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Fundamental design aspects of amphiphilic shell-crosslinked nanoparticles for controlled release applications

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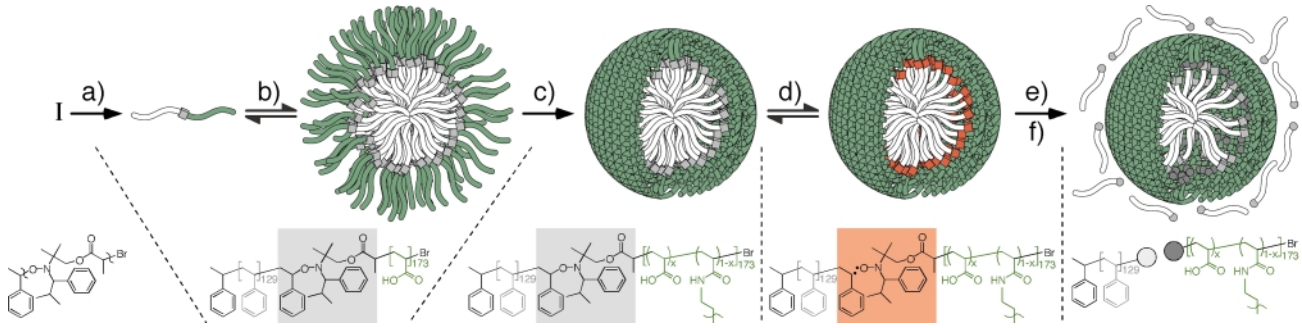
A unique method is developed for the controlled release of the hydrophobic polymer chains from the core of the shell crosslinked nanoparticles (SCKs) by selective cleavage of labile C–ON bonds present at the core–shell interface; this represents a methodology to probe the permeability of nanoscopic membranes and a means for applications in the controlled release of macromolecular species.

Polymeric nanoparticles, engineered with controlled composition, structure and dimensions, have attracted great interest in recent years for their potential applications in medicine and in nanotechnological devices, particularly as drug delivery vehicles.[1,2] Inspired by the nano-sized, amphiphilic core–shell structure of lipoproteins, shell crosslinked nanoparticles (SCKs) with a hydrophobic core, contained within a hydrogel network, were prepared by the self-assembly of amphiphilic block copolymers followed by intramicellar crosslinking between the polymeric chains located within the shell.[3] SCKs are characterized by their structural integrity and available functionality to attach receptor-recognizing ligands on the shell surface.[4] The control over size, shape and composition of these nanoparticles holds great potential in drug delivery applications.[5–7] The permeable crosslinked shell of the SCKs can act as a protective layer for the encapsulated guest molecules and determines the transport into and out of the core of these nanoparticles.[8] The polymer chains constituting the core can be selectively degraded and extracted to generate hollow nanospheres, which makes possible the loading of large quantities of guest molecules.[9]

SCKs with the core composed of bioactive polymeric chains, and covalently attached to the shell, through labile bonds at the core–shell interface, are expected to be of interest for the release of hydrophobic drugs, such as peptides.[2] Such SCKs may be useful as prodrugs with enhanced oral bioavailability for poorly absorbed drugs and may exhibit site-specificity and controlled release, the latter being controlled by the extent of crosslinking of the permeable shell membrane. Therefore, the porosity of these membrane networks, which are only a few nanometers in thickness, must be accurately evaluated. Herein, we report our preliminary results carried out on an SCK model system, based on poly(styrene)-*b*-poly(acrylic acid) (PS-*b*-PAA), possessing labile C–ON bonds present at the core–shell interface. The controlled release of the core PS chains is demonstrated, upon selective thermolytic cleavage of the C–ON bonds.

The synthetic approach for the preparation of the SCK nanoparticles from PS-*b*-PAA and the subsequent release of the PS chains from the core is presented in Scheme 1. SCKs possessing thermally labile C–ON bonds at the core-shell interface were prepared from the amphiphilic copolymer PS-*b*-PAA, which was designed to have a C–ON bond connecting the hydrophilic and hydrophobic blocks. The synthesis of PS-*b*-PAA utilized a combination of nitroxide mediated radical polymerization (NMRP) and atom transfer radical polymerization (ATRP)[10] from a bi-functional initiator, **1**.[11] The alkoxyamine group of **1** was utilized for polymerizing styrene by NMRP and the resulting PS with a terminal bromopropionyl ester group was then used as a macroinitiator for the ATRP of *tert*-butyl acrylate to obtain PS-*b*-P*t*BA.[12] The diblock was made amphiphilic by the hydrolysis of *tert*-butyl ester groups by reaction with TFA in CH_2Cl_2 at rt.[13]

Micellization of PS-*b*-PAA and subsequent shell crosslinking, using 2,2′-(ethylenedioxy)bis(ethylamine) as a crosslinker, were carried out according to the previously reported procedure.[3] To study the effect of shell crosslinking on the structure of the nanoparticles and, in particular, the shell permeability, polymeric micelles were crosslinked to different extents by adding stoichiometric ratios of diamine crosslinker to carboxylic acid moieties that would result in a maximum theoretical crosslinking of 20, 50 and 100%. Upon crosslinking, the C=O absorption band corresponding to free carboxylic acid groups (1710 cm^{-1}) decreased and the amide I and II bands (1650 and 1540 cm^{-1}) with intensity in proportion to the amount of added crosslinker appeared in the IR spectra of lyophilized samples of the SCKs. The effective Stokes, volume-weighted, mean hydrodynamic diameters (by dynamic light scattering (DLS) measurements) were 54 ± 3 nm for the micelles, and the SCKs were respectively, 55 ± 3, 28 ± 2 and 35 ± 3 nm for 20, 50 and 100% crosslinking.[14]



Scheme 1 The synthetic approach for the preparation of SCK nanoparticles by a combination of self-assembly and intramicellar crosslinking reactions followed by the subsequent release of core polymer chains upon the cleavage of C–ON bonds present at the core–shell interface. (a) *See* ref. 12 and 13; (b) and (c) *see* ref. 3; (d) thermolysis of C–ON bonds (125 °C, 24 h in water); (e) lyophilization and (f) resuspension in THF.

The irreversible termination of the PS· radicals, generated upon the thermal homolytic cleavage of the C–ON bonds at the core–shell interface[15] leads to the detachment of PS chains from the crosslinked shell. The addition of ascorbic acid during this process facilitates the irreversible termination of the carbon-centered radicals by reducing the aminoxyl counter radicals to the corresponding hydroxylamine.[16] Thermal decomposition studies were carried out first with the amphiphilic diblock copolymer PS-*b*-PAA, by heating at 125 °C for 24 h in a sealed Schlenk tube (in toluene–acetic acid, 80 : 20 v/v) in the presence and absence of ascorbic acid. The isolated PS (pure by ^{1}H NMR) accounted for 58.5% (ascorbic acid) and 20.7% (no ascorbic acid) of the theoretical amount of PS expected. The polydispersity index (PDI) decreased from 1.22 (for PS-*b*-PAA) to 1.10 (for the PS obtained upon decomposition). The GPC trace of the PS obtained was monomodal and the peak retention time coincided with that of the PS macroinitiator.

The amount of PS isolated (20.7% in 24 h) by the homolytic cleavage of C–ON bonds in PS-*b*-PAA was significant even in the absence of radical quencher, ascorbic acid. Hence, for simplicity, the cleavage of the C–ON bonds present at the core–shell interface of the SCKs were carried out by heating the aqueous solution of SCKs (125 °C in a sealed Schlenk tube for 24 h) in the absence of ascorbic acid. The kinetics of PS chains released from the core was followed by suspending the lyophilized SCKs in THF (at rt) with stirring and analyzing aliquots removed at different time intervals, after filtration through a 20 nm filter (Whatman), by GPC using a PS standard (M_n = 2450) of known weight as an internal reference. Representative GPC traces of PS released as a function of time, for SCKs with 20% crosslinking, are given in Fig. 1(a) and the data for % PS released as a function of time for SCKs with different extents of crosslinking are plotted in Fig. 1(b). In each case, the GPC retention time of the PS released and that of the PS macroinitiator was identical. Based on the nearly monodisperse GPC chromatograms of the released PS, it appears that bimolecular termination between the PS· radicals is not a major termination process. However, an alternative argument for the lack of observable PS dimer is that the molecular weight of the dimer may be sufficiently great as to prevent its permeation through the shell of the SCK. An inverse relationship was observed between the amount and rate of PS release with the extent of shell crosslinking. The amount of PS released (92 h) was 28.7, 6.8 and 3.5% for the SCKs with 20, 50 and 100% crosslinking, respectively.[17] The PS released during the initial period was rapid ('burst effect'), likely due to the increase in osmotic pressure with the addition of THF, followed by a decreasing rate of release, consistent with passive diffusion.

The amount of PS released from the nanoparticles was larger (28.7% in 92 h, 20% crosslinking) than that isolated (20.7%) from PS-*b*-PAA under similar decomposition conditions (125 °C, 24 h). A decreased efficiency of the recombination reactions between the PS· radicals in the core and the aminoxyl counter radicals located at the inner surface of the shell, leading to more dead PS chains, is consistent with the observed results for SCKs.

The experimental data reveal that the free PS chains, present in the core, can penetrate the hydrogel-like shell membrane. However, when the shell is highly crosslinked such penetration is inhibited. The controlled release of polymer chains from the core by adjusting the crosslink density of the shell opens the possibilities of designing polymeric nanoparticles with specific shell permeabilities, capable of delivery of large guests. This approach may provide a solution to some of the delivery problems posed by biologically active molecules, such as peptides and proteins, genes and oligonucleotides. The results of this study also provide a foundation for better understanding of the porosity of the crosslinked shell of SCKs.

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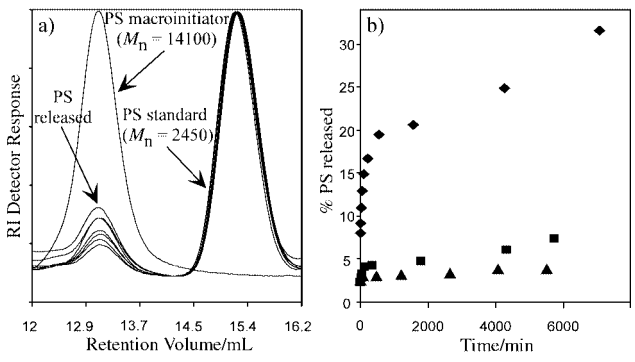

Fig. 1 (a) GPC traces of PS (M_n = 14100) released from the core of SCKs (20% crosslinking) as a function of time. The PS standard (M_n = 2450) provided concentration quantification. (b) Release kinetics of PS from the core of SCKs crosslinked to different extents (◆ 20%, ■ 50% and ▲ 100% crosslinking).

Notes and references

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11 The initiator, **1**, was synthesized by reacting 1-hydroxy-2,2,5-trimethyl-3-(1-phenylethoxy)-4-phenyl-3-azahexane (HAA) with excess bromoisopropionyl bromide in the presence of triethylamine. The crude ester was purified by column chromatography eluting with hexane–ethylacetate (4 : 1). ^{1}H NMR ($CDCl_3$, 300 MHz, diastereomers) δ 7.5–7.1 (m, 20H), 5.0–4.8 (m, 2H), 4.5–2.3 (m, 8H), 2.0–1.8 (m, 8H), 1.66 (d, 3H, J = 6.6 Hz), 1.58 (d, 3H, J = 6.6 Hz), 1.37 (d, 3H, J = 6.3 Hz), 1.36 (d, 3H, J = 6.3 Hz), 1.26 (s, 3H,), 1.05 (s, 3H), 0.98 (d, 3H, J = 6.3 Hz), 0.61 (s, 3H,), 0.59 (d, 3H, J = 6.6 Hz), 0.26 (d, 3H, J = 6.6 Hz). HRMS mass calculated for $C_{25}H_{34}BrNO_3$ $[M + 1]^+$ 476.17, found 476.2. For the preparation of HAA *see* D. Benoit, V. Chaplinski, R. Braslau and C. J. Hawker, *J. Am. Chem. Soc.*, 1999, **121**, 3904.

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13 To 6.05 g of PS-*b*-P*t*BA was added 150 mL of CH_2Cl_2 followed by 15.0 mL of TFA. The reaction mixture was stirred at 25 °C for 40 h, concentrated *in vacuo*, and precipitated (3×) in hexane from THF solution. The PS-*b*-PAA product was dried under vacuum.

14 The effect of shell crosslinking upon the hydrodynamic diameter is being studied in detail for these and other shell crosslinked nanostructures.

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17 In a blank experiment, performed upon 20% crosslinked SCKs without heating, 3% of the PS chains were extracted (in 92 h) from the core, presumably these were macroinitiator contaminants.