Silicate Matrix with Implanted Peptide Self-Assemblies for Smart Sensor Electrodes and Coated Glass Systems

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Abstract: Objective: Long-term stable and highly versatile and technologically important sol-gel derived material’s synthesis, characterization and some of its potential applications are presented.

Methods: Multifunctional sol-gel precursor is added with 3,5-bis(trifluoromethyl)benzylamine derived peptide self-assembled nanotubes (BTTPNTs), to form a core-shell, BTTPNTs@SiO2 matrix. The prepared material is characterized by UV-vis absorbance (UV-vis), Diffuse Reflectance Spectra (DRS), X-Ray Diffraction (XRD), Field Emission-Scanning Electron Microscopy (FE-SEM), Confocal Raman Spectral and Imaging (CRS & CRM), Atomic Force Microscopy (AFM) and Transmittance Electron Microscopy (TEM).

Result: The obtained results clearly revealed the expected BTTPNTs implanted core and SiO2 as shell in the formed nanocomposites.

Conclusion: Cyclic Voltammetry (CV) studies explore the sensing activity and UV-vis spectroscopy presents the smart UV protective ability of the coated glass systems.

Keywords: Photo-physical properties, core-shell nanocomposites, FT-Raman, CRM, smart glass system, biosensors.

1. INTRODUCTION

Sol-gel technology is the well-known process for the production of nano-structured materials and hence for several industrial and biological applications [1-6]. The capability of the self-assembly processes is an efficient methodology on finding out the simplest biomaterials like silicate entrapped biomolecules [2]. One example is the recognition of Alzheimer’s sickness and its treatment option by bionano materials [4, 5]. Phe-Phe nanotubes (NTs) show several special properties like consistent homogeneity, and stability at high temperatures [6-8]. The sol-gel formation mechanism is due to the ability of hydrolytically labile precursors that forms a solid from an aqueous medium. This procedure is majorly used to entrap enzymes and to develop biosensors [7]. Peptide Nanotubes (PNTs) were anchored/entrapped in the silicate matrix [8-11] with different perspectives. A wide range of reported results depicts the use of self-assembled nanostructures, formed from simple peptide units in the form of nano-spheres, -composites, -vesicles and nano-tubes [12-16]. New materials emerging from these bio-inspired processes, offer a new way for bionanotechnological innovations. Nowadays, new synthetic routes to create bimolecular assembly with highly ordered, nanostructures have been reported. Peptide nanomaterials (PNM) are also studied for their optical and electrochemical properties that cannot be generally achieved by using normal biological materials [17-21].

Numerous phenomena like physical adsorption, covalent attachment and hydrogen bonding in silicate matrix are used to attain a reproducible technique of the entrapment [18]. Silicate surface promotes a wide range of surface reactions to produce an active peptide based catalyst [22]. The physical confinement of biomolecules within the porous matrix, provides applications in bioanalytical, biotechnological and biomedical industries [23, 24]. The results are focused on the shielding of biomolecules to protect them and to create a single entity [22]. Recently, new class of sol-gel derived materials have been introduced with the mentioned rationalization on making porous matrix, which is compatible with the biomolecules [25-34]. Silicate matrix serve as reservoir for water, and it increases the ability to maintain the biological activity of enzymes, anti-bodies, and body fluid cells etc., [35-39]. These porous silicate matrices obtained by the low-temperature sol-gel method act as promising host matrix for the encapsulation of biomolecules [1, 5]. So as a result, pore-size motif is one of the key elements which enables to accomplish the entrapment of wide range of bio-molecules and opens up different applications [40-48]. This work reports such potent core-shell mesoporous silicate entrapped peptide nanotubes for smart glass coating applications and to create new smart sensor electrodes.

UV-A protecting activity and sensor electrode applications are explored with the newly synthesized tyrosine derived BTTPNTs implanted silicate matrix.

2. EXPERIMENTAL SECTION

2.1. Chemicals

3,5-Bis(trifluoromethyl)benzylamine has 98.0%; Boc-AA-OH (AA is= Tyr;) HATU is (1.0 equiv., 0.125 mmol, 47.5 mg);
Acetic acid (1.0 equiv., 0.125 mmol, 17 mg) and DIEA is (4.0 equiv., 0.500 mmol, 0.087 mL) purchased from AAPPTEC, USA. KH\(\text{SO}_4\) has 99.0%; (3-aminopropyl)-triethoxysilane (APTES) is 98.0%; DCM has 98.0%; TFA is 98.0%; 5% Nafion solution and other reagents and solvents were bought from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) and Tetraethoxysilane (TEOS) 98.0%, Alfa Aesar) were used without additional purification. All aqueous solutions were nanopure. All equipments and other glasswares were washed with acetone, rinsed with Deionized Water (DIW) and dehydrated with air hot oven at 100°C, and then used.

2.2. Sample Preparation

2.2.1. Preparation of SiO\(_2\) Nanopowder

SiO\(_2\) nanopowder was synthesized from TEOS and APTES by mixing in a 5:1 ratio. The solutions were dissolved in 5 mL of distilled ethanol [16]. The ensuing content was stirred for 30 min at room temperature; the obtained sol-gel collection was placed in a hot-air oven for 12 h at 100°C. Finally, the completely dried product was made into a fine SiO\(_2\) nanopowder by grinding.

2.2.2. Synthesis of 3,5-Bis(Trifluoromethyl)Benzylamine Derivatives of Tyrosine Peptide (BTT Peptide)

The detailed synthetic procedure was adopted from the reported methods [1, 4, 5] and the same is shown as in Scheme 1.

2.2.3. Preparation of Self-Assembled BTT Peptide Nanotubes and Vesicles (BTTPNTs)

The procedure was adopted from the reported method [1, 4] 10 mL of an aqueous solution of tyrosine peptide is poured in a 100 mL reaction vessel. The reaction vessel containing the solution was placed in pre-warmed silicon oil bath (65°C) and gentle stirring was applied for 30 min, heating was stopped and it was cooled at room temperature with moderate stirring over a period of 3 h (Scheme 2a).

2.2.4. Preparation of BTTPNTs Entrapped with Silica Nanopowder (BTTPNTs@SiO\(_2\))

Loading of PNTs is described in Scheme 2b. In brief, TEOS (5 mL) and APTES (1 mL) were dissolved in the distilled ethanol (5 mL), it was stirred for 30 min at room temperature. As-synthesized BTTPNTs (1 mL) was added into the reaction mixture. The mixture was stirred for 30 min at room temperature to form the BTTPNTs@SiO\(_2\) matrix, which resulted in the formation of dense sol-gel matrix having BTTPNTs@SiO\(_2\). The solid was dried at 100°C for 12 h [5, 30]. Finally, the sol-gel product was ground well and used for further studies.

2.2.5. Preparation of Cleaned Quartz Glass Plates

Briefly, 1×1 cm sized cleaned quartz glass plates were taken and as-synthesized 1 g of BTTPNTs@SiO\(_2\) composites was dissolved in 5 mL of distilled ethanol. The resulted sol was coated on the glass plate by using simple dip coating method.

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*Scheme 1.* Schematic illustration for solid phase synthesis of 3,5-bis(Trifluoromethyl)-Benzylamine derivative of tyrosine peptide (BTT).  

*Scheme 2.* a) Self-assembly of BTT peptide, b) Sol-gel approaches of core-shell BTTPNTs implanted SiO\(_2\) matrix (BTTPNTs@SiO\(_2\) matrix).
dissolved in 5 mL of distilled ethanol. The resulted sol was coated on the glass plate by using simple dip coating method. The coated glass plates were dried in desiccator for an hour, and then UV-vis and UV-DRS studies were carried out.

2.3. Characterization Methods

UV-vis spectrum was recorded in the range of 600-200 nm [16] by using Shimadzu UV-1650PC spectrophotometer. The analyte was filled in a cuvette of 1 cm path length. Confocal Raman microscopic investigation was done by using STR micro Raman spectrometer (Model STR 300) equipped with 532 nm (100 mW) laser source. Reference spectrum was obtained from silicon using 532 nm laser (power 100 mW) at 600 g mm\(^{-1}\) grating for 1 s and imaged with lens of ×50 objective resolution [1, 34].

Atomic Force Microscopic (AFM) analysis was carried out by using Agilent-N9410A series 5500 microscope operating in tapping mode with a standard silicon nitride tip. This instrument uses silicon nitride tip with a radius of 20 mm and low resonance frequency cantilever that has spring constant of 35-65 nm. Scan of 2×2 μm be obtained for each sample. Images were recorded in non-conduct mode, and made in air under ambient condition to produce 2D and 3D images. Sample for AFM was added in small quantity with carbon tetrachloride, sonicated for 30 min in a "fast-clean" ultra-sonic cleaner. Then this solution was spread out on the silica glass plate. The plate was dried at 100°C for 5 min. The morphology of the synthesized material was visualized by using Supra 55-Carl Zeiss (Germany) Field-Emission Electron Microscopy (FE-SEM) with field emission filament operating at 20 kV. This device has a resolution of 1.5 nm, and is operational with Through-the-Lens Detector (TLD) functioning in Ultra-High Resolution (UHR) mode.

High resolution transmission electron microscopy (HR-TEM) images were recorded using a JEOL 3010 high resolution transmission electron microscopy with an Ultra-High Resolution (UHR) pole-piece operate at an accelerate voltage of 300 kV [16]. HR-TEM image was enhanced by image J viewer software. The analyte for HR-TEM study was prepared by depositing a drop of the synthesized sample on carbon coated Cu grid and allowing it to dry [1]. The UV-vis-DRS of SiO\(_2\) and BTTPNTs@SiO\(_2\) matrix was recorded with UV-3600 Shimadzu (Japan) spectrometer in the range of 600-200 nm. The samples were prepared for the measurement by pilling a small amount of barium sulphate powder and it is used as standard, subsequently, the sample was spread into thin uniform layer by using glass rod [28, 30, 39].

X-Ray Diffraction (XRD) was recorded on Inel Pro model X-ray diffractometer from analytical instruments operate at voltage of 40 kV and current of 30 mA with Cu Kα radiation. The average crystalline size (L) of synthesized material was calculated by Debye-Scherrer formula. Cyclic Voltamograms (CVs) were performed by using CHI 842B electrochemical analyzer (CHI instruments Inc., Austin, TX). A conventional three-electrode cell was used, which includes Ag/AgCl (saturated KCl) electrode as a reference electrode, Pt wire serves as counter electrode, and glassy carbon electrode coated with the prepared sol as a working electrode. Glassy Carbon Electrode (GCE) was successively polished before the experiments with 1.0, 0.3 and 0.05 micron gamma alumina powder slurry on micro cloth pads [1, 42]. To fabricate a working electrode, 0.5 mL of the prepared sol was suspended with 1 mL of distilled ethanol and sonicated for 30 min. 2 drops of this solution was put on to the surface of GC electrode and dried it for 15 min at room temperature. GC electrode was coated with the prepared sol and 0.1 mL of 5% Nafion solution was placed onto the GC electrode and dried for 15 min at room temperature. Nafion membrane helps loaded sol to stick on the electrode surface and works as proton exchange membrane. All solutions were passed with high purity nitrogen gas before obtaining the CV measurements [1, 30].

3. RESULTS AND DISCUSSION

3.1. UV-vis Analysis

UV-vis absorbance spectra of BTTPNTs, BTTPNTs@SiO\(_2\) is shown in Fig. (1). The wide optical absorption peak at 273 nm (4.53 eV) and peaks at 265 (4.68 eV), 259 (4.79 eV), 253 (4.90 eV) and 248 nm (5.0 eV) were noted for the BTTPNTs as similar to the reported spectra of simple Phe-Phe NTs [1, 5]. The energy gap between the two adjacent peaks was reported as the same (0.10-0.11 eV). But when UV-vis spectra of BTTPNTs@SiO\(_2\) is recorded in the sol-gel derived silicate matrix, only the large absorption band at 273 nm is observed instead of the three peaks as noted as above [1, 28] and it is due to the entrapment of BTTPNTs in the silicate matrix. Reduction in the intensity is also shown by the BTTPNTs implanted SiO\(_2\) matrix. So when BTTPNTs implanted inside the silicate shell it is showing a broad absorption band [23].

![Absorption spectra of a) BTTPNTs and b) BTTPNTs@SiO\(_2\) matrix.](image)

**Fig. (1).** Absorption spectra of a) BTTPNTs and b) BTTPNTs@SiO\(_2\) matrix.

3.2. CRM and CRS Studies

CRM and CRS analysis is executed for BTTPNTs and BTTPNTs@SiO\(_2\) matrix (Figs. 2a and 2b). The scattering images validated the BTTPNTs@SiO\(_2\) matrix, by mainly showing tyrosine stretching frequency and Si-O-Si stretching frequencies in the range of 1079 and 1600-1632 cm\(^{-1}\), respectively [31-36]. In addition to that, 1689-1701 cm\(^{-1}\) band presenting carbonyl and amide stretching frequencies is also noted [33]. Confocal Raman spectral images of BTTPNTs and
1. The obtained CRM images look alike with AFM and FE-SEM images, which will be discussed in forthcoming sections.

UV-vis-DRS of the prepared BTTPNTs and BTTPNTs@SiO₂ matrix are given in Figs. 3a and 3b. Reflectance is increased in BTTPNTs@SiO₂ matrix, but bare BTTPNTs is not showing significant reflectance. This is due to the presence of BTTPNTs which is having selective absorption edge. Fig. (3a and b) displays UV-vis-DRS of BTTPNTs and BTTPNTs@SiO₂ matrix. F(R) values are calculated from recorded reflectance (R) by using Kubelka-Munk algorithm \( F(R) = (1-R)^2/2R \). Where, F(R) is Kubelka-Munk algorithm and R is diffuse reflection of powder planes [30, 34]. Direct band gap (\( E_g \)) of BTTPNTs and BTTPNTs@SiO₂ matrix is obtained from Tauc plot which is given in Figs. (3c and d). The band gap indicates the difference between top of the valence and bottom of the conduction band. Fig. (3c and d) depicts \( [F(R)h\nu]^2 \) versus photon energy (h\nu) which gives direct band gap of the synthesized BTTPNTs and BTTPNTs@SiO₂ matrix as 4.20 and 5.75 eV, respectively. UV-vis-DRS analysis further reveals the increase in the direct band gap of BTTPNTs@SiO₂ matrix when compared to the BTTPNTs alone [1, 12]. These results predict that the BTTPNTs@SiO₂ matrix is useful in effective UV-A protecting applications.

3.4. XRD Analysis

XRD pattern of bare SiO₂ and implanted BTTPNTs@SiO₂ matrix is analyzed. Crystalline size is calculated by using Debye Scherer’s formula which is given by eqn. (1):

\[
\text{Crystalline size} = \frac{K \lambda}{\beta \cos \theta}
\]

where \( \lambda \) is the wavelength of X-rays, \( \beta \) is the full width at half maximum of the diffraction line, and \( \theta \) is the Bragg angle.

Table 1. Confocal Raman vibrational assignments of BTTPNTs and BTTPNTs@SiO₂ matrix.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>BTTPNTs</th>
<th>BTTPNTs@SiO₂</th>
<th>*Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>492.6</td>
<td>557.0</td>
<td>( \omega )C-F bending (C₁₉)</td>
</tr>
<tr>
<td>2</td>
<td>720.9</td>
<td>-</td>
<td>CH₂ symmetric stretching (C₇)</td>
</tr>
<tr>
<td>3</td>
<td>862.0</td>
<td>-</td>
<td>( \mu )N-H oop bending (N₁)</td>
</tr>
<tr>
<td>4</td>
<td>1001.0</td>
<td>-</td>
<td>Phenyl(Tyr) breathing mode</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>1079.6</td>
<td>Si-O-Si symmetric stretching</td>
</tr>
<tr>
<td>6</td>
<td>1256.1</td>
<td>-</td>
<td>( \pi )CH₂ bending (C₁₀)</td>
</tr>
<tr>
<td>7</td>
<td>1293.8</td>
<td>1327.9</td>
<td>( \omega )C-C bending (C₁₃)</td>
</tr>
<tr>
<td>8</td>
<td>1409.6</td>
<td>-</td>
<td>Aromatic ring symmetric stretching</td>
</tr>
<tr>
<td>9</td>
<td>1632.1</td>
<td>1600.2</td>
<td>Tyrosine and phenyl ring stretching</td>
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<td>10</td>
<td>1689.7</td>
<td>1701.0</td>
<td>CO-NH symmetric stretching</td>
</tr>
<tr>
<td>11</td>
<td>2457.3</td>
<td>-</td>
<td>C-C asymmetric stretching (C₁₁)</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>2731.0</td>
<td>( \pi )Si-NH bending (N₁)</td>
</tr>
<tr>
<td>13</td>
<td>2923.6</td>
<td>2959.3</td>
<td>( \rho )N-H bending (N₂)</td>
</tr>
<tr>
<td>14</td>
<td>3062.1</td>
<td>-</td>
<td>( \pi )CH₂ bending(C₁)</td>
</tr>
<tr>
<td>15</td>
<td>3331.6</td>
<td>-</td>
<td>O-H stretching vibration</td>
</tr>
</tbody>
</table>

\( \omega \) - wagging; \( \mu \) - twisting; \( \pi \) - scissoring; \( \rho \) - rocking.

*From the references of [29-33].
D = 0.89λ/βcosθ ...

Fig. (3). UV-vis-DRS of a) BTTPNTs, b) BTTPNTs@SiO2 matrix and Tauc plots of c) BTTPNTs, d) BTTPNTs@SiO2 matrix.

Where, λ is the wavelength of X-rays, β is full width at half maximum of X-ray profile and θ is the Bragg angle [16, 28, 30]. Fig. (4a) and b) depicts XRD of SiO2 and BTTPNTs@SiO2 matrix. The observed broad peak represents the amorphous [43] SiO2 (2θ = 25-30°) is shown in Fig. (4a). The peaks observed at 37.3, 38.9 and 64.6° (Fig. 4b) are corresponding to (111), (220) and (222) crystal planes, respectively. This indicates the amorphous SiO2 is implanted with the BTTPNTs (JCPDS Card No. 04-0836). The size of BTTPNTs@SiO2 matrix is calculated by using the Debye Scherrer’s eqn. (1), and found to be 23.9 nm, which indicates the large surface area to volume ratio of the obtained BTTPNTs@SiO2 matrix.

Fig. (4). XRD of a) SiO2 and b) BTTPNTs@SiO2 matrix is amorphous in nature.

3.5. AFM Analysis

The morphology of the synthesized SiO2 and BTTPNTs@SiO2 matrix is examined by AFM. Fig. (5a) reveals the AFM image of the SiO2 implanted with BTTPNTs showing tubular like structure with the width of ~23.0 nm [30, 41]. Morphology of the nanosized BTTPNTs@SiO2 matrix is clearly shown in the three-dimensional (3D) image (Fig. 5b). It corresponds to the equally distributed self-assembled entities of BTTPNTs, with some imperfections as shown in Fig. (5c). Large aggregation is noticed in the BTTPNTs@SiO2 matrix. Thus, the AFM result confirms the structural activeness and surface morphology of the synthesized BTTPNTs@SiO2 matrix.

Fig. (5). a) AFM 2D image of BTTPNTs@SiO2 matrix, b) AFM 3D image of BTTPNTs@SiO2 matrix and c) plot profile diagram of implanted BTTPNTs@SiO2 matrix.

3.6. FE-SEM with EDX Survey

The core-shell structure of BTTPNTs@SiO2 matrix is analyzed by FE-SEM analysis. Fig. (6a) shows FE-SEM images of BTTPNTs@SiO2 matrix at various magnifications. The
obtained images distinctly show the BTTPNTs@SiO₂ matrix which is having well-aggregated silicate matrix [1, 41, 42]. Elemental mapping of the prepared BTTPNTs@SiO₂ matrix is shown in Fig. (6b). Elemental survey from EDX clearly reveals the Si and O contents in the obtained core-shell BTTPNTs@SiO₂ matrix.

3.7. HR-TEM Studies

Core-shell morphology of BTTPNTs@SiO₂ matrix is further analyzed by HR-TEM. Fig. (7) shows HR-TEM images of the synthesized core-shell BTTPNTs@SiO₂ matrix. The prepared BTTPNTs@SiO₂ matrix shows that the spherical shape tubes are implanted. Fig. (7a-d) clearly reveals that the composite matrix is showing spherical tubular structure. The grating edge orientation in HR-TEM image also confirmed this observation (Fig. 7d). It shows the amorphous nature of the BTTPNTs@SiO₂ matrix, which reveals that BTTPNTs are present in the core of silicate matrix shell [1, 5]. The highlighted NTs size is measured by using image J analysis.

3.8. Cyclic Voltammetry (CV) Studies and Sensor Electrode Preparation

Fig. (8a-d) shows CVs of various modified electrodes in 0.1 M KCl as a supporting electrolyte at scan rate of 50 mV s⁻¹ with a) bare GCE, b) Cu NPs/GCE, c) SiO₂/GCE and d) BTTPNTs@SiO₂/GCE. This significant peak shows the presence of implanted BTTPNTs, which is attached through electrostatic interaction through N-H with SiO₂. CV 8c shows the absence of characteristic anodic as well as cathodic peak, it represents only SiO₂ matrix coated on GCE [28]. The current density of the prepared modified GCE shows positive sweep potential \( i_p = 0.1838 \, \text{mA cm}^{-2} \) and negative sweep potential \( i_p = -0.0981 \, \text{mA cm}^{-2} \) which is highly suppressed, when compared to the bare electrodes as shown by CV of 8a and 8b [1, 43-48]. These results indicate the core-shell BTTPNTs@SiO₂ matrix showing better sensing behavior.
for copper nanoparticles. The interaction of BTTPNTs and Cu nanoparticles are well established.

3.9. UV-A Transmittance Studies

BTTPNTs@SiO2 coated glass slide and bare is subjected to the UV-vis absorbance and UV-DRS analysis. It is found that the UV transmittance is low in the coated glass substrates than bare. It is due to the presence of BTTPNTs in the silicate matrix [1, 4 and 44]. It is found that the monomer of PNTs degrades while it is stable in the case of BTTPNTs formed by the self-assembly of the same monomer [4]. By gaining interest of this work, it is extended to the proposed smart coatings on glass window systems. The transmittance of the coated and bare materials is compared and found to be UV-A protective (Fig. 9A and B). So it is concluded that the UV protective effect of the implanted silicate will open up many technological applications.

CONCLUSION

Sol-gel technique is used to prepare the highly stable BTTPNTs@SiO2 core-shell nanomaterials. The characterization by UV-vis and UV-DRS clearly reveals that as-synthesized core-shell BTTPNTs@SiO2 matrix shows an excellent UV-vis (UV-A) resistive/protecting activity. The core-shell form of BTTPNTs@SiO2 matrix is further analysed by FE-SEM coupled EDX mapping, AFM and HR-TEM. UV-vis-DRS indicates the increase in the direct band gaps and UV-A protecting activity. CV results showed the achievement of the better sensing ability for copper nanoparticles. It is proposed that BTTPNTs@SiO2 matrixes can be used as an effective UV-A protecting coating for smart glass windows and similar systems.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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