Controlled release of Capreomycin sulfate from pH-responsive nanocapsules

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Abstract: The synthesis of polymeric nanocapsules loaded with the antituberculosis drug Capreomycin sulphate is presented in this work. The pH-responsive polymeric nanocapsules were successfully synthesized using the RAFT-based vesicle templating approach in the presence of Capreomycin sulphate. Anionic small polyelectrolytes comprising randomly distributed butyl acrylate and acrylic acid units were adsorbed on cationic vesicles of dimethyldioctadecyl ammonium bromide (DODAB). N,N-(dimethylamino)ethyl methacrylate (DMAEMA) and methyl methacrylate (MMA) or tertiary butyl methacrylate (tBMA) and MMA were used for the formation of the polymeric shells. Subsequently the tBMA was hydrolyzed to obtain negatively charged nanocapsules. The nanocapsule morphology for both approaches was characterized by DLS and TEM. The most stable hollow latex particles were obtained with the monomer composition of DMAEMA:MMA = 1:1. These nanocapsules were found to be colloiddally stable and pH-responsive. The entrapment efficiency of drug determined by UV-Vis-spectroscopy was 70 %. The drug release rate from nanocapsules at pH 6.5 was found to be relatively fast (substantial release within one hour already), however we believe slower release can be achieved by using a thicker polymer shell.

Introduction

The improvement of the efficiency of the drugs which are used for the treatment of long-term diseases (tuberculosis, AIDS, cardiovascular and tumor diseases) is an important task of modern medicine. As in most cases high levels of drug administered into the body spread out throughout the whole organism within several hours and therefore the efficacy of therapy decreases considerably. Therefore the development of new formulations for the drugs with improved efficiency is required.

Partially these new formulations should have improved targeting to the desired parts of the body and partially the release rate should be optimized. Targeting of the drugs depends amongst others on the particle size. The results of recent investigations have shown the improved efficacy of the use of principally novel forms of drug formulations using colloidal nanoparticles and nanocapsules [1-3]. Polymeric

nanocapsules have attracted great attention owing to their versatile properties and have wide application in different fields of science and technology. They are also of great importance in medicine as colloidal drug delivery systems [1-6]. During the synthesis of polymeric nanocapsules for drug delivery systems it is desirable to avoid the contact of the drug with the reactive species in the polymerization medium as some drugs are not stable under polymerization conditions (pH, presence of radicals, heating, etc.). Therefore it is preferable to load the polymeric nanoparticles with drug afterwards. However this is mainly accomplished by adsorption of the drug on the surface of polymer particles which is less effective in comparison with entrapping the drug inside polymeric capsules. In some cases it is possible to minimize the drug degradation by having the polymerization take place in another phase than the location of the drug. Therefore the synthesis of nanocapsules in the presence of the drug could be the best option. This can be realized by using “soft templates” such as emulsion droplets, liposomes or vesicles. Drug release can be controlled by creation of the system where drug release occurs as a result of polymer degradation or by encapsulation of the drug in stimulus-responsive shell where the drug releases to the medium depending on outer triggers (pH, temperature, ionic strength, magnetism, UV-light, ultrasound, etc.). The main feature of pH and temperature responsive polymers is that their volume, conformation and solubility can be altered (reversibly) by change in the pH and temperature.

Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization is a controlled radical polymerization technique which now has been developed for its applications in emulsion based systems [7-8]. Contrary to conventional free radical polymerization, RAFT offers an inherent advantage of control over molecular weight and the structure/composition of the polymer. The application of RAFT for the synthesis of controlled morphologies such as nanocapsules already resulted in few very promising approaches [7-8]. Recently, Ali et al. reported a simple RAFT-based templating approach for the synthesis of water filled polymeric nanocapsules using vesicles [8-9]. The approach requires the adsorption of short-chain anionic polyelectrolytes comprising randomly distributed butyl acrylate and acrylic acid units on cationic vesicles of dimethyldioctadecyl ammonium bromide (DODAB) [8-9]. The successful preparation of anionic pH responsive nanocapsules was reported earlier [9]. In this paper we also prepared nanocapsules that in principle can be charged positively and both types of nanocapsules were loaded with Capreomycin sulfate prior to the polymerization step by two methods: Method 1) Polymerization of DMAEMA and MMA to form a shell which can be protonated at lower pH and will then expand and release; Method 2) Polymerization of MMA and t-BMA, followed by hydrolysis of t-BMA to create a shell (MMA/MAA) which can be deprotonated at higher pH and will then expand and release. As a preliminary study the drug release was studied from these capsules at one pH.

Results and discussion

Synthesis and characterization of vesicles

Vesicles were prepared using the surfactant DODAB (Fig.1), by carrying out an extrusion of a 10 mM DODAB dispersion through three-stacked 100 nm pore size polycarbonate filters. This surfactant belongs to a class of synthetic surfactants called “dioctadecyldimethylammonium salts”. It is known that they are capable of producing vesicles in aqueous solutions. DODAB vesicles are stable and do not tend to form

lamellar phases.

Membrane extrusion is a simple, reproducible and widely used method to prepare LUVs. In extrusion the amphiphile dispersion, containing bilayer fragments, is simply forced through small pores of a track-etched polycarbonate membrane filters, using either pressurized inert gas at temperature well above the gel-to-liquid crystalline phase transition temperature (T_m). In extrusion, the vesicle size can be controlled by the pore size of the filters.

Fig. 1. Structure of dimethyldioctadecyl ammonium bromide (DODAB)

Synthesis and characterization of RAFT copolymers

Random copolymers consisting of acrylic acid and butyl acrylate units were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization in solution. Random copolymers compositions were chosen because they are less likely to self-assemble in aqueous phase into micelles which are highly undesirable for our purpose as they can give rise to unwanted secondary nucleation in the aqueous phase during vesicle encapsulation. The reaction pathway is presented in Fig. 2.

Fig. 2. Synthesis of Random RAFT Copolymer

The recipes for the synthesis of RAFT copolymers are presented in Table 1.

Tab. 1. Recipes for RAFT copolymer synthesis

RAFT copolymer	[DBTTC] mM	[AIBN] mM	[Acrylic acid] mM	[Butyl acrylate] mM
$BA_5\text{-co-AA}_{10}$	15.2	1.3	151.3	75.5
$BA_{7.5}\text{-co-AA}_{10}$	12.7	1.2	126.3	95.2
$BA_{10}\text{-co-AA}_{20}$	8.29	0.75	166.3	82.57

The reactions were performed in dioxane at 70 °C by using dibenzyl trithiocarbonate (Fig. 2) as a chain transfer agent (CTA). Synthesized polymers were characterized by ^1H NMR spectroscopy (Fig. 3) and GPC (Table 2). The GPC calibration with PS in principle is not suitable for our copolymers but can still give an order of magnitude for the molecular weights.

It is expected that copolymerization with acrylic acid and butyl acrylate gives random copolymers because of their similar reactivity ratios. Characterization of the resulting RAFT copolymers by ^1H NMR shows that the chain grows from both ends of the RAFT agent resulting in the trithiocarbonate moiety situated in the middle of the chain and benzyl groups are at the chain ends. This is an expected result because of the similar benzyl R and Z groups of the RAFT agent DBTTC (Fig.2) and can be seen from the ratio of 5 for the integral value of aromatic peaks at 7.1-7.3 ppm and S-CH bond peak at 4.5-4.7 ppm. This ratio would have been 10 in case the chain had only grown in one direction.

Fig. 3. ^1H NMR spectra of RAFT copolymer $BA_{9.5}\text{-co-AA}_{18.5}$

Tab. 2. Characterization of RAFT Copolymers

RAFT Copolymer	GPC data		^1H NMR data	
	M_n (g/mol)	PDI	F_{AA}	M_n (g/mol)
$BA_5\text{-co-AA}_{10}$	1652	1.2	0.61	1662
$BA_{7.5}\text{-co-AA}_{10}$	1972	1.3	0.54	2040
$BA_{9.5}\text{-co-AA}_{18.5}$	2893	1.4	0.63	2919

As it is seen from Table 2, experimentally determined M_n values ($M_{n,GPC}$ and $M_{n,NMR}$) correspond well each other. Also they correspond well with theoretically calculated M_n values (not shown). This, together with low polydispersity index values, indicates that the RAFT method worked successfully for the synthesis of RAFT copolymers.

Synthesis and characterization of nanocapsules

Nanocapsules from the monomers DMAEMA : MMA and EGDMA

At present time a number of synthetic monomers have found wide application in different fields including medicine owing to their stimulus-sensitive properties. Therefore DMAEMA with MMA (with 3% of EGDMA) have been chosen as monomers since the copolymer network formed possesses pH-responsiveness.

Nanocapsules were synthesized by using starved feed emulsion polymerization. Starved feed conditions can help to avoid the formation of monomer droplets and secondary nucleation. For the encapsulation reactions DODAB vesicles obtained by extrusion through 100 nm PC filters and RAFT copolymer BA_6 -co- AA_{10} (Table 2) was used. The rational behind the use of this copolymer is that it has previously shown good behavior for the encapsulation reactions performed using different (meth)acrylates feed ratios [8-9]. The chosen RAFT copolymer BA_6 -co- AA_{10} is relatively short-chained, which could also give possibility of avoiding secondary nucleation and give maximum number of RAFT groups on the surface of the substrate to be encapsulated (surfactant vesicles, in our case). The encapsulation route can be divided into 2 simple steps: 1) absorption of RAFT copolymer on DODAB vesicles; 2) feeding with the desired monomer feed composition in the presence of a water-soluble initiator V-501.

To study how different monomer compositions influence particle morphology of the nanocapsules as well as to prepare in the end two different pH-responsive systems, two monomer mixtures were investigated: DMAEMA:MMA and t-BMA:MMA. To preserve the integrity of the nanocapsules during the pH responses also a flexible crosslinker (3% of EGDMA) was added. The recipes of encapsulations are listed in Table 7 (experimental part).

Particle size and morphology

The characterization of particles was done by DLS and TEM. Final z-average particle diameters for three different reactions (listed in Table 3) were measured by DLS. The size of the actual template vesicle, as measured by DLS and cryo-TEM was around 120 nm.

Tab. 3. Z-average diameter of nanocapsules.

Latex	Z-average diameter (nm)
<i>vesicles</i>	120
<i>E-1</i>	138.2
<i>E-2</i>	198.7
<i>E-3</i>	230.3

An increase in particle diameters was observed after the encapsulation reactions which is an indication of successful formation of a thick polymer layer around the surface of the vesicles.

As it is seen from Table 2, the monomer feed composition has considerable effect on the particle diameters of the resulting structures. One possible explanation for these different particle diameters could be the extent of particle aggregation. Therefore we decided to investigate the resulting morphologies by using TEM. Fig.4 a, b and c show the morphology of the resulting products obtained by using monomer feed composition with molar ratios of DMAEMA:MMA of 1:1, 2:1 and 1:2 respectively (entries E1, E2 and E3, Table 2).

Fig. 4. TEM images of nanocapsules obtained by encapsulation of DODAB vesicles using RAFT copolymer BA_6 -co- AA_9 and monomer mixture DMAEMA:MMA with 3% EGDMA: 4a – E₁, 4b – E₂, 4c – E₃.

Successful formation of nanocapsules is evident from the TEM images. As we already concluded from DLS measurements some aggregation of the particles took place (Fig.4 b, c), though the formation of bigger particles also can be seen in these micrographs. The observed primary particle diameters in TEM for all three cases are smaller than that obtained from DLS. As is evident from Fig.4 b and c, there is some particle aggregation which can explain the increase of the z-average particle diameter in the DLS measurements. This also complicates the pH dependent size measurements described in the next section.

pH-responsive behavior of formed nanocapsules

It is well-known that DMAEMA possesses pH-responsive properties. We investigated the effect of changing pH on the behavior of the formed hollow latex particles (Fig.5).

Fig. 5. pH-dependent particle size of nanocapsules obtained by encapsulation E₁(●), E₂(▲) and E₃(■).

The effect of pH on particle size was evaluated by redispersing freeze-dried sample of nanocapsules latex in different pH buffer solutions ranging from pH 1 to 8.

As it is seen from Fig.5, the size of nanocapsules changes appreciably in the pH range from 1 to 4 which correspond to the de-ionization of pending quaternary ammonium groups to neutral amino groups. The presence of crosslinks formed by tetrafunctional monomer suppresses complete dissolution of the polymer shells at low pH.

For the samples E1 and E3 more or less the original particle size as reported in Table 4 were obtained again at neutral pH. For E2 a considerably larger size was seen. This could be caused by partial aggregation of the particles. Still a pH dependent size change was observed (Fig. 5) for all three samples.

Synthesis and characterization of nanocapsules using a monomer feed comprising t-BMA:MMA with 3% EGDMA

Carboxylic acid containing polymers (such as polyacrylic acid and polymethacrylic acid) can show pH responsive behaviour. However it is difficult to use some of the acidic monomers directly during emulsion polymerization due to their very high water solubility. For instance the use of acrylic and methacrylic acids can cause polymerization in the aqueous phase resulting in water soluble polymers which will disturb colloidal stability during emulsion polymerization. To incorporate this monomer in emulsion polymerization, an alternative choice is to use monomers containing protected acid groups, such as monomer containing tertiary ester groups which are liable to hydrolysis, yielding acid groups. Typical examples include tertiary butyl acrylate [9] and tertiary butyl methacrylate (t-BMA). These two monomers are adequately hydrophobic to be used in emulsion based polymerization systems. Once incorporated into desired morphologies (nanocapsules in our case) tertiary butyl

ester groups can be easily and selectively hydrolysed to acid groups resulting in pH-responsive system.

Following the previously described method [9] for t-BA:MMA with EGDMA we tried to obtain nanocapsules of *t*-BMA:MMA with 3% of EGDMA at a somewhat lower ratio of monomers to DODAB.

After polymerization the latex was freeze-dried and redispersed in dioxane. The hydrolysis of *t*-BMA groups was done with trifluoroacetic acid under constant stirring during 12 hours. The hydrolyzed product was dried in the oven under vacuum to eliminate organic solvent, after that the solution was freeze-dried again.

Particle size and morphology

The effect of monomer feed ratios on particle morphology was studied by using DLS (Table 4). All values are in good agreement with the starting particle size of the template vesicle. The observed increase in the particle size values after encapsulation is due to the formation of polymer layer around the vesicles.

Tab. 4. Z-average diameter of nanocapsules.

Latex	Z-average diameter (nm)
<i>vesicles</i>	120
<i>E-4</i>	146.1
<i>E-5</i>	138.3
<i>E-6</i>	142.7

The size of the nanocapsules is also bigger than the original vesicles indicating a polymer layer around the vesicles.

pH-responsive behavior of the formed nanocapsules

After hydrolysis the purified nanocapsules were freeze-dried and also redispersed in different pH buffer solutions (pH from 3 to 8). However the particle size measurements revealed larger values (at least a factor of two) than reported in Table 7 (similar problems to E2 in the previous series) and no consistent size changes with pH could be observed. This is in contrast to the data of Ali et al. [9] where the size before and after hydrolysis was the same at low pH (they used t-BA instead of t-BMA). Redispersibility is an issue with some batches of these particles and will be further investigated. Still these particles are tested for their ability to release drugs as the aggregates can still work as nanocapsules. Previous work of Ali et al [9] with a similar monomer combination but other concentrations of the monomers was successful and the size as function of the pH showed a steady increase going from pH=5 to pH=8. It seems that the freeze-drying step is not always leading to complete redispersibility of the nanocapsules, depending on the recipe of the nanocapsules.

Encapsulation of capreomycin sulfate and drug release experiments

Nowadays one of the most serious diseases is tuberculosis. According to the World Health Organization more than 8 million people are diagnosed with this disease world wide annually and about 2 million of them die [10]. Capreomycin sulfate (Fig.6) is one of the drugs which is used to treat tuberculosis alone or in combination with other

drugs in complex therapy. It is a water-soluble polypeptide antibiotic. The studies of its biological activity show a high activity against *Mycobacterium tuberculosis*. The drawback of this drug is its quite rapid metabolism in the organism resulting in reduced efficacy of the therapy. In this regard, the possibility of obtaining a novel encapsulated form of capreomycin sulfate with a pH-responsive shell was considered in this work.

Fig. 6. The structures of Capreomycin sulfate

Capreomycin sulfate was present during the vesicle preparation by the extrusion technique. The size of the formed vesicles was 130 nm similar to the vesicles not containing drug (120 nm, Table 3 and 4). Purification of the vesicles from excess of drug was carried out by ultracentrifugation. The amount of drug entrapped by vesicles was determined by UV-Vis-spectroscopy and was equal to 70%. Feeding compositions for encapsulation were chosen based on the previous experiments with DMAEMA:MMA and *t*-BMA:MMA. A ratio of 1:1 with 3% EGDMA was chosen in both cases. Some simple experiments, exposing Capreomycin sulfate to decomposing initiators (including V501) at 60 °C, showed no change in the UV-VIS spectrum indicating that the drug remained intact in the presence of small radicals. This does not guarantee that this is also the case in a polymerizing system. For encapsulation of Capreomycin sulfate the monomer mixtures given in Table 8 (experimental part) were used. The size of nanocapsules was measured by DLS and data are given in Table 5.

Tab. 5. Z-average diameter of nanocapsules.

Latex	Z-average diameter (nm)
<i>Vesicles with drug inside</i>	130
<i>VCap -1</i>	206.5
<i>VCap - 2</i>	202.3

As it is seen from table 8 the size of nanocapsules increased with almost 70 nm. It is possible that again some particle aggregation took place.

During the preliminary measurements it was observed that the initial peak of capreomycin sulphate at 268 nm shifted to 310 nm (Fig.7).

Fig. 7. UV-Vis-spectra of capreomycin sulphate before (■) and after encapsulation with DMAEMA:MMA (▲); t-BMA:MMA(●).

This shift was observed in both encapsulations. The shift of the peak in the UV-Vis-spectra indicates either changes in structure or chemical environment of the initial compound. The change in UV spectrum of capreomycin sulphate, also after release, indicated that it is most probably chemically modified. But because the drug is still released, as can be seen from the UV-VIS spectra over time, we believe the drug is not covalently bound to the nanocapsules. It was already observed that the spectrum of capreomycin sulfate does not change when exposed to decomposing V-501. Therefore, it is possible that capreomycin sulfate reacts with polymeric radicals (formed during the polymerization reaction) and as a result it may change the chemical structure of the drug which may lead to reduction of biological activity or even deactivation of the drug. In the case of the RAFT approach small initiator derived radicals, polymeric radicals and radicals on the RAFT agent are present. Under investigation is which of the latter two radicals is responsible for the chemical reaction with the drug. Also other living radical systems are under investigation. In the case of nitroxide mediated polymerization the only type of radicals present are polymeric radicals. Under the right conditions these radicals cannot diffuse through the vesicle bilayer and in this way the drug and the radicals cannot get into direct contact, hopefully preventing deterioration of the drug.

Drug release studies

The next step was to study the release of drug from polymeric nanocapsules, where the VCap-2 particles (t-BMA-MMA, 3% EGDMA) were first hydrolyzed as described before. We expect that we do not lose the drug during the hydrolysis step since capreomycin sulfate is not soluble in dioxane.

Drug release experiments were performed using UV-Vis-spectroscopy. According to the previous studies the size of the particle in case of DMAEMA:MMA increases with a respective decrease in pH. Basically the biggest size can be observed in very acidic conditions. Drug release rates were observed using UV-Vis spectroscopy. As a preliminary study we selected a pH of 6.5 for both systems (Fig.8).

The measurements were done immediately after redispersing the capsules and adjusting the pH (all within 6 minutes). This is the reason why at t=0 we already have partial release of the drug.

Fig. 8. Drug release study of nanocapsules VCap-1(▼) and VCap-2 (▲) over time at pH = 6.5, $\lambda = 310$ nm

As it is seen from Fig.8 the drug release is relatively fast, with an initial fast burst of drug release going to a second slower release profile.

Conclusions

Vesicle-templated nanocapsules loaded with capreomycin sulphate were successfully synthesized. After entrapment of the drug inside of the vesicles the RAFT copolymer based approach was used to form pH-responsive nanocapsules.

The pH responsiveness was successfully monitored for the DMAEMA-MMA-EGDMA nanoparticles not loaded with drugs. However the particle size measurements were indicating some partial aggregation, especially for the MMA-tBMA system. The freeze-drying step intensified this problem even. So for MMA-tBMA particles the particle size vs pH curves could not be studied. The results showed a high entrapment efficiency of the drug (70 %). An initial study on the release of capreomycin sulfate from these polymeric nanocapsules was performed at pH=6.5. It was found that the release is relatively fast in both cases; however we believe the time can be prolonged by increasing the shell thickness of the nanocapsules and the degree of crosslinking.

However as a result of the polymerization reaction in the presence of capreomycin sulfate the peak maximum in the UV-VIS spectrum shifted from 268 nm to 310 nm which was probably caused by reaction between the drug and polymeric radicals generated during the reaction. Investigations are under way to pinpoint which type of radicals are responsible for this reaction and how to separate radicals and drug during the polymerization reaction on the exterior of the drug loaded vesicles.

This type of crosslinked responsive nanocapsules can find possible applications in pharmaceuticals as drug carrier.

Experimental

Materials

Dimethyldioctadecyl ammonium bromide (DODAB, Acros, >99%) was used as received. Methyl methacrylate (MMA, Aldrich, 99%) was distilled under reduced

pressure prior to use. N,N-(dimethylamino)ethyl methacrylate (DMAEMA, Aldrich, 99%) and tertiary Butyl methacrylate (t-BMA, Aldrich, 99%) were purified by passing over a basic aluminum oxide column (Sigma) and inhibitor remover (Sigma). Ethylene glycol dimethacrylate (EGDMA, Aldrich, 99%) was columned (Inhibitor remover, Aldrich) to remove inhibitor before use. The water-soluble azo-initiator 4,4'-azobis(4-cyanovaleric acid) (V-501, Fluka, 98%), ethanol (Biosolve), methanol (Biosolve) and 1,4-dioxane (Biosolve) and tetrahydrofuran (THF, Biosolve) were all used as received.

Instruments and Measurements

The particle size distribution and zeta potential (ζ) were determined by dynamic light scattering (DLS) on a Malvern Zetasizer Nano ZS instrument at 173 ° and 25 °C.

Molecular weight and polydispersity index (PDI) of RAFT copolymers and the composite latexes were measured by gel permeation chromatography (GPC) using a Waters GPC equipped with a Waters model 510 pump and a model 410 differential refractometer. A set of two mixed bed columns (Mixed-C, Polymer Laboratories, 30 cm, 40 °C) were used. Tetrahydrofuran was used as the eluent, and the system was calibrated using narrow molecular weight polystyrene standards (range = 580-7.5·10⁶ g/mol).

¹H NMR spectra were recorded on a Varian 400 MHz spectrometer using dimethyl sulfoxide-d₆ and chloroform-D as solvents.

Absorbance measurements were recorded using a Hewlett Packard Photodiode Array UV spectrophotometer equipped with a Peltier heater/cooler. Samples were measured in a screw-capped quartz cuvette (1 cm optical path length) equipped with a magnetic stirring bar and an immersed thermocouple.

Transmission electron microscopy (TEM) measurements were performed on a FEI Tecnai 20, type Sphera TEM instrument equipped with a LaB₆ filament operating at 200 kV. Images were recorded with a bottom-mounted Gatan CCD camera. A 3 μL sample was applied on a Quantifoil grid (R 2/2, Quantifoil Micro Tools GmbH; freshly glow-discharged just prior to use), excess liquid was blotted away.

Vesicle preparation

Large unilamellar vesicles (LUVs) were prepared by a membrane extrusion method [8]. The preparation procedure of DODAB vesicles and their characterization by DLS and cryo-TEM have previously been described in detail [8].

Synthesis of RAFT Copolymers

RAFT copolymers containing different combinations of randomly distributed acrylic acid (AA) and butyl acrylate (BA) units were synthesized in dioxane using dibenzyl trithiocarbonate (DBTTC) as a chain transfer agent. Table 6 summarizes the structural compositions and recipes used for their synthesis. Briefly, RAFT copolymer containing average 10 BA units and 20 AA units abbreviated as BA_{10-co-AA}₂₀, was synthesized as follows: 10.57 g (83 mmol) of BA, 11.9 g (166.3 mmol) of AA, 2.55 g (8.29 mmol) of DBTTC and 0.12 g (0.75 mmol) of AIBN were mixed in 25 g dioxane in a Schlenk flask. The mixture was degassed by three freeze-pump-thaw cycles and then heated and stirred at 70 °C for 5 hours. After synthesis, RAFT copolymers were characterized by GPC and ¹H NMR.

Tab. 6. Recipes for RAFT copolymer synthesis.

RAFT copolymer	[DBTTC] mM	[AIBN] mM	[Acrylic acid] mM	[Butyl acrylate] mM
<i>BA₅-co-AA₁₀</i>	15.2	1.3	151.3	75.5
<i>BA_{7.5}-co-AA₁₀</i>	12.7	1.2	126.3	95.2
<i>BA₁₀-co-AA₂₀</i>	8.29	0.75	166.3	82.57

Synthesis of nanocapsules

Nanocapsules were synthesized by starved feed emulsion polymerization performed in a 50 ml three-neck flask equipped with a magnetic stirrer bar and a heating bath as described in detail previously [9]. The recipes and the reaction conditions used for the encapsulations are listed in Table 7. For all experiments: [RAFT copolymer] / [DODAB] = 3; 0.23 g of monomer (fed at a rate of 0.01 g/min, reaction temperature 70°C). In all recipes 3% of crosslinker EGDMA was added.

Tab. 7. Recipes for encapsulation.

Latex	Monomer Feed composition		
	MMA (mol)	DMAEMA (mol)	EGDMA (mol)
<i>E-1</i>	0.12	0.12	0.0074 (3 mol %)
<i>E-2</i>	0.12	0.24	0.0111 (3 mol %)
<i>E-3</i>	0.24	0.12	0.0111 (3 mol %)
	MMA (mol)	t-BMA (mol)	EGDMA (mol)
<i>E-4</i>	0.12	0.12	0.0074 (3 mol %)
<i>E-5</i>	0.12	0.24	0.0111 (3 mol %)
<i>E-6</i>	0.24	0.12	0.0111 (3 mol %)

Responsive behavior of nanocapsules

The synthesized vesicle-templated DMAEMA:MMA crosslinked nanocapsules were washed by centrifugation followed by freeze drying to obtained powders which were redispersed in buffer solutions of various pH values (ranging from 1 to 8) in separate vials. To achieve complete dissolution at certain pH, mild bath sonication was applied. After the equilibration time of around 12 hours, the pH induced dimensional changes were recorded using DLS measurements.

Encapsulation of Capreomycin sulfate in the nanocapsules and drug release experiments

In order to encapsulate the drug into vesicles, 50 ml of 1 mM capreomycin sulfate solution was mixed with 50 mL of 10 mM DODAB aqueous pre extrusion dispersion. The resulting dispersion was extruded to obtain drug-loaded DODAB vesicles following the same extrusion procedure described above. The resulting drug loaded

vesicles were separated from the drug in the serum by ultracentrifugation (60000 rpm 10°C) and redispersed in water. The concentration of the drug in the supernatant was analyzed by quantitative UV-Vis-spectroscopy using a calibration curve of capreomycin sulfate at $\lambda_{\text{max}} = 268$ nm. After the completion of the extrusion run, the resulting vesicle dispersion was maintained at 65 °C for more than 12 hours before cooling down to room temperature. The influence of temperature on stability of the drug has already been studied and the drug has proven to be stable when heated.

Drug-loaded vesicles were then used for the synthesis of crosslinked nanocapsules using DMAEMA:MMA and t-BMA:MMA monomer feeds (both with 3% EGDMA). The polyt-BMA-MMA nanocapsules were subsequently hydrolyzed with the following procedure. The latex was freeze-dried and redispersed in dioxane. The hydrolysis of t-BMA groups was done with trifluoroacetic acid under constant stirring during 12 hours. The hydrolyzed product was dried in the oven under vacuum to eliminate organic solvent.

The resulting crosslinked nanocapsules were freeze dried and redispersed in a buffer solution of pH = 6.5. The recipes for each reaction are given in Table 8.

Tab. 8. Recipes for encapsulation.

Latex	Monomer Feed composition		
	MMA (mol)	DMAEMA (mol)	EGDMA (mol)
VCap - 1	0.12	0.12	0.0074 (3 mol %)
VCap - 2	MMA (mol)	t-BMA (mol)	EGDMA (mol)
	0.12	0.12	0.0074 (3 mol %)

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