

# Factors Affecting Adsorption and Pre-Covalent Coupling of Protein to Particles

December 2010

## Key Words:

- Adsorption of protein
- Covalent conjugation
- pH of Buffer
- Ionic strength
- Hydrophobic interaction

Thermo Scientific complete particle technology solutions provide simple protocols for working with particles and concrete data backed by 30 years of proprietary applications research in our labs.

## 1. INTRODUCTION

The following describes the various factors at work in the adsorption of proteins on particles, and the results of experiments conducted using Thermo Scientific particles with three different surfaces in conjunction with two different protein types. These experiments involved the interaction of proteins with Thermo Scientific plain sulfate polystyrene or carboxylate-modified polystyrene particles.<sup>1</sup>

Protein adsorption occurs rapidly and generally precedes covalent coupling. For this reason, understanding the variables affecting adsorption is critical to a covalent coupling strategy. A successful reagent development strategy based on covalent coupling has an equally successful adsorption strategy. Refer to TN-02702 for information on recommended adsorption and covalent coupling procedures.<sup>2</sup>

The main variables at work in the adsorption of proteins to particles are related to binding buffer type, pH, the ionic strength of the wash buffer, buffer concentrations, and changes in ionic strength of the particle reagent.

## 2. MATERIALS

### Particles

The principles governing adsorption were demonstrated using a model system containing three particles with the same nominal diameter but each with a different surface: 1) high acid hydrophilic carboxylate modified (CM), 2) low acid CM, and 3) hydrophobic plain sulfate polystyrene.

Table 1	High Acid CM	Low Acid CM	Plain Sulfate
<b>Diameter</b>	0.281 $\mu\text{m}$	0.282 $\mu\text{m}$	0.272 $\mu\text{m}$
<b>Parking Area</b>	13.8	50.5	Not available
<b>Charge</b>	High	Medium	Low
<b>Surface</b>	Hydrophilic	Hydrophilic/ Hydrophobic	Hydrophobic

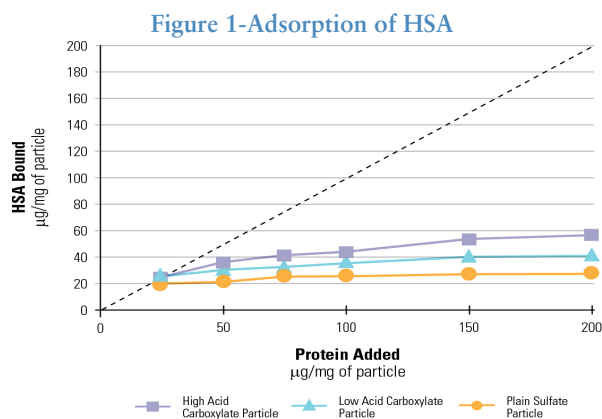
### Proteins

The proteins that were adsorbed to the particles in the model system were also quite different. Human Serum Albumin (HSA) and rabbit IgG were chosen because of their distinct properties to illustrate how the protein characteristics can affect their ability to bind to particles. Rabbit IgG is a large molecule with a high isoelectric point (pI) and a low density of charged groups. HSA is a smaller molecule with a low pI, and a high density of charged groups (high solubility).<sup>3</sup>

Table 2	HSA	IgG
<b>Molecular Weight</b>	66 KDa	150 KDa
<b>Structure</b>	Single Chain	Subunits, Glycosolated
<b>pI</b>	4.7	7.8
<b>Charged Groups</b>	High	Low
<b>Type</b>	Very Flexible	Limited Flexibility

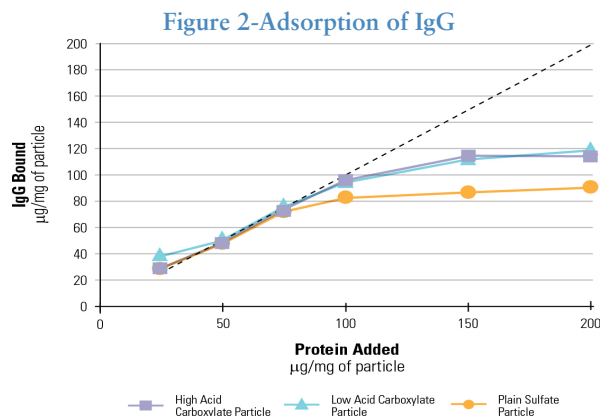
## 3. ADSORPTION ISOTHERMS

Data from a series of adsorption experiments resulted in plots of bound protein vs. added protein (adsorption isotherms) in 50 mM MES, pH 6.1. It was found that an increase in surface acid groups favored adsorption and that IgG adsorbed more readily than HSA for all three particle surfaces. The resulting adsorption isotherm for HSA is shown in Figure 1. Bound protein was determined by BCA assay.<sup>4</sup>



All three particles reached a plateau or saturation level where adding more protein would not result in more bound protein.

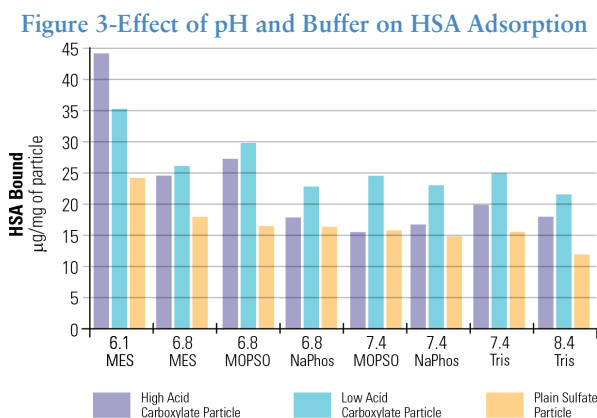
A comparison of the three particle surfaces revealed a difference between the plain sulfate particle and the two carboxylated particles, and between the low acid and high acid carboxylate modified particles. The corresponding adsorption isotherm for IgG is shown in Figure 2. It is apparent that the IgG adsorbed more readily to particles than HSA for all particle surfaces. Both carboxylated particles adsorbed almost equal amounts of IgG and significantly more IgG than plain sulfate particles.



#### 4. BINDING BUFFER TYPE AND pH

The effect of different types of binding buffers and pH on the adsorption of IgG and HSA was also studied.

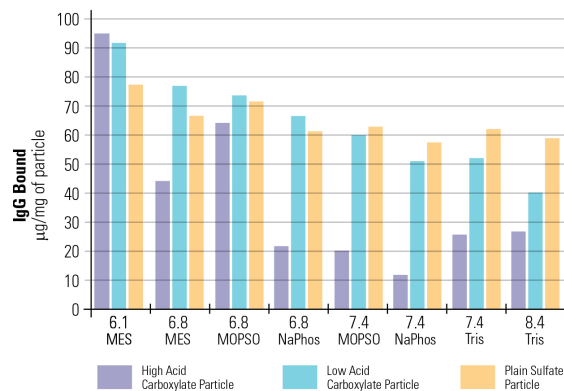
In the HSA experiment in Figure 3, HSA was added at 1 mg/mL or 100 µg/mg particle. The maximum adsorption seen was 44 µg HSA/mg particle on the high acid carboxylated modified particle, which demonstrates low binding efficiency.



In the IgG experiment shown in Figure 4, IgG was added at the same concentration as for HSA: 1 mg/mL or 100 µg/mg of particle. However, the adsorption was far more efficient, with binding of up to 95 µg IgG/mg particle on the high acid particle.

Of the conditions tested, the 25 to 50 mM MES buffer at pH 6.1 yielded the highest efficiency binding of HSA and IgG on all three particles. Using any other buffers/pHs results in lower protein binding efficiency.

**Figure 4-Effect of pH and Buffer on IgG Adsorption**



These studies indicate a general trend of decreasing adsorption with increasing pH. As the pH is raised, the charge on the entire system becomes more negative.

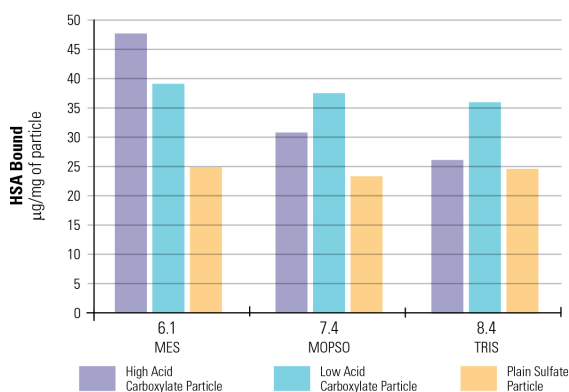
#### 5. pH OF WASH BUFFER

Changing the pH of the wash buffer after adsorption can result in elution of bound protein, the extent of which depends on the properties of the bound protein and particle surface.

To illustrate this, HSA (1 mg/mL) was adsorbed to each particle using 25 mM MES buffer. A pH of 6.1 was chosen to get the highest efficiency binding. After mixing for 1 hour, the coated particles were centrifuged and resuspended in 1 mL 50 mM MES at pH 6.1.

From this, 250 µL were transferred to three “fresh” tubes. The particles were pelleted and resuspended in 0.5 mL of the three buffers indicated in Figure 5.

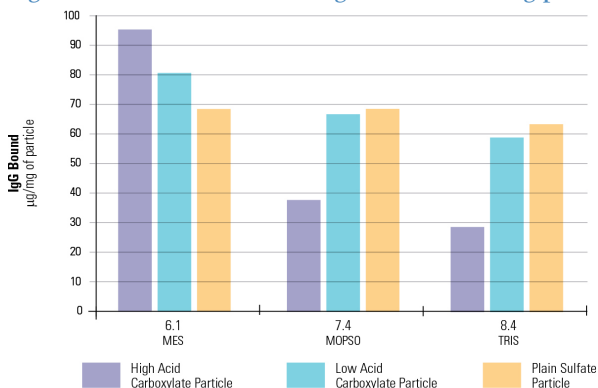
**Figure 5-Elution of Adsorbed HSA with Increasing pH**



After incubating overnight at room temperature, the particles were pelleted and washed with the same buffers and resuspended in 250 µL.

In a separate experiment, IgG (1 mg/mL) was adsorbed to each particle using 50 mM MES buffer, pH 6.1. Subsequent treatment and assay were performed exactly as described for the experiment with HSA. Results are shown in Figure 6.

Figure 6-Elution of Adsorbed IgG with Increasing pH



For both proteins, adsorption was most stable to pH changes on the plain sulfate particles. Increasing pH caused elution of protein from the low acid carboxylate modified particle and significant elution from the high acid carboxylate modified particle.

If the eluted fraction reflected the proportion of the adsorption which is due to electrostatic or ionic interaction, then it appears that the increased protein binding due to electrostatic forces was easily disrupted by changing the pH.

## 6. BUFFER CONCENTRATION

The effect of MES buffer concentration at pH 6.1 on HSA and IgG adsorption is shown in Figures 7 and 8, respectively. The first point on each figure represents “0” MES, or adsorption in deionized water (pH neutral).

Figure 7-Effect of MES Concentration on HSA Adsorption

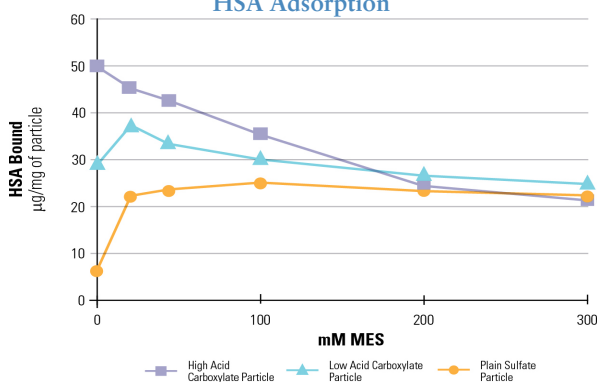
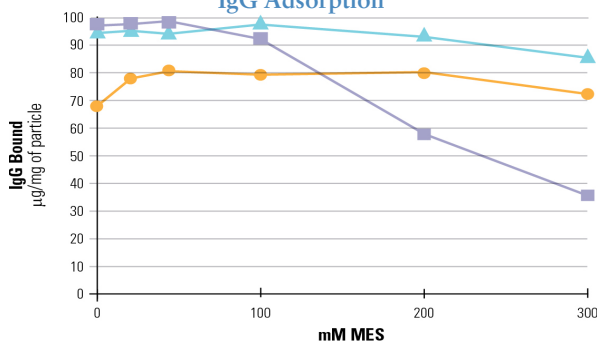


Figure 8-Effect of MES Concentration on IgG Adsorption



HSA was adsorbed to the three particles at 1 mg/mL added protein and varying concentrations of MES from a 500 mM stock at pH 6.1. The final pH did not vary much over this range of concentrations. The HSA-particles were washed and resuspended in 50 mM MES at pH 6.1, and the bound protein was determined by BCA assay.

IgG was adsorbed to the three particles at 1 mg/mL added protein and varying concentrations of MES from a 500 mM stock at pH 6.1 (Figure 8). The particles were washed, resuspended and assayed as previously described for HSA.

In water, the adsorption was protein and particle dependent. Where the adsorption was mainly ionic (high acid carboxylate modified particle, HSA), any buffer strength was detrimental.

Where there was some contribution to adsorption by hydrophobic attraction (low acid carboxylate modified particle or plain sulfate polystyrene, HSA; plain sulfate polystyrene, IgG), some buffer strength was advantageous. However, after a certain point, the effect of buffer strength was minor.

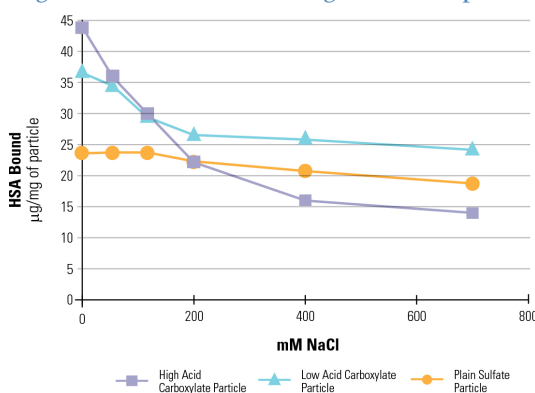
MES buffer concentration alone had a negative effect on adsorption to the high acid CM particle while the effect on the low acid CM particle was intermediate.

## 7. IONIC STRENGTH

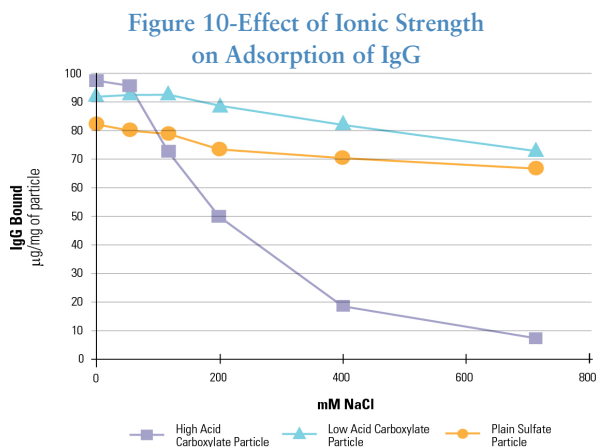
The effects of ionic strength on HSA and IgG adsorption was also studied by varying the NaCl concentration in 50 mM MES, pH 6.1. HSA was adsorbed to the three particles at 1 mg/mL added protein, 50 mM MES buffer, pH 6.1 and varying concentrations of sodium chloride.

In Figure 9, the largest effect was seen with the high acid carboxylate modified particle, which showed a steady decline in HSA adsorption with increasing ionic strength from NaCl. On the low acid carboxylate modified particle, there was a significant effect of increasing ionic strength up to 200 mM NaCl, then only a slight decline. On the plain sulfate particle, there was only a minor effect of ionic strength on adsorption of HSA.

Figure 9-Effect of Ionic Strength on Adsorption of HSA



In **Figure 10**, another large effect of increasing ionic strength was seen with the high acid CM particles with a sharp drop-off in IgG adsorption above 50 mM NaCl. Adsorption to both the low acid CM particles and the plain sulfate particles was level at 100 mM NaCl, and then gradually declined.



It should be noted that the buffer concentration effects described in Section 6 appear to be purely ionic strength effects, as the curves for increasing MES buffer concentration in **Figures 7 and 8** can be overlaid on the added salt curves in **Figures 9 and 10**.

## 8. SUMMARY

The data in this technical note provide insight into the factors that influence protein adsorption to particles. To relate the ionic strength effects back to the adsorption isotherms (see **Figures 1 and 2**), it appears that the “extra” adsorption of HSA to the high acid carboxylate modified particle was due to an ionic interaction of the amino groups of the protein to the carboxyl-rich particle. This binding was considerably reduced by high ionic strength.

On the low acid CM particle which adsorbed an intermediate amount of HSA, the adsorption of HSA was a combination of hydrophobic and salt-reduced ionic interactions. On the plain sulfate particles which adsorbed the least HSA, the adsorption of HSA was predominately hydrophobic or not affected by salt.

The binding of IgG exceeded the binding of HSA onto all three particles, and more IgG was bound to the CM particles than the plain sulfate particles. The nature of IgG binding was different than for HSA, as shown by the patterns of elution with increasing pH. For IgG, the hydrophobic component was greater and the electrostatic component was smaller. Also, the larger size of IgG compared to HSA may explain why it was more difficult to elute IgG from the plain sulfate particles.

Several key points can be taken from the adsorption experiments:

1. The saturation level for a protein on a particle is important information to be gained from doing a protein binding isotherm.
2. MES buffer at pH 6.1 gave maximum adsorption for HSA and rabbit IgG on all three particle types tested.
3. Both ionic and hydrophobic forces played a role in adsorption of proteins to the particles.
4. The CM particles adsorbed more protein than the plain sulfate particles.
5. Increased protein binding due to electrostatic forces was easily disrupted by changes in pH.
6. The binding of IgG exceeded the binding of HSA on all three particle types used in the experiments.

It is important to understand these phenomena and the complexity of the interactions between proteins and particles. Both ionic and hydrophobic forces play a role, but individual protein characteristics sometimes make it difficult to predict the results without doing the actual experiments.

For both HSA and IgG, the same conditions (25 to 50 mM MES at pH 6.1) gave the most efficient adsorption, resulting in less waste of precious proteins. However, these conditions are not recommended for storage and use of particle reagents. Also, if one changes the buffer, there is a risk of desorption or elution of protein. For these reasons, we recommend using covalent coupling because it provides high efficiency coupling and the ability to change the storage/reaction buffers as desired to optimize your particle reagent.

## 9. REFERENCES

1. Griffin, C., Sutor J., Shull B., *Microparticle Reagent Optimization*, Seradyn Inc., Indianapolis, IN, 1994
2. *Particle Reagent Optimization-Recommended Adsorption and Covalent Coupling Procedures*, TN-02702, Thermo Scientific, Fremont, CA, 2010
3. *The Practical Handbook of Biochemistry and Molecular Biology*, CRC Press, Boca Raton, FL, 1989
4. *Particle Bound Protein Assay Quick Elution Technique*, TN-2005.1\_11/10, Thermo Scientific., Fremont, CA, 2010

## 10. ADDITIONAL INFORMATION

Visit [www.thermoscientific.com/particletechnology](http://www.thermoscientific.com/particletechnology) for additional information.

TN-6003.01\_12/10

In addition to our office, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

### Thermo Scientific Particle Technology

46360 Fremont Blvd  
Fremont, CA 94538 USA  
+1 800 232 3342 (USA)  
+1-510-979-5000 (International)  
+1 510 979 5498 fax  
info.microparticles@thermoscientific.com

[www.thermoscientific.com/particletechnology](http://www.thermoscientific.com/particletechnology)

© 2010 Thermo Fisher Scientific Inc. All rights reserved.

All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.