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Research Paper

Effect of Buffer type and pH on adsorption of Protein on Micro Particles for development of Particle enhanced Immunoassay

Biotechnology

KEYWORDS : Micro particles, Immunoassay, Buffer type and pH

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ABSTRACT

Latex particles coated with protein material have many applications in Biology and In Vitro Diagnosis. Particle Enhanced Immunoassay continues to be widely used for the detection of micro level and smaller quantity of Antigen or Antibody from various clinical samples. In present study Physical adsorption of Albumin and Globulin, two distinct protein types on to latex particle surface is analyzed with reference to buffer type and pH, major factor affecting adsorption. Study was carried out on different particle types, plain Polystyrene Micro particles (PS-MP) and Carboxyl modified Polystyrene Micro particles (CM-PS). We have observed trend of decreasing adsorption with increase pH. At pH 6.1 MES buffer gives maximum adsorption for both protein in all Micro Particles.

INTRODUCTION:

Increase in the use of clinical diagnostic methods involving immunological procedures have been seen in recent years because they are specific and have high sensitivity. Agglutination of latex particles continue to be widely used in biology and medicine for the detection of small quantities of an antibody or antigen in a fluid test sample. Some advantages of these assays are that the procedures are simple, widely applicable, non hazardous, and test results are obtained in a very short time. The agglutination reaction involves in vitro aggregation of microscopic carrier particles often called "uniform latex particles". This aggregation is mediated by the specific reaction between antibodies and antigens, one of which is immobilized on the surface of the latex particles to enhance the sensitivity and extend the point of equivalence. (Larsson, Johansson, Hult and Gothe, 1987)

Stable and reproducible binding of antigen or antibodies to a solid phase is prerequisite for most of the high resolution immunoassay. Numerous polymers such as polysaccharide derivatives and a variety of plastic compounds are used for this purpose. The attachment of protein molecules to latex particles can be achieved through physical adsorption or covalent coupling. Covalent linking of ligand to reactive solid phase group is mediated by group specific bifunctional reagent which produces the desired orientation of the ligand to be coupled. The increase in methodological simplicity has brought physical or passive adsorption technique to the forefront. Plastic solid phase of all forms and shapes are also easily available. The mechanism underlying the forces of binding of protein molecule to micro particle has been considered to be mainly hydrophilic interactions (Kapmeyer, 1991).

Binding of protein to solid phase greatly depends on the characteristic of Protein. For example binding of human IgG was found to be wholly pH dependent. IgG binds to latex particles solely by hydrophobic bonds of the van der Waal type, where as the binding of human serum albumin is somewhat pH dependant and electrostatic forces also play a role. This surface active behavior of proteins is utilized in various biomedical and biotechnological applications. Examples are the immobilization of enzymes on solid matrices in biocatalysis (Malcata et al., 1990) and of immunoglobulins in clinical diagnostics (Karlsson, Michaelsson & Matson, 1991).

In view of the increasing importance of Latex particle and ligand coated Latex particle necessitate closer investigation in to the mechanism of IgG and other protein binding by Latex particle. Effective control of any binding processes requires an understanding of the driving force(s) for adsorption, which is a complex process. For instance protein and sorbent hydrophobicity, charge distribution, protein structural stability, solution pH and ionic strength are known to influence the affinity of a protein for a given interface. These findings form the basis of a qualitative theory, originally proposed by Norde and Lyklema (Norde & Lyklema, 1978; Norde & Lyklema, 1979), which indicates that four effects namely, structural rearrangements in the protein molecule, dehydration of the sorbent surface, redistribution of charged groups in the interfacial layer, and protein surface polarity usually make the primary contributions to the overall adsorption behavior.

Thus it is required to expand knowledge of the adsorption mechanism by further experimental approach. The mechanism of adsorption can be studied by systematically changing the physical properties of the protein, the sorbent surface and solution. In this way information is obtained about the nature of the interactions responsible for the adsorption process. A detailed understanding of the influence of the various interactions on the adsorbed state of the proteins is required to optimize the functioning of the immobilized proteins in their applications. Certain additives also influence the performance of the coated particles in the slide agglutination test. Therefore, it is important to determine the optimum conditions for the performance of the test so as to obtain the best sensitivity and specificity. This paper presents the results of a study on the effect of a Buffer type and pH on the passive adsorption of polyclonal antibodies on to polystyrene latex particles. This study was carried out as a first step in the development of CRP slide latex test of good stability, specificity and sensitivity.

MATERIALS AND METHOD:

Experiments were carried out by using two different types of particles with very different surface property but having same diameter (100 micron). Plain Polystyrene particles and Carboxyl- Modified particles were used in the study. Particles were obtained from Magsphear, USA.

Two different proteins with distinct property were selected to study how characteristic of protein can affect the binding to MPs. Rabbit anti human CRP (IgG) is a large molecule with a high isoelectric point, a low density of charged group (low solubility) and limited flexibility in tertiary and quaternary structure. Rabbit anti human CRP (IgG) was obtained from Midland Bioproduct, U.S.A. Human Serum Albumin (HAS) is smaller, has a low PI, a high density of charged group and very flexible tertiary structure. HAS was obtained from Sigma. 1 mg/ml concentration of IgG and HAS was used for the experiment. Eight different buffers MES pH 6.1, MES pH 6.8, MOPSO pH 6.8, MOPSO pH 7.4, PBS pH 6.8, PBS pH 7.4, TRIS pH 7.4 and TRIS pH 7.4 were prepared as per the standard formula; all buffers were used at 50 mM strength.

100 µl of 10% solid stock micro particles was added into 1 ml of working buffer solution. 1 mg/ml concentration of protein solution is added into it. Mix it well for 1 hour and then centrifuge at 10000 rpm for 15 min and supernatant was removed. Protein

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concentration of supernatant was measured at A280 to find out concentration of protein absorb on particle.

RESULTS:

General trend of decreasing adsorption with increase pH is observed. MES Buffer at pH 6.1 shows maximum adsorption for both proteins. We have observed immediate effect of varying buffer conditions with CM-MP and the PS-MP shows the least effect. Same results are observed for both proteins which are very different from each other. In terms of quantity, CM-MPs adsorb more protein than the PS-MP in our experiment.

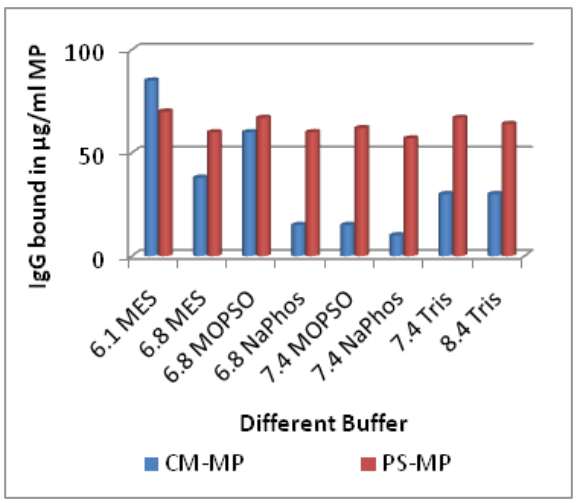

Figure-1: IgG adsorption on Polystyrene and corboxylate-Modified Microparticles

As shown above in Figure 1 maximum adsorption on CM-MP is observed with 6.1 MES buffer and comparatively higher than PS-MP. On CM-MP very less adsorption of IgG is observed with 6.8 NaPhos, 7.4 MOPSO, and 7.4 NaPhos buffer, comparatively more adsorption is observed with PS-MP with all buffers except 6.1 MES buffer. Adsorption of IgG on PS-MP have shown least effect and found more or less consistent with different buffers. With higher pH buffers IgG has shown more affinity to PS-MP as compare to CM-MP.

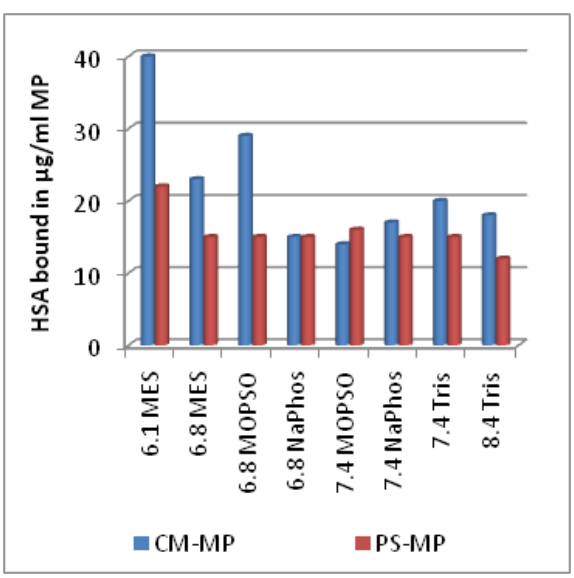

Figure-2: HSA adsorption on Polystyrene and corboxylate-Modified Microparticles

As shown above in Figure 2 maximum adsorption of HSA on CM-MP is observed with 6.1 MES buffer and it was comparatively higher than PS-MP. On CM-MP comparatively less adsorption of HSA is observed with 6.8 NaPhos and 7.4 MOPSO buffers. Unlike IgG adsorption, HSA adsorption has sown similar performance with these buffers. Adsorption of HSA is observed higher with CM-MP as compare to PS-MP.

DISCUSSION:

In our experiment high adsorption was observed with pH 6.1 MES buffer for CM-MP, this could be because the carboxyl groups of the MPs are partially protonated, repulsion is minimized and the electrostatic attraction can act to increase protein adsorption. At higher binding pH this effect is prevented and the increased binding to CM-MP is not seen. For HSA, being a highly charged protein, the electrostatic interaction makes a major contribution to the adsorption. This interaction, presumably between the amino group of the protein and negatively charged group on the MP is proportional to MP negative charge.

In the case of HSA, a large excess of protein is required to get the maximum binding. IgG has very different adsorption characteristics. The adsorption of IgG is very efficient or of high affinity. Most of what is put in to the reaction is bound to the MP up to the saturation of the surface. Protein to protein interaction could be a factor in the different adsorption characteristics of IgG and HSA. IgG is not very soluble due to low net charge. HSA is highly soluble and trend to repel itself. IgG molecule may be more amenable to crowding together and are thus able to saturate the solid phase.

The literature concerning the study of IgG adsorption at solid–liquid interfaces has a long and confusing history (Soderquist & and Walton, 1980; Fair & Jamieson, 1980; Bagchi & Birnbaum, 1981). Experimental adsorption isotherms performed in different laboratories on quite similar systems often conflict; and minor changes in experimental conditions (pH, ionic strength, temperature) may result in major differences in the measured adsorption.

From the adsorption point of view the solubility of a protein is of major importance as the method for determining adsorbed protein amount is based on the difference between the initial and the supernatant concentration. If protein molecules denature in the process, they form aggregates and precipitate in the centrifugation step this amount of protein should be quantified as adsorbed, and it could be a cause of error (Serra et al., 1992). This results in a greater tendency for structural rearrangements of the adsorbing molecules that create a larger surface area per molecule and cause a small amount of IgG to be adsorbed. Furthermore, at pH values away from the isoelectric point of the IgG, there is an increased electrostatic repulsion between adsorbed molecules that leads to a smaller amount of adsorbed IgG. Maximal protein adsorption around the isoelectric point (IEP) has been reported for IgG (Bagchi & Birnbaum, 1981; Serra et al., 1992; Elgersma, Zsom, Norde & Lyklema, 1991), nevertheless, some authors have shown that the maximum appears in the IEP of the immunoglobulin–carrier complex (Elgersma, Zsom, Norde & Lyklema, 1991). Antibody adsorption to and desorption from, adsorbent surfaces is a function of the nature of both antibody and the surface, and can be dependent on time, temperature, ionic strength, pH, protein concentration, and surface tension (Bale et al.,1989). Present study will help to understand influence of pH and Buffer type on binding behavior of protein, similar study to find out influence of other relevant factors is warranted to have complete understanding of Particle enhanced reagent development.

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