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Full Length Research Paper

Phosphotidylcholine as a new strategy in biocompatible material

Reihaneh Sabbaghzadeh

Department of Biology, Tarbiat Moallem University of Sabzevar, Sabzevar, Iran.

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Biomaterial has a wide variety of usages in medicine and chemistry. The biomaterials used in medicine and chemistry are often polymers. Polymers used as biomaterial must be compatible to the environment and they must not be poisonous and in case they are broken down they must not provide poisonous products in biological environment. The biomaterial used for each case has certain and specific chemical and physical characteristics. The proteins are absorbed as form of the biomaterial. This protein layer provides suitable environment to absorb later proteins, lipids and other materials. We have offered a new and effective method to increase the bioadaptability of biomaterial. We studied active chemical groups in order to cover the cases of covalent link production. The protein absorption steps can occur if Gibbs energy is decreased.

Key words: Biomaterial, compatibility, contact lenses.

INTRODUCTION

Planning and synthesis of biomaterial is one of the wide fields of research in medicine and chemistry (Baszkin et al., 1984). One of the primary reasons that biomaterials are used is to physically replace hard or soft tissues that have become damaged or destroyed through some pathological processes (Williams, 1990). Biomaterial has a wide variety of usages in medicine and chemistry including artificial joints, joining vessels in inner organs such as heart the drug carriers, microscopic covering and contact lenses (Fabrizius-Homan and Cooper, 1991). One of the modern usages of the biomaterial is in contact lenses. Using the soft contact lenses to correct the optical is increasingly used because of its abundant advantages. Replacing the glasses for contact lenses especially for athletics, in dust and bad weather condition has a lot of advantages. The most important reason to use the soft lenses is their resistance in biological environment and their adaptability with the biological environment (Baszkin et al., 1984). Using the biomaterial in biological environment is usually with some problems, the most important of which are in compatibility or little compatibility in biological environment (Baszkin et al., 1984). Protein-surface interactions are fundamentally responsible for the bio compatibility of medical devices, or the lack thereof. When a solid material (for example, a catheter, stent, hip joint replacement, or tissue engineering substrate) comes in contact with a fluid that contains soluble proteins (for example, blood, interstitial fluid, cell culture media), proteins rapidly adsorb onto the surface of the material, saturating the surface within a time frame of seconds to minutes. While this is conceptually simple to understand, the numerous types of soluble proteins contained in physiological fluids combined with their structural complexity has made, and continues to make, this an extremely (Tathe et al., 2010). In 1972, Bier defined three surface factors which are important materials in bioadaptability. He called them magical factors. In addition to surface charge, surface combination, surface chemistry, a factor such as the conditions of biological environment in which matter is used is used, namely, circuit speed biochemical

E-mail: reihanehsabb@sttu.ac.ir.

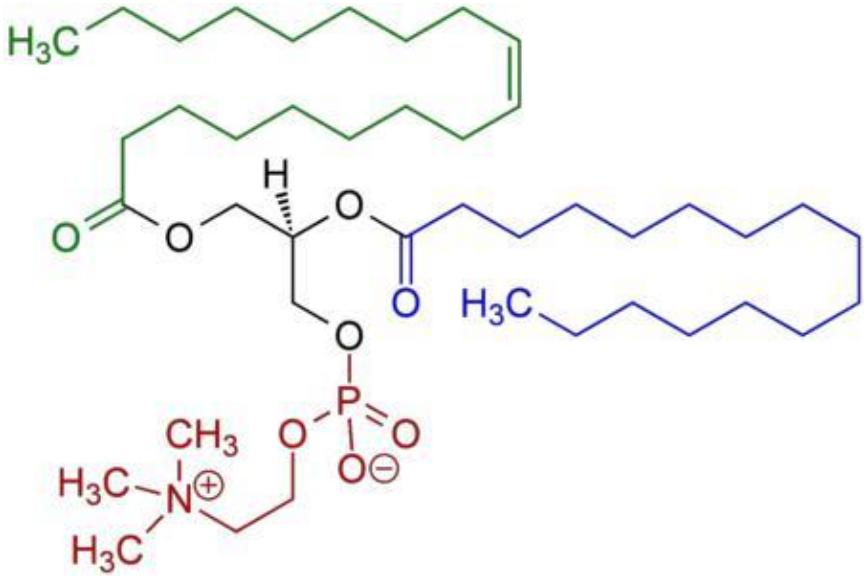


Figure 1. Phosphatidylcholine.

manufacture and the PH of the environment are also considered important (Steadman et al., 1992).

In living systems, blood (plasma) is the first component to come in contact with biomaterials such as a titanium implant during surgery. It is known that immediately after contact with plasma, rapid adsorption of plasma proteins onto the biomaterial takes place, which influences subsequent cell attachment, spreading, proliferation and differentiation (Hexsel and Serra, 2005). The proteins are absorbed as soon as the lenses are put in the eyes and this protein layer provides suitable environment to absorb later proteins, lipids and other materials in tears (Nordc and Lyklenw, 1991). Clearly, the protein and the given solid surface, depending on the specific orientation, react against each other in different ways (Sack et al., 1987). If these interactions are enough regarding energy and number, the absorption interaction cannot be done again. This case can be observed in hydrophobic surfaces such as polyethylene, polyproplin, polystyren, and other biomedicine polymers (Norde and Favier, 1992). Biomaterials for tissue regeneration need to be biocompatible as well as biodegradable *in vivo* (Rena et al., 2002). Lipids play an important role in biomineralization and countless other biological processes, and they are receiving increasing attention for the synthesis of new biomimetic biomaterials. Several emerging strategies in biomaterials research take advantage of phospholipids to compartmentalize and/or template chemical reactions via self-assembled structures such as liposomes and tubules. Still, others exploit the inherent biocompatibility of phospholipids and phospholipid-mimetic materials for use as novel tissue-contacting biomaterials that mimic biological membranes.

In the future, phospholipid-based materials may be increasingly utilized as tools for the manipulation of cell and tissue responses to biomaterials, for controlled drug release, for reconstructive surgery, and as tissue-engineered constructs (Anderson, 2005). In order to fur-ther improve their blood compatibility; lots of modification methods have been developed. Among them, the imita-tion of cell membrane by introducing phosphatidylcholine into 'polyurethanes' is a very effective way (Rena et al., 2002). Phosphatidylcholine (lecithin) is a phospholipid, extracted from various plants and animal tissues apart from the egg yolk (Sahoo et al., 2011). Chemically, phos-phatidylcholine is also known as 1,2-diacyl-:ussn:ueglycero-3-phosphocholine, PtdCho and lecithin (Jimbo et al., 2010). The phosphatidylcholine molecule consists of a head group (phosphorylcholine), a middle piece (glycerol) and two tails (the fatty acids, which vary) (Figure 1). The phosphatidylcholine molecules present in the tissues of organisms may vary depending on the radical of the fatty acid linked to the molecule (Jimbo et al., 2010). Goda et al. (2002) prepared biofouling poly (dimethyl siloxane) (PDMS) with excellent surface hydro-philicity and good oxygen permeability by surface initiated radical graft photo polymerization of 2-metacryloil-oxiethylphosphatydil cholin (MPC)—biomimetic synthetic phospholipid polymer, containing phosphatidylcholine groups (Todorka, 2010). The bioactive biomaterials esta-blish specific interactions in contact with living matter (tissue, blood, cells) and mimic some human functions. They are actively interacting and integrating with their biological environment (Sahoo et al., 2011).

It is experimentally established that an increase in the surface hydrophilicity decreases the cell adhesion. However, low cell adhesion does not certainly mean prevention of the biological activation. Some researchers have

Table 1. Distribution of charged groups and changes in the structure of proteins.

Subdivision	Thermodynamic parameters interference	Factors important parameters
Changes in surface dehydration of protein	$\Delta H\rangle\langle 0, \Delta S\langle 0, \Delta G\rangle\langle 0$	The hydrophobic quality of the surface and protein
The distribution of charge groups	$\Delta H\rangle\langle 0$, $\Delta S\rangle\langle 0$, $\Delta G\rangle\langle 0$	The spreading of charges and fixed dielectrics before are after the absorption
1-Electric part merging of electric fields		Capacity and the size of transferred ions
2-chemical part the environment change through transferred ions	$\Delta H\langle 0, \Delta S\langle 0, \Delta G\rangle 0$	
Changes in the structure of protein	$H\geq 0, \Delta S\geq 0, \Delta G\leq 0$	The structural stability protein molecules

established low platelet adhesion to strong polar surfaces and high thrombin activation and coagulation (Coleman et al., 1982).

MATERIALS AND METHODS

To examine the biological adaptability of biomaterial, the surface of the matter which is synthesized should be considered. Creating a suitable balance between polar and non-polar positions is important in biocompatibility. The phosphatidylcholine polymers (PC) do not deposit as one or two layers, the range of the thickness of the formed layer is from 10 nanometer to numerous micrometers; it depends in the viscosity change of covering layer. Some of these materials have been examined in medicine and they are now used for medical devices. We have offered a new and effective method to increase the bioadaptability of biomaterial. We studied active chemical groups in order to cover the cases of covalent link production with dipping the biomaterial into chloroform solution of phosphatydylcholine. In several cases, a simple step of swallowing, dripping drops one by one and making dry is used in order to provide a cover adaptable to the intended needs. The advantage of using a polymer instead of a small molecule is that the links produced in several points make the surface steadier. Phosphatydylcholine is negatively charged polystyrene latex which prepared particles are a commercial product. Bovine serum albumin (BSA) was purchased from Sigma. Table 1 contains those properties of the protein which may be relevant to its adsorption behaviour.

Buffer solutions were prepared by mixing appropriate volumes of 10 mM Na_2HPO_4 and 10 mM NaH_2PO_4 solutions to give pH 7. Phosphate buffer solution and protein stock solution were added to the sorbent to give a series of samples of constant volume but varying protein concentration. After incubation, the samples protein concentration in the supernatant was measured using UV spectroscopy. Standard used were of analytical grade. Reduced SDS-PAGE (12% gels) was carried out on the elutes. Preparations of purified albumin ranging in concentration from 5 to 250 mg/ml in 2% SDS were used to generate a standard curve. Absorbance was measured at 690 nm using a spectrophotometer. Relative amounts of attached albumin were analyzed by electrophoresis and coomassie blue staining of the gels after solubilization of the disc-associated albumin. Samples were analyzed by 5% SDS-polyarylamide gel electrophoresis followed by coomassie blue staining of the gels. Phosphatidylcholine can be used for covering or other cases. Figure 2 shows the structural similarity between lipid dipalmitil phosphatidyl choline (DPPC) and coplimer methacrylat (MPC). Both of them have hydrophilic and hydrophobic and they can be used on the surface (Nordc and Lyklenw, 1991). It is known from the explanation that the polymer layer pc is consistent, nontoxic, and does not create inflammatory reaction.

RESULTS

Figure 3 shows the comparison of microscopic picture of 'scanning electron microscope', an uncovered vessel craft on the left and a covered vessel graft on the right. The adsorption of BSA on silica and PS_latex particles are presented in isotherms, as shown in Figure 4, where the adsorbed amount (G) is plotted as a function of the protein concentration in solution after adsorption (*c*BSA). The adsorbed amount of BSA (in mass per unit sorbent surface area) at pH 7 is invariant with the sorbent used. The initial slopes of the isotherms do not differ significantly and adsorption saturation is characterised by well-defined plateau values. The adsorption isotherms of BSA on both silica and latex are in good agreement with the results obtained under practically the same conditions by Elgersma et al. (1990), Norde and Favier (1992) and Kondo et al. (1992). One of the new polymeric materials having a phosphotidiylcholine (PC) cover is thought to make the contact lenses. This polymer attracts a lot of water because of the previous polar group (PC). Blood is another case in which the pc polymer can be used effectively. Blood is a complex environment and the biomaterials should encounter a lot of problems in this environment. When the blood contacts a synthetic material, some plasma proteins including fibrinogen are adsorbed rapidly and then blood cloth is formed through some steps. The laboratory studies have shown that the surface covered with phosphotidylcholine (PC) adsorbed less protein than the materials without a cover.

Studying plasma proteins such as fibrinogen, the human serum albumin, and Immunoglobin has proved that the covered cases with PC attracts (as much as 75%) less protein than the on covered surface. As a result of pc, polymers are effective on inflammatory response and

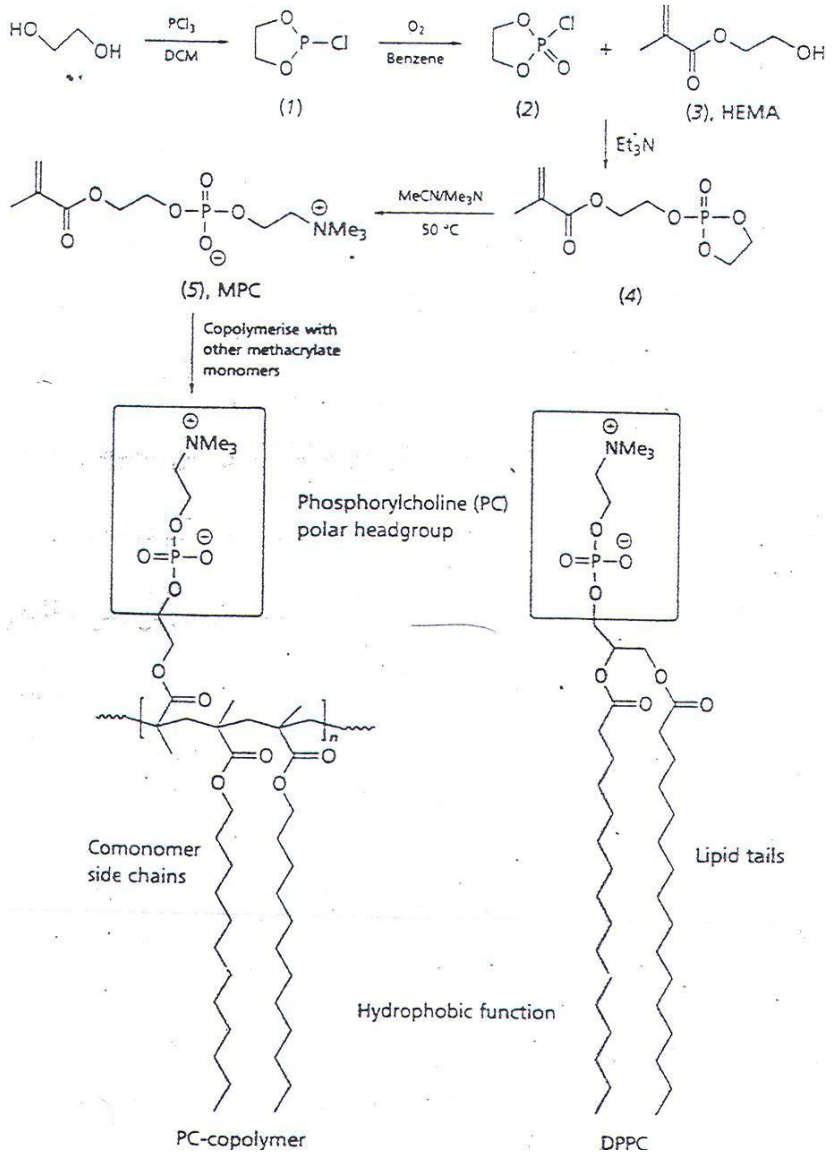
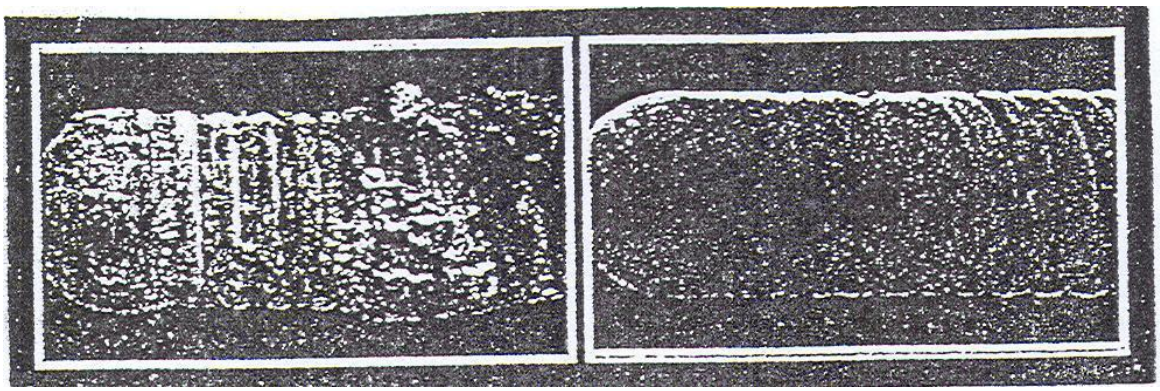


Figure 2. The structure similarity of DPPC and MPC (Baszkin et al., 1984).



(a) (b)

Figure 3. The microscopic picture of SEM. a) Without pc cover, b) with pc cover.

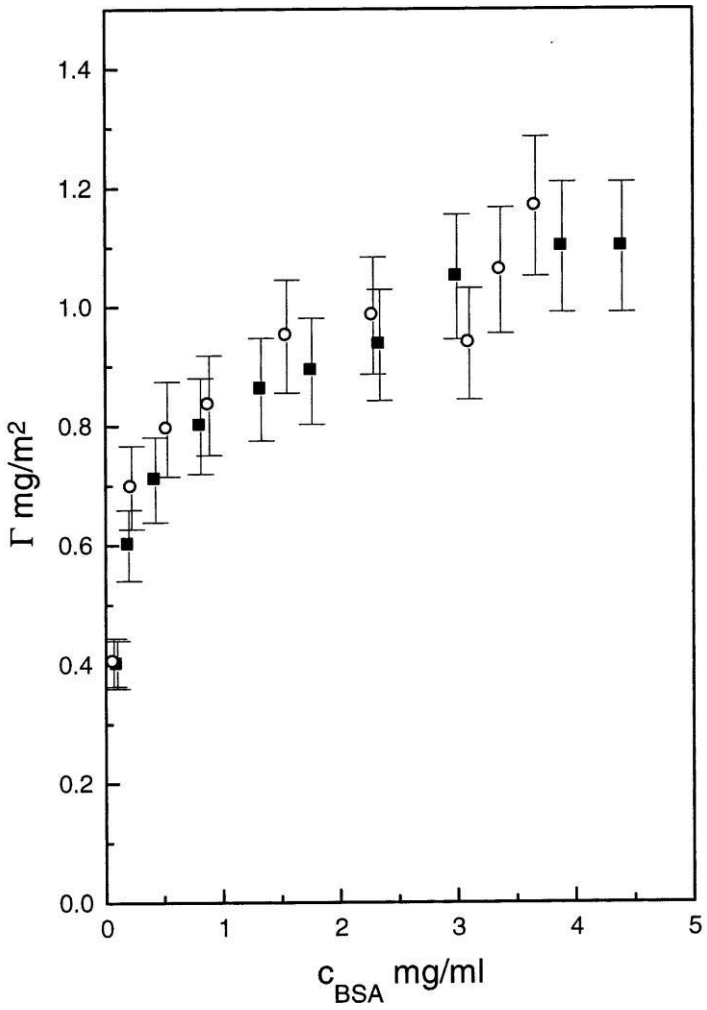


Figure 4. Adsorption isotherms of BSA adsorbed on () silica and on (_) PS_ particles at pH 7 (phosphate buffer 10 mM) and room temperature.

it was proved that these polymers decrease the adhesiveness and activity of the platelet (Fabrizius-Homan and Cooper, 1991). Coomassie dye stained gels of the proteins eluted from the surfaces (Figure 5) showed numerous bands of varying intensity indicating the presence of many different proteins, a band at 27 kDa was clearly visible in all the elutes. Normally, the introduction of PC would improve the hydrophilicity of polymers. This was ascribed to strong affinity between PC and water molecules. Various published papers have proved that the PC groups migrate to the surfaces of polymers when contacted with water and consequently combined with water molecules (Norde and Giacomelli, 2000). Protein molecules of the solution are absorbed to a surface so that it makes a layer of almost (100 to 500) nanogram on square centimeter. Tear is a suitable food producing environment for microorganisms such as bacteria. It has a physiological controlled PH and a suitable temperature. These factors causes the quick growth of the microbes and the deposited protein layer can be a sticking factor resulting in swelling and infection of the eyes. It increases the risk of allergy and swelling reactions. The lens infection results in dehydration of the lens and its instability in the tear layer which ends in pain, discomfort and lack of resistance in water. Furthermore, the joined protein layer to the lens may change the physical qualities of the lens including wet angle and penetration. So, it gives a different physiological activity to the surface.

Lipids especially phospholipids, are an important part of biomembrane. They are bipolar, having a hydrophobic tail and hydrophilic head and they make two layers in water. The bioadaptability of phosphatidylcholine technology (PC) is based on these observations, in which the outer surface membrane erythrocyte is adapted to blood but the inner part makes a cloth in contact with blood. The inner part of membrane is composed of phospholipids with negative charge, while the outer surface is com-posed of two polar phospholipids (Ratner, 2007). How do

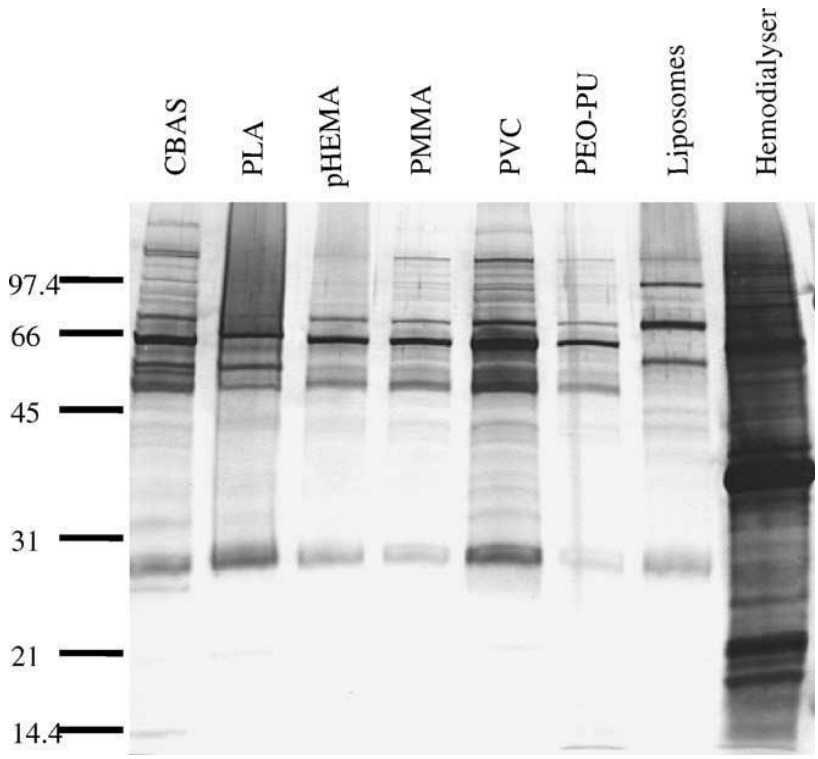


Figure 5. SDS-PAGE (12%, coomassie blu stained, reduced) of proteins eluted from biomaterial surfaces.

PC polymers act? Actually they imitate the external surface of membrane molecules. Several factors such as electrostatic and structural factors are important; the PC group of phosphoric acid and ammonium tetra alkyl are polaric, but on the whole in physiologic conditions, they are natural. Polymers are flexible in form and can adapt to different conditions. The head groups of PC are placed on the surface of water. The water surrounds firmly the head groups and like a security protector hides the substrate and the protein attraction becomes unfavorable regarding of energy production. Using pc in devices such as vessel grafting and dialyze membrane is being studied (Ratner, 2007). However, sometimes it is possible to overcome the situation. For example, if the absorption of irreversible hydrophobic contacts another separator that decreases or destroys the hydrophobic action, what was irreversible will become reversible in the new solution.

The experiments are better to be done on a surface with low density to make it remain irreversible and analyzable using the related models. Long (1916 to 1925) stated the concept of independent principle of surface action by studying the thin surface of organic combinations on water. The nature of atomic groups on every surface decides the wet ability level. The adhesiveness power must be greater than the organic tension power, so some connections must be broken down. When a liquid makes a contact angle (θ) of zero with a phase, the thermodynamic sticking ability is very great (Steadman et al., 1992). Then, the good wetness (with little angle of almost zero) by liquid, provides a concave carve on the surface of air, liquid, so that if the contact angle of a liquid and a harder liquid is little, and specially if the liquid is sticky, it sticks quickly. This thin liquid layer between the two surfaces will attach them. Thus, the largest contact area between the phases will be formed through the widespread liquid layer on the hard surface or each hole or vessel. The protein absorption steps is influenced by surface features protein nature and solution feature.

DISCUSSION

What happens if we have two or more proteins in the solution? The studies of absorption of protein solutions have shown that proteins compete with each other to join especially on a hydrophobic (Kalltorp et al., 2000). Many studies purport to assess blood compatibility by counting adherent platelets and changing the morphology of the platelets on the surfaces of materials. What is more, as soon as the blood contacts a material, the protein starts to adsorb onto the materials surface and change their conformation. Thus, the platelets interact with these and eventually starting adhering and spreading onto the protein layer. The platelets in the state are activated to release blood coagulation factors (Ratner, 2007). This question why proteins are absorbed to the surfaces can

be answered by examining different forms of Gibbs energy in different steps (G). It is proved that sub-divisions of the formation of protein absorption are changes in dehydration, the distribution of charged groups and changes in the structure of proteins (Table 1). The hydrophilic surface usually does not repel protein but it can be moved by being subjected to high pH and high ions power. Electrostatic actions are among protein absorption factors on the surface. The absorption of hard proteins (those which have a strong unity) is usually done through electrostatic distribution. But those protein molecules which have a naturally weak structure may be repelled on the surface of water because of 'electrostatic force'. So, there must be other factors in these proteins to compensate the 'electrostatic repellent force'. This effective force can be the structural rearrangement of protein molecules.

The rearrangement of the protein structure is the case of hard proteins after absorption has some limitations (Norde and Favier, 1992). In non-ionic surfaces, because of little ion ion bound positions, the amount of deposit decreases; therefore, the protein attraction on the surface is non-union. Thanks to the hydrophilic and hydrophobic reactions. These forces will provide the non-ionic amino acids; thus, there will be a heterogeneous mixture of denatured protein on the surface (Sack et al., 1987). When the absorption is done from a complicated mixture, the protein layer is the outcome of high density of proteins and its time of contact (Fabrizius-Homan and Cooper, 1991). In solutions with low density, the protein has no neighbor on the surface, so it can best be absorbed to the surface to maximize the number of attachment reactions. In solutions with high density, an absorbed protein is immediately surrounded by neighbors and probably the structural adaptability with the surface is minimized. Structural changes possible lead to open and increase the number of protein positions to absorb the surface. During the surface absorption time, the structural change results in more enthropy of protein. This re-entropy can be a movement force to attract protein. This re-entropy can be a movement force to attract proteins since the protein absorption on the surface hydrophilic needs heat (Norde and Favier, 1992).

Regarding the impact of density, the proteins with low density in plasma may be very interested in absorbing the protein layer. The first probability is that they come to the surface quickly after the first contact with plasma and attach firmly to the empty absorption places and this absorption is pretty non-retrievable. The next provability is their replacement with the reabsorbed proteins on the surface or probably their attachment to the absorbed proteins on the first layer through the sulfide or non covalent attachments (Fabrizius-Homan and Cooper, 1991). An improved understanding of adsorption of surfactants on solid surfaces is important in many different areas of surface chemistry.

The Gibbs free energy, *G*, gives a measure of the maximum amount of work achievable from a system at constant temperature, pressure and amount of a sub-stance, and is one of the most used potential energy functions in chemistry (Liljeblad, 2007). The protein absorption steps (in fixed heat and pressure) can occur if also Gibbs energy is decreased (Cao et al., 2010). G is comment for Gibbs energy, H enthalpy, T temperature and s entropy.

The primary contributions to protein adsorption on a smooth, rigid surface originate from (a) electrostatic interactions between the protein and the sorbent surface, giving rise to co adsorption of small ions, (b) dispersion interaction, (c) changes in the state of hydration of the sorbent surface and parts of the protein molecule, and (d) structural rearrangements in the protein (Brock, 1985). Proteins adsorb on hydrophobic surfaces because dehydration of the sorbent surface is a strong entropic driving force for adsorption. As a rule, the affinity of the protein molecule for the sorbent surface increases as the hydrophobicity of the surface increases; although, other interactions like electrostatic ones may interfere with this trend. Indeed, according to the adsorption isotherms presented in Figure 4, the negatively charged BSA does not have a high affinity for both the negatively charged hydrophobic PS_ and negatively charged hydrophilic silica surface. It demonstrates that even when electrostatic interaction is not the determining factor in the adsorption process; it still has a great influence on the affinity of the protein for the surface. For both negatively charged surfaces, the initial part of the isotherms has similar slopes. It suggests that in the case of BSA, the adsorption behaviour is not controlled by the hydrophobicity of the sorbent surface. Other literature data also indicate that BSA adsorption is mainly determined by the protein and not by the sorbent surface. It has been quite often observed that the extent of the structural change of the protein resulting from adsorption depends on the surface coverage, that is, it varies along the adsorption isotherm.

At lower protein concentration in solution, corresponding to lower surface coverage, the rate of arrival of the protein molecules at the sorbent surface is slower, so that the molecules are allowed more time to adjust their structure to the new environment before a neighbouring site becomes occupied by subsequently arriving molecules. As a consequence, structural rearrangements are more severe at lower surface coverage. It is well known that BSA adapts its conformation readily and often reversibly to variations in the environmental conditions.

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