AN INSIGHTFUL APPROACH TO UNDERSTANDING THE MECHANISM OF AMINO ACID ADSORPTION ON INORGANIC SURFACES: GLYCINE ON SILICA

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Abstract. The adsorption of glycine on amorphous silica surface has been studied to demonstrate the catalytic activity of silica surfaces towards the formation of peptide bonds on prebiotic earth. Silica nanoparticles were synthesized using a microwave assisted method and the nanoparticles were characterized using SEM. Glycine was adsorbed from aqueous solution on the nanoparticles and the adsorption behavior was characterized using FTIR and TGA analyses. At a glycine concentration of 0.5M and at pH=7, favorable adsorption was observed which obeyed the Langmuir isotherm model. From the FTIR characterization, peptide bond formation was confirmed. It was concluded that the adsorption of glycine occurs via electrostatic interactions as well as hydrogen bonding between the silica surface and glycine molecules.

Keywords: prebiotic, silica, amino acid, glycine, adsorption, peptide.

1. Introduction

The understanding of interactions between biological molecules and inorganic surfaces is important due to its significance in many applications spanned across several fields.1-7 These include aqueous geochemistry, soil science, prebiotic chemistry, bionanotechnology, and drug delivery. In the never-ending discussion on the origin of life, these interactions play a major role as certain minerals are known to catalyze peptide synthesis. There are many studies performed on the surfaces of TiO$_2$, silica and other clay minerals in the attempt to understand the mechanisms of chemical reactions that have contributed to the origin of life on Earth.6,8-14 Some reports suggest that TiO$_2$ may have been present on prebiotic Earth and the studies on TiO$_2$ surfaces claim to reveal mechanistic details about the possible reactions occurred on prebiotic Earth.15 Silica, on the other hand, being the most abundant material in the Earth crust, provides many useful aspects for the prebiotic chemistry of the origin of life.16-19 Further to this, the corresponding studies may contribute to the development of procedures for solid-phase peptide synthesis which is a cleaner procedure compared to the conventional synthesis from solution.20

Bionanotechnology is an emerging field comprising of development of medical implants, drug delivery platforms and biomedical sensors, through the combination of biological chemistry with sensor technology with a glimpse of nanotechnology. In bionanotechnology, nanoparticles are usually used as the anchoring or catalytic support for the biorecognition element on which the sensory reactions take place. Once the sensor is immersed in a biological test solution, the first event would be the adsorption of biomolecules on the sensor surface.3 Similarly, when a medical implant is used in biological systems, the first event would be to form a biofilm on the implant surface by the adsorption of biomolecules (usually peptides, amino acids etc.). In both instances, it is very important to study the interaction of biomolecules with the surface of the inorganic material used in the sensor or the implant.1 In addition to this, drug molecules or biomolecules with pharmaceutical properties maybe adsorbed on inorganic surfaces which can be used as carriers to release the drug/biological molecule on-site through a controlled mechanism.3,21,22 In this application too, the understanding of the interactions at the interface is essential. The initial biofilm formed on these devices determine whether they are accepted or rejected by the biological system in which they are being used.

When the biomedical sensors, medical implants and drug carriers are concerned, the biorecognition element maybe immobilized on the surface of the inorganic material by different methods such as adsorption, covalent attachment, entrapment, encapsulation and cross-linking, among which, adsorption has been known as the mildest method which preserves the structure of the biorecognition element.1,21,23 In order to understand the interfacial phenomena occurring in these devices and the mechanism of catalytic peptide synthesis on mineral surfaces which
may have occurred on prebiotic Earth, amino acid adsorption on the corresponding inorganic surfaces is typically chosen, since the adsorption of amino acids is the first step in the formation of the peptide bonds. For adsorption process, properties of the amino acid/peptide as well as the properties of the substrate are important. Proteins are known to be adsorbed readily on hydrophobic surfaces. At the same time, electrostatic interactions, covalent bonding, and hydrogen bonding between the protein and the inorganic surface also govern the adsorption process. Due to these different adsorption behaviors and the varying charge on amino acid molecules in the aqueous solution depending on the pH, adsorption of amino acids on inorganic surfaces is a difficult process to interpret mechanistically.

The surfaces of TiO$_2$ and SiO$_2$ (silica) are known to catalyze peptide synthesis which may add new knowledge to the interpretations of prebiotic catalysis. Catalytic formation and self-assembly of glycine polymers on TiO$_2$ and SiO$_2$ surfaces have been studied by Martra et al., in which they report for the first time, the catalytic formation of polypeptides up to 16 units long on the inorganic surfaces. Adsorption of amino acids and peptides on silica surfaces serves as a model of bioorganic-inorganic interface systems. Kitadai et al. have studied L-lysine adsorption on amorphous silica and found that lysine adsorption is driven by electrostatic interactions with the negatively charged silica surface. Guo et al. have studied lysine adsorption on fumed silica nanoparticles in which they concluded that the interactions are through hydrogen bonds between the amino acid and the silica surface. Lomenech et al. have done theoretical and experimental studies of glycine adsorption on silica from gas phase in which they discovered that the most favorable bonding of glycine with one surface silanol group is through the carboxyl group, and with two or three silanols, hydrogen bonding also contributes. They ruled out the possible dimerization of glycine to produce diketopiperazine (DKP). Protein and amino acid adsorption on silica surfaces and other mineral surfaces have been studied by several researchers. However, research on nanoparticle surfaces of the mineral materials is a rapidly growing field due to their applications in biomedical sensors, drug delivery systems and medical implants. The ease of functionalization of the surface of silica nanoparticles, and being environmentally friendly, are also advantages of using them in such applications.

This study on adsorption of glycine on silica nanoparticles provides another approach for functionalizing silica nanoparticles with amino acids which may then be used in biorecognition events.

### 2. Experimental

#### 2.1. Materials

Tetraethyl orthosilicate (TEOS; 99.5 %) was purchased from Sigma-Aldrich. Ammonium hydroxide (NH$_4$OH; 28 % v/v) was purchased from Daejung Chemicals and Metals Company Limited, Korea. Ethanol (95 % w/v) was purchased from Lanka Sugar Company Limited, Pelawatte, Sri Lanka. Glycine (purified ≥ 99 %) was purchased from Merck Specialties Private Limited, Mumbai. Double distilled water was prepared in the laboratory.

#### 2.2. Instrumentation

Microwave assisted syntheses were done using a microwave reactor (MAS II, ShangahaiSino Microwave Chemistry Technology Co., Ltd.). FT-IR spectrophotometer (Varian 660-IR, USA) and scanning electron microscope (Zeiss, 51-ADD0048) were used to characterize both synthesized silica nanoparticles and amino acid adsorbed silica nanoparticles. UV – visible spectrophotometer (Jenway 6300) equipped with quartz cuvettes was used for absorption measurements. Thermogravimetric analysis was done using TA instrument – SDT Q600.

#### 2.3. Synthesis of Silica Nanoparticles Using a Microwave-Assisted Method

A volume of 50.00 mL of double distilled ethanol was collected in a three-neck round bottom flask and 3.00 mL of tetraethyl orthosilicate (TEOS) was added to that, followed by dropwise addition of 6.60 mL of ammonium hydroxide. The mixture was quickly set up in the microwave reactor in the magnetic stirring mode. The temperature program used was with 800 W microwave power for the duration of 18 minutes at 30 °C. The resulting turbid solution was kept aside for 25 minutes for gelation. Then the mixture was centrifuged at 5400 rpm and was washed...
three times with a 1:1 mixture of double distilled ethanol and double distilled water. After that the solution was kept overnight for air drying followed by oven drying at 65 °C for 24 hours. The resultant white powder was calcined at 600 °C for 2 hours.

2.4. Adsorption of Glycine on Silica Nanoparticles

2.4.1. Investigating the Effect of Glycine Concentration

Using 1M glycine stock solution, a dilution series having concentrations of 0.5M, 0.2M, 0.1M, 0.05M, 0.02M, 0.01M was prepared. For each experiment, 0.400 g of synthesized silica nanoparticles were used. Measured silica nanoparticles were placed in a 100.00 mL volumetric flask and 20.00 mL of glycine solution was added to it in each separate experiment. The mixture was then stirred using the maximum stirring speed for 5 hours using the magnetic stirrer and was kept aside overnight. After that, the solution was centrifuged at 5400 rpm for 10 minutes. The residue was washed with a 1:1 mixture of double distilled ethanol and double distilled water, and the residue was collected which was dried overnight in a vacuum oven.

2.4.2. Investigating the Effect of pH

To a mass of 0.400 mg of synthesized silica nanoparticles, 20 mL of 0.5M glycine solution was added. Glycine solutions were prepared to have three different pH values (pH 5, 7 and 10) by using buffer solutions. Each mixture, after being stirred for 5 hours, was centrifuged at 5400 rpm for 10 minutes followed by washing with pure ethanol for 10 minutes. The residue was dried in a vacuum oven at room temperature.

2.5. Adsorption Isotherms

Glycine solutions with concentrations of 0.5M, 0.4M, 0.3M, 0.2M, and 0.1M were prepared using the pH 7 buffer. A mass of 0.400 g of silica nanoparticles was used in each experiment. Adsorption experiments were carried out using the same procedure used in section 2.4.1 above. Results were analyzed according to the Langmuir and Freundlich isotherm models.

2.6. Determination of the Adsorbed Amount of Glycine on Silica Nanoparticles

2.6.1. Titrimetric Method

A simple titrimetric method was used to determine the amount of glycine adsorbed on silica nanoparticles. The supernatants discarded from the adsorption experiments detailed in section 2.4.1 above were used. Aliquots of 5 mL of the supernatants for each concentration of glycine were transferred to 25.00 mL volumetric flasks and topped up with distilled water. A volume of 10.00 mL of these solutions was transferred to a conical flask and 2.50 mL of formaldehyde was added. After leaving it for 2 minutes, the solutions were titrated with 0.05M NaOH to a phenolphthalein endpoint. Amount of glycine adsorbed was determined using stoichiometric ratios.

2.6.2. Thermogravimetric Analysis (TGA)

The solid nanoparticles with glycine adsorbed on them, were placed in the TGA balance and purged with nitrogen gas. A temperature program was set in the range of 0–600 °C with increments of 5 °C min⁻¹. The TGA curves obtained were analyzed for weight loss determination through which the adsorbed amount of glycine was calculated.

2.7. Characterization of the Bare and Glycine Adsorbed Silica Nanoparticles

Silica nanoparticles from the synthesis were characterized using Fourier Transform Infrared (FTIR) spectroscopy in the Attenuated Total Reflection (ATR) mode. The particle shape and the size range were analyzed using Scanning Electron Microscopy (SEM) images. All adsorption experiments were analyzed using FTIR spectroscopy results. Thermal analysis was done using thermogravimetric analysis.

3. Results and Discussion

3.1. Synthesis and Characterization of Silica Nanoparticles

Amorphous silica is known to be water soluble and biodegradable in contrast with the insoluble and highly toxic crystalline silica. Therefore, the reactions occurred on prebiotic Earth at the origin of life may very well have occurred on the surfaces of amorphous silica. For the same reason, amorphous silica would be the ideal candidate in the development of biosensors. For these reasons, in this study, amorphous silica was chosen, and nanoparticles were synthesized using a microwave-assisted method. This method was chosen due to the higher yield, less reaction time, and the homogeneity of the particle size, when compared to the nanoparticles obtained from the traditional Stöber method. In the microwave method, the efficiency of the synthesis depends on the ability of the solvent to absorb microwave radiation, and therefore, the choice of solvent matters. Polar solvents work well in
microwave-assisted synthesis. In this study, ethanol was used due to this reason and as a milder solvent which permits the use in biomedical applications due to low toxicity. Fig. 1 shows the SEM image of the synthesized silica nanoparticles along with the particle diameter distribution which illustrate good homogeneity and monodispersity of the particles with an average size in the range of 200 nm – 300 nm.

In this study, tetraethyl orthosilicate (TEOS) with the formula Si(OC<sub>2</sub>H<sub>5</sub>)<sub>4</sub> was used as the silicon alkoxide precursor. While alkoxides with bulky ligands show slow hydrolysis, the smaller tetramethyl orthosilicate (TMOS) with the formula Si(OCH<sub>3</sub>)<sub>4</sub> has high cytotoxicity at inhalation and therefore unsuitable in the synthesis of silica nanoparticles. Therefore, TEOS serves as a good alternative. During the reaction, the alkoxy groups of TEOS get hydrolyzed by water to produce silanol (Si-OH) groups which will then react with each other to produce siloxane (Si-O-Si) bonds through a condensation step. Siloxane bonds are also produced via alcohol condensation of unreacted TEOS with silanol groups. Ultimately the silicates will transform into SiO<sub>2</sub> where each silicon atom is interconnected through Si-O-Si bonds. The silica surface can be hydrophilic or hydrophobic depending on the surface functionalities. A silica surface predominantly having siloxane groups will be hydrophobic whereas a surface predominantly having silanol groups will be hydrophilic. Silica nanoparticles contain surface hydroxyl groups which make them hydrophilic and allow easy chemical modification and functionalization of the surface. Due to both of these reasons silica nanoparticles are biocompatible and can be used in biological applications. Ammonia was added as the catalyst for hydrolysis reaction and its feed rate was chosen to be slow to control the nucleation rate of particles to generate monodispersed and homogeneous nanoparticles in the suspension.

According to the stoichiometry of the reaction happening during the silica nanoparticle synthesis shown below, water : precursor ratio should be at least 2:1 for stoichiometric conversion.

\[
\text{Si(OC}_2\text{H}_5)_4 + 2\text{H}_2\text{O} \rightarrow \text{SiO}_2 + 4\text{C}_2\text{H}_5\text{OH}
\]

We have observed that efficient hydrolysis of TEOS happens when this ratio is significantly higher than the stoichiometric ratio. In our synthesis, we have maintained it at 19.64 by taking 0.264 mol of water and 0.0134 mol of TEOS.

The FTIR spectrum of the synthesized nanoparticles (Fig. 2) agrees with spectra previously reported in literature, having an intense broad band around 1079 cm<sup>-1</sup>.

![Fig. 1. SEM image of the silica nanoparticles synthesized using a microwave-assisted method (a).](image1)

![Fig. 2. The FTIR spectrum of silica nanoparticles synthesized using a microwave-assisted method.](image2)
due to asymmetric stretching of Si-O-Si groups, a band at 798 cm⁻¹ due to symmetric vibrations of Si-O bonds, a band around 443 cm⁻¹ due to the bending of Si-O-Si groups, a band at 1623 cm⁻¹ due to the scissor bending mode of molecular water and a broad intense band in the 3300 to 3500 cm⁻¹ region due to stretching vibrations of O-H groups of adsorbed water.

### 3.2. Adsorption Experiments

Adsorption of glycine from solution phase onto the surfaces of the silica nanoparticles serves as the best experimental method to model the reactions happened on prebiotic Earth to form the first peptide bonds by the self-assembly of amino acids to form protein polymers. Adsorption is also the first event that occurs when a biosensor or a medical implant is introduced into the biological solution. Therefore, here we have studied the adsorption of glycine on silica nanoparticles and the adsorption behavior was studied both qualitatively and quantitatively.

#### 3.2.1. Effect of Concentration of Glycine

Fig. 3 illustrates the adsorption behavior of glycine on silica nanoparticles as a function of glycine concentration. When the spectra are compared with those of bare silica nanoparticles and free glycine, it is clear that glycine adsorption has taken place on the silica surface. Since the extra glycine was removed by a thorough washing procedure, these spectra are clear evidence for the adsorption of glycine on silica nanoparticles.

![Fig. 3. FTIR spectra of glycine adsorbed on silica nanoparticles as a function of glycine concentration](image)

The peaks centered around 460, 800 and 1860 cm⁻¹ are present in both bare silica and glycine adsorbed silica surfaces and they correspond to Si-O-Si bending vibration, Si-O symmetric vibration and Si-O-Si asymmetric vibration, respectively. The broad band around 3300 cm⁻¹ is due to adsorbed water and the hydrogen bonded silanol groups. For isolated glycine, symmetric stretch of C=O of the carboxyl group appears around 1790 cm⁻¹. However, for adsorbed molecular glycine, the corresponding peak (Si-O-CO) has shifted to 1700 cm⁻¹. The peak at 1630 cm⁻¹ may be due to the H-O-H bending as it appears in the spectrum for bare silica surface too. The NH₂ scissoring vibration peak may also be embedded in the same position. For bulk glycine zwitterion, δs NH₂ appears at 1527 cm⁻¹ but it has shifted to 1500 cm⁻¹ for adsorbed glycine. This peak and the presence of a peak at 1410 cm⁻¹ which corresponds to νc COO⁻, confirms the presence of zwitterionic form of glycine. The weak feature at 1340 cm⁻¹ is due to NH₂ twist.

According to Guo et al. who investigated lysine adsorption on fumed silica nanoparticles, peaks in the ranges of 1650–1700 cm⁻¹ and 1530-1560 cm⁻¹ correspond to amide I and amide II bands which are typical of peptide species. Therefore, due to the presence of bands in the FTIR spectrum at 1700 cm⁻¹ and 1500–1600 cm⁻¹ region in our study, it can be concluded that peptide bond formation has occurred on the silica nanoparticle surfaces. Amino acid adsorption on perfectly defined silica surfaces have been studied before. However, there are no reports of the use of silica nanoparticles synthesized by a microwave-assisted method in a mechanistic study of amino acid adsorption. Therefore, the findings reported here will be of advantage for further research involving silica nanoparticles in biomedical applications.

#### 3.2.2. Effect of pH of the Medium

At pH values greater than 3, surfaces of silica are negatively charged and the silanol groups exist in the form of Si-O⁻ allowing electrostatic interactions with the incoming molecules. Between pH 2.14 (correspond to pKa1) and 9.78 (correspond to pKa2), glycine exists in the zwitterionic form. Below pH 2.14 it exists as the glycinium cation and above pH 9.78 it exists as glycinate anion. The isoelectric point of glycine is at pH 5.97. This explains the preferential adsorption of glycine on silica nanoparticle surface at pH 5 and 7 as illustrated in the FTIR spectra in Fig. 4. The characteristic peaks of glycine adsorbed on silica are present in the spectra taken at pH 5 and 7 while they are more prominent at pH 7.

According to some reports, the pH of early age oceans was in the range of 5-11 and peptide formation on silica surfaces must have been preferential according to the results from our study. Physiological pH is also at 7.4 and therefore, preferential adsorption of peptides and proteins will occur when implants and sensors developed using silica materials are used in biological media.
3.3. Determination of the Amount of Glycine Adsorbed on Silica Nanoparticles

3.3.1. Titrimetric Method

According to the results obtained from the titrimetric method, it was found that around 54% of glycine that was used in the adsorption experiments has been adsorbed on the surface of silica nanoparticles. This was calculated from the titration done for the supernatant solution from the adsorption experiment using 0.5M glycine solution.

3.3.2. Thermogravimetric Analysis

In addition to the titrimetric method, thermogravimetric analysis was used to quantify the glycine adsorption and to investigate the thermal behavior of the adsorbed glycine on silica nanoparticles. The weight loss curve from thermogravimetric analysis is shown in Fig. 5. This weight loss curve shows two significant stages of weight loss; one in the range 40-100 °C and the other above 170 °C. The first stage which corresponds to about 4% weight loss maybe due to the evaporation of absorbed and trapped water. The second stage which is a broad feature in the weight loss curve might include multiple steps of decomposition of adsorbed glycine on silica nanoparticles ($T_{\text{decomposition}} = 233$ °C). This stage corresponds to about 2.5% weight loss.

3.4. Adsorption Isotherm Analysis

Adsorption isotherms provide information about the surface behavior of the adsorbate. They also provide information about saturation coverages and equilibrium constants. The data were fitted to Langmuir and Freundlich adsorption isotherm models.

Langmuir adsorption isotherms were studied using the amounts of glycine adsorbed as a function of equilibrium concentration of the solution. Both the equilibrium concentrations and adsorbed amounts were measured using a titration method which involved the titration of supernatant of the glycine/silica mixture with NaOH. The amount of glycine adsorbed per unit mass of silica is found using the following equation.

$$q_e = \frac{KQ^0C}{1 + KC_e}$$

$q_e$ – the amount of adsorbate adsorbed per gram of adsorbent at equilibrium (mmol/g); $C_e$ – equilibrium concentration of glycine after adsorption (mmol/dm$^3$); $Q^0$ – maximum adsorption capacity when monolayer is formed (mmol/g); $K$ – Langmuir isotherm constant (dm$^3$/mmol).

The linear form of Langmuir isotherm was used to check the adsorption behavior.

$$\frac{1}{q_e} = \frac{1}{Q^0K} + \frac{1}{Q^0C_e}$$

A straight-line graph of $1/q_e$ vs $1/C_e$ is proof for adsorption behavior fitting into the Langmuir model.

The linear form of the Freundlich isotherm model is:

$$\log q_e = \frac{1}{n} \log C_e + \log K_f$$

$q_e$ = the amount of adsorbate adsorbed per gram of adsorbent at equilibrium (mmol/g); $C_e$ = equilibrium con-
3.5. Proposed Mechanism of Glycine Adsorption on Silica Nanoparticles

In aqueous solution, adsorption of glycine in zwitterionic form predominates in the pH range between 5 and 7. According to Hassanali et al., silica surface is negatively charged at pH>3 when it is in contact with aqueous solutions because a fraction of silanol groups are dissociated to form siloxides (SiO\(^{-}\)). A research by Emami et al., reports that at pH values 5 and 7.4, silanol group shows a degree of ionization of 9% and 18% respectively, to form siloxide groups.

Therefore, the zwitterions interact with the silica surface via electrostatic interactions as illustrated schematically in Fig. 7. At pH 7 which is not too basic, there can be hydrogen bonding interactions involving the carboxyl moieties as well (Fig. 8). Hydrogen bonding interactions are also possible with the NH\(_2\) moieties on glycine (Fig. 8).

4. Conclusions

Using a unique method of microwave assisted technology, monodispersed and spherical shaped silica nanoparticles were synthesized with good homogeneity in size, in relatively short time compared to the traditional Stöber method. The optimum concentration and pH for the adsorption of glycine on silica surface were 0.5 M and 7, respectively. The adsorption data were well fitted to Langmuir adsorption isotherm model. It was evident that the glycine molecules exist in the zwitterionic forms under the conditions of study which favors electrostatic interaction with the negatively charged silica surface. However, hydrogen bonding interactions with non-charged glycine molecules and silanol surface cannot be completely ruled out at pH 7. The reaction conditions used in this study are comparable with the conditions existed in prebiotic Earth and therefore the results can be confidently used to study prebiotic chemistry, especially the synthesis of the first peptide bonds which led to the creation of life on Earth.

The presence of bands at 1700 cm\(^{-1}\) and 1500–1600 cm\(^{-1}\) region in the FTIR spectra of glycine adsorbed on silica indicates the formation of peptide bond on the silica nanoparticle surface. We therefore conclude that the silica surface facilitates in catalyzing the peptide bond formation.

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Declaration of Competing Interests
There are no conflicts of interest to declare.

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