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Full Length Research Paper

Adsorption of lysozyme unto silica and polystyrene surfaces in aqueous medium

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The adsorption capacity of lysozyme (chicken egg white) from aqueous solutions unto silica and polystyrene interfaces was studied at varying lysozyme concentrations and ionic strength. The studies revealed an increase in adsorption capacity with increase in concentration and with maximum adsorption densities of 1.34 x10^{-6} mol g^{-1} and 1.57 x10^{-6} mol g^{-1} obtained for silica and polystyrene respectively at the maximum concentration studied. The observed adsorption isotherms on both surfaces were well fitted by the Langmuir adsorption isotherm model with maximum adsorption capacities (q_{max}) corresponding to monolayer coverage as 1.60 x 10^{-6} and 2.72 x 10^{-6} mol g^{-1} respectively for silica and polystyrene. Fitting of the data into Langmuir-type isotherm suggested that chemisorption was the predominant mechanism in the adsorption process. Results presented in the limelight of varying ionic strength showed that adsorption capacity decreased with increase in ionic strength onto silica surface while showing no significant effect onto polystyrene surface, thus implying that electrostatic interaction may play a major role in lysozyme adsorption onto the silica surface.

Key words: Adsorption capacity, lysozyme, silica, polystyrene, Langmuir.

INTRODUCTION

Adsorption of protein onto solid surfaces is a process that embraces various disciplines as diverse as fouling of process equipment in the food industries, blockage of filtration membranes in bioseparation processes, bio-fouling of ship hulls and regulation of immunoresponse to foreign materials (Horbett and Brash, 1987). Protein adsorption onto surfaces is a complex phenomenon, a consequence of the large nature of adsorbing particles thus, the surface-protein interactions are usually long range. Due to the large size and the shape of the particles, the interactions between the adsorbed particles on the surface are nontrivial and can be strongly influenced by the fact that the particles may undergo conformational changes upon adsorption (Van der Veen et al., 2004). Size, charge, structure, stability and unfolding rate are some of the properties of proteins that affect their interaction with surfaces. Larger molecules for instance can have more sites of contact with the surface. Molecules close to their isoelectric points generally adsorb quite readily. Less stable proteins particularly those with less intra-molecular cross-linking usually unfold to a greater extent and form larger points of contacts with the surfaces (Andrade, 1985). Some of the properties of surfaces that affect their interaction with proteins include topography, chemical composition, hydrophobicity, surface heterogeneity and potential.

Understanding of these fundamental factors that determine protein adsorption is important in improving our ability to design biocompatible materials, biotech-nological devices and coatings that may improve or inhibit protein adsorption. No laid standard governs this but it is imperative one understands the kinetics and thermodynamics of protein adsorption processes and the consequences of adsorption on the protein conformation and activity. Knowledge of the chemistry of surfaces is also of paramount importance for the adsorption kinetics as protein adsorption is a very important fundamental

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Abbreviations: PS, Polystyrene; **GPPS,** general purpose polystyrene; **ATC,** automatic temperature control; **SSA,** specific surface area; **BSA,** bovine serum albumin; **OVAL,** ovalbumin.

problem that involves large competiting energy scales and conformational statistics that may result in reversible and irreversible processes.

Proteins are flexible chains that have been coiled, folded and bent to assume a particular conformation (three – dimensional structure). Changes in the micro-environment of the proteins, such as pH and ionic strength can alter the conformation of the molecule. Likewise, proteins experience structural alterations during interaction with solid surfaces (Lord et al., 2006). Their conformation may be changed, but adsorbed proteins generally retain at least some of their biological activity. Two modes of conformational changes can occur. First, protein molecules can undergo time-dependent molecular spreading. Initially, the molecules may make contact with a minimal number of binding sites on the surface by interaction of amino acids on the exterior of the protein. As the length of time the molecules resides on the surface (residence time) increases, the protein may unfold, exposing interior functional groups for interaction with additional binding sites. Consequently, a time-dependent increase in the number of contact points between protein and surface is observed (Brands et al., 2006). Overall, desorption becomes quite unlikely to occur as the residence time increases because of the greater number of contacts formed. Secondly, altered conformation can result from changes in the bulk solution concentration. At low concentration, abundant surface area is available for each protein molecule. Without near neighbours, molecules can spread to form multiple contacts with the surface. At high bulk concentrations, the amount of surface per molecule decreases and less unfolding can occur because of adsorbate – adsorbate interactions. Consequently, more proteins may be present on the surface, but with each molecule having fewer contacts (Andrade, 1985).

Dissolved protein molecules adsorb at almost all interfaces. In protein adsorption, the deposition of blood proteins onto medical devices and the subsequent modification of their biological responses, the bacterial fouling of ship hulls and the blockage of filtration membranes in bio-separation processes may be considered as some of the unfavourable aspects of protein adsorption. However, the extensive use of a variety of proteins in food emulsion stabilization and in the fabrication of biosensors are positive applications (Wahlgren et al., 1993). Since protein adsorption on interfaces has some undesirable consequences, an understanding of the protein adsorption process at a molecular level and a determination of the structure of protein adsorbates are needed to optimize these applications or prevent adsorbed proteins if they are undesired.

A common way of portraying the results of adsorption studies on solid surfaces is in the form of an adsorption isotherm, which gives the amount of adsorbed material per surface area at a constant temperature as a function of pressure or concentration in the bulk phase. The isotherm shows the extent of the adsorbed layer, that is the net result of these two competing processes.

In this study, lysozyme (hen egg white) was used as a model protein. This lysozyme was selected for the following reasons: (1) Lysozyme is a globular protein with well-defined molecular dimensions in its native state and its stability is likely to prevent complete unfolding at a surface (Czeslik and Winter, 2001). Therefore, the structure of lysozyme and the unfolding behaviour of dissolved lysozyme are well characterized; (2) at moderate pH values it has a high thermal stability, originating in part from four disulphide bonds (Mitchell and Slaughter, 1986).

The adsorbents used in this study are silica particles (non-synthetic surface) and polystyrene (synthetic surface). The adsorption behaviour of the lysozyme was investigated at low concentrations where protein–protein interactions may be minimized. The equilibrium adsorption of the protein was carried out, effect of varying ionic strength was also studied and an overall picture of maximum adsorbed amount was carefully examined using the Langmuir isotherm model.

The main aim of this study was to assess the degree of adsorption of lysozyme at the silica/water and polystyrene/water interfaces as a function of varying lysozyme concentration and ionic strength. The outcome of this study will be of importance in material selection for the removal of lysozyme from solutions. Optical spectroscopy was employed to investigate the amount of lysozyme adsorbed onto the interfaces.

MATERIALS AND METHODS

Pure crystalline lysozyme (protein from chicken egg white, grade 1, product No L-6876, molecular weight 14,000 g mol^{-1}) used in the adsorption studies was obtained from Sigma Chemical Co. (USA). Chromatographic grade silica particles (mesh size 200μm) from Burgoyne Burbridges and Co. (India) MUMBAI, product No. 07079 and batch No. 21920 was used without any further purification. Polystyrene (PS) – general purpose polystyrene (GPPS) Grade G/26 and lot No. 1211171 FKS – BI was obtained from Dongbu Hannong Chemical Co. Ltd Korea. The polystyrene was crushed with manual blender to smaller particles and sieve analysis was performed using the standard sieve plate to obtain samples of 200 μm size. During crushing, the polystyrene was mixed with glucose to prevent coagulation of the fine particles and afterwards were washed with water to separate the glucose and then dried at a temperature of 80˚C for 6 h. All inorganic salts used were BDH products of analytical grade. Double distilled water was used throughout the experimental work. Digital pH meter CE HI 98127 used for the analysis is a product of Hanna instruments inc. Woonsocket, Rhode Island, USA. The meter measures to an accuracy of 0.1 and has an in-built automatic temperature control (ATC). It was standardized using standard buffer solutions (4.10, 7.10 and 9.18) according to specifications. Visible spectrophotometer (722S spectrophotometer); No. SFZ 1506010514 was used for quantification of the lysozyme (protein). The absorbance was taken and their concentrations determined from the calibration curve. Digital analytical weighing balance X21-0014 KERN 770-15, 15402301, made in Germany was used. The balance measures to an accuracy of 0.0001g and mechanical shaker, versal shaker, type: LE-203/1 from Hungary was used.

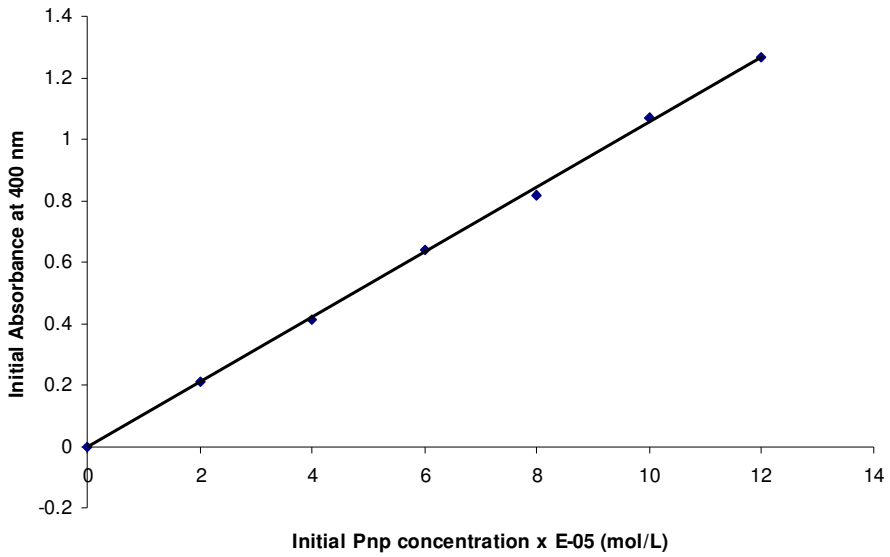

Figure 1. Initial absorbance at 400 nm against initial paranitrophenol concentration (mol L^{-1}).

Methods

The specific surface areas of the silica and polystyrene samples (adsorbent) were determined using the method of adsorption of aqueous solution of paranitophenol onto silica and polystyrene as reported by Gilles and Nakhwa (1962). One gram of each of silica and polystyrene particles was placed in seven test tubes (double set) containing 10 mL of different standard paranitrophenol solutions. At room temperature (30˚C), the tubes were shaken vigorously for 1 h to attain equilibrium adsorption and allowed to settle. The supernatant liquid was decanted and centrifuged for 15 min at 3000 xg. A drop of ethanol was added to reduce the surface tension and this allowed the floating powders to sink. The absorbance of the supernatant liquid was measured at 400 nm and concentration obtained from the calibration curve (Figure 1).

The lysozyme (protein) material under test was dissolved in the buffer (pH 11.0) used to prepare the test solution. Portions of this solution were diluted with the same buffer to obtain seven standard solutions having concentrations between 0.1 and 1.4 g L^{-1}. These concentrations were evenly spaced. The buffer used for the test and standard solutions were also used as blank. pH 11.0 which is the isoelectric pH of lysozyme (Chaiyasut and Tsuda, 2001) was employed for the analysis and at this pH, the net charge on lysozyme is zero. The buffer, pH 11.0 was prepared by mixing 100 mL of 0.025 M K_2CO_3 with 43 mL of 0.025 M $KHCO_3$. The ionic strength was 0.01.

In the experiment, 0.4 g each of silica and polystyrene particles was added to 10 mL of standard lysozyme solution at a pH 11.0 and ionic strength 0.01. The flasks were shaken on a mechanical shaker at a constant temperature of 30˚C at a speed of 6.0 oscillations per second. The flasks were shaken for 16 h and then kept undisturbed for another 4 h. Equilibrium was attained and protein adsorption was completed during this period. At the end of this period, the lysozyme (protein) concentration in the bulk solution was determined by spectrophotometer with the Lowry method (Lowry et al., 1951). The above experiment was also repeated at various ionic strengths. Here 0.4 g each of the pure silica and polystyrene was added to each of the five flasks (double set) containing 10 mL of 7.14 $x10^{-5}$ M lysozyme solutions at a temperature of 30˚C. The ionic strength of the solution was adjusted using varying concentrations of NaCl (0.1M -0.7M NaCl). The flasks were shaken for the same contact time (16 h) to attain equilibrium. At the end of this period, the lysozyme concentration in the bulk solution was determined as previously stated. The concentration of lysozyme in the bulk solution was then read off from the calibration curve.

The quantification of the adsorbed lysozme was carried out using the Lowry assay. To 1.0 mL of each test solutions and the blank, 6.0 mL of alkaline copper reagent was added, mixed and allowed to stand at room temperature for 10 min. 0.5 mL of diluted Folin-Ciocalteu reagent was added, shaken and allowed to stand for another 30 min. The absorbance of the test solution was determined at the maximum wavelength of 750 nm with a suitable visible spectrophotometer, using the solutions from the blank to set the instrument to zero absorbance.

Alkaline copper reagents used for the quantification of the concentration of the unknown adsorbed amount of lysozyme were prepared by mixing the following reagents in equal proportions: Copper sulphate (20 mg L^{-1}); sodium potassium tartarate ($NaKC_4H_4O_6.4H_2O$) (20 mg L^{-1}); sodium hydroxide (40 mg L^{-1}); sodium carbonates (20 mg L^{-1}).

RESULTS AND DISCUSSION

The amount of paranitrophenol adsorbed per gram of the adsorbents was calculated and plotted against equilibrium paranitrophenol concentrations and the specific surface areas were then calculated. From the plots (Figures 2 and 3), the monolayer coverage that is, the quantity of adsorbed paranitrophenol molecules that saturates the surface of 1.0g of the adsorbents (silica and polystyrene) so that all possible sites in the original solid surface are filled were obtained as 6.90 x 10^{-4} and 7.31 x 10^{-4} mol g^{-1} for silica and polystyrene, respectively. The specific surface area (SSA) was evaluated from the relation:

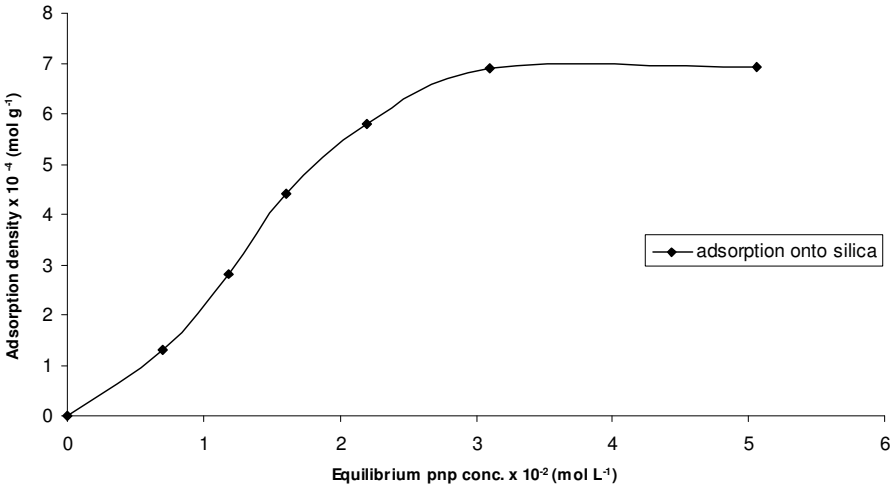

Figure 2. The variation of adsorption capacity (mol g^{-1} silica) with equilibrium paranitrophenol concentrations.

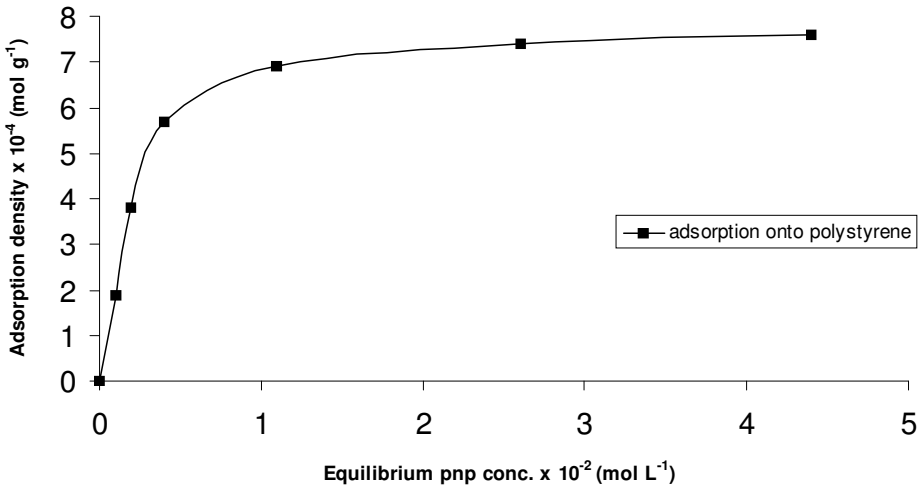

Figure 3. The variation of adsorption capacity (mol g^{-1} polystyrene) with equilibrium paranitrophenol concentrations.

$$SSA = X_m N_A A \quad (1)$$

Where, N_A = Avogadro's number, A = the area of surface occupied by each paranitrophenol molecule. The values of A as given in the Table of cross-sectional areas of adsorbed solute molecules by Gilles and Nakhwa (1962) are 25 x 10^{-20} m^2 for silica and 52.5 x 10^{20} m^2 for polystyrene. Hence, the obtained SSA for silica is ≈104 m^2 g^{-1} while polystyrene is ≈231.1 m^2 g^{-1}.

Effect of concentration

The effect of concentration was investigated as a plot of the variation of the amount of lysozyme adsorbed on both silica and polystyrene surfaces (mol g^{-1} adsorbent) against initial lysozyme concentration in mol dm^{-3} (Figure 4). The plots of adsorption densities of lysozyme on both surfaces were s-shaped with the plateau regions indicating the attainment of equilibrium for the adsorption process and with maximum adsorption capacities (mol g^{-1}) of 1.34 x10^{-6} and 1.57 x 10^{-6} for silica and polystyrene, respectively. The plots show an increase in adsorption with increase in the bulk lysozyme concentration until a saturation limit was reached which probably corresponds to completion of a monolayer (Figure 4).

The plots further revealed that adsorption was sensitive to particle size since the effective adsorption area; SSA, for polystyrene of 231.10 m^2 g^{-1} is higher than that of silica of 103.90 m^2 g^{-1}. The observed difference in the

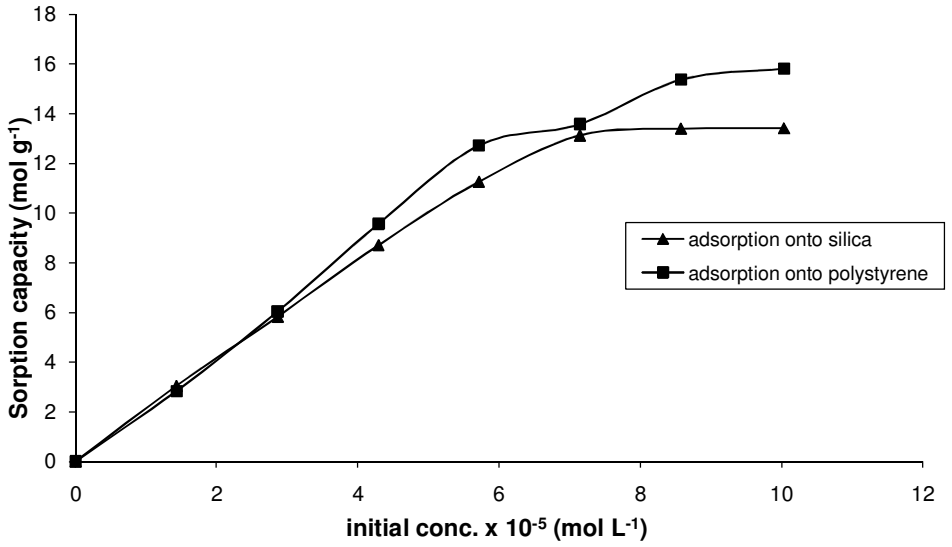

Figure 4. Variation of sorption capacity with initial concentrations of lysozyme.

amount adsorbed may be due to the difference in granulometry of the adsorbents. Kondo and Higashitani (1992) found that at low ionic strength, the adsorption of protein with small molecules such as lysozyme is affected by the surface properties of the adsorbent, whereas proteins with large molecules such as bovine serum albumin (BSA), ovalbumin (OVAL) etc. have maximum adsorption only around their isoelectric points without the size of the adsorbates influencing such adsorption. Therefore, the lysozyme is easily adsorbed on granulated adsorbents, probably because its small molecules penetrate the pores of the adsorbent particles and are retained by occlusion caused by tortuosity.

Adsorption isotherms

The equilibrium established between adsorbed lysozyme onto the surfaces and the unadsorbed in solution were assessed by the Langmuir isotherm model. The Langmuir equation, which is valid for monolayer adsorption onto a surface having a definite number of identical sites that are homogeneously distributed over the adsorbent surface is given by the relation (Bansal et al., 2009; Khani et al., 2006):

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} K_L} + \frac{C_e}{q_{\max}} \quad (2)$$

Where, q_e is the adsorption capacity of lysozyme per gram of adsorbents, C_e is the residual (equilibrium) lysozyme concentration in solution after adsorption, q_{max} is the maximum specific uptake or sorption capacity corresponding to site saturation or monolayer coverage and K_L is the Langmuir isotherm constant (ratio of adsorption and desorption rates). q_{max} and K_L were determined from the slope and intercept of linear plots of C_e/q_e versus C_e of the Langmuir plots (Figure 5). The Langmuir adsorption parameters and the linear correlation coefficients (R^2) for the adsorption process are shown in Table 1. High regression correlation coefficients (> 0.9800 for silica) show that the adsorption onto silica was well fitted by the model. Fitting of data into Langmuir isotherm model suggests that chemisorption is the main adsorption process. The isotherms show that polystyrene is a better adsorbent for protein removal than silica. The essential characteristics of Langmuir isotherm were expressed in terms of dimensionless quantity called separation factor, (or equilibrium parameter) R_L, defined as (Ahalya et al., 2005):

$$R_L = \frac{1}{(1 + K_L C_o)} \quad (2)$$

Where, K_L represents the Langmuir constant (L mol^{-1}) and C_0, the initial concentration of lysozyme (mol L^{-1}). The R_L parameter is usually treated as a reliable indicator for the adsorption process and is given in Table 1. According to Mckay et al. (1982) and Horsfall et al. (2004), R_L values between 0 and 1 indicate favourable adsorption. For unfavourable isotherm, $R_L > 1$; for linear isotherm, $R_L = 1$; for irreversible isotherm, $R_L = 0$ and for favourable isotherm, $0 < R_L < 1$.

The values of R_L obtained for the adsorption of lysozyme on both adsorbents were all less than unity, showing that the isotherms were favourable under the conditions of this study and also indicated that the adsorbents could be good materials for the removal of

Table 1. Langmuir Isotherm constants.

Adsorbent	q_{max} (mol. g^{-1})	K_L (L mol^{-1})	R_L	R^2
Silica	1.60×10^{-6}	1.39×10^{4}	0.50	0.9841
Polystyrene	2.72×10^{-6}	7.71×10^{4}	0.15	0.7934

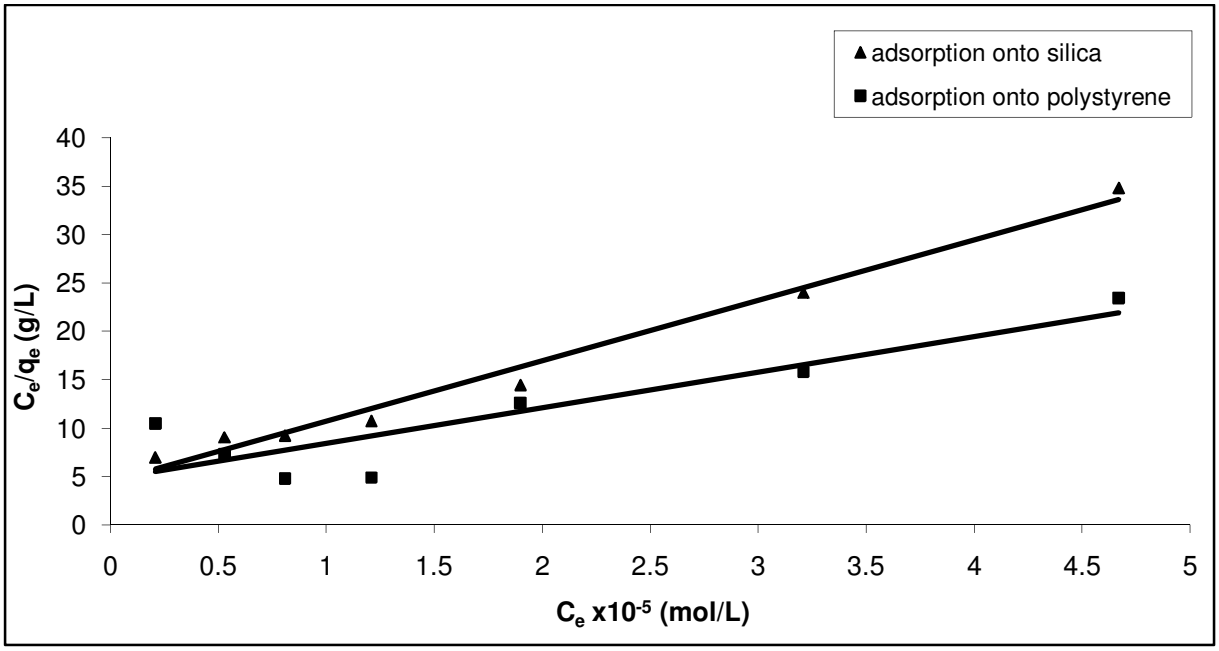

Figure 5. Langmuir isotherm plot of Ce/qe against Ce for adsorption of lysozyme onto the surfaces.

lysozyme from solutions.

Effect of ionic strength

The influence of ionic strength on the adsorption of lysozyme by silica and polystyrene surfaces was studied from the plot of adsorption density as a function of ionic strength as shown in Figure 6. It is evident from the plots that the adsorption of lysozyme decreased with increase in ionic strength for adsorption onto the silica surface. The adsorption densities decreased from 1.33×10^{-6} to 0.71×10^{-7} mol g^{-1} for silica and showed no significant effect for the adsorption onto the polystyrene surface (that is, from 1.35×10^{-6} to 1.30×10^{-6} mol g^{-1}) on increasing the ionic strength from 0.010 to 0.017. Adsorption is sensitive to the change in ionic strength showing that electrostatic interaction plays an important role in the adsorption of the lysozyme onto silica surface since a change in the ionic strength may cause a variation in the surface charge. The increase in ionic strength may have swamped the surface of silica and decreased lysozyme access to the silica surface for adsorption. This is buttressed by the reduced adsorption on the surface of silica and the insignificant decrease in the level of adsorption with increase in ionic strength on polystyrene surface since the surface of polystyrene is inert. According to the theory proposed by Guoy and Chapman (Osipow, 1972), when a solid adsorbent is in contact with adsorbate species in solution, they are bound to be surrounded by an electrical diffused double layer whose thickness is significantly expanded by the presence of an electrolyte. Such expansion inhibited the adsorbent particle (silica) and the lysozyme species from approaching each other very closely and through the decreased electrostatic attraction leads to the decreased uptake of the adsorbate (lysozyme). Addition of salt therefore reduced the amount of adsorption and complete removal of the lysozyme (protein) from the silica surface may be achieved for higher concentrations of the salt. The presence of small ions clearly alters the balance of repulsion between lysozyme (protein) molecules within the adsorbed layer and the attractive force between the surface and the protein. The results further show that hydrophilicity may be the major interaction process involved in lysozyme adsorption onto silica as compared to hydrophobicity observed for adsorption onto polystyrene. Hydophobic interaction observed for adsorption

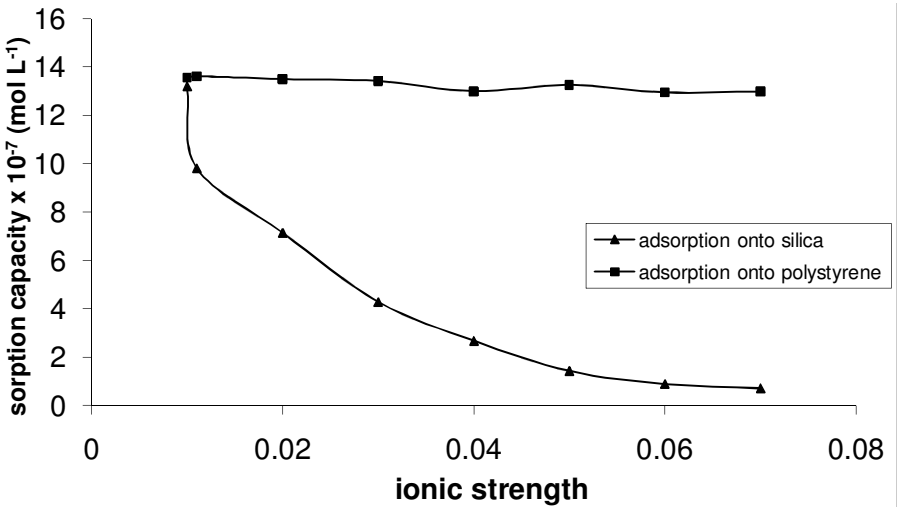

Figure 6. variation of sorption capacity with ionic strength for adsorption onto the surfaces.

onto polystyrene may be associated with entropy changes resulting from the dehydration of the lysozyme molecules or possibly structural changes resulting from the conformation or reorganisation of the lysozyme molecules upon adsorption.

Conclusion

The study of the adsorption capacity of lysozyme as functions of varying concentration and ionic strength was conducted. This study indicates that polystyrene (a synthetic material) could be used as an effective adsorbent material for the removal of lysozyme from aqueous solution than silica (a non-synthetic material). The isotherm obtained in this study indicates that the adsorption data fitted well into the Langmuir-type adsorption isotherm showing that the adsorption of lysozyme on silica is probably by chemisorption as observed by the high R^2 values (Table 1). The results also show that adsorption of small molecules like lysozyme is sensitive to particle size (granulometry of the adsorbent). The higher adsorbed amount of lysozyme of 1.57×10^{-6} mol g^{-1} by polystyrene may have also resulted from high effective specific surface area (SSA) of the polystyrene (231.10 m^2 g^{-1}) as compared to the SSA for silica of 103.90 m^2 g^{-1}.

Particularly relevant to the issues associated with protein fouling (biofouling) is the effect of ionic strength of the lysozyme adsorption. Increase in ionic strength reduced the amount of lysozyme adsorbed onto silica and complete removal of protein from the surface is likely to be achieved at high ionic strength. The presence of small ions may have altered the balance of repulsion between lysozyme molecules within the adsorbed layer and the attractive forces between the surface and the lysozyme.

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