



## Investigation of the Interfacial and Wetting Characteristics of Bovine Serum Albumin with Selected Solvents and Amino Acids

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### ABSTRACT

The interaction of Bovine Serum Albumin (BSA) with various solvents such as water, diiodomethane, glycerol, and amino acids. they play a crucial role in the study of biomaterials and surface chemistry. BSA, being a well-characterized and widely used model protein, provides valuable insight into the adsorption behavior, conformational stability, and surface affinity of proteins under different environmental conditions. Understanding these interactions is fundamental in biomaterials research, as the physicochemical properties of solvents and substrates directly influence protein behavior, surface wettability, and biocompatibility. By analyzing changes in the contact angle, researchers can infer surface modification, protein layer formation, and the degree of hydrophilicity or hydrophobicity induced by protein adsorption.

**KEYWORDS:** Contact angle, amino acids, wettability, biomaterials.

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### INTRODUCTION

Bovine serum albumin (BSA), as the name suggests, is a serum albumin derived from cows. It is made of 607 amino acids of which 18 are what are called signal peptides that are useful in the transportation of peptides. BSA is added in DNA fragmentation reactions to reduce the adhesion of enzymes used to pipettes etc. (Szabo *et al.*, 2002). Adhesion forces are usually measured using Atomic Force Microscopy techniques (Anand *et al.*, 2011). The effect of surface charge of a cationic monolayer on the adsorption of BSA is studied using ellipsometry methods (Watanabe *et al.*, 1986). The adsorption of BSA on hydroxyapatite ceramic is probed by measuring the zeta potential of the hydroxyapatite surface and by varying the pH of the BSA solution (Zhu *et al.*, 2007). The adsorption of BSA on titania as a function of ion concentration has been reported by de Serro *et al.* (Serro *et al.*, 1999). The presence of calcium and phosphate ions caused an increase in the hydrophilicity of the substrate by rapid adsorption of BSA. Also, the adsorption of only a small fraction of BSA on titania is attributed to electrostatic and/or conformational effects. The decrease in  $Y_{SL}$  with time for water/diiodomethane over titanium substrates pre-incubated in Hank's balanced salt solution and BSA has been observed and attributed to protein desorption from the substrate (Serro, 1997). Adsorption and desorption of BSA on various polymers is studied using dynamic contact angle hysteresis using the Wilhelm plate method by Ueda *et al.* (Ueda *et al.*, 1995) and it is concluded that a faster desorption rate from some of the polymers is due to the poor interaction between BSA and the polymer. Adsorption of BSA from an aqueous solution on silicon dioxide and silicon substrates is studied using in situ ATR-IR spectroscopy. Contact angle of water over monolayer BSA is found to be  $53 \pm 1^\circ$  and the film thickness to be 2.0-3.8 nm, assuming that the film is as thick as the bulk protein. BSA is seen to adsorb as a side-on monolayer with flattening of the protein due to denaturing. Furthermore, protein unfolding is seen on hydrophobic surfaces compared to hydrophilic surfaces, with the contact angle being the same (Mc Clellan and Franses, 2005). Stronger adhesion of BSA is seen on substrates that exhibited a water contact angle  $> 60^\circ$ - $65^\circ$  whereas the adhesion is poor for substrates with water contact angle  $< 60^\circ$  (Xu and Siedlecki, 2007). Adsorption of BSA on model hydrophilic (OH) and hydrophobic (CH<sub>3</sub>) surfaces from quartz crystal microbalance studies and grazing angle infra-red studies show that adsorption of BSA is a single step process and has a stronger affinity to CH<sub>3</sub> compared to OH (Roach *et al.*, 2005). While the adsorption of BSA on hydrophilic and hydrophobic substrates is due to different mechanisms, the result is the passivation of the outer layer, identical for both the cases (Swierdyda *et al.*, 2004). In both cases adsorption occurs until passivation of the outer layer as exhibited by a similar contact angle. Water content near the surface is found to be of importance in the desorption of BSA from metal surfaces (Anand *et al.*, 2011).

### MATERIAL AND METHOD

Bovine serum albumin (BSA), were coated using the spin-coating technique. A commercial spin coater and a lab-made spin coater were used for this coating and the results were seen to be identical. Since the access to the commercial spin coater was limited and the lab-model worked well for this work, all studies reported in this paper were performed using the lab-made model. Spin coater is an instrument which is used for coating liquids sample like amino acids, proteins etc over the substrates. The coating of sample film using spin coater is uniform as compare to drop and dry method. Spin coating technique has been applied for the coating of thin film over different substrates. The procedure involves dispensing a small volume of liquid

sample on to the centre of the substrate and then spinning the substrate at a high speed, the centripetal acceleration will cause the sample liquid to coat over the substrate. Thin film accumulation on the substrate depends on the surface properties of substrate and liquid-like surface energy, roughness, viscosity, surface tension and drying rate of sample liquid. One of the most important factors for uniform coating is speed of the spin coater. The speed of the substrate affects the centrifugal force applied to the liquid, there by effect the thickness of the film. The final film will be inversely proportional to the spin speed and spin time. Since there are several factors affecting the coating process like spin speed, spin time and nature of liquid and substrate that effect the coating process. Contact angle measurements were made using Rame-Hart contact angle goniometer, which consists of volume-controlled syringe place over the substrate holder, CCD camera which take the drop image and advanced drop image software for processing of data. This software was used to measure the contact angle between the liquids and the substrate. The experiment was conducted at ambient temperature and the surface energy of the substrate was determined using the multi-liquid tool of the goniometer. Test liquids used for the determination of surface energy of substrates in the present study are water, glycerol and diiodomethane. The contact angle between substrates and the contact angle between substrates and liquids are related by the young equation (Young,1805).

$$\cos \theta = \frac{Y_{SV} - Y_{SL}}{Y_{LV}}$$

Where,  $Y_{SV}$  =solid-vapor interfacial tension,  $Y_{SL}$  =solid-liquid interfacial tension and  $Y_{LV}$  =liquid-vapor interfacial tension.

## RESULTS AND DISCUSSION

The surface energy of amino acids and surface tension of the three liquids are given in table 1 and table 2 respectively. The contact angle made by the three test liquid on a substrate of BSA are shown in fig1, the contact angle show the expected trend. There is no variation of  $\theta$  with time as in the case of amino acid, as seen from fig 2. Hence, no active adsorption or desorption from the surface is expected for these test liquids and the given substrates. However, the contact angles made by the saturated aqueous amino acid drop on BSA surface show interesting results. The data are given in fig.3, while the contact angle made by most of amino acids is in the range of 70-80°. The  $\theta$  value for lysine is  $\sim 35^\circ$ . By looking into table 2 no specific reason for the anomalous value can be found.

**Table 1: Surface tension of test liquids.**

Test liquids	Surface tension (mj/m <sup>2</sup> )
Water	72.46
Glycerol	63.79
Diiodomethane	51.21

**Table 2: Amino acids chosen for the present study and their properties.**

Amino acids	Acid/Basic /Neutral*	Designation (BMS data bank)	Polar energy (mj/m <sup>2</sup> )	Dispersive energy (mj/m <sup>2</sup> )	Total energy (mj/m <sup>2</sup> )
Aspartic acid	Acid/Polar	W	26.97	40.52	67.49
Cysteine	Neutral/Slightly polar	L	27.00	38.63	65.63
Glutamic acid	Acidic/Polar	W	29.90	29.43	59.34
Phenylalanine	Neutral/Non-polar	L	35.11	24.02	59.13
Histidine	Basic/Polar	N	33.91	21.71	55.63
Tyrosine	Neutral/Polar	N	27.90	27.95	55.85
Methionine	Neutral/Non-polar	L	24.65	27.97	52.62
Arginine	Basic/Polar	W	24.54	27.47	52.01
Proline	Neutral/Non-polar	W	12.85	38.02	50.87
Valine	Neutral/Non-polar	L	31.55	18.94	50.49
Threonine	Neutral/Polar	W	25.51	21.25	46.76
Lysine	Basic/Polar	W	13.85	27.76	41.61
Leucine	Neutral/Non-polar	L	7.69	32.50	40.18
Isoleucine	Neutral/Non-polar	L	05.10	35.04	40.14
Tryptophan	Neutral/Slightly polar	L	6.10	33.97	40.07
Alanine	Neutral/Non-polar	N	12.30	27.42	39.72
Serine	Neutral/Polar	W	05.41	33.47	38.89
Glycine	Neutral/Non-polar	W	08.06	30.58	38.64

	Bovine serum albumin	Polar	-	4.76	34.89	39.64	
	Bovine serum albumin (BSA),	Polar	-	34.29	22.91	57.20	
The	W=Hydrophilic	L=Hydrophobic	*Amino acids wikipedia				polar,

dispersive and total surface energies reported in table 4.1 are determined using the multi-liquid tool of Goniometer.

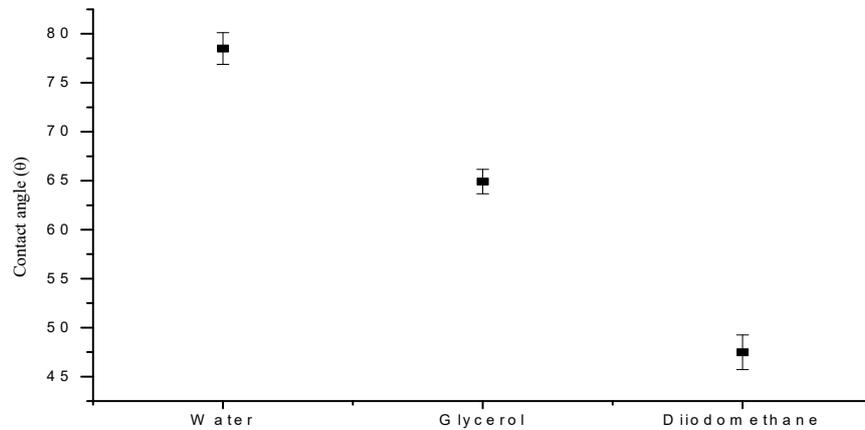


Figure 1: Contact angle of BSA on glass with water, diiodomethane and glycerol.

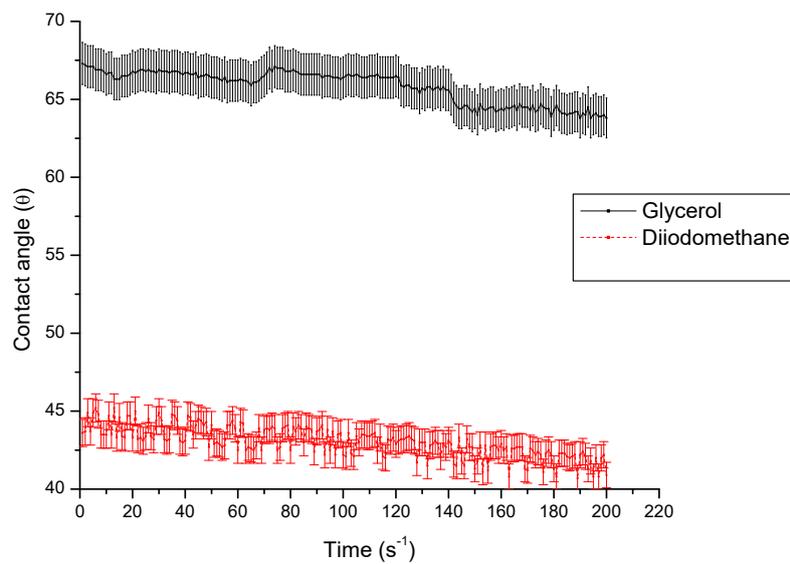
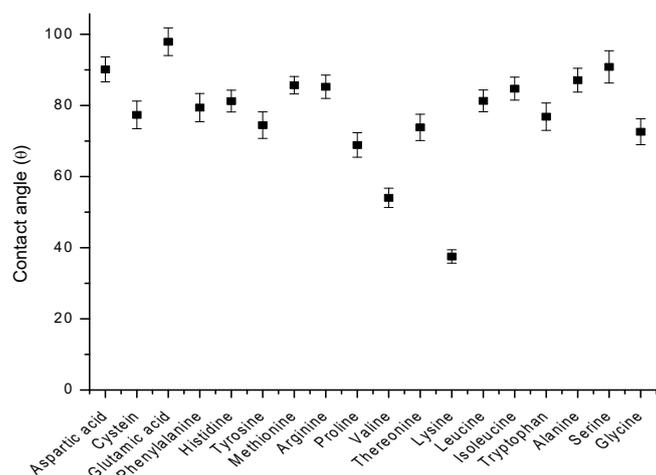


Figure 2: Contact angle of BSA on glass with diiodomethane and glycerol, time variation.



**Figure 3: Contact angle of amino acids solution on BSA coated on glass.**

The contact angles made by solutions of amino acids over BSA coated over glass surfaces is reported in fig. 4.10. The contact angle made by most amino acids is in the range of 70-80° except for lysine where it is ~ 30°. The two figures show the surface charges on either side of the protein structure (Majorek *et al.*, 2011). The charges are obtained from the pdb database. As seen from the surface of BSA is predominantly negatively charged (red) or neutral (white) with a few parts of positively charged areas. Since, there is a dominance of negative charges on the surface, BSA binds readily to positively charged lysine and show a large contact angle for the negatively charged aspartic acid and glutamic acid. The large neutral surface charge is indicated in the lower contact angle of neutral amino acid valine. Since BSA protein has a large size and significant parts of surface that are positive/negative/neutral charged, most amino acids, other than those discussed above, display contact angles between 70-80°.

## CONCLUSIONS

The wetting studies of amino acids the primary conclusions drawn are (a) there is no active adsorption - desorption for the first three minutes (b) positively charged amino acids bind better to the negatively charged surfaces of borosilicate glass and ITO and (c) amino acids with alkyl side chains display a thickness dependent wetting. BSA display wetting characteristics that are consistent with their surface charges. BSA displays a preference in bonding to positively charged lysine, as seen by the small contact angle.

To the best of our knowledge, this is the first time that contact angle measurements are shown ability to detect specific protein-protein interactions. Measurement of contact angle is a fairly simple experiment, although a lot of care must be taken to avoid erroneous results due to presence of contaminants etc. Hence measurement of contact angle can be treated as a first step in understanding protein-protein/surface interactions. Hence understanding the electrostatic attractions in biological systems, especially to surface, is useful with the fabrication of better filtration equipment. Hence by understanding the nature of binding of BSA to other amino acids, and hence altering the surface charge and structure, BSA can be used as a metal sensor.

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