Amphiphilic core–shell nanospheres obtained by intramicellar shell crosslinking of polymer micelles with poly(ethylene oxide) linkers

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Intramicellar crosslinking of the polymer chains within the shells of polystyrene-*b*-poly(acrylic acid) micelles by reaction with difunctional poly(ethylene oxide) afforded unimolecular amphiphilic core–shell nanospheres (50 nm hydrodynamic radius); the resulting surface hydrogel layer gives an approximate fivefold increase of particle volume from the dry state to aqueous solution.

The concept of shell crosslinked knedel-like structures (SCKs)[1] has been applied to synthesize nanoparticles with core–shell structures, composed of poly(ethylene oxide) (PEO) chains as covalent crosslinks binding together the peripheral layer of polymer micelles. The unique combination of size, structure, stability and composition of these nanospheres renders them great potential for drug delivery systems. Extensive efforts have been undertaken by other researchers to understand the structurally related drug delivery properties of the submicrometer-sized polymeric particles.[2–6] For example, the sub-100 nm diameter of PEO-adsorbed polystyrene nanoparticles allowed for evasion of sequestration by the phagocytosis process,[2] and the hydrophilicity of PEO facilitated the transportation of these particles through the interstitum by aqueous channels.[7] Moreover, the surface PEO blocks of polymer micelles sterically stabilized the nanospheres and prolonged the circulation lifetime in the blood.[3]

The shell-crosslinked nanospheres in this study offer some advantageous features, such as the ability to maintain structural integrity upon infinite dilution and mechanical stresses, the stability of covalently bound PEO within the surface domain, and the potential to attach receptor-recognizing ligands to the residual carboxylate side groups on the PAA block. SCKs have previously been prepared with coupling *via* short difunctional oligomeric crosslinkers,[8] in which the chemical composition of the crosslinkers resulted in differences in the nature of the shell. This report demonstrates that PEO can be incorporated into the SCK shell as a polymeric crosslinker to greatly increase the shell thickness and to modify the surface properties[9] of the SCKs (*e.g.* permeability and flexibility), without the evidence of intermicellar reactions occurring.

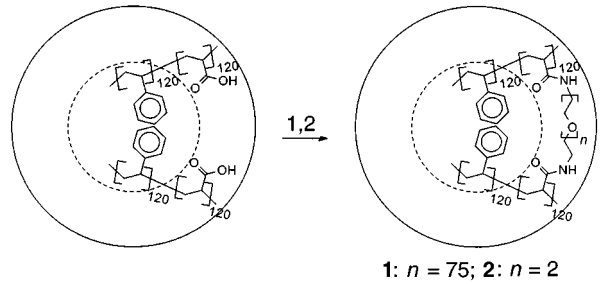
SCKs are essentially unimolecular polymer micelles, which are prepared by stabilization of the basic structure of the spherical micellar assembly through linking together of the hydrophilic portions of the chains within the micelle shell. Therefore, the synthesis of the SCKs involves only three steps: (*i*) preparation of an amphiphilic block copolymer; (*ii*) self-assembly of the amphiphilic block copolymer into polymer micelles; (*iii*) crosslinking through side groups along the blocks occupying the shell of the polymer micelles.

The preparation of the SCKs began from the diblock copolymer, polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA), which was conveniently synthesized by living free radical polymerization.[10] The living free radical polymerization was accomplished by a stepwise atom transfer radical polymerization technique, similar to the procedures reported by Matyjaszewski and coworkers.[11] Micelles composed of PS-*b*-PAA were formed by addition of water to a solution of the polymer in THF,[12] and an aqueous solution of micelles was obtained by dialyzing against distilled water. The crosslinking was accomplished by condensation reactions of diamino linkers with PAA, facilitated by a carbodiimide coupling agent (Scheme 1).

The carboxylic acid groups on the PAA block were first activated by reaction with the water soluble carbodiimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (1 equiv. based upon the acrylic acid groups). The crosslinker, poly(ethylene glycol) diamine (M_w = 3400 Da, 0.5 equiv. based upon the acrylic acid groups), was then added to crosslink the PAA domain by formation of amide bonds[13] and yield SCK **1**. For evaluation of the effects of the crosslinker length, SCK **2** was crosslinked *via* a short tri(ethylene oxide) linker in a similar fashion using 2,2′-(ethylenedioxy)bis(ethylamine).[8] In both cases, the urea by-product was removed by dialysis against distilled water.

^{1}H NMR resonance signals could not be detected in D_2O solutions, due to the colloidal nature of **1** and **2**. Therefore, solid samples of the SCKs were obtained by lyophilization, and the compositions of **1** and **2** were characterized by IR spectroscopy. Upon formation of **1**, absorption bands corresponding to free carboxylic acid groups (3600–2500 and 1710 cm^{-1}) of the polymer micelles attenuated to 10–30% of their original intensity, as amide I and II bands appeared at 1650 and 1540 cm^{-1}. Solid-state NMR experiments are in progress to determine the extent of amidation and the effects on the shell permeability. In contrast, upon formation of **2**, the carbonyl stretch of the carboxylic acid band disappeared in the IR spectrum, and only the amide carbonyl bands were observed. Strong absorption from C–O stretching of the ethylene oxide linkers was observed at 1108 cm^{-1} for both **1** and **2**. The compositions of the SCKs were quantitatively confirmed by elemental analysis.

Transmission electron microscopy (TEM) provided further evidence for structural differences between **1** and **2** (Fig. 1). The cores of **1** and **2** appear to be approximately the same diameter, however, the shell thicknesses are very different. SCK **1** has a distinct corona owing to the poly(ethylene oxide) linker [Fig. 1(*a*), the PEO coronas are the gray areas surrounding the brighter center cores], whereas SCK **2** has a sharp edge [Fig. 1(*b*)]. The average diameters of the nanospheres were determined in aqueous solution by dynamic light scattering (DLS)[8]



1: n = 75; **2**: n = 2

Scheme 1 Amidation chemistry used for crosslinking of poly(acrylic acid) groups located in the shell of Ps-*b*-PAA polymer micelles to form the SCK nanospheres. 1, $CH_3CH_2N{=}C{=}N(CH_2)_3N(CH_3)_3I$; 2, $H_2N(CH_2CH_2O)_n$-$CH_2CH_2NH_2$.

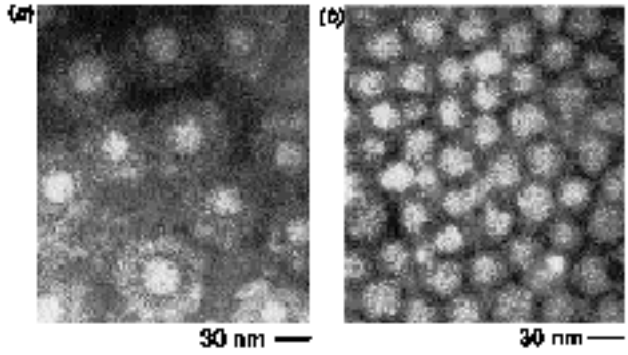


Fig. 1 Negatively stained transmission electron microscopy (TEM) images at 300 K magnification of the SCKs crosslinked by (*a*) PEO linkers to yield a thick hydrogel-like crosslinked shell surrounding and covalently bound to the PS core, and (*b*) tri(ethylene oxide) linkers to yield SCKs possessing a relatively thin shell of oligo(ethylene oxide)-crosslinked polyacrylamide. The TEM samples were prepared by dropping 1 : 1 mixtures of aqueous solutions of the SCK sample and uranyl acetate (2.5% solution), upon a carbon-coated copper grid and allowing to dry.

to be 100 ± 3 nm for **1** and 37 ± 2 nm for **2**. Based on the size of the precursor polymer micelles in the dry state (average diameter is 26 nm, core diameter is 22 nm and shell thickness is 2 nm, determined by TEM and AFM)[8] and the increment of volume added from the linkers, the theoretical[14] diameters of SCKs **1** and **2** in the compact dry state were calculated to be 56 and 29 nm, respectively. Because both **1** and **2** result from the same polymer micelle, with an average core diameter of 22 nm, the shell thicknesses in the compact dry state are calculated as 17 and 3.5 nm, respectively. The extent of swelling of the crosslinked hydrogel peripheral layer when placed within water was determined to be about twofold in thickness, corresponding to an approximate eightfold increase in shell volume. Considering the differences in shell thickness **1** experiences a fivefold increase in overall SCK volume, whereas only a twofold total volume increase occurs for **2**.

Differential scanning calorimetry (DSC) experiments were performed to deduce structural information for the dried samples of **1** and **2**. SCK **1** exhibits a melting transition (T_m) at 45 °C, which corresponds to the T_m of PEO,[15] however no T_g is observable for the PS core. This indicates that PEO in the shell exists as a phase separated crystalline domain, and that the PS content may be too low for detection by our DSC instrumentation. In contrast, a glass transition (T_g) for the PS core at 105 °C is evident for SCK **2**, but no detectable T_m or T_g corresponding to PEO is observed. These results support the compositional and structural differences between **1** and **2**.

The ethylene oxide surface layer covalently attached throughout the SCK shell provides a stable, steric barrier between the hydrophobic core and aqueous media. Poly(ethylene glycol)diamine incorporates a higher degree of ethylene oxide units than 2,2′-(ethylenedioxy)bis(ethylamine), which allows phase separation of the PEO, demonstration of behavior characteristic of pure PEO, formation of a thicker hydrogel-like exterior layer, and greater extents of overall nanoparticle swelling in water. The SCKs thus prepared have many enhanced features that should serve well when applied as artificial drug carriers. Investigations into protein binding for early *in vitro* screening of potential biocompatibility are in progress.

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14 The sizes of SCK particles of **1** shown by TEM cannot be taken as the compact dry-state diameters, owing to distortion of the PEO-containing corona of the nanospheres upon drying on the carbon-coated copper grid substrate. Therefore, the SCK size was predicted by adding the volume of the precursor micelle to the additional volume from the linkers (calculated from the product of moles of linker, linker molecular mass and linker density), followed by conversion to the diameter for a spherical SCK. The 29 nm calculated diameter of **2** agrees with the particle sizes observed in the TEM image.
15 T_m of PEO–$(NH_2)_2$ (M_w = 3400) is 45 °C.

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