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PERSPECTIVE

Layer-by-layer Polymeric Deposition as an Efficient Strategy to Sustain Drug Release

Filippo Rossi[1,*]

[1]*Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, via Mancinelli 7, 20131 Milan, Italy*

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1. INTRODUCTION

The ability to control and sustain the release of drugs avoiding under and overdosing, maintaining the drug concentration within therapeutic range, is a well-established discipline in pharmaceutics with many commercial products and many other challenges that should be overcome [1-3]. Indeed, despite the very good results obtained in many applications, several critical points come from the fact that pure diffusion mechanism is the main phenomenon involved, then drug release is generally too quick due to the high clearance present within animal bodies [4-6]. A common strategy used to decelerate the release involves the formation of covalent bonds between drug molecules and polymeric chains; these are commonly known in literature as biorthogonal strategies [7-9]. This approach guarantees the controlled and sustained release molecules on one hand, but on the other hand, the chemical modification of active principles may change their efficacy. Another interesting alternative is represented by multilayer deposition onto a single device where different coatings one onto another can decrease the release rates of drugs. In this framework, in the last decade, layer-by-layer self-assembly (LbL) technique has shown extremely promising properties in many different biomedical fields [10-12]. It consists of the alternate adsorption of oppositely charged macromolecules over a charged substrate. Here, the good adhesion achieved between the neighboring layers and the surface is so based on electrostatic interactions and the use of macromolecules rather than small molecules guarantees a certain number of ionic bonds. For this reason, this technique is often referred to as electrostatic self-assembly (ESA) [13, 14]. In principle, when the polyionic molecules approach an opposite charged substrate or layer, within a sufficiently small distance (Debye length), the local electric field is so strong that it attracts the polyionic molecules to the surface, starting the adsorption process.

The deposition of the charged material does not only occur until the charge neutralization of the surface, but also until a certain degree of opposite charge appears on the surface, leading to the overcompensation of the charge of the previous layers [15].

This overcompensation has two important consequences: the repulsion of equally charged molecules, and thus, self-regulation of the adsorption and restriction to a single layer and then the ability of an oppositely charged molecule to be adsorbed in a second step on top of the first one. The cyclic repetition of both adsorption steps leads to the formation of a multilayer structure, as visible in Fig. (**1**) [16].

Therefore, the driving force for the assembly is the charge inversion after the deposition of two consecutive layers. The charge inversion degree strongly depends on the specific polyelectrolyte pair and the assembly conditions (ionic strength, pH), but does not show significant dependence on the property of the surface, such as substrate charge density. This is due to the ability of polymers to simply bridge over underlying defects. With respect to other methods for multilayer fabrication, the LbL approach has shown many advantages, as shown in Fig. (**2**).

*Address correspondence to this author at the Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, *via* Mancinelli 7, 20131 Milan, Italy; Tel: (+39) 02 23993145; Fax: (+39) 02 23993180; E-mail: filippo.rossi@polimi.it

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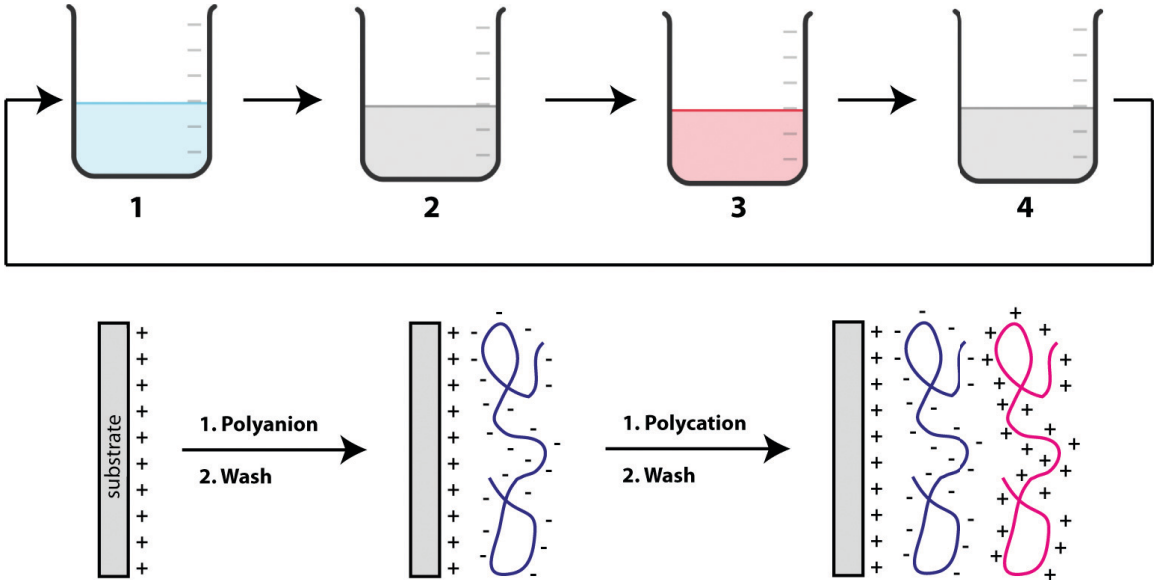

Fig. (1). Schematic of the film deposition process using slides and beakers. Steps 1 and 3 represent the adsorption of a polyanion and polycation, respectively, and steps 2 and 4 are washing steps. The four steps are the basic buildup sequence for the simplest film architecture. The construction of more complex film architectures requires only additional beakers and a different deposition sequence. Reprinted with permission from [16]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

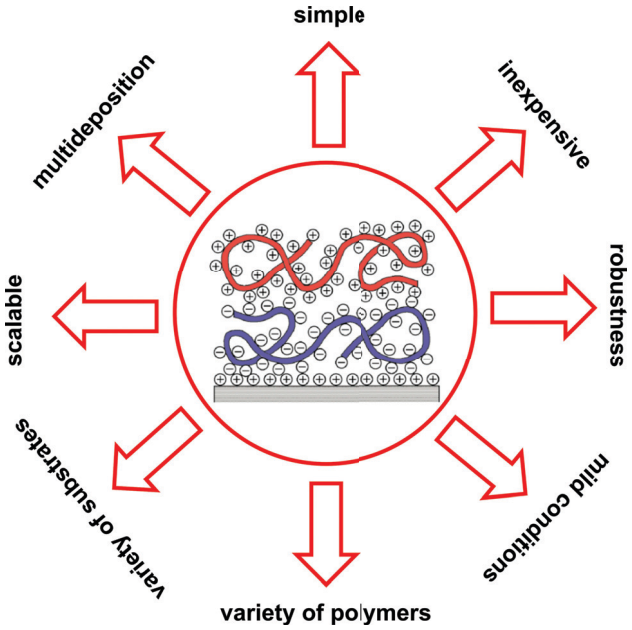

Fig. (2). LbL approach advantages. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The main quality connected to the LbL approach guided by ESA is the possibility to easily tune almost all of the physicochemical properties of the final composite system just by changing some of the starting parameters, such as (*i*) polyelectrolytes type, concentration and charge density, (*ii*) deposition sequence, (*iii*) number of deposited layers, (*iv*) adsorbing time of the opposite charged compounds, and (*v*) environmental conditions, including ionic strength and type of ions, solvent nature, pH, and temperature. In this way, specific film architecture, thickness, roughness, strength as well as macroscopic electrical, optical, magnetic, thermal, permeability and biocompatibility properties may be obtained almost independently from the substrate involved. Moreover, the multilayer assembly can be performed with different methods as dip coating, spin-coating or spray-coating.

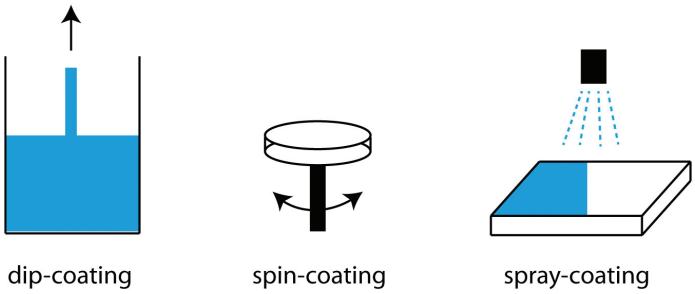

Fig. (3). Different deposition methods for LbL self-assembly. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

In dip coating, the substrate is just dipped, in a sequentially alternating way, into aqueous solutions of the opposite charged macromolecules selected. In this case, the diffusion mechanism in the solution constantly provides the availability of molecules near the substrate surface. One or more washing steps with pure water are usually used after the adsorption of each layer to remove the excess of non-adsorbed polyelectrolyte, thus avoiding the formation of inter-polyelectrolyte complexes in the media during the addition of the subsequent oppositely charged species. The rinsing step also helps to stabilize weakly adsorbed polymer layers [17, 18]. Bernasconi and coworkers using this method coated 3D printed metallized microrobots with alginate/chitosan or alginate/polyallylamine hydrogels that showed better controlled release kinetics of anti-inflammatory drugs with respect to single layer ones [19].

In spin coating, a small amount of coating material is applied over the center of the substrate. The substrate is then rotated at high speed in order to spread the coating material by centrifugal force. The timing and speed of rotation can be varied in order to reach a uniform film with a desired thickness [17, 20, 21]. After an intermediate rinsing by spinning pure water on the template, a second layer can be deposited. The machine used for spin coating is called a spin coater, or simply a spinner. The coating material is applied pre-dissolved in a volatile solvent that spontaneously evaporates during the spinning process. Spin-coating has been used in the development of doxorubicin-releasing thin films made of poly(*N*-isopropylacrylamide-*co*-acrylic acid) and polyallylamine, which exhibited release characteristics dependent on the number of layers incorporated into the film [22, 23]. With respect to dip coating, spin coating is characterized by higher growth rate (more than one order of magnitude), smoother surface because of the shearing effect, and more ordered and compact internal structures (the screening effect of water between the polymer chains is avoided). However, this technique is only effective for simple substrate geometry (*e.g.*, flat substrates).

In the process of spray self-assembly, oppositely charged polyions or nanoparticles are sequentially sprayed onto the substrate within only a few seconds after each step [24, 25]. Between every two depositions, the surface is washed by spraying pure water. This approach is suitable for every shape of the substrate, resulting in a highly uniform multilayer obtained in a short time interval (Fig. **3**). Antibiotic-releasing films (vancomycin) showed a linear release over 40 h after dip coating, while spray-coated samples released >90% of their cargo within 4 h. Films prepared *via* spray coating using polyβ-amino esters (cations) and anionic polymers (alginate, chondroitin sulfate and dextran sulfate) were consistently thinner, smoother, and contained higher drug concentrations than dip-coated films [26].

Between all of the alternatives, ionic interactions remain the most versatile and the most commonly used in biomedical applications, as in this case, where the use of water as a solvent and biopolymers as building blocks is of fundamental importance. Thanks to their anionic behavior, hydrogels, deposited over the microdevice, can effectively serve as a template for LbL ESA. The alternate deposition of different layers over hydrogels could significantly sustain the release of very small drugs by lengthening their diffusion path [6]. Furthermore, the use of charged macromolecules as building blocks could potentially introduce electrostatic interactions with charged drugs, improving the control over the release. In this sense, it would be interesting to find out a way to control also the decomposition of preformed multilayers by changing simple environmental conditions. In this way, without any functionalization, it would be possible to induce a perfectly controlled (and not just sustained) drug release. Recent studies [27, 28], despite the stability of the LbL self-assembly process (Fig. **3**), have found out that factors, such as pH and ionic strength, may influence ESA systems to a large extent, such as complete dissolution. Indeed it was found that exposing these systems to a saline solution with a sufficient ionic strength can cause the film to be completely and rapidly desorbed, baring the substrate [27, 28]. Furthermore, this threshold salt concentration could be reduced by introducing an excess of one of

the two polyelectrolytes involved in the multilayer realization. However, under a certain salt concentration, dissolution does not occur at all, despite exposure to a large excess of polyelectrolyte.

The response of multilayer systems in the presence of saline solution is not the only means of dissolution that has been investigated. Indeed, it is known that liquid-like complexes are also formed by chains with charge density below a critical value. In this sense, the use of one or more polymers bearing weak acid/base functionality affords the possibility, acting on the environment pH, of controlling the average charge per repeat unit, thus extending interaction between charged polymers and so the dissolution of the multilayer complexes. Therefore, if the pH is brought at a specific value, the charges on the polymer chains could be too dilute to maintain cohesive forces throughout the multilayer and it would dissociate. Experiments [27] demonstrate that by varying the pH, it is possible to decrease the thickness of the films, but the loss of polymer is more gradual (and lower) than the one observed in the presence of a high saline solution.

In addition to the high versatility, the high degree of control over the properties of the final composite system, these three easy methods of assembly make it clear that ESA has great potential in a wide range of fields, such as optical coatings, biosensors, development of stimuli-responsive systems, tissue engineering, multistep chemical catalysis, and drug delivery [29, 30]. For this reason, LbL self-assembly technology has been extensively studied in the past few decades. In particular, it has been found out that the electrostatic interactions are not the only driving forces able to achieve LbL self-assembly. Multifunctional polymers, even nonionic, offer the choice of building up layered structures through other types of interactions, such as hydrogen bonding, charge-transfer interactions, chemical bonding (click chemistry), supramolecular complexation, bio-specific recognition, etc. Therefore, the LbL self-assembly can be defined as the process of alternate deposition over a general substrate of building blocks with complementary interactions or structures. Even covalent chemistry can successfully be used for multilayer fabrication. In detail, the covalent layer-by-layer assembly can be performed with two different strategies [31]:

- Post covalent conversion
- Consecutive covalent fabrication

"Post covalent conversion" involves the initial construction of a global multilayer system using non-covalent interactions (most often electrostatic interactions). This step is followed by the application of specific covalent reaction conditions, which are achieved by adding bifunctional reagents, increasing the temperature, or using light irradiation, in order to convert the non-covalent films into covalently woven ones (one-step covalent conversion). Among the chemical reactions that can be used, those between amines and amine-reactive species are the most frequently employed [31].

In "consecutive covalent fabrication" instead, the films are built in each step using covalent reactions, which are performed by applying specific environmental conditions [20, 32]. Table **1** lists the advantages and disadvantages of the two types of fabrication methods.

Table 1. Considerations on covalent conversions in LbL technique.

-	**Advantages**	**Disadvantages**
Post covalent conversion	✓ economic in terms of labor and time	× multiple charged or polar groups required
-	✓ possibility to tune final properties changing experimental parameters	-
Consecutive covalent fabrication	✓ compatible with uncharged blocks	× difficulty in network building
	✓ possibility to include small molecules	-

CONCLUSION

In general, covalent layer-by-layer self-assembly allows to develop system with excellent strength and one that is less susceptible to changes in environmental conditions with respect to ESA systems. However, high-quality multilayer films cannot be reliably obtained with self-assembled films based on covalent chemistry due to the possible formation of side products that remain trapped between the layers. Only a limited number of reactions present exactly 100% yield, which is a prerequisite for the preservation of functional group density in each layer. Moreover, the use of chemical reaction could alter other components present in the global systems, such as drugs in drug delivery applications. In summary, in this perspective, different peculiarities of layer-by-layer strategy have been described together with the possible benefits in drug delivery applications.

CONFLICTS OF INTEREST

Filippo Rossi is the Editorial Board Member of the journal Current Pharmaceutical Biotechnology.

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