Colloidosomes: Synthesis, Properties and Applications

Kate L. Thompson*, Mark Williams and Steven P. Armes*

Dainton Building, Department of Chemistry, University of Sheffield,

Brook Hill, Sheffield, S3 7HF, UK

Abstract

Colloidosomes represent a rapidly expanding field with various applications in microencapsulation, including the triggered release of cargoes. With self-assembled shells comprising colloidal particles, they offer significant flexibility with respect to microcapsule functionality. This review explores the various types of particles and techniques that have been employed to prepare colloidosomes. The relative advantages and disadvantages of these routes are highlighted and their potential as microcapsules for both small molecule and macromolecular actives is evaluated.

Introduction

Colloidosomes are microcapsules whose shells are composed of colloidal particles.[1] In recent years such superstructures have received considerable attention because of their potential importance in the area of microencapsulation. Microencapsulation enables the controlled release of active ingredients in various industrial sectors such as medicine, food, home and personal care products, agrochemicals and cosmetics, enabling the delivery of a range of actives such as drugs, pesticides and fragrances.[2-6] Routes to colloidosomes are most commonly based on the self-assembly of colloidal particles at the interface between two immiscible liquids, typically water and oil. The initial self-assembled structures are known as Pickering emulsions[7] and have been recognized for over a century. Many examples of colloidal particles such as silica sols[8, 9] and polystyrene latexes[10] have been shown to be effective Pickering emulsifiers. Stimulus-responsive particulate emulsifiers have also been developed, with inversion or demulsification being achieved in response to changes in solution pH[11, 12] or temperature.[13] The preparation of colloidosomes requires a mechanism for shell reinforcement at the interface to convert the Pickering emulsion precursor into robust microcapsules that can survive removal of the oil/water template. In this review, we

summarize the various techniques employed to prepare stable colloidosomes and highlight their suitability and potential uses as microcapsules, as well as identifying current technical problems.

The first colloidosome-type structures were reported in a series of three papers by Velev and co-workers in 1996, who employed n-octanol droplets as templates for latex self-assembly[14-16]. Formation of both hollow spherical 'supraparticles'[14] whereby latex particles were adsorbed at the water/oil interface (see Figure 2) and also 'ball-like aggregates'[15] in which the latex particles penetrated the oil droplet interior were described. It was found that using anionic sulfate-stabilized polystyrene latex particles alone did not produce stable emulsion droplets. Instead, the latex had to be 'sensitized' via adsorption of an amino acid (lycine) to promote sufficient interfacial activity. A strong coagulant (casein, HCl and $CaCl_2$), which was known to cause latex aggregation in bulk aqueous solution, was then added to 'lock in' the latex particle monolayer surrounding the droplets. This additional stabilization enabled the resulting microcapsules to survive removal of the n-octanol phase on treatment with excess ethanol. The third paper in the series reported an inverted water-in-oil formulation, whereby the same sensitized anionic latex particles were self-assembled around water droplets[16] suspended in n-octanol. In order to achieve this phase inversion a relatively high volume fraction of n-octanol (0.94) was used. This formulation produced more monodisperse droplets compared to the previous two papers.

Colloidosome preparation via thermal annealing

The term 'colloidosome' was first introduced by Dinsmore et al.,[17] who prepared microcapsules via self-assembly of micrometer-sized carboxylated polystyrene latex particles at the surface of oil-in-water (o/w) or water-in-oil (w/o) emulsion droplets. Once the droplet surface was fully covered by particles, the colloidosome shell was formed by sintering the latex particle monolayer. This was achieved by heating the emulsion to just above the T_g of the polystyrene latex particles (~ 105^oC) in order to fuse them together. Since this latex T_g exceeds the boiling point of the aqueous continuous phase, this approach necessitated the addition of 50 % glycerol to raise the boiling point and hence minimize evaporation. Water-in-oil colloidosomes could be transferred to a water-continuous phase by gentle centrifugation. SEM studies indicated that the interstitial gaps between adjacent fused particles could be controlled by varying the sintering time. The authors suggested that this enabled the permeability of the microcapsules to be tuned, although this aspect was not

demonstrated. Extending this seminal study, Hsu et al.[18] also reported sintering as a means of ensuring the structural integrity of colloidosomes. After heating to 105 ^{o}C for 2 h, individual particles were no longer discernible via SEM, which suggested that the sintering process was complete and a fully-annealed latex shell was formed.

These early examples of colloidosome formation via latex sintering required relatively high temperatures and addition of co-solvent to prevent evaporation of the aqueous continuous phase. However, this approach is likely to be detrimental for the encapsulation of thermally-sensitive actives such as fragrances or drugs. One solution to this technical problem is to replace polystyrene latex with a lower T_g copolymer latex, thus allowing a reduction in the sintering temperature. Accordingly, Routh and co-workers[19, 20] utilized a poly(styrene-co-n-butyl acrylate) latex to prepare 2-5 µm diameter w/o emulsions and, after heating to 35-65 ^{o}C, robust colloidosomes with aqueous cores (see Figure 3). In this particular example, the copolymer latex alone was not an efficient Pickering emulsifier, hence a suitable oil-soluble co-surfactant (Span 80) was required to optimize colloidosome formation.

Laïb and Routh[19] identified a technical problem when preparing colloidosomes via thermal annealing. In addition to the desired intra-shell latex sintering, latex sintering between neighboring colloidosomes can occur, leading to extensive inter-colloidosome fusion. This is particularly problematic at high solids and hence is a potential barrier to scale-up syntheses. However, Salari et al.[21] overcame this problem by adding poly(styrene-block-ethylene-co-propylene) (PS-EP) diblock copolymer micelles to a Pickering emulsion prior to annealing so as to act as an additional steric stabilizer. In this work, the original polystyrene particles used to prepare the Pickering emulsions were synthesized via soap-free emulsion polymerization. This latex was then emulsified with n-decane in the presence of salt to give a water-in-oil emulsion. The PS-EP copolymer micelles were then added to the Pickering emulsion and heated to 50^{o}C. The n-decane oil phase has a plasticizing effect on the polystyrene latex, allowing sintering to occur below its normal T_g. It was found that significant colloidosome fusion occurred in the absence of the PS-EP micelles, whereas particle aggregation was reduced substantially if this diblock copolymer was added prior to latex sintering.

Polyelectrolyte complexation and layer-by-layer deposition

In addition to preparing colloidosomes via latex sintering, Dinsmore et al.[17] also examined electrostatic adsorption of an oppositely-charged polyelectrolyte as a means of reinforcing the

latex superstructure via physical cross-linking. More specifically, a high molecular weight cationic poly(L-lysine) was adsorbed onto the surface of Pickering emulsions prepared using anionic polystyrene latex particles. This approach proved to be very effective: robust colloidosomes were produced which were much more flexible and deformable compared to the relatively brittle microcapsules produced via latex sintering. This polyelectrolyte adsorption method was further developed by Gordon et al.[22] The latex particles were dispersed in toluene as an oil phase and emulsified with an aqueous solution of poly(L-lysine). Latex self-assembly occurred at the aqueous droplet interface and the cationic poly(L-lysine) adsorbed onto the anionic PS latex, locking in the superstructure.[17] The resulting microcapsules were robust enough to survive transfer into an aqueous continuous phase via centrifugation. A series of models were used to determine the structure and mechanical response of these colloidosomes. Addition of salt to the aqueous continuous phase reduces the osmotic pressure of the encapsulated aqueous polyelectrolyte solution. Thus this provides a non-mechanical release trigger for these microcapsules, which become completely deflated when immersed in 1 M NaCl (see Figure 4). This is an important finding, but no encapsulation/release studies were conducted in this particular work. The permeability and encapsulation efficiency of colloidosomes will be discussed later.

Lawrence et al.[23] adsorbed anionic poly(N-isopropylacrylamide-co-acrylic acid) microgel particles around aqueous droplets in n-octanol to form colloidosomes. In this case, the colloidosomes were physically cross-linked by addition of poly(butadiene-N-methyl-4-vinylpyridinium iodide). Although not specifically mentioned in the text, it seems likely that this amphiphilic diblock copolymer is actually present in the form of micelles in the n-octanol continuous phase. This cationic diblock copolymer produced robust microcapsules that survived removal of the droplet interface with ethanol and could be transferred into water. The resulting w/w colloidosomes shrank significantly on heating, suggesting a possible thermally-triggered release mechanism for encapsulated actives.

Caruso et al.[24, 25] reported the formation of microcapsules using a sacrificial colloidal template. Thus 600 nm diameter polystyrene latex was coated using the layer-by-layer technique via sequential deposition of cationic poly(diallyl dimethylammonium chloride) (PDADMAC) and anionic silica nanoparticles of 25 nm diameter. The thickness of the resulting PDADMAC/silica shell depends linearly on the number of pairs of deposition cycles. The colloidal template was then removed either by calcination or dissolution in

organic solvents. Fe_3O_4,[26] TiO_2,[27] and Laponite nanoparticles[28] have also been used to prepare similar hollow inorganic capsules by layer-by-layer deposition onto sacrificial templates. One disadvantage of this technique is that hollow particles can only be produced by template calcination or dissolution, which is inherently wasteful. Moreover, microcapsule fabrication involves a relatively slow multi-step process and loading cannot be achieved in situ. Finally, it is difficult to make such microcapsules at high solids because the PDADMAC tends to cause bridging flocculation under these conditions.

Despite these inherent limitations, Li and Stöver[29] also used PDADMAC in combination with poly(sodium 4-styrenesulfonate) (PSS) to deposit alternating layers onto Pickering emulsions prepared using PSS-coated cationic silica particles (Ludox CL). However, this approach led to poor encapsulation efficiency and extremely porous microcapsule walls that were easily ruptured. However, more robust microcapsule walls could be produced via layer-by-layer deposition using a combination of PDADMAC and anionic silica nanoparticles.

Rossier-Miranda et al.[30] also utilized the layer-by-layer technique to prepare microcapsules. First, a cationic template was created by emulsifying n-hexadecane with cationic whey protein isolate solution. Anionic high methoxyl pectin (HMP) was then adsorbed, followed by more cationic protein. Then a layer of silica particles was adsorbed to reinforce the shell and further pectin/protein adsorption enables the pores between adjacent silica particles to be filled. In principle, such microcapsules may have applications in the food or pharma industries since they are based on inexpensive food-grade materials, and are prepared at room temperature and at low pH. For example, they are expected to survive in the acidic environment of the stomach but later dissolve in the neutral small intestine, suggesting the potential controlled release of actives.

More recently, Laponite has been coated with poly(ethylene imine) (PEI) to form an o/w Pickering emulsion using sunflower oil as the oil phase.[31] In the context of layer-by-layer deposition nanocomposite polysaccharide microcapsules were produced via alternating adsorption of anionic sodium alginate and cationic chitosan onto PEI/Laponite stabilized Pickering emulsions.[32] They use ibuprofen as a model drug to demonstrate that the greater the number of polyelectrolyte layers the slower the rate of release.

Gel trapping technique

An interesting alternative approach to colloidosome stabilization involves using an aqueous gel as the internal phase, as described by Paunov and co-workers.[33, 34] Typically, the aqueous core of a w/o colloidosome is gelled to form a solid-like core. This means enhanced stability and structural integrity for the colloidosomes, which aids their subsequent transfer into the aqueous phase to produce w/w colloidosomes. For example, Cayre and co-workers[33] self-assembled amine-functionalized polystyrene latex onto aqueous emulsion droplets containing 1.5 % agarose at 75^{o}C, with sunflower oil forming the continuous phase (see Figure 6). Gelation of the aqueous droplets occurred on cooling to 20^{o}C. Then the colloidosome superstructure was reinforced by cross-linking the surface amine groups using glutaraldehyde, which binds the particles within the microcapsule shell. Importantly, colloidosomes prepared in the absence of the agarose gelling agent invariably collapsed when attempting their transfer into the aqueous phase via centrifugation. In contrast, colloidosomes with gelled aqueous cores could be successfully transferred from their initial w/o form to produce w/w colloidosomes. The same authors demonstrated that swelling of the polystyrene particles occurred at 75^{o}C when tricaprylin was used as the oil phase. By varying the temperature and also the exposure time of the colloidosomes to the hot tricaprylin, the dimensions of the interstices between adjacent latex particles could be tuned, which might be important for encapsulation/release applications. Noble et al.[34] prepared so-called 'hairy' colloidosomes by the same general method of aqueous core gelation via self-assembly of 'polymeric microrods', while Duan et al.[35] prepared magnetic colloidosomes using magnetite nanoparticles trapped within an agarose gel. This latter study is discussed in more detail in the context of encapsulation and release (see later).

More recently, this agarose gel technique has been used for the formation of 'matrix-assisted' colloidosomes, where almost 100 % encapsulation efficiency of water-soluble model proteins has been demonstrated. This is achieved by using a reverse phase layer-by-layer encapsulation method.[36] An aqueous mixture of agarose, biomolecules (such as glucose oxidase or horseradish peroxidase) and amine-functionalized PS latex was emulsified with mineral oil at 45^{o}C and allowed to cool to form colloidosomes. The reverse phase layer-by-layer deposition process was conducted by placing the colloidosomes in solutions of 1-butanol, first containing an anionic polyelectrolyte and then in a solution containing a cationic polyelectrolyte. The resulting microcapsules were washed twice with ethanol and finally transferred into water via centrifugation. Colloidosome microcapsules dispersed in

water comprising seven polyelectrolyte layers were demonstrated to retain 100% of encapsulated biomolecules for up to one week at 25 °C.

An alternative approach to colloidosomes with gelled cores was described by Wang and co-workers.[37, 38] In this case alginate was used instead of agarose for in situ gelation of the aqueous cores. A shell of porous $CaCO_3$ microparticles was formed around a water-in-oil emulsion. Aqueous D-glucono-δ-lactone was added to the aqueous phase, which caused a gradual lowering of the solution pH as a result of its slow hydrolysis.[39] Ca^{2+} ions were released from the $CaCO_3$ and cross-linked the alginate chains in solution to form gelled cores.

Colloidosome-like structures using silica particles have also been prepared by using molten wax as the oil phase.[40, 41] This approach is analogous to the aqueous gel trapping method. Silica particles dispersed in a molten wax were emulsified with an aqueous solution at 75^{o}C to produce wax droplets in aqueous solution. On cooling, the oil phase crystallized and the silica particles become immobilized at the wax/water interface to produce a colloidosome-type structure. This technique was exploited to prepare so-called 'Janus' particles by in situ modification of the surface of the silica particles that is exposed to the aqueous continuous phase. Once surface modification was complete, the wax droplet phase was removed using chloroform and the Janus particles were isolated for characterization. Although not strictly an example of colloidosomes, this work does serve to illustrate the potential advantages of trapping particles at interfaces.

One advantage of the gel trapping technique is that a wide range of particles can be utilized to form the colloidosome shell. No particular surface functionality is required, since the shell is stabilized via physical gelation of the aqueous droplets on cooling, rather than interactions between the particles and external agents (as in the case with polyelectrolyte complexation). However, a disadvantage is that gel trapping usually requires the emulsions to be prepared at elevated temperature so as to prevent gelation prior to particle self-assembly. This is a potential problem for the encapsulation of various thermally-sensitive species such as proteins or enzymes.

Polymerization after Pickering emulsion formation

In some cases, enhanced colloidosome stability is achieved via subsequent polymerization that takes place either inside or at the surface of the initial Pickering emulsion. This traps the particles at the interface, allowing the original droplet template to be removed. For example, a surface-functionalized silica sol has been shown to act as a Pickering stabiliser for paraffin oil-in-water emulsions. In this case the silica sol had been modified to comprise initiator sites for atom transfer radical polymerization (ATRP).[42] Once a stable Pickering emulsion was obtained, surface-initiated ATRP of 2-hydroxyethyl methacrylate (HEMA) was conducted from the silica particles. HEMA monomer typically contains a dimethacrylate impurity, so cross-linking occurs in situ during ATRP to produce novel PHEMA microcapsules. However, the multi-step protocol required to produce ATRP initiator-functionalized silica particles is time-consuming and not readily amenable to scale-up.

An alternative related approach is to conduct polymerizations within the droplet interior, whereby the oil phase either contains or is itself a vinyl monomer. So-called Pickering emulsion polymerization has been exploited to produce a range of microcapsules.[43-45] Poly(methyl methacrylate) particles have been adsorbed around monomer droplets containing styrene and divinylbenzene and subsequently used as mini-reaction vessels for copolymerization from within the droplet phase.[43] This led to the formation of colloidosomes of 5 to 30 μm diameter reinforced by solid cross-linked polystyrene cores. The same technique has been used to prepare colloidosomes using either TiO_2[44] or ZnO[45] particles. Hollow silica microspheres have also been prepared using silica particles treated with 3-(methacryloxy)propyltrimethoxysilane.[46] In this case the surface-modified silica particles were used as Pickering emulsifiers to produce n-dodecanol droplets containing styrene and divinylbenzene. After copolymerization of the oil phase, thermogravimetric analysis indicated that around 60 % of the copolymer was covalently grafted to the silica shell. Thermo-responsive microcapsules have also been prepared by the copolymerization of N-isopropyl acrylamide (NIPAM) inside aqueous droplets stabilized using hydrophobic silica particles as the Pickering emulsifier.[47] These microcapsules can be dispersed in water and the volumetric contraction (V/V_{max}) above the LCST of PNIPAM was measured as a function of cross-linker density. As expected, a higher degree of cross-linking leads to reduced shrinkage, or a lower V/V_{max} ratio. Polymerization of the aqueous phase has also been demonstrated with gelled aqueous cores with either sodium acrylate or poly(ethylene glycol)

methyl ether methacrylate being reacted with a suitable cross-linker.[48] The enhanced mechanical stability imparted by the gelled cores allows these capsules to be collected as dry powders, which should facilitate their transportation prior to use.

One alternative to interfacial polymerization is to precipitate a preformed polymer onto the inside of the Pickering emulsion in order to reinforce the colloidosome superstructure. This was achieved by Cayre et al.,[49] who prepared an o/w Pickering emulsion using aqueous silica or gold nanoparticles. Here the oil phase comprised linear poly(methyl methacrylate) dissolved in a 9:1 w/w dichloromethane/n-hexadecane mixture. Once formed, the emulsion was then treated by rotary evaporation at 40^{o}C. The dichloromethane was then gradually removed from the emulsion droplets under reduced pressure, producing n-hexadecane cores. This solvent is a poor solvent for poly(methyl methacrylate). Thus this polymer precipitates onto the interior walls of the microcapsules, locking the inorganic particles at the interface. The same authors also investigated the effect of varying the polymer and silica particle concentrations.[50] Reducing the precipitated polymer concentration led to a reduction in the mechanical strength of the dried capsules. Moreover, increasing the particle concentration from 2 to 3 wt % produced visibly less porous colloidosomes as judged by SEM. A slightly different approach was described by Shahidan et al.[51] Here either poly(ε-caprolactone) or polystyrene was precipitated onto a polymeric surfactant-stabilized dichloromethane-in-water emulsion, forming a particle shell during dichloromethane evaporation. One advantage of this strategy is that the colloidosome cores fill up with water after all the dichloromethane is evaporated, thus directly producing a w/w colloidosome without requiring centrifugal transfer. However, loading such w/w colloidosomes with a water-borne cargo is likely to be problematic. The original droplet cores constituted an organic solvent that is unlikely to be a suitable carrier for a water- dispersible active, making loading prior to colloidosome formation somewhat problematic. Moreover, loading the resulting w/w emulsion after dichloromethane evaporation would also be technically challenging, as the active would have to diffuse through the microcapsule shell. Therefore only low encapsulation efficiencies are likely to be achieved via this approach.

Colloidosome Formation via Covalent Cross-linking

Techniques such as thermal annealing, gel trapping and Pickering emulsion polymerization almost always involve a heating step, which may be undesirable for certain applications, e.g.

enzyme encapsulation. In contrast, appropriate selection of the colloidal particles and/or cross-linker can enable covalent cross-linking to be achieved colloidosome formation at ambient temperature (see Table 1).

In 2003 Croll et al.[52, 53] prepared poly-(divinylbenzene-alt-maleic anhydride) microspheres and assembled them at the oil/water interface (see Figure 8A). These microspheres were then cross-linked from the aqueous continuous phase via reaction with various polyamines to form amide linkages and/or protonated amine/anionic carboxylate ionic bonds. More robust microcapsules were obtained when utilizing higher molecular weight polyamine cross-linkers. Conversely, using a small molecule diamine such as tetraethylene pentamine (TEPA) did not lead to microcapsule formation, presumably because this low molecular weight species is simply too small to span the gap between adjacent adsorbed latex particles. The authors do not comment on whether the presence of the polyamine cross-linker in the continuous phase resulted in inter-colloidosome cross-linking under their reaction conditions, which correspond to a microcapsule solids content of 20 % w/w and a polyamine concentration of 1 wt %.

Lin et al.[54] reported covalent cross-linking of CdSe nanoparticles at the planar water/toluene interface. These inorganic nanoparticles were functionalized with pendent reactive vinylbenzene groups, which were oligomerized in situ at 60 ^{o}C using a free radical initiator to lock in the interfacial particulate layer. However, when this protocol was attempted at the curved aqueous droplet interface, some degree of shell cracking was observed. These problems could possibly be due to the elevated temperature required for the free-radical cross-linking. Subsequently, Skaff and co-workers[55] self-assembled norbornene-functionalized CdSe/ZnS core/shell quantum dots at the interface of water droplets in toluene. Ring-opening metathesis polymerization (ROMP) using a water-soluble Ru-based Grubbs catalyst was then used to promote interfacial cross-linking at room temperature in order to 'lock in' the quantum dot super-structure. Using a more hydrophobic catalyst led to problems with inter-colloidosome cross-linking and also produced cross-linked particles throughout the toluene phase, as cross-linking was not confined to the interface. This highlights the potential problems with the covalent cross-linking route. When cross-linking is conducted from the continuous phase, inter-colloidosome fusion is always likely. Moreover, any excess non-adsorbed particles in the same phase as the cross-linker could themselves become cross-linked in suspension, potentially causing cross-linker wastage and/or particle flocculation.

Arumugam et al.[56] prepared 35-60 μm magnetic colloidosomes by assembling terpyridine thiol-functionalized FePt nanoparticles around water droplets suspended in toluene and then ionically cross-linked them via complexation of the terpyridine with Fe(II) ions.

More recently, Weitz and co-workers[57] prepared w/o emulsions using primary amine-functionalized PNIPAM microgels. These microgels were cross-linked from the aqueous phase using glutaraldehyde to produce novel thermo-responsive microcapsules. Presumably, since the cross-linking is spatially confined, no inter-colloidosome fusion can occur, leading to the formation of discrete well-defined microcapsules. Confocal microscopy studies with fluorescently-tagged microgels suggested the presence of non-adsorbed microgels within the droplets. It is not known whether these excess particles become aggregated during the colloidosome cross-linking step. As with the work of Lawrence et al., [23] the resulting colloidosomes exhibit a significant reduction in size when increasing temperature, suggesting a potential thermally-triggered release mechanism.

We recently reported the formation of robust colloidosomes via covalent cross-linking of latex particles adsorbed at the oil-water interface.[58, 59] Bespoke well-defined hydroxy-functional macromonomers[60] were used to prepare near-monodisperse sterically-stabilized polystyrene latexes of either ~100 nm or ~1.0 μm diameter using either aqueous emulsion or alcoholic dispersion polymerization. Such particles stabilize oil-in-water Pickering emulsions using a range of model oils, including n-dodecane, methyl myristate and sunflower oil. Cross-linking was achieved within the droplet phase using a commercially available oil-soluble polymeric diisocyanate cross-linker to produce robust urethane bonds (see Figure 8C). With the cross-linker confined to the oil phase, the possibility of inter-colloidosome fusion (and also the aggregation of excess, non-adsorbed latex particles) is minimized. The resulting colloidosome microcapsules survived removal of the internal oil phase using excess ethanol and also proved to be resistant to the presence of surfactant. In contrast, the precursor Pickering emulsions did not survive such challenges. The same polymeric diisocyanate cross-linker was also exploited to confer covalent stabilization on silica-stabilized and polymer/silica nanocomposite-stabilized Pickering emulsions.[61]

In subsequent work, a well-known water-soluble polymer was used as a more reactive steric stabilizer.[62] Thus poly(ethylene imine) (PEI) was lightly quaternized with 4-vinylbenzyl chloride and then this macromonomer was utilized to prepare sterically-stabilized polystyrene

latexes of 100-200 nm diameter via aqueous emulsion polymerization. The resulting cationic latexes acted as effective Pickering emulsifiers at pH 9 and could be cross-linked at the oil-water interface using three commercially-available bisepoxy polymeric cross-linkers from both inside and outside the oil droplets (see Figure 8D). Unexpectedly, cross-linking from the aqueous continuous phase using a water-soluble bisepoxy poly(ethylene glycol) did not appear to suffer the potential problem of inter-colloidosome fusion, even when conducted at high solids (50 vol %). This unexpected observation may be related to the very different length scales for the colloidosome diameter (~50 μm) and the oligomeric cross-linker (nm). Clay-based colloidosomes have also been developed by adsorbing PEI onto either Laponite[31] or montmorillonite,[63] followed by cross-linking these amine-modified clay platelets at the oil/water interface with either a water-soluble or an oil-soluble bisepoxy polymeric cross-linker. Very recently, PEI-coated fumed silica particles have been used to prepare water-in-oil-in-water (w/o/w) double emulsions and colloidosomes-in-colloidosomes using the same bisepoxy cross-linking chemistry.[64] The surface wettability of the composite particles could be tuned by systematically increasing the PEI/silica mass ratio, hence leading to phase inversion from o/w to w/o emulsions. This approach allows w/o/w double emulsions to be prepared with the two interfaces being stabilized by the same silica particles, coated in different amounts of PEI. Cross-linking could be achieved from either the middle oil phase or the internal/external aqueous phases by using the oil- or water-soluble polymeric bisepoxides, respectively (see Figure 9).

Covalent cross-linking using an alternative reactive steric stabilizer has also been reported by Yuan et al.[65] In this case colloidosomes were prepared using a poly(2-dimethylaminoethyl methacrylate)-poly(methyl methacrylate) diblock copolymer as a steric stabilizer for polystyrene particles, which were subsequently cross-linked at the oil-water interface via quaternization using 1,2-bis(2-iodoethyloxy)ethane (BIEE) (see Figure 8B).

Cross-linking can also be achieved by UV irradiation of particles at the oil/water interface.[66] Ferritin protein cages decorated with poly(N-isopropylacrylamide)/2-(dimethylmaleinimido)-N-ethylacrylamide) (PNIPAM/DMIAAm) were adsorbed at the toluene/water interface. When DMIAAm is exposed to UV irradiation, it undergoes a [2+2] photocyclization reaction. One advantage of this approach is that no additional cross-linker needs to be added to the formulation, thus eliminating the possible presence of excess unreacted cross-linker in either the continuous phase or the droplet phase.

Giant/Pendant Colloidosomes

So far discussion has focused on colloidosomes of the order of tens of microns or smaller. However, so-called 'giant' colloidosomes of ~ 1- 2 mm diameter can be formed with the aid of capillaries. Using a droplet break-off technique, giant colloidosomes with relatively narrow size distributions can be generated.[67, 68] Here a pendant water droplet is formed in an oil phase, stabilized by either polystyrene,[68] poly(styrene-co-acrylamide)[67] or poly(styrene-co-4-styrenesulfonate)[67] latex particles adsorbed at the droplet interface. In the case of Ashby and co-workers,[68] the pendant drop was then transferred through a planar oil-water interface to form a pendant water-oil-water colloidosome. This is an example where colloidosomes can be prepared without the need for sintering or fusing the colloidal particles together; these millimetre-sized colloidosomes were supported by an oil film bridged by a monolayer of particles.

The coalescence behavior of similar covalently cross-linked colloidosomes[69, 70] has been investigated using a high speed video camera. Initially, Thompson et al.[70] observed that individual millimetre-sized Pickering emulsion droplets prepared using poly(glycerol monomethacrylate)-stabilized latex particles readily coalesced when brought into contact. However, a pair of analogous pendant colloidosomes prepared by addition of the oil-soluble polymeric diisocyanate cross-linker to the initial oil bubble could be maintained in close contact for several hours without coalescence. In closely related work, Morse et al.[69] found that the addition of cross-linker to one droplet led to arrested coalescence when the cross-linker was allowed to diffuse from one pendant drop to its neighboring drop. These pendant oil droplet experiments allow the interactions between individual droplets to be examined and may provide useful insights with regard to colloidosome behaviour in the bulk.

Encapsulation and Release from Colloidosomes

As highlighted in this article, there are many successful synthetic routes for the preparation of colloidosomes. The obvious application for these microcapsules is the encapsulation and controlled release of various actives. However, there are few reports focused on the long-term retention of a model payload within colloidosomes. Yow and Routh[20] observed release profiles for fluorescein dye (present in its water-soluble sodium salt form) encapsulated within w/o colloidosomes prepared using a poly(styrene-co-n-butyl acrylate) latex sintered at 49^{o}C for 5, 30 or 60 minutes, followed by careful centrifugation and filtering prior to

dispersion into aqueous solution to produce w/w colloidosomes. Scanning electron microscopy images of the sintered colloidosomes showed that longer sintering times led to smoother microcapsule shells. Release profiles for fluorescein-loaded colloidosomes annealed for 5 or 30 min were almost identical, with ~100 % of the dye being released within just a few hours. The colloidosomes sintered for 60 min released the dye more slowly, but nevertheless all of the dye was released within the first 24 h. Such encapsulation performance is wholly inadequate for most commercial applications, which ideally require retention of actives on time scales of many months or even years. We encountered similar difficulties in our release experiments with covalently cross-linked colloidosomes.[59] In our case, attempted thermal annealing in addition to covalent cross-linking also proved to be unsuccessful in retarding fluorescein dye release.[59] We attribute this disappointing result to the difficulty of closing every latex interstice and also the inherent permeability of an amorphous polymer, which has considerable free volume.

Colloidosomes are prepared from colloidal particles. When closely packed together on a flat surface they will exhibit gaps or interstices between neighboring particles. Taking into account geometrical constraints, perfect hexagonal packing of monodisperse spheres of diameter (d) on a planar surface leads to an estimated interstitial diameter of approximately 0.15d.[35] In principle, such interstices should dictate the permeability of colloidosome microcapsules. However, the curved nature of the colloidosome surface leads to additional intrinsic packing defects. For example, a minimum of 12 pentagonal defects must be present for small monodisperse spheres to pack fully around a larger sphere.[71, 72] These defects are often described as line defects or grain boundary 'scars'[73] and have been experimentally observed during bright field and fluorescence microscopy studies of water droplets stabilized using polystyrene[74] or silica[72] particles, respectively. A pentagonal defect within a packed monolayer of spheres has an estimated size of 0.70d, which is significantly larger than the fundamental interstitial dimensions of 0.15d. Hence only encapsulated species larger than 0.70d are guaranteed to remain within colloidosome shells comprising monodisperse spheres of diameter d. Clearly, in certain cases these interstices can be reduced (e.g. thermal annealing of latex particles), but closing all of these individual holes is a challenge and may explain the poor encapsulation efficiency of the colloidosome systems discussed at the start of this section.[20, 59] In principle, an alternative approach could be to use polydisperse spheres, but this is likely to lead to shells of non-uniform thickness. Alternatively, a bimodal

distribution of small and large spheres could be utilized such that the former are selected to fit within the interstices formed by the latter.[75]

Duan et al.[35] used CdTe nanoparticles as probes in order to examine how packing defects affected the permeability of Fe_3O_4-based colloidosomes. Three types of Fe_3O_4 nanoparticles of 4, 5 and 8 nm diameter were used to form colloidosomes. According to the geometric packing arguments presented above, colloidosomes prepared using the 5 nm Fe_3O_4 nanoparticles should have a size cut-off of 3.5 nm (or 0.7d). To test this hypothesis, CdTe nanoparticles of 2.8 nm diameter (with fluorescence emission at 540 nm) and 4 nm diameter (with fluorescence emission at 650 nm) were placed inside the colloidosomes. The colloidosomes were then repeatedly washed with water and observed by confocal laser microscopy. Only the 2.8 nm CdTe nanoparticles could be removed during washing, while the larger 4 nm CdTe nanoparticles remained encapsulated. Thus this seminal study confirmed that colloidosomes can successfully retain a payload, provided that the active species is larger than the pentagonal defects present within the colloidosome shell. A similar study was also performed by Dinsmore et al.,[17] who used fluorescently-labeled polystyrene particles to probe the permeability of their colloidosomes. Colloidosomes prepared using 1.3 µm polystyrene latex coated with poly(L-lysine) proved to be completely impermeable to 1 µm polystyrene particles present in the aqueous phase but 0.1 µm polystyrene particles were able to pass through the latex shell via its packing defects and hence enter the colloidosome. Similarly, Lee and Weitz[76] reported that low molecular weight fluorescent molecules such as calcein (~ 622 g mol^{-1}) could freely diffuse into colloidosomes prepared from double emulsions, whereas high molecular weight FITC-labeled dextran, (2×10^6 g mol^{-1}) could not penetrate the colloidosome shell. Calcein diffusion could be prevented by including poly(lactic acid) within the encapsulated oil phase, since this component effectively fills the interstitial voids between the silica nanoparticles. Sander and Studart have also demonstrated successful encapsulation and release of nanoparticles larger than those that make up the colloidosome shell.[77]

Biggs and co-workers[65] reported that covalently cross-linked colloidosomes exhibit tunable permeability to fluorescently-labeled dextran (70×10^3 g mol^{-1}).[78] At low pH, the polyamine-based steric stabilizer is protonated and hence highly cationic. Therefore the colloidosome pore size is maximized and the microcapsules are highly permeable. However, at high pH, the stabilizer chains collapse, reducing the pore size and effectively trapping the dextran

within the colloidosome. Stephenson et al.[79] reported release of a range of FTIC-labeled dextrans of varying molecular weight from within colloidomes prepared using 100 nm polystyrene latex particles. The rate of passive release depended on the size of the cargo, with retarded release being observed for dextrans with molecular weight exceeding 1.5 x 10^5 g mol^{-1}.

Rossenberg et al.[80, 81] conducted a series of release experiments from hydrogels covered with a monolayer of spherical particles. They systematically varied the particle diameter in order to achieve different pore sizes and monitored the release of small molecules such as aspirin and caffeine and also higher molecular weight dextran (~ 3-5 x 10^3 g mol^{-1}). It was found that, although mass transport through the colloidal layer was retarded compared to the uncoated hydrogel, the rate of diffusion was largely independent of the particle diameter (and therefore pore size). The only exception was if the particle radius was comparable to the size of the diffusing molecule. In related work, multilayer particulate shells[81] as described by Kim et al.[82] also resulted in retarded diffusion.

Behrens and co-workers recently reported the preparation of stimulus-responsive colloidosomes that completely dissolve in response to a pH trigger.[83] These colloidosomes were prepared using pH-responsive poly(methyl methacrylate-co-methacrylic acid) particles. In this case the colloidosome template was a double emulsion, hence particle multilayers were produced. Firstly, a w/o emulsion was prepared via homogenization of an aqueous dispersion of the copolymer particles with dichloromethane. This initial emulsion was then rehomogenized with a second aqueous dispersion of the same copolymer particles to produce a w/o/w double emulsion. The dichloromethane was then removed by slow evaporation at room temperature; the particles at the original interface remained in contact via Van der Waals forces. The resulting pH-sensitive capsules proved to be stable below pH 7, but on increasing the pH up to pH 8 the microcapsules completely dissolved within 1 min, suggesting a possible pH-triggered release mechanism. However, the authors recognized that these microcapsules were unlikely to be of practical utility, since their permeability prior to triggered release was too high. Hence if a long shelf-life is required, most of the encapsulated small molecules would already be released simply by permeation prior to any triggered release. In view of this, subsequent work[84] focused on reducing microcapsule permeability prior to triggered release. Methods investigated included solvent annealing (or 'ethanol consolidation'), layer-by-layer reinforcement via addition of polyelectrolytes and inclusion of

poly(lactic-co-glycolic acid) within the oil phase prior to evaporation of the dichloromethane. Compared to the unmodified microcapsules, all three methods produced a significant reduction in permeability at pH 6 prior to pH-triggered dissolution of the microcapsules.

Certain w/o colloidosomes have been evaluated for the encapsulation of enzymes. For example, Wu et al.[85] reported enhanced activity when enzymes were encapsulated inside both Pickering emulsions and the equivalent agarose gel-trapped colloidosomes, when compared to activities observed for a macroscopic biphasic reference system. This is attributed to the much higher interfacial area for the emulsion droplets compared to the planar interface, which significantly improves mass transfer and hence catalytic activity. In this particular case the particle interstices are not sufficiently small to prevent enzyme diffusion from the colloidosome cores. Thus centrifugal transfer of the microcapsules from the n-hexane continuous phase into aqueous solution leads to enzyme release. In principle, this unwanted loss of enzyme could be minimized by using smaller particles to prepare the colloidosome shells.

Although some success has been achieved regarding the encapsulation of various actives within colloidosomes, effective encapsulation of small molecules over time scales of weeks/months has yet to be demonstrated. Even if the relatively large packing defects could be blocked, such low molecular weight species could all too easily permeate the smaller interstices that exist between the colloidal particles. In the majority of cases where successful encapsulation has been demonstrated, it has involved a size exclusion mechanism, whereby the encapsulated macromolecular or nanoparticle species is physically too large to permeate the defects within the colloidosome shell. In this context, it has recently been demonstrated that encapsulated yeast cells[86] and bacteria[87] can survive within colloidosome microcapsules.

Recently, small molecule encapsulation has been demonstrated by closing the particle interstices. More specifically, Weitz and co-workers[88] reported that poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (Pluronic L31) dispersed in the droplet phase could reversibly block the gaps between adjacent particles within the colloidosome shell. This polymer reversible adsorbs onto the surface of the colloidal particles and either encapsulates or releases a sulforhodamine B dye in response to temperature. Very recently, Keen et al.[89] described the successful encapsulation of amylase within colloidosomes reinforced with a $CaCO_3$ external shell to seal the particle interstices. This

$CaCO_3$ overlayer can be removed either by dilution or by mechanical shear, which allows triggered release of the enzymatic cargo. Thus this formulation may offer some potential for next generation laundry products, where such enzymes need to be physically separated from the other components (e.g. bleach) to avoid denaturation.

Conclusions and Prospect

In summary, many types of colloidosomes can be prepared from the corresponding Pickering emulsion precursors using various preparation routes. Stabilization of relatively delicate Pickering emulsion precursors to produce robust colloidosomes can be achieved using latex sintering, polyelectrolyte adsorption, in situ particle coagulation (via addition of salt or ethanol), gel trapping, interfacial polymerisation or covalent cross-linking. Some approaches such as latex sintering, gel trapping or interfacial polymerisation require heat treatment, which could be detrimental to the chemical stability of the encapsulated species. In contrast, polyelectrolyte adsorption, in situ particle coagulation or covalent cross-linking are typically conducted at ambient temperature. However, in the latter case, the nature and spatial location of the cross-linker should be carefully selected to avoid unwanted interactions with the encapsulated species. In general, the wide choice of colloidal particles available enables functional colloidosomes to be designed for a particular application. For example, magnetic[35] or temperature-responsive[57] or pH-responsive[83] colloidosome microcapsules have been described in the literature. Although colloidosomes appear to offer exciting new opportunities for microencapsulation, the long-term retention of small molecules still represents a formidable technical challenge. Currently, the encapsulation of small molecules is typically limited to time scales of just a few hours,[20] which is simply not practical for most commercial applications. Whenever small molecules have been successfully encapsulated, usually deposition of a second impermeable layer over the original colloidosomes is required. There is clearly scope for further work, such as evaluating methods for more efficient closure of particle interstices. Even if the problem of small molecule retention cannot be solved, colloidosomes still offer considerable potential for the encapsulation of biological macromolecules or nanoparticles, since such species are often too big to diffuse through the packing defects and/or particle interstices within the colloidosome shells.

Acknowledgments

KLT thanks the EPSRC and the University of Sheffield for a postdoctoral prize fellowship. SPA acknowledges the ERC for a five-year Advanced Investigator award (PISA 320372) and the EPSRC for a Platform grant (EP/J007846/1).

References

1. H. N. Yow and A. F. Routh, Soft Matter, 2006, **2**, 940-949.
2. T.-A. Read, D. R. Sorensen, R. Mahesparan, P. O. Enger, R. Timpl, B. R. Olsen, M. H. B. Hjelstuen, O. Haraldseth and R. Bjerkvig, Nat Biotech, 2001, **19**, 29-34.
3. H. Yoshii, A. Soottitantawat, X.-D. Liu, T. Atarashi, T. Furuta, S. Aishima, M. Ohgawara and P. Linko, Innovative Food Science & Emerging Technologies, 2001, **2**, 55-61.
4. B. M. Choudary, B. P. Prasad and M. L. Kantam, J. Agric. Food Chem., 1989, **37**, 1422-1425.
5. E. Mathiowitz, J. S. Jacob, Y. S. Jong, G. P. Carino, D. E. Chickering, P. Chaturvedi, C. A. Santos, K. Vijayaraghavan, S. Montgomery, M. Bassett and C. Morrell, Nature, 1997, **386**, 410-414.
6. N. A. Peppas and L. Brannon-Peppas, J. Control. Release, 1996, **40**, 245-250.
7. S. U. Pickering, Journal of the Chemical Society, 1907, **91**, 2001-2021.
8. S. Levine, B. D. Bowen and S. J. Partridge, Colloids Surf., 1989, **38**, 325-343.
9. B. P. Binks and S. O. Lumsdon, Phys. Chem. Chem. Phys., 1999, **1**, 3007-3016.
10. B. P. Binks and S. O. Lumsdon, Langmuir, 2001, **17**, 4540-4547.
11. J. I. Amalvy, S. P. Armes, B. P. Binks, J. A. Rodrigues and G. F. Unali, Chem. Commun., 2003, **15**, 1826-1827.
12. E. S. Read, S. Fujii, J. I. Amalvy, D. P. Randall and S. P. Armes, Langmuir, 2004, **20**, 7422-7429.
13. B. P. Binks, R. Murakami, S. P. Armes and S. Fujii, Angew. Chem., Int. Ed., 2005, **44**, 4795-4798.
14. O. D. Velev, K. Furusawa and K. Nagayama, Langmuir, 1996, **12**, 2374-2384.
15. O. D. Velev, K. Furusawa and K. Nagayama, Langmuir, 1996, **12**, 2385-2391.
16. O. D. Velev and K. Nagayama, Langmuir, 1997, **13**, 1856-1859.
17. A. D. Dinsmore, M. F. Hsu, M. G. Nikolaides, M. Marquez, A. R. Bausch and D. A. Weitz, Science, 2002, **298**, 1006-1009.
18. M. F. Hsu, M. G. Nikolaides, A. D. Dinsmore, A. R. Bausch, V. D. Gordon, X. Chen, J. W. Hutchinson and D. A. Weitz, Langmuir, 2005, **21**, 2963-2970.
19. S. Laib and A. F. Routh, J. Colloid Interface Sci., 2008, **317**, 121-129.
20. H. N. Yow and A. F. Routh, Langmuir, 2009, **25**, 159-166.

21. J. W. O. Salari, J. van Heck and B. Klumperman, Langmuir, 2010, **26**, 14929-14936.
22. V. D. Gordon, C. Xi, J. W. Hutchinson, A. R. Bausch, M. Marquez and D. A. Weitz, J. Am. Chem. Soc., 2004, **126**, 14117-14122.
23. D. B. Lawrence, T. Cai, Z. Hu, M. Marquez and A. D. Dinsmore, Langmuir, 2007, **23**, 395-398.
24. F. Caruso, R. A. Caruso and H. Möhwald, Science, 1998, **282**, 1111-1114.
25. F. Caruso, R. A. Caruso and H. Möhwald, Chem. Mater., 1999, **11**, 3309-3314.
26. F. Caruso, M. Spasova, A. Susha, M. Giersig and R. A. Caruso, Chem. Mater., 2000, **13**, 109-116.
27. F. Caruso, X. Y. Shi, R. A. Caruso and A. Susha, Adv. Mater., 2001, **13**, 740-744.
28. R. A. Caruso, A. Susha and F. Caruso, Chem. Mater., 2001, **13**, 400-409.
29. J. Li and H. D. H. Stöver, Langmuir, 2010, **26**, 15554-15560.
30. F. J. Rossier-Miranda, K. Schroën and R. Boom, Food Hydrocoll., 2012, **27**, 119-125.
31. M. Williams, S. P. Armes and D. W. York, Langmuir, 2012, **28**, 1142-1148.
32. H. Liu, X. Gu, M. Hu, Y. Hu and C. Wang, RSC Advances, 2014, **4**, 16751-16758.
33. O. J. Cayre, P. F. Noble and V. N. Paunov, J. Mater. Chem., 2004, **14**, 3351-3355.
34. P. F. Noble, O. J. Cayre, R. G. Alargova, O. D. Velev and V. N. Paunov, J. Am. Chem. Soc., 2004, **126**, 8092-8093.
35. H. W. Duan, D. Y. Wang, N. S. Sobal, M. Giersig, D. G. Kurth and H. Mohwald, Nano Lett., 2005, **5**, 949-952.
36. W. C. Mak, J. Bai, X. Y. Chang and D. Trau, Langmuir, 2009, **25**, 769-775.
37. C. Y. Wang, H. X. Liu, Q. X. Gao, X. X. Liu and Z. Tong, ChemPhysChem, 2007, **8**, 1157-1160.
38. H. X. Liu, C. Y. Wang, Q. X. Gao, X. X. Liu and Z. Tong, Int. J. Pharm., 2008, **351**, 104-112.
39. R. T. Woodward, L. Chen, D. J. Adams and J. V. M. Weaver, J. Mater. Chem., 2010, **20**, 5228-5234.
40. L. Hong, S. Jiang and S. Granick, Langmuir, 2006, **22**, 9495-9499.
41. S. Jiang and S. Granick, Langmuir, 2008, **24**, 2438-2445.
42. Y. H. Chen, C. Y. Wang, J. X. Chen, X. X. Liu and Z. Tong, Journal of Polymer Science Part a-Polymer Chemistry, 2009, **47**, 1354-1367.
43. S. A. F. Bon, S. Cauvin and P. J. Colver, Soft Matter, 2007, **3**, 194-199.
44. T. Chen, P. J. Colver and S. A. F. Bon, Adv. Mater., 2007, **19**, 2286-2289.

45. W. Chen, X. Liu, Y. Liu and H.-I. Kim, Colloid Polym. Sci., 2010, **288**, 1393-1399.
46. D. Yin, Q. Zhang, C. Yin, X. Zhao and H. Zhang, Polym. Adv. Technol., 2012, **23**, 273-277.
47. J. Cejková, J. Hanus and F. Stepánek, J. Colloid Interface Sci., 2010, **346**, 352-360.
48. P. N. Sturzenegger, U. T. Gonzenbach, S. Koltzenburg, J. Martynczuk and L. J. Gauckler, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2014, **447**, 44-50.
49. O. J. Cayre and S. Biggs, J. Mater. Chem., 2009, **19**, 2724-2728.
50. O. J. Cayre and S. Biggs, Advanced Powder Technology, 2010, **21**, 19-22.
51. N. N. Shahidan, R. Liu, S. Thaiboonrod, C. Alexander, K. M. Shakesheff and B. R. Saunders, Langmuir, 2013, **29**, 13676-13685.
52. L. M. Croll and H. D. H. Stöver, Langmuir, 2003, **19**, 5918-5922.
53. L. M. Croll and H. D. H. Stöver, Langmuir, 2003, **19**, 10077-10080.
54. Y. Lin, H. Skaff, A. Böker, A. D. Dinsmore, T. Emrick and T. P. Russell, J. Am. Chem. Soc., 2003, **125**, 12690-12691.
55. H. Skaff, Y. Lin, R. Tangirala, K. Breitenkamp, A. Böker, T. P. Russell and T. Emrick, Adv. Mater., 2005, **17**, 2082-2086.
56. P. Arumugam, D. Patra, B. Samanta, S. S. Agasti, C. Subramani and V. M. Rotello, J. Am. Chem. Soc., 2008, **130**, 10046-10047.
57. R. K. Shah, J. W. Kim and D. A. Weitz, Langmuir, 2010, **26**, 1561-1565.
58. K. L. Thompson and S. P. Armes, Chem. Commun., 2010, **46**, 5274-5276.
59. K. L. Thompson, S. P. Armes, J. R. Howse, S. Ebbens, I. Ahmad, J. H. Zaidi, D. W. York and J. A. Burdis, Macromolecules, 2010, **43**, 10466-10474.
60. K. L. Thompson, S. P. Armes, D. W. York and J. A. Burdis, Macromolecules, 2010, **43**, 2169-2177.
61. Z. An, Q. Shi, W. Tang, C.-K. Tsung, C. J. Hawker and G. D. Stucky, J. Am. Chem. Soc., 2007, **129**, 14493-14499.
62. A. Walsh, K. L. Thompson, S. P. Armes and D. W. York, Langmuir, 2010, **26**, 18039-18048.
63. Y. Cui and J. S. van Duijneveldt, Langmuir, 2012, **28**, 1753-1757.
64. M. Williams, S. P. Armes, P. Verstraete and J. Smets, Langmuir, 2014, **30**, 2703-2711.

65. Q. Yuan, O. J. Cayre, S. Fujii, S. P. Armes, R. A. Williams and S. Biggs, Langmuir, 2010, **26**, 18408-18414.
66. P. van Rijn, N. C. Mougin, D. Franke, H. Park and A. Boker, Chem. Commun., 2011, **47**, 8376-8378.
67. G. R. Yi, V. N. Manoharan, S. Klein, K. R. Brzezinska, D. J. Pine, F. F. Lange and S. M. Yang, Adv. Mater., 2002, **14**, 1137-1140.
68. N. P. Ashby, B. P. Binks and V. N. Paunov, Phys. Chem. Chem. Phys., 2004, **6**, 4223-4225.
69. A. J. Morse, S.-Y. Tan, E. C. Giakoumatos, G. B. Webber, S. P. Armes, S. Ata and E. J. Wanless, Soft Matter, 2014, **10**, 5669-5681.
70. K. L. Thompson, E. C. Giakoumatos, S. Ata, G. B. Webber, S. P. Armes and E. J. Wanless, Langmuir, 2012, **28**, 16501-16511.
71. S. Fortuna, C. A. L. Colard, A. Troisi and S. A. F. Bon, Langmuir, 2009, **25**, 12399-12403.
72. T. Einert, P. Lipowsky, J. Schilling, M. J. Bowick and A. R. Bausch, Langmuir, 2005, **21**, 12076-12079.
73. A. R. Bausch, M. J. Bowick, A. Cacciuto, A. D. Dinsmore, M. F. Hsu, D. R. Nelson, M. G. Nikolaides, A. Travesset and D. A. Weitz, Science, 2003, **299**, 1716-1718.
74. P. Lipowsky, M. J. Bowick, J. H. Meinke, D. R. Nelson and A. R. Bausch, Nat. Mater., 2005, **4**, 407-411.
75. R. T. Rosenberg and N. Dan, Langmuir, 2011, **27**, 8729-8734.
76. D. Lee and D. A. Weitz, Adv. Mater., 2008, **20**, 3498-3503.
77. J. S. Sander and A. R. Studart, Langmuir, 2013, **29**, 15168-15173.
78. O. J. Cayre, J. Hitchcock, M. S. Manga, S. Fincham, A. Simoes, R. A. Williams and S. Biggs, Soft Matter, 2012, **8**, 4717-4724.
79. G. Stephenson, R. M. Parker, Y. Lan, Z. Yu, O. A. Scherman and C. Abell, Chem. Commun., 2014, **50**, 7048-7051.
80. R. T. Rosenberg and N. R. Dan, J. Colloid Interface Sci., 2010, **349**, 498-504.
81. R. T. Rosenberg and N. R. Dan, J. Colloid Interface Sci., 2011, **354**, 478-482.
82. J. W. Kim, A. Fernandez-Nieves, N. Dan, A. S. Utada, M. Marquez and D. A. Weitz, Nano Lett., 2007, **7**, 2876-2880.
83. M. A. San, J. Scrimgeour, J. E. Curtis and S. H. Behrens, Soft Matter, 2010, **6**, 3163-3166.

84. A. San Miguel and S. H. Behrens, Soft Matter, 2011, **7**, 1948-1956.
85. C. Wu, S. Bai, M. B. Ansorge-Schumacher and D. Wang, Adv. Mater., 2011, **23**, 5694-5699.
86. P. H. R. Keen, N. K. H. Slater and A. F. Routh, Langmuir, 2012, **28**, 1169-1174.
87. P. H. R. Keen, N. K. H. Slater and A. F. Routh, Langmuir, 2012, **28**, 16007-16014.
88. S. Zhou, J. Fan, S. S. Datta, M. Guo, X. Guo and D. A. Weitz, Adv. Funct. Mater., 2013, **23**, 5925-5929.
89. P. H. R. Keen, N. K. H. Slater and A. F. Routh, Langmuir, 2014, **30**, 1939-1948.
90. K. L. Thompson, P. Chambon, R. Verber and S. P. Armes, J. Am. Chem. Soc., 2012, **134**, 12450-12453.

Tables and Figures

Table 1. Comparison of the various methods described in the literature to prepare covalently cross-linked colloidosomes

Particle Type	Particle Diameter (nm)	Emulsion Type	Cross-linker/ cross-linking chemistry	Encapsulation/ release data	Ref.
Poly-(divinylbenzene-alt-maleic anhydride) microspheres	600	o/w	Addition of polyamines	no	52
Vinylbenzene-functionalized CdSe nanoparticles	2.9	w/o or o/w	Polymerization of vinylbenzene units with VA-044	no	54
CdSe/ZnS quantum dots	3.5	w/o	ROMP using Ru-based Grubbs catalyst	no	55
Terpyridinethiol-functionalized FePt nanoparticles	7	w/o	Complexation of terpyridine with Fe(II) ions	no	56
Primary amine-functionalized PNIPAM microgels	750	w/o	Glutaraldehyde	no	57
Hydroxy-functionalized PS latex	100-1000	o/w	tolylene 2,4-diisocyanate-terminated poly(propylene glycol) (PPG-TDI)	yes	58, 59
Poly(ethyleneimine)-stabilized PS latex	100-200	o/w	tolylene 2,4-diisocyanate-terminated poly(propylene glycol), poly(propylene glycol) diglycidyl ether or poly(ethylene glycol) diglycidyl ether.	no	62
poly(2-dimethylaminoethyl methacrylate)-block-poly(methyl methacrylate) stabilized PS latex	345	o/w	1,2-bis(2-iodoethyloxy)ethane (BIEE)	no	65

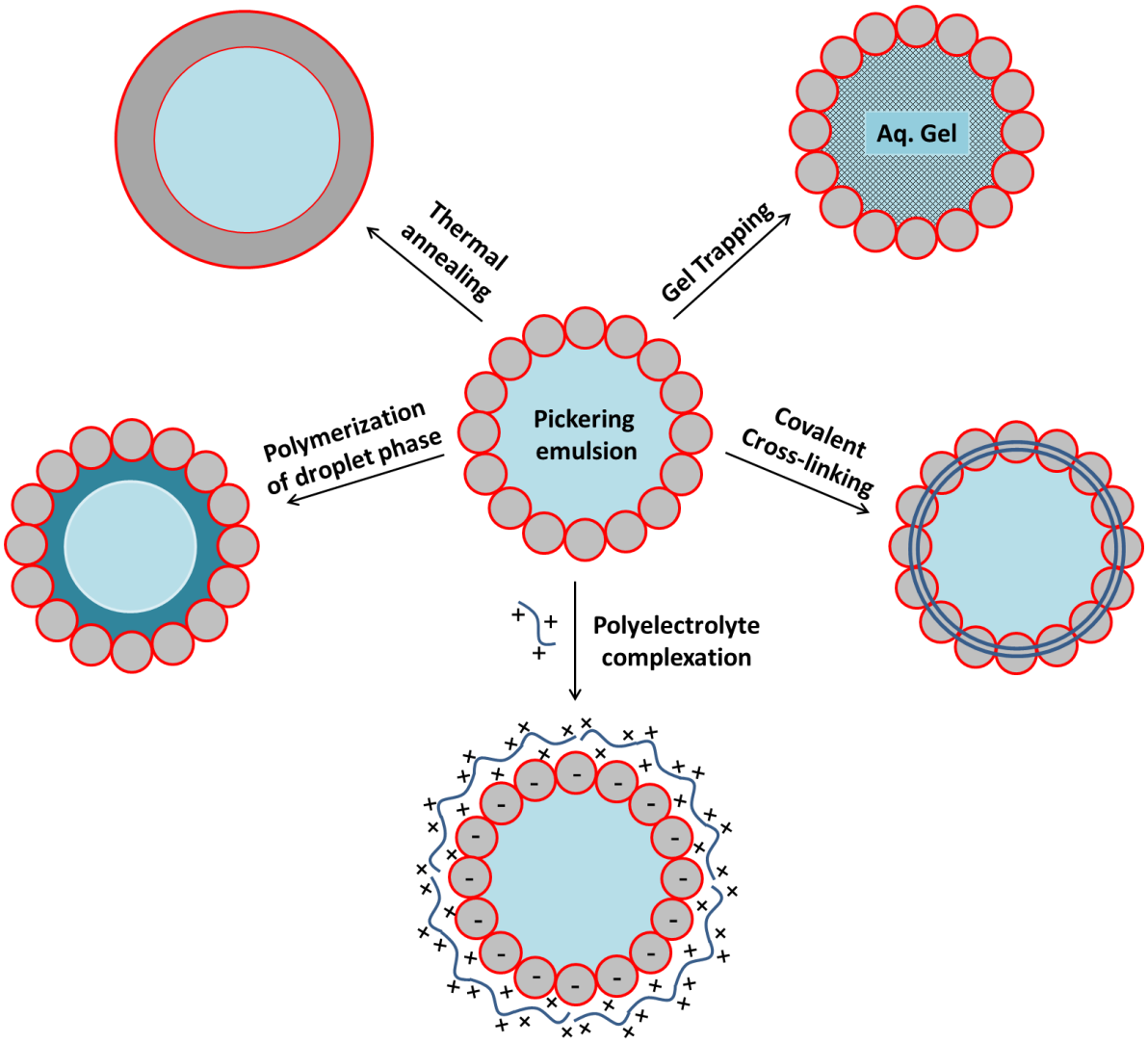

Figure 1. Summary of the various routes investigated for the preparation of colloidosome microcapsules via self-assembly of colloid particles at the oil/water interface.

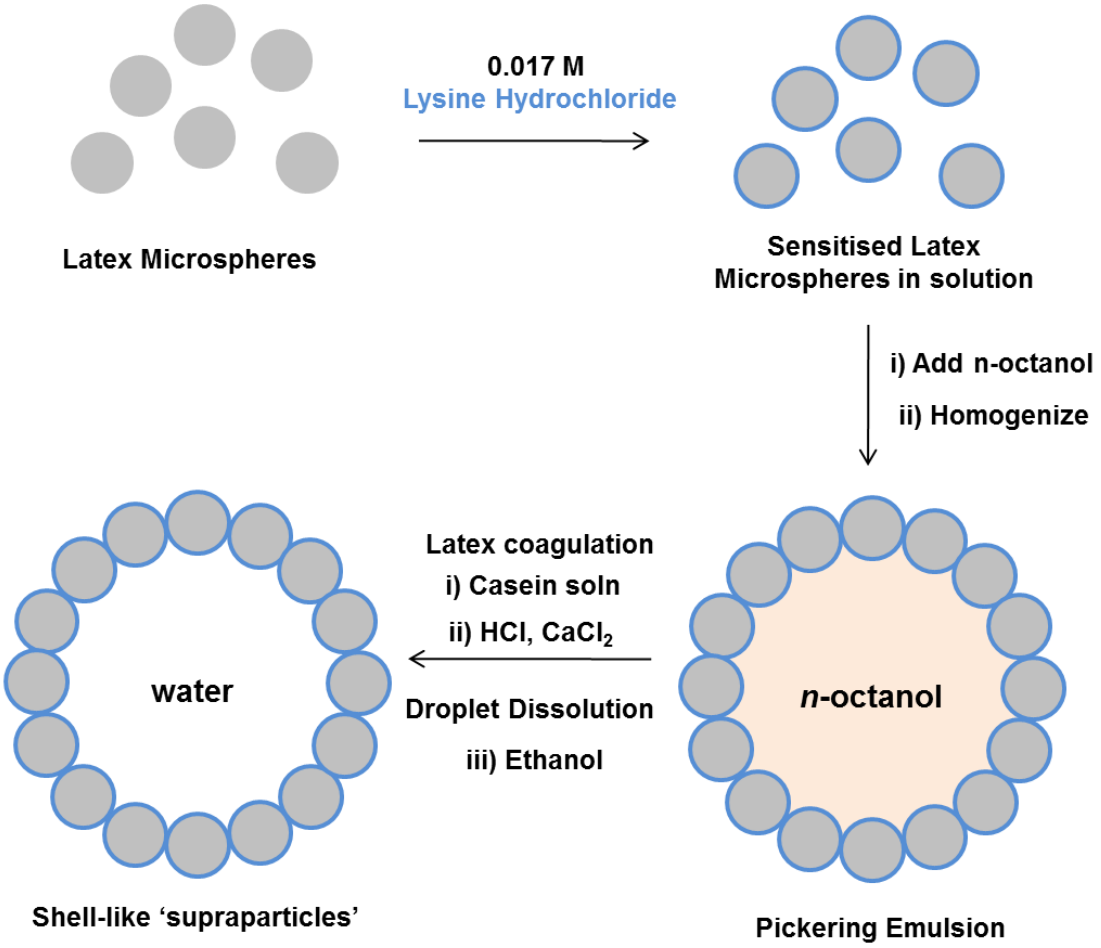

Figure 2. Preparation of shell-like 'supraparticles' formed from a Pickering emulsion comprising sensitized latex particles and n-octanol as the oil phase.[14] The neighbouring latex particles are locked in place using a strong coagulating agent, which allows subsequent dissolution of the n-octanol droplet template in the presence of excess ethanol.

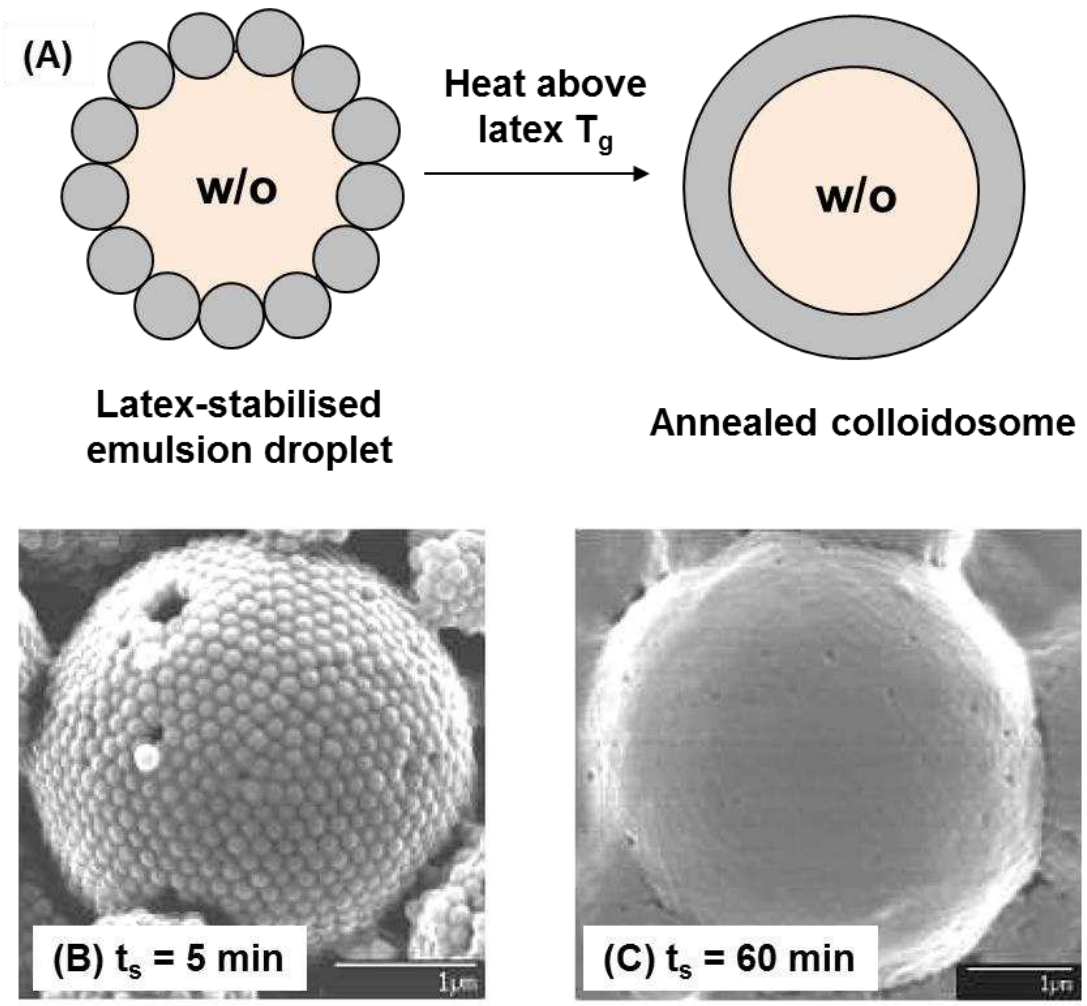

Figure 3. (A) Schematic preparation of colloidosomes via thermal annealing: adjacent copolymer latex particles are locked together by heating above the copolymer T_g to induce coalescence to form a contiguous shell. Scanning electron microscopy images recorded for a colloidosome prepared from a water-in-oil emulsion stabilized using poly(styrene-stat-n-butyl acrylate) latex followed by sintering at 50°C for (B) 5 min. and (C) 60 min. Adapted with permission from ref. 20.

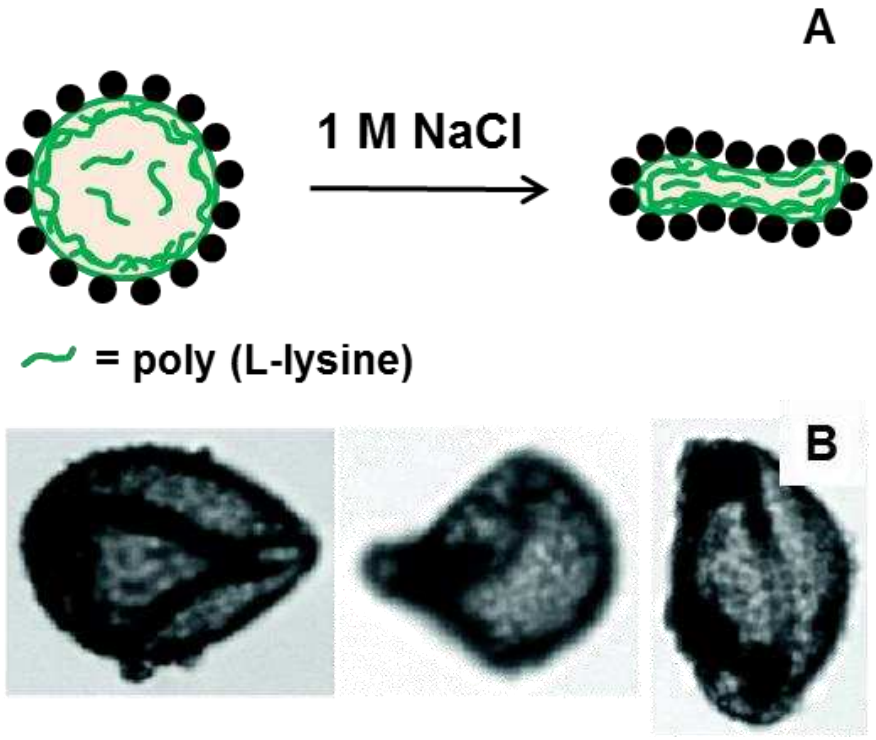

Figure 4. (A) Poly(L-lysine)-stabilized colloidosomes are microcapsules inflated by osmotic pressure that completely collapse on addition of 1 M NaCl. (B) Optical microscopy images of such deflated colloidosomes. Adapted with permission from ref. 22.

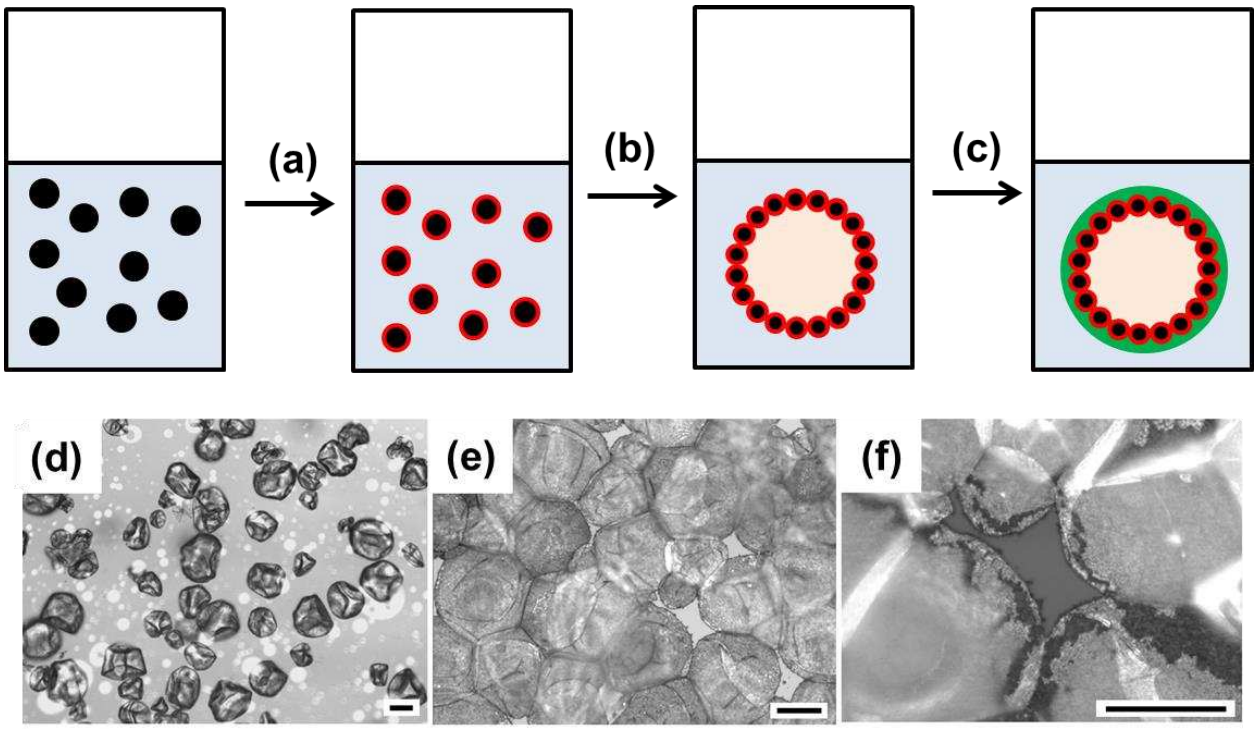

Figure 5. Preparation of colloidosomes using the layer-by-layer (L-b-L) deposition technique. (a) LUDOX CL cationic silica nanoparticles are first surface-modified by addition of poly(sodiumstyrenesulfonate) (PSS), (b) emulsified with an oil to form an o/w Pickering emulsion and (c) subsequent sequential L-b-L assembly of PDADMAC and PSS to build up and reinforce the microcapsule shell. Optical micrographs recorded in the wet state (d) and dry state (e, f) of such microcapsules. All scale bars are 50 µm. Adapted with permission from ref. 29.

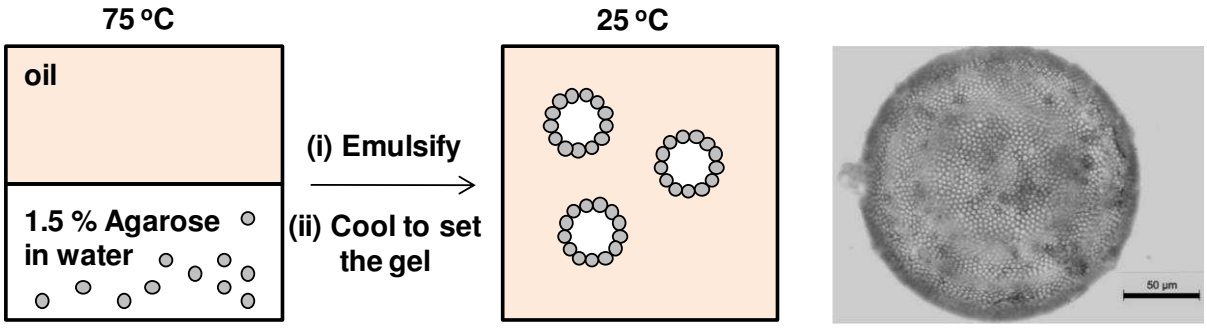

Figure 6. Preparation of colloidosomes via the gel trapping technique. An optical micrograph is shown of a colloidosome prepared with an aqueous phase containing 3.9 µm amine-functionalized polystyrene latex particles and 1.5 % agarose. This mixture is homogenized with sunflower oil at 75^{o}C. The emulsion is then cooled to allow the gel to set and the colloidosomes are transferred from the continuous oil phase into water. Adapted with permission from ref. 33.

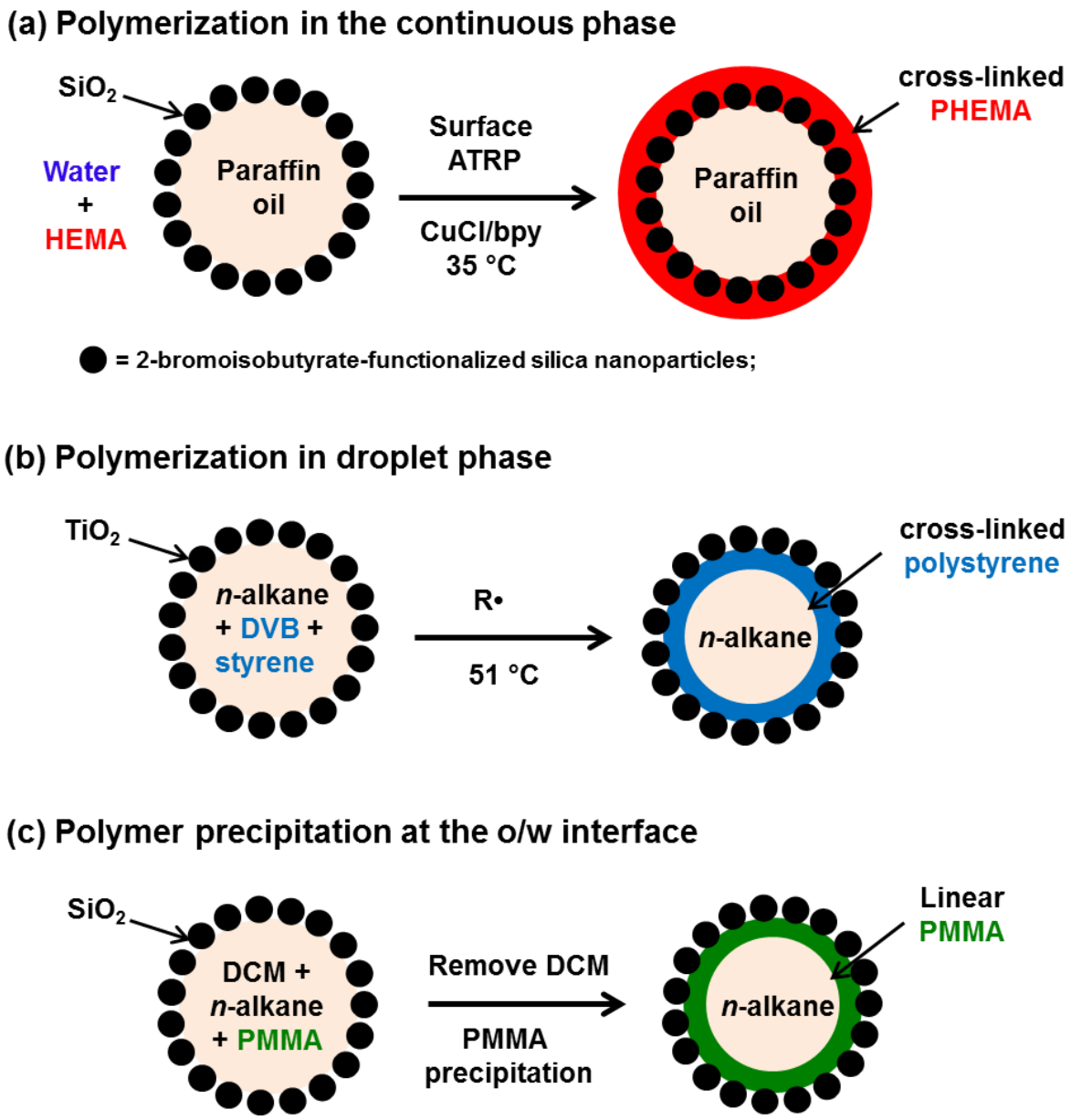

Figure 7. Schematic representation of colloidosome formation via polymerization at the interface of a Pickering emulsion. (a) Silica particle modified with ATRP initiator groups are used to polymerize HEMA from the external aqueous phase,[42] (b) styrene, divinylbenzene and initiator are dissolved the n-alkane oil phase then then polymerized from within the droplet to form cross-linked polystyrene at the o/w interface[44] and (c) a preformed linear PMMA is dissolved in the oil core of a Pickering emulsion, upon removal of the DCM (a good solvent for PMMA) the polymer precipitates at the interface trapping the SiO_2 particles.[49]

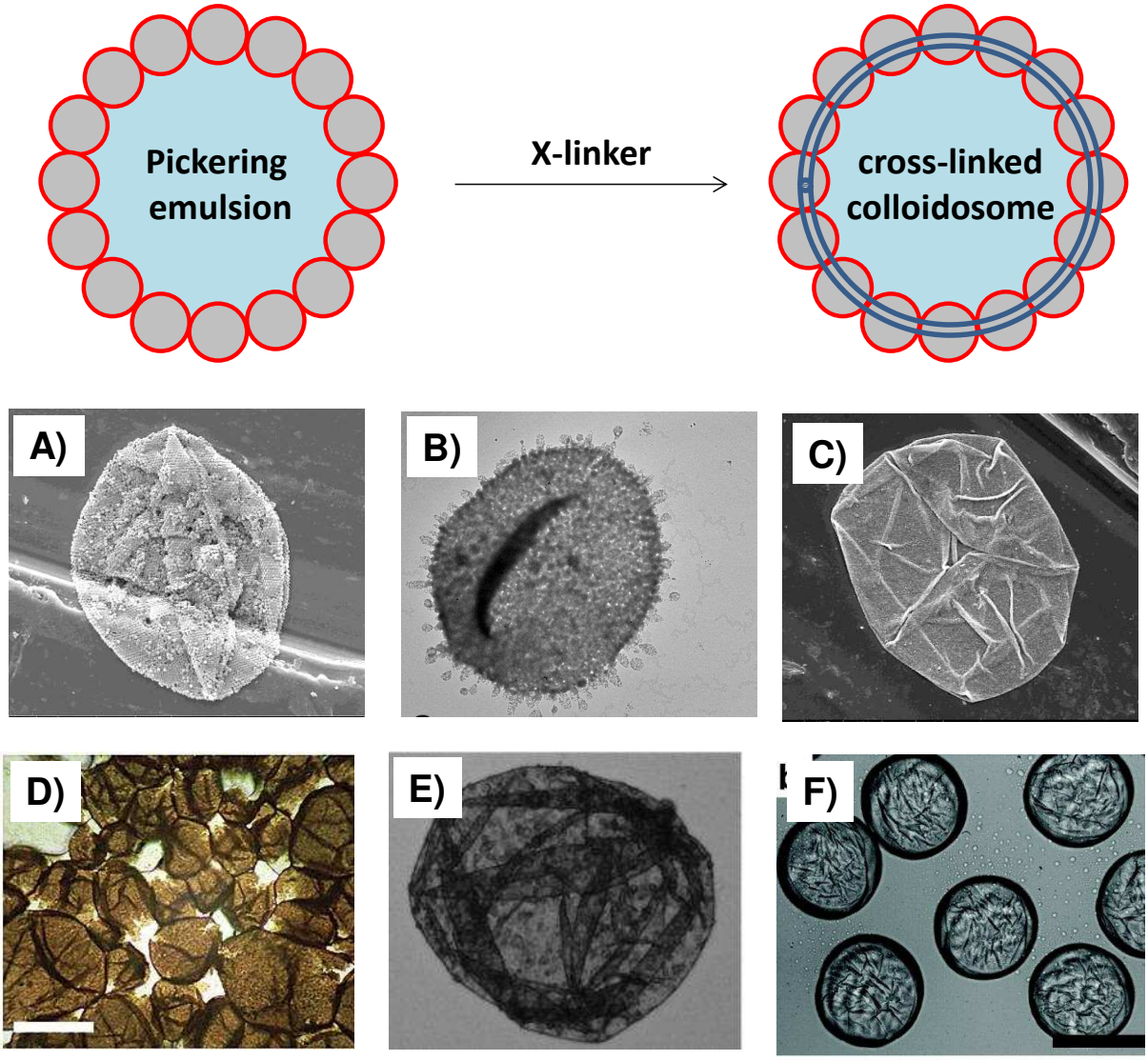

Figure 8. Colloidosomes prepared by covalent cross-linking of colloidal particles at the oil/water interface. Images of colloidosomes cross-linked using a polymeric diisocyanate using (A) poly(glycerol monomethacrylate)-stabilized polystyrene latex[59] and (B) cross-linked poly(glycerol monomethacrylate)-block-poly(2-hydroxypropyl methacrylate) vesicles.[90] (C) polyamine-stabilized polystyrene latex cross-linked using poly(propylene glycol) diglycidyl ether,[62] (D) poly(divinylbenzene-alt-maleic anhydride) microspheres cross-linked from the aqueous continuous phase by addition of polyamines,[52] (E) poly(2-(dimethylamino)ethyl methacrylate)-block-poly(methyl methacrylate) stabilized PS latex cross-linked using 1,2-bis(2-iodoethyloxy)ethane[65] and (F) primary amine-functionalized PNIPAM microgels cross-linked using glutaraldehyde.[57] Adapted with permission from refs. [52, 57, 59, 62, 65, 90].

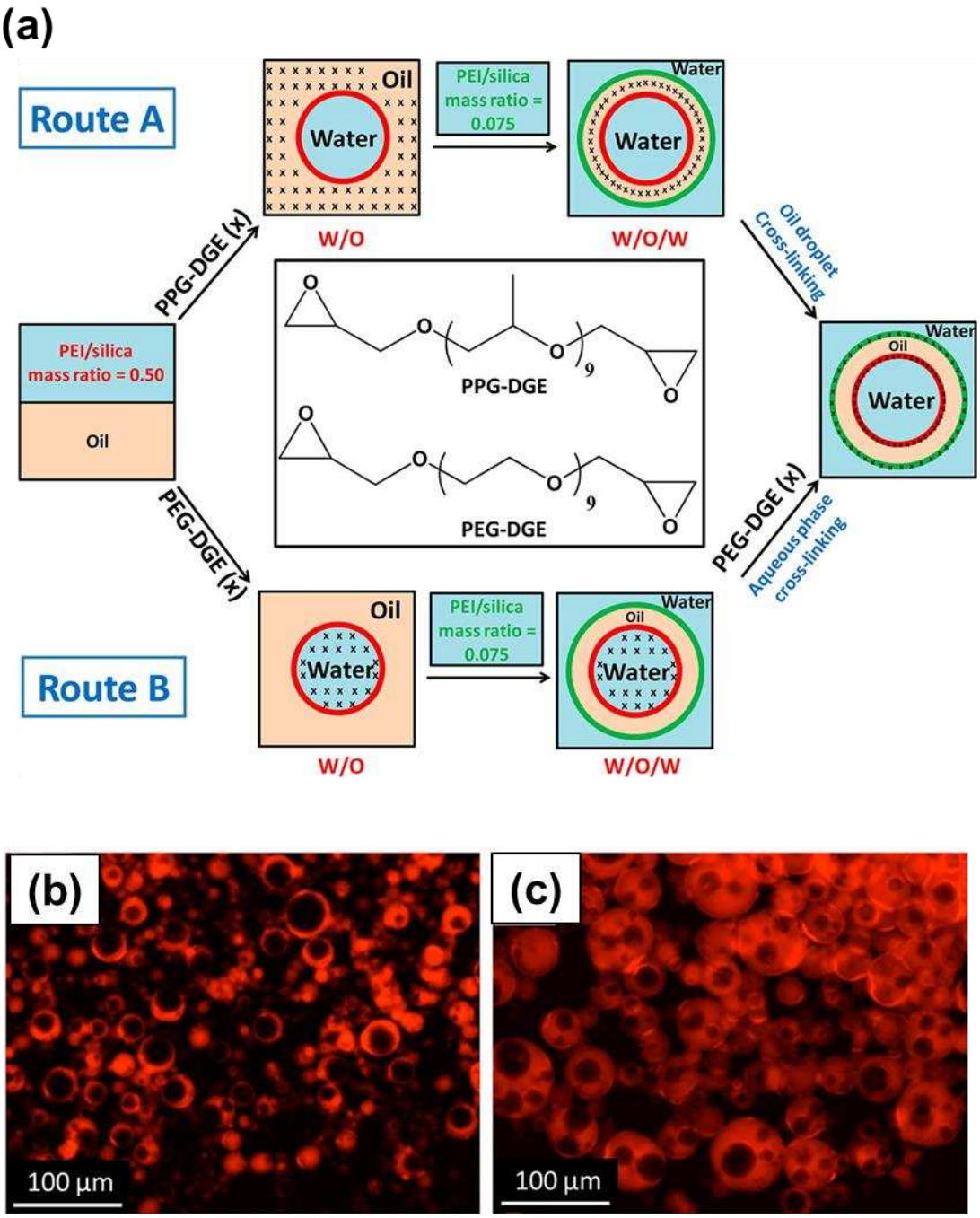

Figure 9. (A) Schematic illustration of the synthesis of novel multicompartment microcapsules using two types of PEI/silica hybrid particles as Pickering emulsifiers at pH 10. Route A depicts the formation of colloidosome-in-colloidosome microcapsules by dissolving an oil-soluble polymeric cross-linker (PPG-DGE) in the oil phase prior to any homogenization. Route B depicts colloidosome-in-colloidosome formation via two separate charges of a water-soluble polymeric cross-linker (PEG-DGE). Fluorescence microscopy images obtained with Nile Red dissolved in the oil phase for (B) w/o/w double emulsion (prepared using an oil volume fraction of 10 %) and (C) w/o/w double emulsion (prepared using oil volume fraction of 25%). Adapted with permission from ref 64.

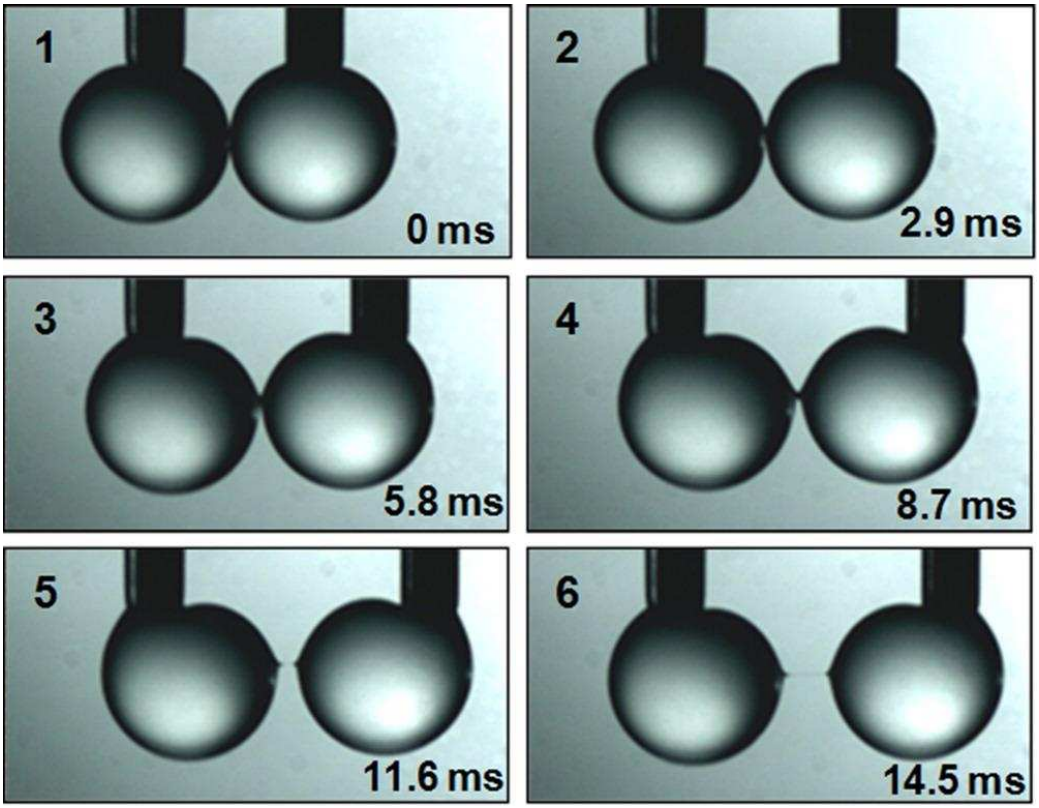

Figure 10. Still images taken from the asymmetric droplet experiment showing two droplets grown in the presence of $PGMA_{50}$–PS particles being moved apart. The left-hand droplet contained pure sunflower oil, and the right-hand droplet contained PPG-TDI cross-linker in sunflower oil. The droplets were aged in isolation for 20 min before being moved into contact. They were left in contact for 2 h and then moved apart. Reproduced with permission from ref. 70.

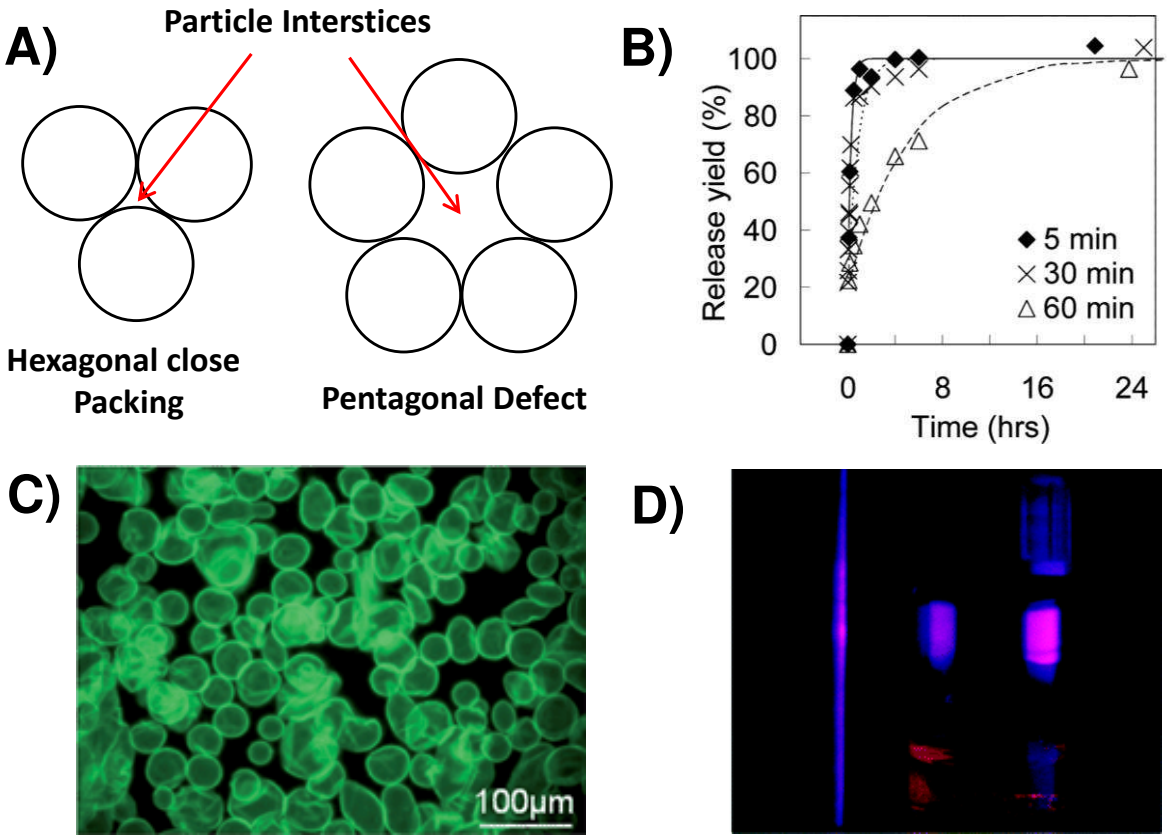

Figure 11. (a) Schematic representation to illustrate the two types of interstices present at the surface of colloidosomes prepared using near-monodisperse spherical particles. Examples of colloidosomes used to encapsulate: (b) a small molecule water-soluble dye (fluorescein),[20] (c) a polymeric dye (70 kDa fluorescently-labeled dextran)[78] and (d) 4 nm CdTe red-emitting nanoparticles trapped inside magnetic colloidosomes prepared using 5 nm diameter Fe_3O_4 nanoparticles.[35] Adapted with permission from refs. 20, 78 and 35.

Table of Contents Graphical Abstract

Colloidosomes: Synthesis, Properties and Applications

Kate L. Thompson*, Mark Williams and Steven P. Armes*

Dainton Building, Department of Chemistry, University of Sheffield,

Brook Hill, Sheffield, S3 7HF, UK