Expert Opinion

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Modulating the release kinetics through the control of the permeability of the layer-by-layer assembly: a review

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The layer-by-layer (LbL) self-assembly technique has emerged as a simple and versatile method for coating biological and non-biological templates for various biomedical applications. A promising avenue of this technique lies in the encapsulation of drugs and other biological substances for controlled release. Fundamental studies of LbL assembly on flat surfaces have provided a sound understanding of film deposition theory and its pertinence to ionic and molecular transport and diffusion through polyelectrolyte multilayer (PEM) films. However, there is a lack of information on the permeability of three-dimensional PEM shell systems. In either PEM films or shells, it has been shown that drug release is a function of the ionic strength, pH and/or multilayer thickness. This report aims to provide an overview of the physicochemical parameters affecting the permeability of two- and three-dimensional multilayer shells, including ionic strength, layer number and pH. Furthermore, their synergic effect on loading and release of biologically active molecules from LbL multilayers are discussed.

Keywords: controlled release of biomolecules, layer-by-layer assembly, polyelectrolyte multilayer film and shell, tuned permeability

Expert Opin. Drug Deliv. (2009) 6(6):585-597

1. Introduction

Surface modification can modulate and improve material surface properties required for a given application. These modification techniques include chemical grafting, nonspecific adsorption and chemisorption through electrostatic interactions. Over the last two decades, Decher *et al.* have contributed significantly to the latter field with the development of layer-by-layer (LbL) electrostatic self-assembly techniques using polyelectrolytes for the preparation of ultrathin films on surfaces of both two-dimensional and three-dimensional templates [1,2]. Potential applications for such films cover a broad range, including biosensing [3], membrane separations [4] and controlled release [5].

The LbL approach involves polyelectrolyte multilayer (PEM) preparation through the sequential adsorption of oppositely charged polyelectrolytes from dilute aqueous solutions onto charged solid substrates [2,6-9]. A crucial step in this LbL build-up involves the removal of free polyelectrolytes after the layer deposition, which is commonly accomplished by means of centrifugation and membrane filtration [10].

The LbL method is versatile as it does not impose restrictions on the types of polymer used. A wide variety of materials, including synthetic polyelectrolytes [11],

10.1517/17425240902967599 © 2009 Informa UK Ltd ISSN 1742-5247

proteins [12], DNA [13], clay materials [14], dendrimers [15], colloidal particles [16] and redox systems [17], are used as polyelectrolytes on various templating supports, namely latex, inorganic crystals, proteins aggregates, organic particles, oil droplets and cells. Hollow capsules are also produced after core material removal by dissolution or by calcination [18,19]. The potential applications of LbL coating are determined by the properties of the polyelectrolytes used.

Polyelectrolytes belong to the category of *smart* polymers. This is because their permeability can be controlled through their response to a stimulus [20]. For example, their molecular structures can be triggered to expand or contract in a controlled fashion by varying conditions such as temperature, pH, ionic strength and the solvent's nature. These stimuli-responsive phenomena are attributed to backbone interactions between the polyelectrolyte molecules within the multilayer. Decreasing the number of interactions between oppositely charged species leads to the loosening of the layer structure and thus promotes enhanced permeability. This behavior is frequently induced to modulate the permeability of PEM/LbL coating materials to ions and other species. There is a great body of literature that has investigated permeability behavior, particularly for hollow capsules [18,19], as well as its relevance to drug delivery carriers, microreactors and catalytic systems. By varying the deposition conditions and polyelectrolyte composition, authors have reported achieving highly permeable, semipermeable, or nearly impermeable PEM films. The permeability requirement varies according to specific applications. For surface protection, an ideal film should be impermeable, whereas for drug release, the films should have adequate permeability in order to attain the proper therapeutic dose release over a given period of time.

Permeability is particularly important when working with cell templates. Previous studies involving the coating of *Escherichia Coli* have shown that cells remained viable and metabolically active following LbL polyelectrolyte deposition using either chitosan (CH)/alginate (AL) or CH/hyaluronic acid (HA) films [21]. Moreover, Veerabadran *et al.* used a poly-L-lysine (PLL)/HA shell that successfully encapsulated stem cells while preserving their morphology and viability for 7 days [22]. These results mainly indicate that cells remain viable because of the films' characteristic permeability, which promotes nutrient and waste exchange.

The permeability of the LbL films' assembly on flat surfaces has been studied extensively by electrochemical methods to assess factors affecting molecular transport and diffusion through the PEM film. Characterization of PEM permeability in three-dimensional colloidal and particulate templates has, however, proved significantly more challenging for both loading and release behaviors of compounds from PEM shells. Therefore, it is beneficial first to analyze critically the effects of experimental parameters on the permeability of two-dimensional films in order to conduct more comprehensive analyses of three-dimensional PEM shells. Major factors influencing the structure of multilayers, and in turn their permeability, include ionic strength, wall/film thickness, pH value and charge balance. This review aims to discuss the aforementioned parameters and their respective effects on the permeability of PEMs deposited on various surfaces and particles, with a focus on drug release modulation and release kinetics, as well as their underlying collective and synergistic relationships.

2. Permeability

For many polymers, the capacity of PEMs to be permeable to a fluid, molecules or ions depends strongly on the pH, polarity and ionic strength of the solution. A change in any of these parameters affects the backbone interactions between the polyelectrolyte molecules within the multilayer and leads to the alteration of the layer structure and overall permeability. Other important factors affecting PEM permeability include the layer number, multilayer porosity and the size of the permeable compound.

For two-dimensional constructs, total internal reflection fluorescence (TIRF) spectroscopy, electrochemistry and *in situ* ellipsometry are the main techniques used to study PEM permeability [23,24], whereas for three-dimensional constructs the main approach consists of encapsulating soluble materials, and measuring and analyzing the release profile from the capsule interior. The main disadvantage of this method is that the capsules are prepared at different equilibrium conditions in relation to the release study. Antipov *et al.* determined the permeability of PEM shells by encapsulating fluorescent crystals and analyzing their subsequent dissolution [25]. The fluorescein dye used does not interfere with multilayer properties and is used in the initial solution without altering the pH. More recently, the molecular beacon (MB) technique was applied in measuring the permeability of PEM capsules [26]. The MB technique uses a molecular probe consisting of a single-stranded DNA molecule with a fluorophore and a quencher at opposite ends [27]. This method involves first immobilizing MBs inside particles, followed by encapsulating the MB-loaded particles within the LbL film to be probed. The encapsulated MB-loaded particles are then incubated with DNA target sequences of different lengths. Permeation of the DNA targets through the capsule shell causes hybridization of the DNA targets with the MB complementary loop region, resulting in an increase in MB fluorescence [27].

A typical dissolution curve of covered particles (**Figure 1**) consists of a linear phase and an exponential phase. During the first phase of dissolution, the core particle is immersed in a saturated solution within the shell. It is then possible to calculate the permeability coefficient using the readily available variables of particle radii and density in lieu of variables such as solution volume and number of particles [28]. Other essential parameters required to calculate the permeability coefficient are the time of dissolution, which can be measured experimentally, and the saturation concentration [28]. The permeability coefficient P is given by:

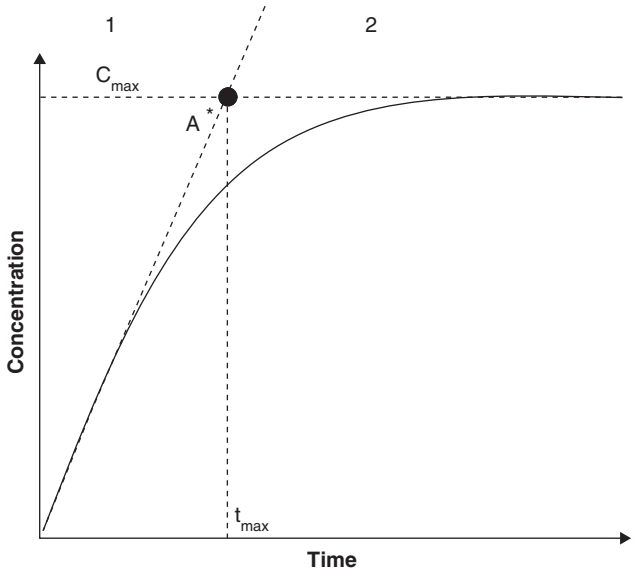

Figure 1. Linear (1) and exponential (2) stages of release in a typical release curve. The extrapolated point A* determines the characteristic time of dissolution t_{max}.
Adapted from [28].

$$P = \frac{C_m V_0}{t_m C_s S} = \frac{2}{3}\frac{\rho}{C_s t_{\max}}\frac{ab}{b + \frac{a}{\varepsilon}\arcsin\varepsilon}$$

where $\varepsilon = \sqrt{[1 - (b^2/a^2)]}$, C_m is the external concentration of the material, V_0 is the volume of the solution, t_m is the time of dissolution, C_s is the saturation concentration, S is the total polyelectrolyte wall surface, ρ is particle material density, t_{max} is the time of dissolution when the external concentration of the material is maximal, and a and b are the particle radii in the case of ellipsoidal particles.

During the second step of the dissolution process, when the solid core is dissolved, the release process is determined by Fick's law [28]:

$$\frac{\partial c}{\partial t}V = -PS\Delta C$$

where the concentration gradient, $\Delta C = C_i - C_e$, consists of C_i and C_e, the concentrations in the interior and exterior of the capsule, respectively. It is important to conduct experiments under a uniform mixing regime in order to obtain a consistent solubility of the core.

3. Permeability of PEMs in two-dimensional structures

3.1 Effect of ionic strength on permeability of PEM films

The ionic strength of the polymer solutions and the washing buffer are of critical importance. The salt concentration during the build-up process dictates the multilayer thickness and film structure. Von Klitzing and co-workers [29] found that the thickness of LbL poly(allylamine hydrochloride) (PAH)/poly(sodium-4-styrene sulfonate) (PSS) (**Figure 2**) is directly proportional to the square root of the salt concentration. Furthermore, it was reported that the addition of more salt to the system after completion of the polyelectrolyte built-up process did not affect the final film thickness [29]. Similarly, Harris and Bruening [24] demonstrated that 20 bilayers of PAH/PSS prepared in the absence of salt had the same thickness as 4 bilayers prepared in the presence of salt. It is also interesting to note that greater film permeability is observed with the addition of salt [24]. With respect to film structure, Caruso and co-workers observed, using atomic force microscopy (AFM), considerable structural changes to the surface of a PAH/poly(acrylic acid) (PAA) (**Figure 2**) film when the ionic strength of the deposition solution was altered through the washing buffer. Moreover, the films produced showed considerable surface roughness [30]. However, relatively smooth multilayer films were formed when the salt concentration was maintained constant during the assembly process and the washing step. For such smooth films, subsequent exposure to pure water led to the introduction of regular, discrete and nanometer-sized pores [30].

When LbL films assembled primarily through electrostatic interactions are incubated in a salt solution, counter-ions and accompanying water molecules are usually incorporated into the films, causing swelling. A higher salt concentration causes a greater degree of swelling, which leads to a change in film morphology and an enhanced film permeability [31], while also contributing to multilayer film decomposition. The resulting dissolution is caused by the competition between polymer-to-polymer interactions, which hold the films together, as well as the polymer interactions with external salt ions [32]. Winnik and co-workers showed that the ionic strength alters film morphology. Increasing the ionic strength induces the reorganization of the surface topography from isolated spherical islets to elongated worm-like features [33]. Therefore, the ionic strength influences not only the range of the electrostatic forces but also their intensities. In many cases, a large increase in ionic strength can lead to dramatic weakening of interactions between polyelectrolyte layers, resulting in the destruction of the inter-polyelectrolyte complex.

With respect to the individual layer thickness, at low ionic strengths, the polymers are elongated at the surface and form thin films; whereas at high ionic strengths, they predominantly form loops and thicker films. It is interesting to note that the transport of rhodamine molecules through layers formed of flat polyelectrolytes, prepared without salt, was reported to be three times faster as compared with those composed of looped polymers [23]. The difference in permeability in this case is attributed to the size distribution of defects in the multilayers, from a few large defects at low ionic strengths to many small pores at high ionic strengths.

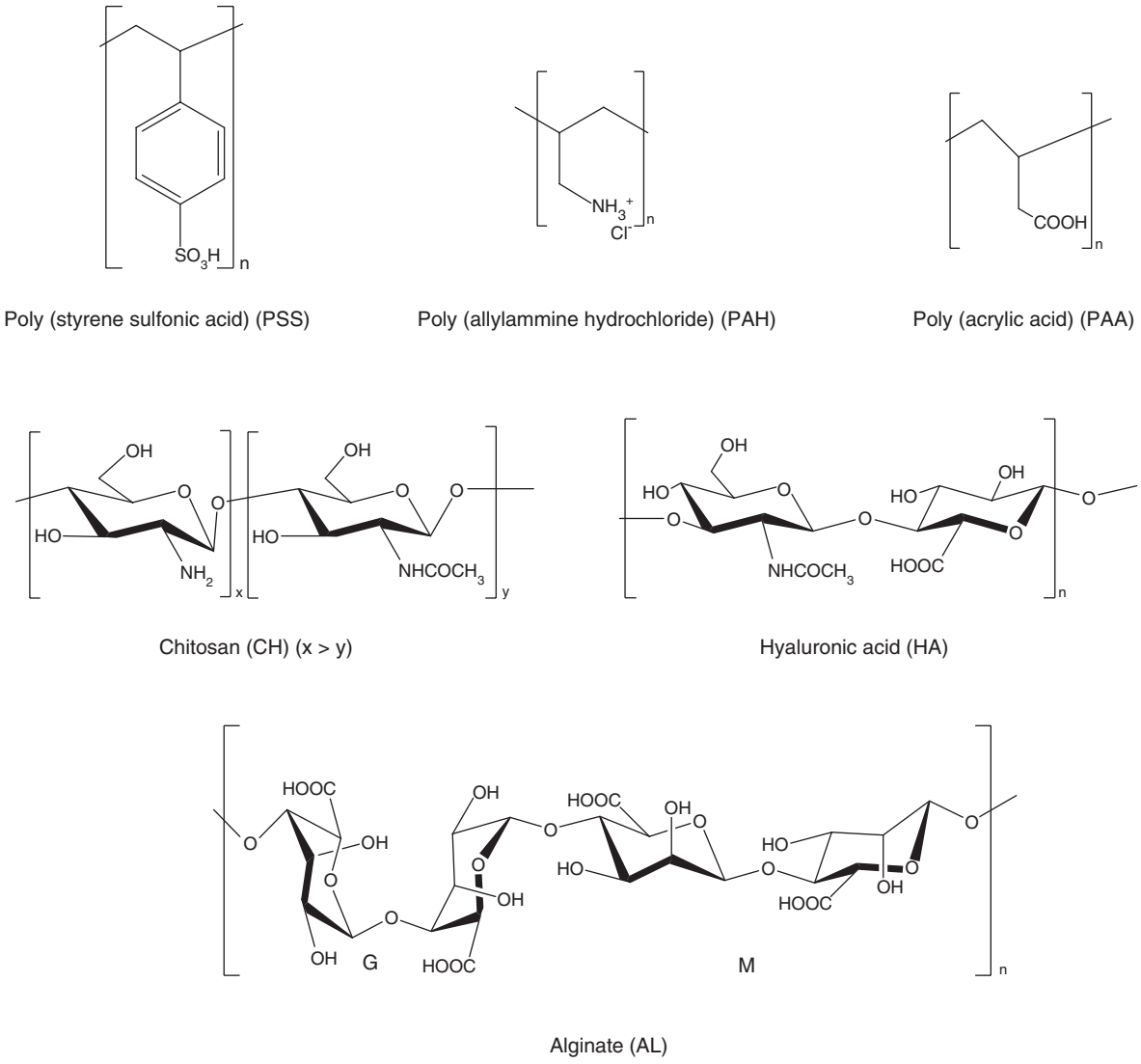

Figure 2. Polyelectrolyte structures.

3.2 Effect of film thickness on the permeability of the PEM films

Lynn and co-workers [34] investigated layer growth in poly(β-aminoesters) and DNA on surfaces modified with 10 bilayers of polyethyleneimine (PEI) and PSS. The results showed that films prepared using plasmid concentrations of 1 mg/ml were thicker than those prepared using concentrations of 0.5 and 0.25 mg/ml. Film thickness was thus modified through DNA concentration and controlled by varying the number of polyelectrolyte layers. The permeability decreases with increasing layer number at a much faster rate than that achieved linearly by an increase in thickness. The growth mechanism involves mainly electrostatic interactions between the polyelectrolytes from the solution and the outer layer of the film [25,35,36]. For an HA/CH multilayer system, an exponential increase of the optical film thickness is measured as the number of deposited bilayers increases [37]. The exponential growth of the HA/CH system relies on the diffusion of polyelectrolytes in and out of the film during each bilayer step. In this system, chitosan is the diffusing polyelectrolyte, whereas HA is the non-diffusing species. Furthermore, it is demonstrated that the multilayer thickness at a given stage depends on the sizes of both CH and HA. The assemblies of high-molecular-mass polysaccharides (HA, 360,000 daltons; CH, 160,000 daltons) were two times thicker than those obtained with their low-molecular-mass counterparts (HA, 30,000 daltons; CH, 31,000 daltons), giving rise to thicknesses of ~ 900 and 450 nm, respectively [38]. Film thickness and thus permeability are affected by polymer concentration and molecular mass as well as by the constituent number of layers.

3.3 Effect of pH and charge on the permeability of PEM films

pH-Responsive or pH-switchable membranes are now applied in filtration systems, membrane-based separations, bio-separations and sensors. pH has a direct effect on the ionization degree of the polyelectrolyte. Each dissociable polyelectrolyte is considered a potential vehicle for a charged ion, as they act in the dissociated state. The diffusion of charged ions through the multilayer is a result of site-to-site hopping on the dissociated polyelectrolytes. Therefore, the ions' permeability is dependent on their respective charges [28]. Moreover, the permeation rates of amino acids [39,40] and ionic drugs [41] through membranes with pH-sensitive chemical groups are greatly influenced by local pH and electrolyte concentration. The ionization state of the chemical groups can generate an excess of either positive or negative charges, giving rise to anion or cation-permselective membranes, respectively. Several research groups [41-43] have conducted theoretical and experimental studies of these ion-permselective membranes. Martin and co-workers [44-48] reported a pH-responsive transport membrane system consisting of gold nanotubules capable of altering between cation-transporting, non-ion-permselective, and anion-transporting states. This multi-bipolar architecture makes polyelectrolyte multilayer membranes especially suitable for ion separations. Krasemann and Tieke [4] showed that polyelectrolyte multilayer membranes composed of 10 – 60 bilayers of PAH/PSS have Cl^-/SO_4^{2-} selectivity factors as high as 45 in diffusion dialysis experiments. They suggested that Donnan exclusion resulting from fixed charges in the membrane is responsible for this selectivity. The strength of interactions is proportional to the charge density of the permeating ions and of the polyelectrolytes constituting the membrane. Elzieciak *et al.* studied the permeability of electroactive molecules (1,2-naphthoquinone 4-sulfonic acid sodium salt and 9,10-antraquinone-2,6-disulfonic acid disodium salt) to PEI/PSS films formed at pH 6 and pH 10.5 using an electrochemical technique [49]. They found that PEI is weakly charged at pH 10.5 but strongly charged at pH 6 and concluded that permeability of the film formed with strongly charged PEI is lower than that built with weakly charged polycation [49].

3.4 Effect of crosslinking on the permeability of PEM films

To control permeability through ion-transport selectivity in multilayered polyelectrolyte films, Bruening and co-workers [50-53] investigated the combined effect of crosslinking, hydrophobicity and charge density modification. Crosslinking was achieved by means of heat-induced amidation followed by hydrolysis of PAH and PAA polymers. Both the hydrophobicity and charge density of PAH/d-PAA films were controlled by partial esterification of PAA (d-PAA) [54]. The authors showed that the variation of the ester functionalities in PAH/d-PAA films, along with subsequent crosslinking and hydrolysis, yielded stable and highly dense -COO– groups in the films. As a result, a higher permeability for positively charged $Ru(NH_3)_6^{3+}$ ions was obtained than for $Fe(CN)_6^{3-}$ ions. They also studied controlled ion transport through PEM membranes by increasing the net fixed-charge density in the films [55]. Advincula and co-workers studied the effects of pH and crosslinking [56] on pH-responsive membranes and successfully induced film permselectivity by varying the pH of either the solution or the film itself (*ex situ*) following crosslinking. The crosslinking serves to stabilize the structure, while responsiveness to pH variation could be maintained. This approach is particularly versatile, as it allows for the preparation of weak polyelectrolyte capsules with stable structures for use in various applications including microcontainers, delivery vehicles, biosensors and bioreactors.

4. Permeability of PEM shells built within three-dimensional templates

4.1 Effect of ionic strength on the permeability of PEM shells

As discussed previously, the increase in salt concentration results in the weakening of electrostatic interactions between polyelectrolytes owing to the competing salt ions [57]. In three-dimensional constructs, the intercalation of salt ions promotes swelling of the capsule wall through the osmotic storage of water. The wall structure and interconnectivity is rendered loose and the mesh widths become larger, thus resulting in an increased diffusion rate of the encapsulated molecules. However, this effect is reversible. After the removal of salts, the capsules become impermeable to the probe macromolecules. This reversible permeability switch, induced by modifying salt concentrations, has been exploited to achieve capsule loading with macromolecules [57].

In other studies, the release of encapsulated fluorescein microcrystals from PSS/PAH capsules was strongly enhanced by increasing salt concentration [28]. More measurements at low concentrations of different salts showed that this effect could be attributed purely to electrostatic effects, but may also depend on further specific interactions of the salts with the capsule wall. However, in a different study, the release of ibuprofen (IB) from PEI/PSS and PAH/PSS multilayers decreased with higher salt concentrations [58]. In this case, as the IB solubility is reduced in salt solutions, a progressive and controlled release rate is observed as salt concentration is increased.

Generally, for PEM systems in which the pH is maintained, it has been suggested that the permeability obeys a power law $P \sim k\,[\text{salt}]^n$, where n is the ion charge. The ionic strength in the capsule wall may in principle be the sum of ionic strengths of the component parts: the buffer and the permeate [28]. Moreover, it is believed that permeation through polyelectrolyte multilayers is a function of the free energy of the inter-polyelectrolyte interactions and can be controlled by parameters responsible for the inter-polyelectrolyte complex formation. In a system with variable pH, it has been shown

Table 1. Loading methods of drugs and molecules in a polymer multilayer by means of LbL self-assembly technique.

Method of loading	Drugs/molecules	Polymers	Ref.
Core for LBL coating	Ibuprofen	(PEI/PSS), (PAH/PSS)	[58]
		(CH/DEXs)	[63]
	Furosemide	(PSS/PDDA) + (PSS/gelatin)	[59]
	Dexamethasone	(PDDA/PSS)	[61]
		(PDDA/gelatin)	
	Indomethacin	(PSS/PDDA)	[64]
Chemical attachment to polymer before coating	Paclitaxel	(HA-paclitaxel/CH)	[85]
Within nanoparticles	DNA/PEI nanoparticle	Poly(L-glutamic acid)	[86]
Embedding	BSA	(CH/AL)	[65]

that the permeability coefficient has a nonlinear dependence on the salt concentration. A pH shift changes the ratio between positive and negative charges within the multilayer and therefore loosens the PEM structure.

4.2 Effect of film thickness on permeability of PEM shells

Drug encapsulation with the LbL is conducted in conditions where the drug is not soluble. The encapsulated drugs are then subjected to appropriate soluble conditions that allow for their controlled release. Drugs are generally encapsulated within the outer layers and their release rate is a function of the encapsulating shell thickness. An increased number of layers in the LbL shell serves to increase the bioavailability of the poorly soluble drugs and maintain a sustained release in the host system [59].

Several studies have reported on the controlled drug release from LbL/PEM shells (**Table 1**). In one study, fluorescein dye microcrystals coated with PSS and PAH were used as a model system. Mohwald and co-workers showed that increasing the layer number resulted in decreased shell permeability and prolonged microcrystal dissolution [60]. Similar trends were observed with furosemide [59], dexamethasone [61] and IB [62] microcrystals encapsulated within a multilayered film (**Table 2**). In the case of the furosemide microcrystals, the reported release rate was 300-fold slower than the uncoated system [59]. For encapsulation, both shell thickness and diameter can be controlled with high precision, within a few nanometers, by varying the polyelectrolytes used in the assembly process [61]. The diffusion-controlled release mechanism of the microcrystals is a two-step process: first, diffusion of the bulk solution into the capsules to dissolve the drug crystals; and second, diffusion of the dissolved drug molecules out of the capsules (**Figure 3**) [61,63]. It is important to note that the core, or drug, dissolution is accompanied by an increase in osmotic pressure that alters polyelectrolyte multilayer shell properties [64].

Studies have also demonstrated the ability to design systems with multiple release kinetics. This has been shown recently by Tabrizian and co-workers using bovine serum albumin protein (BSA) encapsulated in liposomes coated with CH and AL [65]. As expected, the LbL structure retards the release rate of the protein. Furthermore, BSA is found to be encapsulated between the layers and inside the liposomes (**Figure 4**). As a result, the rate of release showed two distinct phases: an initial release from the shell, lasting 0 – 7 days; and a terminal phase with a core release of > 11 – 30 days [65]. Similar release trends were achieved with bone morphogenetic protein-7 (BMP-7) [66].

4.3 Effect of pH and charge on permeability of PEM shells

Li and co-workers showed that the permeability of L-R-dimyristoylphosphatidic acid (DMPA)/human serum albumin (HSA) microcapsules can be tuned by changing the pH value of the media [67]. In this system, HSA molecules take a different conformation and charge at low pHs of 2.5 – 4.5 and at higher pHs ranging from 5 to 7 [67,68]. At lower pHs, positively charged HSA is more likely to be exposed to the outside of the capsules and interact with the DMPA solution. At pH > 5, HSA is negatively charged and the interaction between HSA and DMPA is mainly repulsive. The latter less ordered structure shows an increase in permeability.

The use of pH changes to modify the charge of a molecule is generally applicable to both small and large molecules. A small change in pH for a molecule bearing multiple weak acid functionalities may have a significant impact on the diffusion of this molecule through PEMs. PEM shell systems can thus be designed to target the release of bioactive agents transported in this manner for targeted release in either an acidic or a basic environment [5]. For example, an amine-bearing drug molecule should be released more quickly in the host's intestine (higher pH) than in the stomach (lower pH) because it is neutral in the former and charged (protonated) in the latter. Chen and Lin showed that indomethacin crystals (**Figure 5**) encapsulated with poly(diallyl dimethylammonium) chloride (PDDA) and PSS displayed slower release in simulated gastric fluid (pH 1.4) compared with simulated intestinal

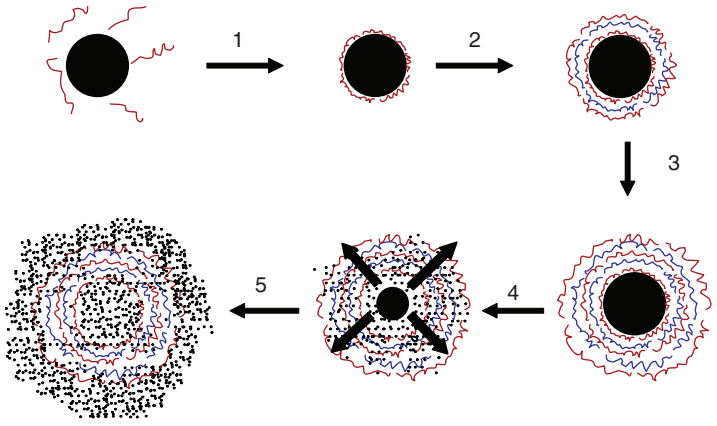

Figure 3. Scheme of the polyelectrolyte multilayer assembly and of the subsequent core dissolution. The initial steps (1 – 3) involve stepwise shell formation on a microcrystal core. After the desired number of polyelectrolyte layers is deposited, the coated particles are exposed to conditions where the core is dissolved (4), resulting finally in fully dissolved cores and remaining hollow capsules (5).
Adapted from [63].

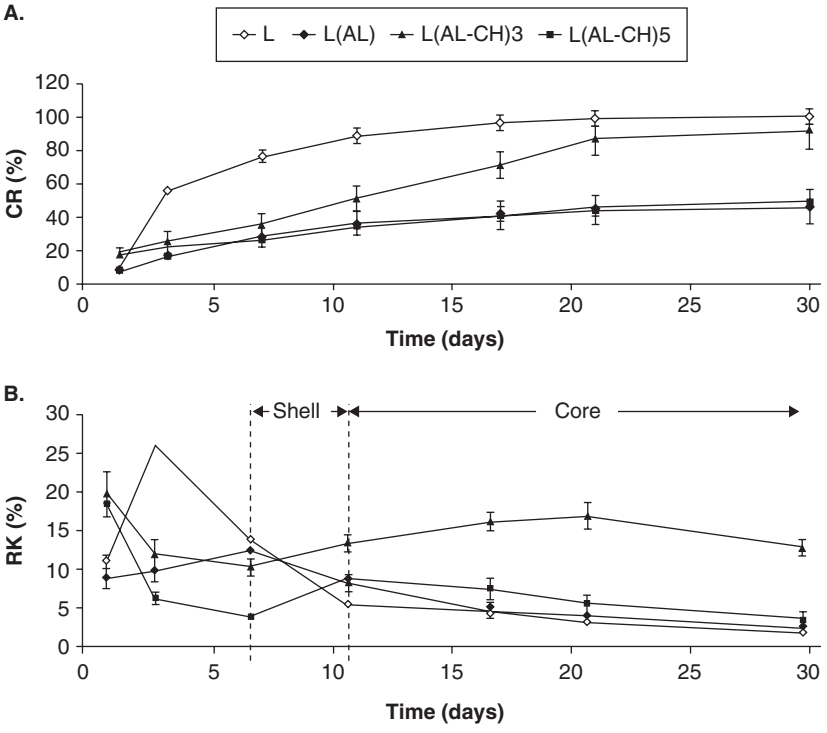

Figure 4. A. Cumulative percentage *in vitro* release profile (CR) of BSA-loaded uncoated and coated liposomes. **B.** Absolute percentage *in vitro* release (RK) of BSA-loaded uncoated and coated liposomes at each time point in UPW. Data represent mean plus or minus standard error of the mean [65].
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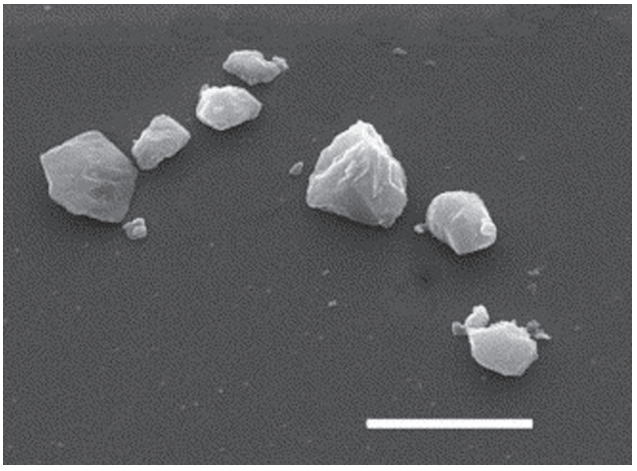

Figure 5. Scanning electron microscope image of indomethacin microcrystals. The scale bar corresponds to 10 μm [78].
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fluid (pH 6.8) (**Table 2**) [64]. Wang *et al.* demonstrated that PEM coating of $CaCO_3$ microparticles loaded with IB have a higher release at acidic pH where IB is not soluble [69]. At a neutral pH, these coated microparticles with five bilayers of PRO/PSS possess a slower release when compared with uncoated IB crystals [68].

Mohwald and co-workers [63] exploited the pH-dependent solubility of IB encapsultated with CH/AL, CH/dextran sulfate (DEXs) or CH/carboxymethyl cellulose (CMC) shell to vary concentration gradient. By raising the bulk solution pH, the release rate of IB increased with the solubility of IB. At pH 7.4, the solubility of the drug was very high and IB was released very rapidly (**Table 2**). At pH 1.4, where the IB solubility was very low, the release of the drug was prolonged (**Table 2**). This supports a model consisting of two release channels in this system; one by diffusion at pH 1.4 and the other by pressure through pore-like struc-tures at pH 7.4. Such pores were observed for thin, flat PAA/PAH films. Furthermore, at the molecular scale, it can be concluded that polyelectrolyte films are not smooth and may contain pores that are influenced by changes in pH or salt content [11,23]. Although pH plays a key role, it is important to note that drug release behavior is primarily governed by shell thickness.

Moreover, variation in pH was used to control the permeability of the weak polyelectrolyte (PAH) in PSS/PAH capsule walls in order to encapsulate macromolecules. PSS/PAH capsules were prepared at neutral pH [25]. Capsules could then be switched between open and closed states by varying the pH value; this influenced the interactions in the PAH/PSS wall and created local charges and defects in the film. Macromolecules could penetrate into the capsules at low pH, but are excluded at pH > 7. This reversible switching behavior, attributed to the protonization/deprotonization of the weak PAH, provides the opportunity to encapsulate different substances under mild conditions.

4.4 Effect of temperature and crosslinking on permeability of PEM shells

In drug delivery applications, LbL multilayer capsules are required to provide protection to the encapsulated drug from enzymatic decomposition in the human body while reducing drug toxicity to the non-targeted human organs [70,71]. Critically, drug activity must remain intact before reaching the target tissue. So far, few reviews have presented an overview of the development of new multilayer films and an understanding of their physical and chemical properties [2,72-74]. However, most of the polyelectrolytes used for drug delivery systems are biodegradable. This biodegradability damages the PEM multilayer and affects their permeability, and consequently the drug release kinetics [75-77].

To enhance the stability of AL/CH multilayer films, it is important to limit enzymatic desorption. This was accomplished by raising the deposition temperature in order to slow enzymatic activity effectively and in turn desorption and release rate. It was shown that increasing the deposition temperature from 20 to 60°C efficiently reduced the release rate of encapsulated indomethacin microcrystals (**Figures 5** and **6**) owing to the increase in the thickness and a better organization of the AL/CH multilayer film [78]. Moreover, crosslinking the AL/CH multilayer film with 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) significantly reduced the enzymatic desorption and the drug release rate [79]. Therefore, increasing deposition temperature and crosslinking neighboring layers are effective methods by which to protect LbL-assembled multilayer films from enzymatic erosion, as well as to prolong the release of the encapsulated drug.

5. Discussion and conclusion

Among the features governing PEM film characteristics, permeability plays a key role in drug release applications. However, owing to the lack of techniques to study the shell permeability of three-dimensional systems, investigations are mostly orientated towards understanding the parameters that affect the permeability of PEM films in two-dimensional systems. Ionic strength seems to be the most important parameter affecting directly and by various means the LbL structure and morphology. A high ionic strength enhances the PEM permeability to molecules, whereas a low salt concentration decreases the permeability [28]. Film thickness can also be controlled by varying the ionic strength of the solution and by the number of layers of polyelectrolyte deposited as well [23]. At low ionic strength, the polymers are elongated at the surface, forming a thinner individual layer thickness and thereby a thinner film, whereas at high ionic strength they predominantly form loops and consequently a thicker PEM film [80]. There is

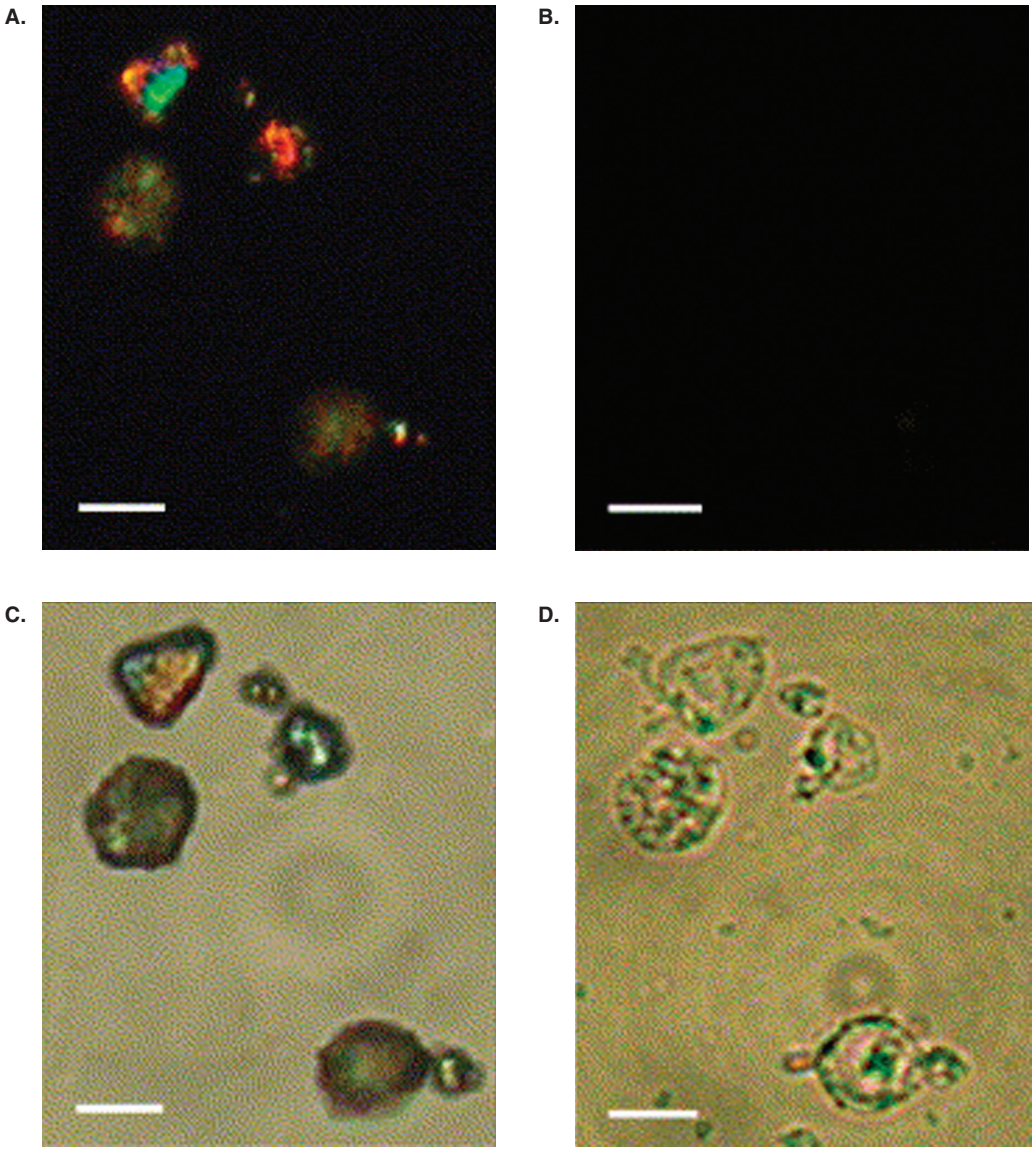

Figure 6. Optical microscope images following dissolution of the encapsulated indomethacin (IDM). Encapsulated IDM microcrystals (**A.**) and hollow capsules (**B.**) observed with crossed polarizer and analyzer. Encapsulated IDM microcrystals (**C.**) and hollow capsules (**D.**) observed under the normal light. All the scale bars correspond to 5 μm [78].

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also a linear increase of film thickness with the square root of salt concentration depending on the nature of polymers [81] at a given pH.

By modifying the pH and the ionization degree of polyelectrolytes, interactions inside the multilayered shell are altered, affecting the permeability. Strong polyelectrolytes possess more charge and in turn form stable multilayers. Weak polyelectrolytes, on the other hand, can be treated by means of high temperature or crosslinking in order to arrive at a functional stability.

The collective outcome of parameters affecting the permeability and controlled release of drugs is much more complex for polyelectrolyte LbL build-up on particulate templates. The drug release rate from these stable nanoshells is dependent on the solubility and the size of the drug, the number of layers and thickness of the shell, as well as on the type of polyion used in the LbL assembling process [58,59].

To discuss effectively the parameters influencing the permeability, one should also consider other factors, such as the nature of polyelectrolytes. As demonstrated by Lvov and co-workers, the use of gelating/polyelectrolyte bilayers displayed better sustained and controlled release of furosemide when compared with shells constructed from PSS/PDDA [59].

Table 2. Half-life release of several drugs encapsulated in polymers multilayer by means of LbL self-assembly technique.

Encapsulated	Conditions/polymers	Half-life drug release ($t_{1/2}$)	Ref.
Ibuprofen	pH 1.4/$(CH/DEXs)_{10}$	8 min	[63]
	$(CH/DEXs)_{20}$	18 min	
	$(CH/DEXs)_{30}$	30 min	
	pH 7.4/$(CH/DEXs)_{10}$	4.5 s	[63]
	$(CH/DEXs)_{20}$	7 s	
	$(CH/DEXs)_{30}$	9 s	
	$(PEI/PSS)_5$	8 – 9 s	[58]
	$(PAH/PSS)_5$	36 s	[58]
Furosemide	pH 1.4/$(PSS/PDDA)_2$ + $(PSS/gelatin)_6$	3.3 min	[59]
	$(PSS/PDDA)_2$ + $(PSS/gelatin)_4$	1.8 min	
	pH 7.4/$(PSS/PDDA)_2$ + $(PSS/gelatin)_6$	12 min	
	$(PSS/PDDA)_2$ + $(PSS/gelatin)_4$	1.2 min	
Dexamethasone	PBS, pH 7.2,/$(PDDA/PSS)_4$/PDDA	3.3 min	[61]
	$(PDDA/gelatin\ B)_4$/PDDA	45 min	
Indomethacin	PBS, pH 6.8/$(PSS/PDDA)_4$	2 h	[64]
	$(PSS/PDDA)_8$	5 h	
Insulin	pH 7.4/$(PMA/WSC)_8$	1 h	[87]

Filled capsules can serve not only in controlled drug release vehicles but also as bioreactors and biosensors [82,83]. Furthermore, the encapsulation of enzymes and proteins provides protection from inhibitors, proteases and bacteria that are unable to permeate the capsule wall. Encapsulated enzymes have also demonstrated higher activity when compared with their chemically immobilized counterparts, which retain only 50% or less of their optimal activity. Encapsulated glucose oxidase enzymes were able to maintain 60% of their activity as well as retain their native protein conformation [84].

6. Expert opinion

The controlled modification of permeability through LbL build-up of polyelectrolyte multilayers can be used effectively in applications within diverse areas, including drug delivery systems, biosensors, catalyst research and biotechnology. By adjusting the permeability through parameters such as ionic strength, pH, crosslinking, thickness, nature and molecular mass of polyelectrolytes, many issues associated with drug formulation and release could be addressed.

LbL assembly has the ability to modify a large range of surfaces at the nanometer scale, as well as the capacity to incorporate a wide array of macromolecules. This characteristic allows the LbL technique to be extended to biomaterials and biomedical device design. Current research interests involve surface modifications of stents and hip implants through the coupling of LbL techniques with biocompatible polymers in order to enhance implant biocompatibility and limit side effects. Continuing research is focused predominately on the investigation of coated endovascular devices by drugs, growth factors and bioreceptors embedded in multilayer films for site-specific release of drugs. For such an application, a sound understanding of PEM/LbL permeability and in turn macromolecule release is critical. In three-dimensional systems, PEM shell development is directed extensively towards drug encapsulation and controlled release by means of infrared, ultrasound or magnetic field activation. By using these external stimuli, the permeability of the PEM/LbL can be modified to hold or release the encapsulated drug or molecule.

Another application that exploits the adaptability of PEM permeability is the development of selective permeability that acts to exclude large macromolecules from interaction with a given template while permitting smaller molecules. This is particularity interesting and beneficial when living cells are used as templates. A potential application of this property is the immunocamouflage of red blood cells or encapsulation of pancreatic cells for insulin production. For example, live red blood cells can be encapsulated within a natural and biocompatible shell where immune system molecules are excluded while nutrient and ions permeate freely. The multilayered shell provides crucial immunoprotection, which serves to prolong survival and functionality.

These are a few examples of the many applications of LbL assembly, which holds great potential in the biomedical and biological fields of research. The versatile, biocompatible and controllable nature of LbL coating techniques, and the

underlying permeability in both two- and three-dimensional systems, are undoubtedly great assets for future development and optimization of a variety of biomedical devices.

Acknowledgement

S Mansouri acknowledges a postgraduate scholarship from the FRSQ.

Declaration of interest

This work is supported by the National Research and Engineering Council of Canada, Canadian Institutes for Health Research-regenerative Medicine/Nanomedicine and the Fonds Québécois de la Recherche sur la Nature et les Technologies in the framework of grants to the Centre for Biorecognition and Biosensors.

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