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Polyelectrolyte multilayers: preparation and applications

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The review concerns the results of studies on the synthesis of polyelectrolyte coatings on charged surfaces. These coatings represent nanostructured systems with clearly defined tendency to self-assembly and self-adjustment, which is of particular interest for materials science, biomedicine and pharmacology. A breakthrough in this area of knowledge is due to the development and introduction of a new technique, so-called layer-by-layer (LbL) deposition of nanofilms. The technique is very simple, *viz*., multilayers are formed as a result of alternating treatment of a charged substrate of arbitrary shape with water-salt solutions of differently charged polyelectrolytes. Nevertheless, efficient use of the LbL method to fabricate nanofilms requires meeting certain conditions and limitations that were revealed in the course of research on model systems. Prospects for applications of polyelectrolyte layers in various fields are discussed.
The bibliography includes 58 references.

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1. Introduction

Rapid progress in fundamental and applied nanotechnology of polymeric materials is driven by increasing interest of researchers in polyelectrolyte coatings and practical applications of these systems in various fields, mainly in medicine and pharmacology, in order to design novel systems for diagnosis and treatment of diseases.[1–12] It is difficult to overestimate the importance of such coatings for the design of systems for targeted delivery of drugs, vaccines and other biomaterials that can carry active drug substances to the lesions without adverse effects on healthy cells. Researchers not only conduct a search for new drugs, but also design improved forms of known biologically active preparations. Often, it is much simpler to improve the efficiency of a known drug by improving its pharmacokinetic properties, bioavailability, by changing the dose amount and dosage form rather than to design a new pharmaceutical product.

In spite of high research activity, the problems mentioned above are still to be solved. In this connection, a promising strategy is to use polyelectrolyte multilayers (PEM). They can be prepared using a well-developed technique called layer-by-layer electrostatic self-assembly (LbL ESA, or simply LbL) which uses step-by-step adsorption of oppositely charged polyelectrolytes to fabricate nanofilms.[13] The approach was for the first time proposed by Iler in 1966 [14] and then developed by Decher *et al*.[15–17] in the early 1990s and by the authors of more recent studies [18–22] including those concerning methods for PEM preparation.

In the early 21st century, alternating adsorption of oppositely charged polyelectrolytes from their solutions on a substrate surface became a very popular technique [2–13, 23–25] which is now widely used for surface func-

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tionalization and for engineering of multilayer films and capsules. The results of basic research into these novel nanostructured materials and possible fields of their application are available in numerous review articles (see, *e.g.*, Refs 26 and 27).

In the past fifteen years, much work has been done to establish factors influencing the formation and stability of PEM. Initially, the emphasis was placed on apparent simplicity of the formation of model PEM on charged substrates and on the rapidly extending range of polyelectrolytes suitable for the preparation of PEM. Many authors attracted the attention of readers to these two features, leaving the mechanisms of PEM formation and degradation out of consideration, although the lack of corresponding information deprives the LbL technique from predictive power. For instance, most of the early studies were concerned with PEM fabricated using only two polyelectrolytes, *viz.*, polystyrenesulfonate sodium (PSS) polyanion and poly(allylamine hydrochloride) (PAA) polycation. The reasons for the beneficial effect of these two polymers were unclear for long.

Currently, a new stage of research on PEM begins. Indeed, studies on general regularities in this field are almost completed and the emphasis is now placed on the development of practically valuable applications. Of course, this cannot be done without reliance on the results obtained to date. Besides, information on the potential of the LbL technique is often fragmentary while interrelations between the physicochemical properties of components and the functional parameters of products are not always interpreted unambiguously. In this connection, we felt it necessary to discuss hidden barriers and problems in the fabrication and applications of PEM. Therefore, consideration of modern publications is accompanied by a brief analysis of the pioneering studies on the title topic.

2. Mechanism of spontaneous formation of multilayers

Polyelectrolyte multilayers can be prepared using a very simple and versatile procedure that is applicable to almost all pairs of synthetic polyions or charged biopolymers. The formation of PEM films (Fig. 1) is based on the electrostatic interaction between a charged substrate and oppositely charged chains that occurs as the substrate is immersed in a dilute solution of a polyelectrolyte. The electrostatic interaction causes neutralization of (*e.g.*, positive) charges of the substrate and the formation of a monolayer of a polymer (polyanion). Owing to the steric factor, the addition of the equivalent (in terms of charges) number of chains almost never leads to complete neutralization of charges that are localized on the substrate at different distances from one another. As a consequence, a portion of the substrate charges remain uncompensated, so an additional amount of charged chains is required for their neutralization. Thus, the first stage of adsorption leads to recharging of the (initially positive) surface and to the appearance of 'loops' and 'tails' bearing an excess amount of (negative) charges on the surface. Clearly, these charges preclude further adsorption of the polyanion from the solution onto the substrate due to the action of Coulomb repulsive forces, *i.e.*, the layer buildup spontaneously ceases.

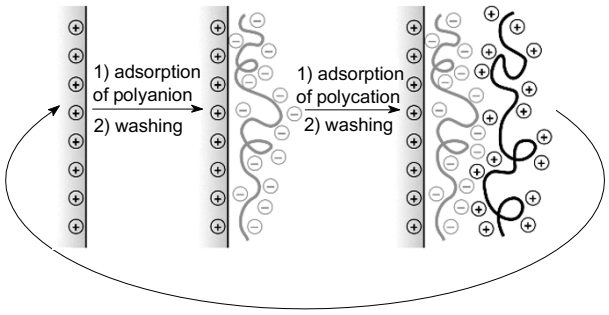


Figure 1. PEM formation and growth: a schematic.

The process is driven by recharging since it ensures the occurrence of the second and subsequent stages of PEM formation, when the substrate with the adsorbed polymer (the polyanion) is immersed in a solution of an oppositely charged polyelectrolyte (the polycation) after washing and removal of the excess polymer. The alternating multiple adsorption provides the formation of stable multilayers with different properties, which are strongly dependent on the nature of the oppositely charged synthetic and natural polymeric components used in the experiments. The LbL technique allows one to control the surface properties of microparticles that are governed by the outer (last adsorbed) polyelectrolyte layer.[22]

The LbL method is of increasing interest because it allows one to control the thickness of multilayer thin films on the nanometre scale both in the course of film fabrication and when such films are used for coating planar substrates or substrates of more complex shape. This makes it possible to design functional ultrathin stimuli-responsive systems for utilization as components of various structural materials.[27]

Limitations of the LbL method are few. In particular, no multilayer formation occurs if the polymer charge density is lower than a certain critical value.[28, 29] Dissolution of PEM may also result from pH- and salt-induced disruption of interpolyelectrolyte binding.[30 – 34] Yet another reason for the degradation of multilayer films is interpolyelectrolyte exchange (see below). For instance, if a substrate coated with a PEM is immersed in a solution of one of the two polyelectrolytes, this polyelectrolyte may be transferred from the substrate to the polyelectrolyte being added. Such a transfer may be governed by the concentration difference between the polyion being added and the polyelectrolyte in the PEM, as well as by the thermodynamically favourable formation of water-soluble nonstoichiometric polyelectrolyte complexes (NPEC). As a result, the multilayer film degrades.

3. Growth of polyelectrolyte multilayer films and phase diagrams of polyelectrolyte complexes in solutions

Efficient use of multilayer coatings depends on relevant choice of the field of application taking account of the results of fundamental research. Practical application of PEM is also limited by certain factors including low rate of polymer rearrangements owing to small amounts of the material and possible influence of the substrate surface on the film formation.

The available PEM growth theories describe the film formation process as irreversible one.[35,36] However, from the standpoint of thermodynamics rather efficient adsorption of multilayer films on the surface and the possibility of being involved in subsequent layer deposition process contradicts numerous data obtained in the studies of soluble NPEC.[37] For instance, in each adsorption step the top layer of the PEM contacts a large excess of charged groups in the solution as compared to the charge on the film surface. In other words, from the standpoint of thermodynamics one can expect that the film growth will be completely suppressed because the environmental conditions favour degradation of the PEM owing to the formation of soluble NPEC.

This fact was emphasized in a review.[38] It was found that the trends revealed in the studies of PEM are due to the phase behaviour of the solutions of corresponding NPEC. In particular, polycations with high density of primary amino groups (*e.g.*, PAA) and polyanions bearing sulfate or sulfonate groups in the molecule [potassium poly(vinyl sulfate), heparin, PSS] provide the strongest interpolyelectrolyte binding, which hampers inter-chain exchange within the polyelectrolyte complex (PEC) and(or) PEM and even can make it impossible. As a consequence, the adsorbed polyelectrolyte cannot participate in inter-chain exchange and remains strongly bound to the multilayer even after subsequent addition of the oppositely charged, equally reactive counter-polymer. Clearly, this is the best situation for the formation and growth of PEM films.[27] Pairs of weakly bound polyelectrolytes, *e.g.*, polycations bearing quaternary amino groups, as well as carboxylate or phosphate polyanions, readily form water-soluble NPEC, which leads to erosion and degradation of PEM.

To find a way of avoiding PEM degradation, one should construct full phase diagrams of polycation–polyanion–salt (NaCl) aqueous mixtures[38] and analyze how the solution pH or ionic strength can be used for controlling the PEM formation on the substrate surface. Information on the interrelations between the formation mechanisms and properties of PEC and PEM allows one to fabricate platforms for controlled growth or erosion of the PEM using a wide range of weak or partially charged polyelectrolytes on the substrate surface.

The observed PEM erosion can be explained by the formation of negatively (region L′ in Fig. 2) or positively (region L″ in Fig. 2) charged NPEC in the course of film growth upon the addition of the polyanion or polycation, respectively. It is noteworthy that the L region corresponding to the deposition of the complex and to the formation of stable layers (see Fig. 2) is bell-shaped and that its base extends as the ionic strength of the solution decreases, thus being indicative of the extension of the precipitation region.

From the phase diagram shown in Fig. 3 it follows that phase separation in the solutions of oppositely charged polyelectrolytes can obey a much more complicated, namely, fan-shaped pattern compared to that schematically shown in Fig. 2 for PEM. This is in good agreement with the previously studied behaviour of NPEC in aqueous salt solutions, *viz.*, the efficiency of layer buildup increases in the solutions with moderate concentrations of the low-molecular-mass electrolyte.

Emphasize that competitive removal of polyelectrolytes from PEM to NPEC occurs under conditions where the polyelectrolyte chains remain strongly associated in both

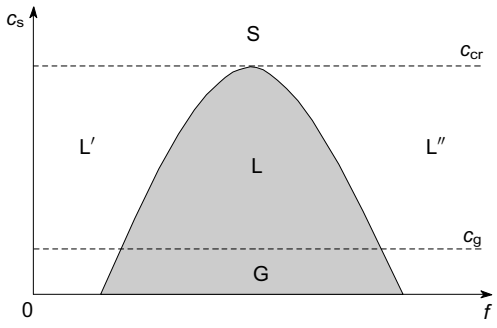


Figure 2. A schematic of the stability diagram of adsorbed multilayers consisting of oppositely charged polyelectrolytes.
Horizontal axis: the composition of the mixture given as mole fraction of cationic species (f).
Vertical axis: the ionic strength of the solution or the salt concentration (c_s)
The L and G regions correspond to the multilayers in the liquid and glassy state, respectively; the L′ and L″ regions correspond to soluble polyelectrolyte complexes. In the S region, no complexation occurs. [Taken from D.Kovačević, S.Van der Burgh, A.de Keizer, M.A.Cohen Stuart. *Langmuir*, **18**, 5607 (2002)].

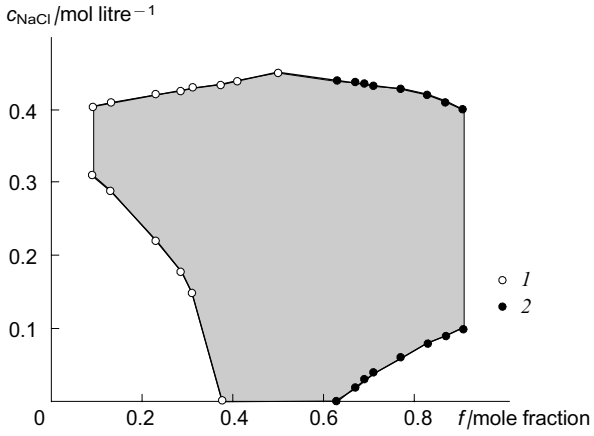


Figure 3. Phase diagram of PEVP–PMAA–NaCl mixture in aqueous solution at pH 8.4 supported by 0.01 mol litre^{-1} phosphate buffer. The concentrations of polymers were 0.001 mol litre^{-1}.[38]
Excess of polyanion (*1*) and excess of polycation (*2*).

the PEM and NPEC; however, the chain redistribution favours the formation of water-soluble NPEC and eventually results in PEM erosion. Understanding the PEM erosion mechanism is of particular importance because this helps to construct a PEM 'growth map' to avoid the instability regions that appear during film deposition. The incorporation of a charged chain into soluble NPEC is a spontaneous process. As the number of NPEC species in the solution increases, one deals with a gain in the translational and configurational entropy and much higher mobility of the soluble polyelectrolyte chains.

For instance, no PEM formation occurred for the pair alkylated poly(*N*-ethyl-4-vinylpyridinium bromide)

(PEVP) – poly(methacrylic acid) (PMAA) at pH 8.4, when both components were strongly charged.[38] The same was observed for the pair PMAA – globular protein lysozyme at pH 8.4, when the polyacid was charged, while the positively charged protein was completely removed from the surface to the solution owing to the formation of a water-soluble complex in the excess of the polyacid. Various examples of PEM erosion by this mechanism are available in the literature. They illustrate the importance of possible formation of soluble complexes in the course of film growth.

4. Factors influencing the formation of polyelectrolyte multilayers

The formation of PEM can be controlled by varying certain parameters including the type and length of the interacting chains as well as the solution pH and ionic strength; changes in these parameters can favour the PEM growth or erosion.[38]

The type of the polyelectrolyte can strongly affect the binding energy and the equilibration time. The rapidly equilibrating systems, *e.g.*, mixtures of polycations bearing tertiary or quaternary amino groups and polyanions having carboxylate or phosphate groups, are characterized by spontaneous formation of water-soluble NPECs representing thermodynamically stable structures that readily undergo polyelectrolyte exchange reactions involving molecularly dispersed complexes.

Systems whose components differ insignificantly in the PEC stability against the destructive effect of the low-molecular-mass electrolyte added are characterized by marked selective binding effects.[37] For instance, the PEVP polycation added to a mixture of solutions containing PMAA of different degree of polymerization preferentially binds to the longer chains. Another type of selectivity is observed in ternary systems, *e.g.*, in a mixture of a polyphosphate anion and a soluble NPEC composed of PMAA and PEVP. The equilibrium in this reaction is controlled by the low-molecular-mass electrolyte. As the polyphosphate anion is replaced by native DNA molecule, the selectivity of binding persists, but the system becomes highly sensitive to the nature of the salt, *viz.*, at the same concentration of KCl and LiCl, the equilibrium of competitive binding of DNA and sodium polyphosphate by the polycation is almost completely shifted to one or another direction.

Complexes containing macromolecules with sulfate or sulfonate groups behave in fundamentally different manner. Even small amounts of these groups (3 mol.% – 5 mol.%) provide strong electrostatic binding of such polyanions to polycations, despite the presence of strongly ionized polycarboxylate anions in the system. This leads to abnormally high stability of ion pairs composed of the polycations bearing quaternary amino groups and the polyanions having $-SO_3^-$ or $-SO_4^-$ groups even if PSS oligomers were used. In particular, a complete dissociation of the complexes formed by PSS oligomers with ten and twenty repeating units in the chain requires NaCl solutions with a salt concentration of 2.3 and 3.4 mol litre^{-1}, respectively,[39] which is ten times higher than the critical salt concentrations for the polycarboxylate and polyphosphate complexes or DNA complexes.[40]

Strong binding of polyanions containing $-SO_3^-$ or $-SO_4^-$ groups imposes kinetic restrictions on interpolyelectrolyte exchange or exchange between complexes of the same chemical identity, which favours PEM growth. Other polyanions containing these groups, like PSS, preferentially bind to polycations, while their antagonists namely, polyamines bearing the primary amino groups (*e.g.*, PAA), exhibit strong binding to polyanions. The order of polyamines with respect to their affinity to DNA is as follows: from weakly bound chains with quaternary amino groups to the polyamines with tertiary, secondary and, eventually, primary amino groups. This order is due to the increase in the steric accessibility of nitrogen atom in these compounds. Dispersive, nonelectrostatic forces may also contribute largely to the binding energy in the case of the most reactive PSS and cationic polymers with primary amino groups.

Very high stability of PAA – PSS films is due to the formation of stable and irreversibly bound ion pairs. These systems, as well as the PAA – PMA pair, are promising for fabrication of PEM.

Thus, the formation of PEM and the pH-dependent changes on their surface are to a great extent governed by the phase behaviour of the polyelectrolyte complexes in solution. Obtaining detailed information on competitive interactions on the substrate surface and in solution requires the use of high-precision methods that are mainly developed for the design of structural nanomaterials. Using the results obtained in the studies of the interrelations between PEC and PEM, one can predict and control the deposition of multilayers composed of weakly or strongly charged polyelectrolytes, globular proteins and nanoparticles on various surfaces. In other words, the possibility appears to design stimuli-responsive PEM films with the desired type of response to small changes in the environmental conditions. This feature is of great importance for various applications of the PEM films, in particular, for fabrication of micro- and nanocapsules.

5. Polyelectrolyte micro- and nanocapsules

Among practically valuable applications of the LbL method, there is the possibility of fabrication of capsules. As applied to colloidal particles, the technique gives capsules with controllable molecular mass, size, composition and functions.[1–3] The key factors of the encapsulation process include technological features, the efficiency of encapsulation, the amount of encapsulated agent in the capsules, the capsule size, *etc.* Encapsulation involving the LbL technique (Fig. 4) meets these requirements. Microcapsules are formed at room temperature in aqueous media and the process does not require organic solvents, surfactants or special equipment. The role of universal micromatrices can be played by aggregates of the insoluble complex of the polyanion or polycation with the oppositely charged coun-

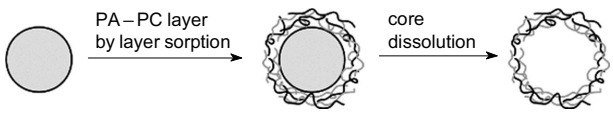


Figure 4. A schematic of formation of LbL capsules. PA stands for polyanion and PC stands for polycation.

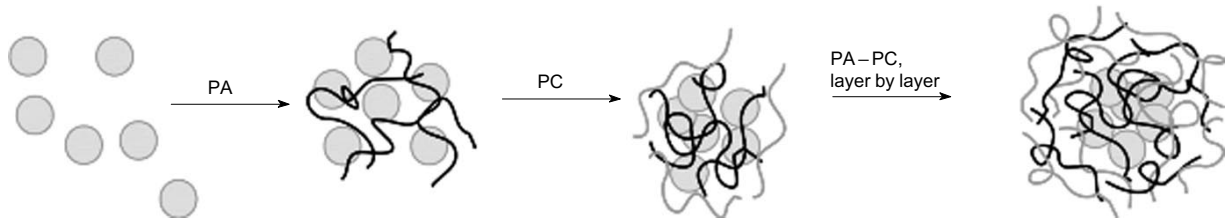


Figure 5. A schematic of formation of protein capsules. PA stands for dextran sulfate polyanion and PC stands for chitosan polycation.

terpart (including proteins) which can be either micro- or nanoparticles.

It is important that the microencapsulated agents fabricated by the LbL technique be highly sensitive to changes in the environmental conditions. Since polyelectrolyte capsules are regarded as promising microscopic tools for targeted drug delivery, considerable attention is paid to the studies on the mechanism of controlled drug release from the capsules into solution. In this connection, of particular interest are stimuli-responsive LbL materials that can be used in many practical applications, *e.g.*, for loading with and controlled release of therapeutic agents upon minor variation of the environmental characteristics, for suppression of inter-chain interaction, as well as for degradation of the LbL films under the action of physical factors and owing to phase transitions or polymer rearrangements within LbL films.

Recent studies on the potential of LbL assemblies based on biopolymers (including polysaccharides, polypeptides and proteins) for drug delivery have been analyzed in a review.[1] The emphasis was placed on drug reservoirs consisting of multilayer planar films and capsules with low toxicity and good biocompatibility, which are suitable for loading and protecting toxic or unstable drugs, and for targeted drug release from the carriers at the desired site and at the appropriate time.

The authors of yet another review[2] noted that the surface modification of biomaterials is a well-known approach to enable an interface between the implant and the surrounding tissue. Since its discovery the LBL method has become an increasingly popular and attractive technique for functionalization of the biopolymer surface and for the engineering of various systems (*e.g.*, capsules, hollow tubes and membranes) with controllable physicochemical properties. Versatility of systems enables the incorporation of so-called nanostructured building blocks, including biopolymers, which represent biomimetic multilayer compositions similar to human tissues.

In the last decade, attention is being increasingly focused on application of the LbL technique in biology, in particular, for cell encapsulation, as an alternative approach to overcome the drawbacks of the well-known bulk hydrogel methods. Nanostructured multilayer materials are used for modification of the surfaces of single cells and cell aggregates. They can also be transformed to hydrogels or other biosystems suitable for fabrication of hollow capsules. A review by Mano and co-workers[3] concerns various applications of the LbL method for solving particular problems in various fields including cell therapy, regenerative medicine and biotechnology.

Chitosan-based polyelectrolyte micro- and nanocapsules bearing encapsulated proteins (*e.g.*, insulin) are being intensively studied as promising carriers to improve the bioavailability of dosage forms. The authors of a review[13] summarized and analyzed the results of studies on the fabrication and properties of protein microparticles with controlled stability that were formed by the LbL method. They considered different variants of using proteins as adsorbed polyelectrolytes, methods for the incorporation of proteins into matrices, as well as immobilization of proteins into pre-formed PEM due to a change in the permeability of their shells. Particular attention was paid to the biocompatible and biodegradable microparticles possessing the depot functions, *i.e.*, the ability to reliably protect biologically active substances from aggressive media in the human body and to quantitatively release protein agents (hormones, enzymes, peptides) into a solution as a certain acidity of the solution is attained; this is of particular importance for the design of oral means of protein delivery.

As a particular example, Fig. 5 presents different steps of formation of protein LbL capsules. To a solution of a positively charged protein (insulin, a hormone), an excess amount of negatively charged dextran sulfate (a natural polyanion) was added. The interaction of these components results in a suspension of insoluble protein – polyelectrolyte complexes, which was centrifuged and the supernatant was removed. The precipitate that formed was miltiply washed and negatively charged microparticles were obtained. To a suspension of these microparticles in the same buffer solution, an excess of a solution of chitosan (a natural polycation) was added and positively charged microparticles were obtained and treated analogously.[13]

The layer deposition process was repeated many times and each step was completed with spontaneous formation of a new monolayer that imparted the corresponding charge and characteristic properties to the entire microparticle. For instance, with chitosan as the top polyelectrolyte layer the particle acquired a positive charge and mucoadhesive properties. Emphasize that the insulin-containing microcapsules thus prepared remained quite stable in acidic media (in stomach), but degraded at neutral pH (in the digestive tract) and released insulin. Yet another important advantage of the LbL technique is the possibility of incorporating other substances, *e.g.*, protease inhibitors into microcapsules. In this case, microencapsulated insulin released into solution at neutral pH is protected from the action of proteases, which improves its bioavailability.

There are three methods for the incorporation of proteins. First, the protein can act as polycation or polyanion,

thus being immediately involved in the fabrication of multilayers. Next, it can be used to form the primary aggregates, which act as the substrate for subsequent LbL adsorption of biopolyelectrolytes. Eventually, the protein can be incorporated into pre-formed polyelectrolyte microparticles or microcapsules due to a change in the permeability of the polyelectrolyte shell.

6. Biopolyelectrolyte multilayers, their properties and specific features

A huge number of polymeric materials were studied to establish their appropriateness for the formation of biopolyelectrolyte multilayers, *i.e.*, PEM composed of charged biopolymers (mainly proteins, enzymes and nucleic acids) since the first publications on multilayer films. The most promising compounds for the development of novel targeted drug delivery systems include synthetic polymers and biopolymers, such as polysaccharides, poly(lactic acid), poly(glycolic acid), polylactides, polyacrylates, acryl polymers, poly(ethylene glycol) and its analogues.

Polymeric materials possess valuable properties necessary for drug transport, *e.g.*, biocompatibility, biodegradability, functional compatibility and low toxicity.[41] Among the materials, there are chitosan and chitosan sulfate,[42] as well as protamine and dextran sulfate.[43] Certain applications of PEM based on chitosan and its derivatives in medicine and pharmaceutic industry for encapsulation of biologically active substances and drugs, as well as the physicochemical, biological and environmental properties of corresponding polyelectrolytes have been reviewed.[44]

Thin polymeric nanofilms can release the active substance when triggered by changes in the environmental parameters (pH, temperature, ionic strength of solution, electric and magnetic field). Stimuli-responsive polymeric materials can adapt to external stimuli, control transport of ions and molecules, change their wettability and adhesion, transform the action of chemical and biochemical factors to optical, magnetoelectric, thermal and mechanical signals. The mechanical strength and elasticity, as well as the electrical, optical and other properties of polymers make them unique building blocks for the design of nanocomposite materials possessing valuable properties that are necessary for drug transport.

Nanomaterials represent promising building blocks for the development of next-generation therapeutics. At present, research in this field is at an intermediate stage corresponding to transition from simple questions 'Can this particle eradicate cancer type cells?' to much more sophisticated problems, *e.g.*, 'Can we design a particle to preferentially deliver cargo to a specific cancer type cells?'. Answers to these questions in a review[4] (see†) open a new era in the development of nanosystems for targeted drug delivery.

As mentioned above, increasing interest in the LbL method for fabrication of multilayer thin films is to a great extent due to the possibility of (i) controlling the film thickness on the nanometre scale and (ii) using such films for coating planar or complex-shape substrates. This allows one to prepare functional stimuli-responsive ultrathin films for various structural systems.[5]

Drug administration implies efficient pre-encapsulation and protection of drugs from the environmental factors, followed by controlled release as the target is reached. A review[6] covers the prospects for the use of LbL structures as drug reservoirs for transport of physiologically active substances with the emphasis on nano devices of various geometry from flat surfaces to fibres and capsules in order to solve particular problems in tissue engineering. The technique enables encapsulation of various compounds (antibiotics, growth factors and biosensing substances), is not limited to water-soluble substances and includes hydrophobic drugs.

Researchers pay much attention to the design and production of nanostructured materials for biomedical applications. At present, more than 1000 scientific papers are published *per annum*, tens of patents are deposited and certain products based on the LbL method become commercially available. A critical analysis of problems and advances in functional LbL biomaterials for biomedical applications, in particular, for tissue engineering was reported.[7] The main advantages of the LbL method are as follows:

— a precise control of the surface properties;

— environmentally friendly, mild conditions and low-cost manufacturing;

— versatility for coating all available surfaces; and

— possibility of synthesis of uniform films with controllable thickness; and

— incorporation of biomolecules and drugs into multilayers and controlled release from them.

Initially, the technique mainly developed as 2D substrates and hollow capsules for fabrication of multilayer compositions. In the last decade, there has been great progress in LbL technique. A review by Picart and coworkers[8] concerns three major aspects:

— new methods and original substrates for engineering LbL films and controlling cell processes from adhesion to differentiation;

— major ways to achieve temporal control by chemical, biological and physical triggers; and

— combining the LbL method, cells and substrates for regeneration of 3D tissues including cardiovascular and neuroprosthetic devices and bone implants.

There is also a review[9] devoted to LbL assembly of PEM of biopolymers with thermo- and pH-responsive micro- and nanogels based on a synthetic copolymer of *N*-isopropylacrylamide and poly(acrylic acid) or biodegradable hyaluronic acid. It was shown that the structure and properties of biocompatible multilayer films can be finely tuned by confinement into thermo-responsive gels, which offers great prospects for biomedical applications including controlled release of biomolecules, fabrication of biosensors and tissue engineering.

At present, single cell assay (SCA) is a powerful tool for diagnostics and classical cell biology studies. Utilizing the LbL method to produce PEM for SCA may offer new prospects owing to the triple role of the multilayer film, namely:[10]

† The authors run a Website at
http://bionano.eng.unimelb.edu.au/knowledge_base/
as the most efficient tool for systematization of the published, in-press and in-preparation materials as the first step in the process of organization in this huge area of multidisciplinary research.

— high capacity for various biomolecules;
— modelling of signal molecule diffusion to a cell; and
— the possibility of cell patterning.

The technique for producing materials with nanometre accuracy, based on self-assembly of multilayers, is in demand in various fields of chemical science, namely, organic, physical, analytical and colloid chemistry, biochemistry, interfacial chemistry and supramolecular chemistry. Although the LbL design of novel materials successfully develops as key avenue of research, fabrication of 3D nanostructures is still a hard-to-reach goal. The formation of controlled multilayers using LbL self-assembly is a promising simple, fast and cheap approach.

Researchers from Japan and Taiwan reported a review [11] focussed on the architectonics of LbL assemblies and on the evolution and innovations of the LbL approach. Examples presented in the review can be divided into two main groups:

— development of methodology (technological, material and phenomenological aspects which expand the concept); and
— advances in practical applications (physical, chemical, biological and biomedical aspects).

The LbL technique was initially treated as a surface modification method based on step-by-step adsorption of at least two different materials onto a flat surface. In the last two decades, the method was extended to substrates of more complicated shape and higher functionality. The potential utilization of LbL films in biomedical engineering, the modification of substrates and improvement of their biointegration by extrapolation of LbL to 3D were reviewed.[12] These aspects are of great importance for protein engineering, for the design of biosensors, microreactors and targeted drug delivery systems.

Immobilization and(or) encapsulation of enzymes in LbL structures has a great potential for the design of various structures including those suitable for encapsulation of multiple-enzyme systems where the catalytic function is realized through sequential activity of the encapsulated enzymes.[45]

Simplicity and versatility of the LbL technique underlie intensive investigation of the method for both drug and gene delivery applications. Recently, particular attention has been paid to the development of stimuli-responsive LbL materials. Responsiveness plays an important role in many practical applications. There are four main action mechanisms of stimuli-responsive systems, namely:

— disruption of layer interactions,
— degradation of the LbL films,
— film destruction under the action of physical stimuli, and
— phase transitions or polymer rearrangements within the LbL films.

These mechanisms and recent advances in this field have been analyzed.[46]

In the last decade there has been rapid progress in the studies on new methods of synthesis, functionalization and application of microcapsules for biomedical applications, in particular, for targeted drug delivery and for the design of biosensors and bioreactors. The authors of a critical review [47] summarized and discussed recent studies on multilayer capsules, considered the advances in LbL assembly and the properties of capsules stabilized by hydrogen bonds, through covalent cross-linking and biospecific interaction. Shape transformation of capsules under the action of external stimuli, biomedical applications of multilayer capsules suitable for drug delivery to cells, as well as problems in the design of biosensors and bioreactors were also outlined.

At present, more than 40% of new pharmacologically active compounds are poorly soluble or even insoluble in water. This requires the development of new methods for their administration and subsequent delivery to targets. One of the most promising strategies for solving this problem is to use the LbL technique for encapsulation of drugs. Publication [48] concerns the application of LbL for the design of emulsions, nanoparticles and capsule-based delivery systems for lipophilic drugs.

Yet another review [49] is devoted to technological aspects of polymer-based microparticles and possible applications of these systems in tissue engineering and regenerative medicine (from research to clinical trials).

Film-like LbL assemblies and their appropriateness for drug delivery applications were assessed using selected recently investigated systems.[50] A number of examples of the delivery of low-molecular-mass physiologically active compounds, nucleic acids, peptides and proteins are given and analyzed and recent advances in the studies of film structures based on organic/inorganic hybrids were pointed out.

An overview of the use of chitosan to prepare nanostructured films using the Langmuir–Blodgett and LbL techniques is available.[51] An emphasisis place on prospects for practical applications.

Currently, there is lack of information on the permeability of 3D PEM shell systems. It is only known that drug release from them may depend on the environmental conditions. The physicochemical parameters affecting the permeability of the 2D and 3D films, namely, the number of layers and the solution pH and ionic strength were analyzed.[52] Synergistic effects on loading and release of physiologically active compounds from LbL multilayers were discussed.

The LbL technique allows one to prepare multilayers with strongly bound polymer chains and to fabricate highly swollen capsules and hydrogel-like films. Progress in this area was described,[53] different approaches to the preparation of ultrathin hydrogel materials were compared and possible applications were outlined.

Recent advances in the synthesis of microcapsules were reviewed.[54] The composition of polyelectrolyte multilayer capsules was optimized and prospects for their application in medicine were discussed.[55]

Polyelectrolyte capsules are treated as new microscopic carriers having high potential in the field of medicine (see above). A review [56] is devoted to detailed consideration of the LbL technique used to fabricate polyelectrolyte capsules, as well as to methods employed to obtain controlled drug release from the capsules to the environment.

The last fifteen years were marked by rapid progress in the LbL technique, which was used to prepare thin films with unique physical and chemical properties. As applied to colloidal particles, the technique enables fabrication of hollow capsules with controllable molecular mass, size, composition and functions (see a review [57]).

7. Conclusion

Undoubtedly, the available fundamental knowledge of the behaviour of PEM can underlie practically important developments,[58] in particular, targeted surface modification or the design of nonviral vectors for cell transfection. Detailed information on the action mechanisms of PEM may be useful to establish the factors governing their behaviour, which is necessary for further progress in this promising avenue of research.

Summing up, it should be stated that the LbL self-assembly technique still has a huge impact on chemistry, physics, materials science and biology. Among the most intensively developing investigation lines, of particular interest is engineering of stimuli-responsive PEM films that exhibit specific response to changes in the environmental conditions. This approach is to a great extent governed by versatility and adaptability of the LbL products. In the text above we considered the fundamental principles of PEM engineering, which depend on various factors, first of all, changes in the solution pH, ionic strength and temperature.

Further progress in fabrication of stimuli-responsive PEM and studies on their properties require understanding of the principles of the formation of multilayers, interaction of charges within the films and competition between different types of interaction in the environment of the PEM films. Advancement in this area may lead to formulation of a new strategy in the design of stimuli-responsive coatings that can, *e.g.*, simultaneously respond to several signals and provide controlled targeted delivery of different active molecules to the desired sites.

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