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Mini-Review

Charge Matters: Electrostatic Complexation As a Green Approach to Assemble Advanced Functional Materials

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formed from biomolecules and for salt-controlled rheology, underwater adhesiveness, and interfacial spinning. Finally, we present an outlook of electrostatic complexation from the colloidal chemistry perspective.

1. INTRODUCTION

Our society strives to adopt the concepts of the circular bioeconomy, which implies the emergence of new materials and green processing routes. Related breakthroughs are escorted by a systemic understanding of the matching between resources and products, from the macroscale to the molecular dimensions. In this respect, colloidal systems are expected to be important contributors because their assembly can be governed by noncovalent interactions. They allow structures to be tailored toward fine-tuned properties and novel functions.

Generally, "colloidal" material assembly exploits (i) hydrophobic (e.g., self-assembled liquid crystals and mesophases), (ii) chemical (e.g., covalent and coordinative), and (iii) physical (e.g., electrostatic complexation) interactions. The latter is ubiquitous in nature; e.g., it is involved in membraneless organelles and adhesive sandcastle worms. Electrostatic complexation also stands out for being greener. In fact, it can be carried out in aqueous media, does not require harsh chemicals, and is easy for scale up. These characteristics are in line with the ever-growing demand for large-scale production of sustainable materials. The layer-by-layer technique (LbL), for instance, is a relevant method for assembling and functionalizing materials for a number of applications, from batteries and solar cells to functional fabrics and tissue engineering.¹ Importantly, electrostatic complexation is reversible and facilitates stimuli-responsive phenomena, as we discuss henceforth in a concise, though contentwise comprehensive overview. We place electrostatic complexation in perspective by weighing its advantages and drawbacks over

hydrophobic and chemical routes. We discuss the most relevant electrostatically complexed materials and offer an outlook from a colloidal chemistry perspective.

2. ELECTROSTATIC COMPLEXATION

Electrostatic complexation involves strong association of species bearing opposite electrostatic charges, sometimes accompanied by phase separation, in aqueous solutions or suspensions. This is applicable to fully extended macro-molecules (e.g., polyelectrolytes) or colloidal particles (e.g., hard nanoparticles, soft micelles, nanofibers, etc.)—or virtually any combination of these—carrying ionic or ionizable groups.

Although this complexation can be considered as purely electrostatic in its origin, the free energy of complex formation in water has been demonstrated to be actually driven by other factors: enthalpic—via the strong Coulombic attraction between unlike charges as well as local water perturbations— and entropic—by the release of counterions that take part in the electrical double layers of the previously unbound species as well as the diminished degrees of freedom in the bound state.² While entropy drives the complexation among strong (poly)electrolytes (i.e., those that are completely ionized when

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in solution), enthalpy dominates in the case of their weak analogues (i.e., those whose ionization extent is controlled by pH and ionic strength, just to mention a few factors).³

The dependency of the charge of weak (poly)electrolytes on the condition of the surrounding medium allows their electrostatic complexations to be inherently responsive to external variables (or stimuli) and also tunable. To exemplify, if the association between polyelectrolytes is weak, the formed polyelectrolyte complexes (PECs) may remain soluble. On the other hand, if the complexation is strong, one would expect a macroscopic phase separation into a polyelectrolyte-poor phase and a polyelectrolyte-rich counterpart, the latter being traditionally referred to as coacervate. This latter system refers to an associative liquid-liquid phase separation of oppositely charged polyelectrolytes. The associative complex coacervates are spontaneously formed, provide microcompartmentalization to selectively encapsulate biomolecules, and, for this reason, can be used for confined biochemical reactions. Due to the ability of coacervates to sequestrate RNA, they have been hypothesized to play an important role in the origin of life, as was postulated as prebiotic membraneless compartments.4

3. ELECTROSTATIC VERSUS COVALENT ASSEMBLIES

Compared to covalent chemistries, reversibility and responsiveness to stimuli (e.g., ionic strength, hydration level, and pH) are the main features of electrostatic assemblies. For instance, electrostatic complexes (ECs) arise from the chemical nature of the complexed species as well as electrostatic interactions. Understanding the role played by different variables is crucial for tailoring the EC properties and to achieve the best conditions for EC preparation, opening up a wide range of applications, as detailed below.

3.1. Role of Ionic Strength/Doping Level. Ionic strength is certainly the most important variable affecting the properties of ECs, regardless of the chemical nature of the constituents. Such an effect originates from the charge screening promoted by small counterions that break the ion pairs of the macro ions. Among the studies on the role played by the ionic strength on PECs, the effect of KBr in solution has been investigated for poly(styrenesulfonate) (PSS)/poly-(diallyldimethylammonium) (PDADMA) PECs.⁵ The association between these polyelectrolytes leads to a solid precipitate that can be made tough and dense after extrusion. NaCl, the residual salt resulting from complexation, can be washed out by water prior to "doping" with KBr. The fraction of ion pairing between the small ions and the charged groups of the poly ions defines the doping level. Therefore, after washing, i.e., in the absence of counterions, the PECs are termed undoped. An alternative protocol to prepare PECs, without counterions, is by direct titration of acid and basic forms of the poly ions, followed by water removal. This procedure was proposed by Picullel et al.⁶ for the preparation of polymer-surfactant complex salts and can be extended for PECs.

An interesting feature is the hydration level with the extent of PEC doping. As the concentration increases, salt breaks the ion pairs of poly ions, making the PEC increasingly more hydrated, until complete dissolution. Changes in the appearance and rheological behavior of the polyelectrolyte assemblies are also relevant: at low salt concentrations, the PEC is opaque and has a soft rubber-like consistency, whereas at higher salt concentration it displays clearer appearance and a fluid-like behavior (see Figure 1 for PSS and PDADMA). This trend has been widely observed not only for PECs but also for polyelectrolyte/surfactant complexes.



Figure 1. Effect of doping level on the properties of a polyelectrolyte complex (PEC) formed by poly(styrenesulfonate) (PSS)/poly-(diallyldimethylammonium) (PDADMA): (a) macroscopic and (b) proposed molecular evolution from a solid- to a liquid-like PEC upon sequential KBr additions, followed by solubilization. Reprinted with permission from Wang, Q.; Schlenoff, J. B. *Macromolecules* **2014**, *47* (9), 3108–3116.⁵ Copyright (2014) American Chemical Society. Further permissions related to the material excerpted should be directed to the American Chemical Society.

By plotting the doping level as a function of salt concentration, different regions can be used to describe the given morphologies: initially, in the solid region, the doping level increases almost linearly (but slightly) with salt concentration. In this region, the precipitate is glassy. Subsequently, as salt concentration increases, the precipitate undergoes a glassy-to-rubbery transition (although there is no clear threshold) until it is converted into a coacervate, i.e., an elastic liquid that is accompanied by an abrupt increase in the doping level. Finally, upon further salt addition, the PEC becomes completely soluble.

An important question that Wang and Schlenoff addressed is whether a critical transition exists between the solid-like precipitate and the liquid-like coacervate (Figure 1). It was observed that the mechanical properties of complexes were remarkably affected by the doping level. For the studied complex, rheological measurements (0.1 Hz and shear stress of 25 Pa) showed decreased storage (G') and loss (G'') moduli as well as viscosity at increased salt concentrations. Although no critical transition was observed, compared to the selection of a certain viscosity, the crossover point (i.e., the concentration at which G' is equal to G'') was suggested as a more suitable means of defining the boundary between a solid precipitate and a liquid coacervate.

We will discuss below how the changes in rheological behavior affect the processability of these so-called saloplastics. The observations highlight the importance of controlling the properties of PECs by varying the ionic strength. The PSS/PDADMA pair discussed so far is particularly relevant because of the variation in the appearance and rheology observed upon salt addition or removal. Of course, not all PECs behave like the PSS/PDADMA pair because many of them are not strong enough and phase separate as liquid coacervates, requiring lower salt concentrations to completely dissolve. In fact, the amount of electrolyte required for PEC dissolution is proposed as a parameter to assess the strength of interaction between poly ions.⁷

3.2. Relationship between Electrolyte and Hydration Levels. Assemblies resulting from electrostatic interactions generally do not phase separate as dry precipitates. Because they continue interacting with the ion pairs, water molecules may act as plasticizers and, as such, change their thermal behavior, as indicated by changes in their glass transition temperatures (T_g) .⁸ In an analogy to the aforementioned relationship between salt and hydration levels and the properties of PECs, there are important implications of salt and hydration levels on the plasticization of PECs. To shed light on this, PSS/PDADMAC multilayer complexes were investigated through molecular dynamic simulations and modulated differential scanning calorimetry (MDSC).⁹

A decreased number of contacts between the ionic charges of PECs, hydration level of PSS (measured by the connections between oxygen from PSS and hydrogen from water), and water mobility (as shown through diffusion coefficient calculations) were demonstrated upon increasing salt content. These results revealed a dual effect of small ions: they weaken the binding between the ion pairs, a behavior that favors plasticization, and on the other hand, small ions attract water molecules to their hydration shells, reducing the plasticization by water.

Experimentally, MDSC measurements indicated T_g values at varying hydration levels and salt concentrations (one of the factors was kept constant, while the other was changed and vice versa) and showed that the hydration level plays an important role in anticipating the T_{gr} i.e., in providing the poly ions with increased mobility. However, T_g does not vary significantly for different salt concentrations for a single hydration level, suggesting that, as far as plasticization is concerned, water is more important than salt. Although salt doping could negatively contribute to plasticization, this effect is compensated by hydration.⁵ Put in another way: salt is suppressing the plasticizing influence of water when the hydration level is low, whereas this suppression does not seem to be relevant at high hydration levels.

3.3. Role of pH. Many of the species involved in the electrostatic complexation are sensitive to pH because of the presence of acidic or basic groups. As a result, the formed aggregates present distinct properties at different pH values because charged groups favor complexation, which would be much weaker otherwise. To illustrate how relevant the role of pH is, the complexation between two weak polyelectrolytes, poly(methacrylic acid) (PMAA) and poly(allylamine hydrochloride) (PAH), was investigated.¹⁰ The PEC was prepared at a charge stoichiometry of 1:1, pH 7, and 0.6 M NaCl. Charge imbalance was induced by changing the pH to 3, 4.5, 7, and 9

(anything beyond this range would lead to dissolution). Two hypotheses for the restoration of the change balance were given: (i) PEC composition is changed through the release of polymer chains and (ii) both the morphology and concentration of counterions are modified.

Attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectra of the PECs evidenced excess PMAA at pH 3 and excess PAH at pH 9; i.e., the polyanion is more abundant at acidic pH, whereas the polycation dominates in alkaline conditions. This observation implies deviations from charge equimolarity due to the release of polymer chains, supporting the first hypothesis. On the other hand, should chain release be strong enough to preserve the charge balance, one would expect that the COO⁻/NH₃⁺ ratio within the PECs remains constant regardless of pH. Indeed, the results show a wide variation, suggesting a counterion compensation within the PECs. This observation, in turn, agrees with the second hypothesis. Na⁺ concentration followed the same trend of COO⁻/NH₃⁺ ratio; i.e., it was progressively increased as carboxylate groups were in excess. Such a behavior suggests that Na⁺ ions compensate the negative charges from the PEC. Differential scanning calorimetry (DSC) profiles show that the $T_{\rm g}$ values of the PECs are smaller at extreme pH values; i.e., the polymer chains are more mobile when the functional groups are not completely ionized. Such results are important evidence on how chain mobility is related to cross-linking density promoted by polyelectrolyte ionization.

PECs have been imaged by scanning electron microscopy (SEM), and their pores are found to be remarkably dependent on pH: pore-rich cross sections were obtained at lower pH, while no porosity was observed at higher pH values. Controlling both porosity and pore size is essential in purification membranes and adsorption of transition metal ions at acidic pH—for the latter, the high specific surface area provided by pores at low pH is compatible with both adsorption phenomena, which take place essentially at surfaces or interfaces, and with the fact that many metal ions precipitate at higher pH values. Altogether, pH can be conveniently used to modulate the microscopic and macroscopic properties of PMAA/PAH complexes and PECs in general, which can therefore be made sensitive to environment acidity and to produce materials featuring responsiveness to pH. Pore confinement, in particular, has been correlated with the surface chemistry of functional porous polyelectrolyte-assembled materials.¹¹

3.4. Self-Healing Ability. Self-healing soft materials formed by electrostatic complexation can potentially overcome the limitations of soft matter such as the lack of mechanical robustness. The self-healing property derives from ion pairs that form reversible cross-links in such a way that a deformation is restored to the initial state. An example is the biomass-derived sodium lignosulfonate (L-SO₃Na), which complexes with three polycations, PDADMAC, poly-(ethylenimine) (PEI), and poly(allylamine) (PAA).¹² The L-SO₃Na/PDADMAC complex displays the highest toughness, and after splitting it into two pieces, they self-heal after 24 h at 60% relative humidity (RH), with total recovery of the original mechanical strength after equilibration at 50% RH. This example also highlights an additional feature of electrostatically assembled materials, which according to Ushimaru et al. have prolonged lifetime. Based on the same concept, Merindol et al.¹³ produced nanocomposite LbL films using cellulose nanofibrils (CNF) and cationic poly(vinyl amine) that display





Figure 2. Insoluble biocatalytic film of complex coacervate core micelles (C3M) prepared by using a coating and photo-cross-linking. Adapted with permission from Sureka, H. V.; Obermeyer, A. C.; Flores, R. J.; Olsen, B. D. *ACS Appl. Mater. Interfaces* **2019**, *11* (35), 32354–32365.¹⁶ Copyright (2019) American Chemical Society. Further permissions related to the material excerpted should be directed to the American Chemical Society.

reversible mechanical properties and self-healing ability. The self-healing property will be discussed later in light of adhesion phenomena.

4. STATE-OF-THE-ART IN ELECTROSTATICALLY COMPLEXED MATERIALS

4.1. Complexation Involving Biomolecules. The associative separation of polyelectrolytes into coacervates enables a compartmentalization tool, which in turn leads to increased local concentration of various biomolecules due to the high partitioning level. As an example, biomolecules (e.g., enzymes and substrates) are compartmentalized, and this confinement favors enzymatic reactions for increased local concentration.⁴ Besides the ability to sequestrate RNA, membraneless compartments support polymerization, enhancing ribozyme reactions. However, RNA is negatively charged, and its interactions with polycations may lead to decreased activity. Raghav et al.¹⁴ hypothesized that, as other polyanions also complex with polycations, there ought to be a competition between polyanions and RNA for polycation sites, which could eventually enhance ribozyme catalysis. To support this hypothesis, Raghav et al. studied different polyanions and ribozymes and demonstrated the universal characteristic of polyanions to increase ribozyme reaction in complex coacervates. They also optimized ribozyme catalysis by choosing the specific size, amount, and identity of polyanions in complex coacervates, culminating in a reaction rate that was increased by 12-fold. This systematic investigation enabled establishing a set of compositions that influenced reactions involving ribozymes and served as a starting point for the use of other polyanions present in organisms.

Proteins and other biomolecules can also be encapsulated within complex coacervates. This is an area of increasing interest due to the potential applications, such as biocatalysis, compartmentalization, drug delivery, and biosensing. Electrostatic complexation between polymers and proteins, for instance, has been successfully used to encapsulate biomolecules with the advantage of not involving covalent modification. Furthermore, encapsulation is likely to increase protein stability, preserving its biofunctionality at varying conditions. However, as proteins are weak polyelectrolytes, the encapsulation process requires a minimum quantity of net charge to favor complexation.

McTiguea et al.¹⁵ reported a ternary system comprising oppositely charged polyelectrolytes (namely, poly(L-lysine) and poly(D,L-glutamate)) and one of the following proteins: bovine serum albumin, human hemoglobin, or hen egg white lysozyme. The combinations between these species allowed testing a wide range of molecular weights and electrostatic charges in addition to ionic strength and pH. Based on the obtained results, relevant general guidelines for encapsulating proteins into PECs were proposed, as summarized below.

Both salt and pH influence the complexation and therefore are important parameters to modulate encapsulation. It has been demonstrated that the charge density of an interacting protein and a polymer, which can be modulated by the solution pH, plays an important role in protein uptake, although complex coacervates are able to encapsulate even weakly charged proteins.¹⁵ In addition to the net charge, the charge distribution over the protein surface has a huge influence on its complexation and encapsulation. McTiguea et al.¹⁵ verified that proteins with patchy charge distribution present higher levels of encapsulation in comparison to those featuring homogeneously distributed charges.

The encapsulation of biomolecules is also feasible with the use of ionic-neutral diblock copolymers. In this case, the formation of complex coacervate core micelles (C3M) is involved, associated with high protein uptake and increased thermal and chemical stability.¹⁶ Thus, enzyme immobilization has a great utility to enhance protein lifetime. The encapsulation strategy can also be applied for catalysis. The activity of biomolecules is associated with several factors, including shape and structure. Lindhoud et al.¹⁷ reported a higher lipase activity, involved in reactions of water-insoluble substrates, when incorporated into C3M compared to that of the free molecule.

The active sites of most lipases are hidden by a lid that can be opened by several factors. Generally, in a hydrophilic system, this lid is maintained closed, and lipase is inactive. However, the lid is opened in the presence of an interface between hydrophobic and hydrophilic phases or compounds. Following this, Lindhoud et al.¹⁷ hypothesized that micelles induce lid opening and enzyme activation. Therefore, it was postulated that the activity of complexed lipase may increase because of the substrate accumulation. Additionally, it was demonstrated that both ionic strength and PEC composition also influence the enzymatic activity. As the ionic strength is increased, enzymes are released, and their activities are lessened.

Surface immobilization is a typical approach to produce biocatalysts and biosensors. Sureka et al.¹⁶ developed an insoluble biocatalytic film through a two-step protocol: coating to form a charged copolymer—protein complex and photo-induced chemical cross-linking, producing an insoluble film (Figure 2). Alkaline phosphatase was used as a model enzyme/ protein for incorporation into the C3M structure. To enhance the aggregation, the biomolecule was genetically modified in order to produce a protein with higher anionic character. The authors found that the complex was sensitive to Zn^{2+} at very low concentrations (50 ppm), even in the presence of other cations, making the material suitable for application as a Zn^{2+}

sensor. The sensor presented low leaching and sensing response for almost one month. The generality of this C3M immobilization method, which has been demonstrated for proteins, can be extended to a wide range of charged biomolecules, reducing the need for any chemical or genetic modifications.

LbL assembly represents an alternative method to immobilize biostructures and increase their uptake and mobility by using a three-dimensional matrix rather than a two-dimensional surface. LbL assemblies are hierarchical structures formed by functional polyelectrolyte multilayers that allow the incorporation and delivery of different guest molecules, such as drugs. LbL provides great versatility as a biocompatible platform that can be applied, for example, to protect and guide cargo substances to a specific target. This platform can also be constructed around a charged polymeric core substrate, forming nanoparticles (LbL-NPs). This colloidal structure has been recently used as a simple, precise means for loading and delivering siRNA to hematologic cancer cells, both in vitro and in vivo.¹⁸ The delivered siRNA stops the expression of some specific genes of B-cell lymphoma (BCL-2), a pro-survival protein, by degrading them. However, to reach an efficient delivery of siRNA, the LbL-NP is required to protect nucleic acids from bloodstream enzymes. This platform also allows the selective delivery and access of siRNA to a specific cell to stop the evolution of hematologic cancer. Polyelectrolyte nanolayers were sequentially deposited onto the negatively charged and polymeric core. BCL-2 siRNA molecules were placed as a negative polyelectrolyte layer on the top of a positively charged amino acid polymer. The outermost layer was formed by hyaluronic acid, acting as a CD44 ligand covalently bonded to CD20 antibodies. CD20/ CD44 dual targeting provided a specific binding to blood cancer cells, and it successfully facilitated the uptake and delivery of BCL-2 siRNA molecules. Overall, LbL-NP has been demonstrated as an important and general tool for targeted siRNA technology as a way to treat hematologic cancer. It is important to emphasize that these results can also be applied as a support for targeting other diseases, establishing LbL as a versatile strategy for transporting therapeutics into different hematopoietic cells.

4.2. Materials with Salt-Controlled Rheology (CoPEC). In an analogy to thermoplastics, which are traditionally processed in the molten state upon heating, compacted PECs, named by Schlenoff and co-workers as "saloplastics", stand out as interesting materials whose rheological properties are modulated by salt loading. As discussed above, saloplastics can be plasticized by salt addition, similar to the response of thermoplastics to temperature. PECs display saloplasticity due to their ion-pairing cross-links that could be broken by salt and combined with an increased water content,¹⁹ making them suitable for many applications.

An important breakthrough in PEC processing was brought about by extrusion (Figure 3),²⁰ the most common technique to process thermoplastics. Extruding PECs enables reconformation because, unlike chemical cross-links that typically undergo nonreversible breakdown, disrupted electrostatic cross-links can be restored. Shamoun at al.²⁰ reported that, although temperature is not essential to extrude PECs, it is possible to optimize it with salt and hydration levels to obtain high-quality materials with improved cost-effectiveness.

In order to be formed homogeneously, PECs are required to be stoichiometric and to be devoid of porosity. Fortunately,



Figure 3. Schematic illustration of the extrusion of a polyelectrolyte complex (PEC) into different geometries (e.g., flat or tubular film, solid or hollow filament)—depending on dye shape—and properties—as affected by pH, hydration, and doping levels.

extrusion is an efficient means of producing highly compacted, pore-free complexes, allowing shaping of PECs into different morphologies, such as tapes, rods, and tubes. Once extruded, PECs are still responsive to ionic strength by showing increased hydration levels, length contraction, and decreased equilibrium modulus as salt content increases. An interesting point is that multiple extrusions do not lead to losses in equilibrium moduli, enabling PECs to be recycled with virtually no degradation. We envisage that PEC extrusion is a promising approach for the development of new materials, including nanoparticle-, nanofiber-, and carbon-filled composites. Additionally, PECs are manufactured and processed in aqueous environments, making them suitable for biological applications. A series of studies on extruded saloplastics have been carried out recently to develop several properties and applications as well as to shed light on fundamental questions.⁵

4.3. Underwater Adhesives. Contrary to the trade-off between adhesion and hydration, numerous situations can be listed where adhesiveness is observed in moist environments, including those involving living organisms such as marine mussels (*Mytilus edulis*) and sandcastle worms (*Phragmatopoma californica*). The secretion from the latter binds mineral particles to form a protective shell, while the feet of the former secrete a glue to attach to surfaces. In the case of mussels, movable organisms, it is particularly interesting that the cohesive forces are suppressed at a certain point. Led by electrostatic complexation, a range of noncovalent interactions give rise to strong, yet reversible, adhesion to surfaces in order to allow motility.

The adherence of these organisms is a successful example of interfacial chemistry. Stewart et al.²¹ suggested that the major mechanism enabling adhesiveness to low-energy (due to adsorbed water, ions, ionic complexes, polymers, and biofilms) underwater native surfaces is the displacement of adsorbed species by the side chains of adhesive proteins through an ion/ligand exchange process. Indeed, removal of weak boundary layers is required for a good adhesion, which then involves adhesive proteins comprising mostly histidine, phosphoserine, and L-3,4-dihydroxyphenylalanine (DOPA) residues. Interestingly, these proteins play surface-adsorbing roles mainly through electrostatic complexation with the negatively charged submarine surfaces, whose anionic character is brought about by biogenic polymers that serve as organic preprimers, such as humic and fulvic acids.²¹ In this phenomenon, instead of



Figure 4. Setups for interfacial complexation into filaments following a scheme (a) and actual photo (b) with a suspension of TEMPO-oxidized cellulose nanofibrils (TO-CNFs), in blue, going through complexation with polycations in solution (chitosan or poly(diallyldimethylammonium)), in pink. The complex is spun into filaments by moving the stage in a direction opposite to the drawing tweezers. Interaction between the protonated primary amino groups of chitosan and the deprotonated carboxyl groups of TO-CNF is shown in (c). The complexation between cationic cellulose nanocrystals (GT-CNCs), in blue, and negatively charged carboxymethyl cellulose (CMC), TO-CNF, and dicarboxylated cellulose nanocrystals (DC-CNC) upon contact of droplets of suspensions or solutions of oppositely charged species, followed by drawing with a pair of tweezers (d). (a) and (b) Reprinted with permission from Toivonen, M. S.; Kurki-Suonio, S.; Wagermaier, W.; Hynninen, V.; Hietala, S.; Ikkala, O. *Biomacromolecules* **2017**, *18* (4), 1293–1301.²⁴ Copyright (2017) American Chemical Society. (c) Reprinted with permission from Grande, R.; Trovatti, E.; Carvalho, A. J. F.; Gandini, A. *J. Mater. Chem. A* **2017**, *5* (25), 13098–13103.²³ Copyright (2017) Royal Society of Chemistry. (d) Reprinted in part with permission from Zhang, K.; Liimatainen, H. *Small* **2018**, *14* (38), 1801937.²⁵ Copyright (2018) John Wiley and Sons. Further permissions related to the material excerpted should be directed to the copyright owners.

jeopardizing cohesive forces, water becomes actually essential for allowing species to attain electrical charges and to complex.

Inspired by nature, several biomimetic efforts have been made toward the formulation of underwater adhesives based on different supramolecular interactions, as reviewed elsewhere.²² These include electrostatic complexation-based adhesives, which have been achieved by the following strategies: (i) adhesion of mfp-3 protein isolated from mussels onto hydroxyapatite in mica; (ii) complex coacervation between hyaluronic acid and the mfp-5 protein isolated from recombinant Escherichia coli or the mimicking proteins mfp-151 and mfp-131 (recombinant and synthetic mfp proteins were converted into DOPA by added tyrosinase, an enzyme that is naturally present in mussels); and (iii) PECs formed by a range of synthetic polycations and polyanions and even polyampholytes and toughened by ions (mostly divalent metals). The natural occurrence of several polyelectrolytes (e.g., the polyanion hyaluronic acid) in the human body offers an opportunity. Related biomimetic strategies have led to advanced medical materials serving as adhesives for sealing

urinary fistula and uterine fetoscopic defects as well as bone graft binding.

4.4. Interfacial Complexation in Filaments. Filaments have been produced via the interfacial polyelectrolyte complexation (IPC) using numerous combinations of polyelectrolytes. At this point, it is clear that most of the literature on electrostatically complexed materials relies on the association of polyelectrolytes. Particles, in the context of complexation, are different from true polyelectrolytes since they do not bear the quasi-infinite aspect (i.e., length-to-width) ratio, for example, of a fully extended polymer chain. In the latter case, a high degree of freedom and mobility coexist. Although challenging, complexes of polyelectrolytes and colloidal particles or between particles have been demonstrated.

Grande et al.²³ and Toivonen et al.²⁴ produced filaments through the interfacial complexation between cellulose nanofibrils (CNFs) subjected to 2,2,6,6-tetramethylpiperidine-1oxyl (TEMPO)-mediated oxidation—to impart carboxylic functionality and therefore anionic character—and a polycation, either chitosan (both studies) or PDADMAC (second study). The spinning setups are illustrated in Figure 4. This approach is particularly relevant because the produced filaments could benefit from the structural role of the multilevel hierarchical assembly of the cellulose fibrils. Indeed, the obtained mechanical properties were quite competitive, with an ultimate tensile strength of 200–220 MPa and Young's modulus of 15–22 GPa. These attributes were further increased upon predrying stretching.²⁴

More recently, a similar approach was used to produce polymer/particle and particle-only IPC filaments.²⁵ Cationized cellulose nanocrystals (CNCs), obtained via Girard's reagent T chemistry, were complexed with different anionic celluloses, namely: carboxymethyl cellulose (soluble anionic cellulose derivative), TEMPO-oxidized CNF (anionic cellulose particle of high aspect ratio and intermediate crystallinity), and 2,3dicarboxylated CNC (anionic cellulose particle of intermediate aspect ratio and high crystallinity).²⁵ Droplets of suspensions or solutions of oppositely charged systems were put in contact, and the interface was continuously drawn into a filament. The produced filaments presented a cross-sectional diameter of 20-45 μ m. The highest and lowest ultimate tensile strengths and Young's moduli were, respectively, (+)CNC/(-)CNF (ca. 153 MPa and 8 GPa) and (+)CNC/CMC (ca. 115 MPa and 5 GPa), with (+)CNC/(-)CNC showing intermediate performance. As expected, filament extensibility followed the descending order of cationic CNC combined with anionic CMC > CNF > CNC. Finally, the role of particle aspect ratio was demonstrated in filaments formed by particle-polyelectrolyte complexation using chitin nanofibers (+) and alginate (-)²⁶ These investigations shed light on the electrostatic complexation of oppositely charged colloidal systems and pave the route for assemblies into materials other than filaments.

5. FINAL REMARKS AND OUTLOOK

We discussed complexation as a means to build systems by exploiting electrostatic interactions. They apply to polyelectrolytes that self-assemble into larger structures but can also consider colloids, including renewable particles and fibrils. A question that remains for further consideration is the role of counterions and water structuring. Such topics, at least to a large extent, remain largely unexplored. However, they are quite important if one thinks about the nature of materials relevant to the future bioeconomy and green chemistries. They include plant-based polymers and their supramolecular constructs, which extend to those derived from other renewable resources. Among these, cellulose, chitin, heteropolysaccharides, and lignins are quite important. As far as the design of advanced materials, another consideration is the process itself: how to scale up and make any process continuous, for example, by exploiting electrostatic complexation? LbL assembly, coacervate formation, and dry spinning are just a few examples that relate to such efforts, which have found little impact in scale-up manufacturing. Any attempt of practical significance needs to factor in aspects related to the time scale, e.g., the diffusion, mass transport, and kinetics. They are all relevant in manufacturing and better classified within the framework of nanomanufacturing. Finally, charge matching and molecular architecture are quite relevant too. Both aspects apply to proteins and other biomolecules, which offer an excellent opportunity to fine-tune stimuli-responsive systems.

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Notes

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Marcos V. A. Queirós achieved his BSc (2016) and MSc (2018) degrees in Chemistry at the Federal University of Ceará, Brazil. In 2018, he joined the PhD program of the Institute of Chemistry at the University of Campinas/UNICAMP, Brazil, under the supervision of Prof. Watson Loh. Nowadays, his PhD deals with the phase behavior and structure of electrostatic complexes involving polyelectrolytes and surfactants.



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Prof. Watson Loh obtained his BSc in Chemistry from the Federal University of Rio Grande do Sul, Brazil (1986), and his PhD in Chemistry from the University of Campinas/UNICAMP, Brazil (1992). He is Professor at the Institute of Chemistry of the University of Campinas, and since 1994 he has led a research group devoted to studying the structural and energetic aspects of polymer and surfactant colloids (including nanocellulose, metallic nanoparticles, and petroleum colloids) using techniques such as light and X-ray scattering, scanning, and titration calorimetry among others.

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