Layer-by-layer coating of degradable microgels for pulsed drug delivery

B.G. De Geest, C. Déjugnat, E. Verhoeven, G.B. Sukhorukov, A.M. Jonas, J. Plain, J. Demeester, S.C. De Smee

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium
Institut de Chimie Séparative de Marcoule (ICSM) CEA/CNRS 2926, 30207 Bagnols-sur-Cèze Cedex, France
Max Planck Institute of Colloids and Interfaces, Am Muehlenberg 1, D-14476 Potsdam, Germany
IRC/Materials, Queen Mary University of London, Mile End Road, E1 4NS, London, UK
Unité de Physique et de Chimie des hauts Polymères, Université catholique de Louvain, Croix du Sud, 1-8134 Louvain-la-Neuve, Belgium
Laboratoire de Nanotechnologie et d’Instrumentation Optique, Université de Technologie de Troyes 12, rue Marie Curie, 10010 Troyes cedex, France

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Abstract

Recently, we reported on “self-rupturing” microcapsules which consist of a biodegradable dextran-based microgel surrounded by a polyelectrolyte membrane. Degradation of the microgel increases the swelling pressure in the microcapsules which, when sufficiently high, ruptures the surrounding polyelectrolyte membrane. The membrane surrounding the microgels is deposited using the layer-by-layer (LbL) technique, which is based on the alternate adsorption of oppositely charged polyelectrolytes onto a charged substrate. In this paper, we characterize with confocal microscopy, electrophoretic mobility, scanning electron microscopy and atomic force microscopy in detail the deposition and the properties of the LbL coatings on the dextran microgels. We show that by fine-tuning the properties of both the microgel core and the LbL membrane the swelling pressure which is evoked by the degradation of the microgel is indeed able to rupture the surrounding LbL membrane. Further, we show that the application of an LbL coating on the surface of the microgels dramatically lowers the burst release from the microcapsules and results in massive release at the time the microcapsules rupture.

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1. Introduction

Due to the ever increasing amount of biopharmaceuticals, there is a growing need for advanced drug delivery systems [1]. Instead of sustained drug release, pulsed drug delivery could be attractive to deliver certain therapeutics [2]. With the term pulsed drug release, we mean that after administration initially no drug release occurs. Only after a well defined period the therapeutics are released. Pulsed release could be advantageous for, e.g., drugs that develop biological tolerance when they are constantly present at their target site or for drugs that require dosing at night [3–6]. Also, microparticles that could suddenly release antigens at well defined times after injection could show potential for ‘single-shot vaccination’ [7]. For this purpose, the injectable device should consist of different types of microparticles, each type of particle suddenly releasing its encapsulated antigen at a well defined time after injection. Devices which show pulsed release upon applying an external trigger such as pH [8,9], electric field [10,11], IR-light [12–14], etc. have been described. Also implants for pulsed drug delivery [15,16] have been reported; however, injectable microparticles could offer substantial advantages towards patient compliance as they are less invasive.

Recently, our group introduced a new type of microparticles which we termed “self-rupturing microcapsules” [17]. These microcapsules are able to rupture without the use of any external trigger. This is advantageous compared to devices which needs an external trigger to induce drug release. Our ultimate goal is to develop a formulation consisting of different populations of self-rupturing microcapsules, each population exploding at a different time after injection. This would offer the possibility to
generate multiple drug pulses after a single injection. The self-rupturing microcapsules consist of a biodegradable microgel core surrounded by a suitable membrane (Fig. 1). It was shown that upon degradation of the microgels the swelling pressure of the degrading microgels was able to suddenly rupture the membrane [18]. In this system, the membrane surrounding the microgels plays a major role as it has to be (i) permeable to water, (ii) impermeable to the degradation products of the microgels (upon degradation the gels turn into a polymer solution) and (iii) rupture when the swelling pressure reaches a critical value.

We applied a membrane at the surface of the microgels by “layer-by-layer electrostatic self-assembly (LbL-ESA)” which is a very promising technology for the coating of microparticles [19–21]. First developed by Decher et al. on planar substrates [22,23], this technique has been extended to the coating of colloidal particles by Sukhorukov et al. [24]. Briefly explained, as illustrated in Fig. 1, this approach is based on the alternate adsorption of oppositely charged polyelectrolytes (PE’s) on a charged surface, driven by the electrostatic interaction at each step of adsorption. It has been shown that a wide variety of colloidal particles such as organic latex particles [24,25], inorganic particles [26,27], dye and drug crystals [28,29], protein aggregates [30,31] and biological cells [32] can be coated by polyelectrolyte multilayer deposition. Also LbL coating of respectively alginate and poly(N-isopropylacrylamide) (pNIPAAm) microgels has been reported [33]. A major advantage of the LbL technique is the possibility to tune the layer thickness on the nanometer scale and thus control the mechanical properties and the permeability of the polyelectrolyte shell.

As the properties of the LbL membrane of the self-rupturing microcapsules has a major impact on the release features of this new type of microparticles, this paper especially aimed to characterize in detail the LbL films deposited on the surface of the microgels. We also aimed to show that choosing an optimal LbL coating significantly reduces burst release and makes release to occur at the time the microcapsules rupture.

2. Materials and methods

2.1. Materials

Dextran (Mw 19 kDa), fluorescein isothiocyanate-dextrans (FITC-dextran, Mw respectively 20 and 150 kDa), tetramethyl rhodamine B isothiocyanate-dextrans (TRITC-dextran, Mw 158 kDa), N,N,N’,N’-tetramethylenediamine (TEMED), methacrylic acid (MAA) and dimethyl aminoethyl methacrylate (DMAEMA), rhodamine B isothiocyanate (RBITC), sodium poly(styrene sulfonate) (PSS, 70 kDa) and poly(allylamine hydrochloride) (PAH, 70 kDa) were purchased from Sigma-Aldrich-Fluka. Potassium peroxodisulphate (KPS) and poly(ethyleneglycol) (PEG, 20 kDa) were purchased from Merck. RBITC labelled PAH was synthesized as reported in literature [34]. The buffers at pH 7 were 0.1 M phosphate buffers and the buffers at pH 9 were 0.1 M carbonate buffers.

2.2. Synthesis of dex-HEMA

Dextran hydroxyethyl methacrylate (dex-HEMA) was prepared and characterized according to a method described elsewhere [35]. Dextran with a number average molecular weight of 19 kDa was used. The degree of substitution (DS, the number of HEMA groups per 100 glucopyranose residues of dextran) was determined by proton nuclear resonance spectroscopy (1H-NMR) in D2O with a Gemini 300 spectrometer (Varian) [36]. The DS of the dex-HEMA used in this study was 2.5.

Fig. 1. Schematic representation of a microgel coated by the LbL technique. Before degradation, the polymer chains (dextran chains in this study) are connected into a three-dimensional network by chemical cross-links (●). (I), (II) and (III) show the coating of the microgels by sequential adsorption of oppositely charged polyelectrolytes. The microgels described in this paper degrade by hydrolysis of the cross-links. As degradation proceeds, the cross-link density decreases and free polymer (dextran) chains arise. (IV) At the end of the degradation process, the core of the particles consists of a dextran solution. When the swelling pressure of the core exceeds a critical value, the membrane suddenly ruptures (V).
Preparation of dex-HEMA microgels. Dex-HEMA microgels, with an initial water content of 70% (w/w), were prepared according to Franssen and Hennink [37]. In detail, 71 mg dex-HEMA was dissolved in 1.577 ml water and subsequently emulsified by vortexing with a 3.35 ml 24% (v/v) aqueous PEG solution. Radical polymerisation of the aqueous dex-HEMA emulsion droplets was initiated by adding TEMED (100 μl pH neutralized with 4 N HCl) and KPS (9 mg). The reaction was carried out at room temperature for 1 h. Afterwards, the obtained microgels were washed three times with pure water to remove PEG, KPS and TEMED. Finally, the microgels were suspended in 5 ml pure water and stored at −20 °C. The size distribution was measured by laser diffraction (Mavern Mastersizer). To prepare negatively and positively charged dex-HEMA microgels [38], respectively methacrylic acid (MAA, 25 μl) or dimethyl aminoethyl methacrylate (DMAEMA, 35 μl) was added to the dex-HEMA/PEG mixture just before vortexing it. In this paper, “dex-HEMA-MAA microgels” and “dex-HEMA-DMAEMA microgels” refer to negatively charged and positively charged microgels, respectively. FITC/TRITC-dextrans were incorporated in the microgels by adding 100 μl of a FITC/TRITC-dextran solution (50 mg/ml) to the dex-HEMA/PEG mixture just before the vortexing step.

2.3. LbL coating of the dex-HEMA microgels

Dex-HEMA microgels were coated by the consecutive adsorption of oppositely charged polyelectrolytes using the centrifugation technique [24]. The microgels (500 μl from the original suspension) were dispersed in 1 ml of polyelectrolyte solution (2 mg/ml in 0.5 M NaCl). The polyelectrolytes were allowed to adsorb for 15 min, under continuous shaking. The dispersion was then centrifuged at a speed of 300×g for 3 min. Subsequently, the supernatant was removed and the microgels were redispersed in Milli-Q water to remove the non-adsorbed polyelectrolytes. This washing was repeated twice before the second polyelectrolyte solution was added. The process was repeated until the desired LbL coating was reached.

2.4. Release experiments

TRITC-dextran containing dex-HEMA-DMAEMA microgels were prepared and LbL coated as described in the paragraphs above. However, the scale on which the LbL coating was performed was 10 ml instead of 1 ml. After preparation, the uncoated and (PSS/PAH)3 coated dex-HEMA-DMAEMA microgels were centrifuged, the supernatant was removed and the microgels/microcapsules were redispersed in centrifugation tubes containing 50 ml 0.1 M carbonate buffer (pH 9). The centrifugation tubes were thermostatised at 37±0.5 °C and the microgels/microcapsules were kept in suspension by mechanical agitation. 1 ml samples were withdrawn at 2.5 h time intervals (by a VenKel Industries 800 automatic sampling station) and filtered to remove the microcapsules. The fluorescence intensity of the samples was measured with a Wallac Victor 2 (Perkin Elmer) plate reader. The measured fluorescence values were normalized against the fluorescence values measured at the end of the release experiments. It was verified that the measured fluorescence values belonged to the range in which a linear relation exists between the concentration of the TRITC-dextran solutions and their fluorescence.

2.5. Confocal laser scanning microscopy

Confocal micrographs of the LbL coated microgels were taken with a MRC1024 Bio-Rad confocal laser scanning microscope (CLSM) equipped with a krypton-argon laser. An inverted microscope (Eclipse TE300D, Nikon) was used which was equipped with a water immersion objective lens (Plan Apo 60X, NA 1.2, collar rim correction, Nikon).

2.6. Electrophoretic mobility

The electrophoretic mobility of the LbL coated microgels was measured using a Malvern Zetasizer 2000 (Malvern Instruments). The ζ-potential was calculated from the electrophoretic mobility (μ) using the Smoluchowski relation: ζ = μη/ε where η and ε are the viscosity and permittivity of the solvent, respectively. Since the largest microgels did not allow ζ-potential measurements (they sedimented too quickly in the cuvette of the instrument and may disturb the measurement), we measured the ζ-potential of the smallest microgels (approximately 1 μm). Therefore, the dex-HEMA microgel dispersions were centrifuged (1 min at low speed (100 g)) and the ζ-potential measurements were done the dex-HEMA microgels which remained in the supernatant. Therefore, we took 50 μl sample of the supernatant and diluted it with 2 ml water.

2.7. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) measurements on (LbL coated) microgels were carried out using a Zeiss DSM 40 instrument operating at an accelerating voltage of 3 kV.

2.8. Atomic force microscopy (AFM)

Experiments were performed on air-dried (LbL coated) microgels deposited onto microscope glass slides. Images were obtained in tapping mode under ambient conditions with an AutoProbe CP system (Park Scientific Instruments) using a 100 μm scanner. Si3N4 cantilevers (spring constant about 0.1 Nm⁻¹) with integrated pyramidal tips were used. The AFM-tip was positioned on top of rather large microgels (diameter of 15 μm) in order to avoid the effect of the curvature on the image.

3. Results and discussion

3.1. Preparation of neutral and charged dex-HEMA microgels

To synthesize biodegradable dextran hydrogels [39], methacrylate moieties were linked to the dextran backbone by hydrolysable carbonate esters (Fig. 3A). Radical polymerization of the methacrylate moieties cross-links the dextran chains. The
fabrication of dex-HEMA microgels using the water-in-water emulsion technique is based on the immiscibility of the PEG and dex-HEMA solutions. Microgels with an average diameter of 7 μm were obtained (Fig. 2 gives the size distribution of the obtained microgels. It could be expected that charged dex-HEMA microgels would be more suitable for LbL coating than neutral ones. Van Tomme et al. recently reported that charged dex-HEMA microgels can be prepared by copolymerization of dex-HEMA with MAA (pK_a 4.5) (Fig. 3B) or DMAEMA (pK_a 8.4) (Fig. 3C) [38]. The incorporation of these charged groups into the microgels was verified by measuring the ζ-potential of the dex-HEMA microgels (Fig. 4A). Indeed, a negative ζ-potential (−30 mV) was measured in case MAA was used, while a positive ζ-potential (+28 mV) was observed in case DMAEMA was used, indicating the successful charge loading of the dex-HEMA microgels.

We observed that the charge of the microgels strongly influences their degradation rate. While the positively charged dex-HEMA-DMAEMA (70%, DS 2.5) microgels were completely degraded within 5 days (at 37 °C and pH 7.4), it took up to 30 days to completely degrade the negatively charged dex-HEMA-MAA (70%, DS 2.5) microgels. It has been reported that the degradation rate of alkaline ester hydrolysis can be influenced by neighbouring ternary amine groups [40,41]. After nucleophilic attack of the hydroxyl ion on the ester group, the intermediate is stabilized by resonance stabilisation which promotes the alkaline hydrolysis of the ester group thus increasing the degradation rate of the dex-HEMA-DMAEMA microgels. On the other hand, in case of dex-HEMA-MAA microgels, the presence of the carboxyl group of the MAA will destabilize the intermediate and thus defavourize the alkaline hydrolysis of the ester group, resulting in a slower degradation of the dex-HEMA-MAA microgels, which was indeed observed.

3.2. LbL coating of the dex-HEMA microgels

To perform the LbL coating of the microgels we used poly(allylamine hydrochloride) (PAH) as polycation and sodium poly(styrene sulfonate) (PSS) as polyanion. This polyelectrolyte pair is well studied for the coating of both flat as well as...
colloidal templates [28,42–44]. Initially, we deposited three PSS/PAH bilayers onto the microgels. Fig. 4A–B show the results of ζ-potential measurements on uncoated and LbL coated dex-HEMA microgels. Before coating, the ζ-potential of neutral, dex-HEMA-MAA and dex-HEMA-DMAEMA microgels were respectively 0 mV, −30 mV and 28 mV. The ζ-potential values proved the successful incorporation of respectively MAA and DMAEMA. Fig. 4A and B clearly show that the charge of the microgels changes upon submerging them in PSS and PAH solutions, indicating that multilayer build-up takes place. The ζ-potential profile observed upon exposure of the microgels to PSS/PAH solutions agrees well with literature data on PSS/PAH coating of other types of particles [24].

Fig. 4B shows that LbL coating of the neutral dex-HEMA microgels is also possible. The obtained ζ-potential profile is however quite irregular. For charged microgels (Fig. 4A), electrostatic interactions between the microgels and the polyelectrolytes are the main driving force for polyelectrolyte adsorption. However, for neutral microgels other interactions such as hydrophobic interactions [45–47] and physical entanglements [48,49] between the polyelectrolytes and the microgels most likely play a role. These interactions seem to be strong enough because otherwise the adsorbed layers would be removed upon adsorption of the next polyelectrolyte layer.

3.3. Microscopy on LbL coated dex-HEMA microgels

Fig. 5 shows confocal microscopy images of (A) dex-HEMA-DMAEMA microgels coated with (PSS/PAH)₃, (B) dex-HEMA-MAA microgels coated with (PAH/PSS)₃, and (C) dex-HEMA microgels coated with (PSS/PAH)₃. Rhodamine labelled PAH (PAH-RITC), which is positively charged, was used to visualize the LbL coating. In case of dex-HEMA-DMAEMA microgels (Fig. 5A) a distinct ring PAH-RITC is observed while in case of dex-HEMA-MAA microgels (Fig. 5B) both a ring as well as inwards diffusion of PAH-RITC is observed. In case of neutral dex-HEMA microgels (Fig. 5C), a homogeneous filling with PAH-RITC is observed. These observations indicate that during the LbL coating of the charged microgels only the polyelectrolytes...
oppositely charged to the microgels will diffuse inside the microgels, forming a complex with the MAA, respectively DMAEMA groups, while the polyelectrolytes with equal charge as the microgels will only adsorb on the microgel surface during the multilayer build-up.

To get a rough estimation on the thickness of the LbL coating, scanning electron microscopy (SEM) and atomic force microscopy (AFM) were performed on both the coated and uncoated microgels. The SEM images in Fig. 6 reveal that the surface of the uncoated particles is rather smooth compared to the coated ones, which show a more granular structure. Especially when PAH was the outermost polyelectrolyte layer a remarkable “brain-like” structure appeared. A similar morphology was observed using AFM by McAloney et al. [50,51] when studying the deposition of PSS/PAH multilayers on planar substrates at high salt concentrations. The difference between uncoated and PSS/PAH coated dex-HEMA-DMAEMA microgels was confirmed by AFM measurements. Uncoated microgels (Fig. 7A) show irregularities ranging from 2 to 5 nm, while PSS/PAH coated microgels (Fig. 7B) show irregularities ranging from 40 to 100 nm. The irregularities in the morphology of the LbL coating are highly likely due to the differential drying between the gel core and the LbL coating. From these data, we estimated the thickness of the LBL membrane to be several tens of nanometers.

3.4. Permeability of the LbL coating

As outlined in Fig. 1, the rupturing of the microcapsules is triggered by the swelling pressure of the degrading microgels. To exert a sufficient pressure, it is important that the degradation products of the microgels, i.e. 19 kDa dextran chains, do not diffuse through the coating during the degradation of the microgels. In a previous paper, we reported that the permeability of the (PSS/PAH)₃ membrane seems to be pH-dependent as self-rupturing capsules could only be obtained upon incubation of the microcapsules at pH 9 (thus suggesting that at pH 9 the coating is impermeable for 19 kDa dextran chains), while upon incubation at pH 7 the microcapsules’ membrane remained intact (thus suggesting that at pH 7 the coating is permeable for 19 kDa dextran chains) [17]. To further investigate the permeability of the (PSS/PAH)₃ coating, we used 20 kDa FITC-dextran as its molecular weight corresponds well to the molecular weight of the dextran chains the microgels are composed of.

The (PSS/PAH)₃ coated dex-HEMA-DMAEMA microgels were incubated in a 1 mg/ml FITC-dextran solution. The

![AFM images of uncoated (left) and (PSS/PAH)₃ coated dex-HEMA-DMAEMA microgels (right) recorded in tapping mode. (A1) and (B1) show the topology of the surface. (A2) and (B2) show the roughness of the surface along the line marked on (A1) and (B1). (A3) and (B3) are the 3D images of the surface.](image-url)
experiment was performed at pH 7 and pH 9. From the confocal images in Fig. 8, it is clear that at pH 7 the capsules' wall is permeable to the 20 kDa FITC-dextrans while at pH 9 it is impermeable. When the number of polyelectrolyte bilayers is increased from 3 to 6, we observed that also at pH 7 the coating becomes impermeable. As a control, when non-coated dex-HEMA-DMAEMA microgels were incubated in a FITC-dextran solution they appeared to be permeable to the FITC-dextrans (data not shown). As PAH is a weak polyelectrolyte (pK_a 8.5 [52]), it is not surprising that the properties of PSS/PAH polyelectrolyte multilayers are pH-dependent. The pH-dependent stability [53] and permeability [42] of PSS/PAH-based polyelectrolyte capsules has indeed been shown. Antipov et al. reported that PSS/PAH-based capsules become impermeable to high molecular weight species upon increasing the pH from 7 to above 8 [42]. Apparently, an analogue phenomenon is observed in our case.

3.5. Behaviour of the microcapsules during degradation of the microgel core

To evaluate the effect of the degradation of the microgel core on the integrity of the polyelectrolyte membrane, the microcapsules were incubated at 37 °C and pH 7 (for 10 days) and at pH 9 (overnight). It was observed with confocal microscopy that upon degradation of the microgel core at pH 9 only remnants of broken (PSS/PAH)_3 microcapsules could be detected, while the (PSS/PAH)_6 microcapsules appeared to maintain their integrity upon degradation of the microgel core at pH 9. Apparently, the increase in number of bilayers from 3 to 6 not only decreases the permeability but also increases the mechanical strength of the microcapsules, preventing them from rupturing upon degradation of the microgels core. The (PSS/PAH)_3 and (PSS/PAH)_6 microcapsules which were degraded at

Fig. 8. Permeability of the LbL coating surrounding the dex-HEMA-DMAEMA microgels to 20 kDa FITC-dextrans. The LbL coated microgels are immersed into a buffered solution containing 1 mg/ml FITC-dextrans and are visualized by confocal microscopy.

Fig. 9. Confocal images after degradation of the microgel core at pH 7 of (A) empty dex-HEMA-DMAEMA microgels coated with (PSS/PAH)_3 and (B) 150 kDa FITC-dextran (green colour) loaded dex-HEMA-DMAEMA microgels coated with (PSS/PAH)_3. The PAH was fluorescently labelled with RITC (red colour). The scale bar represents 10 μm. (C) and (D) show the SEM images corresponding to, respectively, (A) and (B).
pH 7 also maintained their integrity and none of them ruptured upon degradation of the microgel core. The higher permeability of the LbL membrane at pH 7 most likely causes the outwards diffusion of the degradation products of the microgel core which prevent the build-up of a swelling pressure, thus preventing the rupturing of the microcapsules. Fig. 8A–B show confocal microscopy images of (A) hollow and (B) FITC-dextran (i.e. 150 kDa FITC-dextran was used as model drug) filled (PSS/PAH)₃ microcapsules obtained after the degradation of the microgel core at pH 7. Note that in case of FITC-dextran filled microcapsules (Fig. 8B) the microgels were loaded with FITC-dextran during their synthesis and thus before the LbL coating. The fact that the FITC-dextrans remain inside the capsules proves that no rupturing of the LbL coating occurred during the degradation, as this would lead to the release of the FITC-dextran as shown in our previous work [17]. Fig. 9C and D are SEM images corresponding to the microcapsules shown in respectively Fig. 9A and B. The collapsed state of the microcapsules clearly proves that indeed the microgel core is degraded. In case FITC-dextran were encapsulated, the microcapsules showed a more granular structure due to the precipitation of the FITC-dextran upon drying of the sample. A similar morphology of filled capsules after drying has been observed by Sukhorukov et al. [54].

To visualize the rupturing of the (PSS/PAH)₃ coated dex-HEMA-DMAEMA microgels, it was investigated up to which pH the (PSS/PAH)₃ coating was stable. Dejugnat and Sukhorukov have reported on the pH responsive properties of hollow PSS/PAH-based capsules and from these results it can be concluded that the PSS/PAH coating remains intact until a pH of 11 whereas at a pH above 12 the multilayer membrane becomes irreversibly destroyed [53]. Therefore, the PSS/PAH₃ coated dex-HEMA-DMAEMA microgels were incubated in a solution buffered at pH 11 and the degradation process was monitored by confocal microscopy. Fig. 10A1–A5 shows snapshots of the

**Fig. 10.** (A1–A5) CLSM images of (PSS/PAH)₃ coated dex-HEMA-DMAEMA microgels during the degradation at pH 11. The dex-HEMA-DMAEMA microgels were loaded with 150 kDa FITC-dextran (green colour) and the PAH was labelled with RITC (red colour). The scale bar represents 10 μm. (B) Increase in microcapsule diameter during the degradation of the microgel core (n = 10).

**Fig. 11.** Cumulative release curves of 158 kDa TRITC-dextran from uncoated dex-HEMA-DMAEMA (open symbols) and (PSS/PAH)₃ coated dex-HEMA-DMAEMA microgel (closed symbols). The data points are interconnected with a cubic B-spline. The experiments were run in duplicate.
microcapsules during the degradation. Initially, they start to swell and at a certain moment they rupture leading to the release of the encapsulated material, which was 150 kDa FITC-dextran. Fig. 10B shows the evolution of the microcapsules’ diameter during the degradation of the microgel core. As can be observed, the microcapsules gradually swell until an almost two-fold increase in diameter followed by the rupturing of the membrane.

3.6. Release from uncoated and \((\text{PSS/PAH})_3\) coated dex-HEMA-DMAEMA microgels

In a next step, the effect of the \((\text{PSS/PAH})_3\) coating on the release of 158 kDa TRITC-dextrans from dex-HEMA-DMAEMA microgels, degrading at pH 9, was investigated. We expected to observe a pulsed release for the following reasons. First, at pH 9, the \((\text{PSS/PAH})_3\) coating surrounding dex-HEMA-DMAEMA microgels should not be permeable to 158 kDa TRITC-dextrans as it is even impermeable to 20 kDa dextrans (Fig. 8). In other words, the 158 kDa TRITC-dextrans should not leak through the membrane during degradation of the microgels. Second, at pH 9, the \((\text{PSS/PAH})_3\) microgels are self-rupturing, i.e. the membrane breaks due to the increase in swelling pressure of the microgels. Fig. 11 shows that the release of 158 kDa TRITC-dextrans from uncoated and \((\text{PSS/PAH})_3\) coated dex-HEMA-DMAEMA microgels differs significantly. Uncoated microgels show a substantial burst release followed by a continuous release until the microgels are completely degraded after 20 h. A burst release does not occur in case of \((\text{PSS/PAH})_3\) coated dex-HEMA-DMAEMA microgels, the 158 kDa TRITC-dextran release remains even low during the first 10 h of degradation. After this initial phase, the majority of the encapsulated 158 kDa TRITC-dextran molecules are released in a couple of hours allowing to conclude that \((\text{PSS/PAH})_3\) coating of the dex-HEMA-DMAEMA microgels causes the release of the 158 kDa TRITC-dextrans to be much more pulsatile.

One could, however, wonder why the observed release pulse is less steep than one would expect when all the microcapsules would simultaneously rupture. This may be partially explained by Laplace’s law:

\[
p = \frac{2 \gamma}{r}
\]

With \(p\) the pressure, \(\gamma\) the membrane tension and \(r\) the radius. Microcapsules will rupture when the swelling pressure of the microgels evokes a tension in the membrane which exceeds the tensile strength of the membrane. Laplace’s law states that by increasing the microcapsules’ radius, the pressure required to evoke the same membrane tension, decreases. This implies that larger microcapsules require less swelling pressure to rupture and will thus rupture earlier than smaller ones. As the microgels reported in this paper are polydisperse in size, one could indeed expect that not all the microcapsules will self-rupture at the same time but the time of rupturing will show a distribution. Recently, we reported on the fabrication of highly monodisperse dex-HEMA microgels using a microfluidic emulsification device [55]. Future work will focus on the use of these monodisperse microgels to produce a highly uniform population of microcapsules. Another aspect which should be addressed in future work is how to modify the properties of the LbL coating surrounding the microgels in order to render this coating sufficiently impermeable under physiological conditions without increasing the mechanical strength of this coating to a point where it can no longer be ruptured by the swelling pressure of the degrading microgel.

4. Conclusions

Dextran-based microgels, with different surface charges, were used as template for the LbL assembly of the polyelectrolytes PSS and PAH. \(\zeta\)-potential measurements and CLSM proved that polyelectrolytes can be sequentially adsorbed onto the surface of neutral, positively as well as negatively charged dextran microgels leading to microcapsules. It was observed that the positively charged dex-HEMA-DMAEMA microgels were the most promising as template for LbL assembly. The permeability of PSS/PAH-based LbL coatings surrounding dex-HEMA-DMAEMA microgels was investigated. It was found that three bilayers of PSS/PAH rendered the microcapsules impermeable to 20 kDa FITC-dextrans at pH 9 while they were still permeable at pH 7. Increasing the number of polyelectrolyte bilayers to 6 rendered the microcapsules impermeable also at pH 7.

We showed that upon degradation at pH 9 the dextran microgels were able to rupture their surrounding \((\text{PSS/PAH})_3\) coating, resulting in ‘self-rupturing microcapsules’, while when degraded at pH 7 the \((\text{PSS/PAH})_3\) coating did not rupture, leading to hollow \((\text{PSS/PAH})_3\) capsules. This was explained by the pH-dependent permeability of the \((\text{PSS/PAH})_3\) coating to the degradation products of the microgels. When degraded at pH 9, the release of high molecular weight TRITC-dextran, encapsulated in \((\text{PSS/PAH})_3\) coated microcapsules, was significantly more pulsatile compared to the TRITC-dextran release from uncoated dextran microgels.

The concept presented in this paper may be promising towards biomedical applications, especially in the field of pulsed drug delivery, as the time of rupturing is determined by the degradation rate of the microgels, which can be tailored from days to several weeks by varying the cross-link density of the microgels [39]. Our further research will focus on LbL coating of degradable microgels making use of biocompatible polyelectrolytes with the aim to obtain microcapsules which are under physiological conditions impermeable to both the encapsulated drugs as well as the degradation products of the dextran gels, two major requirements to obtain pulsatile delivery from this type of micromaterials.

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