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# Emerging trend in nano-engineered polyelectrolyte-based surrogate carriers for delivery of bioactives

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***Importance of the field:*** In recent decades a new colloidal drug delivery system based on layer-by-layer (LbL) technology has emerged, which offers promising means of delivering bioactive agents, specifically biological macromolecules including peptides and DNA. Nano-engineered capsules specifically fabricated from biocompatible and biodegradable polyelectrolytes (PEs) can provide a better option for encapsulation of cells thereby protecting cells from immunological molecules in the body, and their selective permeability can ensure the survival of encapsulated cells.

***Areas covered in this review:*** This review encompasses a strategic approach to fabricate nano-engineered microcapsules through meticulous selection of polyelectrolytes and core materials based on LbL technology. The content of the article provides evidence for its wide array of applications in medical therapeutics, as indicated by the quantity of research and patents in this area. Recent developments and approaches for tuning drug release, biocompatibility and cellular interaction are discussed thoroughly.

***What the reader will gain:*** This review aims to provide an overview on the development of LbL capsules with specific orientation towards drug and macromolecular delivery and its integration with other drug delivery systems, such as liposomes.

***Take home message:*** Selection of PEs for the fabrication of LbL microcapsules has a profound effect on stability, drug release, biocompatibility and encapsulation efficacy. The release can be easily modulated by varying different physicochemical as well as physiological conditions. Scale-up approaches for the fabrication of LbL microcapsules by means of automation must be considered to improve the possibility of application of LbL microcapsules on a large scale.

**Keywords:** colloidal template, layer-by-layer technology, nano-engineered microcapsules, polyelectrolyte, stimuli responsive, zeta potential

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## 1. Introduction

Ever since their introduction in 1998, micrometer-sized capsules fabricated by layer-by-layer (LbL) assembly of polyelectrolytes (PEs) have found application in diverse areas, in particular for controlled release and targeted transport of active pharmaceutical ingredients, biotechnology to catalysis, synthetic chemistry and in diagnostic kits for

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**Article highlights.**

- Capsules prepared by means of the LbL technique have attracted particular interest, largely because of the ability readily to tailor their properties (e.g., size, composition, porosity, stability, surface functionality and colloidal stability), enabling control and triggered release of various therapeutic molecules by means of physicochemical triggers.
- Selection of template core has a crucial role in the determination of the stability of hollow capsules as well as their loading and release characteristics. Cores that are commonly used comprise those composed of synthetic and natural polymers, inorganic cores, biological cells and drug microcrystals.
- Polyelectrolyte capsules sensitive to small (and physiologically relevant) changes of pH, salt concentration, glucose concentration and redox potential should be fabricated for potential applications for *in vivo* systems. Self-exploding microcapsules have potential application of vaccine delivery.
- Click chemistry allow the fabrication of stable microcapsules without compromising the biocompatibility of polymers. The cores of LbL capsules can also be modified by incorporating micelles. In addition to this, thermosensitive microcapsules were also fabricated.
- Surface modification of LbL microcapsules can be done by the use of pre-modified polymers for LbL assembly or by modification of polymers covalently or electrostatically after fabrication of microcapsules.
- Recently, LbL technology has been used for coating of emulsion droplets, liposomes, and for fabrication of capsosomes, which combines the advantages of two systems, liposomes and polyelectrolyte capsules, and is expected to find diverse applications in biomedicine. LbL microcapsules have also been evaluated as adjuvant in vaccine delivery.

This box summarizes key points contained in the article.



various disorders (**Table 1**). Micro- and nanoencapsulation by LbL assembly can offer many advantages, such as controlled release, site-specific drug delivery, minimizing side effects, and protecting sensitive drugs [1,2]. The vast majority of studies concerning the LbL technique have used macroscopically flat charged surfaces as substrates for multilayer film formation [3].

Capsules prepared by means of the LbL technique have attracted particular interest, largely because of the ability readily to tailor their properties (e.g., size, composition, porosity, stability, surface functionality and colloidal stability), enabling control and triggered release of various therapeutic molecules by means of physicochemical triggers [4]. The fabricated capsules are particularly useful for delivery of proteins, enzymes [5], DNA [6] and siRNA [7]. In addition, the stepwise formation of these capsules allows the introduction of multiple functionalities, thus providing opportunities to engineer a new class of materials with unprecedented structure and function [8].

In addition, LbL capsules can also be assembled from a wide array of materials, including synthetic and natural PEs, nanoparticles biomacromolecules, dendrimers and metal colloids [6]. A range of colloidal particles with sizes varying from nanometers to many micrometers, and composition spanning biological cells, inorganic and polymeric particles to biomacromolecules or low-molecular-mass species, can be used as the particle templates [9].

In this review, various aspects of fabrication of micrometer-sized capsules by the LbL technique and their application in drug delivery, including controlled drug release and drug targeting, are focused on. The driving force for fabrication of LbL-based capsules could be electrostatic, electrodynamic and/or hydrophobic. The fabrication of these capsules is initiated with interaction of colloidal templates that are used as core and PEs that are used to coat the templates. Therefore, the selection of core material and the type of PEs are determining factors for biocompatibility and stability of these micrometer-sized capsules.

Despite all these advantages, there is not a single formulation in the market based on LbL technology, mainly because of the difficulty in scaling up the whole process and time and cost considerations as compared with other multiparticulate delivery systems, such as liposome nanoparticles and polymeric microparticles, as they are fabricated easily, leading to less loss of drug as well as fabricating materials.

## 2. Fabrication of polyelectrolyte capsules

Fabrication of microcapsules is based on the principle of the LbL self-assembly technique [1]. The LbL method permits the fabrication of multilayer thin-film assemblies on solid supports by the spontaneous sequential adsorption of oppositely charged species from dilute aqueous solutions onto charged substrates. The driving force for the multilayer film build-up is primarily due to the electrostatic attraction and complex formation between the charged species deposited [10].

Fabrication of capsules by LbL assembly of polyelectrolyte onto colloidal particles can be done by three different methods.

- The adsorption is carried in an excess of polyelectrolyte and the uncoated polyelectrolyte is removed by centrifugation or filtration.
- The concentration of polyelectrolyte added at each step is just sufficient to form a saturated layer and hence there is no need of separation of coated colloidal particles.
- The one-step approach is slow heterocoagulation of polymers in the presence of a colloidal-particle suspension.

The first method enlisted is one of the widely used methods for fabrication of microcapsules by the LbL technique [5,9,11]. The separation of coated core particles by centrifugation has drawbacks such as inconvenience in scale-up and automation. Practical problems such as difficulty in resuspension of particles, excessive loss of polyelectrolyte, difficulty in

**Table 1. Biomedical applications of LbL technology.**

| Application | Summary of work | Ref. |
|---|---|---|
| Diagnostics | Method for direct detection of DNA sequences. The capture DNA is immobilized onto the surface of a silica optical fiber tip by means of the LbL electrostatic self-assembly technique. Hybridization of target DNA with complementary capture DNA increases the optical thickness of the fiber tip | [119] |
| Diagnostics | Carbon nanotube thin film based device as a high-resolution acetylcholine sensor. Carbon nanotube and acetyl cholinesterase thin films are self-assembled on a silicon substrate as conducting and sensing layers, respectively. Both films are deposited using a LbL self-assembly process | [120] |
| Diagnostics | Glucose oxidase encapsulated by the LbL technique and immobilized onto a solid support for the construction of a biosensor demonstrating very fast sensor response | [121] |
| Diagnostics | Glucose oxidase immobilized in LbL films, adsorbed alternately with PAH layers, onto an ITO substrate modified with a PB layer. The ITO/PB/GOD-PAH heterostructures were tested in amperometric glucose biosensors, with a high sensitivity | [122] |
| Diagnostics | Proteoliposomes incorporating antigenic membrane proteins of *Pasteurella multocida* have been successfully immobilized in LbL films. Detection was carried out using a new strategy based entirely on capacitance measurements, and to enhance sensitivity | [123] |
| Glucose-responsive systems | Phenylboronic acid was used to prepare a glucose-sensitive polyelectrolyte. This polyelectrolyte was used in combination with PSS to fabricate hollow polyelectrolyte capsules. Capsule decomposition would occur only in those cases where threatening glucose levels were reached | [124] |
| Biocompatibility enhancement | LbL films comprised of HA/collagen promote cortical neuron adhesion on glass; these HA-based multilayer PE films or similar build-ups could thus be used in the future as a way to modify surfaces for nerve scaffolds and neuron-based chips | [38] |
| Biocompatibility enhancement | Collagen/poly(acrylic acid) bilayers were added to semiconductor NPs CdTe/polycation LbL films to produce porous collagen bilayers. Such stratified multilayer systems showed successful cell attachment and survival, reducing toxicity of CdTe/polycation LbL films | [125] |
| Biocompatibility enhancement | Surface engineering of titanium was successfully achieved via LbL deposition of chitosan/silk fibroin pairs, and enhanced its cell biocompatibility | [126] |
| Biocompatibility enhancement | LbL deposition of chitosan and dextran sulfate on thermoplastic polyurethane film. The coated film resisted the platelet adhesion and HPF adsorption, thereby prolonging effectively the blood coagulation times | [127] |
| Biocompatibility enhancement | LbL coating by PLL/PAH on a contact lens composed of polysiloxane to increase hydrophilicity and enhance wearer's comfort and ocular health | [128] |
| Biocompatibility enhancement | PEMs electrostatically constructed from PAH and PSS with gelatin, fibronectin and PSS promoted the attachment and further growth of smooth muscle cells, and that this property is dependent on the number of layers in the underlying multilayer film architecture | [129] |
| Biocompatibility enhancement | Surface modification of stents by the LbL technique to increase its biocompatibility as well as hemocompatibility | [125] |
| Tissue engineering | Multilayer nanofilms, formed by the LbL adsorption of positively and negatively charged polyelectrolytes, promoting function of hepatocytes | [130] |

HA: Hyaluronic acid; HPF: Human plasma fibrinogen; LbL: Layer-by-layer; NPs: Nanoparticles; PB: Prussian blue; PE: Polyelectrolyte; PEMs: Polyelectrolyte multilayers.



sedimentation of low-density particles and length of time required for each coating may also reduce acceptability of the method. Another method suggested for separation of coated core particles is by means of filtration [12]. Filtration can easily be applied to large-scale processing, but one has to keep the particles suspended over the total time interval of adsorption and all washing cycles. Aggregation and/or filter cake formation have to be prevented or minimized under all circumstances. A fraction of the microcapsules, at least their outermost layers, is destroyed during resuspension of aggregate by long-lasting stirring and/or ultrasound. This could result in partial recharge of a fraction of microcapsules or a fraction of the surface of the microcapsules, promoting aggregation with other microcapsules possessing the original charge of the undamaged microcapsules. Interaction of PEs and polyelectrolyte-coated particles with materials used for fabrication of filters is of great concern in this context.

In other methods, which involve sequential addition of PE in exact concentrations onto the core particle dispersion, the step of removal of excess of polyelectrolyte is eliminated. The

advantage of this process is faster fabrication of polyelectrolyte capsules and reduction in loss of polyelectrolyte [13].

## 3. Colloidal templates

Selection of the template core for the fabrication of polyelectrolyte microcapsules by LbL technology is a crucial step in the successful fabrication of LbL microcapsules and will have a crucial role in the determination of stability of hollow capsules as well as their loading and release characteristics.

A suitable colloidal core template must have the following properties.

- It should be removed completely after fabrication as required.
- The conditions required or the chemicals used for removal of core should not affect the chemical nature or physical properties of coated PEs.
- The core template used for fabrication of LbL should not lose its stability under the condition of fabrication of microcapsules.

According to chemical nature and biodegradability, the core can be classified into the groups in the following subsections.

### 3.1 Synthetic crosslinked polymers as templates

Synthetic polymers provide a variety of materials that can be used for the fabrication of LbL microcapsules. The most widely used template of this class belongs to weakly crosslinked melamine formaldehyde (MF) [14]. These templates are available commercially and are stable at pH > 5 so PE coating can be done easily at neutral pH. The templates can be dissolved by using 0.1 N HCl solutions. These cores possess positive charge on the surface so the first coating applied is usually of anionic polyelectrolyte. The core decomposition products were protonated melamine oligomers, which were found to be toxic [15]. The main disadvantage with MF cores is that complete removal of melamine oligomers is very difficult owing to the large size and strong positive charge of the degradation product. The residing MF oligomer-generated osmotic stress occurring on dissolution of MF cores may cause rupture of the PE multilayer shell. This also restricts the number of PE layers to be adsorbed on the MF core. It has been observed that the capsule stays intact during the dissolution process only if it is assembled of not more than 8 – 10 polyelectrolyte layers [9]. Colloidal polystyrene (PS) latex has also been used for fabrication of LbL capsules. The PS latex is removed by using tetrahydrofuran (THF). The swelling of polystyrene in THF leads to a large volume increase that is directly responsible for capsule fractures. In addition, the use of organic solvents is not considered suitable for drug delivery applications [16].

A biodegradable polymer core consisting of polylactic acid/polylactic-*co*-glycolic acid (PLA/PLGA) has also been used as a template for fabrication of capsules by LbL technology, which can be removed by using an acetone/*N*-methyl-2-pyrrolidinone mixture. The main disadvantage is that monodispersity is low with PLA/PLGA as core templates and residue remains after removal of the core [17]. Soft and porous temperature-sensitive poly(*N*-isopropylacrylamide) (PNiPAM) microgel has been coated by the LbL assembly of polyelectrolyte multilayers. In addition to this, positively charged dextran-hydroxyethyl methacrylate/dimethyl aminoethyl methacrylate (dex-HEMA-DMAEMA) microgel has also been used as a template that shows pH-dependent bursting of PE microcapsule [18].

Other synthetic polymers that can be used as a template for the fabrication of LbL microcapsules must have biocompatibility and biodegradability as important properties owing to the non-sacrificial nature of those templates; some examples are microspheres fabricated from PLGA microparticles eudragit and ethylcellulose microparticles for oral delivery of therapeutic agents.

### 3.2 Natural polymers as templates

A wide variety of natural polymers can be used as templates, spanning from ionically crosslinked polymers to covalent and heat-stabilized microspheres. Natural polymers have the added advantage of biocompatibility with macromolecular drugs such as enzymes and DNA and also offer high encapsulation efficiency.

Chitosan microspheres were used as a template for the fabrication of chitosan/poly(styrene sulfonate) (PSS) polyelectrolyte capsule loaded with heparin [5]. Polyornithine-coated alginate microcapsules were also used as core to deposit PE multilayers, which has resulted in the successful formation of robust and stable microcapsules around cell spheroids prepared from an immortal cell line [19]. Other polymers that can be used for the fabrication of microspheres and ultimately for application templates for the fabrication of LbL microspheres are crosslinked gelatin, starch, hyaluronic acid, condritin sulfate and alginate microspheres, providing a biocompatible template encapsulating a wide variety of therapeutic agents, ranging from small-molecule to macromolecular therapeutic agents. In addition, various charged solid lipid microparticles can also serve the function of templates effectively, which can effectively be used for lipophilic therapeutic agents.

### 3.3 Inorganic templates

The use of inorganic core templates for the fabrication of polyelectrolyte microcapsules has increased in the current scenario owing to the fact that these inorganic templates can easily be removed and removal does not affect the integrity and chemical structure of the PE coating. As a consequence, these capsules can comprise a large number of layers and have a permeability coefficient significantly lower than that of the latex-templated capsules. The mechanical stress during core removal was found to be negligible.

At present, different carbonate particles ($CaCO_3$, $CdCO_3$ and $MnCO_3$), $SiO_2$ and calcium phosphate particles have been used as templates for PE multilayers. The advantage of

$SiO_2$ particles is that they are available with a broad size range and are monodisperse in nature. The removal of $SiO_2$ core particles is done by hydrofluoric acid and results in the formation of $[SiF6]^{2-}$, which can readily leave the capsule shell without any problem because of its smaller size. Other inorganic templates that can be used as a sacrificial template are ZnO, $ZnCO_3$, zinc oxalate, $Al_2O_3$ and calcium oxalate microparticles, which can easily be removed by acid treatment and specifically can be used for topical and oral administration.

Inorganic carbonates are also widely used for ($CaCO_3$, $CdCO_3$ and $MnCO_3$) owing to their ease of removal under mild conditions, thus preserving the activity of encapsulated macromolecules such as enzymes, proteins and DNA. The inorganic carbonate core can easily be removed by using EDTA solution or 0.1 N HCl [20]. Size-controlled, low-dispersed calcium carbonate microparticles were synthesized in the presence of the amphiphilic block copolymer polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA) by modulating the concentration of block copolymer in the reactive system. These types of hybrid microparticle have acid-resistant properties, but these templates can be removed by trisodium-EDTA solution (pH 7.28) [21].

### 3.4 Biological templates

Biological cells represent a monodisperse and naturally very cheap source of templates for capsule production. Human erythrocytes are widely used as a biological template. They have several advantages over conventional templates as they have a non-spherical shape, they are economical, and monodispersity is also very high; but they have a very fragile membrane, which can rupture during fabrication of LbL microcapsules, so the membrane is stabilized by crosslinking with glutraldehyde [22]. The decomposition of the biological templates can be achieved by sodium hypochlorite solution (pH 12). However, it was found that, under these harsh conditions, the polyelectrolyte wall is also oxidized. This oxidation causes the loss of all the positive charges and of some polyelectrolyte material; in addition, it results in a crosslinking of the wall materials, so the changes in chemical nature of PE microcapsule are rather unpredictable. In addition to red blood cells (RBCs), yeast cells [23] and *Escherichia coli* [24] are also used as templates for the fabrication of LBL microcapsules. The main drawback for using biological cells as templates is that it is very difficult to retain stability and activity of therapeutic moieties, specifically of biological molecules during the core removal process. The other types of biological template that can be used are platelets derived from blood, but the main disadvantage with them is their irregular size.

### 3.5 Drug microcrystals as templates

Drug microcrystals have also been used as a core for fabrication of polyelectrolyte microcapsules, which results in modulation of release characteristics and stability of drug microcrystals. Naproxen microcrystal has been coated with PEs such as gelatin A and dextran sulfate, which resulted in prolongation of release of drug in dissolution media. Similarly, furosemide microcrystals (5 μm) were encapsulated with 55 – 125-nm-thick gelatin/polyanion multilayer shells to achieve sustained drug release [25]. Dexamethasone [26], ketoprofen [27], ibuprofen [28] and indomethacin [29] have also been used as templates for the fabrication of colloidal LbL assemblies. PSS/PAH polyelectrolyte capsules were fabricated using fluorescein particles as templates at pH 2 followed by core dissolution at pH 8 [30]. Other drug crystals showing pH-dependent solubility, such as glibenclamide, *p*-amino salicylic acid, valproic acid and a variety of sulfonamides can also be used, and removal of drug core can be accomplished by treating with pH 8 buffer; or drug crystal that can be dissolved by varying temperature or salt concentration can also be used for the fabrication of polyelectrolyte capsules. The authors have tried LbL coating of hesperitin microcrystals with PS and sodium alginate (SA) bilayers. Other flavanoids can also be used as templates for LbL coating. Other drugs insoluble in water such as felodipine can also be used as templates; vitamins A and E can also be used for coating by the LbL technique in the form of emulsions.

## 4. Polyelectrolytes used for coating of core by LbL technology

Polyelectrolytes are polymers having ionically dissociable groups, which can be a component or substituent of the polymer chain. Polyelectrolytes for fabrication of LbL-based micrometer-sized capsules should have at least 50% charged monomer species. Linear or branched PEs can be used for coating of colloidal particles. Branched PEs can lead to less compact polyelectrolyte multilayers having a higher degree of wall porosity [31]. Polyelectrolyte molecules can be crosslinked within or/and between the individual layers, for example, by crosslinking amino groups with aldehydes, increasing capsule stability [32,33]. A strong polyelectrolyte with high charge density can form microcapsules with better colloidal stability, whereas weak PEs are more coiled and can form microcapsules with better encapsulation efficiency and temperature stability [34].

The structure of the polyelectrolyte layers assembled by LbL technology is fuzzy in nature and molecules experience a high level of interpenetration and disorder. Their main zones could be distinguished in the structure of the multilayers. The first zone is composed of the two to three layers that are next to the surface and is characterized by a high level of order and narrow thickness. Zone 2 forms the bulk of the layers. Zone 3 is the interfacial region in contact with the solvent and is characterized by a low level of order and the presence of chains extending into the solvent [35].

Polyelectrolytes that are mainly used for the fabrication of LbL microcapsules can be classified as in the following subsections.

### 4.1 Synthetic polyelectrolytes

Synthetic PEs are widely used in the construction of LbL films on various templates, including planar surfaces, colloidal templates and intravascular stents. Synthetic PEs can be either biodegradable or non-biodegradable. Synthetic PEs have the advantage of having reproducible properties in terms of charge density and monomer composition, but their biocompatibility with tissues and blood has not been studied extensively. Some synthetic polymers have been reported to be cytotoxic.

PSS is an anionic polyelectrolyte and it is widely used in the coating of colloidal and planar substrate by LbL methodology [36]. PSS is a larger, bulkier PE and produces five times thicker layers than protamine sulfate. The added thickness and mass potentially lead to the greater surface charge observed in the case of PSS-coated particles in comparison with layers comprising protamine sulfate in PSS/protamine sulfate microcapsules [37]. Stable hollow polyelectrolyte capsules were created by using PSS along with poly(allylamine) hydrochloride (PAH) [38,39], and poly(diallyldimethyl ammonium chloride) (PDADMAC) [40] and gelatin [25].

PSS/PDADMAC-coated polyelectrolyte microcapsules are sensitive to annealing and cooling, as well as to electrolyte exposure in comparison with hollow polyelectrolyte capsules composed of alternating PSS and PAH, owing to the porous microstructure and weak bonds of the ion pair between PDADMAC and PSS. PSS/PDADMAC-coated polyelectrolyte microcapsules were found to have a larger capsule size than the dimension of the templates, whereas the PSS/PAH capsules always had a similar size to that of the template. The former also have a rough surface texture as compared with PSS/PAH capsules [40]. The special swelling–shrinking behavior of the capsules may benefit the loading of substances such as nanoparticles and macromolecules with rigid structure or larger molecular size. This could result in capsules for versatile applications as micro-carriers in the fields of drug delivery and controlled release, catalysis, as well as artificial cells [41,42]. Hollow capsules prepared from PDADMAC/PSS on MF core were found to rupture during the core removal process owing to an increase in osmotic pressure inside the capsule. It was found that a smaller colloidal template (3.8 μm) produced more stable capsules, whereas if the size of template was increased to 5.6 μm, the capsules ruptured [40]. In addition, PSS/PAH capsules were fabricated and used for encapsulation of urease enzyme for the fabrication of microreactor capsules [15].

When PSS was used as the first polyelectrolyte on colloidal template, capsules were filled with negatively charged PSS, buffering the interior of the capsules at pH 2 – 2.5 [39], which could be used for selective precipitation of acidic drug or macromolecules that precipitate at acidic pH (**Figure 1**). Anticancer drugs daunorubicin and doxorubicin were successfully incorporated into the multilayer microcapsules having negatively charged PSS molecules, causing entrapment of drug inside hollow microcapsules owing to lower pH [20]. Spontaneous deposition was observed as a general phenomenon for polyelectrolyte capsules templated on MF particles, rather than being an occasional result, regardless of positively charged species displaying a more prominent tendency for self-deposition. The existence of the PSS/MF complex provides an extra driving force that induces water-soluble substances, especially positively charged species, to penetrate through the capsule wall and deposit around the complex in aggregated form, so that the concentration of the substance within the interior of the capsule is always lower than in the bulk [43]. PSS/MF complex may also cause degradation of drug and proteins susceptible to acidic pH. Another drawback with sodium PSS is that it can cause digestive complications such as intestinal necrosis or perforation [44].

The LbL adsorption process for the preparation of a PSS/PAH PE multilayer membrane on the conventional polyornithine-coated alginate microcapsules has resulted in the successful formation of robust and stable microcapsules around cell spheroids prepared from an immortal cell line [19].

PAH is a weak cationic polyelectrolyte widely used in the coating of colloidal and planar substrate by LbL methodology along with PSS. Polyelectrolyte capsules of PSS/PAH bilayers prepared on weakly polymerized MF colloidal particles have been incubated in solutions of different salts. The most remarkable effect on size, wall thickness and permeability of the capsules was found after incubation in solutions of carbonate salts. The capsules became almost impermeable for FITC-labeled dextran of low molecular mass and fluorescein. Also, a reversal of the surface charge from positive to negative was measured for all samples treated with carbonates [45].

The stiffness of both 'hollow' and 'filled' (with PSS) microcapsules composed of PSS/PAH multilayers was found to be largest in water. It decreases with NaCl concentration up to 3 mol/l and becomes quasi-constant in more concentrated solutions [46]. A polyelectrolyte capsule fabricated on human erythrocytes by using PAH and PSS preserved both the size and shape of the cell templates [47].

Disulfide crosslinked polypeptide capsules of 12 bilayers were fabricated using cysteine-containing positive and negative peptides. It was proposed that the biocompatibility and immunogenicity of polypeptide microcapsules will be more favorable for biomedical applications of LBL structures than those made from more usual organic PEs, for example, PAH and PSS [48]. New designed peptides where having reduced tendency to form secondary structure owing to the presence of a large number of glycine residues are more suitable because of reduced immunogenicity as compared with PLL/polyglutamic acid multilayers for the formation of thin films by LbL [49].

Addition of PEG300 to the polypeptide adsorption solutions significantly increased the efficiency of protein loading in nano-engineered polypeptide microcapsules [50]. PAH/PSS polyelectrolyte-coated hollow capsules can be prepared using erythrocytes as templates that can be used for the delivery of drugs or enzymes and DNA [51]. Hollow

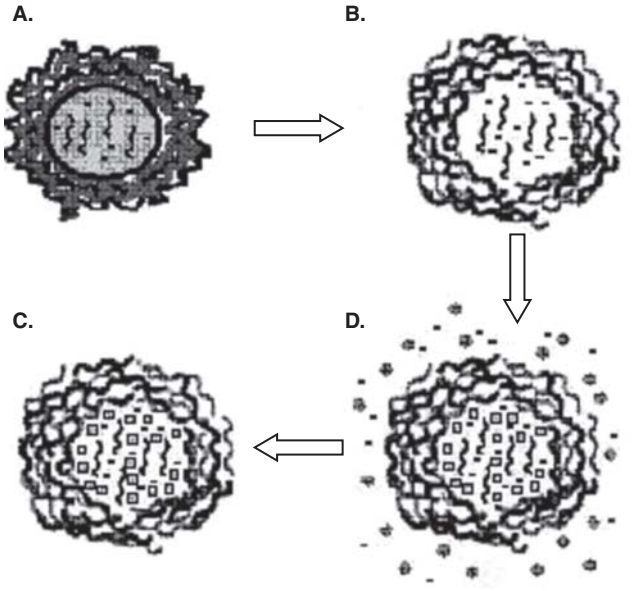


**Figure 1. Spontaneous loading of acidic drugs. A.** Polyelectrolyte capsules having core containing strongly anionic polyelectrolytes (for ex PSS). **B.** Hollow microcapsules containing strongly anionic polyelectrolytes after dissolution of core. **C.** Spontaneous loading of therapeutics in precipitated form inside hollow capsules by virtue of lower pH inside capsules. **D.** Capsules loaded with drug after removal from drug solution.

polyelectrolyte capsules composed of PSS/PAH multilayers were found to be permeable to low-molecular-mass charged molecules and impermeable to high-molecular-mass PSS [52].

The shell-wall permeability of small polar molecules through PAH/PSS LbL microcapsule templated on erythrocytes was controlled by adsorbing lipids along with PE multilayers [53]. Caruso *et al.* [54] fabricated protein multilayers by exposure of the polyelectrolyte-coated PS latex particles to protein solution. FITC-BSA was deposited onto (PDADMAC/PSS/PDADMAC)-precoated PS latex particles, and IgG onto $(PAH/PSS)_2$ precoated particles for proposed immunological detection methods and for flow cytometry application [55].

A LbL system with self-disintegrating microcapsules has been fabricated by encapsulating a highly active mix of proteases (pronase) into polypeptide LbL microcapsules. Pronase was co-precipitated into $CaCO_3$ microparticles that were subsequently coated with poly(L-arginine)/poly(L-aspartic acid) multilayers [56]. The enzyme was released into the capsules' interior, a consequence of core removal triggering digestion of polypeptide capsule walls. The biggest advantage of this method is that, by varying the amount of encapsulated pronase, lifetimes of such self-disintegrating capsules can successfully be adjusted from seconds and hours to days, thus the sustained release of the co-encapsulated DNA or other macromolecule can be adjusted.

PVP/PMA multilayer capsules based on hydrogen bonding have been prepared by the LbL approach and used to encapsulate and release rifampicin. Removal of the silica core has been done using a buffer of ammonium fluoride and HF at ~ pH 3, which was found to produce better capsules than HF alone [57]. An eight-layered capsule had a wall thickness of 20 nm. Release studies showed a burst release, and maximum release was obtained at > pH 7, where the capsules disintegrate rapidly, thereby releasing the drug in a short period.

### 4.2 Natural polyelectrolytes

Naturally occurring PEs are found to be more biocompatible, hence they are used widely for the fabrication of nano-engineered microcapsules for drug delivery and tissue targeting. They are generally of microbial or animal origin. The anionic PEs such as dextran sulfate and sodium alginate are of microbial origin, whereas cationic PEs such as protamine sulfate, gelatin A and chitosan are of animal origin **Table 2**. The main disadvantage with natural PEs is that their properties vary widely depending on the source or other processing conditions; but they are generally cheaper than synthetic PEs and easily available. Hyaluronic acid (HA)-based PE films were found to have the property of being able to support neural cell adhesion and neurite development, especially for the polycation-ending films. It is suggested that HA-based multilayer PE films or similar build-ups could thus be used in the future as a way to modify surfaces for nerve scaffolds [38].

Stable polyelectrolyte capsules were produced through LbL assembling of biodegradable PEs, dextran sulfate (DS) and protamine sulfate. It was observed that by increasing pH from 3 to 8, the permeability as well as the size of capsules were increased, thereby increasing loading of horseradish peroxidase enzyme [58]; encapsulated peroxidase showed a high activity (57%), which remained stable for 12 months.

Natural PEs have a profound effect on capsule permeability owing to their molecular mass and charge density, which ultimately determine the conformation of PEs. Gelatin is a bulky, high-molecular-mass protein having low charge density and isoelectric point ~ pH 8; therefore, it has a small positive charge at pH 7.4, resulting in the formation of a loopy conformation at coating conditions (pH 5.0), consequently leading to adsorption of several monomolecular protein layers, resulting in increased layer thickness. This was not the case when chitosan was used with DS or PAA [59]. The surface charge on gelatin would preferentially be reduced when switched from condition of assembly (pH 5.0) to conditions of dissolution experiment (pH 7.4), resulting in shrinking of the bulky molecule, thus closing the probable defects and pores. Lack of repair of the LbL multilayer in combination with reduced thickness resulted in faster release rates for the chitosan-polyanion-coated naproxen microcrystals as compared with that of gelatin A-polyanion-coated microcrystals.

Similarly, when 5 µm furosemide microcrystals were microencapsulated with 55 – 125-nm-thick gelatin/polyanion

**Table 2. List of naturally occurring polymers used as polyelectrolytes for fabrication of LbL microcapsules.**

| Name of polymer | Properties | Ref. |
|---|---|---|
| Hyaluronic acid | Alternating copoly(β-D-glucuronic acid-β-D-*N*-acetyl-glucosamine. Chemically grafted polyelectrolyte multilayer consisting of the natural biopolymers CHI and HA is resistant to a range of proteins when the multilayer is terminated by HA. Such surfaces are believed to resist nonspecific protein adsorption owing to the highly hydrated state of the uppermost HA layer | [38] |
| Protamine sulfate | A polypeptide positively charged owing to a high content of arginine, which is up to 70% of the total amount of amino acids. It is of natural origin hence widely used in LbL assembly | [26,37,58,110] |
| Chitosan | Chitosan is a linear polysaccharide composed of randomly distributed β-(1 – 4)-linked D-glucosamine and *N*-acetyl-D-glucosamine. Insoluble in basic media, high charge density at pH < 6.5; excellent mucoadhesive and used for LbL capsule formation for a variety of applications | [5,24,25,28,59,65] |
| Sodium alginate | Sodium alginate consists chiefly of the sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic acid. A weakly anionic polyelectrolyte widely used as material for fabrication of LbL coating owing to its biocompatibility; cannot be used for fabrication of LbL microcapsules at pH < 3 | [22,29,62] |
| Dextran sulfate | Sulfated polysaccharide of microbial origin widely used as a polyelectrolyte for LbL coatings in drug | [28,58,59] |
| Gelatin | Bulky, high-molecular-mass protein having low charge density and isoelectric point ~ pH 8, resulting in formation of a loopy conformation at coating conditions (pH 5.0), consequently leading to adsorption of several monomolecular protein layers, resulting in increased layer thickness | [59] |
| DNA | Consists of two long polymers of simple units called nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds. Owing to phosphate groups, they are negatively charged | [6,47,56,60,61,119] |
| λ-Carrageenan<br>ι-Carrageenan | A linear polysaccharide made up of of β-D-galactopyranose and α-D-galactopyranose residue. It contains sulfated residues. ι-Carrageenan keeps its helical conformation within the films whereas λ-carrageenan chains are in random coil conformation in LbL films | [131] |
| Chondroitin sulfate sodium salt | Chondroitin sulfate is a sulfated GAG composed of a chain of alternating sugars (*N*-acetylgalactosamine and glucuronic acid). It is usually found attached to proteins as part of a proteoglycan. Promotes condrogenesis | [132] |

CHI: Chitosan; GAG: Glycosaminoglycan; HA: Hyaluronic acid; LbL: Layer-by-layer.



multilayer shells, sustained drug release was achieved when compared with dissolution of bare furosemide microcrystal [25]. Encapsulation of ibuprofen with chitosan/DS (15 bilayers) also resulted in minimal sustained release [28]. Adsorption of two to six gelatin/PSS bilayers on PDDA/PSS-coated ketoprofen microcrystals resulted in a corresponding capsule wall thicknesses ranging from 41 to 111 nm, resulting in a drastic decrease in release of drug (at pH 7.4) as compared with uncoated ketoprofen [27].

Nucleic acids are negatively charged and are used as coating materials. DNA/PLL (polylysine) capsules were used as dual drug carriers for concurrent delivery of DNA and FITC-dextran molecules by salt-triggered capsule decomposition. The larger the salt concentration, the more DNA and drug is released [60]. DNA and drug (dextran) release from DS/PLL microcapsules was triggered by enzymatic degradation using α-chymotrypsin that was incorporated into the colloidal template, and drug payload was effectively discharged by degradation of PLL [61].

Ciprofloxacin HCl-loaded ultrathin capsules were prepared by coating sodium alginate/PAH multilayer in a solid core (calcium phosphate or RBC) to achieve a prolonged action of the drug. The sodium alginate/PAH capsules were found to be very sensitive to the environmental condition, for example, temperature, salts (electrolytes and non-electrolytes) and even protein. For optimal stability of ultrathin capsules, 0.154 M NaCl and 5.29 M mannitol solution was found to be necessary [22].

Shao *et al.* used chitosan (CS) microspheres as templates for microcapsules prepared by LbL self-assembly technique using PSS and chitosan. This type of microcapsule may provide a new and effective system of heparin delivery [5]. A new type of dual-coated polyelectrolyte shell has been fabricated by coating sodium alginate-coated $CaCO_3$ microparticles (prepared by a non-solvent addition process) with SA/chitosan or DS/chitosan bilayers [62]. Owing to the presence of a sodium alginate inner layer, however, only two pairs of polyelectrolyte bilayers (required to prevent interparticulate adhesion) were able to retain the protein load.

Fast separation of superparamagnetic nanoparticles containing liposomes encapsulated by a LbL technique from unbound PEs was done by using magnetic fields. The presence of magnetic nanoparticles and the polyelectrolyte shell opens the possibility of their magnetic manipulation and targeting by applying an external magnetic or electric field [63]. Encapsulation of lipase B into a polyelectrolyte multilayer system involving PAH/PSS using LbL technology resulted in an increase of the catalytic performance [64].

A general and versatile method for the encapsulation of an electrically uncharged organic substance in polymeric capsules by using a layer-by-layer approach was described by Manna and Patil. The SDS micellar solution of pyrene (uncharged organic substance) in water was then deposited on colloidal particles with chitosan as an oppositely charged polyelectrolyte. The capsules showed potential as a drug delivery system, which is suggested by the slow release (pseudo-second order) of entrapped dye by concentration-dependent diffusion in isotonic saline solution [65].

Polyelectrolyte multilayer films were prepared through LbL self-assembly using polysaccharide, SA and chitosan. After incubation in an enzyme pepsin solution, the multilayer film was partially destroyed because of the enzymatic degradation of chitosan. The enzymatic desorption was also observed from the microcapsule wall made of the SA/chitosan multilayer film directly deposited on indomethacin microcrystals. After erosion by pepsin, the indomethacin release from the microcapsules accelerated because of desorption. Increasing the layer number and raising the deposition temperature effectively slowed down the enzymatic desorption and release rate. In particular, increasing deposition temperature was more effective because of producing a more perfect structure in the SA/chitosan multilayer film. Crosslinking the neighboring layers of SA and chitosan with 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide in the SA/chitosan multilayer film significantly reduced the enzymatic desorption and release rate [29].

Hillberg and Tabrizian used chitosan, alginate, hyaluronic acid and oligonucleotides as PEs to encapsulate individual *E. coli* cells using LbL technology. The cellular activity was not affected by the presence of polyelectrolyte multilayers (PEMs) [24]. Gaserod *et al.* demonstrated that one coating of alginate/chitosan bilayer deposited on preformed alginate-chitosan capsules had only a minor effect on the permeability of immunoglobulin G (IgG), whereas four alginate/chitosan bilayers limited the diffusion of IgG to a greater extent. The decrease in permeability was attributed to the complexed structure of the polyelectrolyte bilayers [66].

*In vitro* degradability of chitosan/HA PE multilayers has been demonstrated by incubating planar films containing LbL coating in plasma. Resistance to degradation was found to increase as the extent of crosslinking by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was increased *in vitro.* The films when cultured with macrophages resulted in degradation of native as well as crosslinked films, but crosslinked films were found to be more resistant. In addition, chitosan was engulfed by macrophages. In *in vivo* conditions, native LbL film was almost completely degraded after 6 days, whereas all crosslinked films were degraded to a much lower extent but macrophage activation was found to be more pronounced in the case of crosslinked films, the highest EDC concentration leading to the development of fibrous tissues [67].

*In vivo* cellular uptake, degradation and biocompatibility of polyelectrolyte microcapsules produced from alternating DS/PLA layers on a $CaCO_3$ colloidal template have been demonstrated by subcutaneously injecting the microcapsules into mice. A moderate tissue reaction was observed after subcutaneous injection of polyelectrolyte microcapsules in mice. Within 16 days of subcutaneous injection, most of the microcapsules are internalized by the cells and start to be degraded. The stability of the microcapsules after cellular uptake was found to be dependent on the number of polyelectrolyte layers [68].

## 5. Tuning of loading and release of therapeutics from LbL microcapsules

Drug delivery systems releasing their payload in response to internal or external triggers may offer great advantages [4]. The potential of the LbL technique to make bioresponsive drug delivery systems has been evaluated. In most of the cases PE capsules respond only to extreme stimuli that do not occur or cannot be applied *in vivo*; as a result, only the IR-sensitive and the biodegradable polyelectrolyte capsules seem sufficiently attractive to be studied further for *in vivo* drug delivery [69]. Polyelectrolyte capsules sensitive to small (and physiologically relevant) changes of pH, salt concentration, glucose concentration and redox potential should be fabricated for potential applications for *in vivo* systems.

The tuning of biological activity has been demonstrated by LbL film on a planar substrate using PLL/PGA bilayers. In between the multilayers protein A was embedded, which has been shown to promote TNF-α secretion from macrophages. It has been shown that by changing the D/L enantiomer ratio of PEs and depth of embedded protein the timing of protein activity can be accurately tuned [70].

Self-exploding microcapsules composed of dex-HEMA-DMAEMA biodegradable microgels (150 μm) coated with four bilayers of DS/PLA, which can release their content in a pulsed fashion after a certain incubation time at physiological conditions, have also been reported. When the microgel core degrades, the swelling pressure increases and finally ruptures the membrane, leading to release of the encapsulated materials. The explosion time and subsequent release of latex nanoparticles (used as a model) can be tailored by varying the crosslinking density of the microgels. These microcapsules have been proposed for use as a vehicle for soluble antigen delivery [71].

The dex-HEMA microgels have also been coated with four layers of PSS/diazoresin and crosslinked by light irradiation,

resulting in a crosslinked multilayer structure. Degradation of dex-HEMA microgel ultimately leads to bursting of crosslinked multilayered structure. By ejection from the microcapsules, the nanoparticles travelled almost 800-fold faster in water than by Brownian motion [72]. This feature could be especially attractive in those situations in which the released species have to cross a viscous/low-permeable (biological) medium such as, for example, mucus covering epithelia, or vitreous in the eye. pH-sensitive polyelectrolyte microcapsules fabricated by LbL assembly of PAH/PMA onto PSS-doped $CaCO_3$ particles followed by core removal has been reported for delivery of water-soluble proteins [73]. These microcapsules induced spontaneous deposition of BSA at pH below its isoelectric point of 4.8, where it was positively charged. These capsules showed reversible pH-dependent open and closed states to FITC-BSA. Initially, burst release was observed for FITC-BSA in the first 0.5 h, which was sustained up to 5 h.

The mechanism of protein release from biocompatible polyelectrolyte multilayer microcapsules coated with SA/polyarginine and DS/polyarginine has been examined using two different approaches of protein encapsulation: i) preloading tetramethylrhodamine isothiocyanate-labeled bovine serum albumin (TRITC-BSA) into $CaCO_3$ particles followed by multilayer assembly; and ii) postloading of TRITC-BSA in preformed empty capsules templated on pure $CaCO_3$ particles. Regardless of the protein encapsulation approach, the capsule's shell composition and thickness have a major effect on the amount of encapsulated protein. The protein entrapping capability of SA/polyarginine multilayers was observed to be better than DS/polyarginine during preloading as well as during postloading of hollow capsules with TRITC-BSA [74]. At the same time, postloaded microcapsules of each investigated composition released protein faster than 'preloaded' ones as a result of the different mechanism of protein distribution across the capsule shell. TRITC-BSA release from SA/polyarginine or DS/polyarginine microcapsules has zero-order kinetics, with hourly medium refreshment.

The effects of relative density and location of amine functionality in two different side-chain functionalized poly(β-amino esters) on release and erosion of ultrathin multilayered films fabricated from plasmid DNA and side-chain functionalized poly(β-amino ester) have been demonstrated by Lynn and co-workers [75-78]. Poly(β-amino esters) having high densities of amine functionality provided the means to prevent large-scale film decomposition while still permitting gradual smooth and uniform film erosion and released DNA over 2 weeks, whereas films fabricated from poly(β-amino esters) having lower densities of amine functionality erode and release DNA over 2 days, undergoing complex nanometer-scale physical transformations [77] when incubated in phosphate-buffered saline. The apparent surface-type erosion of films fabricated from poly(β-amino esters) having high densities of amine functionality and plasmid DNA permitted the fabrication of ultrathin films that provide control over the timing and the order in which two different DNA constructs are released from surfaces [78].

A set of strategies has been applied for the production of compartmentalized, or stratified, films capable of releasing complex molecules in tuned release profiles by physically separate multiple components within a LbL film, thus blocking interlayer diffusion. It has been demonstrated that covalently crosslinked barriers can effectively block interlayer diffusion, leading to compartmentalized structures, whereas ionically crosslinked barrier layers were not able to block interlayer diffusion [79].

An approach to control the biological activity of cells in contact with multicompartment films made of different polyelectrolyte multilayers deposited sequentially on the solid substrate has been reported. Exponentially growing PLL/HA multilayers, used as reservoirs, alternated with a biodegradable layer consisting of PLGA acting as a barrier for PLL chains that diffuse within the PLL/HA reservoirs. Bone marrow cells seeded on these films ending with a PLL/HA reservoir rapidly degraded it and internalized the PLL chains confined in this reservoir. Then after 5 days of seeding the cells, the PLGA barrier was locally degraded, and the PLL localized in a lower (PLL/HA) compartment was internalized by the cells [80]. Therefore, by changing the thickness of the PLGA layer, the time delay of degradation may be tuned.

## 6. Modification of coated polyelectrolyte layers and templates

Click chemistry offers the possibility of carrying out covalent reactions with high selectivity and yield under extremely mild conditions. Fabrication of LbL microcapsules by means of click chemistry has the advantages of being stable and rather nontoxic owing to the fact that polycations are not generally used. LbL capsules have been fabricated by 'click' chemistry, using alkyne and azide-modified dextrans on $CaCO_3$ templates [81]. The introduction of carbonate esters, which link the alkyne and azide groups to the dextran chains, resulted in clicked multilayers and capsules that were hydrolytically degradable.

The biodegradable polymers PLL and PGA have been modified with alkyne and azide moieties and were used for fabrication of LbL multilayers on colloidal silica templates via click chemistry. Covalently stabilized PLL films have been post-functionalized by depositing a monolayer of heterobifunctional poly(ethylene glycol) (PEG), providing low-fouling properties and enhanced specific protein binding [82].

Biodegradable click single-component $PGA_{Alk}$ capsules with DOX-loaded multilayers have been fabricated using $PGA_{Alk}$ and DOX-conjugated alkyne-functionalized poly(L-glutamic acid) $PGA_{Alk + DOX}$/poly(*N*-vinyl pyrrolidone) (PVPON) bilayers by means of hydrogen bonding on colloidal silica templates. The films were subsequently covalently stabilized using diazide crosslinkers, and PVPON was released from the multilayers by altering the solution pH to disrupt hydrogen bonding and subsequent removal of the sacrificial

template. The drug-loaded capsules could be degraded enzymatically, resulting in the sustained release of active DOX over ~ 2 h. Cellular uptake studies demonstrated a decrease in viability of cells incubated with DOX-loaded $PGA_{Alk}$ capsules [83].

'Micelle-enhanced' polyelectrolyte capsules were fabricated by means of the LbL technique [84], templated on hybrid $CaCO_3$ particles with built-in polymeric micelles based on polystyrene-*b*-poly(acrylic acid). Owing to the presence of a large number of negatively charged micelles inside the polyelectrolyte capsule, this type of capsule could selectively entrap positively charged water-soluble substances. The encapsulation efficiency of positively charged substances was dependent on their molecular mass or size. In addition, *in vitro* release study suggested that the encapsulated compounds could be released in a sustained manner to a certain degree.

Polyelectrolyte nanocapsules were fabricated by means of layer-by-layer adsorption of (PAH and PSS) on an yttrium$^{3+}$/PSS coated MF particles. A solution of a poorly water-soluble drug in an organic solvent (e.g., acetone) is then mixed with a water suspension of the capsules and the organic solvent is then allowed to evaporate. The presence of the free polyelectrolyte molecules in the core results in a higher water concentration within the core, relative to the bulk. Because the concentration of water is higher in the core than in the bulk, the drug precipitates within the core due to low solubility, producing a drug-loaded nanocapsule [85].

PNiPAM, a thermosensitive polymer, is neutral and forms multilayers by virtue of hydrogen bonding, which were investigated in conjunction with polyelectrolyte multilayers. Quinn *et al.* found that multilayers consisting of PNiPAM could adsorb rhodamine, and on heating release the absorbed dye [86]. Glinel *et al.* used block copolymers, including PNiPAM, to construct capsules with the traditional electrostatic interaction [88]. The capsule permeability was greatly reduced on heating. In both of these systems, however, the PNiPAM did not show full thermosensitive behavior, as the PNiPAM movement was seriously restricted [87].

## 7. Surface-modified microcapsules

The surface of polyelectrolyte microcapsules can be modified either by using PEs previously modified with the surface-modifying group or by functionalizing the preformed microcapsules containing reactive functional group by antibodies or antigens. The polyelectrolyte making up the outer layer of the nanocapsule can be provided with reactive functional groups, or the surface of the nanocapsule can be treated with a reagent that places chemically reactive groups on the surface. These groups can then be used to bond directly or indirectly the ligand of interest (e.g., an integrin) to the nanocapsule by means of reactive groups that are commonly found on the same, such as amines, alcohols, carboxylic acids and thiols.

Surface modification of LbL microcapsules is also necessary owing to the fact that, because their polyionic nature, they are very susceptible to protein adsorption, leading to clogging in the blood capillaries as well as opsonization and scavenging by macrophages [89]. Hence, improved stealth strategies need to be developed in order to allow the specific targeting of microcapsules either by magnetic guidance or by antibody-mediated recognition.

Integrins are the preferred class of ligands for functionalizing the surface of the nanocapsules prepared by using LbL technology. Integrins recognize a wide variety of extracellular matrix components and cell surface receptors, including collagen, fibronectin, vitronectin, laminin, fibrinogen, and adhesion molecules, including intracellular adhesion molecules and vascular adhesion molecules [90].

Copolymers composed of poly(ethylene imine) and poly (ethylene glycol) PEI 25k-PEG 5k (1:1, 1:5 and 1:10) have also been used as outermost layers for shell assembly to modify the surface properties of LbL microcapsules. All surface charges remained positive. Compared with PEI-covered shells, the change of surface charge for PEI-PEG-covered shells after serum incubation is much less. Shells covered with the PEI 25k-PEG 5k (1:10) copolymer resulted in the lowest cell uptake of 33% at 4 h and 41% at 24 h, possibly owing to the PEG's stealthing property, which has been widely reported [91].

PEG modification has been also done by coating PEG-grafted PLL (PLL-*g*-PEG) onto the PSS-terminated multilayers to impart protein resistance to the surface. The best protein resistance was achieved by using PLA cores and coating the microcapsules with PLL-*g*-PEG after core removal. PLL-*g*-PEG end-functionalized with biotin further specifically recognize and immobilize controlled amounts of streptavidin on the capsule surfaces [92]. PLL-*g*-PEG effectively blocked the phagocytosis of the coated microcapsules [93].

Microcapsules with biologically designed targeting activity has been fabricated by using sythesized carbohydrate branches PEs. A new cationic D-galactose-branched copolymer (poly(vinyl galactose ester-*co*-methacryloxyethyl trimethylammonium chloride) [PGEDMC]) was alternated with PSS to form thin multifilms through the LbL technique with PS microparticles. The microcapsules were found to have recognition abilities with peanut agglutinin rather than concanavalin A [94].

Donath and co-workers demonstrated that phosphatidylserine-coated LbL core-shell microparticles or capsules fused with rubella-like particles (RLPs) can facilitate the membrane fusion. Cell culture results confirmed that the particles integrated with RLPs can retain their biological activity and enhance the cell membrane passage [95].

Naturally occurring lipid, dimyristoylphosphatidic acid, sodium salt (DMPA), was deposited onto the PDDA/PSS-coated particles through electrostatic interaction. The DMPA membranes delaminated from the particle surface at low surfactant concentrations and low ethanol content aqueous

solutions [96]. Surface modification of LbL microcapsules fabricated by sodium alginate and protamine sulfate as cationic and anionic PEs and protamine as the last coating was done by using PE-PEG 2000, resulting in greater resistance to serum proteins thereby masking LbL capsules being phagocytised by the reticuloendothelial system; but the cellular uptake was also reduced compared with unmodified LbL microcapsules [97].

Gupta *et al.* used protamine sulfate and SA to fabricate LbL microcapsules for targeting doxorubicin to the *Leishmania donovani*-infected macrophages. The auto-gelling property subsequent to core removal inside the nanomatrix resulted in high payload efficiency of DOX (i.e., > 70%). The approach allowed preferential uptake of microcapsules by macrophages and release of drug in macrophages. The matrix was completely internalized into macrophages (**Figure 2**), showing improved efficacy ($IC_{50}$ of formulation is almost 1.9-fold as compared with plain drug, $p < 0.05$) against intracellular amastigotes [98].

Adhesion of PAH/PSS and PDADMAC/PSS capsules through electrostatic and specific interactions has been investigated, and it was found that adhesion of unmodified capsules to the surface was through electrostatic interactions, which were spontaneous and strong. Capsules functionalized with PLL-*g*-PEG did not show significant adhesion (as determined by the adhesion area) to streptavidin-coated substrates, whereas capsules functionalized with biotinylated PLL-*g*-PEG showed a significantly larger adhesion area [99].

Humanized A33 monoclonal antibody (huA33) against human A33 antigen expressed by 95% of all human colorectal tumor cells has been used to modify LbL capsules' surface. On binding of the huA33 to the A33 antigen, the cellular internalization mechanism is activated, providing a mechanism for particles to be taken up. Polyelectrolyte capsules coated with huA33 are readily internalized by colorectal cells expressing the A33 antigen, whereas colorectal tumor cells that do not express the A33 antigen fail to take up the particles [100].

## 8. Recent advances in microcapsules fabricated by LbL technology

Recently the permeability of PSS/PAH-based LbL coatings surrounding positively charged dex-HEMA-DMAEMA microgels has been investigated. On degradation at pH 9, the dextran microgels were able to rupture their surrounding $(PSS/PAH)_3$ coating, resulting in 'self-rupturing microcapsules', whereas when degraded at pH 7, the $(PSS/PAH)_3$ coating did not rupture, leading to hollow $(PSS/PAH)_3$ capsules. This was explained by the pH-dependent permeability of the $(PSS/PAH)_3$ coating to the degradation products of the microgels [18]. Soft and porous temperature-sensitive PNiPAM microgel was coated by the LbL assembly of polyelectrolyte multilayers, resulting in permeation of PEs within the porous microgel [101]. Shell crosslinked HA/PLL polyelectrolyte microcapsules fabricated on disulfide crosslinked HA microgels showed much enhanced physical stability against freeze–thaw cycles and acidic pH conditions compared with the uncrosslinked ones. BSA release profiles from the microcapsules could be readily modulated by varying medium pH values or adding an HA digesting enzyme (hyaluronidase) in the incubation medium [102].

A polycation-free encapsulation method has been reported recently to obtain high concentrations of uncomplexed, short oligonucleotide chains confined within monodisperse, degradable microcapsules. This encapsulation method exploits amine-functionalized silica ($SiO_2^+$) particles to adsorb oligonucleotides, followed by the assembly of thiol-functionalized poly(methacrylic acid) ($PMA_{SH}$) and poly (vinylpyrrolidone) multilayers. The key advantages of this method include: the ability to attain high loadings of oligonucleotides (> $10^4$ chains per capsule); and the ability to release DNA under reducing conditions (as occurs in cells) that degrade the capsules [103].

Emulsions have also been tried recently for encapsulation by LbL technology. PSS/PAH-coated of PSS/PAH-coated thermotropic liquid crystal (LC); 4′-pentyl-4-cyanobiphenyl (5CB) (oil)-in-water emulsions droplets were subsequently treated with ethanol to dissolve the LC cores to form hollow capsules. The production of stable capsules confirmed the growth of the multilayer films and the integrity of the film formed [104,105]. LbL assembly of PSS/PAH multilayers has been performed at the interface of cyclohexane-in-water emulsions below the freezing point (< 6.5°C) of cyclohexane. Frozen oil droplets resulted in increased stability of the emulsion against droplet coalescence, decreased loss of volatile phase as well as facilitating handling of the template like any other solid templates for LbL assembly, enabling utilization of microfiltration and centrifugation for removal of excess PE [106]. Mohwald and co-workers have also used the LbL method to coat dodecane droplets with PSS/PDADMAC PE layers using creaming-based separation of excess PEs [107].

Recently, microemulsion stabilized by complex of ionic surfactant AOT (docusate sodium salt) and biocompatible polycation PLL has been coated with PLL/PGA bilayers and the outermost coating was done with PGA-*g*-PEG copolymer. Hydrophobic drugs such as β-carotene and vitamin A were successfully encapsulated in a single step; the PEGylated outermost layer of the capsules' shell improved their biocompatibility and inhibited serum protein adsorption and nonspecific bindings to cells. The nanocapsules were found to be non-toxic with negligible nonspecific binding to blood cells [108].

Layer-by-layer technology has also been applied successfully for the development of a multiplex suspension array with distinguishable microbeads coated with authentic viral surfaces to catch and quantify virus-specific antibodies in a flow cytometric analysis [109].

Recently, LbL technology has been used to enhance stability and the ability to control the release of therapeutic


**Figure 2. *In vitro* phagocytic uptake of LbL-DOX after 24 h by amastigote-infected J774A.1 macrophages using fluorescence microscopy. A.** Represents phase contrast microscopy of infected cell having amastigotes at the surface, indicated by yellow arrow. **B.** Image showing scattered fluorescence of DOX within gel-like structure revealing penetration after core removal. **C.** Infected cell showing complete internalization of LbL-DOX in macrophages indicated by yellow arrow.



agent of liposomes as well as to compartmentalize drugs by using capsosomes. Liposomes (size ≥ 200 nm) composed of egg phosphotidylcholine and brain phospotidylserine have been used along with PLL for deposition on colloidal templates using LbL technology, resulting in the combination of superior encapsulation properties of liposomes and enhanced stability of polyelectrolyte shells [110]. The main difficulty with immobilizing intact liposomes comes from vesicle destabilization or fusion leading to leakage of vesicle cargo on contact with most solid–fluid interfaces. To protect liposomes against disruption on contact with polyelectrolyte film, they were sterically stabilized by PLL coating [110], yielding PLL-coated liposomes, allowing the vesicle integrity to be kept and conferring a high positive surface charge density on the liposome surface.

Unilamellar negatively charged liposomes encapsulating magnetic nanoparticles have also been used as templates for deposition of PAH/PSS multilayers by the LbL technique, thereby increasing thermal and detergent stability of liposomes [111]. The entrapped magnetic nanoparticles ensured easy separation of coated liposomes from PE solution.

Recently introduced capsosomes, which combine the advantages of two systems, liposomes and polyelectrolyte capsules, are expected to find diverse applications in biomedicine, in particular for the creation of artificial cells or organelles where the performance of reactions within a confined environment is a prerequisite [112-115]. Capsosomes have been fabricated by means of LbL technology, which involved the coating of a polymer precursor layer onto a sacrificial colloidal template, followed by an alternating assembly of liposomes and polymer separation layers. Cholesterol-modified polymers have been used as a precursor, separation and capping layer for the stable incorporation of liposomes into the polymer film [112,113]. Unsaturated lipid containing negatively charged liposomes ($L^{u,-}$) and saturated lipid containing negatively charged liposomes ($L^{s,-}$) have been adsorbed using cholesterol-modified PMA ($PMA_c$) and PLL as a separation layer in a similar amount during the first two deposition steps; the $L^{u,-}$ adsorption during the third and subsequent adsorption steps resulted in 'boost-loading' of liposomes in capsosomes. $PMA_c$ has been used for capping the liposome (multilayer) assembly. Subsequently, PVP/$PMA_{SH}$ (thiol-modified polymethacrylic acid) bilayers were adsorbed to form the membrane of the carrier vehicle; crosslinking of the thiols in the polymer film and removal of the template core along with PVP resulted in capsosomes [112].

Capsosomes are fabricated by using a multilayer film assembly of PEs PSS and PAH and liposomes (50 nm, DOPC) on silica particles. On removal of the silica template core, stable capsosomes, containing one or two layers of intact liposomes as cargo, were obtained. A second layer of liposomes was incorporated and the amount of adsorbed liposomes increased with increasing number of polyelectrolyte separation layers between the two liposomes layers [115].

LbL microcapsules composed of two layers of DS/poly-L-arginine pair were evaluated as adjuvant for parenterals as well as pulmonary route specifically for soluble antigens, which often fail to illicit CD8 cytotoxic T-cell responses, which are crucial for killing virally infected cells and tumor cells alone. The LbL microcapsules were found to be permeable to proteases, resulting in degradation of antigen into small peptides, allowing superior presentation to both CD4 and CD8 T cells when compared with soluble antigen. It was also demonstrated that LbL microcapsules after engulfment by dendritic cells are taken to lysosomes, where they are ruptured by lysosomal proteases [116].

In another study, DS/poly-L-arginine PE microcapsules on intratracheal instillation were not only been found to be efficiently taken up by antigen-presenting cells (APCs), but also enhanced activation status of APCs. Pulmonary adaptive immune responses were characterized by the induction of a strongly Th17-polarized response [117]. When compared with a mixture of soluble Ag with empty microcapsules, Ag encapsulation significantly enhanced the strength of this local mucosal response.

The major hurdle with LbL technology has been the difficulty in automating the whole coating process. However, a technique comprising two reservoirs each for cationic and anionic PEs, an electromagnetic valve controlling the flow of polyelectrolyte solutions alternately controlled by a square pulse generator, a flow cell with a volume of ~ 20 µl containing a solid support for the deposited LbL structure, and a peristaltic pump [118] was used for deposition of up to 100 bilayers.

The automation of fabrication can also be done by using advanced separation techniques for the separation of coated microcapsules from the remaining PE solution, such as by using a magnetic core template that can be efficiently removed during core dissolution, so that the whole process can be done efficiently and can be automated and performed in a single vessel.

## 9. Conclusion

LbL technology in the fabrication of new cargo for drug delivery has been applied very successfully in recent decades. A wide variety of templates are used, ranging from natural templates, to inorganic templates to templates composed of crosslinked environment-responsive polymers. The goal of spatiotemporal placement of drug in the body can be approached successfully by using LbL polyelectrolyte capsules. This strategy will be specifically helpful in the delivery of indigenous biochemicals such as hormones as well as other proteins and peptides for the treatment of metabolic disorders needing chronotherapy. LbL microcapsules are also evaluated as adjuvant in the delivery of vaccines and can be viewed as a promising carrier for oral as well as parenteral delivery of vaccines. The application of this platform for the development of thermoresponsive or environment-responsive systems will enhance further the targeting capability by using simple and low-cost techniques. This system can be applied to various metabolic abnormalities such as osteoporosis by using $CaCO_3$ core particles so the dual benefits of calcium supplementation and anti-osteoporotic agent delivery can be approached.

## 10. Expert opinion

The LBL-based microreservoirs have shown great promise, and in less than a decade their application in drug delivery and diagnostics has progressed rapidly. LbL technology provides an excellent platform owing to the avoidance of organic solvents and other harsh conditions for fabrication and loading of bioactives. LbL technology coated microcapsules can also be used as a combined cargo for the delivery of two or more therapeutic agents of different physicochemical and structural properties, for example a therapeutic protein and a synthetic molecule. Coating of emulsions composed of volatile oils can also be coated through LbL technology, resulting in sustained release microcapsules containing volatile oil. It has been demonstrated that these systems can modify the release pattern of drugs and other bioactives according to external environmental conditions, so they can prove to be an excellent bioresponsive delivery system specifically for the delivery of proteins and peptides. Polyelectrolyte capsules sensitive to small (and physiologically relevant) changes of pH, salt concentration, glucose concentration and redox potential should be fabricated for potential applications for *in vivo* systems. Efforts to automate the process should be developed specifically for the fabrication of LbL microcapsules, which will increase the industrial acceptability of the technology. It can be accomplished by using more advanced techniques for the separation of coated PE microcapsules from uncoated PE solutions. Targeting of these systems can easily be achieved by coating ligand-attached PEs. Despite these successes, efforts in the future need to concentrate on developing more effective and safer delivery systems with better specificity for target sites, by attaching antibodies covalently to the LbL capsules. It can be a very good platform for antigen delivery and as adjuvant for various antigenic epitopes, specifically in the case of soluble antigens. Capsosomes can also provide a very good platform for drug delivery, combining the advantages of both liposomes and polyelectrolyte capsules, and can be used for delivery of more than one therapeutic moiety separated physically from each other. A lot of work has yet to be done on the selection of biocompatible cores and PEs, so that the issue of toxicity can be addressed effectively. It has been envisaged that with the increasing development of suitable formulations and reliable preparation procedures, LbL-based microreservoirs will play a significant role in the treatment of various human diseases in the near future.

## Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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