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Adsorption Density and Spectra Distribution of Adsorbed Lysozyme as a Function of pH and Temperature

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Abstract: A study of the adsorption density and spectra distribution of adsorbed lysozyme (protein) from aqueous solutions unto silica and polystyrene surfaces has been carried out as a function of pH and temperature respectively. The maximum adsorption of lysozyme on both surfaces occurred at the pH range of 10.0 to 12.0 with a peaked value at 11.0. This behaviour showed that lysozyme (protein) has maximum adsorption around its isoelectric point of pH 11.0. The spectra of the adsorbed lysozyme were observed for varying temperature values on the two surfaces. The spectra showed maximum absorbance of 0.8020 at 20°C for silica and 0.7860 at 50°C for polystyrene at the same wavelength of 500 nm. The minimum absorbance occurred at the same wavelength of 600 nm and temperature of 40°C corresponds to the minimum absorbance of 0.004 for silica and 0.028 for polystyrene. Comparatively, minimum absorbance occurred at the same wavelength and temperature for both surfaces, but in contrast, maximum absorbance, though occurred at the same wavelength, but at different temperature; a phenomenon attributed to the non-synthetic nature of silica and synthetic nature of polystyrene surfaces.

Key words: Spectra distribution, adsorption, wavelength, absorbance, temperature

INTRODUCTION

Adsorption is a process where by atoms or molecules move from a bulk phase (that is solid, liquid, or gas) onto a solid or liquid surface. It involves separation of a substance from one phase accompanied by its concentration at the surface of another (Slejko, 1985). It is basically the formation of a layer of gas, liquid, or solid on the surface of a solid or, less frequently, of a liquid (Alan, 2000). A typical example of the adsorption phenomenon is the filtration of impurities from liquids or gases by their adsorption on a surface of high surface area such as activated charcoal. Another examples include the segregation of the surfactant molecules to the surface of a liquid, the bonding of reactant molecules unto solid surface of a heterogeneous catalyst and the migration of ions to the surface of a charged electrode.

Adsorption is thus different from absorption; a process in which material is transferred from one phase to another (e.g., liquid) and permeates the bulk of the absorbing substance (Alan, 2000). Sorption is a more general expression that encompasses both processes.

At the molecular level, adsorption is due to attractive interactions between a surface and the species being adsorbed. Adsorption is classified according to the magnitude of adsorption forces.

A common way to portray the results of adsorption studies on solid surfaces is in the form of an adsorption isotherm. Such a diagram gives the amount of adsorbed material per surface area at a constant temperature as a function of pressure or concentration in the bulk phase. An adsorption isotherm represents a chemical equilibrium in which molecules are simultaneously adsorbing to and desorbing from the surface. The isotherm shows the extent of the adsorbed layer, that is, the net result of these two competing processes.

The association of two freely translating and rotating molecules to form a complex, in the gas phase or in solution, generally involves a loss of entropy. Thus, the complex will survive as stable species only if its formation is favoured on enthalpic grounds. That is, the potential energy of the bound complex must be lower than that of the well-separated molecules. In solution it is the potential of mean force that must be lower (Ben-Tal *et al*., 2000).

Adsorption of proteins unto solid surfaces is a process that influences phenomena within many disciplines and as diverse as fouling of process equipment in the food industry, blockage of filtration membranes in bioseparation processes and regulation of immuno-response to foreign materials (Wahlgren and Arnebrant, 1990). Most protein containing fluids are mixtures of many different proteins, which most likely

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influence each other's adsorption behaviour and rate. There are no general rules for determining the adsorption of proteins from a mixture. However the investigations of blood showed that the presence of small proteins adsorbed initially when the concentration is high. This was later replaced by proteins present in lower amounts but with higher surface activity and higher molecular weight. Other factors such as hydrophobicity, charge density, flexibility and conformation may also determine which of the proteins will adsorb.

In order to completely characterize and predict protein adsorption, one seeks the information about adsorption isotherms, adsorption kinetics (in situ), conformation of adsorbed proteins (Andrade, 1985), number and character of surface bound protein segment and the physical parameters describing the adsorbed protein layer (Norde, 1986). All these information can be obtained using different protein adsorption experimental techniques. The combination of such information can answer questions such as the mechanism of protein adsorption unto and desorption from surfaces of which one approach to this problem is the analysis of protein adsorption kinetics (József, 2002).

Due to the polyelectrolytic nature of proteins, adsorption usually occurs via coulombic interaction between the charged surface and the oppositely charged amino acids (proteins). For instance, on a hydrophilic solid surface, the electrostatic attraction between a charged surface and an oppositely charged protein molecule is often the driving force for adsorption from solution unto solid surfaces. A balance between these electrostatic interactions within the adsorbed layer may then determine the amount adsorbed. A further driving force for protein adsorption is entropic changes normally associated with dehydration of the protein or a structural rearrangement of the protein molecules. Strong surface interactions may damage the original state of a protein molecule and this may lead to a loss of its coherent structure (denaturation). The extent of such structural deformation on adsorption depends on the nature of the surface and the relative stability of the protein structure. Adsorption is an important feature of surface reactions, such as corrosion and heterogeneous catalyst and the property is also utilized in adsorption chromatography (Alan, 2000).

In this study, lysozyme (protein from chicken egg white) is used as a model protein. There are several reasons for choosing this protein. For instance, it is a globular protein with well-defined molecular structure and its stability is likely to prevent complete unfolding at a surface. This suffices to say that the structure of lysozyme and the unfolding behaviour of dissolved lysozyme are well characterized. Again, at moderate pH values, it has a high thermal stability, which has its origin in part from four disulphide bonds.

The adsorbents used for this experiment are silica particles, a non-synthetic surface and polystyrene, a synthetic surface. The equilibrium and non- equilibrium adsorptions of the protein were carried out. The effect of varying temperatures on the spectra distribution of adsorbed lysozyme was studied and an overall picture of protein adsorption kinetics carefully examined in the light of varying pH.

MATERIALS AND METHODS

Materials/equipments: Pure crystalline lysozyme (from chicken egg white, grade 1, product No L-6876, mol. Weight 14,000 g mol^{-1}) used in this study was obtained from Sigma chemical Co., USA. Chromatographic grade silica particles (mesh size 200 μm) 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- B1 was obtained from Dongbu Hannong chemical Co. Ltd., Korea. The polystyrene was crushed with manual blender to smaller particles and sieved 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 was washed with water to separate the glucose and then dried at a temperature of 120°C. All inorganic salts used were BDH chemicals and were of analytical grade.

The digital pH meter CE HI 98127 used for the analysis is a product of Hanna instruments inc. Woonsocket, Rhode Island, 02895 USA, (PH, EC /TDS water proof family) and measures to accuracy of 0.1 with an in-built automatic temperature control (ATC) which, was standardized using standard buffer solutions (4.10, 7.1 and 9.18) according to specifications. Visible spectrophotometer, 722 S spectrophotometer; No SFZ 1506010514 was used for the quantification of the protein (lysozyme). The absorbances were taken and their concentration determined from the calibration curve. Digital analytic weighing balance, X21-0014 KERN 770-15,15402301, made in Germany, which measures to an accuracy of 0.0001g and Mechanical Shaker, Versal shaker, type: LE-203/1 made in Hungary were used throughout the study.

Sample preparation: Five different buffers, pH 5.0, 7.0,10.0,11.0 and 12.0 were prepared with each having ionic strength of 0.01.

For pH 5.0, 8 mL of 0.1 M solution of acetic acid was mixed with 35.2 mL of 0.1 M Sodium acetate.

For pH 7.0, 100 mL of 0.05 M solution of KH_2PO_4 was mixed with 55 mL of 0.05 M NaOHF. For pH 10.0, 160 mL of 0.025 M Na_2HPO_4 was mixed with 5 mL of NaH_2PO_4.

For pH 11.0, 100 mL of 0.025 M K_2CO_3 was mixed with 43 mL of 0.025 M $KHCO_3$.

For pH 12.0, 100 mL of 0.05 M Na_2HPO_4 was mixed with 53.8 mL of 0.05 M NaOH.

Methods: The lysozyme (protein) material under test was dissolved in the buffer 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}, with the concentrations evenly spaced. The initial time of addition of the protein to the standard flasks was noted. The flasks were shaken vigorously. At different intervals of elapsed time each flask was taken out of the shaker, the particles were allowed to settle, filtered and 1.0 mL of the protein solution was withdrawn. Using Folin-Ciocalteu reagent, the concentration of the protein solution after adsorption at a time t was determined spectrophotometrically using the Lowry method. The flasks were shaken for 16 h to attain equilibrium and at the end of this period, the lysozyme concentration (C_p) in the bulk solution (normally read off from the calibration curve) was determined by spectrophotometry using the standard method.

To determine the amount of protein adsorbed at equilibrium, T^e_p, the flasks were shaken at constant temperature, 30°C for 16 h and then kept undisturbed for 4 h. This was done to ensure equilibrium attainment and for protein adsorption to be completed during this period. The procedure was also repeated at lower and higher temperatures of 20 and 40°C and pH 7.0. The amounts adsorbed at different intervals of the elapsed time as a function of temperature were calculated.

RESULTS AND DISCUSSION

Results: The above experiment was carried out at various pH and temperatures. To determine the effect of pH, buffer solutions of varying pH, 5.0, 7.0, 10.0, 11.0 and 12.0 were prepared as discussed earlier. 0.4 gram of each of the pure silica and polystyrene was added to each of the five flasks (double set) containing 10 cm^3 of 7.143×10^{-5}M lysozyme solutions at an ambient temperature of 30°C. The effect of temperature on the adsorption equilibrium of lysozyme was studied by varying the temperature from 20 to 50°C using the same initial concentration above and at pH 11.0 as to enable the study of the spectra distribution.

Table 1 shows the adsorption densities at varying pH values for silica and polystyrene while Table 2 shows the

Table 1: Adsorption density at varying pH values onto silica and polystyrene surfaces at ambient temperature of 30°C

Initial lysozyme concentration × 10^{-5} (mol L^{-1})	Varying pH values	Equilibrium absorbance at 750 nm	Equilibrium concentration × 10^{-5} (mol L^{-1})	Amt of lysozyme adsorbed from 1dm^3 of solution ×10^{-7} (mol g^{-1})	Adsorption density ×10^{-9} (mol m^{-2})
Silica					
7.143	5.0	0.291	4.429	6.776	6.531
7.143	7.0	0.214	3.291	9.633	9.278
7.143	10.0	0.190	3.000	10.346	9.964
7.143	11.0	0.123	1.856	13.203	12.082
7.143	12.0	0.156	2.358	11.954	11.510
Polystyrene					
7.143	5.0	0.282	4.2143	7.331	3.174
7.143	7.0	0206	3.071	10.180	4.409
7.143	10.0	0.198	2.930	10.545	4.565
7.143	11.0	0.114	1.713	13.569	5.879
7.143	12.0	0.170	2.572	11.430	4.951

Table 2: Maximum wavelengths of the visible spectra of adsorbed lysozyme on silica and polystyrene surfaces as a function of temperature at pH 11.0

Wavelengths (nm)	Absorbance at 20°C	Absorbance at 30°C	Absorbance at 40°C	Absorbance at 50°C
Silica				
450	0.074	0.036	0.004	0.037
500	0.802	0.750	0.729	0.759
550	0.636	0.570	0.559	0.585
600	0.481	0.405	0.401	0.442
650	0.597	0.344	0.517	0.548
700	0.423	0.344	0.343	0.378
750	0.205	0.123	0.119	0.152
800	0.115	0.051	0.041	0.073
Polystyrene				
450	0.041	0.008	0.028	0.055
500	0.771	0.716	0.687	0.786
550	0.618	0.551	0.536	0.625
600	0.464	0.391	0.385	0.481
650	0.587	0.570	0.506	0.603
700	0.416	0.339	0.333	0.422
750	0.186	0.116	0.110	0.199
800	0.101	0.040	0.029	0.112

maximum wavelengths of the visible spectra of adsorbed lysozyme on silica and polystyrene surfaces as a function of temperature at its isoelectric pH (pH 11.0).

DISCUSSION

It is observed as shown in Table 1 that as the pH value increases, adsorption densities of lysozyme on both surfaces increased rapidly reaching a maximum at 12.082×10^{-9} mol m^{-2} for silica and 5.879×10^{-9} mol m^{-2} for polystyrene at a pH 11.0; the isoelectric pH of lysozyme with a decrease on either side of the isoelectric pH for both surfaces. This behaviour shows that lysozyme exhibits maximum adsorption in the neighborhood of its isoelectric pH. Major interaction involved in lysozyme (protein) adsorption at this isoelectric pH is hydrophobicity, which may be due to entropic changes associated with dehydration of the protein or a structural rearrangement of the protein particles.

Yoon *et al*. (1998) showed that hydrophobic interaction is the most important aspect of protein adsorption and increased hydrophilicity results in a low level of adsorption. The slight increase in adsorption with increasing pH value on the polystyrene surfaces shows that the surface is inert.

Also the low adsorbed amount onto silica at pH 5.0 and 7.0 shows that silica has net positive charges at those pHs. The electrostatic repulsion between the charged lysozyme solution and the charged silica surface led to decreased rate of adsorption.

In general, lysozyme exhibits a maximum adsorption density near or at the isoelectric pH mainly due to entropic contributions originating from the dehydration of hydrophobic surface areas and adsorption-induced conformational changes of the protein.

The spectra distribution of lysozyme on both surfaces showed a markedly similar response in the minimum absorption range at a wavelength of 600 nm and temperature of 20°C. The absorbance of 0.8020 at 20°C for silica and 0.7860 at 50°C for polystyrene both corresponding to a maximum wavelength of absorption of adsorbed lysozyme, 500 nm as shown in Table 2.

The adsorbed lysozyme on silica had uniform variation in absorbance with wavelength (450 to 500 nm) for a given temperature. The maximum absorbance occurred at 0.8020 when the wavelength was 500 nm and decreased sharply to 0.4010 at wavelength of 600 nm. The absorbance had maximum value 0.5970 again at the wavelength 650 nm, after which it decreased to about 0.0410 at the wavelength of 800 nm. Similarly, the adsorbed lysozyme on polystyrene surface showed the same behaviour like that of silica, though; the maximum absorbance was more for that of silica.

From this result, one observes that these materials could be used as filter material within the optical region of the electromagnetic spectrum.

CONCLUSIONS

This study indicates that silica (a non-synthetic material) could be used as an effective adsorbent material for the removal of lysozyme from aqueous solution than polystyrene (synthetic material). The adsorption of lysozyme onto silica is found to be time, pH and temperature dependent. The maximum amount adsorbed occurs between the pH ranges of 10 to 12 for both surfaces –the isoelectric pH of lysozyme. This behaviour shows that lysozyme exhibit maximum adsorption in the range of its isoelectric pH. The major interaction at this pH is hydrophobicity, which is due to entropic changes associated with the dehydration of the lysozyme molecule or a structural rearrangement of the protein.

The spectra distribution shows that the adsorbed lysozyme on silica and polystyrene surfaces could be used as filter materials in the optical region since maximum absorbance occurred around 500nm-a typical wavelength range of visible light.

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