Journal of Applied Polymer Science

REVIEW

Theoretical and Practical Considerations in Electrostatic Depositioning of Charged Polymers

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ABSTRACT: An interfacial engineering technology, based on the electrostatic deposition of charged polyelectrolytes onto surfaces of oppositely charged templates is reviewed with an emphasis on practical applications in the food, pharmaceutical and personal care industries. On interfaces of disperse systems consecutively deposited polymers provide major advantages in terms of physical and chemical stability of dispersions against superimposed stresses (pH, temperature, ionic strength, freezing, chilling, dehydration, lipid oxidation). The controlled deposition of multiple layers allows for a controlled and triggered release of incorporated functional components. This review highlights the basic principles of the layer-by-layer (LbL) electrostatic deposition method as well as some major advantages and drawbacks of this approach. An overview of several systems that can be used as templates for the deposition including emulsion droplets, liposomal vehicles, colloidal aggregates, and planar surfaces is given. Suitable substrates for the deposition are presented with a focus on charged biopolymers such as proteins or polysaccharides since they play an essential role in the formulation and stabilization of food, pharmaceutical and personal care applications. Issues and difficulties associated with implementing the technology on a larger, industrial scale are discussed. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40099.

KEYWORDS: biopolymers and renewable polymers; colloids; lipids; adsorption; coatings

Received 16 September 2013; accepted 21 October 2013

DOI: 10.1002/app.40099

INTRODUCTION

The design of novel structures having specific macroscopic properties such as texture, appearance, aroma, flavor, chemical and microbiological stability, and pharmacological of physiological effectiveness continues to be a major challenge for modern food, pharmaceutical and personal care technologists. Depending on the structure created, a unique set of properties arises that may or may not fully fulfill the requirements set by the intended user.[1] Because of that numerous studies have focused on the development of a wide range of fabrication methods including both top-down and bottom-up approaches. These fabrication methods can be used to build a "structure library" allowing for the selection of a specific structure that is best suited for the task at hand. The top-to-bottom approaches involves a decomposition of existing structures to create new ones, while the bottom-to-top approach involves the synthesis of existing structures to create new ones. The latter in particular has gained an enormous interest amongst manufacturers and technologists because it allows for structures to be created on various length scales including the nano-, colloidal-, and microscopic level.[1,2] There, interactions between molecules are controlled in such a way that specific structures self-assemble. This self-assembly is driven by thermodynamics i.e. the systems gains free energy when it assembles in this specific way. If the environmental conditions governing this thermodynamic behavior were appropriately chosen, the process will occur spontaneously. Structural design approaches based on self-assembly are therefore very cost effective, but require fundamental knowledge of (i) the interaction between molecules and (ii) the interaction between the structural entities subsequently created. This complex interplay of interactions then leads to the formation of hierarchical structure levels with specific macroscopic performances.[2,3]

The formation of ultrathin films based on the electrostatic layer-by-layer (LbL) deposition of charged polyelectrolytes onto oppositely charged template surfaces is one such self-assembly technique. In this technique, the way that the charged molecules are added to the template makes a difference. For example, if molecule A was added to a solution containing a template structure B, a different structure will arise than if molecule A was in solution and template structure B was added. The technique has therefore also been referred to as a "directional" self-assembly, that is the user controls the assembly process by controlling the interactions throughout the process e.g. by controlling the order of addition of components and the rate of change of environmental conditions. Because of the great variety

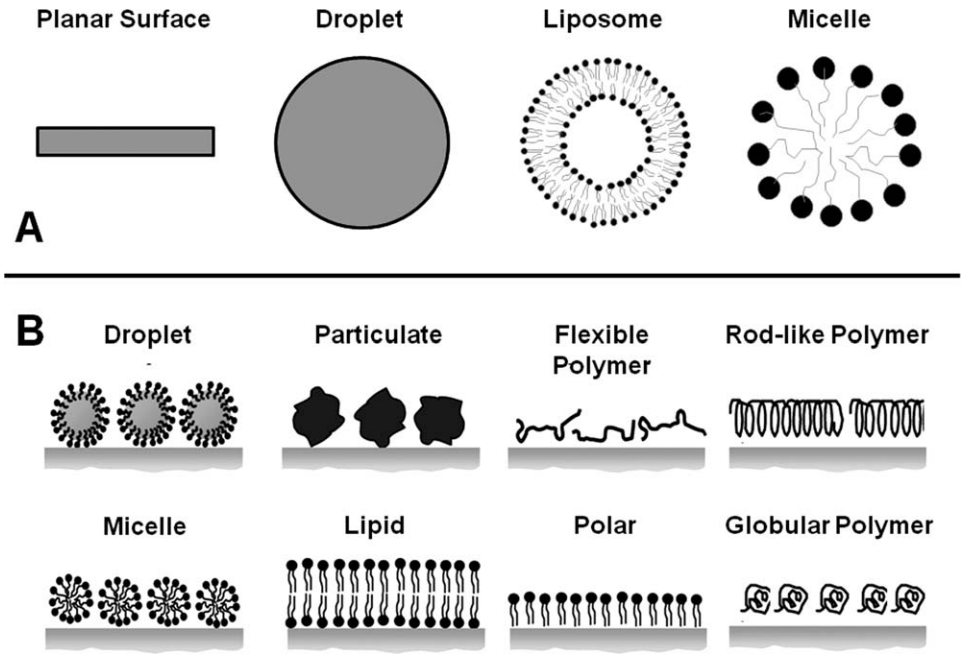

Figure 1. Possible template (A) and substrate (B) structures that could be used to assemble multilayered coatings based on layer-by-layer electrostatic deposition.[27]

of structures that can be created this way, the technique has attracted broad interest and is increasingly used to manufacture a wide variety of products.[2,4,5]

As mentioned above, the LbL technique involves most commonly the use of substrate molecules (mostly polymers) that are directed to accumulate at the surface of a template structure. The technique can be used with many different template structures including oil droplets, liposomes, micelles, or to simply coat planar surfaces (Figure 1). The latter constitutes the first reported use of the technique, and was done by Iler in 1966.[4,6–14] Aside from using just molecules as substrates, charged colloidal entities can also be deposited, a fact that has been demonstrated with the deposition of surfactant micelles, protein aggregates, and silica particles on glass surfaces.[5] In theory, the process can be repeated many times over so as to create multilayered films. There, substrates of opposite charges are sequentially added to a charged base template at appropriate concentrations resulting in the formation of thick films.[5,7,15] The LbL techniques is thus a powerful tool that can be used to carefully control the composition, charge, thickness, and permeability of films. This allows one to for example modulate the functional properties of a particular delivery system such as release kinetics, dissolution rate or integrity during oral processing.[2,5,16]

This article highlights some key insights that we have gained over the past years when using the LbL approach to create applications that may be of commercial interest. We mainly focus in this review on the use of naturally derived polymers as substrates rather than synthetic ones since those are the most commonly used constituents to generate delivery systems such as suspensions, biopolymer particles, simple and multiple emulsions, gels, liposomal vesicles, solid matrices, and association colloids for the food, personal care and pharmaceutical industries (Figure 1).[3,16–22] While charged synthetic polymers have often superior performances, they might also have high toxicity levels and are therefore not approved for use; especially in the food industry.

THEORETICAL ASPECTS OF ELECTROSTATIC DEPOSITION

General Considerations

In the layer-by-layer electrostatic deposition technique a polyelectrolyte layer is formed on a charged surface by adding one or more oppositely charged polymers. The charged polymers translocate from the solution to the surface since they experience strong electrostatic attraction which arise due to Coulomb forces (Figure 2).[23,24] The adsorption of a high amount of charged polymers on the template surface causes a reversal of the net charges or the template surface either from positive to negative or vice versa.[25] This process can be repeated in order to create so called "secondary" (double layered); "tertiary" (triple layered) derivatives of the base template structure. Templates derivatives with up to 6 layers have been reported, albeit the fabrication of these structures became increasingly difficult and the structure of the multilayer apparently less defined (see below).[4,14] The electrical net charge of multilayer-coated templates is determined by the outer layer.[25,26] It is known that about one third of the charges of the terminating layer are neutralized by the underlying polyelectrolyte layer, whereas the remaining charges are compensated by counterions which can be released upon adsorption of the next layer.[26] Initially, it is the charge of both the template and the biopolymer that governs the electrostatic deposition process. After the first deposition though, it is the charge of the biopolymers alone that affect the electrostatic interactions responsible for the attractive forces that lead to their deposition.[21]

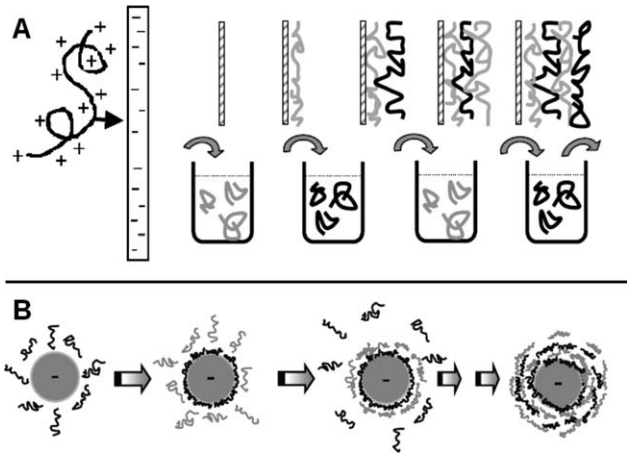

Figure 2. Schematic representation of the layer-by-layer (LbL) electrostatic deposition method using a charged template structure to adsorb oppositely charged polyelectrolytes: (A) planar surface, (B) colloidal particle.[28]

Charge reversal, a prerequisite for the deposition of multiple layers, occurs after adsorption of charged polyelectrolytes onto the surface when the total number of charges is greater than those required to neutralize the oppositely charged surface.[5,26] Charge reversal is also the reason why polyelectrolytes tend to form only monolayers since upon saturation, excess polyelectrolytes in solution will experience electrostatic repulsion preventing their adsorption (Figure 2).

Measurements of the adsorption kinetics of biopolymers onto template surfaces suggest a three-step process in which: (i) polyelectrolytes initially rapidly diffuses close to the template surface, so that adsorption via electrostatic attraction can take place; (ii) adsorbed polyelectrolytes slowly rearrange to optimize their interaction; (iii) positively and negatively charged segments intermingle in such a way that an irreversible complexation of charges occurs.[26]

Templates for Deposition

The following section gives a brief overview of the different template structures that may be used as targets of the layer-by-layer electrostatic deposition technique. All systems mentioned can be manufactured and are stabilized by the use of charged surfactants and/or polymer molecules approved for use in the food, pharmaceutical, cosmetic, and chemical industry (Figure 1).

Planar Surfaces

The formation of films or coatings on planar surfaces has been reported to build moisture, lipid, and gas barriers to protect a variety of foods.[27] In addition, such coatings have been shown to improve the textural properties of foods or serve as carriers of functional agents such as colours, flavors, antioxidants, nutrients, and antimicrobials.[27] Planar surfaces are also used as template materials to construct biological or chemical sensors (e.g., electronic nose), and to custom tailor membranes used in separation technologies.[26] For laboratory studies hydrophilic surfaces such as glass, silica or mica have mostly been used. Their use involves thorough cleaning prior to the deposition.[28] Moreover, Weiss et al. (2006) already proposed that multilaminates can give food scientists some advantages for the preparation of edible coatings and films over conventional technologies which may have a number of important applications within the food industry.[27] A variety of edible coatings and films already protect foods, including fruits, vegetables, meats, chocolate, candies, bakery products, and French fries.[29–32]

Emulsions

Emulsions are dispersions composed of two partially or completely immiscible liquids, with one liquid being dispersed in the other in the form of small droplets. A variety of ionic or ionizable emulsifiers including proteins and surfactants can be used to from oil-in-water emulsions. The charge of these droplets depends on the concentration and type of emulsifier as well as environmental conditions such as pH and ionic strength.[3,5] Typically, the emulsifier is dissolved in the aqueous phase and then blended with the oil phase using a high shear mixer. This premix is then further processed with a homogenizer (microfluidizer, high pressure homogenizer, membrane homogenizer, ultrasonicator, etc.) to further decrease the mean droplet size.[3,33] During the homogenization process, the emulsifier rapidly adsorbs onto the freshly formed droplet interfaces, thereby lowering the interfacial tension between the oil and the water phase. This facilitates further droplet break up and disruption. The emulsifier molecules form a protective layer preventing the droplets from coalescing and aggregation when coming into close proximity. It should be noted that the mechanism of adsorption onto the oil-water interface is however not of electrostatic origin—in contrast to potential subsequent LBL depositions of charged polymers. Rather the amphiphilic properties of surfactants drive their translocalization to the interface. Hydrophobic moieties of surfactants are able to interact with the oil phase while hydrophilic moieties can continue to interact with the polar solvent water. This process is thus enthalpy driven and leads to an overall reduction of the free energy.

The enthalpy-driven adsorption of ionic or ionizable emulsifiers causes the interfacial membrane of the droplets to be electrostatically charged—a fact that is then used for the preparation of multilamellar coatings surrounding the oil droplets (Figure 3). Emulsions containing charged droplets stabilized by an ionic surfactant are known as primary emulsions. In a second step, the primary emulsion can be coated by mixing it with an oppositely charged polyelectrolyte to from a double-layered emulsion (secondary emulsion). The process may be repeated to create

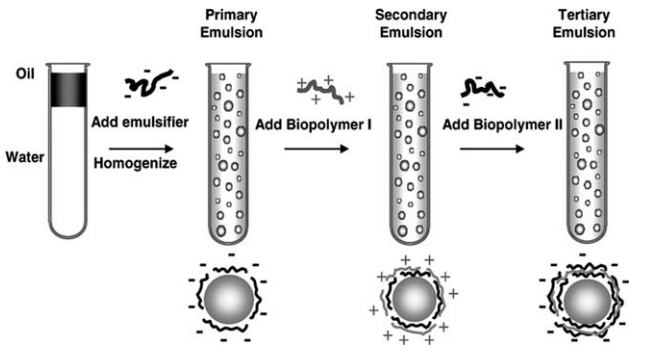

Figure 3. Layer-by-layer technique to produce multilayered oil-in-water emulsions.[10]

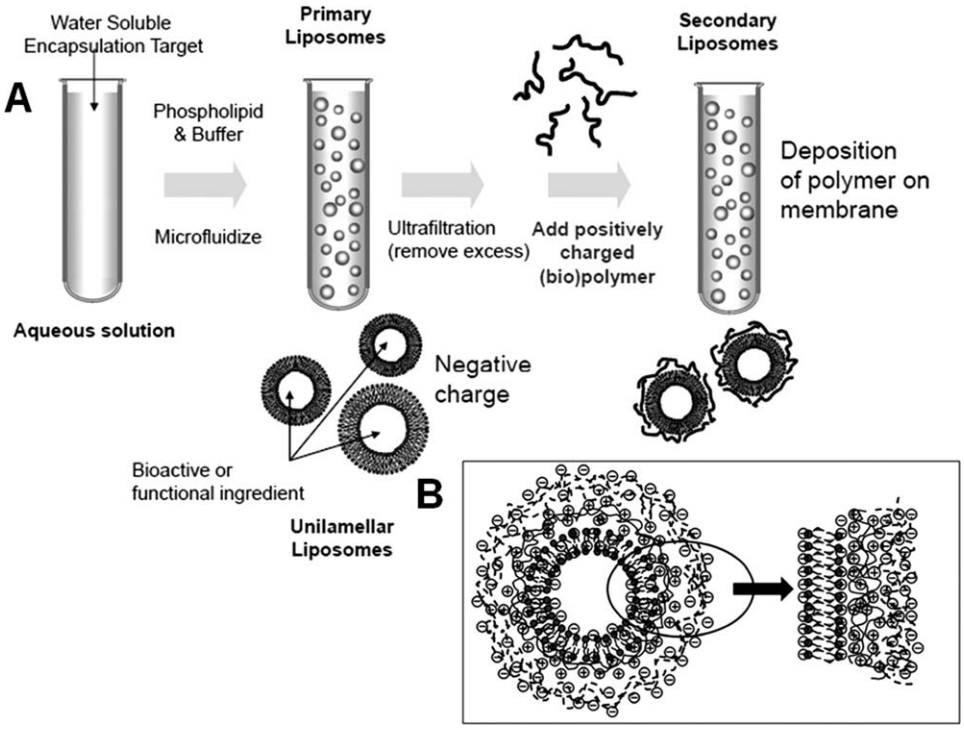

Figure 4. Structure of single liposome sequentially coated with various electrostatically charged polymers (A); Insert (B) gives a detailed structure of the coated membrane complex.[14]

"multilayered" emulsions having similar bulk physicochemical properties such as viscosity and appearance as conventional emulsions with similar particle characteristics (e.g., concentration, size distribution, and net charge) but being significantly more stable to superimposed stresses such as freezing, drying, and heating.[5,34]

Oil Bodies

Recently, oil bodies extracted from soybeans have gained attention due to potential uses in foods, cosmetics, and pharmaceuticals.[35,36] These lipid storage organelles are naturally found in the seeds of many plants.[37–39] Oil bodies consist of a lipid core which is surrounded by a phospholipid-olesin membrane.[40,41] The olesin membrane forms a natural, electrically charged barrier that is able to protect the oil bodies against environmental stresses (moisture, temperature, oxidative reagents). Oil bodies may be used as a template structure to deposit additional charged polymers due to their electrical charge characteristics. Such systems could be used as pre-emulsified oils in food products such as dressings, sauces, dips, beverages, and desserts to improve stability during food processing, storage, transport, and utilization.[42,43]

Liposomes

Liposomes or lipid vesicles that are composed of a bilamellar membrane that surrounds an aqueous core. While such structure self-assemble when polar lipids or mixtures of polar lipids with cholesterol or ergosterol are dispersed in an aqueous solvent, they are often further homogenized to adjust their size.[22,44] The specific molecular structure of polar lipids with two hydrophobic fatty acid tails and one charged hydrophilic head groups causes them to assemble in this specific bilayer membrane structure in polar solvents such as water.[45] The shape of liposomes is typically spherical consisting of single (unilamellar) or multiple layers (multilamellar) of amphiphilic polymer membranes.[22] The size of liposomes can be as little as 30 nm to as large as 50 μm depending on the phospholipid characteristics (e.g., type, concentration), manufacture conditions (e.g., mixing, homogenization) and extrinsic parameters (e.g., pH, ionic strength, temperature). One of the most frequently used polar lipids is phosphatidylcholine (PC), a phospholipid whose head group choline consist of a polar phosphatidyl residue ester linked to the glycerol backbone.[46] Most industrial applications of liposomes however do not use a single, pure phospholipid but rather employ crude phospholipid fractions, also known as lecithins. Lecithins are complex mixtures of a variety of phospholipids and the charge of liposomes manufactured from them hence depends on the exact composition of the extract, and various studies have recently shown that liposomes made from lecithin may serve as suitable template structures for the deposition of polyelectrolytes (Figure 4).[22]

Micelles and Microemulsions

Amphiphilic molecules composed of a hydrophilic head and a hydrophobic tail spontaneously self-assemble under appropriate environmental conditions (e.g., temperature, ionic strength, and pH) to form association colloids such as micelles, bilayers, and reversed micelles.[45] The molecular properties of the surfactant (e.g., geometry of head and tail group, polarity, charge), the properties of the solvent (e.g., pH, ionic strength, dielectric constant), the presence of any co-surfactants, as well as the overall (co-) surfactant concentration, and the temperature determine the structure of the generated association colloids.[2] The formation of association colloids is driven by the hydrophobic effect which causes the system to minimize all unfavorable contact

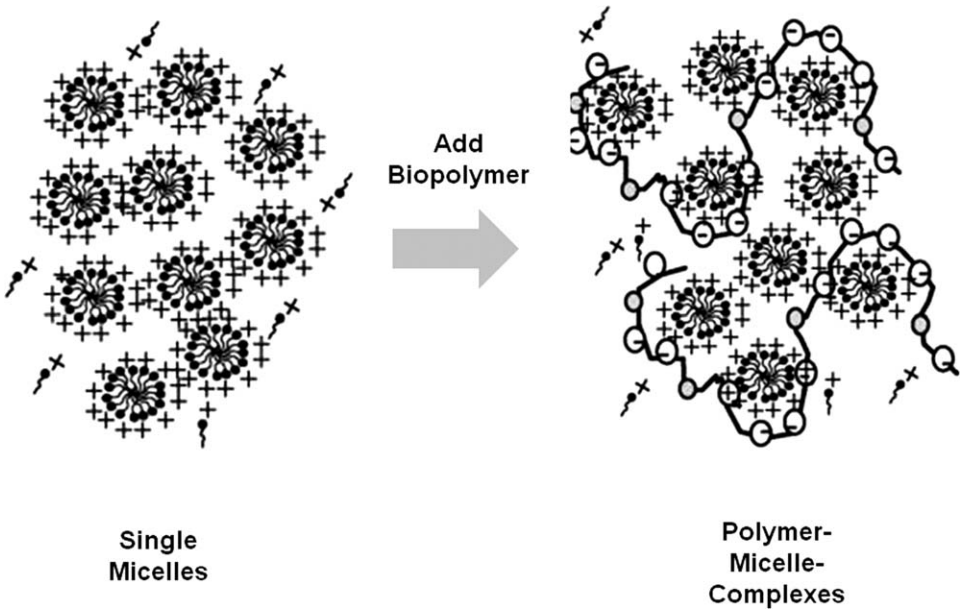

Figure 5. Schematic structure of polyelectrolyte-micelle-complexes.[49]

areas between the nonpolar tails of surfactant molecules and the aqueous solvent.[2] Surfactant molecules exist as monomers in solution and tend to spontaneously associate into thermodynamically stable aggregates if the concentration exceeds a critical value known as the critical micelle concentration (CMC) (Figure 5).[27] Association colloids may be used to incorporate and deliver both polar and nonpolar functional components.[47,48] Lipophilic ingredients may be solubilized into the hydrophobic core of the micelle or become part of the micellar membrane, also referred to as the palisade layer. Such lipophilic molecules carrying association colloids are also known as "swollen micelles" or "microemulsions".[2,45] Addition of a charged polymer to ionic micelles has shown to lead to the formation of polymer-micelle complexes.[49] To date, the precise structure of these complexes is not fully known. Depending on the size and charge characteristics of the polymer and the association colloid, so called bead-on-string structures may be generated. Studies have shown that ionic micelles have an altered capability of solubilizing lipophilic molecules if they are combined with charged polymers. In many cases, the solubilization kinetics for example is accelerated, which may be advantageous in industrial applications. The creation of polymer-micelle complexes is currently under active investigation and is clearly a promising approach that can lead to a modification of the functional properties of micelles.

Polyelectrolytes for Deposition

In the LbL approach, polyelectrolytes are predominately used to create thin films or coatings around or on the surface of a deposition template. More broadly speaking though, any charged entity may be used to create a coat around a template or on the surface of a template, as long as the charge characteristics and the dimensional ratios of the substrate and the template are appropriate and both systems are kinetically stable (Figure 1). Substrates that have been successfully used include for example charged lipids (surfactants, lecithins), biopolymers (proteins, polysaccharides), nucleic acids, enzymes, and organic or inorganic colloidal particles (micelles, droplets, vesicles, biopolymer complexes).[5,50–58] The most commonly used substrates for food applications are proteins (casein, whey protein, soy, and gelatin) or charged polysaccharides (pectin, gum arabic, modified starch, chitosan). One should note that polysaccharides may vary greatly in their molecular, physical and chemical characteristics and may thus be more or less suitable to construct multilamellar films. This is in contrast to synthetic polymers which have in general a more well-defined structure and molecular weight. This holds true unless expensive size and structure separation techniques are employed to fractionate the polysaccharides. In the case of proteins, one often employs crude extracts in commercial applications. One such crude extract is for example whey proteins, which contain β-lactoglobulin, α-lactalbumin ($\sim$25%), bovine serum albumin, and immunoglobulins.[59]

For each application an appropriate polymer type, and polymer/template concentration ratio must therefore be selected in order to ensure that the surface of the template can be fully saturated. The concentration required also depends on the size of substrate and the template. For example if the deposition is done with charged polysaccharides as substrates, the concentration required to saturate a template of a given size and concentration decreases the higher the molecular weight of the polysaccharide. A key parameter to determine is therefore the saturation concentration (c_{sat}). The saturation concentration is the concentration required to fully cover all surfaces of the templates. At concentrations above and below the saturation concentration, heavy aggregation and a complete breakdown of template structures may occur (Figure 6). The major goal is therefore to add just the right amount of substrate. One should also note that if too little or too much substrate is present, it does also create a problem when a second coat is to be deposited. Then, soluble or insoluble substrate and template complexes may be generated that interfere with the subsequent film formation.[26] We will come back to this point when considering limitations and pitfalls of the technology.

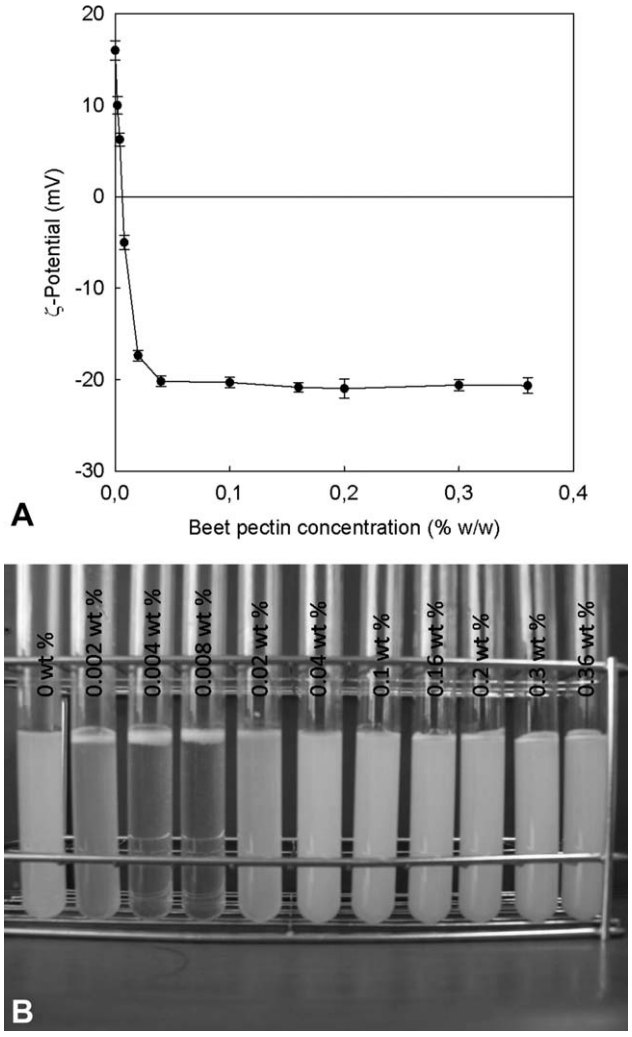

Figure 6. ζ-potential (A) and creaming behavior (B) of fish gelatin-stabilized oil-in-water emulsions as a function of sugar beet pectin to technically determine the saturation concentration.[7]

Analytical methods to determine the saturation concentration typically involve an initial separation of the coated templates from solution by either centrifugation of filtration and a measurement of the excess concentration of substrate in the surrounding aqueous phase.[5] Another option is to measure the charge of the templates as a function of concentration. At the saturation point, the charge of the template has been fully reversed and little or no further charge changes occur if more substrate is added (Figure 6). Each of these method has its own advantages and limitations depending on the system used. In a review by Guzey et al. (2006) a more in-depth discussion can be found detailing the advantages and disadvantages of the different methods.[5]

A theoretical analysis of the influence of the various factors affecting the stability of multilayered colloidal systems was carried out by McClements (2005), who suggested that the stability of particles can be divided into different regimes as a function of substrate concentration c (Figure 7).[25]

i. $c = 0$: This is the initial state of the template system. If the system is kinetically stable, no aggregation between single particles occurs since the electrostatic repulsion between them is high enough.
ii. (ii) $0 < c < c_{sat}$: The templates cannot be completely covered (c_{sat}) with substrate, thus bridging aggregation occurs. Two or more templates may become linked together by a substrate bridge leading to the formation of large aggregates that rapidly cream or sediment.
iii. $c_{sat} < c < c_{dep}$: In this concentration regime saturation of template surfaces occurs; this is therefore the concentration window in which a successful coating can be carried out. One must nevertheless ensure that the time in which particles are saturated with substrate (τ_{Ads}) is appreciably shorter than the time between template-template collisions (τ_{Col}); otherwise bridging flocculation can still occur. This depends on the process. For example, if the templates were added to a substrate solution, the concentration of substrate would change in a step-wise, instantaneous manner. In contrast if the substrate was added to a solution containing the templates, the concentration would change gradually, which may cause problems.
iv. $c > c_{dep}$: In this concentration regime, depletion aggregation can occur since there is now excess substrate in the aqueous phase. The larger the excess concentration (c_{dep}) in the aqueous phase the more pronounced the aggregation, unless the excess concentration is so high that a substantial increase in solution viscosity occurs limiting template-template interaction. Depletion flocculation occurs since substrate molecules are excluded from the surface of the templates due to geometric restriction causing a concentration gradient. The system reacts by aggregation of templates minimizing the area from which the substrate molecules are excluded.[33]

To determine these regimes, a basic template system e.g. an emulsion or a liposomal dispersion is prepared and mixed with substrate solutions having different concentrations. Then, the change in the electrical charge (ζ-potential) of the template system is measured as a function of substrate concentration. To give a practical example, one may consider a recent case conducted in our laboratories. Here, we prepared a double-layered emulsion where oil droplets were stabilized by a fish gelatin-sugar beet pectin membrane. The objective there was to investigate the ability of laccase to crosslink the pectin in these multilayered emulsions.[7] In the absence of pectin, the electrical charge of primary emulsion was +18 mV, indicating that the gelatin membranes had a positively charge at pH 3.5. The electrical charge on the droplets became increasingly negative as the pectin concentration in the emulsion was increased which suggested that the negatively charged pectin molecules adsorbed to the surface of the positively charged oil droplets forming a gelatin – pectin membrane. The ζ-potential became constant at a value of around −20 mV when the pectin concentration exceeded about 0.04% (w/v) indicating that the droplets became fully saturated with beet pectin (Figure 6). An empirical model based on the change in ζ-potential was used to estimate the saturation concentration (c_{sat}) under prevalent conditions:

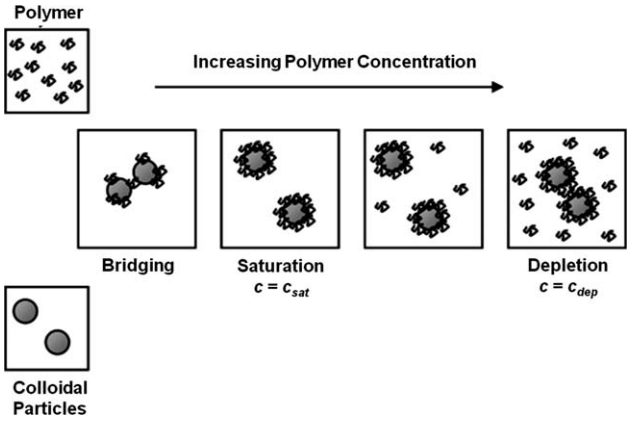

Figure 7. Stability of colloidal particles containing charged droplets as a function of added polyelectrolyte concentration: bridging, saturation, and depletion.[25]

$$\frac{\Delta\zeta(c)}{\Delta\zeta_{sat}} = \frac{\zeta(c)-\zeta_{sat}}{\zeta_0-\zeta_{sat}} \approx exp\left(-\frac{c}{c^*}\right) \approx exp\left(-\frac{3c}{c_{sat}}\right) \quad (1)$$

where $\zeta(c)$ is the potential of the template structure (oil droplet, liposome, micelle) at a given polymer concentration c, and ζ_0 and ζ_{sat} are the potentials in the absence of polymer and when particles are fully saturated with polymer. c^* is the polymer concentration where the change in ζ-potential is $1/e$ of the total change in ζ-potential at saturation: $\Delta\zeta = \Delta\zeta_{sat}/e$. The variable c_{sat} can be estimated by determining the polymer concentration at which the ζ-potential has increased or decreased by 95%. $c_{sat} = -c^* ln(0.05)$ or $c_{sat} \approx 3c^*$.[60] Moreover, the concentration of polyelectrolyte required to completely saturate the surface is given by:

$$c_{sat} = \frac{6\Phi\Gamma_{sat}}{d_{32}} \quad (2)$$

where Φ is the volume fraction of the particles, d_{32} the volume-surface diameter (m), and Γ_{sat} the surface load at saturation (kg/m^2).[25] Knowledge of the surface load enables one to calculate the minimum amount of polyelectrolyte required to prepare stable multilayered particles.[3]

APPLICATION SCENARIOS

Multilayered Planar Surfaces

The formation of ultrathin multilayer films on planer surfaces was first introduced by Decher et al. (1992).[4] In this study, a solid substrate with a positively charged planar surface was immersed in a solution containing anionic and cationic polyelectrolytes. Various studies since then focused on the use of planar surfaces as templates to adsorb one or more enzymes which can be applied to viable biosensors.[61,62] Hen egg white lysozym (HEWL) as an antimicrobial agents was successfully incorporated into multilamellar edible films composed poly-L-glutamic acid/HEWL layers and therefore inhibiting the growth of Micrococcus *luteus*.[63] Moreover, the LbL depositing technique was used to modify the surface of fruits. Kittitheeranun et al. (2012) demonstrated that mangoes fruits were coated by sequential dipping in solutions of either Poly(diallyldimethylammonium chloride), (PDADMAC) or Poly(styrene sulfonate sodium salt) (PSS) to modulate the surface hydrophobicity.[64] Fresh-cut watermelon was coated with a mixture of alginate, pectin, beta-cyclodextrin, and cinnamonaldehyde as antimicrobial and tested against total coliforms, yeasts, and molds.[65] It was shown that the shelf-life of LbL-coated fruits extended from 7 up to 12 days. In general, this approach could be used to encapsulate bioactives within the film such as antimicrobials, antibrowning agents, antioxidants, enzymes, flavors, and colors to increase the shelf life and quality of coated fruits.[27]

Multilayered Oil-in-Water Emulsions

It has been shown that coating of oil droplets by protein-polysaccharide complexes increases stability to environmental stresses due to changes in charge, structure, and thickness of interfacial films surrounding the droplets.[66,67] In particular, resistance of emulsions to changes in environmental conditions such as pH, ionic strength, and temperature (heating or freezing or heat-freeze-cycling) can be improved when membranes are reinforced with the LbL deposition technique.[9,11,15] For example, Littoz et al. (2008) demonstrated that double layered emulsions consisting of β-lactoglobulin-pectin membranes were more stable to droplet aggregation and creaming.[68] A number of authors found a better stability of multilayered emulsions to thermal processing (30–90°C), e.g. for emulsions with β-lactoglobulin-pectin membranes, emulsions with SDS-chitosan membranes, and emulsions with SDS-fish gelatin membranes.[11,15,50] Lecithin-chitosan-coated oil droplets formed at pH 3 were stable to aggregation at $\leq$ 500 m*M* $CaCl_2$, whereas single layered lecithin-coated emulsions aggregated at $\geq$ 300 m*M* $CaCl_2$.[15] We recently carried out a series of studies to further improve the functionality of multilayered emulsions by enzymatically crosslinking the interfacial membranes.[6,7,68] In our studies, we were able to demonstrate that emulsions containing a layer of electrostatically deposited pectin had significantly improved abilities to resist addition of salts or changes in pH after treatment with laccase.[6,7] Moreover, we demonstrated that the rate of Ostwald ripening in *n*-alkane-in-water emulsions can be retarded by manipulating the properties of the interfacial membranes surrounding the oil droplets by the use of electrostatic deposition of additional polymers.[69] Overall, application of enzymes is considered to be a mild processing technology and could be implemented using conventional processing technologies such as mixing or homogenization.[6,70] In addition, multilayered oil-in-water emulsions can be used as carrier systems for volatile organic compounds (VOC) under various environmental conditions (pH and salt). It was also shown that release kinetics of VOC can be changed by manipulating the thickness and structure of the interfacial complex.[71] The study showed that changes in pH (3–6) or ionic strength (0–500 m*M* NaCl) could act as a trigger to detach the pectin from the interface and release VOCs in the surrounding medium.[72]

Naturally dispersed oils stabilized by a phospholipid-protein membrane are known as oil bodies which are physically unstable when dispersed in media containing salts or acids. Therefore, oil bodies extracted from soybeans were coated with one layer of pectin using the LbL approach. Coated oil bodies had similar or even better stability compared to uncoated ones when exposed to high salt concentrations, changes in pH, and temperature fluctuations.[42]

Multilayered Liposomes

Liposomes have attracted considerable attention in the biochemical, food, and agricultural industries in recent years because they

are biocompatible, biodegradable, nontoxic, and have the ability to act as targeted release-on-demand carrier systems for both water- and oil-soluble functional compounds such as antimicrobials, flavors, antioxidants, and bioactive compounds.[22,73,74] In recent studies they have shown to be particularly well suited to carry polyphenolic compounds due to the high compatibility of this class of compounds with the polar lipid membrane of liposomes. Unfortunately, liposomes have shown to be relatively fragile, which can be attributed to the fact that often they consist of a single, thin bilayer membrane, which may be easily disrupted. When suspended in aqueous systems for a prolonged periods, vesicle fusion, aggregation, and leakage of entrapped material over time may occur.[75,76] Subjected to mechanical stresses, they may be disrupted or may coalesce e.g. during pumping and mixing, in particular if such processes were to be conducted under acidic conditions.[44] In addition, liposomes generally carry a negative surface charge due to the prevalence of phosphatidylcholine (PC) as a raw material. Manufacturing of positively or neutrally charged liposomes requires use of positively charged polar lipids such as phosphatidylethanolamine (PE), which is associated with higher costs.[44]

Therefore, deposition of a polymer coat around liposomes increased their physical and chemical stability. Laye et al. (2008) showed that chitosan-coated liposomes had better stability to aggregation than uncoated liposomes when stored at ambient temperatures for 45 days.[44] Similar results were obtained by Chen et al. (2013).[74] Liposomes coated with 4 oppositely charged biopolymer layers composed of chitosan and citrus pectin were physically stable during storage up to 150 days.[76] A further increase in physically stability of liposomes was observed by a combination between the LbL approach and spray drying. There, the coating of the liposomes was in fact an essential prerequisite to ensure that no collapse of the vesicle structure during drying occurred. In that spray draying process, chitosan-coated liposomes were combined with low and medium weight maltodextrins serving as a wall material to yield powders that contained intact liposomes, which could in turn be rehydrated to yield the original liposomal dispersions back.[75] Recently the formation of liposomes surrounded with 6 biopolymer layers was demonstrated. The particle size increased linearly with each successive deposition up to four layers; however, the particle size increased to several micrometers when a fifth and sixth layer was deposited indicating that aggregation may have occurred (see also below pitfalls and limitations).[14] As a side benefit, polymer-coated liposomes carrying polyphenols e.g. grape seed extract appear to have a reduced interactions with proteins allowing for example to add polyphenols to protein-rich foods such as milk or minced meat without incurring the risk of destabilizing the entire food system. The reduced interaction was shown by a decreased reaction with the Folin–Ciocalteu reagent, which also suggests that for example bitterness of polyphenols (which depends on interaction with bitterness receptors on the tongue) may also be reduced.[76]

Electrostatically Stabilized Polymer-Micelle-Complexes

In recent studies, the LbL approach is now also applied to ionic micelles. There, a charged nonsurface active polysaccharide is added to ionic micelles to modify their functionality. Such systems are of great interest to the food industry since ionic micelles have very interesting functionalities in itself (e.g., antimicrobial activity), but readily interact with other charged compounds in complex media. Where micelles to be coated with a charged polysaccharides the resulting complex may not exhibit this interaction therefore providing better functionality. This is also of importance if they are to serve as carrier vehicles of other functional ingredients such as for example antioxidants, flavors, or colors. Lauric arginate (LAE) is a novel generally recognized as safe antimicrobial, that due to its amphiphilic nature readily forms micelles. It is active against a wide range of food pathogens and spoilage organisms. However, the use of LAE is very limited because of its interaction with proteins and charged polysaccharides, its sensitivity to pH, ionic strength, and temperature changes and its bitter taste; all properties that have been associated with its cationic nature.[49] It was shown that mixed micelle/pectin complexes were stable to aggregation and formed clear solutions. These systems were much better able to remain stable in the presence of salts and upon changes in pH. Moreover, storing LAE under refrigeration conditions leads to precipitation over time, while LAE-pectin complexes remained in solution, an important property when using them in for example beverage applications. In addition, Bonnaud et al. (2010) investigated the interactions between LAE and various food grade biopolymers with different charge characteristics including pectin, alginate, carrageenan, xanthan, dextran, and chitosan.[77] Isothermal titration calorimetric and turbidity measurements confirmed that the polymer type significantly impacts the aggregation and binding behaviour with LAE. It was shown that cationic LAE only bound to anionic polymers. In general, the forces acting between oppositely charged polymers and surfactants depend on both electrostatic and hydrophobic interactions, whereas a variety of other factors might influence these and thus the structure of the biopolymer-micelle-complex such as molecular weight, degree of branching, charge density, backbone rigidity, and concentration of the polymer, as well as polar head, chain length, and concentration of the surfactant.[78]

LIMITATIONS AND PITFALLS

We have already mentioned that a sequential deposition of substrates onto a template structure while theoretically feasible is in practice not as simple to implement. The use of two or even more substrates to form multilamellar coatings on the one hand provides benefits such as increased stability, on the other hand, costs increase since additional process operations are required. Below, we briefly highlighted the reasons, why the adsorption of two or more layers becomes increasingly difficult.

Substrate Characteristics

As pointed out above, the most frequently used substrates are naturally occurring charged biopolymers that by their very nature often vary greatly in chemical and physical properties such as molecular weight, conformation, concentration, charge density. This fact makes it difficult to always fully saturate the surface of a template. Specific contributing factors to this are: (i) the distribution of charges along the biopolymer backbone is typically not homogeneous (ii) not each binding site on the template may be occupied by a functional group of the oppositely charged polymer, and (iii) the molecular weight of most

polymers is distributed. As a result, deposition may lead to the creation of progressively heterogeneously charged surfaces.[14]

With each deposition step, the size of the template structure increases. Therefore, increasingly higher concentrations of polymer must be used to cover the surfaces of particles. Simultaneously though, the polymers may also begin to entangle causing the morphology of the substrate layer (density, porosity, charge distribution) to become exceedingly complex. Finding the appropriate polymer concentration thus becomes increasingly more difficult.[14]

In addition, the use of biopolymers that are an exact match in charge density to that of the template and to that of any subsequent biopolymer is difficult. This is however an important prerequisite of the layer-by-layer deposition method—especially if multiple layers are to be formed, since a mismatch can for example cause charge reversal to occur without having all sites occupied. This may become even more of an obstacle if amphoteric molecules such as proteins are used for the deposition. Their charge magnitudes depend not only on their molecular architectures, but on environmental factors as well. Therefore, depending on the environmental pH, a different matching biopolymer should theoretically be selected, which may in practice be very difficult.[14] The formation of multilayer assemblies is mainly driven by electrostatic forces acting between oppositely charged polymers. However, secondary attractive interactions between polymer segments can also play a role. One such interaction that can play a substantial role depending on the nature of the template and the polymer are hydrophobic interactions which are known to be highly temperature dependent.[26]

Particle Concentration and Polymer Particle Ratio

McClements (2005) demonstrated in a mathematical approach that the number of template structures plays a major role in the formation of stable multilamellar coatings.[25] He theoretically calculated that at low template concentrations it should be possible to prepare stable multilayered systems without incurring flocculation since the adsorption of substrates occurs more rapidly than template structure collision. However, at higher template concentrations i.e. at higher oil droplet concentrations in the case of emulsions, this becomes increasingly unlikely since collisions occur more frequently. This means that the LbL electrostatic approach is often only applicable to dilute systems having low template concentrations. In emulsions, this critical concentration is often around 5%, meaning that the technique is only applicable to milk-like systems or beverages.

The formation and stability of multilayered coatings can be challenging since extensive droplet flocculation and aggregation may occur, even under conditions where polyelectrolyte saturation has been achieved.[10,67,79] Two major mechanisms can generally be observed when multilayered coatings are formed: bridging or depletion flocculation. Bridging flocculation occurs at low substrate and template concentrations due to charge neutralization and bridging effects, whereas depletion flocculation occurs when non-adsorbed (excess) substrate is present at a certain level causing an osmotic pressure gradient due to the exclusion of polymer molecules from the surface of the templates.[3,80] Depending on the base system and the bioactive encapsulated, flocculated particles may be disrupted by additional mechanical agitation such as ultrasonication, blending, or homogenization.[10,66,67] Even under saturation conditions one might observe aggregation between template structures and biopolymers due to other attractive forces involved such as hydrophobic, hydrogen bonding or van der Waals interactions. In addition, biopolymers used to from multilamellar coatings are often heterogeneously composed in terms of molecular weight distributions, protein fractions (in case whey proteins or caseinates), as well as contain impurities such as salts and might therefore adsorb more or less rapidly to the template surface leading to flocculation.

Solvent Properties

The pH and ionic strength play a key role in the assembly of multilayered membranes since they influence the degree of ionization of the charged functional groups on the respective substrate (e.g., amino and carboxyl groups).[21] We recently conducted a study to assess the influence of buffer type and ionic strength on the formation of primary, secondary, and tertiary emulsions stabilized by fish gelatin-sugar beet pectin membranes. We were able to demonstrate that using an acetate buffer a three-layered emulsion could be prepared, whereas use of a citrate buffer led to a failure, that is the system heavily aggregated.[81] Solutes such as ions may sequester water molecules to prevent them forming favorable hydrogen bonds with the protein or polysaccharide surface. Consequently, the biopolymer molecules prefer to interact between themselves instead of the surrounding water molecules, thus leading to increased polymer–polymer interactions and aggregation.[82] Moreover, the magnitude and range of electrostatic interactions between a polyelectrolyte and a droplet decrease as the ionic strength of the solution increases since counter-ions accumulate around the surfaces leading to a compression of the diffuse double layer having a tremendous effect on the electrostatic interaction between charged surfaces and polyelectrolytes.[3,5]

Mixing Method

The formation of multilaminar coatings can be achieved by a simple mixing process, nevertheless, as indicated above, the mixing method can have a tremendous impact on the stability. In particular, an inappropriate order of mixing, mixing speed, and locally turbulent flow profiles may induce heavy flocculation or aggregation.[25,66] Guzey et al. (2004) demonstrated that stable secondary emulsions could be formed by mixing emulsion droplets and biopolymers at a pH value where initially one of the species (emulsion droplet or biopolymer) was uncharged.[66] She then adjusted the pH to values where both species became oppositely charged. In this case, polymers and droplets were evenly distributed throughout the continuous phase prior to deposition ensuring that when interactions began to take place, the adsorption occurred uniformly which reduced the tendency to aggregate.[5] Additionally, the order of mixing biopolymer solution and emulsion can strongly affect aggregation behavior. For example, the addition of emulsion droplets into a biopolymer solution under conditions, where an attractive interaction between polyelectrolytes and particles prevailed results in less aggregation than vice versa.[5,7]

Physical Stability of Multilayered Coatings

Successful deposition of charged substrates onto a charged template relies predominately on electrostatic interactions, but other

colloidal and molecular interactions such as Van der Waals, hydrophobic, depletion, and hydration interactions also play a role. These physical interactions are highly dependent on environmental conditions such as pH, salt concentrations, and temperatures and affect the stability of the generated multilayer system.[2,3] One may thus often be able to create a multilayered system but then discover that the deposition has created a surface that is more hydrophobic and thus prone to aggregation, or a surface where polymer coils extend into the aqueous phase allowing for Flory-Huggins driven attractions to take place, especially if the solvent is or becomes a poor solvent (e.g., if temperature changes or an additional solvent is added). The opposite may also be true though. Substrates at the surfaces may rearrange to form more dense layers or substrates may form intramolecular binds (such as for example protein–protein disulfide bridges) which may lead to an improved stability over time. It is therefore advisable that users of this technique also conduct kinetic experiments ensuring that their systems are stable for the duration of the use. One way to improve the stability of multilayers is to induce crosslinking reactions either by chemical or enzymatic means to deliberately crosslink substrates. Such treatments should be carried out shortly after the polymers have been deposited on the surface. Enzymes such as laccase, horseradish peroxidase, or lactate oxidase, transglutaminase have shown to be quite suitable to this task.[6,7,61,62,83]

CONCLUSIONS

This brief review highlighted the layer-by-layer (LbL) electrostatic deposition technique which enables food manufacturers and technologists to manipulate and control the interfacial properties of dispersions such as for example emulsions, liposomes or suspensions used as delivery and encapsulation systems. Recent studies have shown that fundamental knowledge of template properties, substrate characteristics, and environmental conditions are essential to from stable multilaminar coatings without incurring aggregation. While a successful coating of a template structure may involve quite a bit of effort on the part of the manufacturer, significant improvements in stability and functionality may be achieved. Manufacturers are therefore advised to conduct a cost benefit analysis on a case-by-case basis. In addition, while at the moment predominately charged polymers are used to coat surfaces, many different kinds of charged entities can be deposited on the surface. This approach is thus for example an alternative to the Pickering emulsion approach, where solid particles at used to stabilize liquid dispersions. Having solid particles are an emulsion interface provides for some substantial benefits as oxygen migration can be significantly reduced. However, in Pickering emulsions, solid particles must have very specific surface energies in order to ensure adsorption. Electrostatic deposition in contrast offers an alternative that allows a much larger variety of solid particles to be adsorbed. Some ongoing efforts also currently look at the digestion behavior of multilayered emulsions and initial promising results suggest that digestion times and caloric intake can be regulated using this technology. To bring this approach however fully into the commercial realm, more scale-up and processing oriented studies will be needed. For some applications, the layer-by-layer technique is already commercially used to encapsulate probiotics in food where the biopolymer coating serves as a protective shell when exposed to the harsh conditions in the human gastrointestinal tract. In many cases, however, proteins and polysaccharides are nowadays used to be assembled to complexes having surface active properties. The formation of these coacervates is less challenging and therefore commercially favoured.

REFERENCES

1. Aguilera, J. M. *J. Sci. Food Agr.* **2006**, *86*, 1147.
2. McClements, D. J.; Decker, E. A.; Park, Y.; Weiss, J. *Crit. Rev. Food Sci.* **2009**, *49*, 577.
3. McClements, D. J. Food Emulsions: Principles, Practice, and Techniques; CRC Press: Boca Raton, **2004**.
4. Decher, G.; Hong, J. D.; Schmitt, J. *Thin Solid Films* **1992**, 210–211 (PART 2), 831.
5. Guzey, D.; McClements, D. J. *Adv. Colloid Interfac.* **2006**, *128*, 227.
6. Zeeb, B.; Fischer, L.; Weiss, J. *J. Agr. Food Chem.* **2011**, *59*, 10546.
7. Zeeb, B.; Gibis, M.; Fischer, L.; Weiss, J. *Food Hydrocolloid* **2012**, *27*, 126.
8. Gu, Y. S.; Decker, A. E.; McClements, D. J. *Langmuir* **2005**, *21*, 5752.
9. Gu, Y. S.; Decker, A. E.; McClements, D. J. *J. Agr. Food Chem.* **2004**, *52*, 3626.
10. Ogawa, S.; Decker, E. A.; McClements, D. J. *J. Agr. Food Chem.* **2004**, *52*, 3595.
11. Ogawa, S.; Decker, E. A.; McClements, D. J. *J. Agr. Food Chem.* **2003**, *51*, 5522.
12. Decher, G. *Science* **1997**, *277*, 1232.
13. Iler, R. K. *J. Colloid Interf. Sci.* **1966**, *21*, 569.
14. Chun, J.-Y.; Choi, M.-J.; Min, S.-G.; Weiss, J. *Food Hydrocolloid* **2013**, *30*, 249.
15. Aoki, A.; Decker, E. A.; McClements, D. *J. Food Hydrocolloid* **2005**, *19*, 209.
16. McClements, D. J.; Decker, E. A.; Weiss, J. *J. Food Sci.* **2007**, *72*, R109.
17. Branco, M. C.; Schneider, J. P. *Acta Biomater.* **2009**, *5*, 817.
18. McClements, D. J.; Rao, J.*Crit. Rev. Food Sci.* **2011**, *51*, 285.
19. Matalanis, A.; Jones, O. G.; McClements, D. J. *Food Hydrocolloid* **2011**, 1865.
20. Maherani, B.; Arab-Tehrany, E.; Mozafari, M. R.; Gaiani, C.; Linder, M. *Curr. Nanosci.* **2011**, *7*, 436.
21. Schmitt, C.; Sanchez, C.; Desobry-Banon, S.; Hardy, J. *Crit. Rev. Food Sci.* **1998**, *38*, 689.
22. Taylor, T. M.; Davidson, P. M.; Bruce, B. D.; Weiss, J. *Crit. Rev. Food Sci.* **2005**, *45* (7-8), 587.
23. Caruso, F.; Trau, D.; Möhwald, H.; Renneberg, R. *Langmuir* **2000**, *16*, 1485.
24. Sukhorukov, G. B.; Donath, E.; Davis, S. *Polym. Advan. Technol.* **1998**, *9* (10-11), 759.

25. McClements, D. J. *Langmuir* **2005**, *21*, 9777.
26. Renard, C. M. G. C.; Lahaye, M.; Mutter, M.; Voragen, F. G. J.; Thibault, J. F. *Carbohyd. Res.* **1997**, *305*, 271.
27. Weiss, J.; Takhistov, P.; McClements, D. J. *J. Food Sci.* **2006**, *71*, 9.
28. Schönhoff, M. *J. Phys: Condens. Matter* **2003**, *15*, R1781.
29. Rhim, J.-W.; Ng, P. K. W. *Crit. Rev. Food Sci.* **2007**, *47*, 411.
30. Cagri, A.; Ustunol, Z.; Ryser, E. T. *J. Food Protect.* **2004**, *67*, 833.
31. Cha, D. S.; Chinnan, M. S. *Crit. Rev. Food Sci.* **2004**, *44*, 223.
32. Morillon, V.; Debeaufort, F.; Blond, G.; Capelle, M.; Voilley, A. *Crit. Rev. Food Sci.* **2002**, *42*, 67.
33. P. Walstra and W. Walstra, Physical Chemistry of Foods, Marcel Dekker Inc, **2003**.
34. McClements, D. J.; Li, Y. *Adv. Colloid Interfac.* **2010**, *159*, 213.
35. Peng, C. C.; Lin, I. P.; Lin, C. K.; Tzen, J. T. C. *Biotechnol. Progr.* **2003**, *19*, 1623.
36. Chen, M. C. M.; Chyan, C. L.; Lee, T. T. T.; Huang, S. H.; Tzen, J. T. C. *J. Agr. Food Chem.* **2004**, *52*, 3982.
37. Murphy, D. J.; Hernández-Pinzón, I.; Patel, K. *J. Plant Physiol.* **2001**, *158*, 471.
38. Murphy, D. J.; Hernández-Pinzón, I.; Patel, K.; Hope, R. G.; McLauchlan, J. *Biochem. Soc. T.* **2000**, *28*, 710.
39. Shimada, T. L.; Hara-Nishimura, I. *Biol. Pharm. Bull.* **2010**, *33*, 360.
40. Tzen, J. T. C.; Cao, Y. Z.; Laurent, P.; Ratnayake, C. Huang, A. H. C. *Plant Physiol.* **1993**, *101*, 267.
41. Tzen, J. T. C.; Huang, A. H. C. *J. Cell Biol.* **1992**, *117*, 327.
42. Iwanaga, D.; Gray, D.; Decker, E. A.; Weiss, J.; McClements, D. J. *J. Agr. Food Chem.* **2008**, *56*, 2240.
43. D. Iwanaga, D.; Gray, D.; Fisk, I. D.; Decker, E. A.; Weiss, J.; McClements, D. J. *J. Agr. Food Chem.* **2007**, *55*, 8711.
44. Laye, C.; McClements, D. J.; Weiss, J. *J. Food Sci.* **2008**, *73*, N7.
45. Weiss, J.; Gaysinsky, S.; Davidson, M. McClements, J. D. Global Issues in Food Science and Technology; Academic Press: New York, **2009**.
46. Koynova, R.; Caffrey, M. *Bba-Rev. Biomembranes* **1998**, *1376*, 91.
47. Flanagan, J.; Singh, H. *Crit. Rev. Food Sci.* **2006**, *46*, 221.
48. Garti, N.; Spernath, A.; Aserin, A.; Lutz, R. *Soft Matter* **2005**, *1*, 206.
49. Asker, D.; Weiss, J.; McClements, D. J. *J. Agr. Food Chem.* **2011**, *59*, 1041.
50. Guzey, D.; McClements, D. J. *Food Biophys.* **2006**, *1*, 30.
51. Gu, Y. S.; Decker, E. A.; McClements, D. J. *Food Hydrocolloid* **2007**, *21*, 516.
52. Sukhorukov, G. B.; Möhwald, H.; Decher, G. Lvov, Y. M. *Thin Solid Films* **1996**, 284–285, 220.
53. Sukhorukov, G. B.; Montrel, M. M.; Petrov, A. I.; Shabarchina, L. I.; Sukhorukov, B. I. *Biosens. Bioelectron.* **1996**, *11*, 913.
54. Fendler, J. H.; Meldrum, F. C. *Adv. Mater.* **1995**, *7*, 607.
55. Keller, S. W.; Kim, H.-N.; Mallouk, T. E. *J. Am. Chem. Soc.* **1994**, *116*, 8817.
56. Schmitt, J.; Decher, G.; Dressick, W. J. *Adv. Mater.* **1997**, *9*, 61.
57. Ichinose, I.; Senzu, H.; Kunitake, T. *Chem. Lett.* **1996**, *10*, 831.
58. Caruso, F.; Schüler, C. *Langmuir* **2000**, *16*, 9595.
59. Dickinson, E. *Colloids Surfaces B: Biointerfaces* **2010**, *81*, 130.
60. Guzey, D.; McClements, D. J. *J. Agr. Food Chem.* **2007**, *55*, 475.
61. Kim, D. C.; Sohn, J. I.; Zhou, D.; Duke, T. A. J.; Kang, D. J. *ACS Nano* **2010**, *4*, 1580.
62. Rauf, S.; Zhou, D.; Abell, C.; Klenerman, D.; Kang, D. J. *Chem.l Commun.* **2006**, *16*, 1721.
63. Rudra, J. S.; Dave, K.; Haynie, D. T. *J. Biomater. Sci.-Polym. E* **2006**, *17*, 1301.
64. Kittitheeranun, P.; Dubas, S. T.; Dubas, L. *Appl. Mech. Mater.* **2012**, 229–231, 2745.
65. Sipahi, R. E.; Castell-Perez, M. E.; Moreira, R. G.; Gomes, C.; Castillo, A. *LWT - Food Sci. Technol.* **2013**, *51*, 9.
66. Guzey, D.; Kim, H. J.; McClements, D. J. *Food Hydrocolloid* **2004**, *18*, 967.
67. Moreau, L.; Kim, H. J.; Decker, E. A.; McClements, D. J. *J. Agr. Food Chem.* **2003**, *51*, 6612.
68. Littoz, F.; McClements, D. J. *Food Hydrocolloid* **2008**, *22*, 1203.
69. Zeeb, B.; Gibis, M.; Fischer, L.; Weiss, J. *J. Colloid Interf. Sci.* **2012**, *387*, 65.
70. Minussi, R. C.; Pastore, G. M.; Durán, N. *Trends Food Sci. Tech.* **2002**, *13* (6-7), 205.
71. Benjamin, O.; Silcock, P.; Leus, M.; Everett, D. W. *Food Hydrocolloid* **2012**.
72. Benjamin, O.; Leus, M.; Everett, D. W. *Food Res. Int.* **2012**, *44*, 417.
73. Mozafari, M. R.; Khosravi-Darani, K.; Borazan, G. G.; Cui, J.; Pardakhty, A.; Yurdugul, S. *Inter. J. Food Prop.* **2008**, *11*, 833.
74. Chen, B.; Li, H.; Ding, Y.; Rao, J. *Food Res. Int.* **2011**, *44*, 1468.
75. Brown, M. A.; Zhao, Z.; Grant Mauk, A. *Inorg. Chim. Acta* **2002**, *331*, 232.
76. Phatak, L.; Chang, K. C.; Brown, G. *J. Food Sci.* **1988**, *53*, 830.
77. Bonnaud, M.; Weiss, J.; McClements, D. J. *J. Agr. Food Chem.* **2010**, *58*, 9770.
78. Langevin, D. *Adv. Colloid Interfac.* **2009**, 147–148 (C), 170.
79. Dickinson, E.; Pawlowsky, K. *J. Agr. Food Chem.* **1997**, *45*, 3799.
80. Dickinson, E. *Food Hydrocolloid* **2009**, *23*, 1473.
81. Karadag, A.; Özçelik, B.; Sramek, M.; Gibis, M.; Kohlus; R.; Weiss, J. *J. Food Sci.* **2013**, *78*, E206.
82. Curtis, R. A.; Lue, L. *Chem. Eng. Sci.* **2006**, *61*, 907.
83. Dickinson, E. *Trends Food Sci. Tech.* **1997**, *8*, 334.