Recent advances in shell cross-linked micelles

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Received (in Cambridge, UK) 25th January 2007, Accepted 15th February 2007
First published as an Advance Article on the web 20th March 2007
DOI: 10.1039/b701217a

The field of shell cross-linked (SCL) micelles is briefly reviewed. Important advances over the last two years are emphasized, potential application areas are discussed and current technical problems with these fascinating nanoparticles are highlighted. Particular attention is paid to (i) the development of new cross-linking chemistries and (ii) the adsorption of SCL micelles at interfaces.

Introduction

Supramolecular structures formed by the spontaneous self-assembly of AB diblock copolymers in selective solvents have been the focus of increasing academic interest over the last decade. The recent development of new so-called 'living radical polymerisation' techniques[1–7] has provided polymer chemists with powerful and versatile tools for the synthesis of a wide range of functional copolymers with controllable block lengths. The molecular dimensions influence the self-assembly and ultimately dictate the length scales of the colloidal aggregates, typically producing nanoparticles in the 10–50 nm range. It is usually difficult to obtain such small particles by either emulsion or dispersion polymerization, although this length scale can be accessed by microemulsion polymerization. Various morphologies such as micelles, cylinders, rods, vesicles and lamellae have been reported.[8,9] In addition, non-centrosymmetric cross-linked micelles (so-called 'Janus' micelles) have been reported by Müller and co-workers[10–14] but in this article we will pay particular attention to spherical 'core-shell' type micelles (Fig. 1).

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One fundamental problem with block copolymer micelles is their spontaneous dissociation at concentrations below their critical micelle concentration (CMC). However, in 1996 Wooley's group[15] reported that cross-linking micelle coronas (or shells) at high dilution led to the formation of robust nanoparticles known as Shell Cross-Linked (SCL) micelles (Fig. 2).[16] These new nanoparticles were prepared from an AB diblock copolymer based on hydrophobic polystyrene (PS) and hydrophilic 4-(chloromethyl)styrene-quaternized poly(4-vinylpyridine) (QP4VP). Shell cross-linking of the precursor micelles was achieved *via* radical oligomerisation of the pendent styrenyl groups on the coronal P4VP blocks in a THF–water mixture. Unlike conventional micelles, these new

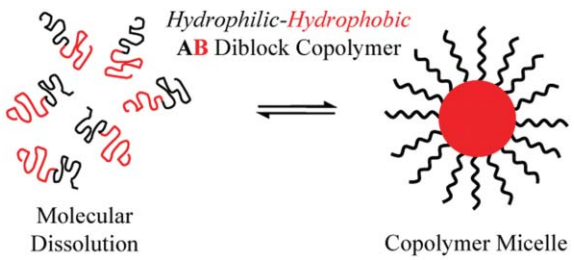

Fig. 1 Schematic representation of the self-assembly of amphiphilic diblock copolymers into core–shell micellar structures.

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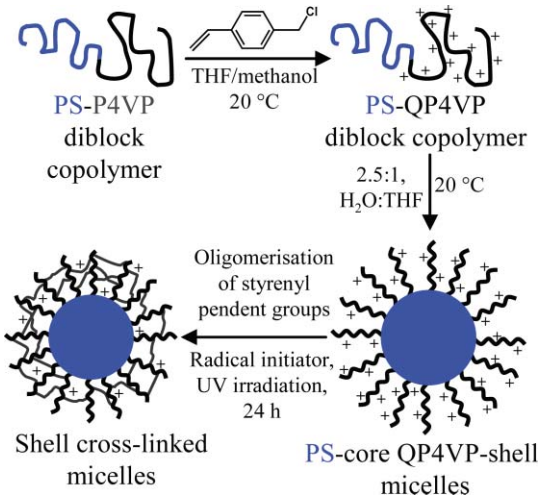

Fig. 2 Schematic illustration of the first example of shell cross-linked micelles from PS-QP4VP diblock copolymer micelles.[15]

covalently-stabilised SCL micelles were stable with respect to infinite dilution. In principle, cross-linking the micelle cores will also achieve this goal, but this alternative approach is beyond the scope for this article and the reader is referred to the literature for further information.[17–20]

Since this first example of SCL micelles, many other cross-linking strategies have been developed. Wooley's group have tended to favour carbodiimide-mediated cross-linking in their subsequent studies,[21–24] although their more recent work has utilized so-called 'Click' chemistry.[25] Alternatively, Ding and Liu reported the synthesis of SCL micelles from AB diblock and ABC triblock copolymers bearing photocross-linkable cinnamoyl groups.[26] Until recently, our own group favoured the use of bis(2-iodoethoxy)ethane (BIEE) and divinyl sulfone (DVS), which allow cross-linking *via* quaternization, esterification or Michael addition under mild conditions in aqueous solution. However, these reagents are mutagenic and less toxic alternatives are clearly desirable. Recently, we reported the successful use of polyelectrolyte complexation for physical (or ionic) cross-linking and we note that this approach has now been extended by McCormick and co-workers.[27,28] Another noteworthy general trend is the increased activity in SCL micelle syntheses in aqueous solution,[21,23,29,30] no doubt to take advantage of the potential biomedical benefits that these nanoparticles may offer for either drug delivery and/or diagnostic imaging.[31–34]

SCL micelles prepared from AB diblock copolymers are generally prepared at high dilution (typically below 0.5% solids) in order to avoid undesirable *inter-micellar* cross-linking, which inevitably results in micelle fusion. This limitation is clearly a major problem for the potential use of SCL micelles on an industrial scale. Our group has overcome this difficulty to produce well-defined SCL micelles at much higher copolymer concentrations by pioneering the use of ABC triblock copolymers, rather than AB diblock copolymers. To date, these copolymers have been typically prepared by atom transfer radical polymerization (ATRP) using a poly(ethylene oxide)-based (PEO) macro-initiator.[35] This PEO block acts as a steric stabiliser and ensures that cross-linking is confined to the inner shell (*i.e.* the B block) of the triblock copolymer micelles (Fig. 3), thus preventing inter-micelle fusion. Appropriate ABC triblocks typically comprise a permanently hydrophilic A block (*e.g.* PEO), a cross-linkable B block and a stimulus-responsive (*i.e.* tunably hydrophobic) core-forming C block. Depending on the nature of the C block, this strategy has been used to prepare SCL micelles with cores whose hydrophobicity can be tuned by varying either the solution pH[35] or temperature.[36,37]

One of the aims of this review article is a critical appraisal of the many cross-linking strategies described in the literature in terms of their commercial viability. The ideal cross-linking chemistry should be non-toxic, cost-effective, facile under mild conditions (*i.e.* preferably at ambient temperature and in aqueous solution), generate no small molecule by-products (to

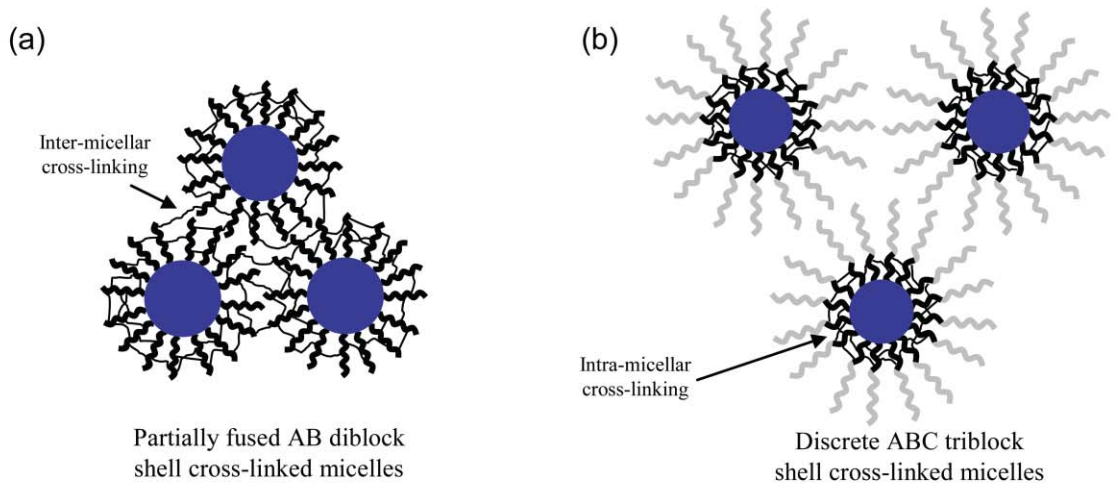

Fig. 3 Schematic representation of the inter-micellar and intra-micellar cross-linking for (a) AB diblock copolymer and (b) ABC triblock copolymer micelles at high copolymer concentrations (>1% solids).[35]

minimise purification) and also be potentially reversible. The ability to assess the degree of cross-linking (or at least monitor the progress of the cross-linking reaction) is also highly desirable. New cross-linking chemistries will be assessed in the light of these demanding, and perhaps conflicting, criteria. Promising applications for SCL micelles will also be briefly highlighted.

Radiation-induced cross-linking

In principle, the preparation of SCL micelles by radiation-induced cross-linking is very attractive, since there is no need for the addition of any small molecule cross-linkers. Covalent stabilisation typically occurs rapidly on irradiating reactive sites within the micellar shells. Ding and Liu were the first to exploit this method to cross-link the shells of a polystyrene-*block*-poly(2-cinnamoylethyl methacrylate) (PS-*b*-PCEMA) diblock copolymer micelles in a THF–acetonitrile mixture.[26] Efficient cross-linking occurred after 1–2 h exposure to UV light, which induces the cycloaddition of the cinnamoyl groups. The minimum extent of reaction required for successful micelle cross-linking was shown to be around 10%. This Canadian group went on to publish a series of elegant articles based on this approach.[19,38–42] Unfortunately, such copolymers are too hydrophobic to allow UV cross-linking to be conducted in aqueous solution. However, inspired by this earlier work, a Chinese group recently reported the successful uv-induced cross-linking of hydrophilic ABC triblock copolymers partially esterified with pendent cinnamoyl groups.[43] This required the synthesis of a poly(ethylene oxide)-*block*-poly(glycerol monomethacrylate)-*block*-poly(2-(diethylamino)ethyl methacrylate) (PEO-PGMA-PDEA) triblock copolymer *via* ATRP. Some of the pendent hydroxyl groups on the central PGMA block were then reacted with cinnamoyl chloride. Up to 50% mono-esterification could be achieved without compromising the water solubility of the functionalised copolymer. The resulting SCL micelles were pH-responsive due to the polybasic nature of the PDEA chains in the cores, which has a pK_a of around 7.3 (Fig. 4).

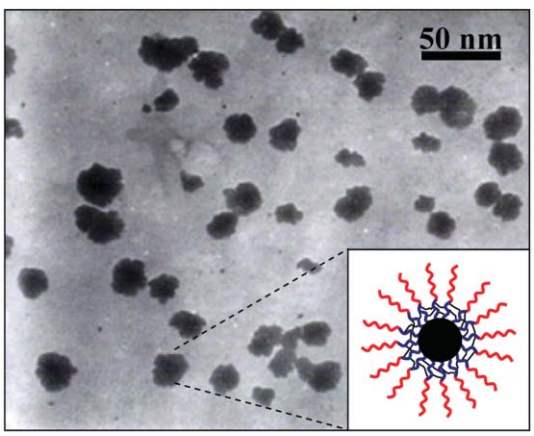

Fig. 4 Transmission electron micrograph and schematic representation of 50% cinnamoylated PEO_{113}-$PGMA_{50}$-$PDEA_{65}$ shell cross-linked micelles at pH 3.[43]

Reaction with bifunctional cross-linking agents

(a) Carbodiimide coupling

Wooley's group have used water-soluble diamines to cross-link poly(carboxylic acid) blocks in aqueous solution using carbodiimide coupling (Fig. 5(a)).[21,23,44–47] SCL micelles were typically prepared *via* a three-step procedure. First, a polystyrene-*block*-poly(*tert*-butyl acrylate) (PS-PtBuA) diblock copolymer precursor was prepared by either anionic polymerisation or ATRP, followed by acid hydrolysis of the *tert*-butyl groups to produce a polystyrene-*block*-poly(acrylic acid) (PS-PAA) diblock copolymer. This amphiphilic diblock copolymer was then dissolved in THF and micellisation was induced by the addition of water, which is a non-solvent for the PS block. Shell cross-linking of the PAA chains in the micelle coronas was achieved by activation of the carboxylic acid groups with a water-soluble carbodiimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide, followed by the addition of a 2,2′-(ethylenedioxy)bis(ethylamine) cross-linker. Unfortunately, this method not only requires expensive reagents but also suffers from time-consuming purification (*e.g.* dialysis) to remove small molecule by-products and THF co-solvent. However, one advantage is that typically only a fraction of the acrylic acid groups are required for efficient cross-linking, leaving the remainder available for subsequent functionalisation, see later.[24]

Fujii *et al.*[48] prepared a PEO-PGMA-PDEA triblock copolymer *via* one-pot ATRP and then esterified the hydroxyl groups on the central PGMA block using succinic anhydride to generate a polyacid block. This pH-responsive copolymer formed PDEA-core micelles in aqueous solution on adjusting the solution pH and the polyacid block was then cross-linked to produce SCL micelles. This route has the advantage that no protecting group chemistry is required and the SCL micelles are prepared in purely aqueous solution. Moreover, such SCL micelles also proved to be very interesting pH-responsive nanoparticles in the context of Pickering emulsions, see later.

Harrisson and Wooley group have prepared AB diblock and ABA triblock copolymers comprising styrene-*alt*-(maleic anhydride) and styrene in a 'one-pot' synthesis using reversible addition fragmentation chain transfer (RAFT) polymerization.[49] On addition of water, polystyrene-core micelles were formed and the anhydride groups in the coronal chains were then cross-linked using a diamine. In addition, carbodiimide coupling has also been reported for cross-linking peptide-containing diblock copolymer micelles from poly(butadiene-*block*-L-glutamic acid) (PB-PGA).[50]

(b) BIEE

In 1998 our group reported the synthesis of shell cross-linked micelles with tunable hydrophilic/hydrophobic cores.[29] Diblock copolymer micelles comprising partially quaternized poly(2-(dimethylamino)ethyl methacrylate-*block*-2-(*N*-(morpholino)ethyl methacrylate) (PDMA-PMEMA) was cross-linked using BIEE in aqueous solution at 60 °C (Fig. 5(b)). This bifunctional reagent selectively quaternised the unreacted tertiary amine groups on the PDMA blocks located in the micelle coronas, leaving the thermo-responsive core-forming

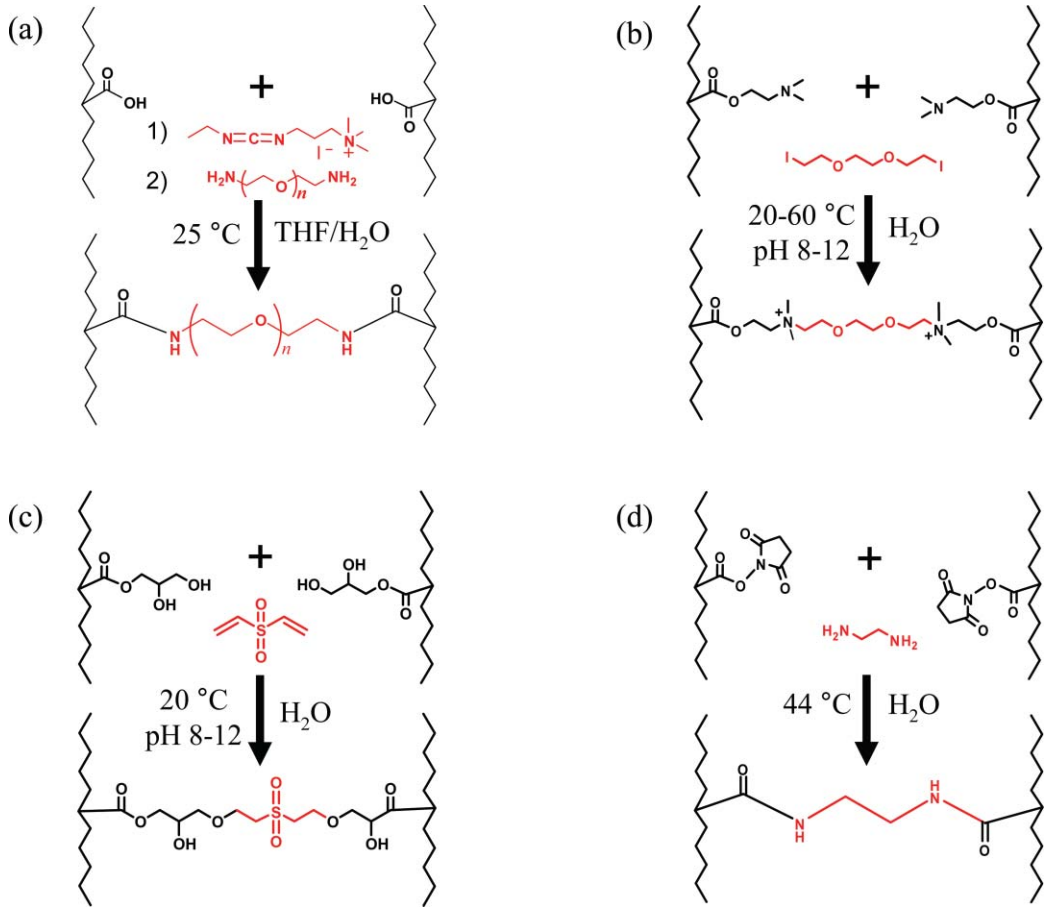

Fig. 5 Cross-linking chemistry of small molecule cross-linkers and appropriate monomers: (a) diamines in the presence of a carbodiimide catalyst,[21] (b) BIEE,[29] (c) DVS[30] and (d) activated esters.[61]

PMEMA block untouched. On cooling to 25 °C, the PMEMA chains pass through their lower critical solution temperature (LCST) and hence become rehydrated. These SCL micelles thus contain micelle cores that can be reversibly hydrated or dehydrated, depending on the solution temperature (and also the electrolyte concentration). This approach offers potential applications for the controlled uptake and release of actives. It is also worth emphasising that BIEE cross-linking leads to an *increase* in the hydrophilic character of the PDMA chains, which enhances the colloid stability of the SCL micelles. Most other cross-linking chemistries lead to a *reduction* in hydrophilic character, which means that relatively high degrees of cross-linking can sometimes compromise the colloid stability of the SCL micelles.

BIEE has also used for the preparation of zwitterionic SCL micelles.[51] A poly[2-(dimethylamino)ethyl methacrylate–2-tetrahydropyranyl methacrylate] (PDMA-PTHPMA) diblock copolymer precursor was synthesised by group transfer polymerisation (GTP) and subsequently converted to a zwitterionic poly[2-(dimethylamino)ethyl methacrylate-methacrylic acid] (DMA-PMAA) diblock by selective acid hydrolysis of the PTHPMA under mild conditions. Two types of SCL micelles could be prepared from this diblock precursor, depending on the *sequence* of reaction steps. **Type I** micelles comprising anionic PMAA cores and cationic PDMA coronas were formed by self-assembly of the PDMA-PTHPMA diblock precursor in an aqueous solution containing 5% THF co-solvent. This protocol produced well-defined micelles with hydrophobic PTHPMA cores.[52] The second step involved cross-linking the PDMA chains using BIEE at pH 10 and 25 °C prior to removal of the THPMA protecting groups *via* acid hydrolysis.[53,54] In contrast, **Type II** micelles were prepared by removing the THPMA groups first to yield the zwitterionic diblock copolymer. This copolymer underwent micellar self-assembly in aqueous alkaline solution at elevated temperature (*i.e.* above the LCST of the PDMA block) to form PDMA-core micelles with anionic PMAA coronas. Finally, shell cross-linking was achieved by reaction with BIEE at 60 °C. It is perhaps noteworthy that cross-linking in this latter case is achieved by esterification, rather than quaternisation. Moreover, the BIEE is also selective, since the rate of esterification of the PMAA blocks is significantly faster than the rate of quaternisation of the PDMA blocks. Thus the same reagent can be used for the formation of two types of zwitterionic SCL micelles using different aqueous cross-linking chemistries. These zwitterionic SCL micelles also possess isoelectric points (IEPs) and hence precipitate from aqueous solution at a certain critical pH.[51] This suggests the possible use of zwitterionic SCL micelles as readily isolable and recoverable nanoparticles.

As already mentioned, our group favours the preparation of SCL micelles from ABC triblock copolymers, rather than AB diblock copolymers, in order to avoid extensive inter-micellar cross-linking.[35] A 'proof-of-concept' route to well-defined SCL micelles in moderately concentrated copolymer solution (10% solids) was published in 2000. This involved using a poly[ethylene oxide-*block*-2-(dimethylamino)ethyl methacrylate-*block*-2-(*N*-morpholino)ethyl methacrylate] (PEO-PDMA-PMEMA) triblock copolymer synthesised by ATRP. Self-assembly into three-layer 'onion' micelles was achieved in aqueous solution at 20 °C on addition of Na_2SO_4, which selectively salted out the PMEMA block.[29] ^{1}H NMR spectroscopy studies conducted in D_2O indicated that the PMEMA chains formed the micelle cores, the PDMA blocks were located in the inner shells and the PEO blocks acted as the stabilising outer shells or coronas. DLS studies indicated an intensity-average micelle diameter of 68 nm. Shell cross-linking of the PDMA chains was achieved *via* quaternisation using BIEE.[29,55] Since water is a good solvent for the coronal block, PEO-PEO interactions are much less energetically favourable than PEO-water interactions. Thus although inter-micelle collisions are frequent due to Brownian motion, interpenetration between reacting micelles was minimised by a steric stabilization mechanism. To test this hypothesis, cross-linking was performed in the absence of the PEO block: macroscopic gelation was observed when attempting to cross-link the analogous PDMA-PMEMA diblock micelles. In contrast, essentially no inter-micelle fusion occurred during cross-linking of the PEO-PDMA-PMEMA micelles at 10% solids. We believe that this is an important result if SCL micelles are to realise their commercial potential.

In 2001 we reported[36] a facile, one-pot synthesis of thermo-responsive SCL micelles. A new poly[propylene oxide-block-2-(dimethylamino) ethyl methacrylate-*block*-methoxy-capped oligo(ethylene glycol) methacrylate] (PPO-PDMA-POEGMA) triblock copolymer was synthesized by bulk ATRP of DMA using a PPO macro-initiator, followed by aqueous ATRP of OEGMA after dilution of the reaction solution with water. *In situ* self-assembly was induced at 40 °C to produce PPO-core micelles (the LCST of PPO is around 15 °C). BIEE was then added to cross-link the PDMA chains. On lowering the temperature to 5 °C, DLS studies indicated that micelles were still present, as expected. The observed reduction in dimensions of the SCL micelles was attributed to dehydration of the PPO cores. Moreover, the spent ATRP catalyst was readily removed from the final SCL micelle reaction solution by silica chromatography.

Synthesis of ABC triblock copolymers *via* oxyanion-initiated polymerisation has also been employed to prepare SCL micelles *via* BIEE cross-linking.[56] A novel PEO-PDMA-PBAE [where PBAE = poly(*tert*-butylaminoethyl methacrylate)] triblock was prepared *via* sequential monomer addition using a PEO-based macro-initiator. This triblock self-assembled in aqueous solution to form three-layer 'onion' micelles. These SCL micelles could also be prepared at moderately high solids (up to 25%).

Model SCL micelles with pH-responsive cores were synthesised by Liu *et al.*[57] A PEO-PDMA-PDEA triblock copolymer was prepared *via* ATRP. At low pH, this triblock copolymer dissolved molecularly as single chains (also known as 'unimers'). Self-assembly to form PDEA-core micelles occurred above pH 7.3 and subsequent BIEE cross-linking for three days at ambient temperature led to robust SCL micelles, since no dissociation was observed at low pH. In principle, the abrupt change in core hydrophilicity that occurs between pH 7 and 8 should allow the pH-triggered release of hydrophobic drugs from such SCL micelles. The block copolymer composition and target degree of cross-linking had a significant effect on the pH-dependent (de)swelling of the SCL micelles. Longer PDEA blocks and lower target degrees of cross-linking led to increased degrees of swelling, as expected.[57]

Bell and co-workers have reported using BIEE to cross-link the PDMA coronas of poly(solketal methacrylate)-*block*-poly(2-(dimethylamino)ethyl methacrylate) (PSMA-PDMA) micelles.[58] Following cross-linking in a 95 : 5 water–THF mixture, the PSMA micelle cores were selectively hydrolyzed under acidic conditions to produce hydrophilic poly(glycerol monomethacrylate) (PGMA) chains. The resulting 'nanocages' have no hydrophobic component: their colloidal dimensions are retained solely due to the cross-linked PDMA coronas. In 2006 Bütün *et al.* synthesised novel 'schizophrenic' PDEA-PDMA-PMEMA triblock copolymers by group transfer polymerisation (GTP).[59] These water-soluble copolymers formed PMEMA-core micelles at pH 6.5 and PDEA-core micelles at pH 7.6. In both cases the PDMA chains located in the inner shells could be selectively cross-linked with BIEE to 'lock in' the micellar structures.[57] Unfortunately, BIEE is most likely too toxic to be employed for biomedical applications of SCL micelles.

(c) DVS

This reagent has been used to covalently cross-link pH-responsive SCL micelles (Fig. 5(c))[30] formed from hydroxyl-functional copolymers such as either poly(ethylene oxide)-*block*-glycerol monomethacrylate)-*block*-2-(diethylamino)ethyl methacrylate (PEO-PGMA-PDEA) or poly(ethylene oxide)-*block*-2-hydroxyethyl methacrylate-*block*-2-(diethylamino)-ethyl methacrylate (PEO-PHEMA-PDEA). This cross-linking is an example of Michael addition and hence in principle it does not generate any small molecule by-products. However, DVS is slowly hydrolysed at neutral pH (and more rapidly in alkaline solution), which generates unwanted side-products.[30] Although cross-linking of the PEO-PGMA-PDEA chains is reasonably efficient, the reaction of DVS with the PHEMA-based copolymer was much less effective since a large excess of DVS was required to form SCL micelles. One practical advantage of DVS is that its incorporation (and hence extent of reaction) can be assessed by sulfur microanalyses. DVS has also been used to cross-link another ABC-type triblock copolymer, poly[2-(methacryloyloxy)ethyl phosphoryl choline-*block*-glycerol monomethacrylate-*block*-2-(diethylamino)ethyl methacrylate (PMPC-PGMA-PDEA), which was synthesised *via* methanolic ATRP.[30] This triblock dissolves molecularly at pH 2 and micellar self-assembly occurs above approximately pH 7, with the formation of PDEA-core onion micelles. Cross-linking of the hydroxyl groups of the central GMA block was

achieved at ambient temperature by reaction with DVS for 2–3 h at pH 12. The same triblock copolymer also formed PMPC-core micelles in a 4:1 THF/methanol mixture; these 'inverted' micelles could also be cross-linked with DVS. Using sulfur as a unique elemental marker for DVS, X-ray photoelectron spectroscopy (XPS) studies[30] were conducted on these two types of SCL micelles in order to examine the extent to which the core–shell morphology of SCL micelles is retained in the solid state. The typical XPS sampling depth is 2–10 nm, hence this technique is relatively sensitive to the coronal chains of the SCL micelles, rather than the chains located in the micelle cores. Reasonable evidence was obtained for the partial retention of the original core–shell morphology in the absence of solvent.

(d) Glutaraldehyde

Lecommandoux and co-workers synthesised a polypeptide-based diblock copolymer,[50] poly(isoprene-*block*-L-lysine) (PI-PLys), which was self-assembled into PI-core micelles at neutral pH. Glutaraldehyde was added to this aqueous micellar solution to cross-link the primary amines within the PLys block. Conductivity measurements were used to monitor the rate of imine formation and also estimate a mean degree of cross-linking.

(e) Activated esters

Reversible addition-fragmentation chain transfer (RAFT)[60] polymerisation has been used to prepare a thermo-responsive triblock copolymer.[61] A PEO-based chain transfer agent (macro-CTA) was used to statistically copolymerise *N*,*N*′-dimethylacrylamide (DMAA) with *N*-acryloxysuccinimide (NAS). The resulting macro-CTA was then used to polymerise NIPAM to produce an ABC-type triblock copolymer. Above the LCST of the PNIPAM chains, PNIPAM-core micelles were formed in aqueous solution, as expected. The PDMAA-*stat*-NAS statistical block was then cross-linked in situ using ethylenediamine at an ethylenediamine : NAS molar ratio of 0.50 : 1 (Fig. 5(d)). This appears to be the first example of the use of an activated ester (NAS) to form SCL micelles. The cross-linking reaction was monitored *via* ^{1}H NMR by following the formation of a soluble by-product. At solution temperatures below the LCST of the PNIPAM chains, the resulting SCL micelle cores become solvated and therefore swollen, with DLS studies indicating an increase in size from around 38 to 72 nm depending on the target degree of cross-linking.

(f) Disulfide-based cross-linkers

Thermo-responsive PEO-(PDMAA-*stat*-NAS)-PNIPAM micelles[61] with PNIPAM cores have also been cross-linked at 45 °C using cystamine.[62] Subsequent cleavage of the disulfide bonds within the cystamine-based cross-links using either tris(2-carboxyethyl)phosphine (TCEP) or dithiothreitol (DTT) caused dissociation of these SCL micelles to produce molecularly dissolved triblock copolymer chains. However, further addition of excess cystamine induces cross-linking of the micelles *via* thiol–disulfide exchange. This 'reversible cross-linking' concept might prove to be useful for drug delivery applications, since the degraded copolymer chains can be excreted from the body *via* the kidneys, whereas in principle the much higher molar mass SCL micelles should be retained. Control over the rate of release of a model cardio-vascular drug, dipyridamole (DIP), from these SCL micelles was also demonstrated by the same research team, see later.

Metal-catalysed cross-linking

A polystyrene-*block*-poly(neopentyl-*p*-styrene-*co*-*p*-(1-methylsilacyclobutyl)styrene)-*block*-poly(*p*-styrenesulfonate) (PSt-P(St-SBS)-PSSPen) triblock copolymer precursor was synthesised *via* nitroxide-mediated living radical polymerisation.[63] This copolymer formed micelles in cyclohexane comprising PSSPen cores, PSt outer shells and St-SBS inner shells. Cross-linking of these micelles was achieved by Pt-catalysed ring-opening of the silacyclobutyl groups on SBS. The PSSPen block were then converted to sulfonic acids (PSS) *via* thermolysis. Core cross-linked micelles have also been prepared using this chemistry.[64] However, given the cost and toxicity of the Pt catalyst, the synthetic utility of this approach seems to be rather limited.

Click cross-linking

A novel cross-linking strategy based on Click chemistry was reported by Wooley and co-workers in 2005.[25] More specifically, acetylene groups present in the shells of PAA-PS diblock copolymer micelles were cross-linked with azide-functionalised dendrimers (Fig. 6).[25] Unreacted azide termini on the cross-linked dendrimer were used for the subsequent addition of fluorescent labels, which are useful for confocal microscopy studies. Carbodiimide-mediated coupling was used to introduce the acetylene groups into the PAA micelle coronas.[65] It was found that only first generation dendrimers were useful for cross-linking; attempts to cross-link with second and third generation dendrimers failed. Evidence for successful Click cross-linking was provided by ^{1}H NMR spectroscopy: a new proton signal corresponding to the triazole groups formed by the acetylide-azide reaction was observed at 7.6 ppm. In principle, UV spectroscopy could also be used to monitor the Click cross-linking *via* the triazole chromophore, although this approach has not yet been reported.

One major problem with all the literature data on SCL micelles is that it is very difficult to assess the *actual* degree of cross-linking achieved for these nanoparticles. The *target* degree of cross-linking is readily calculated from the molar ratios of the reagents but, even if complete reaction of a small molecule cross-linker occurs, the actual degree of cross-linking is almost certainly less than that targeted. Consider the example of BIEE being used to quaternise PDMA chains within a micelle. It is quite possible for all of the BIEE to react with the tertiary amine groups. However, there is no guarantee that each BIEE molecule produces an *inter*-chain cross-link, since the possibility of *intra*-chain (*i.e.* both iodoalkyl groups reacting with the *same* PDMA chain to form a 'loop') reaction cannot be excluded. In reality, it is also quite possible that one iodoalkyl end of the BIEE may react, while the other end does not react (or, perhaps more likely, this second iodoalkyl end is

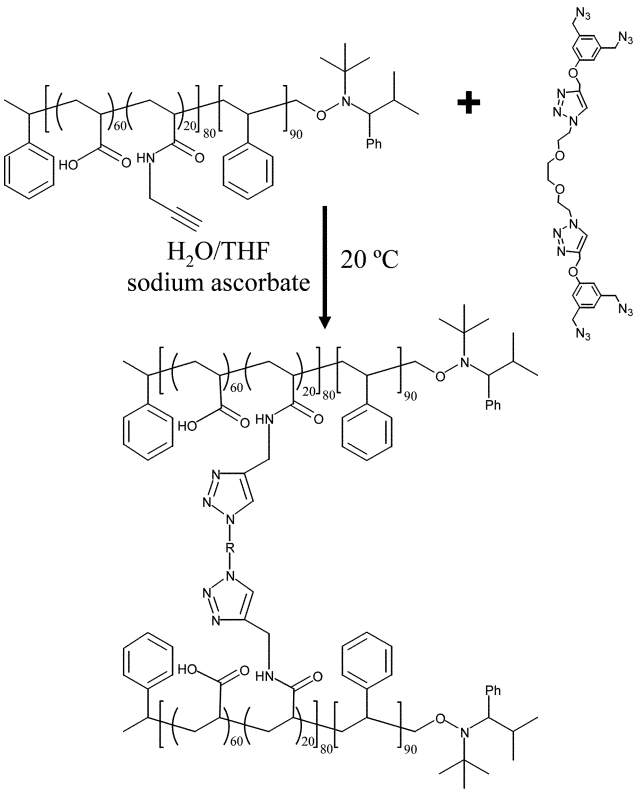

Fig. 6 Click cross-linking of neighbouring alkyne functional groups to a bifunctional azide dendrimer (R represents dendritic functional groups).[25]

hydrolysed before it can quaternise a second tertiary amine group). In the case of BIEE, ^{1}H NMR spectroscopy can be used to estimate the mean degree of quaternisation, but this value can only be taken as an upper limit for the actual degree of cross-linking. As far as we are aware, this fundamental uncertainty in determining the actual degree of cross-linking is generic: it applies not only to SCL micelles but also to most, if not all, other examples of gelation.

Cross-linking *via* polyelectrolyte complexation

In 2004, our group developed a new method of cross-linking based on polyelectrolyte complexation.[27] Complexation of oppositely-charged polyelectrolytes is very well-documented, as recently exemplified by layer-by-layer deposition studies[66] and also by DNA condensation.[67] Polyelectrolyte complexation offers many advantages over other cross-linking methods: (1) most polyelectrolytes exhibit low toxicity; (2) physical cross-linking is relatively fast and should ensure that there is no chemical modification of guest molecules; (3) apart from the counter-ions that are released, no small-molecule by-products are formed so purification is straightforward; (4) in principle, such 'ionic' cross-linking can be reversed on addition of salt.

First, a cationic ABC triblock copolymer, poly(ethylene oxide)-*block*-2-(dimethylamino)ethyl methacrylate-*block*-2-(diethylamino)ethyl methacrylate (PEO-PDMA-PDEA), was synthesised by ATRP. The PDMA block was then selectively quaternised using methyl iodide in THF to create a permanently cationic central block. DLS studies confirmed

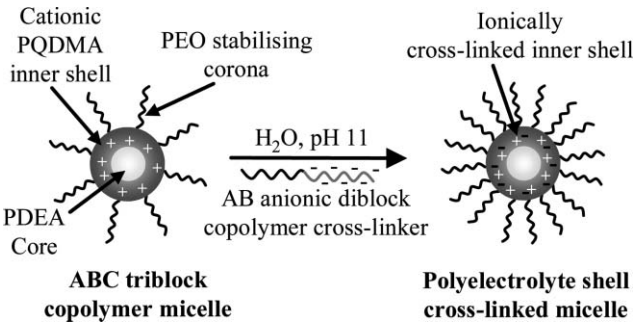

Fig. 7 Schematic representation of shell cross-linked micelles *via* polyelectrolyte complexation between and an anionic AB diblock copolymer cross-linker and cationic ABC triblock copolymer micelles.[27]

that this triblock was molecularly dissolved at low pH, but above pH 7 it formed PDEA-core micelles. Ionic cross-linking was initially attempted at high pH using an anionic poly(sodium 4-styrenesulfonate) (PNaStS) homopolymer. However, this cross-linking was unsuccessful: the large increase in size indicated by DLS indicated significant inter-micelle bridging flocculation.

Thus an alternative PEO-PNaStS *diblock copolymer* was evaluated as a cross-linker (Fig. 7). The PEO chains of this cross-linker reinforced the micelle coronas and, as a result, bridging flocculation was suppressed (see Fig. 7). Unlike the non-cross-linked precursor micelles, these ionically cross-linked micelles were stable with respect to dilution at pH 2. Moreover, the ionically cross-linked micelles proved to be surprisingly salt-resistant: up to 1.0 M NaCl was required to induce micelle dissociation at pH 3.3. However, it is worth noting that *excess* anionic cross-linker was required for robust cross-linking, hence additional complexation with the protonated PDEA cores occurred at pH 2. Current work in our group seeks to overcome this problem. One possible solution has been reported by McCormick's group, who have succeeded in preparing SCL micelles with PNIPAM-based thermoresponsive cores using a *homopolyelectrolyte* cross-linker.[28] In this study, a triblock copolymer, poly(*N*,*N*-dimethylacrylamide)-*block*-(*N*-acryloylalanine)-*block*-poly(*N*-isopropylacrylamide) (PDMAA-PAAL-PNIPAM) was first prepared by RAFT chemistry. Above the LCST of the PNIPAM chains, this triblock copolymer formed PNIPAM-core micelles. Successful ionic cross-linking was achieved by addition of a cationic homopolymer poly[(ar-vinylbenzyl)trimethylammonium chloride] (PVBTAC). It was also shown that cross-linking was reversible, since micelle dissociation occurred on addition of 0.4 M NaCl. In addition, the same group have also recently reported the preparation of block copolymer vesicles *via* polyelectrolyte complexation.[68]

Adsorption of SCL micelles at interfaces

Ma and Wooley have reported the preparation of SCL micelles from a poly(acrylic acid)-*block*-poly(methyl acrylate) (PAA-PMA) prepared *via* ATRP using *tert*-butyl acrylate as the protected monomer for the PAA block.[69] The amphiphilic diblock copolymer obtained after selective deprotection using

anhydrous trifluoroacetic acid was cross-linked *via* carbodiimide chemistry, as already discussed.[21] AFM studies confirmed that these SCL micelles formed 2D arrays with long-range order when deposited onto mica from aqueous solution.[70] It was shown that this long-range order could be tuned by varying the micelle diameter and the micelle charge density. AFM height-image measurements indicated that some flattening/spreading occurred when these SCL micelles were allowed to dry on solid substrates. A similar phenomena was observed with PCL-PAA [where PCL = poly(ε-caprolactone)] SCL micelles,[46] but in this case flattening could be controlled by varying the temperature due to the semi-crystalline nature of the PCL core.[71] Below the melting temperature (T_m) of the PCL block, disk-like shapes were observed due to the rigid crystalline core. Raising the temperature above the T_m resulted in a reversible reduction in height as judged by AFM. In contrast, when dispersed in solution, disks were again observed below the T_m but spheres were obtained above the T_m, as judged by angular-dependent DLS studies. This last observation was attributed to the molten fluid-like core adapting to the surrounding environment. A similar study was conducted on PI-PAA SCL micelles.[72] The PI cores have a relatively low glass transition temperature (T_g) of −63 °C. Thus these SCL micelles have hydrophobic fluid-like cores at ambient temperature, whereas significant deformation occurred after adsorption onto mica. However, if the PI block was reacted with HCl (with up to 90% hydrochlorination being achieved according to ^{1}H NMR), the T_g of the micelle cores significantly increased to 33 °C. Thus deposition of these HCl-treated SCL micelles onto mica at 22 °C initially resulted in negligible deformation of their spherical morphology due to the glassy nature of these cores. Raising the temperature to above the T_g resulted in significant flattening of the adsorbed SCL micelles. It was also shown that core cross-linking prevented shape deformation, since only spherical morphologies were observed above and below the T_g.

Wooley and co-workers used confocal microscopy to show that SCL micelles adsorb onto a mineral surface (halite).[73] The SCL micelles acted as a model for organic-based 'protocellular material' and were excluded (rather than incorporated) from the halite during its crystallization from hot brine. Remarkably, these results were claimed to shed some light on the origin of life.

We recently prepared novel PPO-PDMA-PGMA triblock copolymers in a one-pot synthesis by ATRP.[37] This triblock copolymer displayed a critical micellisation temperature (CMT) at approximately 12 °C. Above this CMT, PPO-core micelles were formed. Variation of the solution temperature at which the micelles were cross-linked using BIEE allowed the SCL micelle diameter to be varied from 30 to 70 nm. The resulting *cationic* SCL micelles were then electrostatically adsorbed onto near-monodisperse silica particles, which served as a model *anionic* colloidal substrate. Scanning electron microscopy (SEM) images confirmed that the SCL micelles uniformly decorated the surface of the silica sol and aqueous electrophoresis studies indicated that surface charge reversal had occurred, as expected (Fig. 8). These hydroxy-functional SCL micelles share some similarities with dendrimers in that they have large numbers of surface functional groups available for further reactions. As their synthesis is far more facile, SCL micelles conceivably offer a practical alternative to dendrimers if multifunctional nanoparticles are desired. The closely-related PPO-PGMA-PDMA triblock copolymers were also synthesised by ATRP and subsequently cross-linked with DVS.[74] These SCL micelles could also be used to modify silica surfaces.

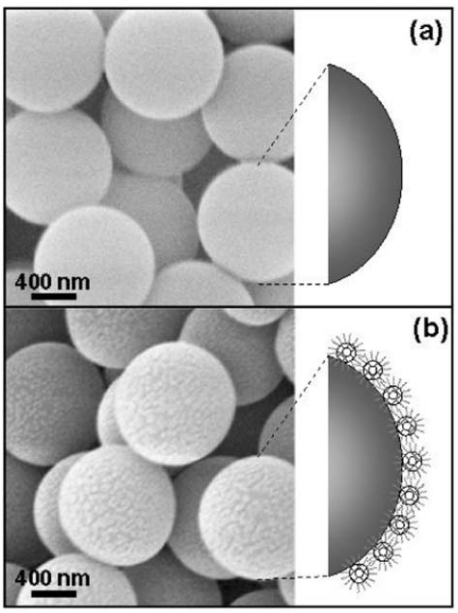

Fig. 8 Scanning electron micrographs and schematic representations of (a) bare Monospher 1000 nm silica particles and (b) the same silica particles decorated with shell cross-linked micelles (2.5 wt% micelle loading, from thermogravimetric analyses).[37]

Our group has recently shown that pH-responsive SCL micelles can be adsorbed at the oil/water interface and hence act as stimulus-responsive 'Pickering'-type emulsifiers.[48,75] A PEO-PGMA-PDEA triblock copolymer precursor was synthesized *via* sequential monomer addition using ATRP in methanol at 20 °C. The central PGMA block was then esterified using excess succinic anhydride (SA) to give a zwitterionic PEO-PSAGMA-PDEA triblock. Near-monodisperse PDEA-core micelles were formed by this copolymer at pH 9 with anionic inner shells and PEO outer shells. Ambient temperature cross-linking of the anionic SAGMA chains was achieved using carbodiimide chemistry by addition of 2,2′-(ethylenedioxy)bis(ethylamine) in the presence of 1,3-(dimethylamino)propyl)-3-ethylcarbodiimide at pH 9. The resulting SCL micelles were colloidally stable from pH 2 to pH 9, swelling slightly at low pH due to protonation of the PDEA cores.

Aqueous dispersions of SCL micelles were homogenised using an equal volume of 1-undecanol as a model polar oil. A stable oil-in-water emulsion was obtained at pH 8–9, as confirmed by conductivity measurements and the standard drop test. Light diffraction and optical microscopy studies indicated spherical, polydisperse 1-undecanol droplets with a mean diameter of 18 μm (Fig. 9). Lowering the solution pH of this emulsion to approximately pH 3 with aqueous HCl resulted in macroscopic phase separation due to desorption of

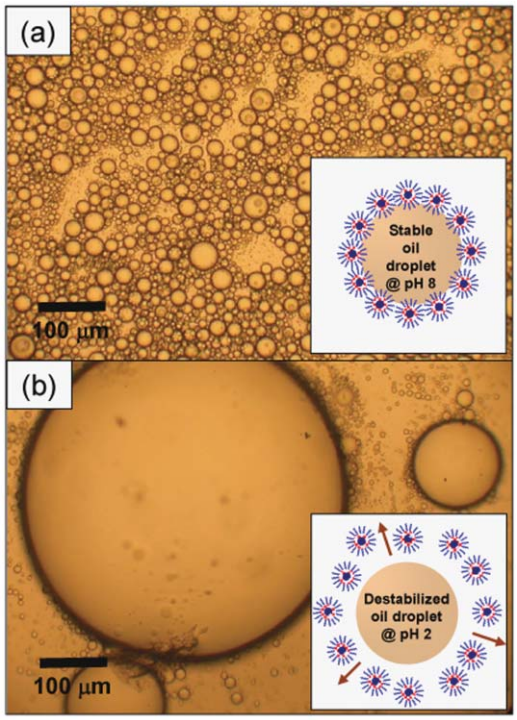

Fig. 9 Optical micrograph showing (a) 1-undecanol-in-water emulsions prepared at pH 9 using 100% shell cross-linked micelle at 20 °C and (b) rapid demulsification after one droplet of 1 M aqueous HCl solution was added to the emulsion on the microscope slide.[48]

the SCL micelles from the surface of the oil droplets. This system is reversible: rehomogenisation resulted in oil droplets being formed with a similar mean diameter. It is also worth emphasising that our control experiments showed that *non-cross-linked* micelles were much less effective than the SCL micelles, since only partial demulsification (60%) occurred on addition of acid in the former case. This was the first report of the use of SCL micelles as pH-responsive particulate emulsifiers, although this particular application may not be commercially viable given their relatively high cost compared to other 'smart' particles such as nanocomposite microgels.[76–79]

Encapsulation

In principle, the cores of SCL micelles are well suited for the uptake and release of hydrophobic drugs for drug delivery applications; the possible sequestration of metabolites has also been suggested.[32,34,47,80] In collaboration with Schaeffer and co-workers, Wooley's group has demonstrated that carbodiimide cross-linked PS-PAA SCL micelles can be loaded with 6-fluorotryptophan (6-FT), which was shown to be distributed throughout the micelle structure by $^{13}C\{^{19}F\}$ rotational-echo double resonance (REDOR) solid-state NMR studies.[81,82] It was found that the relatively hydrophilic 6FT marker was located exclusively within 10 Å of the interface between the micelle cores and the cross-linked shells; there was no spectroscopic evidence for this species within the micelle cores. The same group also used this REDOR technique to examine the spatial location of cholic acid sequestered by SCL micelles, with similar results.[32] In contrast, a hydrophobic guest, 4-(trifluoromethyl)benzophenone, was located within the micelle cores, albeit close to the core–shell interface.[47]

Wooley's group also performed an elegant analysis of he controlled release of hydrophobic polystyrene chains from SCL micelle cores.[83] The key to this pioneering study was the use of a nitroxide-based initiator to prepare a PS-PAA precursor that contained a labile C–ON bond between the PS and PAA chains. Thus after preparing carbodiimide cross-linked SCL micelles from this diblock copolymer precursor,[21] selective thermolytic cleavage of this C–ON bond at 125 °C led to the detachment of the PS chains within the micelle cores, The diffusion of these PS chains through the cross-linked PAA shells was monitored by gel permeation chromatography (GPC). Higher target degrees of cross-linking significantly hindered the release of the PS chains: this appears to be essentially a 'mesh size' effect (Fig. 10). However, even nominally 100% cross-linked SCL micelles were unable to prevent the partial permeation of PS chains of 14 100 g mol^{-1}. Thus these experiments suggest that shell cross-linking is unlikely to offer a useful barrier to the diffusional release of small hydrophobic molecules (most drug entities have molecular weights below 1000 g mol^{-1}), but may well be useful for the delivery of higher molecular weight species such as proteins or DNA.

It has been claimed that their amphiphilic character may allow SCL micelles to mimic the behaviour of lipoproteins, viruses and globular proteins. For example, an analogy has been made between histone core proteins and SCL micelles in the context of DNA packaging.[80] It is well known that cells can condense long strands of DNA *via* electrostatic complexation. In this study, the SCL micelles comprised PS cores and cross-linked quaternized PVP shells[15] and were described as acting like 'synthetic cells'. AFM and DLS techniques

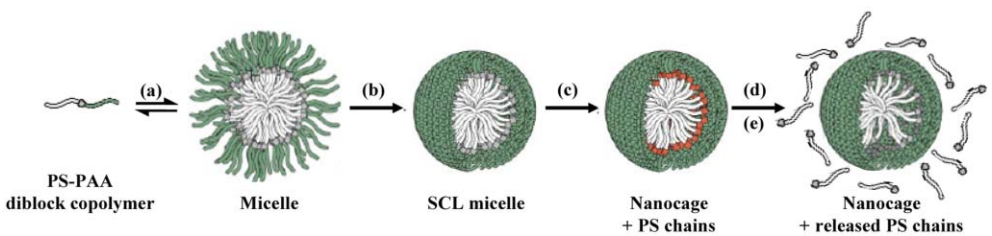

Fig. 10 Synthetic approach for the preparation of SCL micelles followed by the subsequent release of core polymer chains after the cleavage of C–ON bonds present at the core–shell interface (a) self-assembly of poly(styrene)-*block*-poly(acrylic acid) diblock copolymers into micelles, (b) cross-linking *via* carbodiimide chemistry,[21] (c) thermolysis of C–ON bonds (125 °C, 24 h in water), (d) lyophilization and (e) resuspension in THF. Reproduced from ref. 83.

confirmed that these cationic SCL micelles could condense DNA and protect this genetic material with respect to enzymatic digestion. DNA release was also demonstrated, indicating potential gene therapy applications.

The preparation of reversibly cross-linked thermo-responsive SCL micelles has been reported.[62] A PEO-P(DMA-*stat*-NAS)-NIPAM triblock was synthesized *via* RAFT, as already discussed.[61] Lowering the solution temperature induced the release of dipyridamole (DIP) from these SCL micelles and higher degrees of cross-linking retarded the rate of release of DIP compared to non-cross-linked micelles. Thus this study appears to contradict the earlier model studies by Wooley's group, although a direct comparison is difficult since the actual degrees of cross-linking achieved in each case are not known. It is also noteworthy that the addition of TCEP or DTT led to disulfide bond cleavage, and hence micelle dissociation, which also increased the rate of DIP release (Fig. 11). In principle, the *in situ* cleavage of these SCL micelles could occur *via* glutathione-induced disulfide cleavage, since this naturally-occurring oligopeptide is found in human cells at up to millimolar concentrations.[84] This could provide a mechanism to allow the renal excretion of the degraded (and dissociated) SCL micelles after drug delivery.

Hollow SCL micelles

Ozonolytic,[42,85] hydrolytic[46] and photochemical degradation[86] of the hydrophobic cores of SCL micelles has enabled the preparation of hollow nanospheres or so-called 'nanocages'. In some respects, these 'nanocages' can be considered nano-sized analogues of (cross-linked) block copolymer vesicles[87,88] and they show some potential for uptake/release applications. Both Wooley and co-workers and Liu's group have increased the inner volume of SCL micelles by 'excavating' the core chains (Fig. 12).[42,85] In both cases the SCL micelle cores comprised polyisoprene chains. On exposure to ozone, the double bonds within the polyisoprene backbone underwent oxidative scission to form ozonides, prior to reduction to produce either ketone or carbonyl groups and removal of small-molecule by-products *via* dialysis. Liu's group synthesised a novel

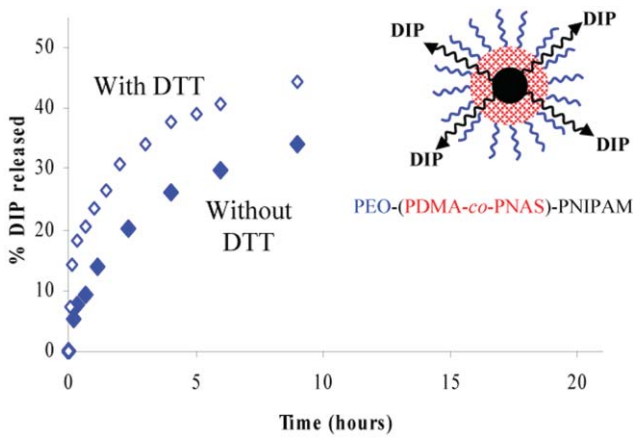

Fig. 11 Cumulative dipyridamole (DIP) release from PEO-(PDMA-*co*-PNAS)-PDPA shell cross-linked micelles (see inset) in the presence or absence of dithiothreitol (DTT) at 37 °C.[62]

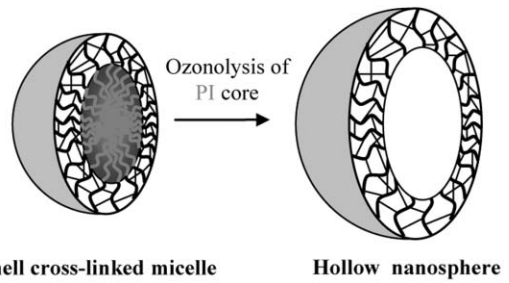

Fig. 12 Schematic representation of hollow nanospheres or 'nanocages' obtained after ozonolysis of the cores of shell cross-linked micelles.[42,85]

triblock copolymer, poly-[isoprene-*block*-poly(2-cinammoyl-ethyl methacrylate)-*block*-poly(*tert*-butyl acrylate)] (PI-PCEMA-P*t*BA),[42] and photocross-linked the PCEMA coronal chains of these PI-core micelles.[17,19,26,89] These hollow nanospheres might be useful as nanoreactors, but their hydrophobic character precludes their use for drug delivery applications. It should be noted that the same research group also used the same technique to form cross-linked vesicles that became water-dispersible on conversion of the polyisoprene chains to poly(2,3-dihydroxyl-2-methylbutane) chains.[38,90]

Wooley's group prepared nanocages[85] using water-soluble PI-PAA diblock copolymers that had been cross-linked by carbodiimide coupling.[21] After ozonolysis,[85,91] DLS studies confirmed a significant increase in size, from around 27 nm to 133 nm. Presumably, this swelling is due to the ingress of water: the 'nanocages' acquire hydrogel character after removal of the hydrophobic core chains. Obviously, removal of the hydrophobic cores of SCL micelles also destroys their amphiphilic character, hence the resulting 'nanocages' have no affinity for hydrophobic guest molecules, although a hydrophobic component can be re-introduced by covalent attachment of phosphatidylethanolamine-based lipids using Schiff-base chemistry.[92] DLS studies confirmed that the resulting lipid-functionalised nanoparticles were around 32 nm, which was smaller than the original nanocages (45 nm). Use of a 7-nitrobenz-2-oxa-1,3-diazole (NBD) labelled lipid proved that these lipid-linked nanospheres had hydrophobic cores. A dye assay based on visible absorption spectroscopy confirmed that the lipid-functionalised nanoparticles had a greater dye capacity than the original SCL micelles, with the hydrophilic 'nanocages' taking up the least dye, as expected.[92] Similar results were obtained with the anti-metabolite methotrexate.

Sakurai's group developed another method for core removal,[86] which is much milder than ozonolysis. The polysilane cores of carbodiimide cross-linked poly(1,1-dimethyl-2,2-dihexyldisilene)-*block*-poly(methacrylic acid) (PMHS-PMAA)[93] micelles were removed by exposure to UV irradiation. Chain scission was illustrated by UV absorption spectroscopy by a continuous blue shift during irradiation. The final hollow 'nanocages' had an intensity-average diameter of 650 nm, which was more than three times larger than the original SCL micelles. This indicated a high degree of swelling for the hydrophilic cross-linked poly(methacrylic acid) layer, which could be used to encapsulate a water-soluble fluorescent dye.

Core removal was also achieved under mild conditions using a biodegradable polyester, poly(ε-caprolactone) (PCL), by Zhang *et al.*[46] Ring-opening polymerisation (ROP) of ε-caprolactone was followed by the ATRP of *tert*-butyl acrylate and subsequent deprotection of the *tert*-butyl groups to give a PCL-PAA diblock copolymer. Following micellar self-assembly, shell cross-linking was achieved by amidation chemistry[21] and degradation of the PCL cores was carried out by selective hydrolysis. The amide cross-links in the shell are more hydrolytically stable than the ester linkages of the PCL, which enabled selective degradation of the PCL at pH 12. Hydrolytic degradation was followed by ^{1}H NMR over a two-week period by monitoring the appearance of a new signal due to a small molecule degradation product, sodium 6-hydroxylhexanoate. In principle, the cores of these SCL micelles should be hydrolytically degraded at physiological pH over longer time scales, which may allow slow release applications. However, diffusional release of a small molecule payload is likely to occur over much shorter time scales and it is not clear whether removal of the core-forming chains would actually aid renal excretion of the 'nanocages' after their use.

One limitation of such routes to 'nanocages' is that chemical degradation of the core-forming PI or PCL chains of SCL micelles is, like protecting group chemistry, inherently atom-inefficient. In principle, a more attractive strategy is to prepare SCL micelles using stimulus-responsive core-forming polymer chains. This route was pioneered by our research group using pH-responsive polymers such as PDEA and has been extended to include thermo-responsive polymers such as PDMA, PMEMA, poly(propylene oxide) and poly(*N*-isopropylacrylamide) by both ourselves[29,35–37,51,74] and McCormick's group.[28,61,62] The advantage of this alternative approach is that the core-forming chains can be rendered either hydrophilic or hydrophobic depending on the solution pH or temperature and that this physical (rather than chemical) transformation is reversible. Although we have no direct evidence, we believe that when the core-forming chains become hydrophilic, they mingle with the cross-linked coronal chains, thus creating a temporary 'nanocage'.

The formation of hollow 'nanocages' can also be achieved without the use of diblock copolymers.[94,95] In this case non-covalently connected micelles are formed by interpolymer complexation of a binary homopolymer/copolymer pair; there are no chemical bonds between the core and shell. For example, poly(styrene-*co*-[*p*-(1,1,1,3,3,3-hexafluoro-2-hydroxylpropyl)-α-methylstyrene]] (PS-OH) forms a hydrogen bonded complex with poly(4-vinylpyridine) (P4VP). Micelle formation was achieved by first solubilising this binary pair in $CHCl_3$, which is a good solvent for both components. Then nitromethane was added, which is a non-solvent for PS-OH. In the absence of P4VP, precipitation of PS-OH was observed. However, in the presence of P4VP, DLS studies indicated the formation of stable aggregates of 138 nm, with no evidence for any precipitation. These PS-OH aggregates were stabilized by P4VP due to hydrogen bonding between the vinylpyridine rings and the pendent acidic hydroxyl groups on the PS-OH. These micelles were then cross-linked with 1,4-dibromobutane, which quaternises the P4VP chains, followed by addition of DMF. In the absence of any cross-linking both polymers should become solubilised. In contrast, after cross-linking only the PS-OH core became soluble, producing a stable hollow 'nanocage' of 194 nm diameter as judged by DLS. However, it seems that this clever hydrogen bonding self-assembly is much less likely to be successful if attempted in aqueous solution.

Surface-functionalised SCL micelles

There is increasing interest in the conjugation of biologically-important motifs to synthetic nanoparticles. So far, conjugated SCL micelles have been prepared by either functionalisation of pre-formed SCL micelles or by the synthesis of functionalised diblock copolymer chains prior to their micellar self-assembly and covalent stabilisation. Wooley's group prepared conjugates of fluorescently-labelled SCL micelles with the protein transduction domain (PTD) peptide sequence derived from HIV, which rendered the final hollow 'nanocages' biologically active and hence able to permeate cell membranes.[96] However, this method was limited, as only one peptide sequence could be conjugated per 'nanocage'. Nevertheless, these particles exhibited binding to cell surfaces and some preliminary evidence of transduction was obtained. Such a biological recognition strategy may allow specific delivery or sequestration of guest molecules in the future.

Another approach for enhancing cellular uptake efficiency was developed by the same group. In this case, the peptide was conjugated to the PAA chains in the SCL micelle coronas *via* carbodiimide coupling.[97] Varying amounts of PTD (and also a fluorescent marker) could be grafted as judged by UV-visible spectroscopy. The binding interaction between these conjugated SCL micelles and mammalian cells was assessed using fluorescence microscopy. Accumulation of the functionalised SCL micelles within the cells was observed in all cases. The biocompatibility of these PTD-functionalised SCL micelles was assessed by enzymatic and apoptosis assays to determine the likely effects on the exposed cells for *in vivo* applications.[98] The same method was used to prepare peptide nucleic acid (PNA) surface-functionalised SCL micelles.[99] PAA-PS micelles were cross-linked *via* carbodiimide coupling and then functionalised with either T-rich or A-rich complementary sequences. Some evidence for base-pairing aggregation was obtained by AFM for a mixture of the T-rich and A-rich SCL micelles.

Novel nano-sized delivery vehicles that target cancer cells have been developed by conjugating folate receptor ligands[100] and integrin $\alpha_v\beta_3$-targeting ligand to SCL micelles.[101] Folic acid is a known receptor-specific ligand for the folate receptor marker that is expressed by tumours. PI-PAA micelles were cross-linked *via* carbodiimide chemistry and some of the remaining carboxyl groups were coupled with folate-PEG-amines.[100] Surface derivatisation of SCL micelles with PEG has been shown to greatly improve their blood circulation times *in vivo*.[31] An endothelial cell-specific delivery vehicle has also been prepared.[101]

A second approach to the functionalisation of SCL micelles is to derivatise the diblock copolymer chains prior to micellar self-assembly. This technique was employed to prepare PTD-conjugated SCL micelles. Functionalised block copolymers were prepared by sequential polypeptide growth and nitroxide-mediated radical polymerisation (NMRP) from initiator sites

on the chain terminus of the polypeptide while it is still attached to a solid support.[102] Subsequent cleavage from the solid support yielded the hybrid polypeptide–synthetic block copolymer. This method appears to be extremely versatile and it seems likely that virtually any peptide sequence and most living radical polymerisation conditions can be utilised.

Other peptide sequences have also been conjugated to SCL micelles. Tritrpticin end-functionalised diblock and triblock copolymers have been synthesised *via* ATRP and NMRP on an initiator-loaded resin.[103] Tritrpticin has strong antimicrobial activity against various pathogens.[104] First, tritrpticin was prepared on a Wang's resin[105] and then functionalised with a fluorine-labelled alkoxyamine to yield an NMRP initiator for subsequent chain polymerisation. The final tritrpticin-PAA-PS diblock copolymer self-assembled into micelles in aqueous solution, but cross-linking of these micelles has not yet been reported. However, the enhanced antimicrobial activity of these new colloidal particles has been confirmed. Thus this conjugation seems to be promising for the attachment of both biologically-active ligands and also small molecule drugs.

Another approach to the chain end conjugation of SCL micelles is the use of an appropriate functional ATRP initiator for the targeted amphiphilic diblock copolymer. This approach was first reported for antigen-decorated SCL micelles.[106] Multivalent binding of an antigen such as 2,4-dinitrophenyl (DNP) to a cell receptor is known to trigger an immune response. A DNP-functionalised ATRP initiator was used to prepare a poly(*tert*-butyl acrylate-*block*-methyl acrylate) (P*t*BA-PMA) diblock precursor. After cleavage of the *tert*-butyl groups, the PAA-PMA diblock copolymer was self-assembled to form micelles in aqueous solution. A 'mixed micelle' approach was used such that stoichiometric amounts of the same non-functionalised diblock were incorporated, thus allowing control over the surface concentration of DNP end-groups. Carbodiimide coupling was used to cross-link and hence stabilise the structures.[21] The surface availability of the DNP was assessed by titrations with the fluorescently-labelled IgE antibody. Successful surface expression of the DNP and its binding with an antibody IgE caused degranulation, which was confirmed by an appropriate assay. It was suggested that these virus-like antigenic carriers may have some potential for the development of synthetic vaccines. Using the same method, shape-adaptable mannose-functionalised[33] and biotin-functionalised[107] SCL micelles have been described. Mannose is a saccharide that is known to selectively interact with receptors expressed by Gram negative bacterial cells such as *E. Coli.* whereas the biotin-avidin ligand-receptor pair is widely used in the biomedical field.[108]

Most of the methods discussed so far for the preparation of SCL micelles involve complex syntheses with varying degrees of control over the incorporation of functional groups.[109] In an attempt to develop a more versatile, reliable and robust strategy for the functionalisation of SCL micelles, O'Reilly *et al.* have investigated the use of Click chemistry.[65] This versatile method involves the incorporation of either azido- or alkynyl-functionalised groups in either the core or shell. Click chemistry is highly orthogonal: these two species are completely unreactive towards most biological molecules[110] yet react selectively with each other under mild conditions in aqueous solution to enable the facile attachment of functional groups to SCL micelles. Functionalisation of both the hydrophilic shells and the hydrophobic micelle cores was investigated.

Amidation chemistry was employed to functionalise some of the acrylic acid residues of a PS-PAA diblock copolymer with amine-functionalised azido or alkynyl moieties prior to micellisation. Then cross-linking was achieved *via* carbodiimide coupling of the remaining acrylic acid groups. Click-functionalisation of the core-forming PS chains required the copolymerisation of 4-vinylbenzyl chloride with styrene in order to incorporate appropriate reactive groups. Addition of excess NaN_3 produced azide-functional micelles, which were then shell cross-linked. Finally, alkynyl functionalisation of the micelle core was achieved, but unfortunately subsequent hydrolysis of the *tert*-butyl groups led to loss of functionality.

Click chemistry has also been demonstrated on SCL micelles.[25] Thus copper-catalysed cycloaddition led to triazole formation using both alkynyl and azido-functionalised dyes. This method allows facile labelling of SCL micelles with biologically active ligands, which is important for both their detection and also tracking their fate under *in vivo* and *in vitro* conditions. This method may lead to the attachment of other useful entities such as reporter molecules, therapeutic agents and biologically active ligands.

Inorganic SCL micelles

SCL micelles have been used as 'nanoreactors' to prepare metal nanoparticles.[111,112] For example, our group synthesised DVS cross-linked triblock copolymer micelles with pH-responsive cores starting from a PEO-PGMA-PDEA triblock copolymer.[111] $HAuCl_4$ was then used to protonate the PDEA cores, leading to the selective loading of $AuCl_4^-$ ions within the micelle interior. *In situ* reduction of the Au(III) to Au(0) using $NaBH_4$ resulted in the formation of gold nanoparticles within the SCL micelles with the retention of colloidal stability. Subsequently, Sakurai's group used their polysilane SCL micelles[93] to prepare gold and also palladium nanoparticles using the same approach. Kowalewski and co-workers reported using poly(acrylonitrile-*block*-acrylic acid)-based SCL micelles as templates for the formation of well-defined carbon nanoparticles by high temperature pyrolysis.[113]

Hollow PI-PAA 'nanocages' have been coated with ultrathin layers of calcium phosphate.[114] This was achieved by sequestration of Ca^{2+} ions by the carboxylic acid groups on the surface of the PAA shells and subsequent calcium phosphate nucleation on addition of Na_2HPO_4. The rate of uptake of β-carotene depended on the thickness of the calcium phosphate shell, which was typically 10–20 nm. Given that calcium phosphate is chemically stable, non-toxic and biocompatible and that these new hybrid nanocapsules are stable in water, they show some promise for drug delivery and bioimaging applications.

A University of Toronto team has reported several examples of novel organic-organometallic hybrid SCL micelles.[115–118] The first example was based on poly(isoprene-*block*-ferrocenylphenylphosphine) [PI-PFPP], which formed spherical

PFPP-core micelles in *n*-hexane.[115] The PI chains were cross-linked using a combination of UV radiation and an AIBN free radical initiator. The analogous poly(isoprene-*block*-ferrocenyldimethylsilane) (PI-PFS) was prepared by living anionic polymerisation and self-assembled into colloidal aggregates in *n*-hexane. The PI chains in the cylinder shell were cross-linked by hydrosilylation using a Pt-based catalyst to produce the first examples of shell cross-linked *cylinders* (Fig. 13).[116] This unusual particle morphology was attributed to the crystalline nature of the organometallic PFS block. These highly anisotropic particles could be aligned within microfluidic channels and magnetic ceramic replicas could be obtained by controlled thermal degradation of the organic components under a nitrogen atmosphere at 600 °C. Retention of the original cylindrical morphology was only achieved if shell cross-linking had been performed prior to pyrolysis. A similar approach was taken to prepare redox-active SCL cylinders with tunable swellability based on a related diblock copolymer, poly(ferrocenylsilane-*block*-poly(methylvinylsilane) (PFS-PMVS).[117] In this particular case, the redox-active PFS cores were subsequently used to reduce Ag^+ *in situ* to produce silver-loaded SCL cylinders.[118]

Very recently, block copolymer micelles have been stabilised using a biomineralisation approach. Thus Huo *et al.* have demonstrated that micelles formed by poly(ethylene oxide-*block*-propylene oxide) Pluronic-type triblock copolymers become coated with an ultrathin overlayer of silica during the in situ hydrolysis of tetramethyl orthosilicate (TMOS).[119] Such copolymer-silica hybrid nanoparticles did not dissociate at high dilution, suggesting that silicification causes inorganic cross-linking of the micelles. The copolymer–silica nanoparticles were loaded with various fluorescent molecules and the release of these model compounds was monitored against time. One disadvantage of this silicification route is that the hydrolysis required rather acidic conditions (around pH 1). However, our group has recently shown that using *cationic* block copolymers, rather than non-ionic block copolymers, allows silicification to be conducted under much milder conditions (pH 7.2, 20 °C).[120] The cationic precursor was a PDMA-PDPA diblock copolymer in which the tertiary amine groups on the PDMA block had been partially (and selectively[121]) quaternised using methyl iodide. Thus the PDPA chains formed the hydrophobic micelle cores, with the cationic PDMA chains being located in the micelle coronas. Under appropriate conditions, silicification could be confined to the micelle coronas, hence this biomimetic approach[122–129] leads to well-defined copolymer-silica nanoparticles of 30–35 nm diameter with core–shell morphologies (see Fig. 14). Typical silica wall thicknesses were around 5–10 nm, as judged by small-angle X-ray scattering studies. ^{1}H NMR spectroscopy studies confirmed that protons can diffuse into the PDPA cores, rendering these chains hydrophilic. This suggests that these new copolymer-silica nanoparticles may have uptake/release applications. Biomineralisation offers several advantages over covalent cross-linking: inorganic cross-linking occurs relatively fast in aqueous solution under mild conditions, the TMOS precursor is relatively cheap and the silicic acid by-product is readily removed by dialysis. Moreover, such inorganic overlayers should offer a much better barrier than covalent cross-linking to the diffusion of both small molecules and polymers. Finally, silica is an extremely inert, naturally-occurring material that has been assigned 'food-grade' status in certain forms. It remains to be seen whether these copolymer-silica nanoparticles will prove to be similarly benign.

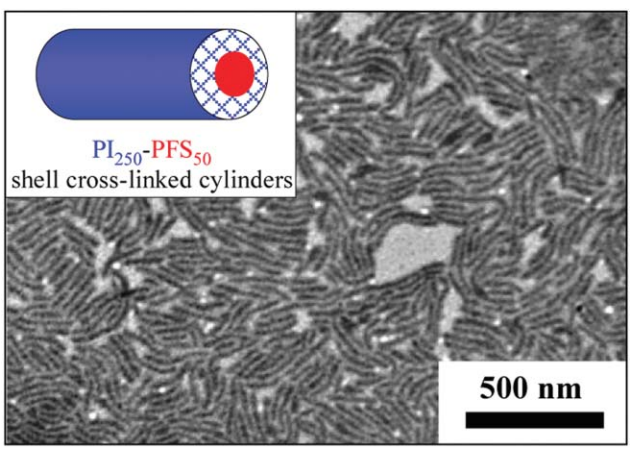

Fig. 13 Transmission electron micrograph of PI_{250}-PFS_{50} cylindrical micelles cross linked *via* Pt(0)-catalyzed hydrosilation dried from hexane solution (micrograph courtesy of Dr X. S. Wang, University of Leeds, UK).

Conclusions and outlook

Since their initial discovery in 1996, SCL micelles have become a fascinating new class of organic functional nanoparticles. Micelle cross-linking strategies include radical chemistry, carbodiimide coupling, UV-induced coupling, Michael addition, quaternisation, esterification, Click chemistry, disulfide/thiol chemistry and polyelectrolyte complexation. The use of ABC triblock copolymers, rather than AB diblock copolymers, has provided a promising route to the preparation of SCL micelles at high copolymer concentration and their structural diversity has broadened significantly as the increasing number of academic groups working in this area has led to fresh perspectives and new ideas.

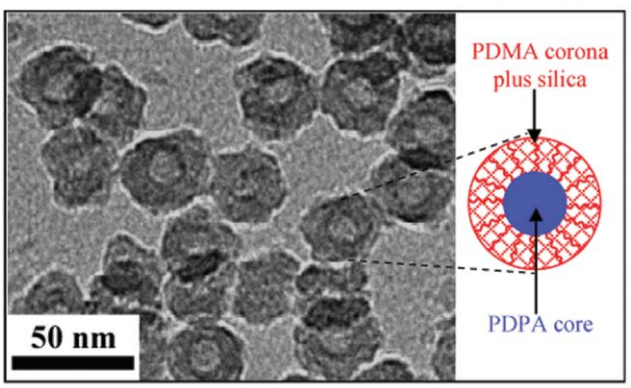

Fig. 14 Transmission electron micrograph of hybrid $QPDMA_{68}$-$PDPA_{23}$ copolymer-silica micelles after redispersion in acidic solution at pH 2. Silification also leads to cross-linking in this case.[120]

Functionalisation of these robust nanostructured particles with peptide sequences and/or other biological molecules (*e.g.* sugars) offers a wide range of potential biomedical applications. The ability of core encapsulation has been demonstrated for polymeric species, which augurs well for the use of SCL micelles as carriers for DNA and proteins. In addition, chemical (or physical) removal of the hydrophobic cores leads to the formation of 'nanocages'. There is also considerable current interest in the surface activity of SCL micelles at both the solid/water and oil/water interface.

Future work is likely to focus on improving the biocompatibility and biodegradability of functionalised SCL micelles for their use in biomedical applications. New, less toxic and more commercially viable cross-linking chemistries are still highly desirable. Rapid cross-linking is particularly important if SCL micelles are to be physically loaded with hydrophobic actives, since significant diffusional loss of such actives can occur if cross-linking proceeds too slowly. In addition, new characterisation techniques that will enable the actual degree of cross-linking to be assessed would be extremely beneficial to the development of this fascinating sub-field.

Acknowledgements

Dr S. Liu, Dr. Y. Li and Dr X. S. Wang are thanked for providing data and micrograph images for the figures. S. P. A. is the recipient of a five-year Royal Society-Wolfson Research Merit Award. Unilever Research (Colworth, UK) is thanked for CASE support of a PhD studentship for ESR.

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