

# Protein Mediated Silica Particles with pH Controlled Porosity and Morphology

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## Abstract

**Background:** The silica leaching activity of some of the mystifying non-pathogenic BKH1 bacteria present in the cluster of hot springs (temperatures range 35°C - 80°C) at Bakreshwar (West Bengal, India, 23°52'48"N; 87°22'40"N) has provided some significant advancements in the field of nanotechnology. The present investigation was designed to synthesis the silica particles using bioremediase protein at different pH conditions. **Methods:** A secretary bacterial protein bioremediase (UniProt Knowledgebase Accession Number P86277) isolated from a thermophilic non-pathogenic bacterium BKH1 (GenBank Accession No. FJ177512) has been used to synthesis the silica particles at different pH conditions (pH at 3.0, 5.0, 8.0, 10.0, and 12.0 respectively). The silica particles were synthesized by the action of bioremediase protein on Tetra-ethyl-orthosilicate (TEOS) under ambient condition. Morphological and compositional studies of the biosynthesized silica particles were characterized by Field emission scanning electron microscope (FE-SEM) equipped with Energy dispersive X-ray analyser (EDX). **Results:** The Fourier transformed infra-red (FTIR) spectroscopic analysis confirmed the nature as well as occurrence of several functional groups surrounded on the silica particles. The amorphous nature of the prepared silica particles was confirmed by X-ray diffractometer (XRD) study. The Zeta potential ( $\zeta$ ) study revealed the stability of silica particles in neutral pH environment. The Brunauer-Emmett-Teller (BET) surface area measurement confirmed the porosity variation in all biosynthesized silica particles prepared at different pH conditions. Raman spectra analytically depend on their respective specific surface (BET) area. Thermogravimetry tool was used to monitor the effects of the thermal treatment on the surface properties of all the samples. **Conclusions:** The method for the synthesis of silica particles at different pH condition using the protein bioremediase has a special implication as it is an environmentally benign, cost-effective and facile technique which may have conceivable application in chromatographic packing. In addition, controlling of size as well as porosity of the silica particles can be achievable by pH as an only variable.

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## Keywords

Bioremediase Protein, Tetraethyl Orthosilicate, Green Chemistry, Silica Particles, Porosity

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## 1. Introduction

Porous materials have stimulated growing interests due to their wide-ranging prospective utilizations in several applications fields e.g., as catalysis, drug delivery, chemical sensors, chromatography, micro reactor and biological images [1]-[6]. Silica particles have been established as probable promising material due to its some distinctive properties [7] [8]. On the other hand, the use of silica-based chromatographic packing is usually popular because of the mechanically, thermally and chemically stable bonded phases in the pH range of about 2 to 8. The pH value can be extended up to 11 using double end-capped silica-based bonded phases with organic buffers below 313 K [9]. At low pH value, the chemically bonded ligands are slowly detached from the surface via the process of hydrolysis of siloxane linkages [10]. On the other hand disbanding of the silica matrix will take place at higher pH value. Due to the low stability of silica-based materials at low and high pH values, other porous inorganic oxides like alumina, zirconia, titania etc. are often used. But the commercial availability of inorganic oxides like alumina, zirconia, titania etc. are limited as compare to silica-based materials [11]. So, now a day it is more desired to prepare materials with controlled shape and pore size having commercial importance. Silica is most usable and commercially available inorganic oxide for chromatographic packing applications. In general, the technique used for synthesis of silica particles is sol-gel methods. Apperence in the sol-gel processing of ceramic and glass materials on Silica gels preparation was done [12]. Stober proposed first mono-disperse and nonporous silica spheres with the hydrolysis of tetra-ethyl-orthosilicate (TEOS) in strongly basic medium. Stober and Fink well established theaction of mechanism involved in the formation of silica nanoparticles ( $\text{SiO}_2$ -NPs) [13] [14]. Bogush and Zukoski procured mono-dispersed  $\text{SiO}_2$ -NPs with controlled hydrolysis of TEOS in ethanol [15]. Sung Kyoo Park provided  $\text{SiO}_2$ -NPs from TEOS in ethanol by using a semi-batch process in order to control the particle properties [16]. Butnow it becomes a high demanding field to focus on the more eco-friendly and green facile synthesis technique of  $\text{SiO}_2$ -NPs. In the present work, we recommended a single step, rapid and simple technique for the formation of amorphous, along with thermally and chemically stable silica particles with controlled size using a microbial secretary protein (bioremediase) at different pH conditions and its plausible application in the field of chromatographic packing.

## 2. Material and Methods

### 2.1. Preparation of Materials

#### 2.1.1. Reactants

The bacterial strain BKH1 (Gen Bank accession number FJ177512) was obtained from

the Biophysics Laboratory, Department of Physics, Jadavpur University [17] [18]. The analytical grade TEOS was purchased from the Merck, USA. All other fine chemicals were purchased from Spectro. Chem. Pvt. Ltd. India.

### 2.1.2. Isolation and Purification of the Bioremediase Protein

Bioremediase protein is secreted by the bacterium BKH1 in the growth medium while growing in the medium at pH 8.0 and temperature 65°C. The bacteria are cultured a sealed glass pressure vial anaerobically (in presence of CO<sub>2</sub> atmosphere) [17]. The growth medium consists of Fe(OH)<sub>3</sub>—0.1 M, Na<sub>2</sub>HPO<sub>4</sub>—0.6 g/L, KCl—0.33 g/L, Na<sub>2</sub>CO<sub>3</sub>—2.5 g/L, yeast extract—0.02% and peptone—0.5%). The pH of the medium is kept at 8.0 and the temperature for optimum growth is maintained at 65°C temperature [17]. The enzyme was purified from 6 to 7 days old bacterial culture medium similarly as described by Biswas *et al.* [18]. The bioremediase enzyme present in the growth medium was concentrated by lyophilization technique and the concentrated protein was loaded on Sephadex G-100 column for purification. Double step 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 [18].

### 2.1.3. Biosynthesis of Silica Particles

For the biosynthesis of silica particles, the purified bioremediase bacterial protein (1 mg/ml) was put drop wise in a desired amount organic silica rich substrate (TEOS, 0.1 mol/L) solution in a 5 ml plastic vial and kept at ambient temperature for 24 h. Same experiment was performed by making the reaction mixture at different pH conditions (at 3, 5, 8, 10 and 12). After adding the protein, a spherical structure of silica particles was produced 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°C temperature in vacuum desiccators for 24 h. Finally, the dried balls were crushed by mortar pestle to get fine powder for further auxiliary characterizations. Fineness of the powder was formed mechanically as far as practicable.

## 2.2. Particles Characterizations

### 2.2.1. Optical and Electron Microscopy

The as-prepared silica particles powder sample was dispersed in DI-water and the optical characterizations were performed with UV-Vis spectrophotometer (UV-3101PC, Shimadzu). The surface morphology images and compositional compositions of the biosynthesized silica particles were investigated in Field Emission Scanning Electron Microscopy (FE-SEM, FEI INSPECT F50) equipped with Energy dispersive spectrometer (EDX, Bruker System).

### 2.2.2. Zeta Potential

The synthesized silica particles powder was dispersed in Milli-Q water and the particles size as well as zeta potential experiments were characterized through DLS (Zeta Sizer,

Nano ZS 90, Malvern) experiment.

### 2.2.3. X-Ray Diffraction

XRD measurements of as-prepared powder silica particles sample was carried out on a Bruker, D8 Advance, X-ray diffractometre instrument operated at a voltage of 40 kV and a current of 40 mA with Cu-K<sub>α</sub> radiation.

### 2.2.4. FTIR and Raman Spectra Analysis

FTIR was used to identify the types of chemical bonds and functional groups presents in silica particles. The prepared silica particles 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<sup>-1</sup>. The Raman spectroscopy for the as-prepared silica particles 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.

### 2.2.5. Thermo-Gravimetric Weight-Loss Analysis (TGA)

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

### 2.2.6. Nitrogen Adsorption-Desorption

An adsorption-desorption isotherm of nitrogen on prepared silica particles was performed by Brunauer-Emmett-Teller (BET) surface area analyser, SA 3100, Beckman Coulter, Switzerland.

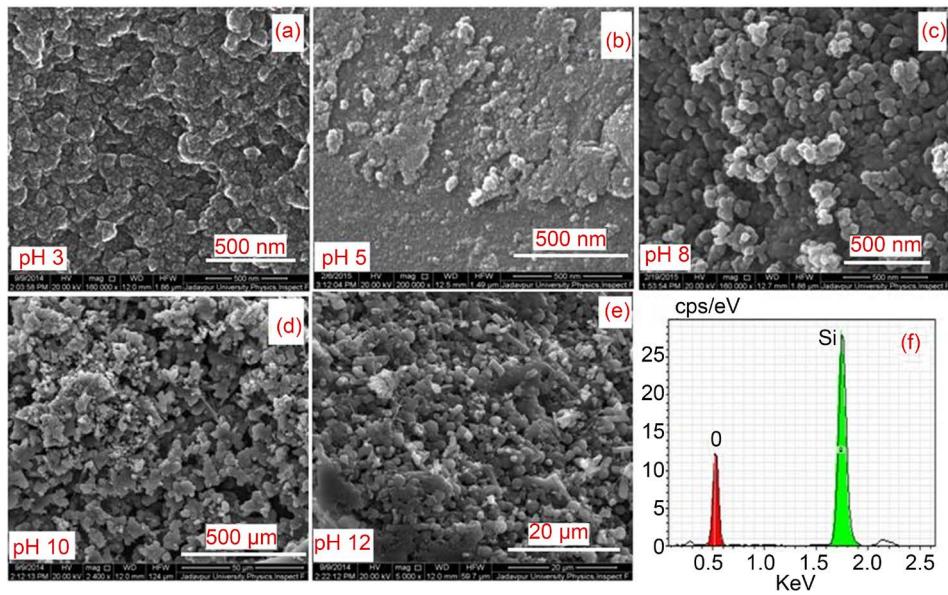
### 2.2.7. Statistical Analysis

For each experiment all prepared samples were tested repeatedly. Each experiment was repeated at least three times. Data were presented as average and  $\pm$  SD where ever is possible.

## 3. Results and Discussions

### 3.1. Morphological and Compositional Characterization of Silica Particles

**Figures 1(a)-(e)** specified the FESEM images of the as prepared silica particles formed by the interaction between bioremediase protein and TEOS solution at different pH values. It confirmed the formation of Silica particles. The size of the particles varied from few nanometres to few micrometres which were shown in **Table 1** ( $n = 100$ ). FESEM images also concluded the fact that the variation of shape as well as the size of the prepared Silica particles directly depend on the condition of pH of the reaction medium. The synthesized particles were quite regular in shape and uniformly distributed for sample prepared at pH 8.0. EDX analysis (**Figure 1(f)**) of all the prepared silica particles indicated the two strong peaks which could be attributed to Silicon (Si) and Oxygen (O<sub>2</sub>) species. The results of EDX and FESEM analyses for the size of the silica particles were presented in **Table 1** ( $n = 3$ ).



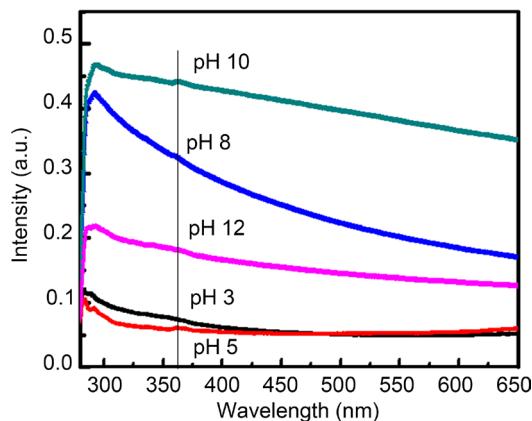
**Figure 1.** FE-SEM image (a)-(e) and EDX spectrum (f) of silica particle formed by the Interaction of bacterial protein with TEOS at different pH. The experimental setup was repeated thrice and photo-micrographs were taken at different magnifications.

**Table 1.** Average particle size of Silica particles.

Sample name	Average size	Elementary analysis by EDX (%)
S <sub>1</sub> (pH 3)	40 ± 10 µm	Si-66.67, O <sub>2</sub> -33.33
S <sub>2</sub> (pH 5)	1.5 ± 0.5 µm	Si-66.67, O <sub>2</sub> -33.33
S <sub>3</sub> (pH 8)	15 ± 5 nm	Si-66.67, O <sub>2</sub> -33.33
S <sub>4</sub> (pH 10)	3 ± 0.5 µm	Si-66.67, O <sub>2</sub> -33.33
S <sub>5</sub> (pH 12)	10 ± 2 µm	Si-66.67, O <sub>2</sub> -33.33

### 3.2. Optical Properties

The UV-Vis absorption spectra of silica particles suspended in DI-water exhibited an absorption peak at ~360 nm (**Figure 2**). The estimated band gaps ( $E_g$ ) of Silica particles were tabulated in **Table 2** ( $n = 3$ ), resembling an earlier reported value [19].  $\text{SiO}_2$ -NPs have been widely considered owing to several appealing optical phenomena caused by point defects generated from  $\text{SiO}_4$  continuous network system, including both vacancies of oxygen and silicon. This network system is denoted as a neutral (diamagnetic) oxygen vacancy that comprises a simple oxygen vacancy and twofold-coordinated silicones. Theoretical studies using *initio* molecular orbital calculation showed that non-paramagnetic defect is caused by a singlet-singlet transition [20] [21] [22] [23] [24]. Whereas, silica in pure state was observed as UV-inactive and the value of  $E_g$  is ~5 eV. But as-prepared silica particles exhibited  $E_g$  as  $3.4 \pm 0.1$  eV, which concluded the fact that silica particles were UV-inactive.



**Figure 2.** Absorbance spectra of the silica particles dispersed in aqueous medium. The experimental setup was repeated thrice and no differences in results were found.

**Table 2.** Band gap calculation of Silica particles synthesized at different pH values.

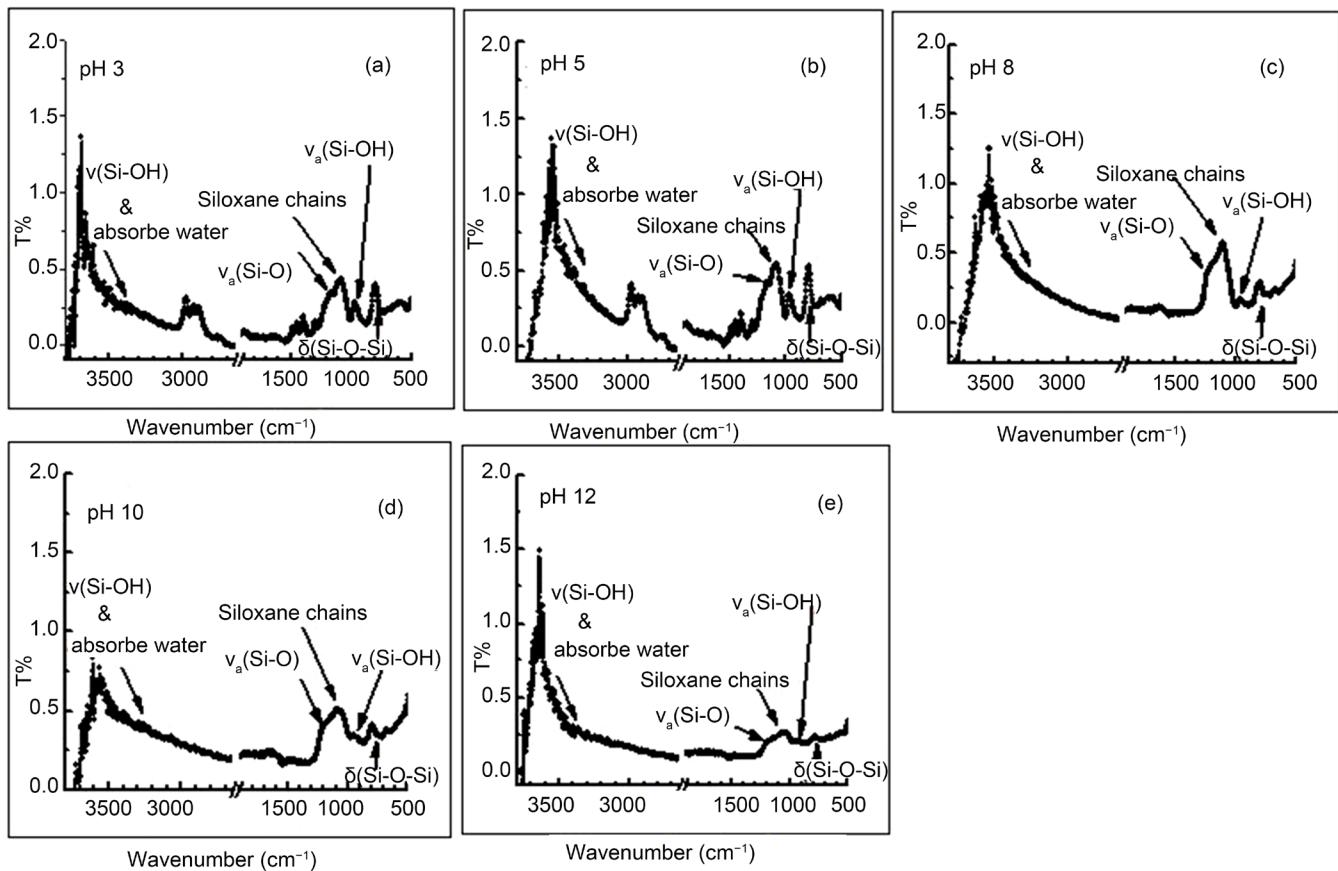
Sample name	Absorption Peak (nm)	E <sub>g</sub> value (eV) (n = 3)
S <sub>1</sub> (pH 3)	363	3.43 ± 0.1
S <sub>2</sub> (pH 5)	363	3.43 ± 0.1
S <sub>3</sub> (pH 8)	360	3.40 ± 0.1
S <sub>4</sub> (pH 10)	362	3.43 ± 0.1
S <sub>5</sub> (pH 12)	363	3.43 ± 0.1

### 3.3. FTIR and XRD Study

FTIR spectra (**Figure 3**) indicated the successful synthesis of silica particles by bacterial protein assisted route. Two peaks around  $1097\text{ cm}^{-1}$  and  $790\text{ cm}^{-1}$  were the strong evidence for presence of silicon dioxide ( $\text{SiO}_2$ ) in all prepared samples. The absorption bands between  $800$  and  $1269\text{ cm}^{-1}$  had been attributed due to the superposition of various  $\text{SiO}_2$  peaks, Si-OH bonding and residual organic groups. Water molecules observed a strong characteristics absorption band between  $3300\text{cm}^{-1}$  and  $3600\text{ cm}^{-1}$  conveyable to O-H stretching in H-bonded water. The scissor bending vibration of water molecule at around  $1630\text{ cm}^{-1}$  band also concurred with the above information [19]. All characteristics absorption bands are tabulated in **Table 3** (n = 3). Absence of any sharp crystalline diffraction peak in XRD pattern suggested the amorphous nature of silica particles (**Figure 4**).

### 3.4. Stability of Silica Particles

The stability of the as prepared silica particles were determined by observing the Zeta potential ( $\zeta$ ) of the silica particle as tabulated in **Table 4** (n = 3) in neutral pH environment (**Figure 5**). It suggested that silica particles surrounded with negative type of surface charges were quite stable. The negative nature of Zeta potential prevents from agglomeration and promotes stable dispersion in neutral surroundings.



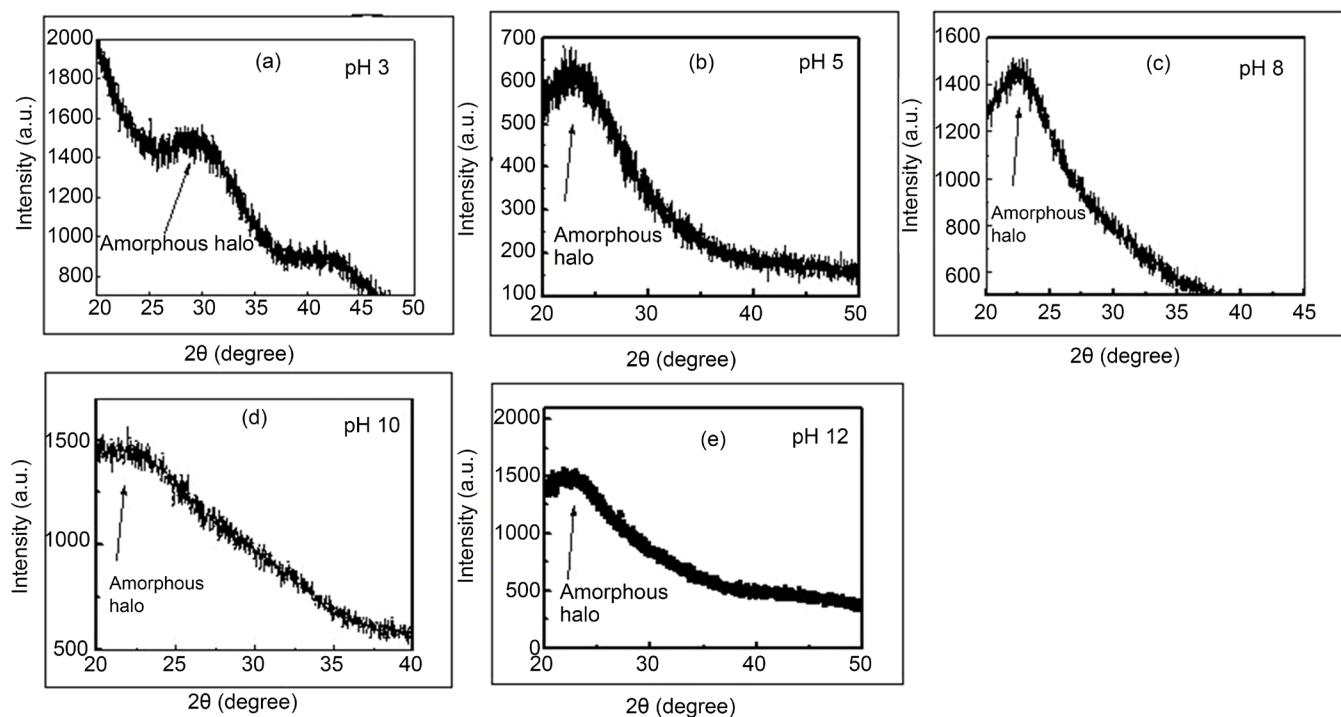
**Figure 3.** FT-IR spectra (a)-(e) of the silica particle formed by the interaction of bacterial protein with TEOS at different pH. The experimental Setup was repeated thrice and no significant errors were noted.

**Table 3.** FTIR bands formation of Silica particles synthesized at different pH values.

Sample name	Si-O-C Bonding (nm)	(ν <sub>as</sub> , Si-O) (nm)	(ν <sub>s</sub> , Si-O) (nm)	Si-OH Bonding (nm)	O-H stretching (nm)	scissor bending (nm)
S <sub>1</sub> (pH 3)	1145	1087	786	964	3606, 3691	Not detected
S <sub>2</sub> (pH 5)	1164	1068	790	964	3494, 3610	Not detected
S <sub>3</sub> (pH 8)	Not detected	1107	794	948	3533	1627
S <sub>4</sub> (pH 10)	Not detected	1107	790	925	3610	1631
S <sub>5</sub> (pH 12)	Not detected	1087	790	Not detected	3637	Not detected

### 3.5. Thermo-Gravimetric Weight-Loss Analysis

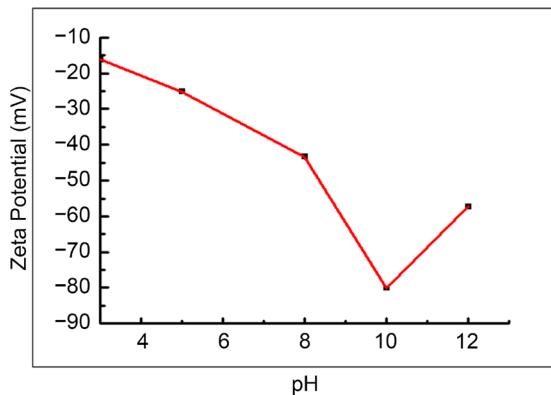
**Figure 6** indicated the thermal properties of biosynthesized silica particles measured from room temperature ( $30^{\circ}\text{C}$ ) to a very high temperature ( $800^{\circ}\text{C}$ ) by using TGA. TGA mainly used for characterizing the structural properties as well as for confirmation of the thermal stability of the materials. A ceramic ( $\text{Al}_2\text{O}_3$ ) crucible was used for heating and measurements were carried out in  $\text{N}_2$  atmosphere at the heating rate of  $10^{\circ}\text{C}/\text{min}$ . The diminishing of mass due to thermo-desorption of the surfactant used during



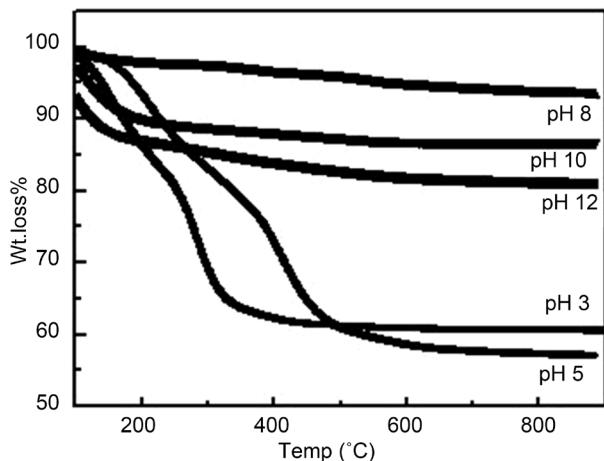
**Figure 4.** XRD patterns (a)-(e) of the silica particle formed by the interaction of bacterial protein with TEOS at different pH. The experimental Setup was repeated thrice and no significant errors were noted.

**Table 4.** Zeta Potential of Silica particles synthesized at different pH values.

Sample name	Zeta potential ( $\zeta$ ) value (mV)
S <sub>1</sub> (pH 3)	(-) 16.1 ± 0.1
S <sub>2</sub> (pH 5)	(-) 25.2 ± 0.3
S <sub>3</sub> (pH 8)	(-) 43.4 ± 0.2
S <sub>4</sub> (pH 10)	(-) 80.1 ± 0.2
S <sub>5</sub> (pH 12)	(-) 57.4 ± 0.3



**Figure 5.** Zeta Potential ( $\zeta$ ) curve of synthesized silica particle formed by the interaction of bacterial protein with TEOS at different pH in neutral environment. The experimental setup was repeated thrice and the average with Standard Error of Zeta potential was presented.



**Figure 6.** Thermo-gravimetric weight-loss curves for all synthesized silica particle formed by the interaction of bacterial protein with TEOS at different pH. The experimental Setup was repeated thrice and no significant errors were noted.

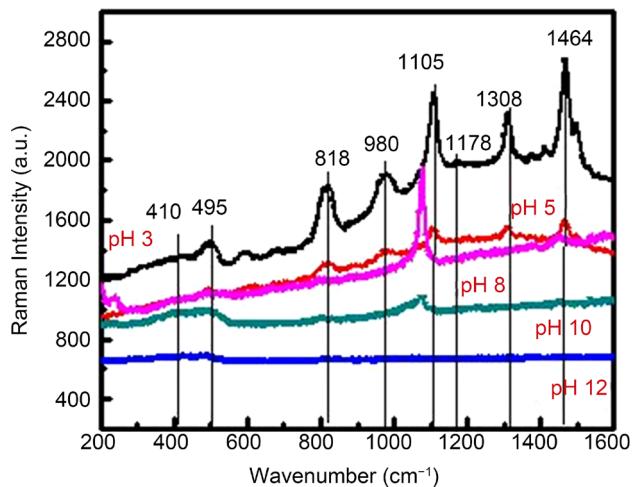
preparation at low pH value ( $\text{pH} = 3.0$  and  $5.0$ ) occurred at below  $300^\circ\text{C}$ . It is known that all the water molecules of the surfactant are removed when temperature is raised nearly equal to  $130^\circ\text{C}$  [11]. Between  $250^\circ\text{C}$  and  $650^\circ\text{C}$  temperature range the mass of the silica particles was almost remained constant for all samples (Figure 6). This implied that the as prepared silica particles could be used in chromatographic packing for its stability at higher temperature.

### 3.6. Raman Spectra Analysis

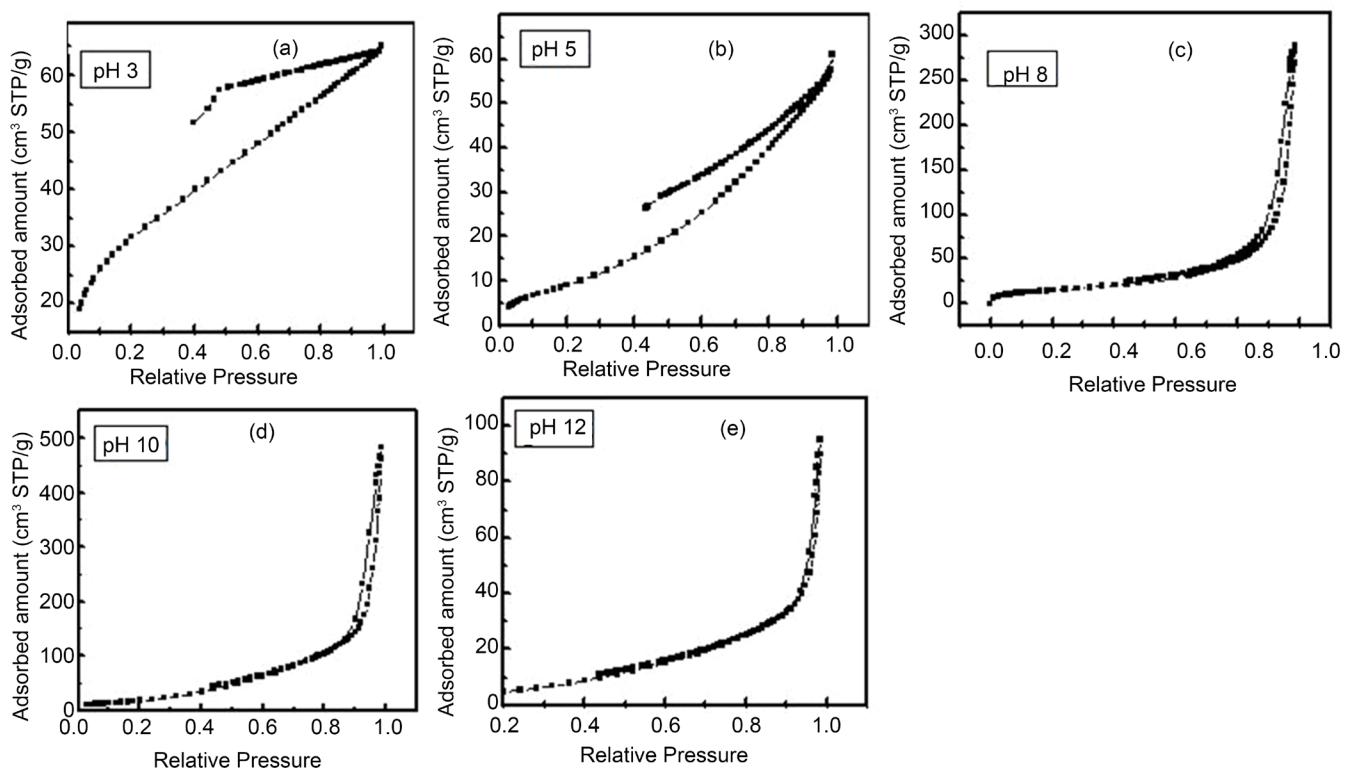
Figure 7 shows the Raman spectra of the as synthesized materials and conformation of the typical property of silica particles, prepared at different pH values. In particular, the characteristic peaks at  $410\text{ cm}^{-1}$  was the due to the contribution of bending mode of oxygen in n-membered rings ( $n > 4$ ) and it was well known as R line; again peaks at  $495\text{ cm}^{-1}$  was the breathing mode of 4-membered rings, known as  $D_1$  line; peaks at  $818\text{ cm}^{-1}$  was the contribution of Silica network optical mode; vibrational mode due to  $(\text{OH})$  - group with admiration to Si contributed the peaks at  $980\text{ cm}^{-1}$ . However, the amplitude of all Raman peaks systematically depends on their specific surface areas [25]. In more elaborately, the highest and lowest values of BET area found at pH 3 and pH 12 samples, where the intensities of all probable Raman peaks were maximum and minimum respectively. Raman peaks intensities were increasing on increasing of the specific surface as well as the value of pH.

### 3.7. Adsorption-Desorption Isotherm

Figure 8 shows the nitrogen adsorption-desorption isotherms of all silica samples tabulated in Table 5 ( $n = 3$ ). They can be easily recognized by type IV adsorption isotherms with a hysteresis loops. Type IV isotherms contributed the fact that the material was mesoporous in nature. In details, for  $S_1$  the maximum (43%) size of pore diameter (nm) was evaluated as less than 6 nm and BET surface area was  $119\text{ m}^2/\text{g}$  which was



**Figure 7.** Raman spectra analysis for all synthesized silica particle formed by the interaction of bacterial protein with TEOS at different pH. The experimental Setup was repeated thrice and no significant errors were noted.



**Figure 8.** Nitrogen adsorption isotherms (a)-(e) for all synthesized silica particle formed by the interaction of bacterial protein with TEOS at different pH. The experimental Setup was repeated thrice and no significant errors were noted.

concluded the fact that sample also had few micro-pores. Similarly, for all samples the BET measurement revealed the conclusions as indicated in tabulated **Table 5** ( $n = 3$ ). From BET measurement, it was revealed that the BET surface area decreased with increasing the pH which caused an increase in pore size and change its nature of porosity.

**Table 5.** Porosity measurement (BET) of Silica particles synthesized at different pH values.

Sample name	Treatment temperature (K)	BET surface area ( $\text{m}^2/\text{g}$ )	Total pore volume ( $\text{cm}^3/\text{g}$ )	Conclusion
S <sub>1</sub> (pH 3)	473	119	0.10	Micro porous (43%)
S <sub>2</sub> (pH 5)	473	35	0.09	Micro porous (32%) Mesoporous (50%)
S <sub>3</sub> (pH 8)	473	56	0.42	Mesoporous (80%)
S <sub>4</sub> (pH 10)	473	67	0.68	Mesoporous (70%) Macro porous (30%)
S <sub>5</sub> (pH 12)	473	18	0.13	Macro porous (70%)

## 4. Conclusion

A potentially gratifying non-pathogenic bacterium arbitrates green synthesis of silica particles from organic silica precursor (TEOS) at different pH is successfully established presently. The as-prepared samples were thoroughly characterized by different techniques and tools including UV-Vis spectra, XRD, FE-SEM, EDX, BET, Zeta Potential, Raman spectra and FTIR spectra. The values of Zeta potential ( $\zeta$ ) of the silica particles implies the stability of synthesized synthesised silica particles in neutral pH atmosphere, preventing it from the auxiliary agglomeration. As a conclusion from BET measurement, it is revealed that the BET surface area decreases with increasing pH which causes an increase in pore size and change its nature of porosity. Peaks intensities are increasing on increasing of the specific surface as well as the value of pH as reveal from Raman spectra. In conclusion, silica leaching bacterial plausible proteins called bioremediaseis responsible for such process. The formation of silica particles via a simple biocompatible protein mediated way indicates that with this green methodology, uniform spherical and significant reduced particle size with plausible potential application as chromatographic packing having variable porosity can be obtained.

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## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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