On Some Peculiarities in the Adsorption of Natural and Synthetic Macromolecules*

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This paper discusses some conflicting issues in the field of macromolecular adsorption. First, the adsorption of human serum albumin (HPA) on polystyrene latices is discussed. For this system at specified pH values the effect of overall charge is contrary to expectation in that negative proteins adsorb spontaneously on negative surfaces and do so in amounts increasing with increasing electrostatic repulsion. Coadsorption of small cations is one of the main clues for the solution of this. Polymer adsorption reversibility is the second poser. For flexible random homopolymers it is shown that conflicting experimental data are only apparent in that all discrepancies can be attributed to heterodispersity.

Among the various branches of colloid and interface science, the adsorption of macromolecular substances is still relatively new and unexplored. For the researcher, this fact offers several challenges, especially if observations are made that seem to be at variance with basic tenets of physical reality. Closer inspection is then mandatory and if all goes well, it will eventually lead to an improved insight.

In this article, two of such observations will be discussed.

The first has to do with the adsorption of proteins. In the course of an extensive systematic study on the attachment of human plasma albumin (HPA) molecules at polystyrene latex particles, Norde observed that under certain conditions negatively charged HPA molecules adsorb spontaneously onto negative latex particles. Subsequent measurement of the enthalpy of adsorption revealed that sometimes $\Delta_{\text{ads}}H$ is nevertheless exothermal. This is contrary to general experience according to which work has to be done to merge two negative charges.

The second observation stems from work by Cohen Stuart, Scheutjens and Fleer on the adsorption of synthetic polymers. The central problem here is the existence of conflicting experimental evidence on the reversibility of this adsorption. A number of observations plead for reversibility, for instance the (sometimes) easy exchangeability of adsorbed polymers. However, other

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observations seem in downright conflict with reversibility, for example the impossibility to desorb polymers by diluting the solution.

The discussion below will suggest answers to these problems. It will appear that in both cases more factors must be taken into account, but once this has been done a satisfactory picture of the adsorption emerges.

**HPA ADSORPTION ON POLYSTYRENE LATTICES**

**General Features**

At a previous opportunity we already discussed some aspects of this process. At present, a restriction will be made to the adsorption plateau values. These values are well-defined and the trend therein as a function of pH is satisfactorily repeatable between different authors. Figure 1. collects this trend.

![Figure 1. Plateau adsorption of human serum albumin on negative polystyrene latex particles. The latex surface charge is indicated. T = 22 °C. Electrolyte 0.06 M KCl.](image)

The first noteworthy feature is the «illogical» course of \( I_m \) as a function of pH. To the left, where the protein is positively charged, the overall electrostatic interaction between adsorbent and adsorbate is attractive, whereas it is repulsive at high pH. However, \( I_m \) (pH) is not a decreasing function, so that other than simple over-all electric interactions must prevail. The very coincidence of the adsorption maximum with the bulk i.e.p. of the protein rather suggests that the observed behaviour is dominated by inter- or intramolecular interactions of the protein. We suggested that at the i.e.p. the HPA adsorbs more or less in its bulk conformation but that it unfolds progressively with increasing distance (in either direction) between the pH of adsorption and the i.e.p.\(^1,2\).

The effect of the latex surface charge \( \sigma_0 \) is also contrary to simple electrostatic reasoning, at least at pH > i.e.p.: increasing the negative surface charge, i.e. increasing the overall electrostatic repulsion, leads to an increased adsorption and not to a reduction of it. This is not so simple to explain.
Thermodynamic Aspects

Figure 2. gives some typical curves for the adsorption enthalpy $\Delta_{ads} H$, obtained microcalorimetrically. Curves like these cured us from trying simple explanations. On electrostatic considerations, $\Delta_{ads} H$ is expected to be exothermal at low pH, and this is indeed found. However, the trend as a function of $\sigma_0$ is opposite to expectation. At higher pH, $\Delta_{ads} H$ passes through a maximum that coincides with the i.e.p. at $\sigma_0 = -15.5 \mu$C cm$^{-2}$ but not at $\sigma_0 = -2.3 \mu$C cm$^{-2}$, so that the first coincidence is fortuitous. At still higher pH, where the HPA is negative, everything is contrary to expectation. Here $\Delta_{ads} H < 0$, meaning that heat is liberated if the negative adsorbate attaches itself onto the negative adsorbent. Even more surprising is the fact that more heat is liberated if the repulsion between adsorbent and adsorbate increases. On first sight this seems to solve the energy crisis.

However, the observed trends clearly defy simple analysis on the basis of energetical considerations only. For one thing, as in

$$\Delta_{ads} G = \Delta_{ads} H - T \Delta_{ads} S$$

(1)

$\Delta_{ads} G$ is always negative (because the process is spontaneous), $\Delta_{ads} H > 0$ over a certain pH range, $\Delta_{ads} S$ must be positive over at least part of the pH range and may well be positive at any pH. This conclusion means that the adsorption process is entropically driven. The problems are then: (i) why is $\Delta_{ads} S > 0$ and can this be made quantitative? and (ii) why is $\Delta_{ads} H < 0$ at high pH and can this also be made quantitative?

In a recent paper$^3$, we discussed the thermodynamics of protein adsorption in some detail and concluded that the positive value of $\Delta_{ads} S$ has three causes: hydrophobic bonding, electric field overlap and structural alterations in the
protein molecule due to adsorption. Hydrophobic bonding has a positive entropy contribution because of the increased number of configurations of desorbing water molecules. The contribution to $\Delta_{ad} S$ of this process is independent of pH and increases with decreasing $\alpha$, because low surface charge latexes are more hydrophobic. That electrical field overlap contributes also positively to $\Delta_{ad} S$ followed from a model analysis of the temperature dependency of $\Delta_{ad} G$; it could imply that many charges are more mobile or more randomly distributed in the adsorbed state than in the state where latex and protein are separately dispersed in the solvent. The occurrence of structural alterations inside a HPA molecule upon adsorption has not been spectroscopically proven but could be inferred from potentiometric proton titrations and from analysis of the adsorption isotherm, obtained under different conditions. We are not yet able to allocate this last mentioned entropy contribution. For instance, we do not know if it is a property of the protein or of the adhering water, or perhaps of both of them. Anyway, it may be noted that only a fractional increase in the degrees of freedom of the amino acids constituting the HPA molecule suffices to overcome the loss of translational and rotational entropy of the entire molecule upon adsorption.

The second problem is the explanation of the sign of $\Delta_{ad} H$. In our previous work\(^\text{2,3}\) it was shown that co-adsorption of cations is the main factor. We are now in a position to work this out more quantitatively.

**Co-Adsorption of Simple Cations**

If a negative latex particle binds a negative protein molecule, the electric potential well between the two is very deep unless cations accumulate in this region. That protons co-adsorb has been understood by many investigators or, for that matter, in several studies it was realized that all $pK$ values for proton binding shift to the corresponding value at lower pH in negative electric fields. However, systematic studies of the uptake of other cations have not been done to our knowledge. Due to this process, the binding of negative molecules to negative particles becomes energetically feasible. Conversely, partly because of this co-adsorption, the final values for $\Delta_{ad} H$ and other adsorption characteristics depend on $c_{salt}$ and on the nature of the electrolyte. As a matter of routine, buffers are often added to regulate pH, but they have a dual function because they act also as electrolytes. The feature of cation uptake must be of a more general nature and also play a role in, say, cell adhesion\(^4\) and in the assembly of viruses\(^5\).

As a first step to quantify the ion uptake, electrophoresis experiments can be done. In principle, mobilities can be converted to electrokinetic charges. Subtracting the sum of the electrokinetic charges on the protein and the latex before adsorption from the same after adsorption, an overall charge balance is obtained. Model studies are required to split the overall charge transfer into cation- and anion uptake and/or exclusion and to determine the amount of cations absorbed in the interlayer between latex and protein. Direct measurement of the uptake by radiolabelled ions can then be used to confirm the obtained picture.

Figure 3. collects typical results for a latex of high surface charge. The cation incorporation is a minimum around the i.e.p. To the left of this point, it increases together with the uptake of anions (not shown). To the right it
increases strongly, in agreement with the picture set forth above. The direct measurements are difficult to perform. For one thing, as the pH changes during the adsorption process, there is always some uncertainty with respect to the abscissae axis value. An additional experimental problem is that the procedure involves one or more washings which may render the establishment of the ionic strength somewhat uncertain. Anyway, within these experimental limitations the theoretical trend is quite well reproduced. In a forthcoming paper, we hope to show that this is also the case for other surface charges and even for quite different proteins like RNase.

From all of this it seems justified to conclude that a coherent picture of HPA adsorption on latices is developing. Mutatis mutandis, this picture may well be of wider applicability.

POLYMER ADSORPTION: REVERSIBLE OR IRREVERSIBLE?

Some General Experiences

There are several ways to obtain an answer to the question whether or not synthetic macromolecules adsorb reversibly on solid surfaces. One way is to simply dilute the solution and verify if any polymer comes off. Another way is to exchange the polymer for another one, or for a low molecular weight substance or change the solvent. A third approach would be to verify the applicability of polymer adsorption theories: as these theories are based on the assumption of equilibrium, evidence of reversibility would derive from any corroborations between theoretical predictions and experiment.

These three methods are of a different character and, indeed, give sometimes conflicting answers. Often no desorption upon dilution is found, although
the adsorbed polymer is easily exchanged or desorbed by altering the quality of the solvent. Some theoretical predictions are usually moderately well obeyed, others are not. Similar confusing facts can be obtained with other macromolecular adsorbates. For instance, in our experiments with HPA on polystyrene latices, described above, no detectable desorption could be observed upon dilution, even after protracted periods of patient waiting, whereas tracer experiments suggested that adsorbed and dissolved HPA molecules exchanged readily. Hence the problem of reversibility appears to be general.

Below, we shall argue that as far as flexible homopolymers are concerned reversibility may well be the case, even if dilution does not lead to desorption. As a first step it is expedient to consider the shape of polymer adsorption isotherms more closely. We follow thereby an argument set forth in more detail in ref.11

**Polymer Adsorption Isotherms**

Figure 4 compares two types of isotherms: a sharp one and a rounded isotherm. It is immediately seen that if adsorption is reversible, in the case of sharp isotherms dilution of the solution does not lead to desorption, whereas with rounded isotherms it does. Consequently, there is a relation between the

\[ \Gamma_P \]

\[ c_P \]

\[ \Gamma_P \]

\[ c_P \]

Figure 4. Representation of a sharp polymer adsorption isotherm (top) and a rounded one (bottom).
shape of an isotherm and the extent of desorption (as judged from dilution experiments) that must be anticipated.

Experimentally, usually rounded isotherms are obtained, sometimes with a well-defined plateau, in other cases without it. However, all modern theories predict sharp isotherms\textsuperscript{8-11}. In other words, if experimentally in a rounded isotherm no desorption upon dilution is observed, the system follows theoretical prediction for a sharp isotherm. Clearly, the problem of reversibility boils down to the solution of the disparity between theory and experiment.

Before analyzing this discrepancy, some statement on the scale of the abscissa axis in Figure 4 is mandatory. Obviously, if in the lower figure the concentration axis would be considerably compressed, the isotherm would more resemble the upper one. Let us therefore agree that the $c_p$ axis is linear and covers the range from zero to several hundreds of p.p.m., which is the range usually encountered in polymer adsorption studies. If upon dilution no desorption is measured this does not mean that not a single polymer molecule has left the interface, but that the extent of desorption is so low that the ensuing $c_p$ remains below the limit of detectability, which is usually on the order of p.p.m.'s or higher.

Closer inspection of theoretical isotherms reveals that the plateau is not exactly horizontal, but that $I_p$ increases a little with $c_p$. This is a very small increase. For instance, for a polymer of $M \sim 10^4$, adsorbing from a $\Theta$-solvent, $I_p$ increases by less than $10^0\%$ over the concentration range of $10-100$ p.p.m. and for higher $M$ and better solvents this increase is even less\textsuperscript{10}. Virtually this means that, according to theory, extremely (and, in fact, undetectably) low concentrations are needed before any desorption sets in. By way of example, for $M \sim 2500$ this concentration is less than $10^{-3}$ p.p.m. (depending on solvent quality, adsorption energy etc.) and it decreases exponentially with increasing $M$. In other words, theoretically for all except very short polymers no detectable desorption should take place, although there is no argument against full equilibrium between adsorbate and solution.

This solves part of the problem, because it explains that experimentally no desorption upon dilution needs to be observed, even if other experiments (such as exchange studies) indicate reversibility. The problem that is left is: why is the experimental adsorption isotherm rounded (in conflict with theoretical prediction), whereas experimental desorption isotherms follow theory?

\textit{Heterodispersity Effects}

The clue to this last question is that all theories are based on adsorption of polymers of a given $M$, whereas in practice, as a rule, heterodisperse polymers are used. The adsorbability of the various fractions is different. Specifically, with increasing $M$ polymers adsorb progressively stronger and displace molecules of lower $M$. If $c_p$ is increased, more bigger molecules are available to replace smaller ones. As a consequence, the molecular weight distribution of the adsorbate changes with $I_p$ and may differ considerably from that in bulk. Cohen Stuart et al.\textsuperscript{11} worked this out in some detail. Here we shall give a very simplified representation, illustrating the principle.

Consider, by way of example, a polymer in solution which consists of a mixture of small and big molecules with molecular weights $M_s$ and $M_b$ respect-
Let us further assume that both molecules individually obey a sharp isotherm (with a higher plateau for the big ones) and that big molecules completely displace the smaller ones. The isotherm of this mixture has two kinks as illustrated in Figure 5.

The explanation of this particular isotherm shape runs as follows. Because of the initial steep rise, with $c_p$ below the limit of detectability, the first part of the isotherm coincides with the ordinate axis. Over this range, everything adsorbs and hence the composition of the adsorbate is identical to the over-all composition. The vertical portion ascends till the surface area is completely covered. In the figure, point 1 corresponds to monolayer coverage of the mixture. The amount adsorbed in this point depends on the composition of the mixture and on $M_s$ and $M_b$.

Beyond point 1, addition of more polymer to the solution leads to displacement of small molecules by bigger ones. From point 1 to point 2 this results in a gradual and linear increase of $\Gamma_p$; in this region, in the solution only small molecules remain. This displacement process is completed in point 2. Here the surface contains exclusively big molecules and the equilibrium solution solely little ones. Otherwise stated, in this point complete fractionation is obtained.

If beyond point 2 still more of the given polymer mixture is added to the solution, $\Gamma$ cannot increase any more. With rising $c_p$, the composition of the solution changes gradually, although it remains richer in $M_s$ than the original polymer solution.

Dilution of the solution from point 3 or point 2 downward means in the first place that more of the low $M$ component is deleted than of the big molecules, whereas the latter fraction remains on the surface. As for these big molecules desorption takes place only far below the limit of detectability, no such desorption will be observed.

This is the basis of the interpretation. If the polymer mixture would have contained three components, three kinks would have been observed. For a wide distribution, a »rounded« isotherm is obtained for which indeed the shape reflects the $M$ distribution.
Some Consequences

The main impact of this picture is of course that, at least for heterodisperse random polymers, the reversibility issue has been solved. An important offshoot is that a foundation is given for determining $M$ distributions from the shape of the isotherm and for polymer fractionation. Another incidental finding is that for heterodisperse polymers the shape of the isotherm depends on the area/volume ratio $A/V$; this can be understood by realizing that the distribution of polymer over surface and bulk (and the ensuing extent of fractionation) depends on $A/V$. This, in turn, has an important consequence: it is not allowed to simply identify the properties of polymeric adsorbates on macroscopic surfaces (as in monolayers and films or on substrates used in ellipsometry) with those on colloidally dispersed adsorbents.

With several systems all these trends have now been experimentally verified. For instance, Cohen Stuart et al. working with polyvinylpyrrolidone on silica, and Hladý et al. studying dextranes on silver iodide, corroborated the picture of Figure 5 for binodal distributions, and Koopal obtained evidence for the volume/area ratio influence in the adsorption of polyvinyl alcohol onto silver iodide. Finally, it is now understood why polymers can sometimes be desorbed by other polymers but not by dilution.

Under certain restricted conditions, the shape of the HPA adsorption on polystyrene latex is also that of Figure 5. This is unexpected since HPA is monodisperse, although sometimes dimerization has been reported. One is inclined to speculate that under the given conditions the HPA molecule absorbs in two modifications, of which the adsorbabilities are so different that one modification displaces the other. Once the platform has been reached, no desorption by dilution is observable any more. However, it must be added that the shapes of HPA isotherms are still under study, because they depend rather critically on details of the experimental conditions, so that certainly no general conclusion may be drawn. Still, it remains of considerable interest for theory and practice to pursue the biopolymer adsorption reversibility matter more systematically.

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REFERENCES

6. P. van Dulm, W. Norde, and J. Lyklema, to be published.
Iznesen je kritički osvrt na dva opažanja u proučavanju adsorpcije polimera. Jedno od tih opažanja je egzotermna adsorpcija albumina ljudske krvne plazme na lateksu polistirena, iako su obje vrste, u uvjetima eksperimenta, električki negativno nabijene. Drugi problem je tumačenje kontradiktornih opažanja o reverzibilnosti adsorpcije sintetskih polimera, kao npr. polivinilalkohola na srebrnom jodidu. Tumačenje se nalazi u pojavi ko-adsorpcije jednostavnih kationa, u termodinamičkoj interpretaciji kompleksnih adsorpcijskih izotermi, te u efektima uzrokovanim heterodispersnim adsorbensima.

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