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38th International Science Technology Education Arts Management
& Social Sciences (iSTEAMS) Bespoke Conference - Accra Ghana 2024

Towards the Evaluation of the Adsorption of Bovine Serum Albumin onto Silica as a Function of pH

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ABSTRACT

Adsorption of proteins play an important role in many disciplines which includes Biomedical Engineering, Biotechnology and Environmental Sciences. This paper investigated the adsorption of bovine serum albumin (BSA) onto silica with the sole aim of elucidating the rate of adsorption at different P^H of 4.7, 5.0 and 7.0. The specific surface area of silica used in this adsorption study was $2.2m^2/g$ and $47.2m^2/g$. These two surface areas were used in this adsorption study to ascertain if there will be any change in the adsorbed amount of BSA on silica.1.5mg/ml of BSA was prepared in aqueous, isotonic and buffer solutions and the concentration of BSA in the solution was determined form the absorbance value obtained by using Beer Lambert's equation. Chapter one dealt with the introduction of BSA and Silica, chapter two dealt with the experimental and methodology, chapter three is the result and discussion and chapter four is the conclusion and future work.

The findings showed that adsorption on silica surface was governed by the isoelectric point, ionic strength of the solvent being used, hydrogen bonding and to a lesser extent, electrostatic attraction. Highest adsorbed amount of BSA on silica was observed in the range of the isoelectric point of BSA. Results obtained were reported and represented using the Langmuir adsorption isotherm

Keywords: Adsorption, Bovine Serum Albumin, Silica, Beer Lambert's equation, isoelectric point

Proceedings Citation Format
Sani, Z. (2024): Adsorption of Bovine Serum Albumin onto Silica as a Function of pH. Proceedings of the 37th iSTEAMS Multidisciplinary Bespoke Conference. 17th – 19th June, 2024. University of Ghana, Accra, Ghana.
Pp 163-173. dx.doi.org/10.22624/AIMS/ACCRABESPOKE2024P16

1. BACKGROUND TO THE STUDY

Proteins are organic compounds which are made up of amino acids arranged in a linear chain and these chains are joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. Proteins work together to achieve a particular function, and they often associate to form stable complexes. [1] They are relatively large bio molecules and have the tendency to accumulate at the interface between solution and solid surfaces. [2] Proteins are essential part of organism and participate in every process within cells. Many proteins are enzyme that catalyze biochemical reactions and are vital to metabolism. Bovine serum albumin is used as our model protein in this adsorption study because of its medicinal importance, low cost, readily availability and unusual ligand binding properties.

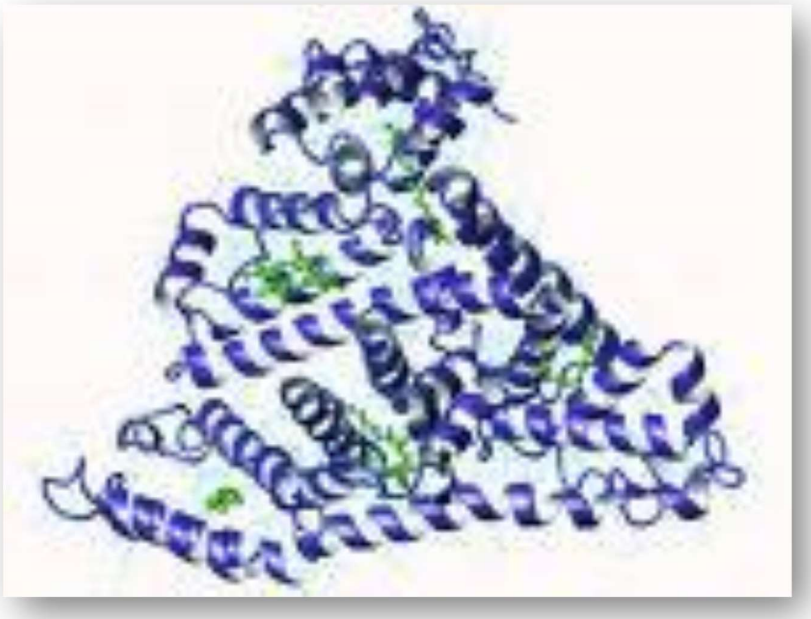

Figure 1: Structure of Bovine Serum Albumin. [3]

Adsorption of proteins plays an important role in many disciplines which includes biomedical engineering, biotechnology and environmental science. [4] Also from a more fundamental research perspective, the protein adsorption process is of interest due to the complex nature of the system and ideally it is important to understand in details how the protein concentration, buffer, pH, ionic strength e.t.c, influence the protein adsorption[5]. Even though the effect resulting from a change in one or more of the above parameters depends to a large extent on the actual protein in question, some general features of the adsorption process have been elucidated. The chemical and morphological property of the substrate on which the protein adsorbs is of crucial importance.

Mechanism of protein adsorption from aqueous solution on a solid surface is in three steps which include: protein transportation form the solution toward the solid surface, attachment of the protein to the surface and conformational change in the protein structure after adsorption. [6]

1.1. Understanding Of Protein Adsorption

The understanding of protein adsorption is an important phenomenon and as such, it has been of great interest to many scientists over the years. Fibrinogen, lysozyme and albumin have been used as model proteins for most adsorption studies [6]. Many experimental results are contradictory. This is mainly due to use of different measurement techniques, surface preparation, hydrodynamic conditions, solution properties i.e. ionic strength, medium pH, electrostatic interaction, surface charge.[7] Several factors can drive the protein adsorption process[8], this includes:

- Electrostatic interactions between protein – protein and surface – protein which may be attractive or repulsive.
- Dehydration of the surface and the protein. This is related to the release of a large number of water molecules during adsorption, which promotes adsorption on a hydrophobic interface while opposing it on a hydrophilic interface.
- Structural changes in the proteins which are a consequence of changes on adsorption in the balance between intermolecular repulsion and intra molecular hydrophobicity.

The hydrophilic character of the surface and conformational protein stability are perhaps, the two main aspects that determine the adsorption process. As a rule, a protein molecule with low conformational stability (soft protein), such as albumin, adsorbs on a great variety of surfaces (hydrophilic or hydrophobic), even under adverse electrostatic condition, in contrast, high stability protein (hard protein) adsorbs on hydrophilic surfaces only under favourable electrostatic interactions.

2. RELATED WORKS

In 2008, Renganathan and co workers successfully studied the binding of TiO_2 colloid with serum albumin. The interaction involved in protein adsorption can be classified as electrostatic, hydrophobic and hydrogen bonding. Protein adsorption has being studied over the decades because of its usefulness in biomedical application, which includes artificial tissue and organ drug delivery system. [9] In this study, bovine serum albumin is being used as a protein model because of its importance in medicine, low cost, readily availability and unusual ligand binding properties

2.1 Bovine Serum Albumin

Bovine serum albumin (BSA) is a globular protein about (66,000 Da) with dimensions 4nm×4nm×14nm [10] and has a great conformational adaptability (it is a soft protein). It consists of a single chain of 583 amino acids residues, and forms sub domain by paired 17 disulfide bonds. The blood plasma protein BSA represents 52-62% of the total plasma protein fraction. [11] The most important physiological function of serum albumin is to maintain the osmotic pressure and pH of blood and transport, a wide variety of endogenous and exogenous compounds which includes fatty acids, metal, amino acids, steroids and drugs [12] The iso electric point of BSA is at pH 4.5-5.0.

This means that in a neutral solution, BSA is negatively charged BSA has the ability to bind substances reversibly especially negatively charged substances. For this reason, it is able to assume roles of transportation. [6]

Some physiochemical characteristics of BSA[13] include:

Parameter	Value
Molecular weight	
From composition	66,267
From physical data	66,700
Molecular dimensions	
Side	8.0nm
Depth	3.0nm
Iso electric point	4.7
Conformation stability	stable

2.2. Efects OF pH ON BSA

When changes are made in pH, bovine serum albumin undergoes reversible conformational isomerization as shown below.

E <------> F <------> N <------> B <------> A

pH of transition:	**2.7**	**4.3**	**8**	**10**	
Name:	expanded	fast	Normal	basic	aged
% helix:	35	45	55	48	48

Fig 2: The relationship of isomeric forms of bovine serum albumin [14]

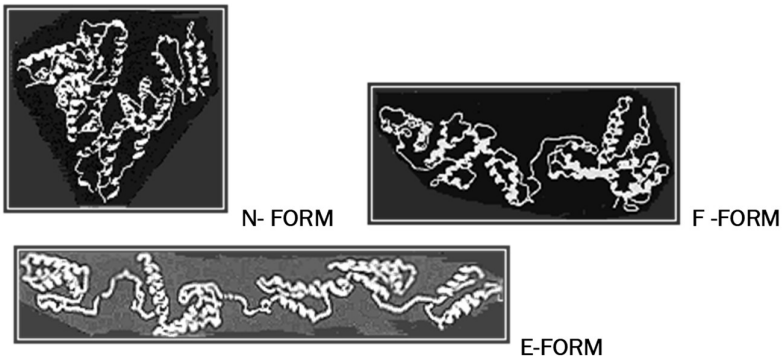

Figure 3: Showing the ribbon diagram of serum albumin in its N, E and F forms [15]

2.3. Adsorption isotherms

Adsorption is an effective separation process which is used for a wide variety of applications. Adsorption can be defined as an accumulation of atoms or molecules on surface of atoms on surface of a material. The adsorption process usually creates a film of the adsorb ate (i.e. the molecules or atoms being accumulated) on the adsorbent's surface. [16].

Adsorption is usually described through isotherms, i.e. the amount of adsorbent as a function of its pressure (if gas) or concentration (if liquid) at a constant temperature. Langmuir equilibrium isotherm equation is widely used to describe equilibrium adsorption phenomena [17]. It was developed for gas adsorption but has been extensively applied to adsorption from solution. The assumption is made that protein binds as a monolayer to equivalent surface binding sites that are independent, that is, binding site at one site does not affect the binding at other sites. The kinetic equation for a single domain protein molecule can be written as

$$\frac{d\theta}{dt} = k_a c (1-\theta) - k_d \theta \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where θ is the fractional surface coverage, t is the time, c is the concentration of the protein in the solution k_a and k_d are the adsorption and desorption rate constant, respectively. When the adsorption reaches equilibrium (i.e. θ constant), the Langmuir isotherm equation results [18]

$$\theta = \frac{kc}{1 + kc} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where k is the equilibrium binding constant

$$K = \frac{k_a}{k_d} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

In this study, the Langmuir isotherm was obtained when the graph of adsorbed amount of bovine serum albumin on silica was plotted against concentration.

3. ULTRA-VOILET VISIBLE SPECTROPHOTOMETRY

The ultra violet visible spectroscopy was used in this study to determine the absorbance value of the bovine serum albumin in the solution. The concentration was calculated from the absorbance value obtained using the beer Lambert equation knowing the molar absorbtivity of bovine serum albumin to be 0.667mL mg^{-1} cm^{-1} [19] and the path length to be 1cm.

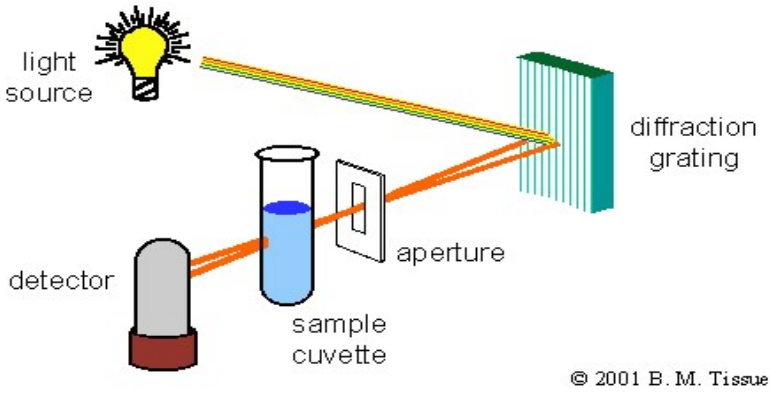

Figure 4: Schematic diagram of the single beam UV/ visible spectrophotometer [20]

3.1 Beer Lambert's law

The beer-Lambert law states that the absorbance of a solution is directly proportional to concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/visible spectroscopy can be used to determine the concentration of the absorber in the solution. Beer Lambert law governs the measurement of light absorption from a solution of molecules and can be written as follows:

$\text{Log } I_o / I_t = A = \epsilon bc$ [21]

Where I_o is the intensity of the incident radiation; I_t is the intensity of the transmitted radiation; A is the absorbance and is the a measure of the amount of light absorbed by the sample; ϵ is the molar extinction coefficient and is the absorbance of a 1M solution of the analyte: b is the path length of the cell in and is usually 1 cm: and c is the concentration of the analyte in moles per litre.

In pharmaceutical products, concentrations and amounts usually expressed in grams or milligrams rather than in moles and thus for the purpose of the analysis of these products, the beer Lambert equation is written in the following form:

$$A = \varepsilon\ (1\%, 1cm)\ bc$$ [21]

Since measurements are always made in a 1cm cell, the equation can be written as:

$$c = \frac{A}{\varepsilon\ (1\%, 1cm)}$$

The above equation gives the concentration of the analyte in g/100ml.
The UV/ visible spectrometer was used in this study as a suitable analytical technique considering the aim of the study which has to do with finding out the amount of bovine serum albumin adsorbed onto the silica surface.

3.2 Buffer Solution

The term buffer, buffer solution, and buffered solution, is often used with reference to hydrogen ion concentration or pH and it refers to the ability of a system , particularly an aqueous solution, to resist a change in pH on adding acid or alkali or on dilution with solvent. [22] If an acid or base is added to water, the pH of water changes markedly because water has no ability to resist change of pH. A very weak acid such as carbon dioxide changes the pH of water, thereby decreasing the pH from 7 to 5.7 when the small concentration of carbon dioxide present in air equilibrated with pure water. This extreme susceptibility of distilled water to change in pH upon adding very small amount of acid or base is often of great concern in pharmaceutical operations. Solution of neutral salts, such as sodium chloride, similarly lacks ability to resist change of pH on adding acid or base.

A buffer can be prepared in two ways.

- known amount of A^- and HA forms may be mixed and diluted to volume
- To a known amount of the HA form (A^- form), a known amount of base (acid) may be added and the mixture diluted to volume.

Due to the pH in which this study is being carried out, (i.e. pH 7), bovine serum albumin being negatively charged, will not adsorb or adsorb in small amount onto silica surface which is also negatively charged due to electrostatic repulsion. Citrate phosphate buffer was used in this study to serve as an electrolyte to enhance adsorption due to the addition of both positive and negative ions from dibasic sodium hydrogen phosphate.

3.3 SILICA

Silica is the major component in the earth crust, and it has a general formula of SiO_2 or $SiO_2.XH_2O$ [23]. It occurs naturally in minerals such as quartz. In chemical applications, most of the silica used is of the synthetic origin. Silica is the major component of most glass and also is found in substances such as clay. Silica has been employed in material sciences and also in the field of engineering for many years. Silica finds wide application in various facets which includes [24]:

- Liquid-liquid chromatography in which silica packaging's are used and they act as a sponge to hold the static liquid phase as an active adsorbent
- Silica being a good adsorbent is used as a catalyst base
- Silica also finds application as a desiccant.
- Silica nanoparticles are by far the most attractive for basic research and clinical trials and hence used for drug/ gene delivery carriers.

The silicon is bonded to four oxygen atoms and each silicon atom is located in the middle of a regular tetrahedron of oxygen atoms as shown in the figure below:

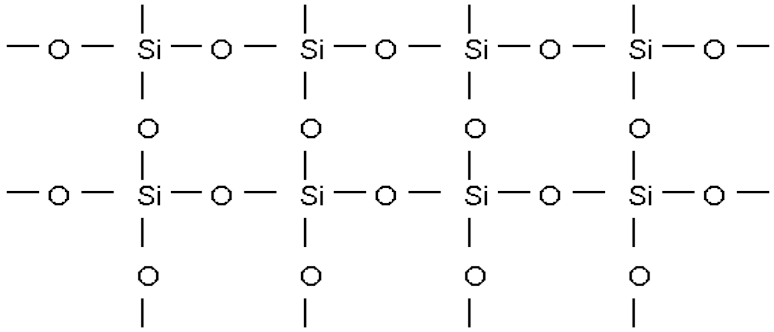

Figure 5: silicon linked through oxygen to other silicon [25]

The silica surface consists of hydroxyl groups and also ethereal linkages and hence the silica surface is said to be negatively charged due to the adsorption of electron deficient species. The surface also consists of various kinds of silanols and siloxanes [26]. The silanols are strong adsorption sites and the siloxane site is regarded as hydrophobic. The siloxane (SiOSi) is characterized by residual valences which react with water and hence, the surface is covered with silanol group at normal temperature. During adsorption onto the silica surface, the mechanism involved is a binding force between a specific atom of the adsorbate and an atom on the surface and once the surface is covered, no other layer is adsorbed. Proteins bind most strongly at their isoelectric points and the binding may be between organic cationic sites and the charged silica surface and also through hydrogen bonding. [27]

In a work carried out by Fakuzaki (1996), the adsorption of bovine serum albumin on silica, titanium dioxide, and alumina as a function of pH was investigated. It was reported that the saturation values of bovine serum adsorption varied with pH which showed maximum saturation around the isoelectric point of bovine serum albumin for all the metal oxides. The maximum amounts of adsorbed BSA (mg/m^2) were 2.2 on silica, 3.4 on titania, 4.2 on Zirconia and 4.7 on alumina. This was due to the surface charge density of the metal oxide. Also in a similar work carried out by Rezwan et al, the adsorption of lysozyme and bovine serum albumin on silica and also silica particles coated with AlOOH was investigated. They found out that at pH 7, in which bovine serum albumin is negatively charged adsorbed nearly 100% on the positively charged surface of AlOOH and the lysozyme which is positively charged adsorbed completely on the negatively charged silica.

From their observation, they concluded that electrostatic interaction governs adsorption process at pH 7 for the proteins they investigated and also the oxide surfaces. In this report, the adsorption of bovine serum albumin onto silica with different surface areas i.e. 47.2 surface areas, and 2.2 surface areas was studied in different media (isotonic, buffer and water) and at different pH i.e. 7, 4.7 and 5. Silica with different surface area was used in this study to ascertain the effect of surface area on the adsorption amount. The results obtained were of similar nature to those obtained above.

4. MESOPOROUS/MICROPOROUS MATERIAL

Microporous materials are defined as materials with pore diameter of less than 2 nm while mesoporous materials are materials with pore size ranging from 2 nm to 50nm. Typical mesoporous materials include some kinds of silica and alumina which have similar sized fine mesopores. [28] much attention has been paid to mesoporous materials with regular geometrics and this is due to their great potentials in practical applications which includes catalysis, separation, adsorption, sensing, medical usage, ecology, and nanotechnology to mention but few.[29] A major break through in the mesoporous materials research came when Mobil scientist disclosed the M41s family of materials, these materials have large uniform pore structure, high specific surface areas and specific pore volumes including hexagonal - MCM-41.

Mesoporous materials are basically prepared through silica formation around template micelle assemblies followed by template removal by appropriate methods of calcinations. Adsorption of proteins to mesoporous materials, especially to mesoporous silica, has been rapidly paid attention to as well as polymeric matrix have been employed as substrates for drug delivery [30]. Owing to high chemical and thermal stabilities, large surface areas and good compatibilities with other materials, porous silica has also found wide application in adsorption, enzyme immobilization and drug delivery.

In the past decade, mesoporous materials have found a lot of applications in separation, catalysis, sensors and devices. Due to stable mesoporous structure and well defined surface properties, mesoporous materials seem ideal for encapsulation of pharmaceutical drug, proteins and biogenic molecules. [28]

4.1 Silica Mesoporous Material

Mesoporous silica has well defined nano channel structures which are formed over templates through self assembly process. Mesoporous materials are well characterised by huge surface areas, which makes them ideal for use as catalysts in chemical reactions.
The discovery of highly ordered mesoporous silica was quickly recognized as a break through that could lead to a variety of important application in host-guest systems [31] Silica –based mesoporous materials have unique structural characteristics, tunable pore size, well modifiable surface area which make them highly applicable in the fields of catalysis, and sensing.

Exploring new application of these silica based mesoporous materials in their composition, textural and structural characteristics was a goal to consider them as promising materials with bone regeneration purposes[32]. However, for such desirable application, silica based mesoporous materials must exhibit a bioactive response.

Several drugs have been introduced into silica-based mesoporous materials to be used as drug delivery systems. Some of these drugs are ibuprofen, amoxicillin, gentamicin, nitrofurazone, erythromycin, vancomycin, naproxen, aspirin, diflunisal, captopril, persantin, etidronate and alendronate. [33] The double perspective of the mesoporous materias, tissue regeneration and drug delivery, has promoted the research of these materials for biomedical application in the last few years.

The regular repeating mesoporous structures of these silica based mesoporous materials has motivated the adsorption in their pores and subsequent release of a large variety of biologically active species such as proteins, polypeptide or amino acids [34]. In this adsorption study carried out, the silica used was with little or no porosity with a surface area of 2.2 g/m^2 and 47.2 g/m^2.

5. CONCLUDING REMARKS

This research evaluated the adsorption of bovine serum albumin on silica surface at different pH of 4.7, 5.0 and 7.0 to ascertain the effect of P^H on the adsorbed amount of BSA on silica surface . Results obtained was represented by the Langmuir adsorption Isotherm. The effect of ionic strength on adsorption was also elucidated by carrying out the experiment in different media such as water, isotonic and buffer solutions. The results obtained showed that adsorption is governed by other factors asides the isoelectric point which includes ionic strength of the solvent used, hydrogen bonding . The highest adsorbed amount of BSA was observed in the range of the isoelectric point of BSA which is 4.7.

Future works will address the methodologies to be adopted and present experimental results

BIBLIOGRAPHY

[1] Walsh, G. (2001), Proteins biochemistry and biotechnology, John Wiley & sons.

[2] Norde, W. (1994) Protein adsorption at solid surfaces: a thermodynamic approach, journal of pure applied chemistry 66, 491-496.

[3] Structure of bovine serum albumin. [Online] available from http: //166.114.99.91/astrojan/protein/pictures/albumin3. [Accessed 20th July 2009].

[4] Yeung, K.M., Lu, Z.j., and Cheung, N.H. (2008) Adsorption of bovine serum albumin on fused silica: elucidation of protein- protein interactions by single – molecule fluorescence microscopy. Journal of colloids and surfaces B: Biointerfaces 69(2), 246-250.

[5] Norde, W. (2nd edition) In: M. Malmsten, editor, biopolymers at interfaces vol. 75, Marcel Dekker, New York. 21

[6] Leuensmann, D and Jones, L. (2008) Albumin adsorption to contact lens material:A review. Journal of contact lens material: 31(4), 179-187.

[7] Kuldeski, A. (2003), influence of electrostatically bound proteins on the structure of linkage monolayers; adsorption of bovine serum albumin on silver and gold substartes coated with monolayers of 2- mercapto ethane sulphonate, 33, 197-204.

[8] Alkan, M., Demirbas, O., Dogan, M., and Arslan, O. (2006) surface properties of bovine serum albumin- adsorbed oxides: Adsorption, adsorption kinetics and electro kinetic properties, Microporous and mesoporous materials. 96 (1-3) 331-340.

[9] Asha Jhonsi,A., Kathiravan, A., and Renganathan, R. (2009) Spectroscopic studies studies on the interaction of colloidal capped cds nanoparticles with bovine serum albumin. Journal of colloid and surfaces B: Biointerfaces 72(2), 167-172.

[10] Mc Clellan, S.J., and Franses, E.I. (2003) Effect of concentration and denaturation on adsorption and surface tension of bovine serum albumin. Journal of colloids surfaces B: biointerfaces 28, 63-67.

[11] Brandes, et.al. (2006) induced conformational changes of proteins onto ceramic particles: differential scanning calorimetry and FTIR analysis. Journal of colloid interface science 299, 56-59

[12] Kathiravan, A., Anandan, S., and Renganathan, R. (2008) Interaction of colloidal TiO2 with human serum albumin: A fluorescence quenching study. Journal of colloids and surfaces A: physiochemical and engineering aspects 333(1-3), 91-95.

[13] Carla et.al. (1997) physiochemical properties of bovine serum albumin. Journal of colloid and interface science 188, 387- 395.

[14] Foster, J.F. (1977). In 'Albumin structure, function and uses' (V.M. Rosenoer, M. oratz, and M.A.Rothschild, eds). 53-84. Pergamon, oxford.

[15] Carter, D.C and Ho, J.X. (1994) structure of serum albumin. Adv. Protein chemistry 45, 153-203.

[16] Henderson, et al. (2009) A novel isotherm, modeling self – Assembled monolayer adsorption and structural changes, Langmuir, 25(2), 931-938.

[17] Shaw, D.J., (fourth edition). Introduction to colloid and surface chemistry. Great Britain. Redwood books.

[18] Hiemanz, P.C., and Rajagopalan, R., (third edition) principles of colloid and surface chemistry. Blackwell: CRC press.

[19] Simi, C.K., and Abraham, E.T., (2009). Nanocomposite based on modified TiO2 – BSA for functional applications. Journal of Colloids and surfaces B: biointerfaces 71, 319-324.

[20] Schematic diagram of UV/ visible spectrophotometer [online]. Available from http://www.files.chem.vt.edu/chem.ed/sped UV-visible/ single beam. Html. [Accessed 20th July, 2009].

[21] Watson, D.G (second edition) pharmaceutical analysis; a textbook for pharmacy students and pharmaceutical chemists. Oxford: Elsevier.

[22] Remington, (21st edition) the science and practice of pharmacy. Lippin Cott William & Wilkins: U.S.A.

[23] Vansant, E.F., Vrancken, V.K and Voort, P. (1995) Characterization and chemical modification of the silica surface. Elsevier.

[24] Liu, Y., Miyoshi, H., and Nakamura, M. (2007) Novel drug delivery system of hollow mesoporous silica nanocapsules with thin shells. Journal of colloids and surface: biointerfaces. 180 – 187

[25] [Online] available from www.hferrier.co.uk/higher/unit1d/unit1d.htm. [accessed 20th July, 2009]

[26] Somasundaran, P. (2006) Encyclopedia of surface and colloid science. USA: CRC press.

[27] Iler, R.K., (1979) the chemistry of silica, Wiley interscience. New York.

[28] Rouquerol, J., et al. (1994) recommendations for the characterization of porous solids. Journal pure and applied chemistry 66: 1739-1758.

[29] Taguchi, A., and Schuth, F. (2005), micoporous and mesoporous material 77 1-4508-311.

[30] Melillo, M. et al. (2004), structural characteristics of activated carbons and ibuprofen adsorption affected by bovine serum albumin, Langmuir 20 2837-2851.

[31] Kresge, et al. (1992) ordered mesoporous molecular sieves synthesized by a liquid – crystal template mechanism. Nature 359 710

[32] Vallet – Regi, M. (2001) chem.mater13 3

[33] Wang, S. (2009) ordered mesoporous material for drug delivery. Microporous and mesoporous materials 117: 1-9.

[34] Rosenholm, M., and linden, M. (2008). Towards establishing structure activity relationships for mesoporous silica in drug delivery applications. 128: 157-164.

[35] Gayathri, k. (2009) adsorption of bovine serum albumin on silica surface. Msc. Thesis, Greenwich University (Medway School of science).

[36] Mudunri, R.S., (2009) Adsorption of bovine serum albumin on silica surface. Msc thesis. Greenwich University (Medway School of science).