Historical perspective

Overcharging and charge inversion: Finding the correct explanation(s)

Wiebe M. de Vos a, Saskia Lindhoud b,*

a Membrane Surface Science, University of Twente, MESA+ Institute for Nanotechnology, P.O. Box 217, 7500 AE Enschede, the Netherlands
b Nanobiophysics, University of Twente, MESA+ Institute for Nanotechnology, P.O. Box 217, 7500 AE Enschede, the Netherlands

Abstract

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Both overcharging and charge inversion denote a general observation that the sign of a surface charge can flip in the presence of interacting species such as surfactants, polyelectrolytes, proteins and multivalent ions. Moreover, charge inversion of proteins through charge regulation, is one explanation for protein adsorption to similarly charged surfaces. While overcharging and charge inversion have been long studied, the explanations for these phenomena are often still debated. Broadly these explanations can be categorized as “chemical” where specific attractive interactions are seen as the cause of charge inversion, and “physical” where purely electrostatic interactions and constraints of geometry are used as explanation. In this review, charge inversion is discussed from a very broad viewpoint, where we draw connections between the various explanations proposed for very different systems. Especially, we highlight the work of Johannes Lyklema, who always carefully balanced between the competing chemical and physical explanations, and demonstrated that only few experimental systems allow just a single explanation.

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1. Introduction

This paper is part of a special issue of Advances in Colloid and Interface Science dedicated to the memory of Johannes (Hans) Lyklema. Through a very long and distinguished career, Hans has been an extremely influential scientist in the field of colloid and interface science. His work on the fundamentals of interface and colloid science [1–5], remains a solid base for the field to build on. Still, we only met Hans Lyklema late in his career, when we joined the lab for Physical Chemistry and Colloid Science as PhD students, at a time that he was already an emeritus professor. Also in this stage of his career, he took great effort to help young scientists take their first steps to become academics. Where we helped him to make the transition from overhead slides to powerpoint presentations, he helped us to develop ourselves and to become better scientists. Especially, his very interdisciplinary and open minded approach to science is something that we still try to emulate in our work to this day.

In this review, we present a broad discussion on the theme of charge inversion, also called charge reversal and overcharging, focusing on the search for explanations for this complex phenomenon. Charge inversion is a general observation where a charged surface becomes oppositely charged due to interactions with species in solution, or due to an external potential. It is a phenomena that is known and has been studied for...
many decades, but it is also a topic that even now leads to strong debates on its exact explanation [6]. Moreover, it is a topic that has many important implications in terms of colloidal stability [7], the formation of polyelectrolyte multilayers [8], and protein accumulation at interfaces [9]. Finally, it is a topic that Hans has contributed to throughout his rich career and a topic that he always spoke and wrote very passionately about.

Charge inversion has been found in a variety of systems as schematically depicted in Fig. 1. Charged surfactant molecules adsorbing to an oppositely charged surface lead to charge inversion at sufficiently high surfactant concentrations [10,11]. Depending on the surface properties, this can stem from a surfactant monolayer, or from the formation of surfactant aggregates, such as hemi-micelles or bi-layers. But charge inversion is also observed for charged polymers [12] and multivalent ions adsorbing to oppositely charged surfaces. Finally, the adsorption of protein molecules to surfaces can lead to charge inversion, while at the same time charge inversion of protein molecules is seen as one explanation for the counter intuitive observation that charged protein molecules can adsorb to like charged surfaces [13]. By discussing these seemingly very different systems together we will see that there are many connections and common explanations for the observed charge inversion.

2. Charge inversion by adsorption of charged surfactants

Surfactants, amphiphilic molecules that typically combine a hydrophilic headgroup with a hydrophobic tail, are amongst the most studied systems in colloid and interface science [1-3]. Their amphiphilic nature allows surfactants to self-assemble into micellar aggregates above the critical micelle concentration (CMC). But another natural consequence of their amphiphilic nature is their adsorption onto interfaces, including gas-liquid, liquid-liquid and solid-liquid interfaces. Many surfactant molecules carry a charged headgroup and adsorption of ionic surfactants to charged surfaces has led to very early observations of charge inversion phenomena [10,11,14]. This can happen in two fashions, as schematically depicted in Fig. 1a. On a hydrophobic surface, sparsely decorated with charges, the surfactant tails will have a strong interaction with the surface (1a, left side), although a few surfactants will also have electrostatic interaction with the surface charges [15]. Adsorption of surfactants to a hydrophobic surface can simply be described by a Langmuir type equation, see also Fig. 2a.

$$\Gamma = \frac{\Gamma_{\text{max}} C}{T + kC}$$

Here the adsorbed amount ($\Gamma$) is determined by the surfactant concentration C and a equilibrium constant (k) related to the interaction between surfactant and surface. The adsorption is limited by a maximum adsorbed amount, that for charged surfactants is not because the surface is completely filled, but rather because the CMC is reached, with every additionally added surfactant leading to the formation of micelles in solution.

It's important to mention here the crucial role of water molecules when we discuss a hydrophobic tail group adsorbing to a hydrophobic surface. The favourable nature of this process stems mostly from the release of water molecules that becomes possible because of the adsorption. Water molecules released from the hydrophobic tail and surface can interact more favourably with other water molecules (hydrogen bonding, dipole-dipole interactions etc) and gain entropy due to their larger degree of freedom. The release of water molecules is the main

Fig. 1. The four types of charge inversion discussed here a) Surfactant adsorption, b) Polyelectrolyte adsorption, c) Charge inversion by multivalent ions, d) Charge inversion of proteins due to a surface potential.
driving force for what we discuss as hydrophobic interaction [16] (although the entropic component can also be relevant for the adsorption of hydrophilic molecules to a hydrophilic surface).

But charge inversion by surfactants is not limited to hydrophobic surfaces. On strongly charged polar surfaces, charge inversion can occur by the formation of surface aggregates such as hemi-micelles or even surfactant bi-layers (Fig. 1a, right side) [11,14,17]. This process can be described in two steps, as was very eloquently done by Hans Nyklema [18]. In the first step, at low surfactant concentrations, the surfactants are mainly individually adsorbed through electrostatic interactions with the opposite charges at the surface. In this first step, the extend of adsorption is mainly determined by the charge density of the interface, with more charges leading to more adsorption. Still, a point made by Hans Nyklema [18], is that one should be wary to rule out non-electrostatic interactions between surface and headgroup, as these can also play an important role. In the second adsorption step, the surfactants aggregate around the primary adsorbed species through hydrophobic interactions to form aggregates such as hemimicelles, or surfactant bi-layers. In Fig. 2b, the adsorbed amount of surfactant is depicted as a function of the concentration and displays the two adsorption plateaus, $\Gamma_{1st}$ and $\Gamma_{2nd}$. Such a two-step adsorption process can be described by a variation of the Langmuir isotherm as proposed by Zhu and Gu [19], with the following equation:

$$\Gamma = \frac{\Gamma_{max} k_1 C^{(1/n + k_2 C^{(n-1)})}}{1 + k_1 C^{(1/n + k_2 C^{(n-1)})}}$$

A critical point to make here is that in the first plateau, the ionic surfactants lower the effective surface charge of the surface, while at the same time the hydrophobic tails make the surface more hydrophobic. For colloidal systems, such as particle suspensions the addition of a small amount of surfactant can in this way lead to flocculation. The strong effects on charge an colloidal stability have very nicely been studied by Rupperecht and Gu [7]. Here negatively charged silica particles in solution were studied as a function of cationic surfactant concentration. Results of their work are schematically shown in Fig. 2b. Already at low surfactant concentration, surfactants adsorb and the electrophoretic mobility becomes less negative. When the charge is fully compensated by surfactants substantial particle flocculation is observed through a strong increase in solution turbidity. However, at higher surfactant concentrations, charge inversion is observed, and the now positive charge on the particles leads to a stable particle suspension.

Clearly charge inversion by surfactant molecules is expected in nearly all cases where a charged surface is brought into contact with an oppositely charged surfactant, given that the surfactant concentration is high enough. The process is well understood, and within the framework of this review it acts as a nice example where non-electrostatic, or “chemical”, interactions can without much debate be seen as the driving force for charge inversion. On a slightly charged hydrophobic interface, the surface tail interaction will lead to surfactant adsorption and charge inversion. On a hydrophobic and charged surface, a first adsorption step at low concentrations is expected to be dominated by electrostatic interactions until the surface charge is compensated. The charge inversion then occurs at higher surfactant concentrations, and is dominated by hydrophobic tail-tail interactions.

3. Charge inversion by specific adsorption of charged polymers

Large molecules, such as polymer chains, adsorb to surfaces in quite a different way than small molecules such as the surfactants described in the previous section. As many polymer segments adsorb together, a polymer adsors to the surface following a high affinity isotherm [20,21]. With so many adsorbing segments, just a small adsorption energy per segment is sufficient to achieve effective irreversible adsorption. Still adsorption also comes with an entropic penalty. Each adsorbing segment loses entropy, and hence a critical adsorption energy per segment is required for adsorption to be possible. The chain also loses configurational entropy during adsorption, something minimized by the formation of loops and tails [21,22]. So even though polymer adsorption to a surface is associated with a high affinity, there is a strong entropic penalty associated with polymers adopting a flat conformation on the surface.

For the adsorption of polyelectrolytes to charged surfaces now also other interactions become important [20,23–25]. Both the charged interface and the polyelectrolyte are surrounded by small counter-ions. When the charged polymer adsorbs to an oppositely charged interface, this allows many of these small ions to be released. The salt concentration plays an extremely important part in this. On one hand it determines the entropy gain of counter-ion release, high at low salinity, but low at high salinity. Water molecules are naturally also released upon adsorption (entropically favourable), but water molecules could also become bound to the released counterions (reducing entropy). On the other hand, the salt in solution will help to screen any excess charge that builds up on the surface during adsorption, allowing more adsorption (and thus overcharging) at increased salt concentrations. Together this double role of salt in solution leads to interesting adsorption behavior as shown in Fig. 3, for PVP+ adsorption to TiO2. At low salinity the strength of adsorption is very high, while the build up of excess charge is unfavourable, resulting in polyelectrolytes lying relatively flat on the surface. The adsorption energy is so high that it overcomes the loss of configurational entropy and few loops and tails are found. Only a very small degree of overcharging is possible. However, at higher salt concentrations excess charge build up becomes possible and the observed adsorption is much higher. Now also loops and tails become favoured and much more overcharging of the surface is observed [12,24]. Finally at very high salt concentrations, the entropy gain from counter-ion release falls below the critical adsorption energy and no chains are adsorbed.
But, a key point, made by Hans Lyklema [20,26] is that there is the danger to only focus on the electrostatic aspects in these systems. Indeed, the behavior above is only observed for polyelectrolyte chains when electrostatic interactions are the only or strongly dominating effect. In Fig. 3, we also show the results of Hoogeveen et al. for the adsorption of PVP⁺ to a silica surface. Here the polymer chain is not desorbed at high salt concentration, indicating favourable non-electrostatic interactions between polymer and surface. Salt does still play a key role as at higher ionic strength as the build up of excess charge becomes less unfavourable and quite high adsorbed amounts are observed (and thus very significant overcharging). Clearly non-electrostatic interactions can substantially strengthen, and at high salt concentrations even dominate the effect of overcharging. For many combinations of polyelectrolyte, solvent and surface non-electrostatic effects will play a role. Only by careful and good experimentation does it become possible to really find out what all the driving forces are [20]. A key experiment is to study conditions where the electrostatic effects are very weak, such as at high salinity or at a pH value where the surface or the polyelectrolyte is completely uncharged. In this way the role of the non-electrostatic interactions can really be understood.

Overcharging through polyelectrolyte adsorption is key to many important applications. For example cationic polyelectrolytes are used on big scales to bind negative dye molecules to negative surfaces in the paper and textile industry [27]. But another application lies in polyelectrolyte multilayers. Here a charged surface is coated alternately with positive and negative polyelectrolytes, something only possible with overcharging taking place at every adsorption step [8,20,28,29]. These systems have found many applications and are now for example used as membranes [30,31].

In the scope of this review, overcharging by polyelectrolyte adsorption is a nice example of a system where overcharging can be fully explained by electrostatic interactions, in combination with the entropic penalty associated with polymer adsorption. The loops and tails needed to retain as much configurational entropy as possible allow adsorption of more charged monomers than required to compensate the oppositely charged surface charges, especially at medium ionic strength. But a key point made by Hans Lyklema is that this does not mean that other interactions are not present. Indeed, attractive non-ionic interactions are expected between many surfaces and polyelectrolytes and can strongly affect the degree of charge inversion. Only by careful experimentation can one unravel the relative importance of electrostatic and non-electrostatic interactions. For example at high salt concentration, electrostatic effects can be sufficiently screened, allowing only the study of non-electrostatic interactions [12,20].

4. Charge inversion by adsorption of multivalent ions

From the previous two examples, charge inversion by charged surfactants and charge inversion by polyelectrolytes, it can be concluded that the origin of this phenomenon can be chemical [7], considering the charged surfactants and physical, i.e., the adsorption of PVP⁺ on TiO₂ purely due to electrostatic interactions [12]. But for other systems, like charge inversion through multivalent ions, it becomes much more difficult to differentiate between “chemical” and “physical” explanations [6,32].

Charge inversion by multivalent counterions was first described by Freundlich in 1906. In the PhD thesis of Troelstra (1941) on Uitvakking en Omlading (Floculation and Charge Reversal) it was first mentioned that charge inversion occurred in the AgI system [33], at that time the most commonly studied system in Colloid Science in the Netherlands. In the seventies it was shown by James, Wiese and Healy that charge inversion of SiO₂ could occur because of the specific adsorption of hydroxylated metal ions [34,35]. For AgI Lyklema and Golub showed in 2007 that the charge inversion of AgI by multivalent hydrolysable ions had a chemical origin [33]. These are just a few of the systems in which charge inversion by multivalent counterions has been observed.

Many experimental systems were interpreted using Poisson-Boltzmann mean field approximation. Here the potential mean force for an ion is simplified to the mean electrostatic potential times the charge of the ion. As a result many features of the system are not accurately taken into account. Therefore the Poisson-Boltzmann theory for planar surfaces i.e., the Gouy-Chapman theory can only be applied to a limited number of systems. Incorporation of the stern layer in the Gouy-Chapman theory widens its applicability. Nevertheless the Gouy-Chapman theory is not exact and therefore it is difficult to judge whether discrepancies between the experimental results and the theory could be the result of limitations of the theory [6,36].

The increase in calculation power of computers meant that it became possible to model ions having a certain volume. This lead to the discovery of a new phenomenon i.e., ion-ion correlations, which could lead to charge inversion [37]. The Gouy-Chapman theory ignores ion-ion correlations, although at high surface charge and multivalent counterions ion-ion correlations could be of importance for charge inversion in these particular systems. Therefore it is not surprising that, when there is a very elegant model which can describe experimental results in terms of ion-ion correlations it is tempting to use this model and forget about specific chemical interactions, which could also be the origin of charge inversion [38–40]. Especially when the counterions used have a high degree of chemical complexity, i.e., it is likely that they can have a certain chemical interaction with the surface [40].

When you have a charged surface in solution it will be surrounded by an electric double layer. Naively one could argue that it is unfavourable for surfaces to have a net charge so the amount of counterions in this double layer should compensate the surface charge. If more counterions are present in the double layer to compensate the surface charge, charge inversion has occurred. The question is why this has happened, so what is the correct explanation for this phenomenon? Here a debate arises and it appears that the academic background of the researcher strongly determines the proposed mechanism for the experimental observations. Chemists have a tendency to explain charge inversion by specific chemical adsorption, whereas physicists have a tendency to explain charge inversion by ion-ion correlations. The next question is whether these two effects mutually exclusive? To put it in Hans Lyklema’s words: “that overcharging can be explained by one of these mechanisms does not mean that it is caused by this way [36].”

Although in most experimental systems specific adsorption plays a dominant role, this does not mean that ion-ion correlations are not of importance too. In fact, there might be systems where ion-ion correlations are the main driving force for charge inversion. This became Hans Lyklema’s quest, the “Quest for ion-ion correlations in electric double layers and overcharging phenomena” [32].

Ion-ion correlation theories agree on one thing, charge inversion should occur at charged surfaces >10 μC cm⁻² and with multivalent counter-ions having a valency of 2 or higher [41]. The cations and anions should be as symmetric as possible in terms of their size and:

• The surface charge should be measurable and high.
• Multivalent counter-ions should not chemically adsorb.
• The hydrolysis state of the ions should be known and measurable by performing pH titrations.

This means that the classical colloid system silver iodide cannot be used for this purpose, its surface charge is too low. What about oxides? Their hydrolysis state can be determined by titrations. The latter is very important because due to hydrolysis the valency can be reduced which in turn might lead to specific adsorption. This makes oxides a very difficult system to study in terms of ion-ion correlations because the surface charge and hydrolysis state both change as a function of pH.

Therefore Lyklema and coworkers investigated the electrophoretic mobility of the clay sodium montmorillonite, polystyrene latex and silica as function of the pH in the presence of different salts at variable concentrations. The aim of this study was to distinguish between chemical...
specific adsorption and ion-ion correlations [41]. It was observed that 1–2 and 2–2 electrolytes do decrease the electrophoretic mobility when their concentrations are increased, charge inversion was not observed. Trivalent cations on the other hand, did show inversion of the charge above a certain critical concentration. Typically this was observed for any of the latices and montmorillonite, which both have a surface charge which is independent of the pH. On close inspection of these systems specific adsorption of the hydrolysed trivalent metal ions appears to be the main driving force for the charge inversion. Only at low pH, where no hydrolysis of the trivalent ions occurs, ion-ion correlations are the most likely explanation for charge inversion. This effect is schematically shown in Fig. 4, based on the data of Jiménez et al. [41]. At pH 3, where nearly exclusively Al$^{3+}$ ions are present a small degree of overcharging is observed, most likely due to ion-ion correlations. However at higher pH values, Al(OH)$^{2+}$ ion concentrations increase, and their specific adsorption to Na-Montmorellonite leads to much more substantial charge inversion.

Fortunately there exists a system which fulfills all requirements for ion-ion correlations being the main driving force for charge inversion, as mentioned above: the mercury/aqueous electrolyte interface. For this purpose mercury drop electrodes are used and by using a Lippmann electrometer, the surface charge density can be deduced from the interfacial tension as function of the applied potential via the Lippman equation. The question is which electrolyte to choose because any kind of asymmetry between the anions and cations of the electrolyte can cause differences in ion-ion correlations. When this asymmetry is sufficiently large the ion-ion correlations can also become specific, meaning that it depends on the type of ion used [36]. It appeared that MgSO$_4$ was one of the only 2:2 salt for which high quality data, needed for this study, were available [42]. Moreover for some of these data it was known that chemical specific adsorption was negligible.

For the mercury/aqueous MgSO$_4$ interface it was found that at for MgSO$_4$ concentrations up to 1.5 M and surface charge densities which were more negative than $-1$ μC cm$^{-2}$ the data were well described by ion-ion correlations and not by Gouy-Chapman theory [36]. This indicates that in this experimental system ion-ion correlations are responsible for the reversal of the surface charge. Nevertheless Hans Lyklema concludes that it would be useful to find more experimental systems in which the data are better described by ion-ion correlations than by Gouy-Chapman theory. He also argued that ion-ion correlation theory could be extended by including specific adsorption of ions [36]. This will help for general better understanding of charge inversion by multivalent ions.

5. Charge inversion by adsorption of proteins

Proteins are natural polymers, their monomers are amino acids which all have their own chemical structure and properties. In general, as a result of the interactions between the amino acids proteins can fold in very specific 3D structures. Therefore the surface of the protein is chemically complex and because of this complexity protein adsorption is a complicated interplay between various attractive and repulsive interactions between the protein and the surface. As we will discuss here, that complexity means that protein adsorption can lead to charge inversion, just as for surfactant or polyelectrolyte adsorption, but also that the charge of the protein itself could become inverted during adsorption.

When proteins adsorb on a smooth surface long range, e.g., electrostatic attraction between the protein and the surface cause the protein to be transported from the bulk to the interfaceal region. Within a certain shorter distance from the surface short range interactions e.g., dispersion, hydrophobic, van der Waals interactions and hydrogen bond formation start playing a role and protein attaches to the surface. These short range interactions are mainly responsible for optimization of the protein surface interactions leading to a structural rearrangement of the protein on the surface, resulting in an increase of conformational entropy [43,44]. Protein adsorption can be seen as very negative, as a first fouling layer that can facilitate the formation of a bio-film [45], but can also be beneficial with an adsorbed protein corona helping to prevent foreign body responses or to help molecular recognition in bio-medical applications [46].

To make it even more complex, the charge of proteins is dependent on the pH of the environment, which means the the electrostatic interactions with charged surfaces are dependent on the pH. Moreover, in the case of proteins interacting with weakly charged surfaces or weakly charged molecules on the surface, the protein and the surface can affect each others dissociation behaviour. This plethora of interactions makes it difficult to correctly explain why proteins can adsorb on surfaces which are similarly charged. Especially the adsorption of Bovine Serum Albumin (BSA) in a like charged polyelectrolyte brush caused some debate in literature and will be discussed in this section.

Before we consider all the details of proteins adsorbing on like charged surfaces, let's first consider the charge of proteins, strong and weakly charged polyelectrolytes as function of the pH. In Fig. 5 the charge as function of the pH for five different species is shown. Strong polycasids and bases have a charge which is independent of the the pH. Weak polybases are fully charged at low pH and uncharged at high pH, whereas weak polyacids are uncharged at low pH and fully charged at high pH. Proteins are positively charged at low pH and negatively charged at high pH, this means that there is a pH at which the number of positively charged amino acids is equal to the number of negatively charged amino acids, at this pH the net charge is zero. This pH value is called the isoelectric point (IEP) and depends on the amino acid composition of the protein.

![Fig. 4. Electrophoretic mobility of Na-Montmorillonite particles as a function of AlCl$_3$ concentration, schematic figure redrawn after data from [41].](image)

![Fig. 5. Charge as function of the pH for weak and strongly charged polyelectrolytes and proteins.](image)
At the end of the 1970's Norde and Lyklema [9] investigated the adsorption of Human Plasma Albumin (HPA), now known as Human Serum Albumin (HSA) on strongly negatively charged polystyrene lattices. This protein is the human variant of BSA a commonly used protein. Since BSA or HSA are serum proteins, their adsorption on bare and coated surface is studied to get insights in the fouling of the surface of interest. In general proteins are first molecules to adhere to surfaces and once they are adsorbed bacteria can adsorb. So for biomedical applications it is mostly desired that proteins do not adsorb on surfaces. Therefore the adsorption of serum albumins is widely studied.

Lyklema and Norde found maximal protein adsorption around the IEP, additionally the adsorption as function of the pH was more or less symmetrical around the IEP [9]. Two straightforward explanations for this phenomenon i.e., that less adsorption is found at pH values other than the IEP are 1) the distance between the adsorbed proteins molecules is larger because of the electrostatic repulsion between the proteins, 2) the molecules change their conformation and have a tendency to spread on the surface [43].

Additionally, the adsorption of BSA at pH values above its IEP is of course a remarkable finding because it implies that BSA, which is negatively charged at pH values above its IEP spontaneously adsorbs on a negatively charged surface. There are several possible scenarios to explain this observation. It could be explained by patchiness of the protein. With this is meant that one specific part of the protein contains a reasonable number of basic amino acids and adsorption arises from electrostatic attraction between this positively charged patch and the negatively charged latex surface. Alternatively there are other favourable interactions between the surface and the protein which, although there is an electrostatic repulsion, cause the protein to adsorb. In the case of HSA Norde and Lyklema proposed that the protein could change its conformation such that the hydrophobic residues which in solution are mainly found in the interior of the protein could be partially exposed upon adsorption and interact with the surface [9].

One way to test this hypothesis is by carrying out hydrogen ion titrations also known as potentiometric protein titrations [47]. Using this technique the number of titratable groups on the surface can be determined, which will give you information about the amount of charged groups which are unavailable, meaning being located at the protein/surface, in this case protein latex interface. Additionally a change in the structure of the protein upon adsorption can expose or shield groups which can be dissociated. For HSA it was found that the titration behaviour of the adsorbed proteins was very different from the titration behaviour of proteins in solution, indicating that upon adsorption a change in conformation occurs [47].

Not only protons are taken up and released during the adsorption of proteins, other cations and anions are absorbed and released as well. The general trend is that at pH values below the IEP, where the protein is positively charged anions are adsorbed, at pH values above the IEP cations are taken up. So at pH values where the protein and surface both are negatively charged small ions might contribute to the adsorption being spontaneous [48,49]. This indicates that during protein adsorption redistribution of small ions plays an important role [50,51]. Finally, also water molecules will be released from both the protein and the surface, which can contribute significantly [16].

From this early work on HSA/BSA adsorption there is one important question which arises, the question whether BSA does adjust its conformation upon adsorption. For BSA in solution ion hydrogen equilibria experiments carried out by Tanford et al. in the 1950s indicated that BSA is present in different conformations as a function of the pH [52]. Below pH 4.3 and above pH 10.5 the protein undergoes a reversible change in conformation, to a more expanded form [52,53]. A compact form of the protein is found between pH 4.3 and 10.5. It was shown by Carter et al., that it was even more complicated than that [54]. In the low pH regime, BSA can in principle be present in three forms. The normal, also called N-form is the compact form which is stabilized by two disulfide bridges. This form is present between pH 4.5 and 8. Between pH 4 and 4.5 the F-form or fast form is present in solution. Below pH 4 an extended conformation (E-form) of the protein is found. Above pH 8 BSA is present in its basic (B-)form [54–57].

Since in solution BSA can adopt different conformations as a function of the pH it is very plausible that the conformation of the protein at surfaces is also different as a function of the pH. At the end of the 1970s the access to techniques to study this was limited. Currently several experimental techniques are available to study whether conformational changes of BSA occur during protein adsorption and it is possible to investigate how BSA adsorbs. From neutron reflectivity it can be deduced that the BSA molecules are adsorbed side-ways on [58]. A combination of hydrogen-deuterium exchange and mass spectrometry indicates that BSA changes its conformation upon adsorption [59]. It appears that whether a conformational change occurs depends on the size of the particle BSA adsorbs on [60]. A combination of QCM (Quartz Crystal Microbalance) and AFM (Atomic Force Microscopy) revealed that BSA does undergo a conformational change upon adsorption [61]. From this same work contact angle measurements below, at and above IEP show a difference in hydrophobicity of the surface when the proteins are adsorbed. At the IEP the contact angle is higher than below and above the IEP. This is not surprising since at the IEP the net charge of the protein is zero, resulting in a hydrophobic surface compared to a more hydrophilic character of the surface when the proteins have a net charge.

So far we have focussed on just a few proteins, HSA and BSA, as these contributed so much to the general understanding of protein adsorption. Still, it can be clear that different protein properties can lead to differences in adsorption behaviour. An important characterization was made by Willem Norde [52] who distinguished between “hard” and “soft” proteins. Hard proteins have a very stable internal structure, while for soft proteins the internal structure can easily be changed. Here BSA and HSA are examples of soft proteins, that undergo a conformational change upon adsorption, to optimize their interaction with the surface. These conformational changes can even result in a gain in configurational entropy [62]. Lysozyme is a commonly used model system for a hard protein. Upon adsorption hard proteins cannot change their conformation, limiting the possibilities for interaction. Indeed for hard proteins, electrostatic attraction is often found to be dominant when protein adsorption is observed [63].

Another key property of protein molecules is their surface charge. Typically a protein molecule has both cationic and anionic groups on the surface, with the charge strongly dependent on the pH (Fig. 5). But the charge does not have to be evenly distributed and many examples exist of proteins with pronounced negative and positively charged patches. A prime example of this is cytochrome C which exhibits a substantial dipole [64]. Such a patchy protein can orientate to allow for the most favourable electrostatic interaction (Fig. 6). Finally, as was discussed before, protein molecules can adapt their charges to other charged surfaces, and especially around the IEP of a protein this can allow charge inversion of the protein molecule to allow adsorption to an oppositely charged interface.

BSA is a commonly used model protein because it is a model for plasma proteins which induce fouling in biomedical applications. Therefore the adsorption of this protein is studied at surfaces with different surface chemistries and functionalities. One of the surface chemistries which was studied and debated in the 1970s was the adsorption of BSA in polyacrylic acid brushes (PAAs), attached to either planer surfaces [13] or nanoparticles with a polystyrene core [65,66]. The adsorption of BSA follows a similar trend compared to HSA/BSA adsorption on negatively charged lattices as was studied by Norde and Lyklema about 25 years before. Also in the polyacrylic acid brushes BSA adsorbs on the “wrong side of the IEP,” so why does BSA adsorb in the brush when both are similarly charged?

There is a substantial difference between the negatively charged polystyrene lattices, studied by Lyklema and Norde in the seventies and PAA brushes. The lattices were strongly charged surfaces, whereas
polyacrylic acids are weakly charged polyelectrolytes, so their charge depends on the pH of the system. In Fig. 5 it can be seen that PAA is uncharged at low pH and fully charged at high pH. It is well-known that in such systems the complex forming molecules, i.e., PAA and BSA can affect each other’s dissociation behaviour. A phenomenon which is called charge regulation and explained in detail in review by Lund et al. [67]. The titratable groups on the surfaces of the BSA and PAA can affect each other in such a way that optimal complexation occurs. This effect strongly depends on the pH of the system.

Charge regulation leading to charge inversion of the protein could be an explanation for the adsorption of negatively charged BSA in a negatively charged brush. On the other hand, it was argued by Dubin et al. that BSA is a patchy protein [68]. At pH 6.5 BSA has a small region of the protein surface which is positively charged and a large region on the protein surface with a net negative charge [68]. This positively charged patch of the protein could of course form a complex with the negatively charged PAA molecules. Because this is an electrostatic interaction its strength should be dependent on the ionic strength of the system. The same is true for charge regulation, so the interaction being dependent on the ionic strength does not tell you which is the correct explanation for protein adsorption on the wrong side of the IEP.

Both charge regulation and patchiness could be a driving force for protein uptake in like charged polyelectrolyte brushes. In fact these effects can be additive [69]. What the “patchiness” and “charge-regulation” supporters fail to discuss is the possibility of specific non electrostatic (“chemical”) interactions playing a determining role in the adsorption of BSA. It has been shown that the protein adopt various conformations as a function of pH. Especially around its isoelectric point BSA can be adopt the E, F and N-form.

Czeslik et al. do report an irreversible conformation change of BSA upon desorption from spherical polyelectrolyte brushes [65]. A similar finding was reported by Norde and Giacometti, they studied the conformation of BSA after desorption at two different solid surfaces. On hydrophilic silica the conformation of BSA before adsorption was the same as after desorption. On the other hand after desorption from negatively charged polystyrene particles an irreversible conformational change of the structure of BSA had occurred [44]. They discuss that this might be due to the interior of the BSA molecules being involved in intramolecular hydrogen bonding [44,70,71]. Additionally Takasu et al. found a decrease in alpha-helical content of BSA was decreased upon adsorption in different kind of polymer brushes [72]. Alpha-helices are stabilized by intramolecular hydrogen bonds, this decrease could be due to the formation of intramolecular hydrogen bonds between the protein and the surface.

In 1984 Hans Lyklema concluded a review on protein adsorption by: “Protein adsorption is a very complicated process, determined by a range of contributions. Overall electrostatic repulsion seldom plays a decisive role; in most cases proteins adsorb spontaneously on adsorbants with the same charge sign” [43]. In the case of BSA adsorption in PAA brushes at the “wrong” side of the IEP, apart from charge regulation and protein patchiness specific chemical interactions between BSA and the polymer brush could play an important role. Very likely hydrogen bonding between the surface and the protein does play a role, but also low molecular weight electrolytes may contribute to the spontaneity of the protein adsorption [51,73].

6. Conclusion

Charge inversion is a phenomenon that over many decades of study has been found to occur for a wide variety of systems, including adsorption of surfactants, polyelectrolytes, proteins and multivalent ions, but also as a result of charge regulation. Still, the exact explanation(s) for charge inversion can be difficult to pinpoint and have been the topic of many scientific debates. Charge inversion due to the adsorption of charged surfactants to an oppositely charged surface is a nice example of a system where the explanation is relatively straightforward. Hydrophobic tail-surface and/or tail-tail interactions, an example of “chemical” interactions, can without much debate be seen as the driving force for inversion. In contrast, charge inversion by polyelectrolyte adsorption can be fully explained from a “physical” perspective. Electrostatic interactions, in combination with the formation of loops and tails to retain as much of the configurational entropy of the polyelectrolyte chain as possible, allows charge inversion. However, in many systems an additional “chemical” affinity between surface and polyelectrolyte will substantially strengthen the effect of charge inversion. Only by carefully studying these systems as a function of the ionic strength, can one really distinguish between the chemical and physical contributions [20].

Possibly the strongest debate on the explanation(s) for charge inversion come from the observation that also the adsorption of simple multivalent ions can also lead to charge inversion. Two very valid explanations arose, a “chemical” explanation (specific adsorption) and a “physical” explanation (ion-ion correlations). However, as strongly put forward by Hans Lyklema, these explanations are not mutually exclusive [36]. Only by very careful design of experimental systems, does it become possible to study the explanations separately [36,41].

For protein adsorption the strongest debate in literature focused not on surface charge inversion itself, but rather on the observation that protein molecules can adsorb to a like charged polyelectrolyte brush [66]. In recent years charge inversion of the protein molecule due to charge regulation was put forward as an explanation for this effect, while proteins having charged patches was proposed as an alternative explanation. This effect was, however, already described by Lyklema and Norde in the eighties [9,47,48,50] for protein adsorption to solid surfaces. They found specific adsorption, often coupled to a change in protein conformation, to be responsible. With this, three very valid explanations become available to explain protein adsorption to like-charged brushes, again they are not exclusive, and can actually work together in unison.
Clearly, charge inversion is a complex phenomenon where the explanations are strongly system dependent. In many systems, multiple effects can even work together to determine the observed degree of overcharging. Hans Lyklema has made a major contribution to this field during his rich and long career. His open minded and interdisciplinary approach, coupled to very detailed experimental work, allowed a unique insight into these complex systems. One aspect that disappointed Hans, was that physicists often only sought physical explanations and chemists only chemical ones. Hans was one of the founding fathers of Molecular Sciences, an interdisciplinary study program at the interface between chemistry, physics and biology. Indeed, Hans always reminded us that there is no clear interface between chemistry and physics but rather that the transition from physics into chemistry is continuous.

Declaration of Competing Interest

The authors declare no conflict of interest.

References