Capsules with external navigation and triggered release

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Encapsulation is an important technology for pharmaceutical industry, food production, etc. Its current level of development requires capsule functionalization. One of the interesting ideas to provide new functionality to the micro- and nanocapsules is Layer-by-Layer deposition of functional species. This technique provides step-by-step adsorption of various species (polyelectrolytes, nanoparticles, proteins) when the layer growth is controlled by electrostatic, hydrogen bonding, hydrophobic forces and forming multilayer shells with nanometer precision. This review article introduces recent achievements of Layer-by-Layer technique attaining external navigation ability and release properties the capsule shell.

Introduction

Delivery vehicles modified in order to respond to a certain external triggers in a defined manner have major role in a new generation of intelligent materials, which enable fine spatial and temporal control in 3D environment and replicate natural events during materials' exploitation time. This is very challenging task with requirements, which depend on the application area, such as encapsulation of molecules keeping of their pharmaceutical or other activity during the encapsulation and storage, controlled and sustained release, release at selected target sites.

Different methods for the design of capsule depot systems have specific advantages and drawbacks concerning the upscaling possibility, performance, feasibility to employ different active materials. Capsules with LbL assembled polyelectrolyte shell were used for encapsulation and release of drugs, DNA, sensor dyes and enzymes [1]; inorganic halloysite nanotubes were demonstrated to be suitable for loading of ferments and inorganic nanoparticles [2]. Mesoporous nanoparticles with polypeptide multilayer shell were used for encapsulation and delivery of enzymes [3]. Hydrogels were used for encapsulation of phospholipids, drugs, as liposome reactors and plant growth media [4]. There are numerous publications on the application of micelles and microemulsions in delivery systems and we

would address several recent reviews to provide better understanding of the current achievements in this area [5,6].

LbL capsules can be fabricated under mild conditions both from water solutions and organic solvents. Moreover, the availability of different polyelectrolytes and other charged materials used as building blocks of LbL assembly, offers interesting prospects for engineering capsules tailored for targeted applications [7].

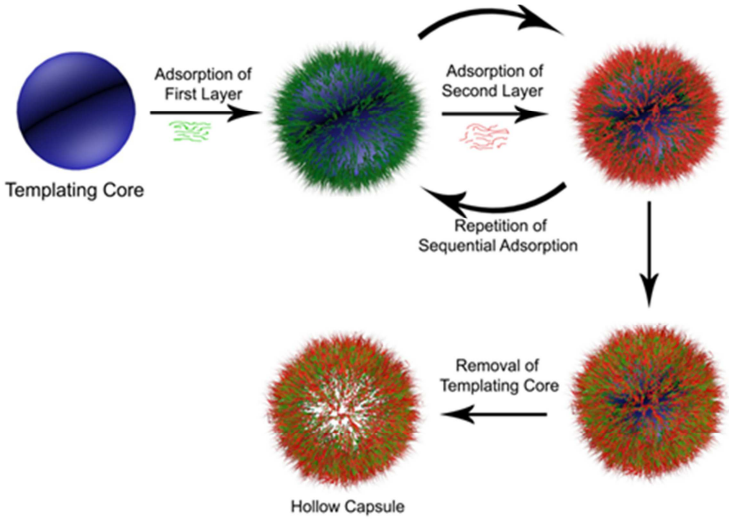

Figure 1. Scheme of the polyelectrolyte capsule formation (from Master Thesis of Simona-Vyara Kolarova, 2014, The University of Liverpool).

As depicted in Figure 1, the template core can be dissolved after applying LbL layers resulting hollow LbL capsules. Initially, organic templates based on polystyrene or melamine formaldehyde were used for capsule fabrication. After deposition of polyelectrolyte multilayers, templates were dissolved by organic solvents and aqueous acidic solutions, respectively, to obtain hollow capsules. However, acidic solutions should be avoided for capsules to be employed for delivery of bioactive material due to the possible inactivation of the latter. Therefore, inorganic template materials were used: silica and carbonates [8,9].

Capsules with external navigation

The best way to receive the external control over capsule movement is to include magnetic nanoparticles inside LbL shell as one or several monolayers. In most cases, chemically inert magnetite and γ-Fe_2O_3 are used [10,11,12]. These oxide nanoparticles possess very good magnetic susceptibility and, at the same time, inert enough to withstand changes of local pH between 3-11. Typical method of shell functionalization with magnetic nanoparticles is as

follows. The capsules are firstly modified with several layers of polyelectrolytes to provide a positively charged surface that provokes subsequent adsorption of magnetic nanoparticles (usually stabilised with acids like citric acid and have, therefore, negative surface charge) from their colloidal solutions. Additional positive polyelectrolyte layer is deposited at the last stage to stabilize Fe_3O_4 nanoparticles in the capsule shell. Figure 2A presents the initial solution containing the suspension of magnetic capsules. When a magnet is placed close to the vial, as shown in Figure 2B, the capsules are forced to the wall of the vial close to the magnet.

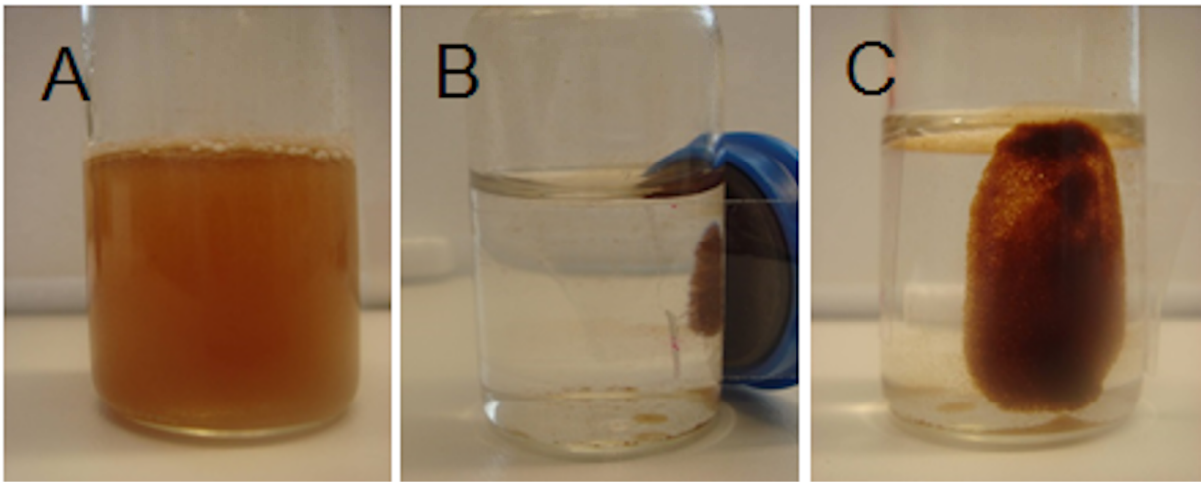

Figure 2. The prepared magnetic capsules in aqueous solution and their targeted movement under external magnetic stimuli [13].

So, capsules with magnetic nanoparticles in the shell can be moved by an external magnet in a desired direction. After removal of magnet, magnetic capsules can be easily redispersed in the solution by gentle agitation. When a magnet is placed on the vial again, the precipitates forms againdemonstrating the ability of magnetic containers can be redirected without loss of the magnetic property. Another way to get magnetic properties is to in situ synthesize magnetic nanoparticles inside capsules. Fe_3O_4 nanoparticles were in situ synthesized inside nanoporous poly(L-glutamic acid)/chitosan (PGA/CS) microcapsules [13, 14]. The carboxylate groups of PGA in the shell could be used as binding sites for the absorption of iron ions for the synthesis of magnetic nanoparticles [15]. Unlike earlier reported ‘magnetic backpacks’ (microconfinements carrying magnetic properties) attached to the cell membrane, the capsules reported here are internalized by cells and could transport a cargo.

Triggered release of encapsulated species

Since LbL method provides high versatility in the shell components, it is possible to fit capsule shell to the certain trigger(s), which remotely and sometimes reversibly open capsule shell. Triggers can be divided into two main groups: (i) internal triggers, where capsule response is based on the intrinsic properties of polyelectrolyte shell and (ii) external ones, where the response is affected by special components (mostly nanoparticles) inside the capsule shell.

Internal triggers are pH, ionic strength of the solution and addition of new solvent. Classic polyelectrolyte shell is permeable for macromolecules and nanoparticles at low pH (<3) and high pH (>8) or high ionic strength while it is "closed" at moderate pH range [16]. An explanation of the permeability properties is influenced by polyelectrolyte interactions in the shell. At the pH of capsule formation, the common charge densities of both polyelectrolytes determine their stoichiometric ratio during adsorption. However, the excess of charging in one polyelectrolyte layercan induce positive (negative) excess of charge in the shell. This may alter the shell morphology by repulsionresulting in defects and pores. The same is valid for the permeability changed at increased ionic strength [17] and addition of a solvent with lower dipole moment [18], which lead to the screening of the interactions between polyelectrolyte layers in the shell followed by shell opening.

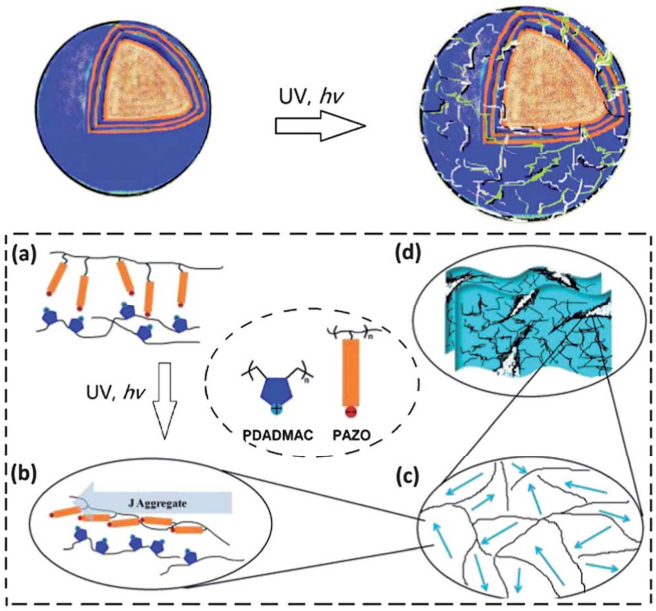

Figure 3. Schematic illustration of poly(diallyldimethyl ammonium) chloride / PAZO microcapsule disruption induced by UV irradiation [23].

External triggers are light, magnetic field, ultrasound, electrochemical and enzymatic degradation [19]. All of them require additional components in the capsule shell sensitive to the trigger impact. Light-responsive capsules are the most developed ones and based on the inclusion of the light-sensitive elements (either dyes or nanoparticles) into the capsule shell. Tao *et al.* have described photoinduced permeability of polyelectrolyte capsules with Congo Red exposed to visible light [20]. Sukhorukov *et al.* demonstrated light-induced encapsulation of active materials in capsules with azo-groups in the shell [21,22]. Upon exposure to a light source, these capsules start to swell and this process continues for several hours. However, long illumination time clearly limits the applicability of the system based on organic dyes. This can be overcome using laser light as a trigger for capsule opening. Skirtach *et al.* developed light responsive capsules with light-sensitive nanoparticles (Ag, Au) inside the LbL shell [23,24]. Upon laser irradiation, the nanoparticles were locally heated causing ruptures in the capsule wall andallowing the release of encapsulated materials. The efficiency of this concept was demonstrated by release of the cargo into the cell cytosol from Ag-containing capsules [25]. Here, near-IR part of the spectrum (800–1200-nm) was used to minimize the absorption of laser light by cells and tissues. Although near-IR light is relatively harmless, it has to be stressed that Ag/Au nanoparticles acting as absorption centers for energy from the laser beam cause substantial local heating due to the small cell volume. By modulating laser power, decreasing illumination time and the concentration of nanoparticles in the shell, cell viability could be improved [26]. The ability to move and open microcapsules by external light has been implemented by simultaneously functionalizing these capsules with magnetic and gold nanoparticles [27].

Other remote forces used for opening of polyelectrolyte capsules are magnetic field and ultrasonic irradiation. Ferromagnetic gold-coated cobalt 3 nm nanoparticles were introduced inside the capsule shell [28]. Resulting 5 micron microcapsules had shell consisting of 9 polyelectrolyte layers and 1 layer of Co@Au. External alternating magnetic field (100-300 Hz and 1200 Oe) was applied to rotate the embedded Co@Au nanoparticles, which subsequently distorted the integrity of the capsule shell drastically increasing its permeability. When the capsules are subjected to ultrasound, a morphological change in the shell occurs due to the shear forces between the successive fluid layers, which results in the disruption of the capsule shell and release of encapsulated materials (even at short (5 s) sonication times) [29,30]. The presence of metal or oxide nanoparticles in the capsule shell is necessary for ultrasonically stimulated release due to the density gradient in the capsule shell perpendicular to the ultrasonic wave. The good conductivity of conductive polymers (e.g., polypyrrole,

polyaniline), chemical stability and mechanical properties allow one to employ them as matrix or shell components for polyelectrolyte capsules combining electric conductivity of the polymer with redox controlled permeability of polyelectrolyte shell [31]. Incorporation of the capsules inside conducting polypyrrole(PPy) results in electrochemically controlled storage and release properties of the capsules. Similar approach was shown for tetrasulfide bridges successfully introduced into the shell, where they can be reduced to yield thiol groups increasing the permeability of the capsule shell [32].

Degradation of capsule shell by biomacromolecules (e.g., enzymes and proteins) requires the presence of the biodegradable polyelectrolytes and enzyme or other catalyst (either inside or outside capsule) to catalyse the degradation of the shell. In this case, the overall polyelectrolyte shell and separate polyelectrolyte layers must be the substrates of the enzymes. Self -disintegrating microcapsules were fabricated by introducing a highly active mix of proteases (Pronase (R)), a mix of proteolytic enzymes isolated from Streptomyces Griseus, into biodegradable polyelectrolyte shell [33]. Pronase was captured by micron-sized calcium carbonate particles that were subsequently covered by onion-like layers of poly(L-arginine) and poly(L-aspartic acid). After dissolution of $CaCO_3$, pronase was released inside the capsule and started to digest polyelectrolyte shell. Lifetimes of thus developed capsules could be successfully adjusted to seconds, hours or days by varying the amount of encapsulated pronase. Being activated, this type of controlled release system becomes autonomic, as it does not require any additional external impact. Similar effect, but after addition of thiol-disulfide exchange reagent dithiothreitol, was observed for hydrogen-bonded multilayer thin films constructed from poly(vinylpyrrolidone) and poly(methacrylic acid) functionalized with cysteamine [34]. The resulting films included thiol moieties that were cross-linked to stabilize the films at physiological pH. Films without disulfide bonds were readily destroyed at physiological pH, while those with disulfide bonds were swollen upon exposure to pH 7 and remained intact. Addition of thiol-disulfide exchange reagent, dithiothreitol (DTT) led to disassembly of the multilayer films. The function of DTT can be also performed by some of intracellular proteins.

Conclusion

The review article described a number of promising directions for application of LbL assembled capsules for targeted delivery and controlled release, but one has to know that there are other parameters that finally decide if LbL capsules can be marketed: mechanical

properties, size, homogeneitylow cost and facile fabrication. On the other hand, the achievement made in the last years is encouraging that one can easily find solutions for many different types of polyelectrolyte micro- and nanocapsules. Additional knowledge is required in understanding the interplay of structural units in multilayered shell. This is also a challenge demanding more sophisticated preparative and analytical methods covering length scales from nanometers in the shell to microns in the capsule lumen.

Acknowledgements

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• of special interest

•• of outstanding interest

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