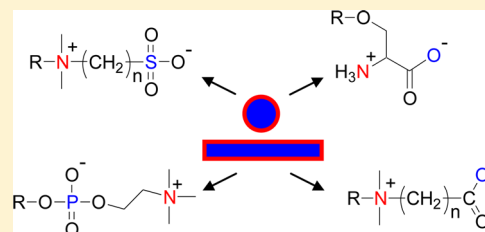


Zwitteration: Coating Surfaces with Zwitterionic Functionality to Reduce Nonspecific Adsorption

Joseph B. Schlenoff

Department of Chemistry & Biochemistry, The Florida State University, Tallahassee, Florida 32306-4390, United States

ABSTRACT: Coating surfaces with thin or thick films of zwitterionic material is an effective way to reduce or eliminate nonspecific adsorption to the solid/liquid interface. This review tracks the various approaches to zwitteration, such as monolayer assemblies and polymeric brush coatings, on micro- to macroscopic surfaces. A critical summary of the mechanisms responsible for antifouling shows how zwitterions are ideally suited to this task.



INTRODUCTION

Reducing the adhesion of environmental molecules and systems to surfaces has long been a goal of applied surface science. The most active areas of current research are at the biological interface: preventing the *in vivo* and *in vitro* adhesion of biomolecules, cells, and bacteria to objects and the fouling of surfaces by marine organisms.^{1,2} Materials for nonfouling coatings have many properties in common. They are usually neutral or weakly negative and well hydrated. Numerous hydrophilic, net-neutral monomers and polymers have been pressed into service,³ including acrylamides, polysaccharides (e.g., mannitol⁴), and, most commonly, polymers or oligomers based on the ethylene glycol, EG, (–CH₂–CH₂–O–) repeat unit, termed PEGs. PEGylation refers, in addition to general nonfouling applications, to the modification of a molecule or surface with EG repeat units to decrease interactions in a biological environment⁵ and therefore enhance the circulation (of molecules and nanoparticles) or residence time (of implants).

The use of zwitterions against fouling was inspired by the external surface of the mammalian cell membrane, rich in phospholipids bearing zwitterion headgroups, notably phosphatidylcholine.⁶ The balance of this surface is made up with neutral or anionic phospholipids. These zwitterions are presented mainly at the extracellular side of the lipid double layer, the cytoplasmic side of the cell membrane having far fewer zwitterions.⁶ The antifouling properties of a single monolayer of a lipid zwitterion are all the more remarkable considering it rests on an extremely hydrophobic blanket of hydrocarbon chains.

Some of the more common zwitterion functional groups are shown in Figure 1.

Around 1980, after the PC headgroup was shown to be nonthrombogenic,⁷ several groups polymerized zwitterionic phosphatidylcholine analogues to create stabilized membranes.^{8–13} Diacetylenes in lipid tails were used in these early works to photopolymerize vesicles or membranes. Synthetic polymer zwitterions were introduced by Ladenheim and Morawetz¹⁴ and Hart and Timmerman.¹⁵

As summarized later, some of the high-performance materials that have met the nonfouling challenge exceptionally well rely on a synergistic combination of surface and polymer science.

Historically (dating back to the late 1970s), research into zwitterion coatings has followed two trajectories: one focused on biocompatible materials and the other on more general nonfouling at interfaces.

While the concept of biocompatibility is often linked to nonbiofouling, the two are not synonymous, even though there is strong overlap in the technology used to implement them.¹⁶ Biocompatibility, implying *in vivo* applications, has more stringent requirements than simple nonadhesion.¹⁷ Ideally, platelets must not be activated.¹⁸ *In vivo* surfaces must not initiate the foreign body recognition system, for example, the tagging of particles by opsonins to be cleared by phagocytes.¹⁹ Of course, a completely nonfouling surface might achieve this, but the point is that proving biocompatibility requires more than proving nonfouling properties.

Conversely, biocompatibility does not necessarily mean or require nonfouling. Many materials, such as polyurethane, polyethylene, siloxane polymers, and titanium, to which proteins rapidly adsorb are classified as biocompatible. In reality, these “medical-grade” materials tolerate fouling for the location and time period for which they are used.

This review begins with a survey of how zwitteration has been implemented at macroscopic or planar surfaces and at the surfaces of nanoparticles. A further breakdown is provided on how zwitterion functional groups have been deployed in two dimensions (monolayers) and three dimensions. After this attempt to link historical threads, a discussion on the poorly appreciated topic of zwitterion interactions is followed by a critical analysis of the mechanisms for nonfouling, with comparison to PEG where appropriate.

Planar Surfaces. Bulk Zwitterion Polymers. The zwitteration of bulk polymeric materials to render them biocompatible

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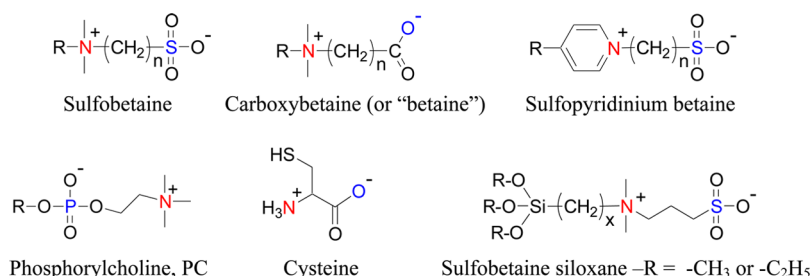


Figure 1. Zwitterionic functional groups and one zwitterinated siloxane.

started with the PC functionality. MPC itself (Figure 2) was invented by Nakabayashi's group in 1977.²⁰ PC polymers have

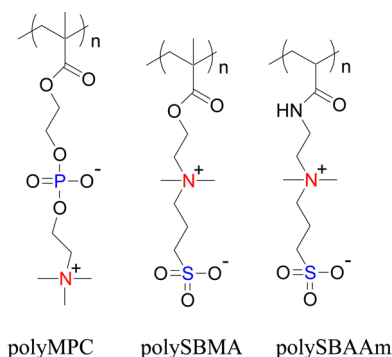


Figure 2. Three examples of zwitterionic polymers: poly-(methacryloyloxyethyl phosphocholine), polyMPC; poly-(sulfobetaine methacrylate), polySBMA; and poly-(sulfobetaineacrylamide), polySBAAm.

poor structural integrity and so are combined with tougher materials such as segmented polyurethanes.²¹ Copolymers with PC units were reviewed extensively at the turn of the millenium by Nakaya and Li²¹ and by Lewis.²² Copolymers of hydroxyethyl methacrylate, HEMA, a common contact lens material, and MPC have been in commercial use for nonfouling extended-wear lenses for some time (as omafilcon A).²³

For these bulk materials, rather than relying on a multistep process of making the polymer article and then coating it with an adhering layer of MPC, the zwitterion is incorporated as a comonomer. The zwitterionic functionality presumably orients to the surface on contact with water²⁴ in a kind of amorphous self-assembly. Zwitterion comonomers enhance the surface hydrophilicity of dimethylsiloxane polymers,²⁵ as illustrated by a reduced water contact angle.²⁴

Despite the manufacturing convenience of adding a zwitterion comonomer, depositing a nonfouling coating is a more versatile strategy, as this approach preserves the optimized bulk property of the coated material, whether it is a polymer, metal, or ceramic, while rendering it biocompatible.

Monolayer. The properties of liposomes prepared from zwitterion phospholipids, including those made stealthy by PEGylation,^{26,27} have been reviewed²⁸ and are not discussed here. Surfaces modified with zwitterion surfactants²⁹ are somewhat unstable, requiring a reservoir of dissolved surfactant to keep them in place (dynamic coating³⁰). The early photopolymerized membrane mimics^{8–13} were more stable but were not employed for practical materials, requiring, for example, assembly and compression at the air/water interface using a Langmuir trough.

Adsorption driven by strong interactions of sulfur with gold was introduced as a new method to organize monolayers at surfaces.³¹ The Regen group extended their earlier work on photopolymerized PC lipids¹⁰ by preparing self-assembled monolayers (SAMs) of a zwitterionic phosphorylcholine thiol on gold.^{32,33} These studies included dithiols, which should yield more stable monolayers,³⁴ and lipoic acid (disulfide) functionalities, similar to those used recently to make zwitterionic monolayers on gold³⁵ and semiconductor³⁶ nanoparticles. The efficiency of zwitterionic SAMs in preventing protein adsorption was later demonstrated by Tegoulia et al.³⁷ and the groups of Whitesides³⁸ and Jiang.³⁹

Films. Lowe et al. described a statistical copolymer of butyl acrylate (anchoring groups) with sulfobetaines which, when adsorbed to plastic discs, reduced the adhesion of bacteria and fibroblasts.⁴⁰ Another way of depositing a polymeric film of zwitterions is to incorporate them into a polyelectrolyte multilayer. We layered SBAAm-co-acrylic acid copolymers with polycations to protect surfaces from cell⁴¹ and protein adsorption.⁴² Interestingly, multilayers presenting both oligoethylene glycol and PC in a pendant group were not quite as efficiently nonfouling as the EG oligomer by itself.⁴³

Zwitterionic polymers brushes may be grafted to⁴⁴ or grafted from^{45–50} surfaces. Though not designed for antifouling, zwitterion polymers, prepared as monoliths or attached to chromatographic support media, were used by the Irgum group to separate ions and proteins.^{51–54} In 2002, Jiang and Irgum reported SBMA polymer brushes (which they called tentacles) grafted from silica particles using surface-bound radical initiators.⁵³ Xu et al.⁵⁵ produced phospholipid analogue brushes at a polypropylene surface by photoinduced graft polymerization of a dimethylamino vinyl monomer followed by the conversion of the grafted polymer to polyzwitterions with oxodioxaphospholanes. With sufficient grafting density, these coatings could reduce the adsorption of serum albumin substantially. Exceptionally low friction in solution was observed between surfaces grafted with zwitterionic brushes.⁵⁶

Because of the increasing number of ATRP (atom-transfer radical polymerization) and other living polymerization tools, grafting—from, which generally yields denser, more volume-excluding brushes, has become popular. Feng et al. grew MPC brushes from silicon wafers using ATRP,⁵⁷ which was shown to decrease protein and cell adhesion significantly.^{58,59} Zwitterion brushes grown from surfaces have been extensively reported by Jiang's group.^{60–62} These coatings demonstrated particularly effective fouling resistance, even from pure serum.⁶³ Bacterial adhesion was also inhibited.⁶⁴ Since about 2009 the number of works employing graft polymerization of polyzwitterions from/to surfaces has increased dramatically, as witnessed by grafting from silicon nitride,^{49,65} polypropylene membranes,⁶⁶ various surfaces using a bioinspired peptide initiator,⁶⁷ indium tin oxide

conducting glass,⁴⁸ gold,⁶⁸ hydrogels,⁶⁹ filtration membranes,⁷⁰ conducting polymers,⁷¹ cellulose membranes,⁷² and polysulfone membranes.⁷³

Nanoparticles (NPs). Nonfouling coatings confer both nonadhesive properties and colloidal stability to nanoparticles. Both are essential to the use of NPs in nanomedicine for diagnostics and/or therapy.⁷⁴ Coatings which prevent aggregation, precipitation, or clearance of NPs allow them to circulate in vivo and accumulate at a specific site via passive (such as a leaky vasculature) or active (e.g., using antibodies or aptamers) targeting.⁷⁵

Monolayers. Cysteine, a zwitterionic amino acid, has been used to decorate nanoparticles such as those made from semiconductors^{76–78} and gold⁷⁹ using the chemisorbing properties of the thiol group. Cysteine is not effective at preventing the salt-induced aggregation of Au NPs⁷⁹ or Ag NPs⁸⁰ whereas semiconductor nanoparticles⁸¹ are stabilized and passivated by a cysteine coating. The difference may be due to a greater stability of the S–semiconductor over the S–Au bond.

The use of synthetic molecules for zwitterionic monolayers on metal NPs followed some time later. Gittins and Caruso⁸² effected the complete transfer of Au NPs prepared in toluene into an aqueous phase using 4-dimethylaminopyridine as a phase-transfer agent, which has partial zwitterionic character when adsorbed to Au. These NPs, produced at high concentrations, were described as indefinitely stable. Tatum and Fujihara used an imidazoliumsulfonate-terminated thiol as a capping agent,⁸³ leading to Au nanoparticles that were not soluble in pure water but were soluble and stable in aqueous solutions at high salt concentrations.

We introduced the sulfobetaine motif for stabilizing Au NPs using a disulfide zwitterion.⁸⁴ The NPs were prepared by simple place exchange of weakly adsorbing citrate ligands with a strongly adsorbing disulfide zwitterion. These zwitterated nanoparticles were very stable, even in aqueous 3 M NaCl. We subsequently extended the sulfobetaine functionality to stabilize silica^{85,86} and (superparamagnetic) iron oxide nanoparticles,⁸⁷ employing siloxane condensation chemistry to bind the ligand to the surface.

Thin Films. In some of the earliest work on zwitterated polymer nanoparticles, Yamaguchi et al.⁸⁸ and Sugiyama and Aoki⁸⁹ reported the emulsion copolymerization of narrow-size-distribution MPC-containing nanoparticles. The MPC was shown to be localized at the surface, and the nanoparticles decreased, modestly, the amount of serum albumin adsorbed to the surface relative to nonzwitterated NPs. However, aggregation was observed.⁸⁹ Emulsion-polymerized methacryloyl-L-serine was also observed to reduce protein adsorption on methyl methacrylate nanoparticles.⁹⁰

Konno et al.⁹¹ prepared poly(L-lactic acid) nanoparticles stabilized by a shell of MPC/butyl methacrylate copolymer which yielded low surface zeta potentials and resistance to serum albumin absorption. Uchida et al.⁹² described the synthesis of styrene nanoparticles with grafted MPC units of about 6000 Da M_n starting with an MPC macromonomer.

In 2003, Chen and Armes reported a versatile method of adsorbing a copolymer with positively charged groups to attach to the surface and R-Br groups from which ATRP could be conducted.⁴⁵ The resulting polyzwitterion brush on silica nanoparticles is one of the few coatings to demonstrate zeta potentials approaching zero mV over a wide pH range, indicating the efficient masking of all of the charged groups

on the silica surface and negligible hydrolysis of the ester functionality. (A monolayer of sulfobetaine siloxane on silica also provides a zero zeta potential.⁸⁵) The grafting-from methodology is now widely used to produce highly stable zwitterion brushes on silica⁹³ or iron oxide⁴⁶ NPs.

Interactions. It is sometimes assumed that because zwitterions prevent fouling they do not interact with other species. This is not the case. There is much evidence for interactions with and between zwitterions on surfaces and in solution. In the former case, as with any weak interaction, multiple (polyvalent) associations between macromolecules amplify the effect. The challenge is to engineer zwitterions to minimize interactions with solution species. In the following treatment, it will be assumed that zwitterion functional groups are in their fully ionized state, which for carboxybetaines means that they are above their pK_a . Protonated carboxybetaines are not zwitterions. Sulfonate and phosphate groups have sufficiently low pK_a to remain fully charged over most working ranges of pH.

Interzwitterion associations depend strongly on the solvent. For example, significant interactions are observed in organic solvents⁹⁴ and calculated for solvent-free systems.⁹⁵ Zwitterionic end groups have been used to attach polymer brushes to surfaces in organic solvents.⁹⁶ Because the nonfouling behavior of zwitterions depends critically on hydration mechanisms, only aqueous solutions will be considered here.

Various inter- and intramolecular ion-pairing scenarios can be proposed (Figure 3) for zwitterions.^{97,98} For example,

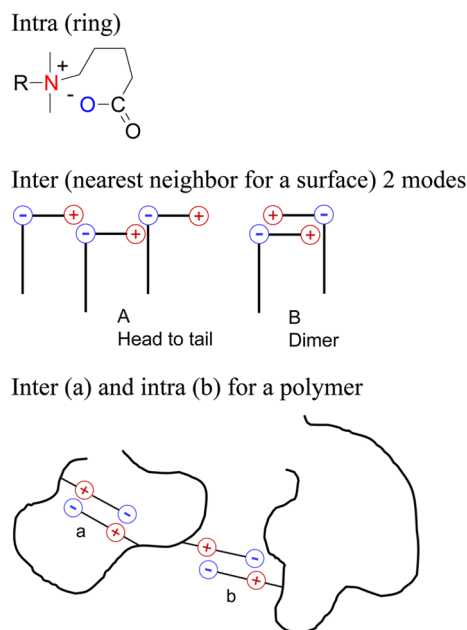


Figure 3. Interaction modes between monomeric and polymeric zwitterions. Added salt breaks interactions.

interzwitterion pairing requires relatively minor contortions and has been suggested to occur among the dense phospholipid headgroups making up the exterior leaflet of the bilayer cell membrane.⁹⁹ This type of pairing is supported by neutron diffraction studies¹⁰⁰ which show the P–N vector close to parallel to the plane of the bilayer. On the other hand, intramolecular pairing requires the bending around of the headgroup to meet the inner charge or neutralization of the charges through space (i.e., without distortion). Molecular

mechanics models show zwitterions to be extended¹⁰¹ with “no evidence of intramolecular ion pairing” (i.e., ring formation) for monomeric zwitterionic surfactants below the critical micelle concentration (CMC).¹⁰¹

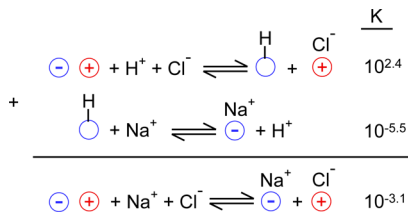
For carboxybetaines, the strength of ion pairing of zwitterion charges can be probed by titrating the carboxylate group: a decrease in pK_a indicates stronger associative interactions (ion pairing) between ammonium and carboxylate (i.e., $-\text{COO}^-$ becomes harder to protonate). For monomeric carboxybetaines, pK_a changes with the distance between zwitterion groups,^{101,102} which was suggested to be a field effect. In contrast, carboxybetaine repeat units on a polyzwitterion exhibit a constant lowered pK_a as a function of intercharge spacing,¹⁰³ causing the authors to invoke a ring-type interaction.

The contradiction between monomeric and polymeric carboxybetaine pK_a behavior may be reconciled by the behavior of zwitterion surfactants such as docosyldimethylammonium hexanoate.¹⁰² Below its CMC, the titration curve of this surfactant is described accurately with a single pK_a , whereas above the CMC the apparent pK_a decreases as the carboxylate is protonated. One interpretation of this phenomenon is that as the ratio of ammonium to carboxylate increases, the carboxylates are more strongly paired with positive charges, making the $-\text{COOH}$ a stronger acid (more difficult to protonate). This supports the idea that condensed or neighboring zwitterions interact.

For the purpose of preventing fouling, surface interzwitterion pairing should not be viewed as a problem and may actually enhance antifouling performance; as long as zwitterions are paired with each other they are not interacting with external species.

A rough estimate of the strength of ion pairing in zwitterions may be made using the titration data for polycarboxybetaines. According to Izumrudov et al.,¹⁰³ the pK_a for polycarboxybetaines is about 2.4 compared to that of free poly(acrylic acid), which is about 5.5. Scheme 1 uses these two results to

Scheme 1. Estimate of the Ion Pairing Strength of a Zwitterion^a



^a K_a for polycarboxybetaines is combined with that of the polycarboxylate to arrive at an estimate of the ion pairing strength of a zwitterion, $1/K_1 = 10^{3.1}$, where the zwitterion carboxylate is represented by \ominus and pyridinium by \oplus .

estimate the equilibrium constant, $1/K_1$, for inter/intra-zwitterion pairing of carboxylate and pyridinium. Pairing between zwitterions is suggested in a fascinating new class of bioadhesive polymers made with zwitterion pendant groups that have the positions of choline and phosphate (CP) reversed compared to those in PC.¹⁰⁴ Adhesion between red blood cells is promoted without rupturing the membranes.¹⁰⁵ If intermolecular zwitterion dimerization plays a role, as the authors postulate, then the open question is why is CP/PC binding stronger than PC/PC binding when both zwitterions

headgroups are so similar in geometry? The comparison is illustrated in Figure 4.

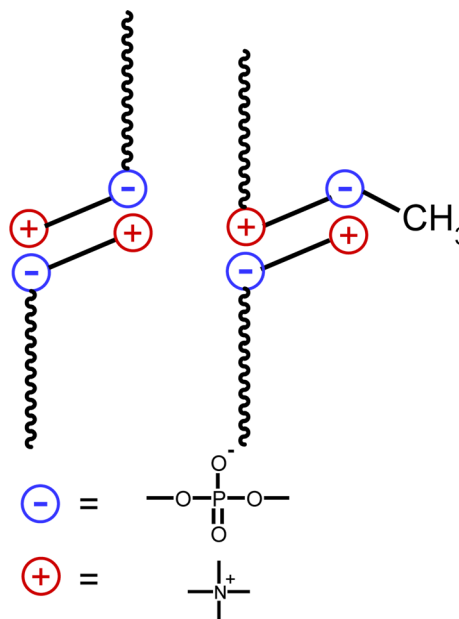


Figure 4. Comparison of PC/PC and CP/PC interactions.

It is possible that the one additional methyl group in the CP is enough to induce stronger binding.

Interactions of solution species with zwitterions have been exploited in so-called hydrophilic interaction liquid chromatography (HILIC).⁵⁴ Chromatographic methods are able to employ weak adsorption because even slight retention leads to resolved separations. Even so, when zwitterated stationary phases¹⁰⁶ are used they exhibit such weak interactions with solutes that the mobile phase often requires an organic modifier.⁵⁴ Zwitterated stationary phases also interact with proteins.⁵²

Ion chromatography reveals the clear retention of both anions and cations on bonded zwitterionic stationary phases in 100% water but only for anions at the less-solvated (chaotropic) end of the Hofmeister series (e.g., perchlorate and thiocyanate salts).⁵¹ NaCl showed no retention, indicating minimal interaction,⁵¹ a sign of strong intra(inter)zwitterion pairing. Anions and cations work cooperatively; for example, a strongly adsorbed anion, such as perchlorate, enhances the adsorption of a cation. On the other hand, monovalent cations in Cl^- are not separated, and only multiply charged cations, such as Zn^{2+} , Ca^{2+} , Ba^{2+} , and Ce^{3+} , are actually retained.⁵¹

Solution properties, such as solubility, depend on what groups make up the zwitterions. Polyvinylimidazolium sulfobetaine requires added salt to dissolve,¹⁰⁷ as do gold nanoparticles decorated with a thiolated vinylimidazole sulfobetaine.⁸³ PolyMPC dissolves in pure water.¹⁰⁸

Interactions between zwitterionic macromolecules are reflected in chain conformations and associations. The “antipolyelectrolyte” effect, expansion of the polymer coil in solution with added salt, is often cited as a property of synthetic polyzwitterions.¹⁰⁹ This effect is not consistently observed, with slight expansion seen for some polymer,^{110,111} or absolutely no change in dimension with added salt.¹¹² PolySBMA expands with added salt only in quite dilute solutions (10^{-4} to 10^{-2} M NaCl), perhaps indicating the breaking of intermolecular

pairing, but the size remains roughly constant from 10^{-2} to 1 M.¹¹³ In fact, around the conditions relevant for nonfouling applications (0.15 to around 1 M NaCl) little change in size is observed.¹⁰⁹

The fact that the antipolyelectrolyte effect in polyzwitterions is mild, if observed at all, is evidence that inter- and intrachain zwitterion pairings are weak. Even 1% intrachain ion pairing should lead to network formation for a polymer of molecular weight $>10^5$. For example, Matsuda et al.,¹¹² using static and dynamic light scattering on polyMPC prepared by ATRP, reported no change in the coil hydrodynamic radius, R_h , of 10.5 nm from 0 to 1 M NaCl and no evidence of aggregation for a polymer with $M_w = 2 \times 10^5$ and $M_w/M_n = 1.50$.¹¹² PolyMPC with such a molecular weight has a weight-average degree of polymerization, DP, of about 660 repeat units, so the fraction of intermolecular dimers must be less than about 10^{-3} . The polyzwitterion had a much larger coil size ($R_g \approx 16$ nm if $R_g = 1.5R_h$) than the negative polyelectrolyte poly(styrenesulfonate), PSS with a DP of 660 in a θ solvent (4.17 M NaCl at 16.4 °C, $R_g = 6.4$ nm¹¹⁴) or in 0.5 M NaCl ($R_g = 12.2$ nm¹¹⁵), or polystyrene, PS, in a θ solvent ($R_g = 7.6$ nm¹¹⁶), or even a good solvent ($R_g \approx 12$ nm¹¹⁷) which does not suggest intersegment attractive forces. The polyMPC R_g is similar to a rather expanded PSS in 0.05 M (16.8 nm).¹¹⁵ These comparisons support the known property that MPC is well hydrated. They are not consistent with intra- or intermolecular interactions. Even nearest-neighbor interactions as in Figure 3 should lead to changes in coil dimension when broken.

Our group found that the interactions between zwitterionic polyelectrolytes and either polyanions or polycations were insufficient for multilayering,^{41,118} as did Kharlampieva et al.¹¹⁹ It was necessary to copolymerize a charged group, such as acrylate, along with the zwitterion repeat unit⁴¹ to provide ion-pairing interactions with a polycation, such as PAH. In contrast, Mary and Bendejacq¹²⁰ reported the multilayering of a polyzwitterion with a polycation, perhaps because their polyzwitterion was partially hydrolyzed to an acrylate.¹²¹

Mechanism. To a first approximation, the antifouling mechanism of zwitterion coatings is intuitively straightforward: they are well hydrated with no net charge. On the other hand, if the extensive and conflicting discussion of the nonfouling properties of PEGylated surfaces is extended to zwitterions, then the mechanism becomes less clear. I will attempt to take the path of least contradiction, starting with the relevant mechanisms, followed by their contributions, ending with whether they are important in monolayer (2-D) versus film (3-D) zwitterion coatings. Both dimensionalities rely on minimal interaction with solution species, but the film coating has the added benefit of an excluded volume effect (entropy penalty), which prevents large molecules from approaching the surface.

Surface Energy Mechanisms—The Watery Surface. All nonfouling surfaces contain a good deal of water. If the surface contains extensive water in a similar state to bulk water, then no free energy can be gained in replacing a protein/water interface with a protein/surface interface by adsorption.¹⁶ In other words, a surface with low interfacial energy with water should discourage adsorption driven by interfacial energy change. Ikada analyzed blood-compatible polymers using this rationale.¹²² Recent measurements by Kobayashi et al. on surfaces coated with MPC and SBMA brushes showed low water-in-air contact angles and high oil-in-water contact angles, indicating such a low surface free energy.¹²³ PolyMPC performed somewhat better than polySBMA in this respect, where the

former showed no dependence on salt concentration, in contrast to the latter.¹⁰⁸ Wahlgren and Arnebrant warned against using simple surface free energy arguments, as the components of many surfaces are mobile enough to respond to the liquid in which they are immersed.¹²⁴ For example, if a surface is transferred from water to an organic solvent then the hydrophobic parts of a molecule or chain may flip out to maximize exposure to the solvent. Polyzwitterions show little surfactant activity¹¹³ (i.e., they do not perturb the surface free energy of water), which suggests that this type of molecular rearrangement may not occur for zwitterion-modified surfaces or polymers. The hydrophobic parts of the polymer chain are probably too small to be able to segregate with themselves (hydrophobic association) at the entropy cost (reduced chain conformations and translational entropy) that this association would entail.

Water Structure Argument. Molecular-level analyses of the nonfouling effectiveness of zwitterions frequently invoke special properties of water, specifically, structural effects on its hydrogen-bonding network.¹⁶ Disrupting the hydrogen-bonding network of water comes with a high energy cost as reflected in a large water cohesive energy density.¹²⁵ At the same time, optimizing the number of hydrogen bonds by the ordering of water leads to a loss of entropy.

Here, the environment for water falls under two broad categories. *Associated* water hydrates the zwitterion charges directly and is also termed nonfreezing. *Unassociated*, or bulk, water would be outside the first hydration shell, maintained within the zwitterion layer (whether 2-D or 3-D) by osmotic pressure. Wu et al. estimated that 7 ± 1 water molecules associate specifically with one sulfobetaine zwitterion,¹²⁶ which is lower than 85% water found by Murphy et al. for an MPC copolymer at the interface¹²⁷ but about the same as the intrinsic water content in a polyelectrolyte multilayer with quaternary ammonium and sulfonate polyelectrolytes in roughly stoichiometric proportion and with charged groups in similar proximity to each other.¹²⁸

Molecular dynamics simulations¹²⁹ show about 7 H₂O around a sulfonate group and 19 H₂O around the quaternary ammonium in a $-N^+(CH_2)_2SO_3^-$ sulfobetaine, slightly more than for a similar carboxybetaine. In comparison, thermal analysis of the MPC polymer¹³⁰ revealed about 58% non-freezable water, corresponding to about 23 H₂O per PC repeat unit.

Is water exceptionally structured by the strong fields around the zwitterion? Zwitterionic stationary phases are ion exchangers only in the presence of hydrophobic (chaotropic or structure-breaking) ions such as perchlorate or thiocyanate.⁵² Yet the evidence shows that zwitterions do not disrupt the H-bonding structure of water at all. In a highly informative set of experiments using Raman^{131,132} and FTIR¹³³ spectroscopy, Kitano et al. measured no disruption of the H bonding of water, including associated water, by zwitterionic polyelectrolytes. In contrast, regular polyelectrolytes induced a net loss of hydrogen bonds. Thus, water is not highly structured around zwitterions. More precisely, water appears to be no more or less structured around a zwitterion than in bulk water. The desirability of this property of zwitterions in promoting nonfouling behavior was noted earlier by Laughlin: "The geometry of hydrogen bonding...must resemble that of water molecules within liquid water."¹⁰²

Other pieces of evidence point to minimal disruption of the water structure by zwitterions. PolySMBA shows negligible

surface activity, indicating that it is perfectly at home in water.¹¹³ (There are no sufficiently nonpolar parts that migrate to the surface, and there is no need to banish the molecule to the water/air interface to preserve the H-bonding network.)

That a material can contain about 50 vol % water and still maintain the hydrogen-bonding network of bulk water is remarkable. How might a zwitterion help maintain such a robust network? Figure 5 compares, in cartoon form, water

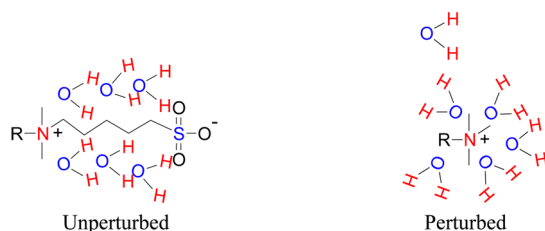


Figure 5. Representation of differences in water molecule ordering around a zwitterion and a single positive charge. The zwitterion allows the H-bonding structure to remain unperturbed (with reference to bulk water), while the single charge reorients the waters to a more disordered and less H-bonded state.

molecules around a zwitterion and a single charge. It is intended to show that the fixed polarity, or proximity, of opposite charges supports the polarity of the water molecules, at least over a correlation length of a few angstroms.

It should be noted that there is no special reason to have a chemical bond between the zwitterion charges in order to observe the effect in Figure 5. Maintaining one tetraalkylammonium and one sulfonate at a fixed distance should set up the same stabilizing field as in Figure 5. Referring again to our work on polyelectrolyte complexes in the form of multilayered films,¹²⁸ which contain almost stoichiometric amounts of paired tetraalkylammonium and sulfonate, we could find no difference in the O–H stretching region of water within a polyelectrolyte complex compared to bulk water.¹²⁸ This nonperturbing charge proximity model would also contribute to the fouling resistance of mixed-charge SAMs³⁸ and amphoteric polymers (which have mixed positive and negative repeat units).

Excluded Volume (Steric) Effects. A neutral, hydrated layer with some thickness, what Ikada¹²² called a “superhydrophilic diffuse surface”, can be extremely effective at preventing adsorption. Polymer brushes may be well defined, with known height, grafting density, and hydration level. Steric repulsive forces on compressing a brush such as PEG grafted to a surface^{134,135} contribute to the resistance of a brush or dense surface layer to invasion by particles (colloid stabilization) or macromolecules.

Entropy changes on the brush during protein sorption include the following: (1) osmotic pressure entropic penalties from displaced water; (2) compression or crowding of the polymer chain, reducing its configurational entropy (steric interactions). Enthalpic considerations originate from (1) the dehydration of hydrophilic repeat units and (2) the contact free energy of the polymer with protein. These entropic and some of the enthalpic components work against sorption to hydrophilic net neutral polymer layers from aqueous solution. From the adsorbing protein’s perspective, it loses translational entropy when it adsorbs, experiences a change in contact free energy on going from solvent to the surface, and possibly gains conformational entropy if it denatures¹³⁶ (but denaturing is not

a condition for adsorption). Reducing the mobility of a liquid or mobile surface also carries an entropic cost.

A barrier of water (sometime further described as structured) is often invoked to explain the nonfouling attributes of zwitterions. As mentioned above, there is no unusual structuring of water and water itself does not form a physical barrier. There is an *energy* barrier, to be sure. Water associated with surface polymers becomes part of the excluded volume portion of the steric mechanism. Water that is not attached is part of the osmotic pressure component of this same mechanism.

Ion-Coupled Driving Forces. Antifouling arguments based only on surface hydrophilicity are quickly derailed. For example, silica is a strongly hydrophilic surface (water contact angle in air of $\sim 0^\circ$), yet silica is a universal adsorber for proteins.¹³⁷ Thermochemical measurements of serum albumin adsorption to silica reveal an endothermic signature.¹³⁸ The electrostatic attraction of a positive patch on the protein to the negative surface should be exothermic. Because adsorption is spontaneous, the driving force must include an entropic component that outweighs the positive enthalpic one.

We recently highlighted an ion-exchange mechanism shown in Figure 6 to account for the entropic net driving force for

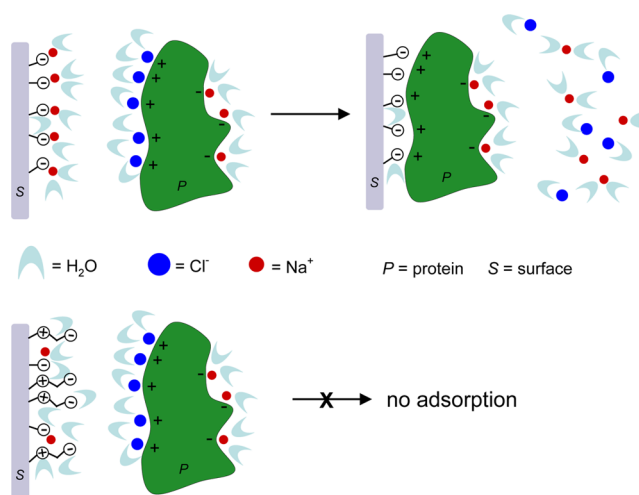


Figure 6. Cartoon for the ion-coupled adsorption mechanism of a protein with a positive charge onto a negatively charged hydrophilic substrate, such as a silicon wafer (where the charge comes from ionized silanols). Upper: The adsorption of protein is facilitated by the release of counterions and the formation of ion pairs between the sorbent and the adsorbate. Lower: A neutral surface (zwitterion or PEG) has no surface ions associated with it. The binding of protein to the surface will not result in a net increase in entropy due to counterion release, and thus adsorption is not preferred. Note that some net charge is still associated with the original surface but is inaccessible due to a steric barrier.

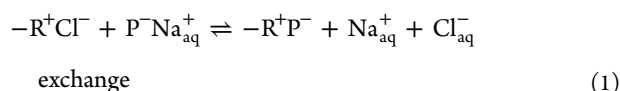
adsorption.⁸⁶ The adsorption of a positive protein, or positive patch of a net negative protein, results in the formation of ion pairs and the release of counterions. Each ion pair formed releases two counterions.

The term counterion evaporation, used to describe ion release from surfaces, unfortunately emphasizes enthalpic contributions.¹³⁹ As we described recently,¹²⁸ ion pair formation as the driving force for athermal or net endothermic complexation also involves the release of water molecules hydrating the counterions. Roughly speaking, the net entropy

gain would be kT for each ion or water molecule released. For a neutral surface, such as a zwitterion or PEG moiety, the surface charge is internally balanced (zwitterion) or neutral (PEG), and the formation of an ion pair with the adsorbate is unlikely, as no ions are available for release from the surface. Using this rationalization for protein resistance, adsorption can be divided into ion-coupled and ion-decoupled mechanisms. The small value (4 kJ) for the enthalpy of dilution of NaCl allows entropic contributions to dominate when Na^+ and Cl^- are counterions (i.e., in vivo).

Although adsorption is prevented by a single zwitterion layer, a steric component of this ion-coupled mechanism is also possible. Also shown in Figure 6 are a few isolated negative surface charges with their counterions. Access to these residual charges is blocked by neighboring surface ligands, preventing the release of counterions. One can envisage the same mechanism with oligo or polymeric brushes of neutral polymer. These bulky coatings are probably more effective at blocking access to surface ions.

This ion-exchange mechanism is accessible through quantitative ion equilibria. The adsorption of a charged species at a charged site may be represented as an ion exchange, for example, a negative polymer or protein charge P^- adsorbing to a positive site $-\text{R}^+$:



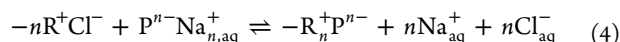
The species are depicted with their counterions condensed on (associated with) the charges. The corresponding equilibrium, using concentrations in lieu of activities, would be given by

$$K_{\text{ads}} = \frac{\text{RP}[\text{NaCl}]_{\text{aq}}^2}{\text{R}[\text{P}^-\text{Na}^+]_{\text{aq}}} \quad (2)$$

where RP is the fraction of occupied (by protein) sites and R is the fraction of vacant sites. Or since $\text{RP} + \text{R} = 1$, at constant $[\text{NaCl}]$, in a Langmuir isotherm format

$$K'_{\text{ads}} = \frac{\text{RP}}{(1 - \text{RP})[\text{P}^-\text{Na}^+]_{\text{aq}}} \quad (3)$$

If the adsorption site on the polymer/protein consists of n charges which adsorb simultaneously

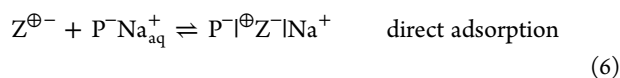


$$K'_{\text{ads}} = \frac{\text{RP}}{n(1 - \text{RP})^n[\text{P}^{n-}\text{Na}_{\text{aq}}^+]_{\text{aq}}} \quad (5)$$

In this case, the isotherm rises more steeply in a so-called high-affinity mode, which is typical for polymers. For simplicity, further analysis is limited to single-charge adsorption (eq 1).

In the absence of specific attractive interactions between $-\text{R}^+$ and P^- , which should be manifest as negative ΔH , the adsorption is driven by the release of counterions. From eq 2, the adsorbed fraction RP should decrease with increasing salt concentration, which is universally observed in experiments.

In comparison, a zwitterionic site, $\text{Z}^{\oplus-}$, has no counterions with which to exchange upon protein adsorption



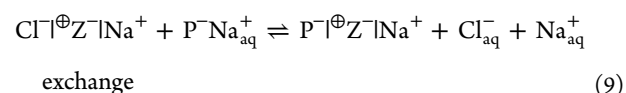
With no counterions to release, there is no corresponding entropic driving force. In addition, the protein charge is competing with an internal zwitterionic charge at a much higher effective concentration.

$$K_3 = \frac{\text{ZP}}{\text{Z}^{\oplus-}[\text{P}^-\text{Na}^+]_{\text{aq}}} \quad (7)$$

K_3 is expected to be small, which means that protein adsorption is minimal. In sufficiently concentrated salt, the zwitterion will be forced to take up salt. The internally ion-paired, or intrinsic, form of the zwitterion, $\text{Z}^{\oplus-}$, opens up into the extrinsic form, $\text{Cl}^-\text{Z}^{\oplus-}\text{Na}^+$, when it pairs with counterions,



The solution species, P^- is now able to exchange with counterions doping the zwitterion and release them



If $\text{Z}^{\oplus-}$, ZP, and $\text{Z}^{\oplus-}$ are defined, respectively, as the fraction of the zwitterions in intrinsic, protein-paired, and counterion-paired forms and $\text{Z}^{\oplus-} + \text{ZP} + \text{Z}^{\oplus-} = 1$, then

$$K_1 = \frac{\text{Z}^{\oplus-}}{\text{Z}^{\oplus-}[\text{NaCl}]_{\text{aq}}^2} \quad (10)$$

$$K_2 = \frac{\text{ZP}[\text{NaCl}]_{\text{aq}}^2}{\text{Z}^{\oplus-}[\text{P}^-\text{Na}^+]_{\text{aq}}} \quad (11)$$

wherein $K_3 = K_1K_2$. The more resistant to ion pairing, the more effective the zwitterion will be at defeating adsorption. The zwitterion efficiency, defined as K_1^{-1} , should be as large as possible (i.e., $K_1 \ll 1$). The zwitterion efficiency should be a function of various parameters such as the solvent, the salt ions (with those ions on the chaotropic side of the Hofmeister series, such as SCN , I^- , and ClO_4^- showing lower efficiency, i.e. better doping), the zwitterion functional groups, and the distance between them (as n in Figure 1 increases, K_1^{-1} decreases).

An interesting consequence of eq 7, which rearranges to $\text{ZP} = K_3\text{Z}^{\oplus-}[\text{P}^-\text{Na}_{\text{aq}}^+]$, is that if the zwitterion efficiency is high and $\text{Z}^{\oplus-}$ stays close to 1 then ZP is essentially independent of salt concentration, in contrast to a fixed-charge site (eq 2).

Guidelines for Effective Zwitteration. For fouling resistance, polyzwitterions have a lot in common with PEG or, for that matter, any neutral hydrophilic polymer. However, for 2-D coverage a monomeric layer of EG or EG-like units, such as methoxy or OH, is not as effective as a layer of monomeric zwitterion,³⁸ probably due to non-ion-coupled interactions such as hydrogen bonding or hydrophobic interactions in the former.

Three-dimensional or film coverage is expected to be more rugged than for a monolayer: if an area of monolayer is compromised, then fouling resistance fails, whereas a brush or film is able to fill in exposed or damaged areas to some extent. Balanced against ruggedness is an increased size (for nanoparticles), more possibility of contamination if the brush detaches, and slower integration into surroundings (e.g., by endothelialization or coating with a noninflammatory extracellular matrix).

At high NaCl concentration EG units collapse, whereas zwitterion units are well hydrated.⁸⁶ In fact, it is one of the properties of monomeric or polymeric zwitterions that they become more hydrated as the NaCl concentration increases, a consequence of the equilibrium in eq 8.

It is difficult to quantify the contribution of each mechanism because of entangled enthalpic and entropic components. However, it is possible to summarize qualitatively, as in Table 1, whether each mechanism operates favorably for a 2-D or 3-D coating.

Table 1. Contributions of Various Nonfouling Mechanisms in 2- and 3-D Zwitterionic, ZW, and EG Coatings^a

	monolayer ZW ^b	brush polyZW	monolayer EG	brush polyEG ^c
excluded volume	x	✓	x	✓
surface energy	✓	✓	x	✓
ion coupled adsorption	✓	✓	✓	✓
water structuring	✓	✓	✓	✓
steric ion coupled effects	✓	✓	✓	✓
surface mobility	x	✓	x	✓
salt resistance	✓	✓	x	x

^ax = little or no contribution, ✓ = favorable contribution. ^bZW = zwitterion. ^cIncludes oligo EG.

Whether perfect nonfouling is needed depends on what type of surface response is sought. In some cases, high-performance nonadsorption at all costs may not really be desirable. Imagine an area of an implant coated with a zwitterion brush which has perhaps been partially hydrolyzed to reveal carboxylic acid groups which in turn activate platelets. The implant remains nonfouled but is now less biocompatible. It may be preferred, in many cases, to integrate without inflammation.

Table 1 shows zwitterion polymers to be the highest performer, with all boxes checked. Zwitterion brushes are bioinspired but not biomimetic (like zwitterion monolayers). If zwitterion brushes are so good at preventing fouling, then why has nature not endowed the cell membrane with them? A cell is not a fortress. A multitude of extracellular macromolecules need to interact in a controlled, specific manner with the cell via its membrane. Nonspecific interactions are discouraged by the zwitterion monolayer. There is a brush component to the cell membrane: the glycocalyx. These are mainly polysaccharides (not zwitterions, their ether linkages place them closer to PEG in structure), and they presumably provide a fine balance between assisting the zwitterion monolayer in repelling nonspecific adsorbers while allowing some access to the membrane and providing specific modes of interaction with themselves via programmed molecular recognition.

CONCLUSIONS: OUTLOOK FOR ZWITTERATION

The use of zwitterions to protect surfaces is expected to grow, at both sophisticated biological interfaces and at less-well-defined environmental ones. An example of the latter category would be protecting membranes from fouling by particles and organics. Because zwitterion interactions are reduced with exposure to aqueous NaCl, they are ideally suited for physiological or marine conditions and applications. Zwitterionic behavior may be hiding in plain sight. As mentioned earlier, stoichiometric polyelectrolyte complexes, such as those

often found in polyelectrolyte multilayers,¹²⁸ have the requisite functional groups in the right spatial proximity. Such systems may be considered to be bulk zwitterions or zwitterosols.

In nanomedicine, conditions and times used to demonstrate the nonaggregating properties of nanoparticles vary widely. A period of about a day is enough to cover most imaging and therapeutic requirements for targeted and/or diagnostic nanoparticles. Thus, the 24 h time point for stability tests in media approaching serum as closely as possible should be used in screening the effectiveness of coatings designed to promote circulation. The use of a rugged nonaggregating coating should make it easier to discern whether possible nanoparticle toxicity is a result of the NP itself or their aggregates.

In planar systems, short-term use for zwitterated surfaces has significant potential. MPC itself is already approved for in vivo applications.¹⁴⁰ Coatings on guidewires and catheters to prevent fouling and bacterial biofilms should be effective for the short term (up to a few weeks). The prospect for long-term in vivo applications of any nonfouling coating is more uncertain. Even biocompatible MPC coatings on coronary stents did not prevent endothelialization in pig models.¹⁴¹ Paradoxically, the same coating that initially reduced biofouling now weakens the interface between the stent and the new tissue, making it prone to mechanical failure. In vivo testing of zwitterated particles and surfaces is urgently needed as is more modeling (simulations, molecular mechanics), which must include water molecules explicitly.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

Biography



Joseph B. Schlenoff is Mandelkern Professor of Polymer Science and Distinguished Research Professor of Chemistry and Biochemistry at Florida State University. After a brief stint at Polaroid Corporation (Cambridge, MA), he completed a Ph.D. in chemistry at the University of Massachusetts, Amherst, in 1987. Following a postdoc in polymer science at UMass, he joined the faculty of FSU in 1988. He has worked on polyelectrolytes at surfaces, in thin films, and in bulk complexes. Other surface science interests include self-assembled monolayers and modifying interactions at the biointerface.

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