	3.961	19.282	18.775	40.305	11.400	1.843	5.288
R^2 : 0.99982								
Adj. R^2 : 0.99981								
SiNP ₇₅	ν	1217.7	1155.7	1093.7	1045.4	964.6	927.8	794.8
	% A	1.481	5.573	6.473	10.158	1.724	2.105	1.451
R^2 : 0.99973								
Adj. R^2 : 0.99971								
SiNP ₁₀₀	ν	1221.0	1165.7	1082.9	1045.7	970.2	935.7	797.5
	% A	1.823	7.537	19.789	8.293	2.163	2.863	2.077
R^2 : 0.99955								
Adj. R^2 : 0.99953								
P34-SiNP ₅₀	ν	1220.6	1150.9	1091.7	1045.5	959.4	923.0	798.1
	% A	6.958	32.894	23.531	41.012	8.575	3.997	7.392
R^2 : 0.99959								
Adj. R^2 : 0.99958								
P34-SiNP ₇₅	ν	1220.7	1154.3	1093.4	1044.0	959.9	923.5	797.3
	% A	5.226	23.074	17.973	35.130	6.520	4.740	5.860
R^2 : 0.99982								
Adj. R^2 : 0.99982								
P34-SiNP ₁₀₀	ν	1224.7	1166.6	1079.3	1047.7	961.8	927.0	799.9
	% A	5.492	20.525	49.024	3.60E+00	4.240	2.215	3.981
R^2 : 0.99962								
Adj. R^2 : 0.99961								

^a ν : wavenumber of maximum absorption [cm^{-1}]; % A: relative integrated area contribution of each fitted component.**Fig. 6.** – Relative contribution of (a) LO-associated siloxane modes and (b) six-membered siloxane ring components calculated from the deconvolution of the FTIR spectra in the $1350\text{--}700\text{ cm}^{-1}$ region as a function of the solvent volume used in syntheses of nanoparticles.

Sharma et al. used 0.57 g of a crude acid-precipitated extract from *Bacillus subtilis* BBK006 and represented it in their proposed mechanism as a “surfactin-type” lipopeptide. However, the chemical composition of that extract was not experimentally characterized in their article, and surfactin was used only as a structural model [22]. Herein, 1 mL of a butanolic extract obtained from *Bacillus velezensis* P34 was used. Similarly, the specific P34 batch used in the present nanoparticle synthesis was not chemically characterized in this work. Instead, the discussion of its composition is based on previous characterizations performed by the group that produces P34 [25], [35]. Previous studies reported that P34-derived preparations contain cyclic lipopeptides mainly associated with fengycin and iturin/bacillomycin families, with surfactin-related production also described under specific culture conditions [25], [35]. Therefore, unlike Sharma et al., who assumed a surfactin-type structure for an uncharacterized crude extract, our interpretation of the P34 system is based on previously reported compositional data for P34-derived

lipopeptides, while acknowledging that the specific batch used here was not directly quantified.

Because the two biosurfactants differ in composition, purity, aggregation behavior, and likely in critical micellar and interfacial activities, their templating effects cannot be interpreted in terms of dosage alone. Instead, the present comparison suggests that the chemical identity of the biosurfactant plays a decisive role in directing particle formation. This is supported by the fact that, under high-dilution conditions without P34 (SiNP₇₅ and SiNP₁₀₀), spherical particles did not form, whereas the presence of P34 consistently enabled nanoparticle generation and controlled morphology.

Although the mechanistic basis of this effect is beyond the scope of this study, and no mechanistic equivalence should be assumed between the two biosurfactants, the results indicate that P34 operates as an effective green structure-directing agent even in conditions where silica condensation alone is insufficient to yield well-defined particles.

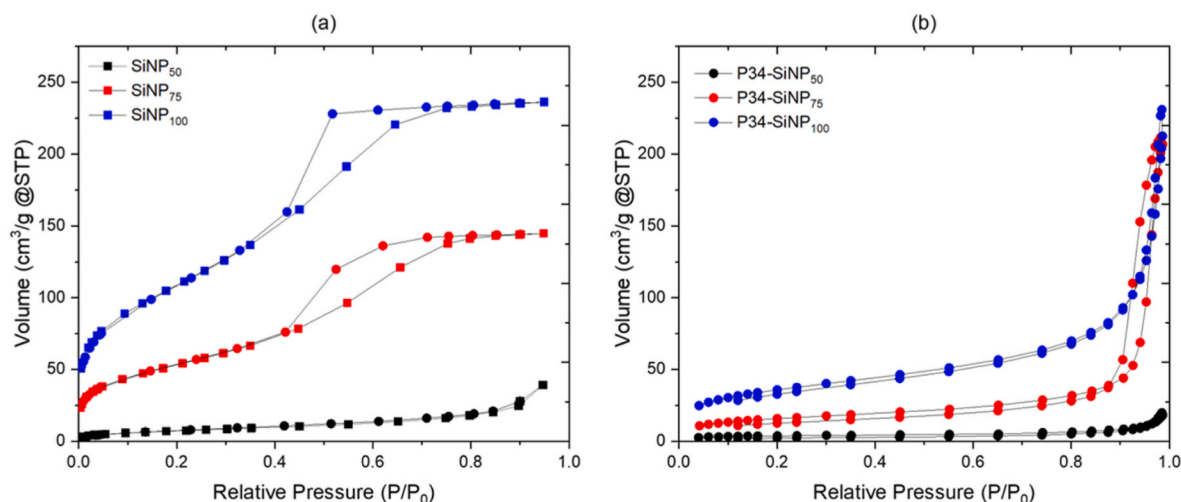


Fig. 7. N_2 adsorption and desorption isotherms for nanoparticles without (a) and with (b) lipopeptide P34.

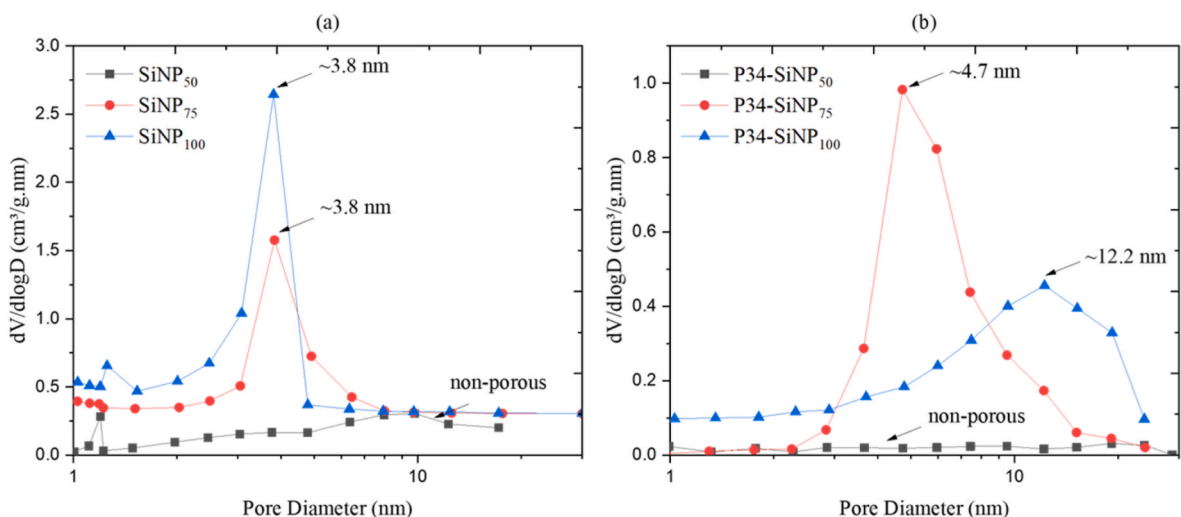


Fig. 8. Pore size distribution obtained by the BJH method by varying the volume of solvent, without (a) and with (b) lipopeptide P34.

Table 4

Specific surface area (S_{BET}), pore volume (V_p), and peak pore diameter (d_p) of silica nanoparticles synthesized with different water volumes, with and without the biosurfactant P34.

Sample	S_{BET} ($m^2 g^{-1}$)	V_p ($cm^3 g^{-1}$)	d_p (nm)
SiNP ₅₀	26	0.068	-
SiNP ₇₅	191	0.249	3.8
SiNP ₁₀₀	392	0.420	3.8
P34-SiNP ₅₀	22	0.035	-
P34-SiNP ₇₅	58	0.328	4.7
P34-SiNP ₁₀₀	131	0.365	12.2
BMSN (550 °C) [22], ^a	8	0.020	14.9

^a Literature data, included for comparison.

To further evaluate the potential of the *Bacillus velezensis* P34-derived lipopeptide extract as a green templating agent, its performance can be contextualized with other alternative biosurfactants reported in recent literature. For instance, Tescione et al. used eco-friendly templates to direct the synthesis of mesoporous silica, achieving distinct structural arrangements [36], while Jovita et al. and Gao et al. demonstrated the versatility of bio-based surfactants in controlling pore characteristics [37], [38]. In the present work, P34-derived lipopeptide extract effectively directed the formation of silica nanospheres under

high-dilution conditions, whereas the corresponding template-free controls did not preserve the same morphological organization. In addition, the use of P34 enabled modulation of the pore size, reaching an average pore diameter of up to 12.2 nm, without requiring hazardous co-surfactants or harsh post-synthetic treatments. These findings indicate that the P34-derived extract can act as an effective bio-based structure-directing agent for the synthesis of mesoporous silica nanoparticles.

It is worth noting that the structure-directing role of the P34 extract appears to be strongly dependent on the solvent volume. At the lowest water volume tested, 50 mL, the templating effect was less pronounced. In contrast, higher dilution conditions, 75 and 100 mL, favored a different organic-inorganic interfacial arrangement during silica condensation. This behavior was associated with an increase in the average pore diameter, reaching 12.2 nm for the P34-SiNP₁₀₀ sample, whereas the template-free control samples maintained a pore size of approximately 3.8 nm. The formation of isolated and less aggregated nanospheres observed by FE-SEM supports the interpretation that the measured porosity is not primarily associated with interparticle voids, but rather with structural features generated within the silica particles. Furthermore, the loss of morphological control observed in the blanks under high dilution indicates that simple variations in the sol-gel water-to-TEOS ratio are insufficient to induce the same structural organization,

thereby supporting the active role of the P34-derived lipopeptide matrix within the processing conditions evaluated.

In this context, although spherical silica nanoparticles can be obtained in plain water systems at low volumes, such systems showed limited structural tunability under the conditions investigated. In contrast, the use of the P34-derived lipopeptide extract as a green template provides a combined advantage: it allows modulation of the internal pore architecture, specific surface area, and surface fractal

features simply by modulating the solvent volume, while simultaneously yielding a functional bio-hybrid material without requiring additional post-synthesis grafting steps.

Fractal dimension analysis

We investigated the porous structure of the particles synthesized with the biosurfactant and varying solvent volumes, as well as their respective controls, by determining fractal dimensions using the

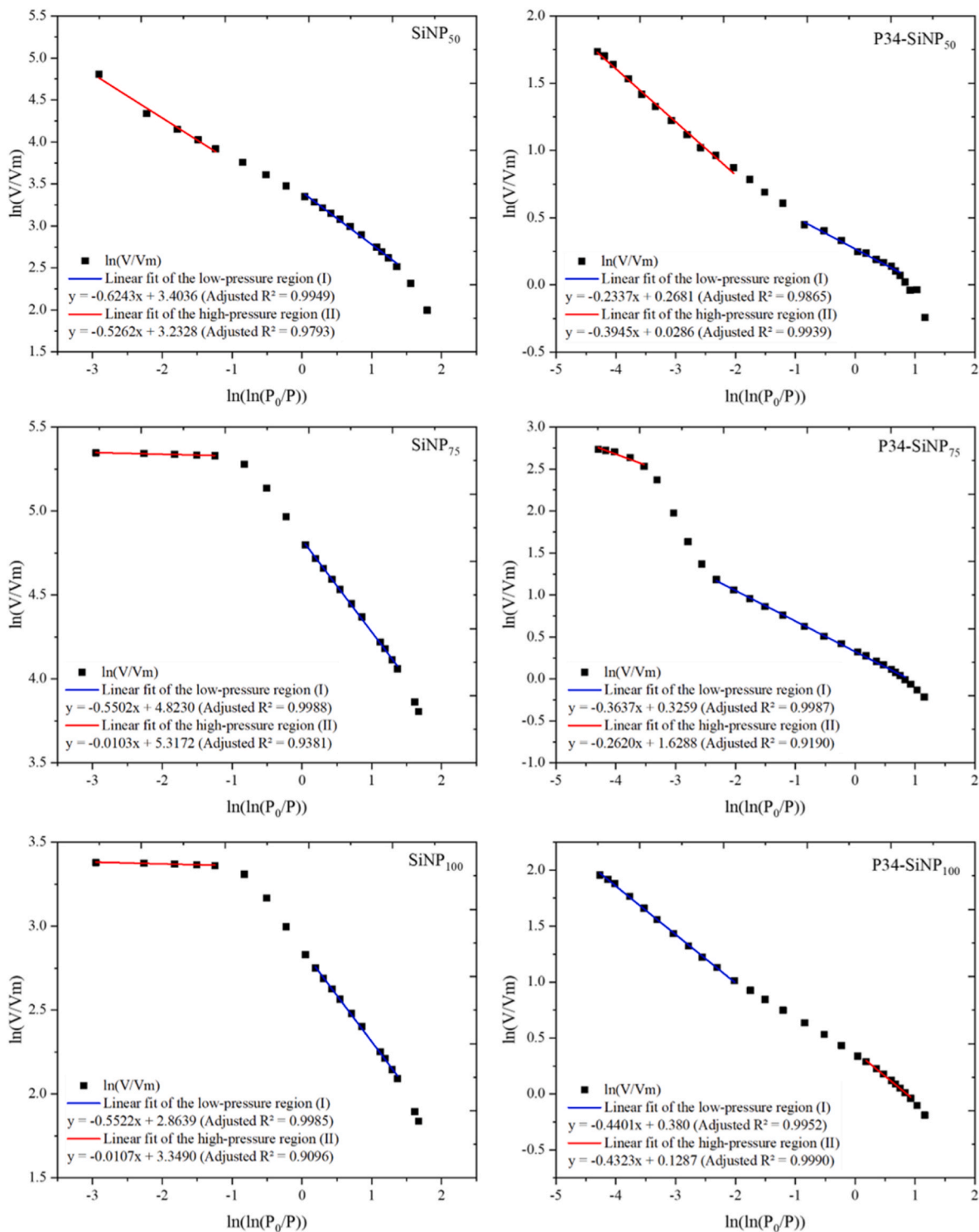


Fig. 9. Fractal analysis of silica nanoparticles synthesized with and without P34, using N_2 adsorption isotherms. Linear fits from the nitrogen adsorption isotherms as $\ln(V/V_m)$ versus $\ln(\ln(P_0/P))$ are shown for the low-pressure region (red) and the high-pressure region (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

modified FHH model. Fig. 9 presents the graphs and fitted data from the nitrogen adsorption isotherms as $\ln(V/V_m)$ versus $\ln(P_0/P)$.

In general, all samples allowed for linear fitting in two distinct regions: low to intermediate pressures (Region I) and higher pressures (Region II). These regions were defined based on the best linear fit, which was also the most reliable method reported in the literature for analyzing another mesoporous material [39]. Table 5 summarizes the results obtained for each sample in both regions, including the slope of the linear fit (α), fractal dimension (D), and adjusted coefficient of determination (R^2).

Region I provided better fittings between the data and the model, with $R^2 > 0.98$ for all samples. This indicates that the surface fractal dimensions (D_1) determined in this region can be more accurately estimated than the volumetric fractal dimensions (D_2) derived from the high-pressure region, where $R^2 > 0.92$. As shown in Fig. 10a, the surface roughness increased with the solvent volume used in the syntheses containing P34, the fractal dimension, varying from 3% to 15%, while the controls exhibited variations of no more than 3%. These results clearly demonstrate the influence of the biosurfactant on particle texture, particularly on the roughness of the microporous surface (represented at low pressures).

In the higher-pressure region (Region II), fractal dimensions that reflect the complexity of mesoporous volume increased by up to 20% with solvent volume for the controls. Conversely, for the syntheses containing P34, these dimensions decreased up to 16%, as shown in Fig. 10b. This reduction in D_2 in the presence of the lipopeptide is consistent with the observed increase in pore volume for the P34-SiNP particles.

These evaluated fractal dimensions provide a quantitative measure of the boundary complexity and surface roughness of the synthesized silica networks. The variation in D values across the series suggests that the presence of the P34-derived lipopeptide extract affected the organization of the silica framework during sol-gel condensation. In general, higher surface fractal dimensions are associated with more irregular and heterogeneous surfaces, which may be advantageous for applications requiring high interfacial area, such as catalyst immobilization or adsorption.

The fractal dimensions obtained from N_2 adsorption were slightly lower than those derived from SAXS. This difference is expected because the two techniques probe different structural features. SAXS is sensitive to electron-density fluctuations and internal interfaces within the silica network, whereas N_2 adsorption reflects the surface of pores accessible to nitrogen in the dried material. As a result, drying effects, pore accessibility, and possible retained organic residues may contribute to the lower apparent surface fractal dimension estimated from N_2 adsorption.

3.6. Zeta potential analysis

To complement the structural analyses, zeta potential (ZP) measurements were conducted. The measured ZP values provide insights into the stability of the suspensions. This property allows an objective evaluation of the stable behavior of particles in suspension. ZP values below -30 mV or above $+30$ mV indicate stable particles, while values

within this range suggest instability, meaning the particles tend to agglomerate and sediment [40].

The ZP values of the silica particles synthesized with the biosurfactant and different volumes of water (P34-SiNP₅₀, P34-SiNP₇₅, and P34-SiNP₁₀₀) were all below -30 mV, as shown in Table 6. The most negative value, -59.1 mV, was observed for particles synthesized under the least diluted conditions (P34-SiNP₅₀). This suggests that higher concentrations of the biosurfactant increased the negative charge of the particles, resulting in a more stable suspension. The greater the magnitude of the ZP, the stronger the electrostatic repulsive forces, leading to enhanced stability. However, all silica particles synthesized with the biosurfactant exhibited ZP values indicative of stable behavior in suspension.

It is noteworthy that the particle with the highest ZP magnitude (-59.1 mV) was also identified as non-porous in the textural analysis. In non-porous particles, surface charge distribution is more uniform, and the zeta potential reflects a homogeneous surface. The value of -59.1 mV for P34-SiNP₅₀ indicates a high density of negative charge uniformly distributed across the surface. For mesoporous particles, the average ZP (in absolute terms) tends to be lower due to the influence of potentials within the pore openings, as the three-dimensional porosity and the relationship between pore openings and specific surface area significantly affect the ZP [41].

Thus, the presence of mesopores introduces local variations in the electric potential, resulting in a lower observed average zeta potential (in absolute terms). The ~ 11 mV reduction in ZP between non-porous and mesoporous particles (P34-SiNP₇₅ and P34-SiNP₁₀₀) can be attributed to the potential variations within the pores compared to the homogeneous external surface. This is consistent with reports suggesting that the average ZP of mesoporous particles can be up to 25% lower than the theoretical value for a flat surface [41].

4. Conclusions

Using a modified Stöber method, this study evaluated the effects of the biosurfactant P34 on the formation of silica nanoparticles. P34 proved to be an effective surfactant and structure-directing agent, enabling the formation of spherical nanoparticles under the tested conditions. In contrast, control solutions without P34 produced nanoparticles only using lower volume of solvent. The results also showed the significant impact of solvent volume on the porosity and morphology of the particles: lower solvent volumes yielded compact, non-porous nanoparticles, whereas higher solvent volumes allowed the formation of mesoporous structures, with pore diameters ranging from 4.7 to 12.2 nm, while employing the biosurfactant. The biotemplate used herein helped in tailoring the structural properties of the nanoparticles. The observed results support the potential of P34 as a green alternative to synthetic surfactants for nanomaterial synthesis. Molecular-level interactions responsible for this templating effect remain to be further elucidated and should be the object of further investigations, physico-chemical mechanisms underlying silica-biosurfactant interactions. Overall, this work demonstrates that P34-mediated synthesis is a promising sustainable route for producing silica nanoparticles with tunable morphology and mesoporosity, reducing the need for

Table 5

Fractal dimensions of silica nanoparticles synthesized with and without P34 at different solvent volumes, calculated using the modified FHH model from N_2 adsorption isotherms. Results are shown for low-pressure (Region I) and high-pressure (Region II) domains.

Sample	Region I				Region II			
	α_1	R^2	d_1	D_1	α_2	R^2	d_2	D_2
SiNP ₅₀	-0.624	0.995	-0.873	2.376	-0.526	0.985	0.315	2.474
SiNP ₇₅	-0.55	0.999	-0.651	2.45	-0.01	0.954	0.95	2.969
SiNP ₁₀₀	-0.552	0.999	-0.657	2.448	-0.011	0.928	0.996	2.968
P34-SiNP ₅₀	-0.234	0.988	0.299	2.299	-0.395	0.995	-0.184	2.606
P34-SiNP ₇₅	-0.364	0.999	-0.091	2.636	-0.262	0.939	0.214	2.214
P34-SiNP ₁₀₀	-0.44	0.996	-0.32	2.56	-0.432	0.999	-0.297	2.568

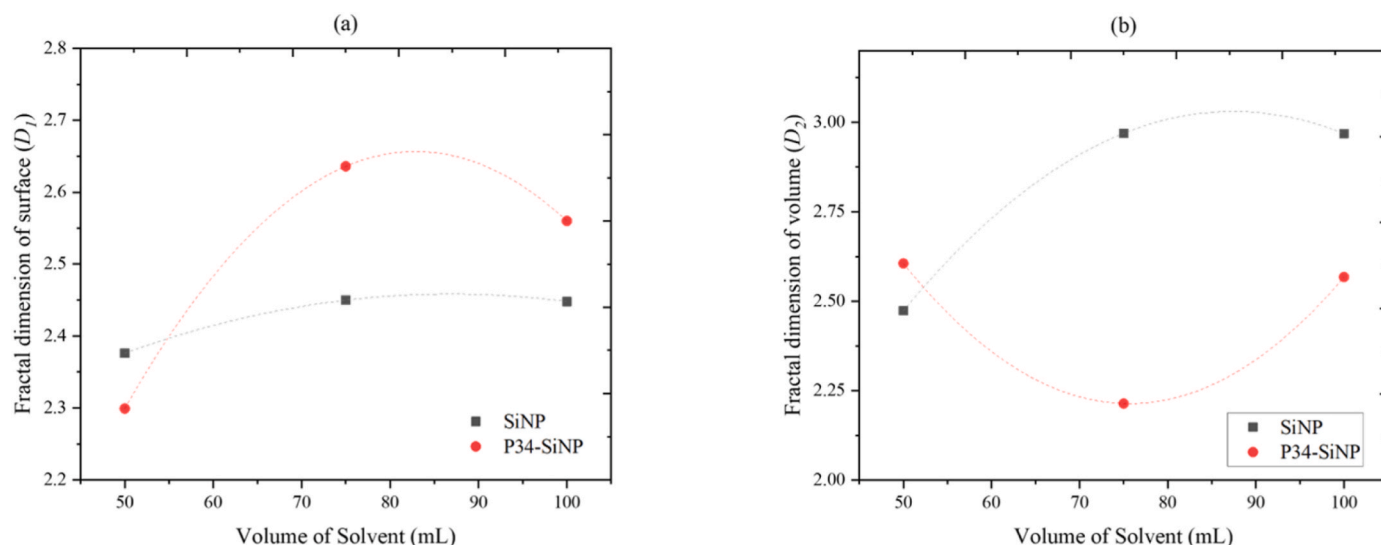


Fig. 10. Fractal dimension of surface (a) and volume (b) of silica synthesized with and without lipopeptide P34 when varying the volume of solvent.

Table 6

Values obtained from Zeta Potential (ZP), electrophoretic mobility (Mob) and conductivity (Cond) of P34-SiNP particles.

Sample	ZP (mV)	Mob ($\mu\text{mcm/Vs}$)	Cond (mS/cm)
P34-SiNP ₅₀	-59.1	-4.636	0.0135
P34-SiNP ₇₅	-41.6	-3.263	0.0096
P34-SiNP ₁₀₀	-42.6	-3.342	0.0122

conventional synthetic surfactants or additional post-synthesis modification steps.

CRedit authorship contribution statement

Fernanda A. Arzani: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Antonella Privitera:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Simone Bernardini:** Data curation, Formal analysis, Investigation. **Sergio Lo Mastro:** Data curation, Formal analysis, Investigation. **Simonetta Tuti:** Data curation, Formal analysis, Investigation, Validation, Writing – review & editing. **Anna Rita Taddei:** Data curation, Formal analysis, Investigation. **Adriano Brandelli:** Data curation, Formal analysis, Investigation, Resources. **Flavio Fonseca Veras:** Data curation, Formal analysis, Investigation, Resources. **João H.Z. dos Santos:** Data curation, Funding acquisition, Resources, Supervision, Validation, Writing – review & editing. **Armida Sodo:** Data curation, Formal analysis, Project administration, Resources, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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