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Computer Model for Fibrinogen Adsorption on Polyurethane Surface

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=Abstract=

Protein adsorption on the polyurethane surface was modelled by a modified random sequential adsorption(RSA) process. In this model, polyurethane surface was modelled as a mixed domain of hydrophobic and hydrophilic parts which was implemented by a 2 dimensional 150×150 lattice in the computer. Protein adsorption was simulated using a small box which represents a particle of the protein, and polyurethane lattice by considering their hydrophobic interaction. In order to validate the model, we performed fibrinogen adsorption on polyurethane surface. Isotherms of the adsorbed protein were calculated and compared to the experimental data. The protein adsorption on the polyurethane surface could be well described using this computer model.

INTRODUCTION

Protein adsorption is known to be the first stage of blood-material interaction. Many researchers have tried to find the basic mechanism of protein adsorption on polymeric surfaces[1-3]. Among hundreds of plasma proteins, three proteins such as albumin, fibrinogen and immunoglobulin G(IgG) have particularly been focused on this research because of their richness in blood and preference to adsorption on the artificial surface. There are many papers which reported the adsorption of these three plasma proteins on various polymeric surfaces and their relationships to antithrombogenicity of biomaterials[4-6]. Since fibrinogen is one of the adhesive proteins activating platelet, it has been mostly focused in thrombosis researches. Relationship between the adsorbed proteins and platelet adhesion has been considered as one of the main factors of thrombus formation in blood-material interaction. Polymeric surfaces also have various complex characteristics according to their composition and fabrication methods. Various techniques for surface modification were tried to improve the antithrombogenicity of biomedical polymers. Therefore, the characteristics of protein adsorption which is closely related to platelet aggregation or thrombus formation is shown in complex patterns according to the characteristics of polymer surface. Only a few investigators have attempted to model the protein adsorption on the polymeric surface. Jeon et al.[7, 8] tried to find the character of protein resistance of polyethyleneoxide(PEO) chains terminally attached to a hydrophobic solid substrate by theoretical consideration of the interaction energy between protein and PEO surface. Sevastianov et al.[9] developed a theoretical model of the reversible and irreversible protein adsorption in a kinetic regime. They introduced the controlling band concept which widely describes the monotonous distributions of the interfacial energetic properties of

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the protein-surface interactions. They explained the kinetics of IgG and human serum albumin adsorption onto a quartz surface using the approximation of the controlling band. Straaten and Peppas[10] presented a new method of calculation of protein adsorption on polymeric surfaces. Thermodynamic equilibrium of a three-component system (water, protein and polymer surface) was considered and the protein concentration profile was described by considering the interaction parameters for protein-polymer, water-polymer and protein-water contacts. In order to calculate these interaction energies, the dispersive forces, the hydrophobic effect and electron-donor-acceptor interaction, which were suggested by Drago et al.[11], were considered. Comparison with experimental results of protein adsorption on various polymeric surfaces give satisfactory results. These models except the model of Jeon et al. were based on the macroscopic characteristics of the protein-surface interaction.

In this paper, computer simulations of protein adsorption on polyurethane surface were described. For the simulations, the conventional random sequential adsorption (RSA) model was modified. Protein molecules were also modelled as adsorbed disks and the amount of the adsorbed protein was calculated at the equilibrium state. In order to validate computer model, we performed protein adsorption with polyurethane beads using ^{125}I labelled fibrinogen.

NUMERICAL SCHEME

Generally, polyurethane consists of two phases; hard and soft microdomains. Diisocyanate is consisted primarily in the hard segment and oligomer such as polyether, polyester, polysiloxane, etc. are consisted in the soft segment. In the aqueous environment, the hard(soft) segment can be considered as the hydrophobic(hydrophilic) domain. According to the chemical composition of polyurethane, its surface was modelled by an 150×150 square lattice which was composed of two segment types; hydrophobic(H+) and hydrophilic(H−) domains. The length of the monomer chain of the hard segment, which is hydrophobic in aqueous environment, is about 100Å. Each point in the 150×150 lattice represents a H+ or a H−, which was distributed randomly as shown in Fig. 1. The ratio of H+/H− points can be adjusted by the weighted random sampling. In order to reduce the finite size effect, a periodic boundary condition was adopted. In this simulation, we concentrated our aim on the fibrinogen which is one of the major plasma proteins. The shape of fibrinogen is fibrous and the length of the long side is about 400Å; the length of the short side is about 70Å. By considering the size of the fibrinogen with respect to that of the hydrophobic segment, fibrinogen is modelled as a small box which is composed of a 3×3 lattice as shown in Fig. 1. The adsorption of proteins and latexes on flat uniform surfaces ccribed by random sequential adsorption(RSA) process. Such a process requires irreversible adsorption and no diffusion of the particles on the surface after they are adsorbed. An occupied site remains occupied, no site may be occupied by more than one square, corresponding to the conditions of irreversible adsorption and no overlapping between particles, is assumed as described in respectively[11~13] . The hydrophobic surface in the conventional RSA process was modified by the randomly mixed surface of hydrophobic and hydrophilic domains. This mixed surface represents the hard and soft segments of the polyurethane surface. The process of the simulation by the modified random sequential adsorption is as follows;

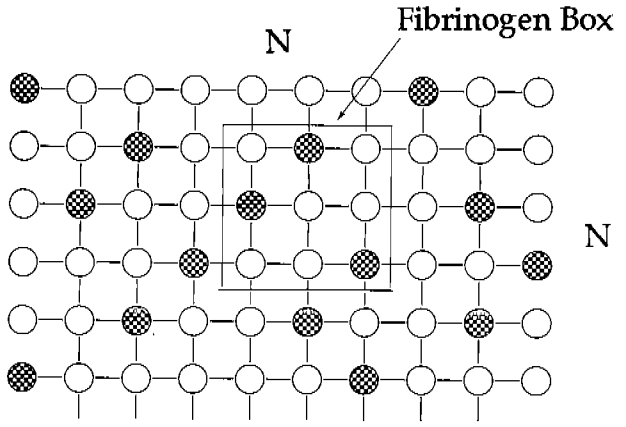

Fig. 1. Polyurethane lattice space, fibrinogen box, and adsorption process(H+ points : 3 → failed adsorption)

1. Select a random position in the 150×150 lattice which is made of randomly mixed hydrophobic and hydrophilic points at a given ratio.

Table 1. Values of the parameters used in the simulations

Parameter name	Values
Polymeric lattice (N × N)	150 × 150
Fibrinogen (m × m)	3 × 3, 3 × 4
Steady condition	300 trials
Adsorption criterion	5~9
H− : H+	1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 0:1

H− : Hydrophilic domain
H+ : Hydrophobic domain

2. Count the number of hydrophobic points in the 3×3 fibrinogen box which is centered at the position selected in the first procedure.
3. Determine success or failure of fibrinogen adsorption by comparing the number of hydrophobic points counted in 2.
4. Equilibrium state of protein adsorption was checked by unabsorbed trials during above three procedures.
5. Calculate the total number of the adsorbed fibrinogen molecules after the equilibrium state is arrived.

The parameters used in this simulation are summarized in Table 1. Various ratios of H+/H− points were tried to validate the characteristics of our simulation. At first, the fibrinogen box was modelled as a square of 3×3. The shape of fibrinogen is more similar to a rectangle. Therefore, the shape dependency of the protein adsorption was tested using a 3×4 fibrinogen box. The threshold of protein adsorption was 5 of 9 points in the fibrinogen box, which means that the hydrophobic interaction is strong enough to adsorb on the polyurethane surface. Equilibrium state of the protein adsorption is one of the important parameters in this simulation. The amount of the adsorbed fibrinogen is not changed at the equilibrium state. Thus, we could detect the equilibrium state of the adsorption process by checking the accumulating number of the successive adsorption failures. We tried to find the appropriate equilibrium condition by changing the equilibrium state threshold in the simulation.

Total schematic diagram of the simulation is shown in Fig. 2. Simulation was performed on IBM PC 386 using Borland C++ version 2.0 language.

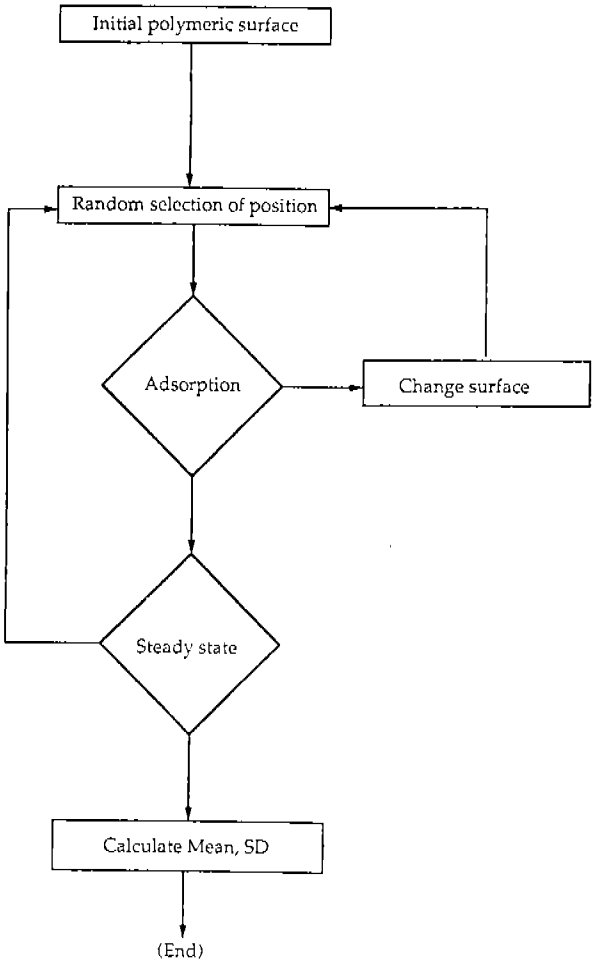

Fig. 2. Diagram of the modfied random sequential adsorption process

FIBRINOGEN ADSORPTION ON POLYURETHANE BEADS

For the quantification of fibrinogen adsorption, human fibrinogen was labelled with Iodine-125 (^{125}I, 2 mCi, Amersham) as sodium iodide in dilute sodium hydroxide solution free from reducing agents (pH 7~11). ^{125}I-labelled fibrinogen was prepared by a modification of the Chloramine-T method[14, 15]. This method is suitable to incorporate carrier-free radioactivity in small quantities of protein rapidly and with good efficiency.

Purified human fibrinogen was dissolved in PBS buffer (pH 7.4) to a concentration of 20 mg/ml, which was diluted with 0.5M sodium phosphate buffer (pH 7.2) to a final con-

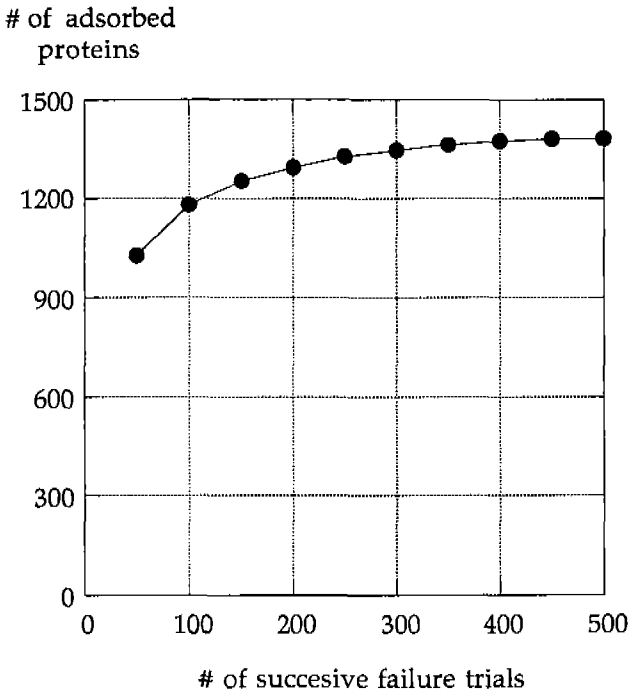

Fig. 3. Adorption threshold test of the modifed RSA

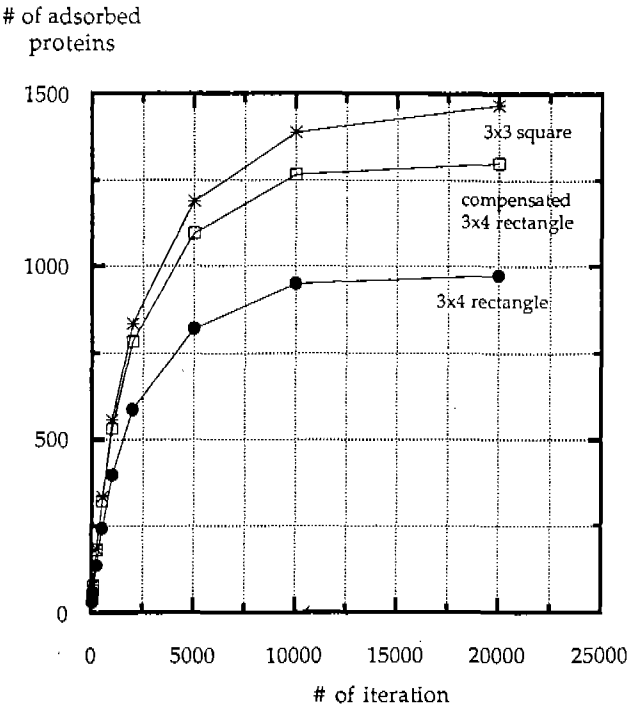

Fig. 4. Test of the protein shape denpendency (adsorption threshold; square $(3\times3)=6-9$, rectangle $(3\times4)=8-12$, compensated (3×4)=rectagle $(3\times4)\times12/9$)

centration of 10 mg/ml. A volume corresponding to 2 mCi of Na-^{125}I was added to 0.1 ml of fibrinogen solution (10 mg/ml). After 10 μl of freshly made Chloramine-T solution (1 mg/ml in PBS buffer (pH 7.4), Sigma Chemical Co., MO) was added as a oxiding agent, the fibrinogen solution was gently mixed for 1 minute and reacted for 10 minutes at room temperature. The reaction was terminated by addition of 10 μl of sodium metabisulfite ($Na_2S_2O_5$, 1 mg/ml in PBS buffer (pH 7.4), reducing agent, Aldrich Chemical Co.), followed by addition of 50 μl of potassium iodide (KI, 1 % (w/v), Aldrich Chemical Co.) and 0.1 ml of 0.5 M sodium phosphate buffer (pH 7.2). The mixture was applied to the Sephadex G-25 column and each 0.5 ml of eluant was collected in a separate polystyrene tube.

The radioactivity of each fraction was observed with gamma counter and the purified ^{125}I-fibrinogen was obtained without free ^{125}I. Polyurethane beads were immersed in 5 ml of ^{125}I-fibrinogen solved PBS buffer at 37℃ for 1 hour. After incubation, beads were removed and washed with PBS buffer (pH 7.4). The fibrinogen contents adsorbed on the sample beads were quantitatively determined by counting in a gamma scintillation counter (Beckmann DP 5,500). Appropriate control tubes were included in all experiments and these blank values were subtracted from test values.

RESULTS

In the test of the threshold of the equilibrium state condition, it is sufficient that the value of threshold is 300 for equilibrium of fibrinogen adsorption as shown in Fig. 3. The adsorption process was slightly dependent on the shape of the adsorbing fibrinogen as shown in Fig. 4. The amount of the rectangular fibrinogen must be compensated by its adsorbed area to compare it to that of the square data because the difference of the area gives the different limiting value of adsorption isotherm. Simulations were performed for several ratios of the hydrophobic and hydrophilic domains and these results are shown graphically in Fig. 5. As the area of the hydrophobic domain was increased, more fibrinogen were adsorbed. Then it went to a limit of 1,565. In Fig. 6, the amount of adsorbed fibrino-

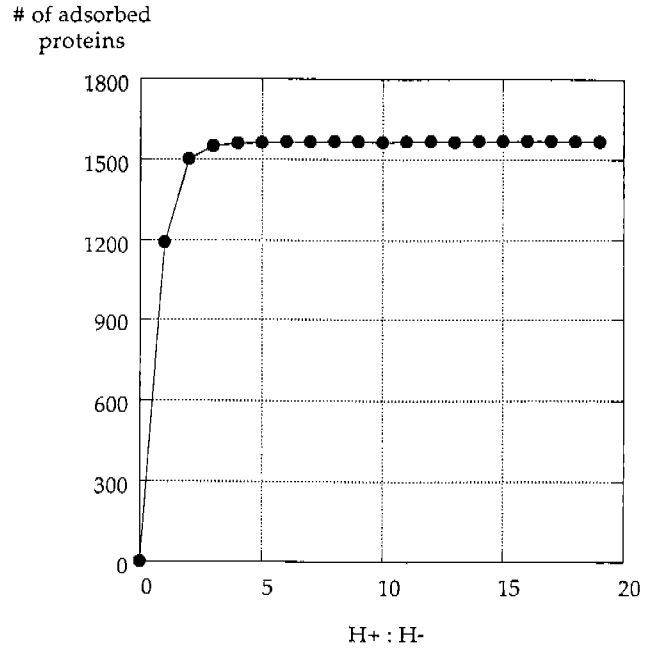

Fig. 5. RSA of fibrinogen on the various polyurethane surfaces with different ratios of hydrophobic and hydrophilic domains (H+/H−)

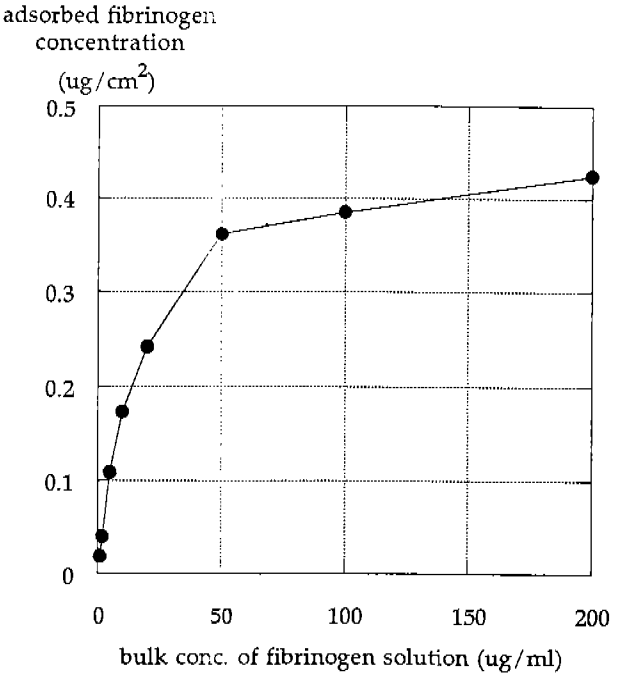

Fig. 6. Fibrinogen adsorption on the polyurethane beads (incubation time=1 hour)

gen was increased as the bulk concentration of the fibrinogen solution was increased. It saturated at about 0.4 μ g/cm^2.

DISCUSSION

The limit when the polymeric surface is fully hydrophobic is known as the jamming limit and its value is 54.6 % when the adsorbing elements are circular disks[16]. Many simulations of random sequential adsorption in two dimensional space were performed with hard circular disks in order to obtain the jamming limit. In our simulation, the mean value of this limit of surface coverage is 62.6 %. The difference between our and other's jamming limit is expected to be caused by the difference in shape and size of the adsorbed particle. In our case, a 3×3 square box was used while others used disk type adsorbed particles[16].

The surface concentration of adsorbed fibrinogen converged to about 0.4 ug/cm^2 in the experimental case. This value has some difference with others'[17~19]. This might be caused from the difference in the incubation time, adsorbing surafce, and the bulk concentration. In the experiments of Brash and Ten Hove's, they used fibrinogen solution of 0.7 mg/ml and performed adsorption for about 3 hours until it becomes a steady state. And then they found the converged value of the adsorbed fibrinogen concentration near 0.7 ug/cm^2 with cuprophane surface[17].

In order to compare this simulation to the experiments, the surface concentration of the adsorbed fibrinogen in this simulation can be calculated. The molecular weight of human fibrinogen is 340 kD, then the weight of a fibrinogen molecule is 5.65×10^{-19} g. The size of the surface area of the polyurethane used in this simulation is $150\times150\times100$ Å^2. Therefore, the surface concentration of adsorbed fibrinogen is about 0.039 ug/cm^2 when 1,565 fibrinogen molecules are adsorbed at equilibrium state on the test lattice. This value is somewhat different from that of the experimental data. There are two major reasons for this inconsistency. One is that the exact size of the hydrophobic (hydrophilic) domain was not adopted in our calculation of the surface concentration in the case of the computer simulation. The other is that we assumed only monolayer adsorption in the computer model. In the experimental case, fibrinogen might be adsorbed in a multilayer on the

polyurethane surface. Therefore, this computer model will be more accurate when the amount of the adsorbed protein is very little and then the protein is adsorbed in a monolayer.

This simulation started from the microscopic considerations between the polyurethane surface and the adsorbing fibrinogen. Thus, it can be easily adaptable to the case of modified surface of polyurethane such as albumin immobilized surface, PEO grafted surface by changing the adsorption threshold. Our simulation is limited to the equilibrium stage of adsorption. Time scale of the adsorption procedure must be defined for the simulation of Vroman effect.

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References

1. Vroman L, Adams AL. *Identification of rapid changes at plasma-solid interfaces.* J Biomed Mater Res, Vol. 3: 43, 1969
2. Bangnall RD. *Adsorption of plasma proteins on hydrophobic surfaces II: Fibrinogen and fibrinogen-containg protein mixtures.* J Biomed Mater Res, Vol. 12: 203, 1978
3. Brash JL, Uniyal S, and Chan BMC. *Interactions of proteins in plasma with various foreign surfaces.* Artif Organs, Vol. 5 (suppl.): 475, 1981
4. Bessinger RL, and Leonard EF. *Plasma protein adsorption and desorption rates on quartz: Approach to multi-component systems.* Trans Am Soc Artif Intern Organs, Vol. XXVII: 225, 1981
5. Wojciechowski P., Hove PT, and Brash JL. *Phenomenology and mechanism of the transient adsorption of fibrinogen from plasma (Vroman effect).* J Coll & Inter Sci, Vol. 111(2): 455, 1986
6. Leimger RI, Huston TB, and Jakcobsen RJ. *Spectroscopic approaches to the investigation of interactions between artificial surfaces and proteins.* Annals of New York Academy of Science, Vol. 561: 173, 1987
7. Jeon SI, Lee JH, Andrade JD, and De genne PG. *Protein-surface interactions in the presence of polyethylene oxide (I. Simplified theory).* J Coll & Inter Sci, Vol. 142(1): 149, 1991
8. Jeon SI, and Andrade JD. *Protein-surface interactions in the presence of polyethylene oxide (II. Effect of protein size).* J Coll & Inter Sci, Vol. 142(1): 159, 1991
9. Sevastianov VI, Kulik EA, and Kalimn IG. *The model of continuous heterogeneity of protein-surface interactions for human serum albumin and human immunoglobulin G adsorption onto quartz.* J Coll & Inter Sci, Vol. 145(1): 191, 1991
10. Van Straaten J, and Peppas NA. *Modelling of protein adsorption on polymeric surfaces.* J Biomater Sci Polymer Edn, Vol. 2 (2): 91, 1991
11. Drago RS, Parr LB, and Chamberlain CS, J Am Chem Soc, 93: 6014, 1971
12. Swedsen RH. *Dynamics of random sequential adsorption.* Phys rev A, Vol. 24(1): 504, 1981
13. Onoda GY, and Liniger EG. *Experomental determination of the random-parking limit in two dimensions.* Phys rev A, Vol. 33(1): 715, 1986
14. WM Hunter and FC Greenwood. *Preparation of iodine-131 labeled human growth hormone of high specific activity.* Nature (Lond), 194, 495-496, 1962
15. GP Tuszynsk, L Knight, JR Piperino, and PN Walsh. *A rapid method for removal of [^{125}I] iodine followed iodination of protein solutions.* Anal. Biochem., 106, 119-122, 1980
16. Hinrichsen EL, Feder J, and Jossang T. *Geometry of Random Sequential Adsorption.* J. Stat Phys, Vol. 44(5-6): 793, 1986
17. Brash JL and ten Hove P. *Transient adsorption of fibrinogen on foreign surfaces: similar behavior in plasma and whole blood.* J. Biomed Mater Res, Vol. 23; 157, 1989
18. Giroux TA and Cooper SL. *FTIR/ATR studies of protein adsorption on plasma derivatized polyurethanes.* J. Coll & Inter Sci, Vol. 146(1); 179, 1991
19. Lu DR and and Park K. *Effect of surface hydrophobicity on the conformational changes of adsorbed fibrinogen.* J. Coll & Inter Sci, Vol. 144(1); 271, 1991