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## Characterization of Silica Particles and Concentration Dependent Adsorption Study of Biomolecules

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### Abstract

Submicron-sized silica particles were initially characterized by scanning electron micrograph, FTIR spectroscopy and XRD analysis. The observations revealed that the highly dispersed amorphous silica particles were relatively monodispersed and the particles surface was covered with hydrogen bonded OH groups. Relatively higher magnitude of adsorption of cationic emulsifier and coagulation of silica particles suggested that particles were negatively charged. A comparative study of adsorption behavior of different biomolecules such as trypsin, albumin and lysozyme was also done at the respective isoelectric pH value. The magnitude of adsorption increased with the increase in the amount of immobilization per unit mass of silica particles.

**Key words:** Silica, Amorphous, Adsorption behavior, Emulsifier, Biomolecules

### Introduction

Monodispersed colloidal particles of any size and shape have become the most significant area of research considering the large surface area and ease to control the surface property. Depending on the surface properties, these particles may find applications in textiles, electronics, chromatography, coating and biotechnology (Ahmad *et al.* 2003, Kang *et al.* 2006, Lin *et al.* 2006, Li *et al.* 2007, Norde 1997, Yang *et al.* 2005). The surface properties also play an important role in determining the colloidal behavior of the latex.

In this investigation submicron-sized silica particles obtained from Nippon Shokubai Co. Ltd. Japan has been taken into account for surface characterization. The adsorption of proteins and emulsifier molecules on the solid surfaces is known to depend on general physicochemical surface properties such as hydrophobicity and surface charge density. Hydrophobic interaction has been found to play an important role in protein adsorption. Krisdhasima *et al.* (1992) studied the adsorption of  $\beta$ -lactoglobulin on chemically modified silicon surfaces and found that the increase in hydrophobicity of the surface increased the amount of adsorption. Several articles (H. Ahmad *et al.* 2004, 2005, 2006, 2007 and 2008) reported similar results in the magnitude of adsorption for different biomolecules on organic polymer latexes. Zoungrana *et al.* (1997) observed that  $\alpha$ -chemotropism and cutinase adsorbed on hydrophilic silica surface retained their native conformation. Vertegel *et al.* (2004) studied the adsorption of chicken egg albumin on sil

ica nanoparticles of various diameters. The adsorption patterns, protein structure and function were strongly dependent on the size of nanoparticles. So far most of the adsorption studies done on silica particles dealt with one type of protein and the silica particles used were not spectroscopically analyzed. The adsorption behaviors of different cationic and anionic surfactants have also been studied in details (Lindman *et al.* 2004, Thibaut *et al.* 2000). Despite the considerable researches dealing with silica particles, it is well known that such adsorption kinetics depending on surface properties is variable with processing condition, dispersity index, size and shape of the particles. Considering this aspect, silica particles obtained from Nippon Shokubai Co. Ltd. Japan have been studied for surface characterization. The modification of such silica particles with organic polymer would be attempted in the next article.

### Materials and method

Silica dispersion donated by Nippon Shokubai Co. Ltd. Japan, was used after centrifugal washing. Styrene of monomer grade (Fluka, Chemika, Switzerland) was distilled under reduced pressure. Poly(vinyl pyrrolidone) (PVP) from LOBA chem. India, and tricapyrylmethyl ammonium chloride (aliquate<sup>336</sup>) from Fluka, Chemika, Switzerland were used as received. Sodium dodecyl sulfate (SDS) and hexadecyl trimethyl ammonium bromide (HTABr) from Fluka Chemika, Switzerland were used as anionic and cationic

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emulsifiers. The biomolecules used were trypsin (TR) from E. Merck, Germany; albumin (AL) from LOBA Chem., India and lysozyme (LZ) from Fluka, Biochemika, Switzerland. Other chemicals used were of reagent grade. Distilled deionized water of conductivity less than 5  $\mu\text{S}/\text{cm}$  was used for all the experiments.

Scanning electron microscope (SEM) (LEO Electron Microscopy Ltd, UK) was used to see the images of PS particles. Helios Gamma Single-beam UV-Visible Spectrophotometer from Unicam, UK and FTIR-8900, Japan were used for the analyses.

#### Preparation of polystyrene (PS) particles by dispersion polymerization

PS particles were prepared by dispersion polymerization of 40 g styrene dissolving in a mixture of 200 mL ethanol and 10 mL water in presence of AIBN (0.6 g) as oil soluble initiator. PVP (1.6 g) and aliquate<sup>336</sup> (0.46 g) were used as stabilizer and co-stabilizer respectively. Polymerization was carried out in a three necked round bottomed flask under nitrogen atmosphere at 70°C for 12 h with stirring rate maintained at 80 rpm. Conversion of styrene to PS was measured gravimetrically and it was nearly 100%.

#### Adsorption of biomolecules

A mixture of 20 mL was prepared from purified silica dispersion (solid 0.1 g) and biomolecules of varying concentrations. In each measurement, for 0.1 g of silica particles 2 mg, 5 mg, 10 mg, 15 mg, 20 mg and 30 mg of biomolecules were used, respectively. The pH value of the mixture was immediately adjusted with phosphate buffer at the respective isoelectric point (TR, pH 10; AL, pH 6; LZ, pH 10.5). The mixture was allowed to stand at 30°C for 45 min, and then centrifuged at 7,000 rpm for 15 minutes. In order to remove the dust particles completely, the supernatant was centrifuged once more at 7,000 rpm. The concentration of the biomolecule in the supernatant was determined by UV-visible spectrophotometer at 280 nm. The magnitude of adsorption was calculated by subtracting concentration of biomolecule in the medium from that of the initial concentration. Calibration curve was used for this purpose. The adsorption of TR on PS particles was also measured under similar conditions.

#### Adsorption of emulsifiers

For each measurement, a mixture of 30 mL was prepared from purified silica dispersion (solid, 0.1 g) and emulsifier (SDS/HTABr) aqueous solution. The concentration of the emulsifier in the mixture was kept below the critical micelle

concentration to avoid the formation of micelle. The pH of mixture was immediately adjusted to 7.0 using dilute KOH solution. The dispersion-emulsifier mixture was then allowed to stand for 2 h at 30°C. In order to examine the adsorption behavior of the emulsifier onto the composite particles the conductance was recorded at 30°C. The magnitude of emulsifier adsorbed was calculated by subtracting the emulsifier concentration in the medium from the initial concentration. The emulsifier concentration was obtained using calibration curves, representing the relationship between the concentration and the conductance, for emulsifier aqueous solution at the pH value of 7.0, assuming that the adsorbed emulsifier molecules don't contribute to the conductance.

#### Results and Discussion:

Fig. 1 shows the SEM photographs of silica particles. The particles are almost spherical. The average diameter and coefficient of variation are 0.52  $\mu\text{m}$  and 3.52%, indicating that particles are relatively monodispersed.

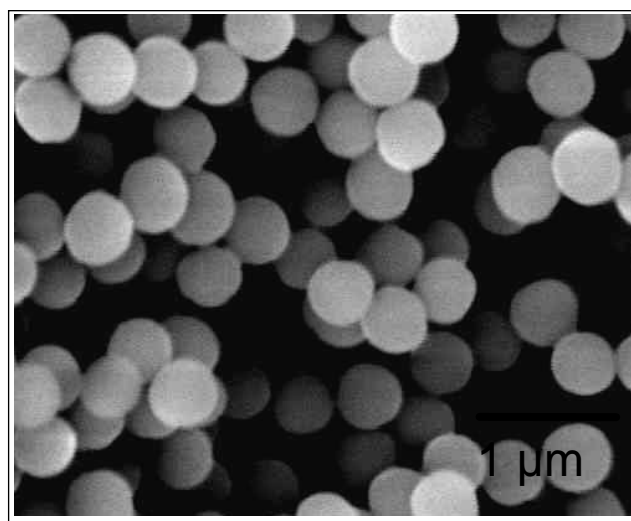
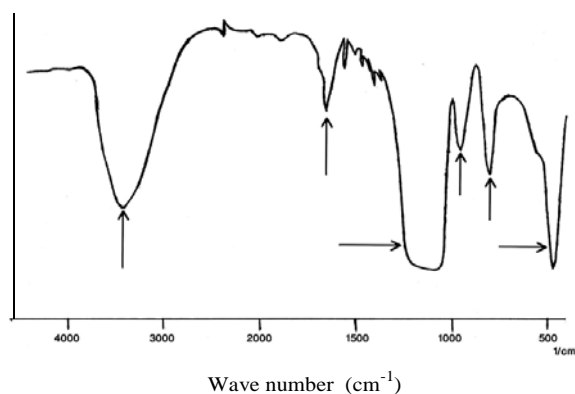


Fig. 1. SEM photograph of silica particles.

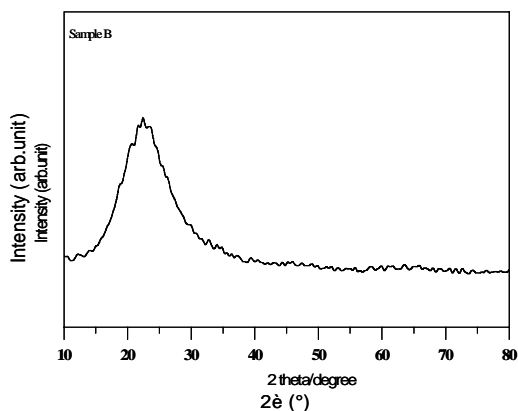
FTIR spectrum of silica particles is shown in Fig. 2. The signal at 1083.9  $\text{cm}^{-1}$  is attributed to Si-O-Si stretching vibration in  $\text{SiO}_4$  units and that at 472  $\text{cm}^{-1}$  is attributed to Si-O-Si bending. The peak at 800.4  $\text{cm}^{-1}$  is assigned to Si-O-Si bond vibration between two adjacent tetrahedra (Ohtsuki *et al.* 1992, Kim *et al.* 1989), occurred in silica gel (Nyquist *et al.* 1991). The signal appeared at 954.7  $\text{cm}^{-1}$  is due to Si-O terminal non-bridging vibration (Costantini *et al.* 2006). The broad signal appeared at 3421  $\text{cm}^{-1}$  can be assigned to bonded OH groups on the surface of silica particles

The XRD pattern of silica particles shown in Fig. 3 exhibits a broad reflection centered at  $2\theta = 22^\circ$ . This broad reflection band suggests that silica particles are substantially amorphous in character.



**Fig. 2.** FTIR spectrum of washed silica particles.

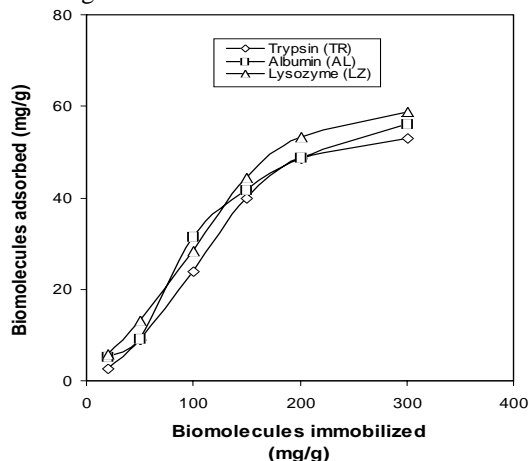
The variations in magnitude of TR, AL and LZ adsorbed on silica particles in presence of different amounts of biomolecules in silica-biomolecule dispersion are shown in Fig. 4. The measurements were carried out at isoelectric point of the respective biomolecule to eliminate the effect of ionic interaction. In each case the magnitude of adsorption increased with the increase in the amount of immobilization and reached almost plateau level beyond 200 mg of biomolecules. The adsorbed biomolecules at the plateau level is



**Fig. 3.** X-ray diffraction spectra of washed silica particles.

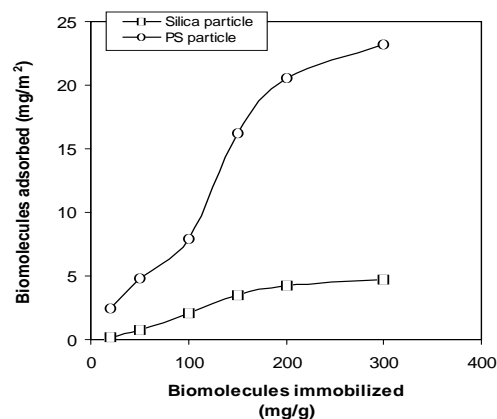
expected to have monolayer coverage on the particles surface. The magnitude of adsorption is influenced by various factors such as properties of proteins, the solid substrate surface and environmental conditions. In this study the solid substrate and environment condition remained the same. The difference in the magnitude of adsorption among different biomolecules is therefore attributed to the difference in properties such as size, flexibility, amino acid composition and steric conformation. The magnitude of adsorption on the hydrophilic surface is usually lower unless there is electrostatic attraction than on the hydrophobic surface. Fig. 5 shows that the magnitude of adsorption of TR on polystyrene (PS) particles is much higher than that on silica particles measured under identical conditions with varying concentra-

tion of TR. This suggests that the surface of silica particles is relatively hydrophilic and is covered with bonded OH groups, which have also been supported by FTIR spectra as shown in Fig. 2.



**Fig. 4.** Effect of concentration of biomolecules in silica-biomolecule dispersion on the magnitude of adsorption at 30°C measured at the respective isoelectric point ((TR, pH 10; AL, pH 6; LZ, pH 10.5).

The magnitude of adsorption for anionic SDS and cationic HTABr emulsifiers were also measured on silica particles under similar conditions. The magnitude of adsorption of cationic HTABr (21.94 mg/g of particles) was higher than that of anionic SDS (4 mg/g of particles). Moreover, contrary to the anionic emulsifier, the silica particles were coagulated in presence of cationic emulsifier. This behavior indicates that silica dispersion is stabilized due to the presence of negative charges on the particles surface and addition of cationic emulsifier neutralized the negative charges which ultimately produced coagulation.



**Fig. 5.** Magnitude of TR adsorbed on polystyrene (PS) and silica particles measured under identical conditions. pH: 10; temperature: 30°C; particles: 0.1 g. TR: variable.

The above study revealed that silica particles are hydrophilic, anionically charged and mostly amorphous in character. These particles can be used in biomedical field or can be modified/ functionalized to diversify the application in biotechnology.

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