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Ultrasound stimulated release and catalysis using polyelectrolyte multilayer capsules

Andre G. Skirtach,* Bruno G. De Geest,† Arif Mamedov,‡ Alexei A. Antipov§ and Gleb B. Sukhorukov**

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Ultrasound has been used to trigger release of encapsulated material from polyelectrolyte multilayer capsules. Sonication was found to destroy both plain and nanoparticle-modified capsules. Cavitation occurs through the collapse of generated microbubbles and the resulting shear forces should cause the destruction of the polyelectrolyte capsules. Application in catalysis is demonstrated in this paper, while further potential usage of ultrasound triggered release is anticipated in bio-medical applications.

Introduction

Among actively growing research and development areas drug delivery takes a prominent position.1,2 Much effort has been focused on the development of well defined drug carriers with tailored physico-chemical and mechanical properties. The advent of the layer-by-layer (LbL) technique has opened the possibility to fabricate hollow capsules by alternate adsorption of charged species, such as polyelectrolytes and nanoparticles, onto an oppositely charged sacrificial colloidal template, followed by the dissolution of this template.3,4 The LbL technique allows the fabrication of microscopic carriers with an unmet degree of functionality as the properties of the capsules’ wall can easily be tailored by the nature and the number of adsorbed layers. Polyelectrolyte capsules find applications in different fields such as catalysis, biotechnology and biosensing.5 The possibility to load various materials into empty capsules provides an opportunity to employ them in medical diagnostic and treatment purposes.6–8 However, to achieve the desired effect and maximum success in such an extensive undertaking it is important that the capsules could release drugs in the vicinity of the target site at a specific time. One possibility is to employ laser light for remote release9,10 which is based on heating of metal nanoparticles by the laser light.11

Here we report on utilizing ultrasound as an external physical trigger to release encapsulated material from polyelectrolyte capsules. Ultrasound12 has been widely applied for fabrication of nanomaterials,13 coating carbon nanotubes14 and noble metals,15 etc. Also ultrasound has been used in the biomedical field for breaking up capsules for drug release,16 destruction and fragmentation of contrast agents,17 gas release, destruction of polymer, albumin or lipid shells of microbubbles.18–20 In this paper we report on the use of ultrasound for destruction of polyelectrolyte capsules.3 We also report on the effect of nanoparticles in the walls of polyelectrolyte capsules on the response of these capsules to ultrasound. The ultrasound affects this denser shell and serves as a ‘trigger’ to release encapsulated material. This ‘trigger’ could be activated from inside as well as outside the body. The aim of this work is to develop a method of remote destruction of capsules under the impact of an external source.

Previously, nanoparticles have been used in combination with ultrasound for biomedical imaging.21 In this paper, nanoparticles are used to increase the density in the walls of the capsules.22 Upon propagation, an ultrasound wave undergoes both viscous and thermal absorption as well as scattering in the surrounding medium.23,24 Cavitation occurs through the collapse of generated microbubbles and the resulting shear forces should cause the destruction of the polyelectrolyte capsules.16–20

Experimental

Materials

Sodium poly(styrene sulfonate) (PSS, $M_w \sim 70000$), poly(-allylamine hydrochloride) (PAH, $M_w \sim 70000$) and FITC-PAH were obtained from Sigma-Aldrich (Germany). Salts and buffer components were purchased from Roth (Germany). Slightly crosslinked melamineformaldehyde (MF) latexes of 5 μm diameter were purchased from Microparticles GmbH (Germany). Gold nanoparticles bearing negative surfaces were fabricated according to Chen and Kimura25 All chemicals were used without further purification. Water used in all experiments was prepared in a three stage Millipore Milli-Q Plus 185 purification system and had a resistivity higher than 18.2 MΩ cm.

Polyelectrolyte capsule preparation

Two types of polyelectrolyte capsules were used in the experiments. The first type was fabricated using MF
microparticles as sacrificial templates following the procedure reported earlier. Briefly, colloidal particles were incubated with each polyelectrolyte of 5 mM monomer unit concentration in 0.5 M NaCl solution for 15 minutes. Triple washing with water and centrifugation followed each adsorption circle. Afterwards, the MF templates were dissolved in a 0.1 M HCl solution followed by several washing and centrifugation steps to remove the MF oligomers. Silver nanoparticles were embedded in the polyelectrolyte coating as previously reported. Silver nitrate ([Ag(NH3)2]NO3) with concentrations of up 0.1 M was added to the capsule dispersion and stirred for 2 hours resulting in the formation of silver nanoparticles. In the next step, two or eight additional bilayers of PSS/PAH were assembled to reduce the chance of contact of silver particles with the surrounding medium, leading to (PSS/PAH)2Ag(PSS/PAH)2 and (PSS/PAH)2Ag(PSS/PAH)8 capsules respectively. Filling of the capsules with FITC-PAH was performed using the earlier reported method. Finally, the capsules were dispersed in pure water.

The second type of capsules was fabricated using CaCO3 microparticles as sacrificial templates. CaCO3 microparticles filled with FITC-dextrans in their pores were fabricated according to Petrov et al. The CaCO3 microparticles were coated with 4 bilayers of PAH and gold nanoparticles in a similar fashion as described above. Hollow capsules were obtained by dissolving the CaCO3 in a 0.2 M EDTA solution buffered at pH 5 followed by several washing and centrifugation steps to remove the dissolved ions. The capsules were then dispersed in pure water.

Destruction of capsules by ultrasound

Fig. 1 schematically illustrates the destruction of the polyelectrolyte capsules by ultrasound. The capsules were subjected to a Branson Sonifier 250 operating at 20 kHz with the microtip in a 0.5 ml Eppendorf tube with the capsule suspension. Also, the capsules were sonicated in a pulse mode with a Bandelin sonoplus HD 200 (Bandelin electronic, Berlin, Germany) in a 1/1 cycle (1 s pulse/1 s pause) using a 3 mm diameter probe operating at 20 kHz. The total power of the ultrasound was equal to 120 W.

In order to reduce as much as possible the effect of heating of the aqueous medium of the capsules, the Eppendorf tube, containing the capsule suspension, was placed in an ice bath during the ultrasound treatment.

Microscopic characterization

Confocal micrographs were taken with a Leica TCS SP, equipped with a 100 x oil immersion objective with a numerical aperture of 1.4. Optical photographs were taken with a Sony 85 MD digital camera. Samples were excited with a 366 nm UV lamp. Atomic force microscopy (AFM) images were taken on a Nanoscope IIIa Multimode SFM (Digital Instruments Inc.) in air at room temperature using the tapping mode. Samples were prepared by applying a drop of microcapsule suspension onto a freshly cleaved mica substrate followed by drying under a gentle stream of nitrogen.

Catalysis experiments

The catalytic reaction conversions were followed by UV-VIS spectrometry on a Cary-50 spectrophotometer (Varian, Inc., Germany). Silver-containing capsules were investigated for their catalytic activity in the reaction of 4-nitrophenol (4-NP) reduction to 4-aminophenol (4-AP) before or after the ultrasound was applied. For each reaction 1 mL of 0.4 M sodium borohydride (NaBH4) and 0.05 M NaOH aqueous solution was mixed with 25 μL of 2.4 × 10−3 M 4-NP aqueous solution. After the addition of 100 μL of capsules, the total volume of the sample was adjusted to 4 mL. The progress of the reaction was monitored by the decrease of the 4-NP peak at 400 nm. UV-VIS spectra were taken each five minutes after capsule addition.

Results and discussion

The influence of the sonication time on the capsules integrity was investigated for the (PSS/PAH)2Ag(PSS/PAH)2 capsules. The capsules destruction is depicted in Fig. 2 showing optical transmission microscopy images of capsules taken at various time intervals. It can be seen from these images that the capsules appear intact before the experiments (t = 0 min). The micrographs taken at 2, 4 and 6 minutes after sonication show the increased breakage of the capsules as evidenced by the absence of regularly shaped capsules and the presence of a larger amount of debris. It can be seen that more than 50% of
the capsules are broken after only 2 minutes of sonication. Four minutes of sonication leads to complete destruction of the capsules—only pieces of the broken capsules are visible, Fig. 2.

Fig. 3A shows an AFM image of a CaCO₃ templated capsule before ultrasound, exhibiting a round shape of the capsule and a height of 447 nm as can be observed on the height profile. This height is due to the presence of precipitated FITC-dextran which was encapsulated inside the capsule. When an AFM image is recorded of the debris after sonication (Fig. 3B) a height of 261 nm is measured. Probably this corresponds to the thickness of a piece of capsule wall which was torn from the capsule due to the sonication. Also, we have observed that the destruction of capsules without metal nanoparticles takes place (although at a slower pace). As the ultrasonic waves propagate from the sonicator probe tip, microbubbles are generated from the air which was initially dissolved in the fluid. The microbubbles start to oscillate due to the ultrasonic waves and finally collapse, so-called cavitation. This cavitation creates strong shear forces in the surrounding liquid, resulting in a dramatic impact on the integrity of the capsules' wall, destroying the capsules completely. Polyelectrolyte capsules are not prone to radicals and therefore we do not suspect the production of radicals, due to the ultrasound, to have a significant impact on the integrity of the capsules.

The release of encapsulated materials (i.e. FITC-PAH) can be seen in Fig. 4, showing a cuvette with fluorescent capsules before and after the exposure to ultrasound. It can be seen that the fluorescent polymer is initially located inside capsules and therefore does not provide a fluorescent signal due to quenching of the fluorescence. Then after the exposure to ultrasound, Fig. 4B, the fluorescent materials left the interior of the capsules and a bright fluorescent signal could be seen filling the cuvette. In subsequent experiments the release was monitored quantitatively. For these studies the kinetics of release of encapsulated polymers was followed from non-nanoparticles containing capsules in comparison with those containing metal nanoparticles. The ultrasound was applied for 0, 1, 2, 4, 6, 10 and 15 minutes. After sonication the capsules were separated from the supernatant by centrifugation. Next, the capsules were dissolved at high pH (i.e. by addition of 1 M sodium hydroxide) to release the encapsulated FITC-PAH. Fluorescence spectra were recorded of dissolved capsules and of the supernatant after the ultrasonic treatment, for both cases the pH of the solutions was equilibrated to pH 7. Quantification was performed by measuring the relative peak heights. Sonication of the capsules without nanoparticles for 15 minutes led to release of 20% of fluorescence, Fig. 5A. After the exposure to ultrasound of metal containing capsules for the same period of time 99% of the polymer was released, Fig. 5B. It can be concluded that release takes place in both cases, although it occurs faster for metal containing nanoparticles because a larger percentage of encapsulated materials was released from these capsules in comparison with non-metal containing capsules after the same exposure time.

Ultrasound triggered destruction of polyelectrolyte capsules could be used for controlled catalysis. The reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) by sodium borohydride (NaBH₄) is catalyzed by silver. In this reaction Ag⁰ is oxidized to Ag⁺ followed by reduction with NaBH₄. The reaction can be monitored with UV-VIS spectroscopy by monitoring the decrease in absorbance at 400 nm (which is the λmax of 4-NP) and the increase in absorbance at 310 nm (which is the λmax of 4-AP) as shown in Fig. 6A. When the reduction of 4-NP into 4-AP is performed in the absence of silver, no reaction occurs, as shown in Fig. 6B (line 1). However, when (PSS/PAH)₂Ag(PSS/PAH)₂ capsules (100 μl containing approximately 4 × 10⁷ capsules) are added to the reaction mixture reaction occurs as the absorbance at 400 nm decreases as a function of time (Fig. 6B, line 2). When the same amount of capsule suspension is added after the capsules were sonicated for 2 min, which is sufficient to destroy them completely (as verified by optical microscopy), a higher reaction rate is observed (Fig. 6B, line 3). These results indicate an influence of the environment of the Ag nanoparticles on their catalytic activity.

To further investigate this hypothesis we repeated the experiments using (PSS/PAH)₂Ag(PSS/PAH)₈ capsules. These capsules have 8 PSS/PAH bilayers on the layer of Ag.
nanoparticles, instead of only 2 PSS/PAH bilayers in the case of (PSS/PAH)\textsubscript{2}Ag(PSS/PAH)\textsubscript{2} capsules. The additional 6 PSS/PAH bilayers should alter the accessibility of the Ag surface and thus alter the reaction rate of the 4-NP conversion. Similarly to the above described experiments, 100 ml of sonicated (6 min sonication to assure complete capsule destruction, as verified by optical microscopy) and non-sonicated capsules were added to the 4-NP–NaBH\textsubscript{4} reaction mixture. When the reaction is monitored over 60 min no reaction occurs when non-sonicated capsules are added. In contrast, when sonicated, i.e. destroyed capsules, are added, reaction does occur. However, a 20 min initial ‘activation’ time during which no reaction occurs is observed. A linear decay of the 4-NP concentration starts only 20 minutes after the addition of the sonicated capsules, as shown by line 3 in Fig. 7.

The existence of the ‘activation’ time before silver starts to catalyze the reaction can be attributed to three factors: (a) the formation of a gel-like structure by PSS inside the capsule, slowing the diffusion of reaction products towards the silver particles, (b) slower diffusion due to the additional layers covering the silver particles layer on the capsules and (c) the surface of silver particles in the capsule interior is oxidized and the reduction of silver oxide by borohydride is limited due to the complexation of the silver surface with PSS. This may also lead to the limited diffusion of the reaction reagents to the silver particles surface. To answer this question, the experiment was repeated with a higher amount of sonicated and non-sonicated capsules (950 ml; the reaction rates are shown in Fig. 8 for non-sonicated capsules (line 1) and sonicated capsules (line 2)), thus providing additional silver particles to enhance the reaction rate by increasing the silver surface. The dispersion of sonicated capsules was aged for one hour before

**Fig. 5** Fluorescence spectra of non-modified (A) and nanoparticles modified (B) capsules taken 15 minutes of sonication.

**Fig. 6** (A) Typical UV-VIS spectra of conversion of 4-NP to 4-AP in the presence of silver catalyst. (B) The decrease in absorbance at 400 nm of 4-NP (1) in the absence of silver, (2) in the presence of (100 \( \mu \)l) non-sonicated (PSS/PAH)\textsubscript{2}Ag(PSS/PAH)\textsubscript{8} capsules and (3) in the presence of (100 \( \mu \)l) sonicated (PSS/PAH)\textsubscript{2}Ag(PSS/PAH)\textsubscript{8} capsules.

**Fig. 7** The decrease in absorbance at 400 nm of 4-NP (1) in the absence of silver, (2) in the presence of (100 \( \mu \)l) non-sonicated (PSS/PAH)\textsubscript{2}Ag(PSS/PAH)\textsubscript{8} capsules and (3) in the presence of (100 \( \mu \)l) sonicated (PSS/PAH)\textsubscript{2}Ag(PSS/PAH)\textsubscript{8} capsules.
its addition to the reaction mixture but even in this case, the 20 minute ‘activation’ period remains, Fig. 8 (line 2). This means that the reaction starts only when the surface of the silver nanoparticles is reduced with borohydride and the adsorption of 4-NP on the silver surface is no longer hindered by the PSS molecules, i.e. after the ‘activation’ time visible on the graphs in Fig. 7 and 8. It was also noticed that conversion of 4-NP in the presence of non-sonicated capsules, where the silver surface is expected to be less accessible to the reaction products compared to sonicated (i.e. destroyed) capsules, takes place (Fig. 8, line 1). However, in this case the ‘activation’ period is twice as long and the conversion reaches an intermediate plateau after 60 min of reaction. Upon leaving overnight, reaction was completely finished in both cases (no color in reaction mixture and no peak in UV-VIS adsorption spectra at 400 nm).

Conclusions
In this paper we have reported on the influence of ultrasound on polyelectrolyte multilayer capsules. Confocal microscopy and atomic force microscopy revealed a dramatic influence of ultrasound on the integrity of the capsules, showing complete capsule destruction upon ultrasonic treatment. Ultrasound triggered release of encapsulated species from these capsules was shown by visualizing the increase in fluorescence of the surrounding solution upon destruction of the capsule containing a fluorophore in a quenched state. Capsules with and without nanoparticles embedded in their shell were examined for their response to ultrasound, showing that in both cases capsules are destroyed, however, nanoparticle containing capsules are more sensitive to ultrasound. Further we have shown that ultrasound can trigger a chemical reaction catalyzed by nanoparticles embedded in the capsule shell.

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