ABSTRACT
Layer by layer technique is based on the consecutive adsorption of polyanions and polycations via electrostatic interaction. The recent idea of LbL was taken from the work of Langmuir and Blodgett’s. LbL technology at present identified as an environmental friendly technology in fundamental and applied research. This system finds vast area interest due to their multifunctionality and responsiveness to a multitude of stimuli. The present article focus on the various aspects of LbL system mechanism (Electrostatic interaction, Hydrogen bonding, Hydrophobic interaction, Van der waals forces). Advances LbL such as core scarification, ultrasonic encapsulation, dissolution assisted by cosolvency followed by nucleation and various stimuli (Chemical, Physical, Biological) that can be used in delivery of drug formulated through LbL. LbL system have application in related to targeted delivery in cancer therapy, biosensory application, sustain release of drug multifunctional nanocarriers and nanotoxicity. This review covers the various US patents on LbL system.

KEYWORDS: Layer by layer self-assembly, Targeted drug delivery, Biosensory application.

INTRODUCTION
Layer by Layer (LBL) technique is based on the consecutive adsorption of polyanions and polycations via electrostatic interaction. LbL self-assembly is defined as adsorbing multiple layers of charged materials, including particles, polymers, and even small molecules through electrostatic interactions. Recently the idea of self assembly was taken from the...
work of Langmuir and Blodgett’s.\textsuperscript{[4]} In 1935 Bigelo et al observed the formation of closed packed arrangements of amphilic molecule, molecule on liquid and solid surface.\textsuperscript{[5]} Iller in 1965 was first developed the LbL technique. The adsorption of invisibley small particles was suggested by him. Iller deposited the alternate layers of positively and negatively charged colloidal particles from solution onto a smooth glass surface. Decher reconnoitered this idea in 1991 by allowing electrostatic polyelectrolytes to assemble in a layer-by-layer fashion for the first time.\textsuperscript{[1,2]} for construct the multilayer films electrostatic attractions can be used was confirmed by Keller and co-workers.\textsuperscript{[6]} Qiu et al. in 2001 introduced the use of LbL self-assembly systems in drug delivery. They deposited polysaccharide multilayer’s on ibuprofen micro particles.\textsuperscript{[7]} LbL has demonstrated wide applications in drug delivery, tissue engineering, implant coating and electronics\textsuperscript{[8]} LbL technology at the present identified as an environmental friendly technology in fundamental and applied research.\textsuperscript{[9]} The surface modification take place by the formation of LbL self-assembly, these modifications are previously not associated with the drugs. The modification that takes place may be in the various properties of materials such as electrical, magnetic, physico chemical and biological properties.\textsuperscript{[10]} Formulation of stimuli responsive LbL construct mainly deals with the inclusion of responsive material Biosensor applications of LbL systems mainly include enzyme biosensors, immnosensors, DNA sensors, protein and nucleic acid sensor.\textsuperscript{[11,12]}

**Advantages of LbL-assembled multilayer’s\textsuperscript{[13]}**

Besides other method LbL self-assembly has certain advances in encapsulation, coating of substances:

1) Stabilization of submicron particles is possible.
2) The wall thickness can be made in the nm–μm range.
3) Easy availability of synthetic/natural colloids for LbL.
4) Conventional technique need higher amounts of colloids ~10% as compared to this technique which need only ~1% of collide to produce a coating.
5) The sequence and location of the layers can be controlled.
6) The thermodynamically unstable micronized particles can be avoided by the use of LbL technique.
Adsortion of any given layer depends on energetic factors such as electrostatic forces, van der waals forces, hydrogen bonds, solvent quality, and entropic factors.

Mechanisms of LbL self-assembly
The major mechanism for formulation of LbL multilayers is electrostatic attraction between the oppositely charged constituents. The other mechanisms for formulation of LbL covalent bonding\cite{14}, hydrogen bonding\cite{15}, hydrophobic interactions\cite{16} and van der waals forces.\cite{17} Supramolecular inclusion\cite{18} charge transfer complex formation,\cite{19} biospecific recognition and stereo-complex formation.\cite{20} These mechanisms can influence morphology, stability, thickness of film and permeation properties particle molecule deposition of film.\cite{17}

General procedure for LbL self assembly\cite{21}
1. The First monolayer for the adsorption of polycation layer, a solid support (e.g. slide) with positive surface charge is incubated in a solution containing polyanions for acertain amount of time, usually 30 min.
2. Next solid supports are rinsed with pure water two or three times to remove excess polyelectrolytes.
3. Then the slide immersed in a solution of cationic polyelectrolytes and a layer is adsorbed.
4. The original surface charge is restored and surface is ready for washing.
5. Washing cycle follows to remove unbound material and contamination.
6. Generally the above mentioned steps are repeated alternately until a film of desired thickness is obtained.
Certain forces that are required for LbL Self assembly are described below.
The self assembly of molecule is governed by the non-covalent intermolecular bonding though the balance of attractive and repulsive interaction leading to formation of layers. The basic principle responsible for layers of self assembly is still challenging because numerous complex and specific interplays between multiple subunits of building blocks. The certain type of forces that are required for LbL self assembly are as followers.

**Electrostatic interaction**
This kind of interaction is one of the reasons for the adsorption and thin film formation as well as interaction between partially doped chains of the polycation and the negatively charged chains of the polyanion.

**Hydrogen bonding**
The dipole-dipole interaction is responsible for the hydrogen bonding. Hydrogen bonding forms when a hydrogen atom covalently bonded to electronegative atom (hydrogen donor and electron acceptor) interact with lone pair of electrons from another electronegative atom (hydrogen acceptor and electron donor). Hydrogen bonding plays important role in carried out layer by layer self assembly. The construction of film of LbL is the hydrogen bonding between hydrogen bond donor (poly acrylic acid)PAA and hydrogen bond acceptor biodegradable (poly ethylene oxide )PEO.

**Hyrophobic interaction**
The apparent repulsion between water and hydrocarbon molecule causes the hydrophobic interaction. This interaction causes formation of physical bond. The presence of
Gunjkar et al. World Journal of Pharmacy and Pharmaceutical Sciences

hydrophobic/hydrophilic nanodomain and surface charge neutrality contributes to the LbL film's resistance to protein adsorption; it has been shown by the Wong et al. The experiment has done by alternating deposition of a hydrophobic N-alkylated polyethylenimine (PEI) and a hydrophilic polyacrylate on thin polyelectrolyte microcapsules (PEM) films. Thus hydrophobic interaction affects on the LbL assembly.\[30\]

Van der walls force

Van der walls force are the weakest and most immanent this force generally caused by repulsive and attractive interaction between two components with random electric charge fluctuation. This fluctuation impel the polarization electron shell and make individual component temporarily polar and causing an oppositely polarization in its neighboring body.\[31,32\] Van der Waals force retains the adsorption of oppositely charged layers so widely used in biomedical, electrical and energy-related fields. Sato and Sano showed that van der Waals (VDW) attraction balances the electrostatic double layer repulsions that exists in acid-treated single-walled carbon nanotubes (SWCNTs) dispersed in water that help in making them kinetically stable.\[33\]

Methods for formulation of layer by layer assembly

Ultrasound encapsulation of insoluble core

The ultrasonic treatment is given to the aqueous suspensions of poorly soluble drugs such as paclitaxel, tamoxifen \[30\], dexamethasone[34] and ketoprofen with particle size in the micron range. The particle size so achieved is in nano range. The stabilization and agglomeration are carried out by application of LbL coating. The LbL coating is achieved by alternate application of polycation and polyanion layers. The particle so produced is of 5 to 50 nm in range.\[20\] The desired thickness can be achieved by controlling the multilayer coating. They are stabilized with first layering of polyelectrolyte to prevent aggregation of formed colloidal NPs.

Core scarification method

The LbL films are formulated by adsorption of oppositely charged polyelectrolytes on the charged colloidal cores and these cores serve as sacrificial templates. The removal of colloid either by calcination or by decomposition induced by exposure to suitable solutions produces hollow shells.\[35\] There is presence of polymer walls and inner cavities that may be loaded with various types of (therapeutic) molecules including enzymes, low-molecular-weight drugs, polymers, protein, antigens and DNA.\[30\]
Dissolution assisted by co-solvent followed by nucleation

When the active molecules used for the formulation are water-insoluble then firstly dissolve it in a water-miscible organic solvent such as ethanol or acetone. Drug nucleation is initiated then by gradual addition of an aqueous solution containing oppositely charged polyelectrolytes under powerful ultra-sonication. The release mechanism of drug under the influence of stimuli is different.\cite{36}

MATERIAL FOR LBL SELF ASSEMBLY

Polyions are the most important material for the building LbL self assembly; especially while multilayers of protein and nanoparticle assemblies.\cite{8}

Polymers

Both natural and synthetic polymers are used as material for LbL assembly.

Natural polymers

Biocompatibility and biodegradability are the great advantages of natural polymers hence they plays important role in LbL self assembly. Most of them are water soluble, which are essential for LbL self assembly. Natural polymers are categorized into three parts.

1. Polypeptides: Polypeptide includes polylys, poly l-glutmic acid, poly aspartic acid.
2. Protein and enzymes: Protamine sulfate, Albumin, Glucose oxidase.
3. Polysaccharides: Involves Dextran, alginate, heparin, chondroitin, carbomethyl cellulose and chitosan.\cite{37}

Synthetic polymers

These synthetic polymers have been used as substrate for cell adhesion for building microcapsules for protein encapsulation. Most commonly used synthetic polymers involves.

Polycations: Such as linier or branched polyethyleneimine [PEI], polydimethylialil ammonium [PDDA], and polyallylammine hydrochloride [PAH].

Polyanions: Polyacrylic acid [PAA] and PSS are synthetic polymer.\cite{38-41} Other material such as organic and inorganic nanoparticles (magnetite, quantum dot, gold) and lipids can also be incorporated. \cite{42}
Stimuli activated release of drug through LbL self assemblies

Stimuli are generally classified into three categories physical, chemical, and biological.

1. Chemical stimuli

These stimuli mainly includes pH, ionic strength, solvent, chemical and electrochemical response. \[17, 43\]

1.1 pH Stimulus

Stomach has strong acidic pH while small intestine and colon is almost neutral. The pH variation in gastrointestinal tract should be considered for the orally administered dosage form. pH sensitive drug delivery relies on the intrinsic pH sensitivity of the LbL layers. In contrast, pH-sensitive drug delivery could be performed on the basis of the pH response of the drug itself or drug conjugates in the films or microcapsules. The pH variation in different cellular compartments is the basis for the pH-sensitive intracellular delivery of drugs. The release of drug through the microcapsules can be triggered either by changes in permeability of capsule membrane or the decomposition of entire capsule; the same mechanism is responsible for release of drug through the LbL films. Thus it is clear that one of the key issues in developing pH-sensitive LbL films and microcapsules is to design appropriate pH-sensitive polymeric materials and use suitable combinations for LbL depositions. The pH sensitive polymer respond to the change in pH as it contain the acidic or basic groups. Ionisable polymers with a pKa value in between 3 to 10 are candidates for pH-responsive systems.\[44, 45\] Certain moieties such as carboxylic acid, amine, azo, phenylboronic acid, imidazole, pyridine, sulfonamide and thiol groups can confer pH-sensitivity by either accepting or donating protons. When these ionisable groups are linked to the polymer structure change produces change in the swelling hence a conformational change produces a change in the swelling behaviour of the hydrogels.\[46, 47\]

1.1.1 Electricstatic bond based system:

The pH-sensitive properties originate from the acid–base equilibrium of the weak polyelectrolyte in the LbL layer. It was found that weak polyelectrolyte-based LbL layers are basically pH-sensitive in terms of swelling, permeability and even decomposition at extremely high or low pH. For example, the permeability of the Fe (CN) \(_6^-\) anion through an LbL film composed of poly (ethyleneimine) (PEI) is significantly dependent on the solution. LbL microcapsules composed of PSS and PAH are reported to be permeable to high-molecular weight compounds such as dextran and albumin at low pH while impermeable at
pH 8 or higher. Dejugnat and co-worker’s observed a significant swelling and enhanced permeability of PSS-PAH microcapsules at pH 11. LbL microcapsules composed of PAH and poly (methacrylic acid) (PMA) is decomposed at pH 2.5 or lower pH and at pH 11.5 or higher. It should be noted that the acid–base equilibrium of weak polyelectrolytes in LbL layers is usually shifted from that in solution. In the PAH-PMA LbL film the apparent Pk values of PAH and PMA are 10.8 and 3.9 in comparison to 8.6 and 6.8 in solution respectively.

1.1.2 Hydrogen bond based system
Polymeric materials employed for the formulation of LbL assembly are a combination of poly(carboxylic acid)s such as PAA and PMA as hydrogen bonding donors and hydrogen bonding acceptors such as poly(ethylene oxide) (PEO) and poly(4-N-vinylpyrrolidone) (PVPON). Deprotonating from poly (carboxylic acids) at higher pH results in breaking of hydrogen bonds in the film. These materials have biomedical applications due to low toxicity and high biocompatibility.

2. Ionic strength
Changes induced in morphology and permeability on multilayer film by salts has been established. Polyelectrolyte multilayers are responsive to ionic strength changes because of screening of electrostatic interaction between the oppositely charged polyelectrolyte polymers by salt ions. In the presence of salt multilayer capsules can thus be filled with molecules of interest. Encapsulated molecules can be later released by similar treatment with salt. The influence of ionic strength on polyelectrolyte multilayer can be traced by measuring dye molecule diffusion through polyelectrolyte multilayer’s as a function of varying salt concentration.

8.1.3 Electrochemical stimuli:
Electrical and electrochemical stimuli are widely used by the researcher. While the electrochemical stimulus is provided to the LbL polymeric film which is electrochemical stimuli sensitive the influx of counterions and solvent molecule take place. This causes an increase of osmotic pressure in the film which results in a volumetric expansion. The electrochemically stimulated systems have biomedical potential and micromechanical applications. Electrochemically-swellable redox-active polyelectrolyte multilayers were also studied. Electrochemical impedance can be used for protecting ferrous metals. Electrochemical also parameters affect multilayer build-up. Electrochemistry control the
polyelectrolyte loading/adsorption of oppositely charged porous materials such as porous microspheres was previously studied. The electrochemical stimuli can be applied to the LbL films for their sensing application.\cite{49}

2. Physical stimuli

2.1 Ultra sound responsive LbL system

The non-invasiveness and controlled drug delivery is an attractive feature of ultrasound-responsive drug delivery.\cite{50} Poly (lactide-co-glycolide) (PLGA) microspheres and poly (HEMA-co-DMAEMA) hydrogels have also been investigated as ultrasound-responsive drug delivery systems. Researchers have investigated that the ultrasonic treatment can be used as a remote trigger for releasing materials encapsulated inside LbL assembled PEMs. Ultrasound also has applications in controlling the permeability of microcapsules. These PEMs can be destroyed and drug can be released using high-power ultrasound (100–500 Watt 20 kHz). The loaded materials form natural biodegradable polyglycolide, polylactide and poly [bis (p-carboxyphenoxy) alkane anhydrides with sebacic acid and non-biodegradable ethylene-vinyl acetate copolymers can be released by ultrasound-assisted release mechanism.\cite{30}

2.2 Magnetic field responsive LbL system

Targeting by magnetic field is one of the main functions of magnetic nanoparticle (NP) incorporated into PEM assembly. The calcination of the core–shell magnetite particles at elevated temperatures produces magnetic particles. The magnetic activation of microcontainers is a good candidate for controlled drug delivery. The long exposure time and the strong magnetic field required for permeabilizing the capsules lead to an increase of temperature.\cite{49} Katagiri and co-workers showed the magnetically induced release from magneto-responsive smart capsules formed with polyelectrolytes, lipid bilayers and magnetic NP.\cite{18} The release takes place by phase transition of the lipid membrane caused by heat of Fe3O4 NP under magnetic stimuli and not by rupture of the capsules. Babincova et al. reported the site-specific release of encapsulated doxorubicin from magnetic liposomes with a static magnetic field which induces a local increase of the temperature resulting in liposome melting.\cite{50}

2.3 Temperature stimuli

The temperature is an external post treatment parameter affecting material properties and polyelectrolyte multilayer formation. Temperature allows producing mechanically strengthened capsules. Kohler et al. showed that shrinking of LbL assembled microcapsules
which can be used for encapsulation and enhancement of mechanical properties in response to thermal treatment.[17] Polyelectrolyte multilayers are kinetically stable, so it means that a temperature increase can provide enough thermal energy to surpass the barrier necessary for polymeric film rearrangements.[51] Bedard and coworkers reported that inserting gold NP within the wall of the microcapsules increases the stiffness of microcapsules.[17] It was found that microcapsules containing gold NP need higher temperatures to shrink to the same diameter compared with those containing no gold NP.

2.4 Mechanical deformation
The need for mechanically stable capsules is accentuated because they are often deformed upon intracellular uptake leading to losses in the amount of delivered material. Enhancement of their mechanical strength can be achieved by functionalizing the capsules with gold NP or carbon nanotubes.[52] If it is encapsulated release depends upon mechanical rupture.[53]

3. Biological stimuli for release and targeting
3.1 Enzyme responsive layer assembly
They do not require an external stimulus for their decomposition and have high potential for the intracellular delivery of therapeutics, other biomimetic applications and in vivo applications. The main disadvantage is due to difficulties of establishing a precise initial time of release. The biosensory application of this LbL assembly is due to the use of the biological components such as enzymes in the system. The amount of adsorbed enzyme and polymer mediator can be controlled by regulating the number of deposited layers. Nucleic acids and proteins can be delivered by biodegradable polyelectrolyte multilayer capsules. [54] The delivery of oligonucleotide sequences has been demonstrated for microcapsules assembled by the polycation-free method. Such a method has the advantage of high retention of encapsulated molecules. De Geest et al. in 2006 demonstrated degradation of capsules by enzymes.[55] Two types of degradable capsules templated on CaCO3 micro particles were reported. The first type of microcapsules responding to enzymatic degradation was composed of pARG as the polycation and DEXS as the polyanion. In the second type of microcapsules poly (hydroxypropyl methacrylamide dimethylaminoethyl) (p(HPMA-DMAE) and PSS were used as the polycation and polyanion respectively. It was shown that polyelectrolyte capsules that contain an enzymatically or hydrolytically degradable polycation (DEXS/pARG and PSS/pHPMA-DMAE) are subject to intracellular degradation in VERO-1 cells while these are made of synthetic PSS/PAH are intact upon intracellular uptake. Initially the degradation
of these microcapsules was demonstrated using Pronase and a mixture of proteases that cleaves proteins and peptides unspecifically. Degradation of microcapsules made of biopolymers after internalization by cells was also demonstrated for hyaluronic acid (HA)/poly-L-lysine (PLL) capsules. The biodegradation process was colorimetrically monitored with UV–vis spectroscopy. Enzyme film biosensors can be classified into two types depending upon their presence or absence of redox properties: oxyreductase biosensors and nonoxyreductase biosensors.\[56\]

### 3.2 Glucose responsive LbL system

Glucose-triggered release can be carried out using glucose-sensitive enzymes such as catalase (CAT) and glucose oxidase (GOx). The first strategy is to use LbL multilayer films as the diffusion barrier for encapsulated drugs. Qi et al. have formulated glucose-sensitive enzyme multilayer shells through LbL assembly. GOx and catalase (CAT) have been assembled on insulin particles for the formulation of assembly which is glucose-sensitive by alternately through GA cross linking using this approach. When external glucose was introduced to this system the release rate of insulin from the protein multilayer increased extensively. Qi et al.in 2009 noticed that the reduction in the pH of the microenvironment due to production of H+ as catalytic interaction of CAT/GOD shells with glucose. Under acidic conditions the C-N bond broken partially and this leads to an increase in the permeability of the capsule wall as well as an increase in solubility.\[57\] Second strategy is to introduce drugs in the LbL membranes as one of the building blocks. By using this strategy the use of Alizarin Red S (ARS) incorporated as a model drug. Ding et al.in 2009 fabricated glucose-sensitive LbL multilayer films. The resultant films display pH-sensitive and thermo sensitive swelling behaviors. The swelling of the films is enhanced when saccharides such as glucose or fructose are present in the solution. The release rate was faster in the presence of glucose due to its competition with ARS for binding sites in the films.\[58\]

### 3.3 Antigen- responsive LBL system

The use of anti-IgG monoclonal antibodies into LbL films was first reported by Caruso et al. The LbL film so formed have interaction with IgG. The use of solid substrate for the immobilization on multilayer bioreceptor assemblies was studied by Brynda et al. He also demonstrated that the use of three dimensional networks of antibodies as surface plasmon resonance biosensors and the sensor with an LbL multilayer of antibodies exhibited an enhanced sensitivity to horseradish peroxidase (HRP) antigens in comparison with two-
dimensional monolayer’s mainly because of the higher number of receptors accessible to the antigens and the better binding properties of antibodies which were not immobilized directly on the sensor surface. The use of lectin, folate and biotin responsive LbL assembly was also considered by the scientist.\cite{30}

**Characterizations of layer by layer self assembly**

The combination of various techniques can be used to study the construction, release of encapsulated content from LbL PEMs. Given below are several most common techniques that are used for the spectroscopic and structural characterization of LbL self-assembly.

**Table 1: It shows various characterization techniques used in studying LbL self-assembly.**

<table>
<thead>
<tr>
<th>Spectroscopic characterization</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV–VIS spectroscopy</td>
<td>Multilayer growth can be observed that determines cumulative absorption attributed to stepwise deposition of UV-active colloids. \cite{1}</td>
</tr>
<tr>
<td>Ellipsometry</td>
<td>Ellipsometry determines the layer thickness or adsorbed mass per layer during each step of LbL assembly formation. \cite{59}</td>
</tr>
<tr>
<td>Cumulative and visible light absorption</td>
<td>Substrate–polyelectrolyte interactions can bestudied by shifting the absorption maxima dependent on coating time, to determine PEM assembly. \cite{17}</td>
</tr>
<tr>
<td>Confocal laser microscopy</td>
<td>Studies in related to, PEM stability and multilayer thickness also scaled proportionally to the fluorescence intensity.</td>
</tr>
<tr>
<td>Infrared analysis</td>
<td>To determine the PEM moisture content, which provide useful information to study the structure of ionizing groups and the permeability of water-leachable materials. \cite{17}</td>
</tr>
<tr>
<td>Raman microscopy</td>
<td>For studying drug interaction with constituent PEM layers\cite{17}</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Structural characterization</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz crystal microbalance studies, (QCM-D)</td>
<td>Quartz crystal microbalance studies and their relate vibration dissipation(QCM-D) as function of the step-wise amount of colloid adsorbed to a quartz crystal surface in real time.\cite{17}</td>
</tr>
<tr>
<td>Electrophoretic particle mobility</td>
<td>For $\zeta$-potential measurements of charged, therefore efficiently coated, surfaces to study charge reversal and colloidal stability.\cite{17}</td>
</tr>
<tr>
<td>X-ray reflectivity</td>
<td>The internal arrangement PEM structure can determined.\cite{17}</td>
</tr>
<tr>
<td>Atomic force microscopy (AFM)</td>
<td>Atomic force microscopy (AFM)\cite{17}</td>
</tr>
<tr>
<td>Spin relaxation NMR</td>
<td>To study the hydration and dehydration properties of multilayer’s or the mobility of polyionic multilayer’s.\cite{17}</td>
</tr>
<tr>
<td>Dynamic light scattering</td>
<td>To study particle size and size distributions. \cite{34}</td>
</tr>
</tbody>
</table>
Application of Stimuli responsive layer by layer technique

Sustained release of drug
Shifeng Yan et.al. Formulated the Layer-by-layer assembly of poly (L-glutamic acid)/chitosan (CS) microcapsules for high loading and sustained release of 5-fluorouracil (FU) [30]. The PLGA/CS microcapsules show high loading capacity of hydrophilic neutral anticancer drug 5-FU by soaking in 5-FU solution. The maximum loading capacity of 5-FU was achieved under conditions of high drug concentration and salt adding. Prolonged 5-FU release is achieved from PLGA/CS microcapsules, in contrast to burst release of bare 5-FU. The release rate and cumulative release amount are both reduced after cross linking the capsule wall by treatment of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) this shows sustained release effects. [60]

As a novel technique in formulation of various formulations
Recently the LbL technique is widely used in the formulation of nanocapsules [61], nanogels [32] as one of the way in drug delivery. C.E. Mora-Huertas et.al. Had reviewed that certain antineoplastic drugs such as Tamoxifen, Artemisinin, Paclitaxel, their nanocapsules had been formulated by LbL technique. [61, 62]

Jeremy P.K. Tan et.al formulated Control of burst release from nanogels via layer by layer assembly, he found that the high initial burst release and short therapeutic time range can be overcome through layer by layer assembly. The initial burst release behavior observed in nanoparticles was minimized and eliminated by the introduction of several PE layers. Through this LBL approach, the permeability of nanogels was altered with each additional PE layer, the time to achieve τD increased linearly with PE layer. The swelling behavior of coated nanogels decreased with increasing PE layers resulting in a slower release of drugs. The colloidal stability of coated nanogel was maintained up to pH 8 for PAH coated system, while it was stable at all pH for PSS coated system. [62]

Stabilization of submicron particle[33]
Layersomes are mainly composed of the layer by layer (LbL) coating of the polyelectrolytes over liposome’s increase stability of the formulation in the biological milieu and long term storage.
Targeted drug delivery

Kaushik Thanki et al. reviewed Oral delivery of anticancer drugs: Challenges and opportunities. About 5.89-fold increase in the oral bioavailability of doxorubicin as compared to the free drug and comparable therapeutic efficacy and toxicity profile were observed as compared to i.v. LipoDox®. Similar results were also observed in case of paclitaxel with about 4.07 fold increase in the oral bioavailability, superior therapeutic efficacy and safety profile as compared to i.v. Taxol.[33] Recently, doxorubicin loaded poly (L-glutamic acid) microcapsules have also been prepared using LbL approach and was evaluated on multi-drug-resistant (MDR) cell lines.[63]

Keun Sang Oh et.al. formulated the multilayer nanoparticles formed by layer by layer approach for cancer-targeting therapy.[64] In cancer therapy, the efficient delivery of anticancer drugs to tumor sites is strongly required and this has been demonstrated by accumulating NPs containing anticancer drugs at tumor site based on the enhanced permeation and retention (EPR) effect. Basic requirement for the accumulation of NPs at tumor site is to prolong their half-life in the systemic circulation. Through LBL approach, appropriate size and surface modification of NPs can lead to achieve this requirement with enhanced target ability.[64]

Magnetic resonance imaging and drug delivery (MRI)

MRI is a powerful non-invasive imaging tool which has shown great value in early cancer diagnosis, implant monitoring, brain functional imaging, vasculature imaging, and drug discovery. The advantages of MRI include, safe imaging without using high-energy radiation, deep tissue penetration, and high resolution (down to 25–100 μm at higher magnetic fields) for soft tissues. Advanced imaging probes helpful to image pathological changes at cellular and molecular level. Besides, application of MRI for drug discovery has proven to be effective, especially when used for characterization of disease models and therapeutic efficacy.[36] When used for drug delivery monitoring, MRI has showed that it can track the drug carriers effectively both in vitro and in vivo. The sensitivity of MRI is much behind nuclear imaging such as SPECT, PET, and optical imaging methods. The introduction of contrast media can improve image contrast and delineate small changes which may be difficult to be discovered under regular scans, and combining of two or three imaging modalities can compensate the disadvantages of each other and provide a lot of information that may not be collected through one modality. Gadolinium (Gd) or manganese (Mn) based
small paramagnetic molecules and super paramagnetic iron oxide (SPIO) based nanoparticles are two major categories of MRI contrast agents approved for clinical applications. Polyelectrolyte capsules developed from layer-by-layer (LbL) self assembly have shown great applications in storing, protection, release, and delivery of different functional agents.\[65, 66,\]

**Multifunctional nanocarriers**

LbL capsules are promising carriers for controlled drug release, combining imaging, targeting and drug delivery functions into one carrier will expand its functionality and provide in-depth understanding of the delivery mechanism, resulting to better design of drug carrier systems.

**Nanotoxicology**

The comprehensive biosafety studies of LbL capsules are the foundations for their biomedical imaging applications. Most LbL capsules are currently studied at *in vitro* environment, which cannot satisfy the preclinical requirements.

**Smart diagnostic probes**

LbL capsules responsive to specific pathological environment such as pH difference, higher macrophage accumulation at vascular plaques, or high matrix metalloproteinase expression resulting to trigger signal intensity change and lead to better contrast.\[65, 66,\]

**Delivery of biotherapeutics (Nucleic acids, antigen, peptides)**

Intracellular delivery of RNA, DNA, oligonucleotides etc is the primal goal for gene therapy, this aim to introduce new genes or replace defective genes.\[37, 38\] Due to its polyionic nature, DNA has frequently been used in the past as building block in LbL films. Through condensation with the small cationic molecule spermidine, Schuler et al. showed the possibility to construct multilayer capsules, however with limited stability under physiological salt concentrations.\[67, 68\]

**Delivery of insoluble (or poorly soluble) drugs**

LbL shells are might be categorized as LbL microshells because their diameters are in the micron to submicron range. One effective formulation strategy for administering insoluble drugs is nano-sizing,\[69\] resulting in to increase in surface area per drug weight, leading to better efficacy in release of insoluble drugs. Because bare insoluble particles easily agglomerate, it would be advantageous to coat the drug nanoparticles with appropriate anti
aggregative materials. Although some strategies have been reported where the loading capacities of those molecular assemblies was very low. Thus, surface-coating of drug crystals using LbL technology is a promising method to improve performance of the oral formulation of insoluble drugs. This LbL strategy allows in formulation of multilayer nanoshells with thickness of 5 to 50 nm and requisite composition. Nano-assembly approaches including design of shells of different components, including ones serving as diffusion barrier and outermost layer containing targeting agents.

Stimuli-free auto-modulated material release
Most artificial stimuli-response systems exhibit a simple on or off response where a single input leads to single output. It is quite different from naturally occurring system where various outputs can be driven by a single trigger through harmonized feedback processes and their hierarchic structures. If artificial functional modules could be incorporated into a hierarchic organization, several mechanisms may operate cooperatively resulting in harmonized and/or non-equilibrated functions in the absence of a complex input. Ariga and coworkers realized this concept by coupling two major nanofabrication methodologies, mesoporous synthesis and LbL assembly, to create mesoporous nanocompartment films. The major components of the mesoporous nanocompartment films are hollow silica capsules containing hierarchical micro- and nano-spaces. These capsule structures are further assembled into hierarchical layered structures through LbL assembly with the aid of silica nanoparticles and a counter polyelectrolyte. An unusual stepwise release of water from the mesoporous nanocompartment films to the exterior without applying an external stimuli was found, which can be regarded as stimuli-free auto-modulated material release.

US PATENTS ON LAYER BY LAYER SELF ASSEMBLY

Table 2: It shows US patent on layer by layer self assembly

<table>
<thead>
<tr>
<th>Title of Invention</th>
<th>Application No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self assembly thin film coating to enhance the biocompatibility of material.</td>
<td>PCT/US/2001/012042 [72]</td>
</tr>
<tr>
<td>Self assembly nanobump array structures and method to fabricate such structures.</td>
<td>PCT/US/2003/011863 [73]</td>
</tr>
<tr>
<td>Self assembly multilayer nanocomposite of graphene and metal oxide material.</td>
<td>US/12/852,794 [74]</td>
</tr>
<tr>
<td>Polar ordering of reactive chromophores in layer by layer non linear optical materials</td>
<td>US#6,953,607 (2005) [75]</td>
</tr>
<tr>
<td>Fiber optic sensor or modulator using tuning of long period grating with self assembled layers</td>
<td>US#7,336,861 (2008) [76]</td>
</tr>
<tr>
<td>Enhancement of second order non-linear optical susceptibility in organic film material Nano-Centrosymmetric nanoparticles</td>
<td>US#7,772,013 (2010) [77]</td>
</tr>
</tbody>
</table>
CONCLUSION

LbL assembly has received much attention over the last few years. This review describes basic mechanism of interaction between layer by layer self-assembly such as electrostatic interaction, hydrogen bonding, hydrophobic interactions, and van der Waals force. Principles of chemical (ionic strength, pH, electrochemical), physical (temperature, electric and magnetic field, ultrasound, and mechanical action), and biological stimuli (enzymes and receptors) helps in understanding the underlying mechanisms of these stimuli which is essential for developing new methods of encapsulation, release, and targeting. Multifunctionality of LbL systems can be formulated by the inclusion of different materials in the multilayer’s i.e. polyelectrolytes, metal oxides, clay nanoobjects and enzymes all of which could be rendered stimuli-responsive. Capsules and vesicles formulated by LbL technique generally shows much higher drug loading capabilities, capsules and nanogels in turn are considered more stable than micelles and vesicles. LbL assemblies will need to be optimized for reproducibility, mechanical stability and resistance to biological media (i.e. high level of salts and proteins).

Future advances are likely to be in the cell surface presentation of peptides and immune system response, cancer treatment, stem cells, neuron signaling, and delivery of therapeutics both in vitro and in vivo.

REFERENCES


73. Gregory W Auner Self-assembled nanobump array structures and a method to fabricate such structures, WO 2003090284 A1.