Release mechanisms for polyelectrolyte capsules

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Received 18th August 2006
First published as an Advance Article on the web 13th October 2006
DOI: 10.1039/b600460c

Polyelectrolyte capsules have recently been introduced as new microscopic vehicles which could have high potential in the biomedical field. In this *critical review* we give an introduction to the layer-by-layer (LbL) technique which is used to fabricate these polyelectrolyte capsules as well as to the different triggers that have been exploited to obtain drug release from these capsules. Furthermore, other types of triggered delivery systems are compared and critically discussed with regard to their clinical relevance. (171 references.)

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Introduction

The field of drug delivery focuses on the development of suitable carriers for therapeutic molecules.[1] The recent availability of many biotechnological therapeutics, such as peptides, proteins and oligonucleotides has challenged and thus stimulated the advanced drug delivery research field as such therapeutics depend on suitable carriers to protect them from extracellular enzymes and to deliver them to the target cells. During the past decades a large variety of micro- and nanocarriers have been developed to serve this purpose. Liposomes were amongst the first nanocarriers studied for the delivery of a large variety of both low and high molecular weight therapeutics.[2]

Since the beginning of the nineties controlled radical polymerisation techniques[3,4] such as atom transfer radical polymerisation (ATRP) and nitroxide mediated polymerisation (NMP) have offered new tools to polymer chemists for the design of well defined polymeric architectures[3] such as block copolymer micelles and polymersomes.[5] The latter supramolecular structures can be seen as the synthetic analogue of liposomes and have, to a certain extent, also been explored for

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the encapsulation of therapeutics. The major advantage of these synthetic structures is that their properties can be tailored by varying their chemical composition. This allows the design of vesicles which respond to specific stimuli (such as pH, redox-potential, magnetic field *etc.*) which may trigger them to release their content at the desired site and time. However, there are several drawbacks to these vesicles. They are not yet commercially available and their fabrication requires complex chemical syntheses and purification, making them expensive to fabricate and hardly accessible to a broad public in the biomedical field

A few years ago, a novel type of vesicle, called polyelectrolyte capsules, were introduced.[6,7] They are fabricated using the Layer-by-Layer (LbL) technique, *i.e.* the self assembly of charged species onto an oppositely charged sacrificial colloidal substrate followed by the dissolution of this substrate. Possible advantages of polyelectrolyte capsules are the absence of hazardous procedures and the use of simple building blocks along with the possibility to introduce a high degree of multifunctionality within their shell. The promising expectations of polyelectrolyte capsules for biomedical applications have evoked a synergistic effect between scientists from different fields such as chemists, material scientists, pharmacists, biologists and even theoretical and experimental physicists.

While other reviews have reported on the physicochemical properties,[8,9] permeability,[10] use as microreactor[11] or biofunctionalisation[12] of polyelectrolyte capsules, this review aims to outline the efforts made towards the development of polyelectrolyte capsules which are 'intelligent' in the sense that they release their content as a consequence of external or internal stimuli. We have especially tried to make known the strengths and weaknesses of intelligent polyelectrolyte capsules *versus* other types of stimuli-sensitive delivery systems and to determine what specific opportunities there are for polyelectrolyte capsules in the field of drug delivery. The discussion brought in this review should stimulate scientists from multidisciplinary fields to focus on the development of polyelectrolyte capsules which could offer a solution to persisting needs in drug delivery.

The Layer-by-Layer technique

The Layer-by-Layer (LbL) technique was introduced at the beginning of the nineties by Gero Decher.[13] Originally this technique was based on the sequential adsorption of oppositely charged polymers (*i.e.* polyelectrolytes) on a charged *planar* substrate. Upon adsorption of a polyelectrolyte layer, charge overcompensation takes place, leading to a reversal of the surface charge, promoting the adsorption of a next, oppositely charged, polyelectrolyte. Fig. 1 is a schematic illustration of the LbL process. A charged substrate, *e.g.* a silicon wafer, is immersed into an aqueous solution of an oppositely charged polyelectrolyte. After a certain adsorption time the substrate is removed and washed with water in order to remove excess polyelectrolyte. In the next step the substrate is immersed into the second polyelectrolyte solution which has a charge opposite to the first polyelectrolyte. This second polyelectrolyte adsorbs onto the layer of the first polyelectrolyte which reverses the surface charge. Again a washing step is performed and the whole procedure can be repeated as many times as one desires. In this way one can easily prepare multilayered films with tuneable physico-chemical properties as both the number of layers as well as their composition can easily be varied.

Several techniques have been used to characterize the multilayer build-up on planar surfaces. Amongst them UV-VIS adsorption,[14] quartz crystal microbalance (QCM)[15,16] and

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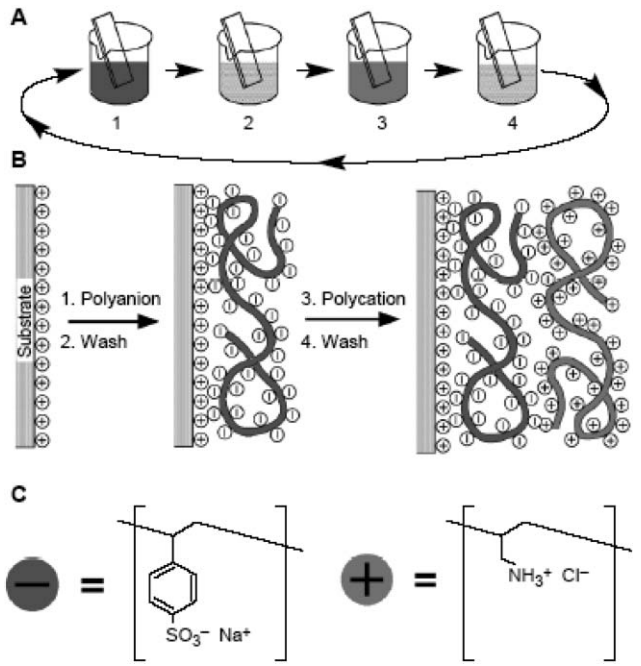

Fig. 1 (**A**) Schematic representation of the deposition of a polyelectrolyte film on a slide. Steps 1 and 3 represent the adsorption of respectively a polyanion and polycation, steps 2 and 4 are washing steps. (**B**) Simplified molecular picture of the first two adsorption steps, depicting film deposition starting with a positively charged substrate. Counterions are omitted for clarity. The polyion conformation and layer interpenetration are an idealization of the surface charge reversal with each adsorption step. (**C**) Chemical structure of poly(styrene sulfonate) (PSS) (left) and poly(allylamine hydrochloride) (PAH) (right), often used polyions to build LbL films. (Reprinted with permission from ref. 13. Copyright 1998, AAAS.)

ellipsometry[17] are the most widespread and allow one to determine both the mass and thickness increment upon adsorption of a single polyelectrolyte layer. The internal structure of the polyelectrolyte films (whether it is ordered or rather 'fuzzy') can be investigated by X-ray techniques[18] while their surface morphology can be adequately analyzed by atomic force microscopy (AFM).[19]

Currently, a large number of components, other than charged polymers, have been used to build multilayered films. DNA,[20] proteins,[21,22] nanoparticles,[23] lipids,[24] viruses[25] *etc.* have been included in the multilayers, yielding thin films with tailor made properties. Besides electrostatic, other interactions such as H-bonds,[26,27] covalent bonds,[28,29] biospecific interactions,[30,31] stereocomplex formation[32,33] *etc.* have also been used in order to accomplish a layer-by-layer build-up. Additionally, pH,[34,35] temperature,[36,37] glucose[38] and biotin[39] responsive LbL films have been made by varying the chemical nature of the polyelectrolyte film.

Most of the applications cited above involve the design of planar films. One of the most eye-catching applications of the LbL technology involves polyelectrolyte capsules fabricated by LbL coating of colloidal templates.[7] The potential use of LbL capsules as drug carriers has extended LbL research in the field of drug delivery[11,40] The following paragraphs give additional information on these capsules.

Polyelectrolyte capsules

Fabrication of polyelectrolyte capsules

Polyelectrolyte capsules, introduced in 1998, are obtained by LbL coating of a colloidal substrate followed by the dissolution of this template as schematically presented in Fig. 2.[6,7,41]

For the fabrication of hollow polyelectrolyte capsules poly(styrene sulfonate)/poly(allylamine hydrochloride) (PSS/PAH) is a very popular polyelectrolyte pair as PSS/PAH multilayer films deposited on planar substrates have been well studied. Also, the preparation of PSS/PAH capsules is reproducible and does not suffer from capsule aggregation or capsule decomposition upon removal of the core template, as often observed in the case of capsules fabricated from biopolymers, having a lower charge density. The physicochemical[10] and mechanical[42,43] properties of PSS/PAH capsules have been investigated by several groups. Initially hollow polyelectrolyte capsules were made using organic templates (like polystyrene (PS) or cross-linked melamine formaldehyde (MF) microparticles) which were dissolved after deposition of the LbL coating using both organic solvents or acidic solutions (0.1 M HCl). However, as discussed below, a main issue during the core removal is the integrity of the capsule wall.[44–46] It is known that organic solvents create pores in polyelectrolyte multilayers allowing the polystyrene to diffuse outwardly from the capsules.[47] However, removal of MF-templates is more difficult as it has been reported that MF oligomers stay complexed to the capsule wall and/or in the capsule interior, even after prolonged incubation in an acidic environment.[44,45,48,49] Therefore MF microparticles became less frequently used as templates, because MF oligomers remaining in the capsule wall lead to a rather undefined structure of the polyelectrolyte capsules and may also be toxic. Although the removal of a PS-core is easier, the fast dissolution of PS in organic solvents creates an osmotic pressure which may

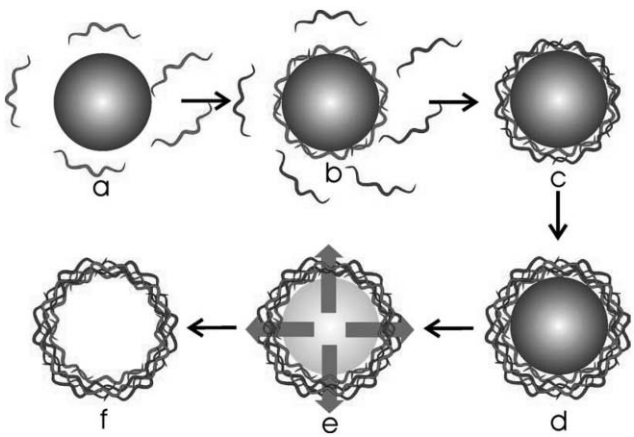

Fig. 2 Schematic illustration showing the preparation of "hollow" polyelectrolyte capsules. The initial steps (a through d) involve stepwise film formation by repeated exposure of the colloids to polyelectrolytes of alternating charge. Between each step the excess polyelectrolytes are removed before the next layer is deposited. When the desired number of polyelectrolyte layers is obtained the core is decomposed (e) resulting in a suspension of "hollow" polyelectrolyte capsules (f).

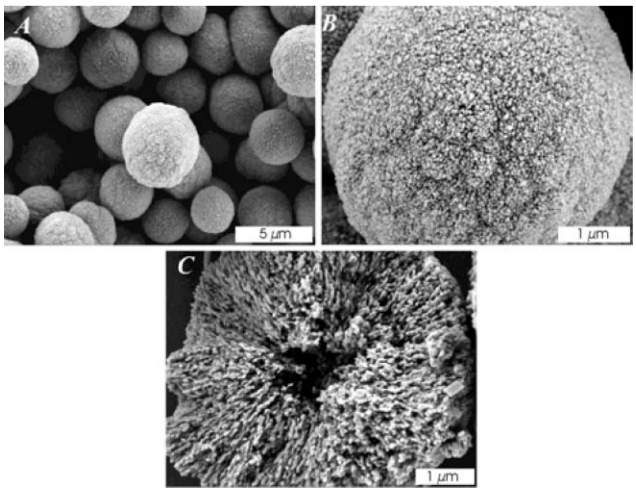

Fig. 3 SEM ages of $CaCO_3$ microparticles: (A) in overview, (B) single particle, and (C) broken particle. (Reprinted with permission from ref. 69. Copyright 2004, American Chemical Society.)

destroy the polyelectrolyte shell.[47] To overcome this inconvenience inorganic carbonates, such as calcium carbonate ($CaCO_3$),[50–54] manganese carbonate ($MnCO_3$) and cadmium carbonate ($CdCO_3$)[50,55] have recently been introduced as a template for the fabrication of hollow polyelectrolyte capsules. These microparticles are easily made by mixing calcium chloride and sodium carbonate and become easily dissolved by EDTA (when $CaCO_3$ is used) or by a low pH (in the case of $MnCO_3$ and $CdCO_3$.). The major advantage of inorganic templates is the low molecular weight of the ions. Polyelectrolyte shells are, generally speaking, known to be permeable to molecules with a molecular weight below 5 kDa[56] and should therefore not undergo an osmotic stress during the dissolution of the inorganic templates.

Loading of polyelectrolyte capsules

$CaCO_3$ microparticles (Fig. 3) have proved to be excellent sacrificial templates not only for the fabrication of hollow polyelectrolyte capsules but also for making "filled" polyelectrolyte capsules since $CaCO_3$ microparticles can be easily loaded with macromolecules (*e.g.* proteins) during[51] or after their preparation. In addition, the mild dissolution conditions do not destroy the encapsulated macromolecules. Silica has also been used as core for the fabrication of hollow polyelectrolyte capsules.[57,58] This approach has advantages as monodisperse non-porous silica particles are commercially available allowing the preparation of monodisperse polyelectrolyte capsules. However, the hydrofluoric acid solution, required to dissolve the silica core, requires extreme caution when handled. This possibility of encapsulating macromolecules is a great advantage over capsules made using PS or MF-templates. Indeed, the latter have to be loaded afterwards by varying the solvent polarity,[59] salt concentration or pH[60] of the medium in order to reversibly create pores to allow the inward diffusion of macromolecules. Subsequently the pores are closed by dispersing the capsules in their original medium as shown in Fig. 4. A variation on this route is filling the capsules with macromolecules followed by a cross-linking of the shell leading to the entrapment of the macromolecules.[61] It is very likely that the conditions used to "post-load" polyelectrolyte capsules will affect the integrity of many therapeutic macromolecules like peptides and proteins. The Caruso group recently reported on the fabrication of mesoporous silica particles which could easily be filled with proteins in their pores and subsequently used as template for the fabrication of protein filled capsules.[62] This pre-loading procedure should offer the same advantages as in the case of coprecipitated $CaCO_3$.

Macromolecular drugs or nanoparticles,[6] lipids,[63] dendrimers,[64–66] enzymes,[67] DNA,[68] and empty viruses[69] can also be incorporated *in* the polyelectrolyte multilayer wall. Finally, polyelectrolyte capsules may be loaded with charged molecules by electrostatic interactions with an oppositely charged matrix present inside the polyelectrolyte capsules.[64,70,71] Clearly, for this purpose the charged molecules should be able to diffuse through the LbL shell. This type of "post-loading" has been used to encapsulate negatively charged species in

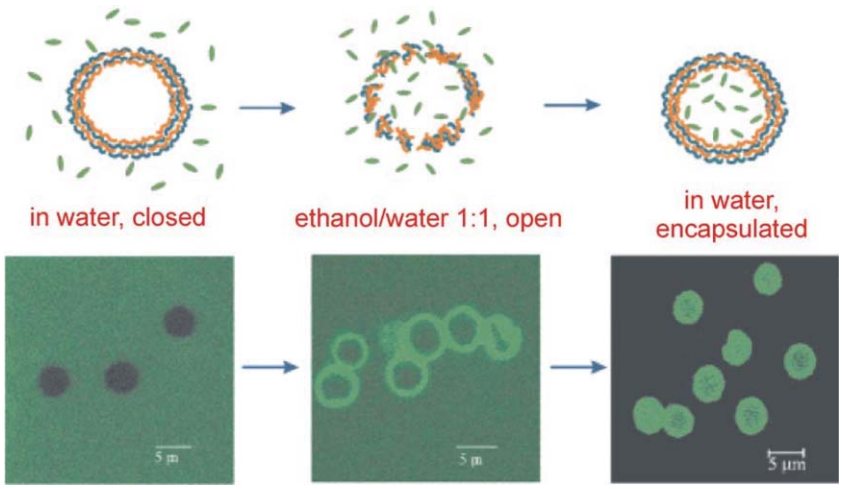

Fig. 4 Loading of (green) fluorescently labelled urease in "hollow" polyelectrolyte capsules. (Reprinted with permission from ref. 72. Copyright 2001, American Chemical Society.)

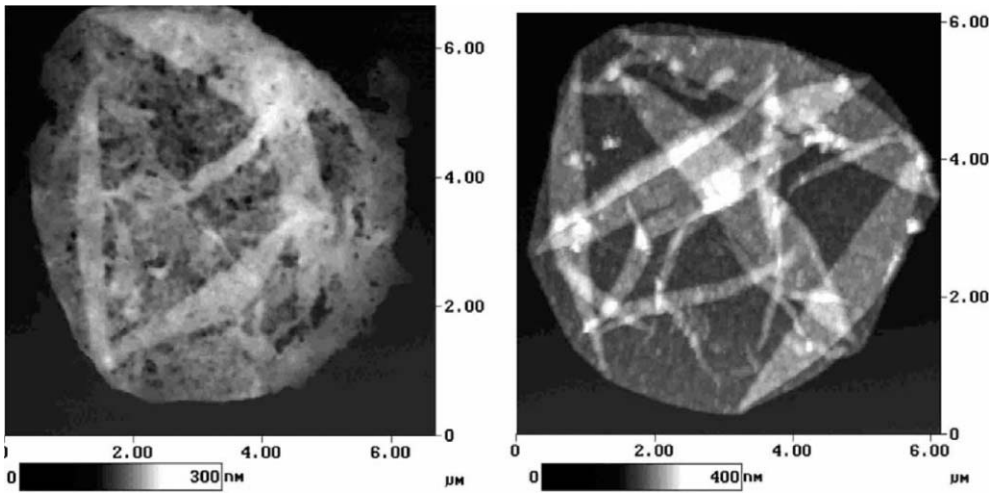

Fig. 5 SFM images of PSS/PAH based polyelectrolyte capsules treated with pH 3.5 (left) and pH 12 (right) buffers before drying. The capsules were prepared on MF particles. The porous structure of the polyelectrolyte shell treated with the acidic solution is clearly visible. (Reprinted with permission from ref. 91. Copyright 2002, Elsevier.)

polyelectrolyte capsules derived from MF templates, through interaction with MF remnants.[48,49] Positively charged species have been encapsulated by using an alginate[71] or PSS[70] matrix inside the capsules.

Drug delivery applications of polyelectrolyte capsules

Polyelectrolyte capsules may find applications in very distinct fields. They may be used as microreactors for the synthesis[11] or separation[72] of materials or they may act as sensors.[73–75] For a couple of years there has been serious interest in exploring their potential as drug delivery vehicles.[40] In the field of drug delivery there is an urgent need for time and space controlled drug delivery systems. Therefore, the LbL technique has recently been applied to prepare stimuli responsive polyelectrolyte capsules for controlled drug delivery. The following paragraphs review stimuli that have been exploited to induce the release from polyelectrolyte capsules.

pH-responsive polyelectrolyte capsules

Generally speaking, polyelectrolyte capsules composed of weak polyelectrolytes are responsive to the pH of the environment. PAH, one of the most popular polyelectrolytes for the fabrication of hollow polyelectrolyte capsules is a weak polyelectrolyte (pK_a of 8.7 in salt free solution). When complexed to PSS the apparent pK_a changes to 10.7 as described by Petrov *et al.*[76] When the environmental pH becomes higher (in the case of a polybase) or lower (in the case of a polyacid) than the pK_a, the polyelectrolytes become uncharged, which in turn, disassembles the capsules.

Rubner *et al.* described the influence of pH on the charge density and morphology of PAH based planar multilayers.[77] Using AFM they observed that pore formation occurs when PAH containing multilayers are placed in an acidic environment, whereas the multilayers exhibit a smooth surface at a higher pH. Also, pore formation seemed to be a reversible process as the pores disappeared when the pH was increased. Antipov *et al.* made similar observations on PSS/PAH-based polyelectrolyte capsules.[78] In Fig. 5 one can clearly see pores in the polyelectrolyte capsules treated with an acidic solution (left image), whereas the shell of capsules treated with an alkaline solution is intact (right image). The pH dependent integrity of the shell makes the permeability of the capsules to high molecular weight substances also pH dependent. As shown in Fig. 6, the capsules are permeable in the acidic region whereas they are impermeable in the alkaline region. Using this reversible pore formation Sukhorukov *et al.* filled PSS/PAH polyelectrolyte capsules with FITC-dextrans (FITC = fluorescein isothiocyanate): dextrans were allowed to diffuse in the capsules at low pH while they became entrapped in the capsules by increasing the pH.[60]

The pH dependent behaviour of polyelectrolyte capsules containing one or two weak polyelectrolytes has been also

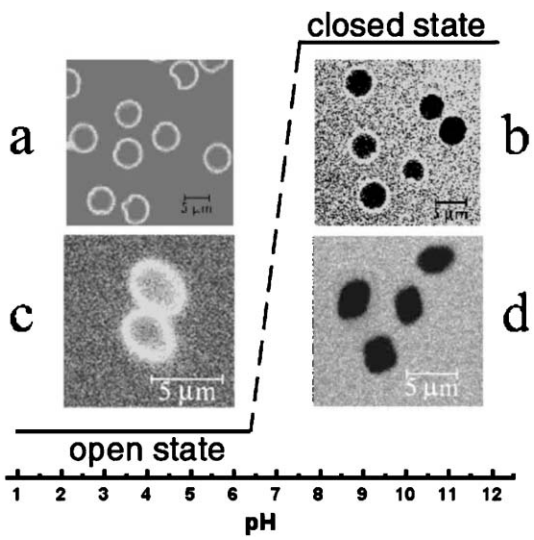

Fig. 6 Open (a, c) at pH 3.5 and closed (b, d) at pH 10 states of polyelectrolyte shells prepared on MF particles (a, b) and $CdCO_3$ crystals (c, d). (Reprinted with permission from ref. 91. Copyright 2002, Elsevier.)

described by Déjugnat *et al.*[47] for PSS/PAH capsules and by Mauser *et al.*[79] for PMA/PAH (PMA = poly(methacrylic acid)) capsules. Both authors reported swelling of the capsules when the pH was shifted towards the pK_a of one of the polyelectrolytes. PSS/PAH capsules started to swell when the pH was above 11 and disassembled when the pH was above 12. Concerning PMA/PAH capsules, swelling, followed by dissolution of the capsules, could be observed both at low and high pH, thus resulting in a dual pH-responsive behaviour of PMA/PAH capsules. The permeability of such dual responsive capsules was studied by Shutava *et al.*[80] for capsules based on tannic acid and PAH: the capsules seemed impermeable to FITC-dextrans at neutral pH but became permeable at both low and high pH.

The reversible pH dependent swelling of PSS/PAH capsules has been used by Déjugnat *et al.*[81] for the encapsulation of high molecular weight compounds such as rhodamine-labelled PSS and FITC-dextrans. By shortly exposing the capsules to a pH above 11 they swell dramatically and become largely permeable to high molecular weight compounds. A rapid lowering of the pH below 11 shrinks the capsules, returns them to their closed state, which entraps the molecules. Similarly, release of high molecular weight compounds can be obtained from the filled capsules by transferring them from neutral pH to a pH above 11.

Pharmaceutical applications of pH-responsive polyelectrolyte capsules may be the delivery of entrapped drugs at locations with a pH lower than that of serum (*i.e.* 7.4). A lower pH is found for example in the stomach,[82] vagina,[83] extracellular matrix of tumours,[84,85] inflammatory and ischemic regions, intracellular vesicles like endosomes, lysosomes and secretory granules. Importantly, except in the stomach, the pH in these tissues and cellular organelles is only *slightly* lower than 7.4. It will therefore be a challenge to design polyelectrolyte capsules that are stable in the blood but release their payload at a pH of *e.g.* $\sim$6.8. None of the present pH-responsive polyelectrolyte capsules fulfil this requirement. This is in contrast with the well studied pH-responsive microgels,[86–92] fabricated from weakly acidic or basic polymers, which swell at lower pH and release encapsulated molecules. Other hydrogels, like the commercially available pH-sensitive SQZ Gel™ (Macromed Inc, USA), shrink upon lowering the pH and squeeze out the drug molecules.[93] The pH at which these hydrogels start to swell or shrink has been fine-tuned to physiologically relevant pH's by playing around with the hydrophobicity of the hydrogel.[94,95] Apart from microgels that show a pH dependent swelling, also microgels[96–99] and liposomes[100,101] which start to degrade or to dissolve at a pH lower than 7.4 have been proposed as a pH sensitive delivery system. Most of the pH responsive hydrogels are prepared by radical crosslinking of derivatives of polyacrylamide, vinylpyridine,[102] vinyl imidazole[103] and amino acids.[93] This is a major drawback as the radical crosslinking may affect the integrity of the encapsulated drugs like proteins or DNA. Therefore, physically cross-linked pH sensitive hydrogels have been recently described.[104,105] Besides pH sensitive microgels and liposomes, pH responsive polymeric micelles have also gained attention as an extra- or intracellular drug delivery system, especially in the treatment of cancer.[106–108] Excellent reviews on pH-sensitive micelles have been recently published.[109,110] Important to note is that intracellular drug delivery by pH sensitive polyelectrolyte capsules will probably be limited to phagocytic and cancerous cells as mostly those cells are able to internalize micrometer-sized particles, which is the size range of most polyelectrolyte capsules studied today.

Salt responsive polyelectrolyte capsules

The first report on polyelectrolyte capsules decomposable by salts was made by Caruso *et al.*[68] They fabricated polyelectrolyte capsules using DNA/spermidine. It is known that DNA–spermidine interactions are reduced at higher ionic strength. When the DNA/spermidine capsules were immersed in a solution containing 5 M of salt, the multilayers completely dissolved leading to the destruction of the capsules. Ibarz *et al.* reported that the permeability of hollow PSS/PAH polyelectrolyte capsules, templated on MF cores, for high molecular weight compounds, sharply improved when the salt concentration exceeded 10^{-2} M. The higher permeability was not due to the formation of pores,[111] this is in contrast to the pH induced pore formation in PSS/PAH capsules as reported above. Föster resonance energy transfer between rhodamine and fluorescein labelled PAH revealed structural changes within the polyelectrolyte multilayers. A sharp decrease in FRET (indicating a longer distance between the polyelectrolyte layers) was observed around a salt concentration of 10^{-2} M, which does correspond with the higher permeability of the capsule wall. The salt induced permeability of the capsules was explained by a shielding of the charges on the polyelectrolytes that lowers the interactions between the oppositely charged polyelectrolytes and facilitates the diffusion of macromolecular substances through the multilayers.

The reversible switching of the permeability of polyelectrolyte capsules through variation of the salt concentration has also been used to encapsulate high molecular weight compounds.[111] (Fig. 7) At higher salt concentration the PSS/PAH capsule are open to macromolecules with a molecular weight of up to 70 kDa while at lower salt concentration the wall closes. Antipov *et al.* reported similar findings on PSS/PAH capsules fabricated on inorganic $CdCO_3$ crystals as sacrificial template.[78]

Although some salt responsive drug delivery systems have been proposed,[112–115] salt responsive polyelectrolyte capsules will probably have no application in drug delivery as ionic strength variations in the human body do not exist. However, the intra- *versus* extracellular concentration of a number of ions differs significantly. For example, the concentration of sodium, calcium and potassium inside cells (respectively $\sim$11 mM, $\sim$230 nM, $\sim$115 mM) is significantly different from the concentration in serum (respectively $\sim$140 mM, $\sim$2 mM, $\sim$4.5 mM).[116–118] Additionally, the intracellular concentration of calcium significantly varies from cell organelle to cell organelle, with the highest calcium concentrations in the mitochondria and sarcoplasmic reticulum. Microgels that swell/shrink upon sensing a specific ion may be suitable to deliver drugs in the cytosol or in certain cell organelles. As an example, polyacrylate hydrogels were described by Horkay *et al.*[119,120] which significantly swell when the calcium

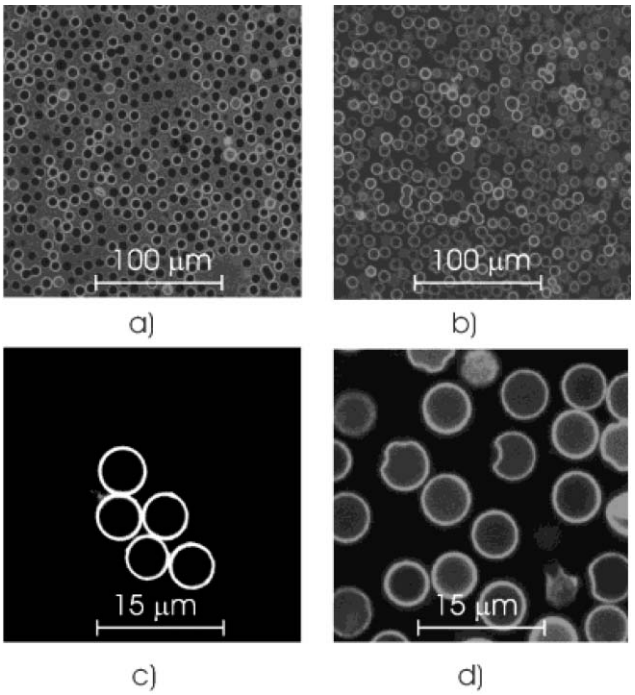

Fig. 7 Confocal microscopy images showing capsules consisting of 8 layers PSS/PAH (a) being impermeable to fluorescent labelled PAH (Mw ~ 70 kDa) in the absence of salt, (b) being impermeable to fluorescent labelled PAH in the presence of 10^{-2} M NaCl, (c) coloured with rhodamine 6G and (d) filled with fluorescent labelled PAH after removal of the excess PAH following a washing/centrifugation step of the capsules in image (b). (Reprinted with permission from ref. 124. Copyright 2001, Wiley.)

concentration in the environment becomes lower than 1 mM (note that the intracellular concentration is ~230 nM). Also, diseases exist which are characterized by a non-physiological plasma concentration of one or more ions. For example, a low calcium concentration in serum has been exploited as a trigger for the delivery of oestradiol in the treatment of osteoporosis.[121] However, the relevance of this system is limited as it is well-known that osteoporosis is not always characterized by low calcium levels in the serum.

Light-responsive capsules

The first report on optically sensitive polyelectrolyte capsules was made by Tao *et al.*[122] They reported on the use of the azo dye Congo red as a constituent of polyelectrolyte multilayers. Irradiation of such polyelectrolyte capsules with visible light (for 120 min) slightly distorted the polyelectrolyte multilayers, enhancing their permeability for fluorescently labelled dextrans with a molecular weight of up to 464 kDa. Both the long irradiation time and the use of visible light (which does not sufficiently penetrate the skin) limit, however, the *in vivo* use of light responsive polyelectrolyte capsules for drug delivery.

Near infrared (IR) laser light is interesting for drug delivery applications as most tissues show negligible adsorption in the 800–1200 nm region, making IR-laser light attractive for inducing structural changes in drug containing vesicles injected in tissues located at the surface of the body. IR-light sensitive polyelectrolyte capsules have been fabricated by incorporating gold nanoparticles in their PSS/PAH polyelectrolyte shell.[123–125] Upon irradiation with IR-light (short pulses of $<$10 ns) the gold nanoparticles absorb the energy and transform it into heat, which locally disturbs the integrity of the polyelectrolyte capsules. The Caruso group was the first to demonstrate the release of encapsulated biomacromolecules upon IR irradiation of polyelectrolyte capsules functionalised with gold nanoparticles.[124] Also Skirtach *et al.* reported recently on near IR sensitive polyelectrolyte capsules.[125,126] Fig. 8a shows confocal and transmission images of a PSS/PAH capsule, doped with gold nanoparticles, which is filled with rhodamine-labelled PSS. Upon irradiation with IR-laser light (Fig. 8b) the capsule breaks open, as can be seen from the strongly deformed structure, leading to the release of the encapsulated material. Fig. 9 shows SEM images of capsules (a) before irradiation, (b) after irradiation at moderate intensity and (c) after irradiation at high intensity; They clearly demonstrate the drastic impact of IR-light on the morphology of the capsules.

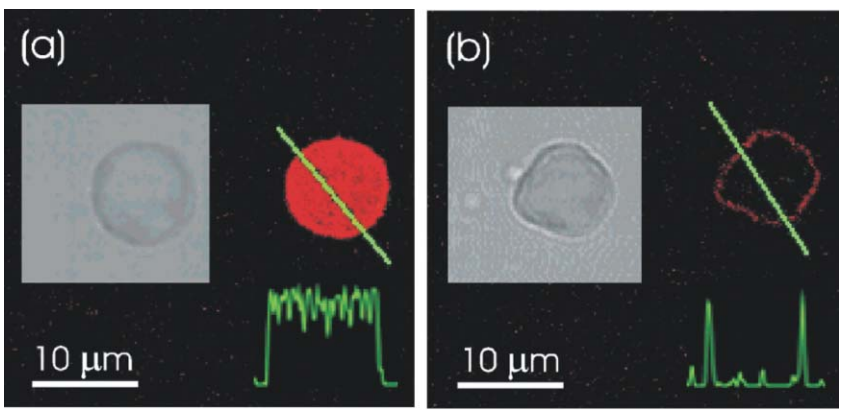

Fig. 8 Confocal microscope images demonstrating remote release of encapsulated rhodamine-labelled PSS polymers from a polyelectrolyte multilayer capsule containing gold sulfide core/gold shell nanoparticles in its walls. Fluorescence intensity profiles along the line through the capsule show that it is filled with fluorescent polymers before (a) and empty after (b) laser illumination. After the release of encapsulated polymers, the leftover fluorescent intensity is observed only in the walls of the capsule, (b). Insets show black and white transmission microscope images of the same capsule. Incident intensity of laser diode operating at 830 nm was set at 50 mW. (Reprinted with permission from ref. 139. Copyright 2005, American Chemical Society.)

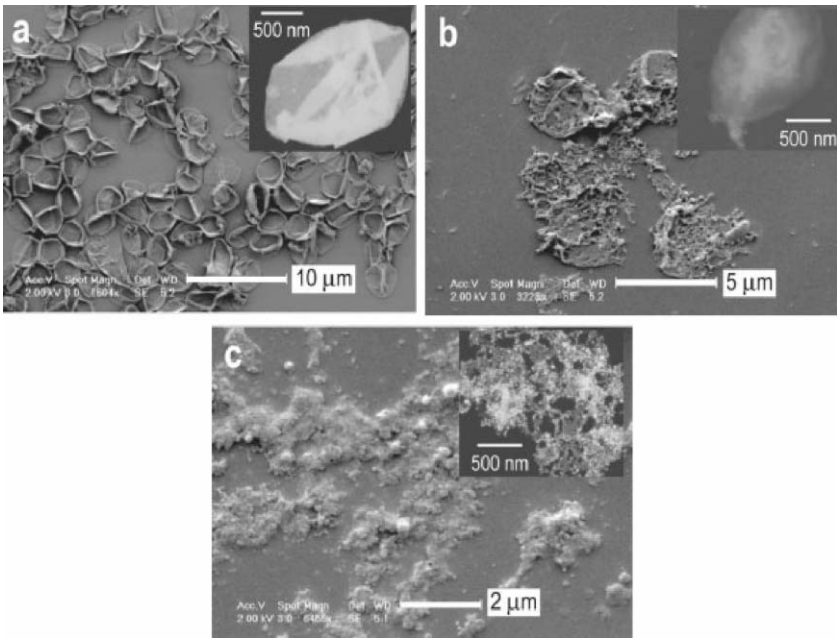

Fig. 9 Irradiation of the capsules with multiple laser pulses. SEM images of the capsules: a) before irradiation; b) after moderate radiant exposure (30 mJ cm^{-2}); and c) after radiant exposure of 50 mJ cm^{-2} and higher. The insets are the corresponding TEM images. (Reprinted with permission from ref. 137. Copyright 2004, Wiley.)

"Photo-controlled" release of drugs remains an attractive approach as it should allow controlling the delivery in time and space. Therefore, IR-sensitive polyelectrolyte capsules may be useful for controlled drug release, because after subcutaneous injection the release of encapsulated material may be triggered by local irradiation of the skin with IR-light. Besides IR-sensitive polyelectrolyte capsules, also photo-sensitive liposomes and photo-sensitive polymer micelles are under development.[127,128] Light-triggered release of drugs from liposomes is due to a light induced destabilization of the lipid bilayer that can occur *via* (1) light sensitized production of reactive species (such as singlet oxygen) that cause fragmentation of the lipids in the liposomes,[129,130] (2) photo-polymerization[131] or (3) photo-isomerization of the lipids.[132] Additionally, local heating upon irradiation of gold nanoparticles incorporated in the liposomes may also induce drug release.[133,134] In case of polymer micelles the release is due to a photo-chemical reaction that alters the hydrophobicity of the micelle-forming polymers, leading to the release of encapsulated material.[135,136] However, most of the photo-sensitive liposomes and micelles are responsive to the shorter wavelengths which limits their application *in vivo*. Indeed, UV-light may damage cells and shows a limited penetration depth (<0.5 mm) in tissues. Therefore, we believe that research on light-sensitive delivery systems should focus on IR-light sensitive systems because IR-light is less harmful and has a much deeper penetration depth in tissues (*e.g.* 8 mm in the liver at a wavelength of 1070 nm).[137]

Magnetic field responsive capsules

Lu *et al.* reported on hollow PSS/PAH-based capsules (using $MnCO_3$ as sacrificial template) which are addressable by a magnetic field due to the incorporation of one layer of positively charged gold coated cobalt nanoparticles (Co@Au) into the polyelectrolyte shell of the capsules.[138] Capsules with a rather thick wall (approximately 250 nm) were observed by AFM. The thick walls are probably due to the aggregation of Co@Au nanoparticles. Upon applying an oscillating magnetic field, the ferromagnetic Co@Au nanoparticles twist, which disturbs the structure of the polyelectrolyte multilayers and, consequently, allows the diffusion of macromolecules through the capsule wall (Fig. 10). Furthermore it was shown that only capsules having one layer of Co@Au nanoparticles could switch their permeability upon application of a magnetic field, whereas capsules having multiple Co@Au nanoparticles layers remained impermeable upon applying a magnetic field. The magnetically induced permeability could be of interest for drug loading and release from polyelectrolyte capsules.

However, one should note that the long exposure time (30 min) and strong magnetic field (1200 Oe, 150 Hz) required to permeabilize the Co@Au capsules described above led to a 30 °C increase in temperature of the capsule suspension which is, highly likely, problematic for the loading of thermosensitive drugs (like *e.g.* proteins) in the capsules. Clearly, magnetically induced drug release from capsules injected in the body would be also problematic. Some other magnetic-responsive delivery systems have been reported as well. The group of Langer *et al.* embedded magnetic beads together with insulin in a ethylene-vinylacetate copolymer matrix and demonstrated, after implantation of the matrix in diabetic rats, that the blood glucose levels drastically decreased each time an external oscillating magnetic field was applied.[139,140] Couvreur *et al.* made alginate spheres containing insulin and ferrite micro-particles and showed a 50-fold increase in insulin release in the presence of an oscillating magnetic field.[141] It was believed that the vibrating magnetic particles induced openings in the

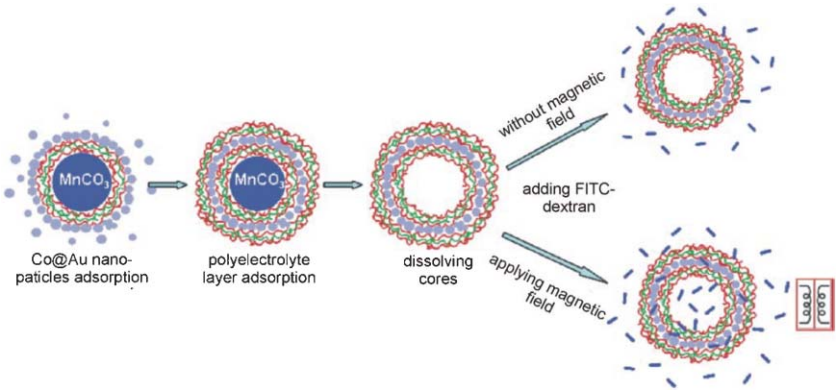

Fig. 10 Assembly and magnetically permeabilization of the polyelectrolyte microcapsules by oscillating magnetic field (1200 Oe, 150 Hz) for 30 min. The molecular weight of the FITC-dextran was 2000 kDa. (Reprinted with permission from ref. 151. Copyright 2005, American Chemical Society.)

polymer matrix facilitating the release of insulin. Currently there is a renewed interest in magnetically controlled drug delivery systems. Babincova *et al.* developed doxorubicin loaded magnetoliposomes which are first targeted into tumours by a static magnetic field and, consequently, massively release the doxorubicin upon application of an oscillating magnetic field.[142] The release occurs due to a local increase in temperature (up to 42 °C) which 'melts' the liposomes.

Glucose-responsive polyelectrolyte capsules

Glucose responsive capsules, encapsulating insulin, could be promising for the treatment of diabetes mellitus patients as the insulin would only be released when the glucose concentration in the blood exceeds a physiological value.

McShane *et al.* fabricated polyelectrolyte capsules containing glucose oxidase within the multilayers.[74] They expected the capsules to be glucose sensitive because the glucuronic acid, occurring from the oxidation of glucose by the immobilized glucose oxidase, would drop the pH at the surface of the capsules and hence modify its permeability. However, glucose did not disassemble the capsules, nor did the authors report on any possible change in permeability of the capsules. Other glucose-sensitive delivery systems for insulin have been described. Indeed, pH-sensitive hydrogels loaded with insulin, glucose oxidase and catalase have been well-studied. In these hydrogels glucose oxidase also generates glucuronic acid when glucose enters the hydrogels. This lowers the pH which swells[143] or shrinks[144] the hydrogel and which leads to the release of insulin. The function of catalase in these hydrogels is to convert the aggressive hydrogen peroxide, occurring from the enzymatic conversion of glucose, to oxygen and water. Unfortunately these systems have important shortcomings. Firstly, they lack a reproducible and rapid response on a long-term basis. Secondly, these hydrogels are often neither biocompatible nor biodegradable. Also, many of the reported systems are only glucose-responsive at very high (4 to 36 mg ml^{-1})[145] (clinically irrelevant) glucose concentrations. Indeed, in a healthy person the blood glucose concentration is around 1 mg ml^{-1}, whereas glucose levels above 2 mg ml^{-1} are already common in diabetic patients. Therefore, glucose-responsive delivery systems should ideally start to release insulin as soon as the glucose level reaches about 2 mg ml^{-1}.

Recently, De Geest *et al.* made use of phenylboronic acid[146,147] to prepare a glucose-sensitive polyelectrolyte.[148] This polyelectrolyte was used in combination with PSS to fabricate hollow polyelectrolyte capsules. In buffer without glucose the capsules remained stable. However, in buffer solutions containing 2.5 mg/ml or 5 mg/ml glucose the capsules dissolved within 5 min (Fig. 11). The mechanism behind the glucose induced dissolution of the capsules is the repulsion between the negative groups on PSS and the borate groups which become negatively charged in the presence of glucose.

Polymers harbouring phenylboronic groups have also been used to prepare glucose-sensitive hydrogels for insulin delivery. The phenylboronic groups form complexes with vicinal hydroxyl groups on polyol polymers (*e.g.* polyvinyl alcohol) resulting in cross-linking and gel-formation. The affinity of glucose for the phenylboronic groups results in a breaking of the cross-links and release of encapsulated insulin. A disadvantage of the phenylboronic acid containing polyelectrolyte capsules and hydrogels described above is that they are glucose-responsive only at a non-physiological pH (*i.e.* pH 9). However, the group of Kataoka developed phenylboronic acid based hydrogels which are glucose-sensitive at pH 7.4.[149]

Degradable polyelectrolyte capsules

For many biomedical applications, especially for drug delivery, biodegradable polyelectrolyte capsules would be preferred to non-degradable ones. Several reports on degradable LbL *films* deposited on planar substrates have been published. Lynn *et al.* reported on degradable polycations (poly-β-aminoesters)[98] to make LbL films for the controlled release of small drug molecules[150] and DNA.[151] Serizawa *et al.*[152] and Picart *et al.*[153] reported on enzymatically degradable polysaccharide-based polyelectrolyte films, which seemed to be degradable in the mouths of rats. Biodegradable polyelectrolyte films could be of interest for the surface modification of implants by rendering them more biocompatible or by making them drug eluting by the incorporation of drug molecules within the multilayers.

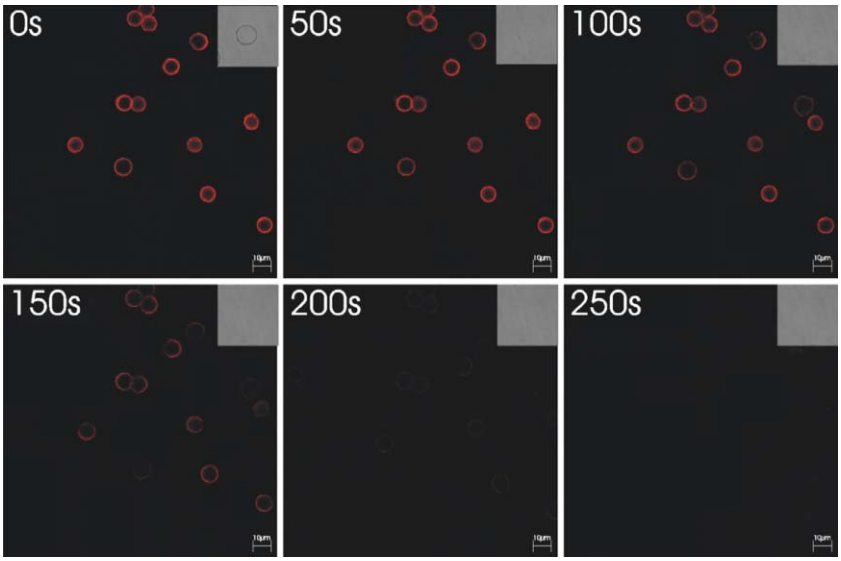

Fig. 11 CLSM images of the dissolution of the phenylboronic acid based hollow polyelectrolyte capsules in the presence of 5 mg ml^{-1} glucose at pH 9. The inset in the upper right corner shows the transmission light image of a capsule that is gradually dissolving. (Reprinted with permission from ref. 161. Copyright 2006, American Chemical Society.)

The first paper on degradable polyelectrolyte *capsules* was published by Mohwald *et al.*[154] Polyelectrolyte capsules were covered with a lipid bilayer rendering them impermeable to low molecular weight compounds such as carboxyfluorescein. When phospholipase A_2 was added, the lipid bilayer degraded resulting in the formation of pores rendering the capsules permeable to carboxyfluorescein.

Several papers reported on the use of biopolymers such as polypeptides,[155] polysaccharides[156] and DNA[157] to fabricate polyelectrolyte capsules. Although the authors did not report on the degradation of these capsules, it is highly likely they will degrade *in vivo*. Recently De Geest *et al.* reported on degradable polyelectrolyte capsules containing one or two polyelectrolytes which can be degraded either enzymatically or through hydrolysis.[158] $CaCO_3$ particles containing FITC-dextrans were prepared by co-precipitation as reported by Petrov *et al.*[51] and applied on cells. The microcapsules were taken up by cells and subsequently degraded and delivered FITC-dextrans in the cells. Fig. 12 shows confocal images of VERO cells which have been in contact for 48 h with the capsules. Non degradable control capsules, made of PSS/PAH, and filled with FITC-dextran, could still be observed intact inside the cells. However, the degradable capsules were no longer visible, indicating that the cells were able to digest them.

A second class of degradable capsules reported by De Geest *et al.*[159,160] were "self-exploding macrocapsules", composed of a biodegradable dextran gel core surrounded by a polyelectrolyte membrane.[161–163] Upon degradation of the dextran microgel its swelling pressure increases which finally causes the polyelectrolyte membrane to rupture.[159,160] The rupturing of the polyelectrolyte film by the swelling pressure of the dextran microgel was first proved using PSS/PAH as a polyelectrolyte pair. The PSS/PAH films broke, however only at pH 9. At a lower pH the PSS/PAH membrane did not rupture due to an increase in permeability. However, when the dextran microgels were coated with negatively and positively charged polypeptides (like poly-L-glutamic acid and poly-L-arginine) the resulting capsules were able to rupture under physiological conditions. The rupturing of these microcapsules, due to increased internal pressure, is demonstrated in Fig. 13.

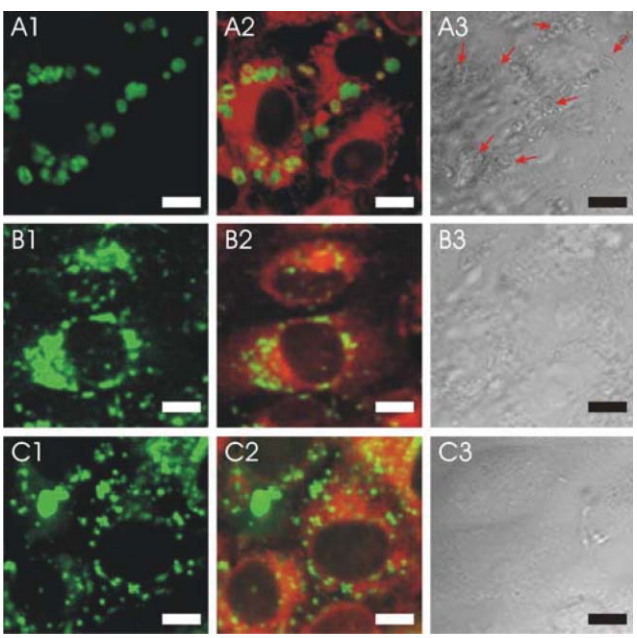

Fig. 12 Confocal microscopy images of VERO cells after 48 h being in contact with FITC-dextran 2000 kDa filled capsules consisting of (A) $(pSS/pAH)_4$, (B) $(pSS/p(HPMA\text{-}DMAE))_4$ and (C) $(DEXS/pARG)_4$. The scale bar represents 10 μm. The images '1' represent the green fluorescence images while the images '2' give the overlay of the green and red fluorescence. The images 'C' give the transmission images. The capsules were fluorescently labelled with FITC-dextran (green colour) while the endosomes of the cells were stained red with Lysotracker. (Reprinted with permission from ref. 171. Copyright 2006, Wiley.)

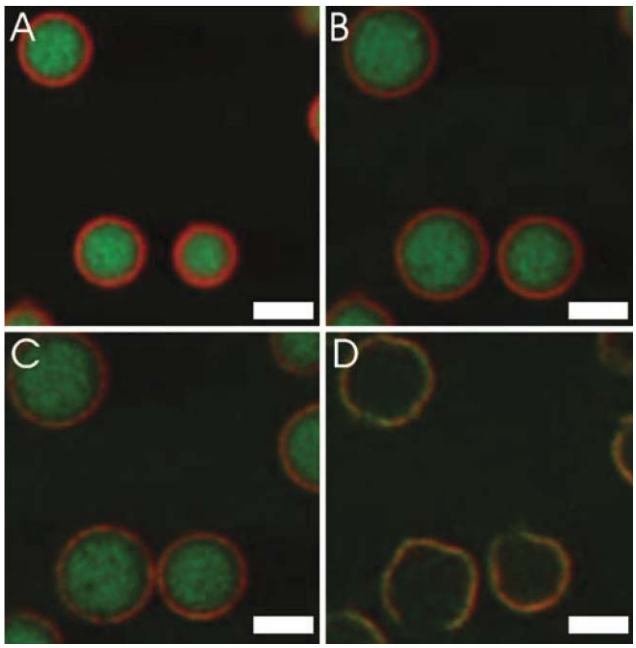

Fig. 13 Confocal microscopy snapshots of "self-exploding microcapsules" after addition of sodium hydroxide to accelerate the degradation process (which would otherwise take up to several days). The time between the consecutive images is 30 s. The microgels were fluorescently labelled with FITC-dextrans (green colour) while the polyelectrolyte membrane was fluorescently labeled using rhodamine labeled poly-L-arginine (red colour). The scale bar represents 10 μm.

Redox responsive capsules

It is well-known that the colon and the cytosol of cells have a more reducing environment compared to other locations in the body. This has been exploited for colon specific delivery of drugs using hydrogels that contain azo-bonds which are reductive-sensitive.[164] Also redox-sensitive polymers containing a disulfide linkage in their backbone have been synthesized.[165] The high redox-potential inside cells has recently been used as a trigger to enhance the intracellular release of plasmid DNA, antisense oligonucleotides, or small interfering (si) RNAs from pharmaceutical carriers containing disulfide linkages.[166]

Polyelectrolyte capsules that are redox-sensitive have recently been reported by Haynie *et al*. They were fabricated from anionic and cationic polypeptides containing cysteine groups.[155] Upon cross-linking of the cysteine's thiol groups (leading to disulfide bonds) capsules were found to be stable at both neutral and acidic pH. However, after reducing the disulfide bonds, the capsules disassembled at a pH lower than the pK_a of the anionic polypeptides as the multilayers were then no longer stabilized by electrostatic or covalent bonds. Although a redox triggered deconstruction of polyelectrolyte capsules is highly suitable for *in vivo* applications, the system reported by the Haynie group requires an acidic pH to disassemble the capsules. At pH 7.4 the capsules will remain stabilized through the electrostatic interactions between the cationic and anionic polypeptides as both are charged.

The Caruso group recently fabricated capsules with a shell composed of multiple layers of thiol-modified polymers, bound to each other through hydrogen bonds and which can be cross-linked *via* disulfide bridges.[167] As described in the introduction of this review it is possible to make multilayers based on interactions other than electrostatic interactions. Hydrogen-bonded multilayers deposited on flat substrates have been well-studied. However, polyelectrolyte capsules fabricated from hydrogen-bonded multilayers are less investigated. The attractiveness of hydrogen-bonded multilayers lies in the fact that some of them can be deconstructed under physiological conditions. For example hydrogen-bonded multilayers containing poly(methacrylic acid) (PMA) as a proton donor are fabricated at low pH in order to have the PMA in its uncharged form, whereas at pH 7.4 the PMA becomes charged and repulsion between the PMA chains will occur, disassembling the multilayer film. Multilayered capsules based on hydrogen bond interactions between PMA and poly(vinylpyrrolidone) (PVPON) were fabricated on protein filled mesoporous silica (note that silica can be dissolved in a hydrogen fluoride (HF) solution).[167] Cross-linking through disulfide bonding was accomplished using PMA functionalized with cysteinamine moieties. Capsules cross-linked through disulfide bonds were found to be stable under physiological conditions. However, when the disulfide bonds were reduced, the capsules disassembled readily and released their content.

Conclusions and outlook

Drug delivery systems releasing their payload in response to internal or external triggers may offer great advantages. The potential of the LbL technique to make (bio)responsive drug delivery systems has recently been discovered. We have reviewed such (bio)responsive polyelectrolyte capsules and compared them with other types of (bio)responsive drug delivery systems which are under development.

In our opinion, at this time only the IR-sensitive and the biodegradable polyelectrolyte capsules seem sufficiently attractive to be further studied for *in vivo* drug delivery. Unfortunately, detailed *in vivo* data on such capsules are currently lacking. As discussed above, most of the so far studied polyelectrolyte capsules only respond to extreme stimuli that do not occur or cannot be applied *in vivo*. One should realize that the physiological processes during which release of drugs from the polyelectrolyte capsules should occur create only subtle physicochemical changes in the human body. Consequently, polyelectrolyte capsules which are sensitive to such subtle changes are required. A clear challenge is to synthesize polyelectrolytes that allow the design of polyelectrolyte capsules sensitive to small (and physiologically) relevant changes of pH, salt concentration, glucose concentration and redox potential. For example, it would be of interest to design polyelectrolyte capsules which could escape from endosomes at the time acidification starts in the endosomes. Subsequently they should release their payload in the cytosol or nucleus in response to the high reductive environment of the cytosol or in response to nucleus specific enzymes.

Other important issues in capsule science are how to promote their cellular uptake and how to target them to cells. Also, it is well known that aspecific adsorption of proteins to the surface of parenterally injected particles should be avoided

to avoid the formation of large aggregates and/or the uptake by phagocytising cells from *e.g.* the immune system and the liver. The issue of 'biofunctionalisation' has been addressed by several groups. Shielding of polyelectrolyte capsules from non-specific adsorption has been performed by pegylation of the capsule surface using a polyelectrolyte grafted with polyethylene glycol (PEG) side chains.[168] Targeting of capsules into tissues using an external magnetic field to concentrate the capsules at a certain location has been demonstrated by Zebli *et al.* using capsules functionalised with magnetic particles.[169] The Donath group has provided capsules with virus functionalities by incorporating rubella like particles in the polyelectrolyte shell.[69,170] Such capsules could hold promise to enhance endosomal uptake/escape or promote the delivery of encapsulated material to the cell nucleus. Lipid coating of capsules was first demonstrated by Moya *et al.*[63] while, recently, the Caruso group coupled antibodies to capsules covered with lipid bilayers and showed that those antibodies could bind the secondary antibodies.[12] This approach is promising towards the selective uptake of capsules by specific cell types.

So far, pharmaceutical technological aspects of polyelectrolyte capsules have not been thoroughly studied. Clearly, to use polyelectrolyte capsules as carriers in pharmaceutical products, capsules stable for extended periods of time (years) should be developed. Freeze drying of dispersions of polyelectrolyte capsules could be considered for this purpose. Also, for the parenteral applications of polyelectrolyte capsules one should investigate how to make such dispersions sterile. Also an issue remains of how to scale-up the production process of polyelectrolyte capsules which are currently prepared on a lab scale. Finally, toxicological and immunological aspects of polyelectrolyte capsules should be further explored.[171]

Clearly, polyelectrolyte capsules are an intriguing new type of vesicles which may offer potential for biomedical applications. Also, the science of layer-by-layer assembly is gaining interest from scientists in a broad field which is a very interesting evolution. This multidisciplinary approach is the prerequisite to finding effective applications for polyelectrolyte capsules. It is very important that people with different backgrounds combine their efforts in order to come up with new applications for these capsules in medicine. Finally, keeping in mind that other concepts and devices do exist, the major challenge for polyelectrolyte capsules research is also to determine precisely for which applications they offer distinct advantages, compared to other vesicles.

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