Principles and Characteristics of Polymerization-Induced Self-Assembly with Various Polymerization Techniques

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A chemical reaction that drives a physical polymer self-assembly process, namely, polymerization-induced self-assembly (PISA), combines block copolymer synthesis and nanoparticle formation efficiently at high polymer concentrations. Various nanoparticle morphologies such as spheres, worms, and vesicles can be prepared readily in polar and nonpolar media. PISA has been well developed in combination with reversible addition-fragmentation chain transfer (RAFT) polymerization. Notably, developments with other polymerization methods are also achieved. In this report, first, we discuss the general principles of RAFT-PISA and the nanoparticles generated from this method. Specifically, new insights into polymer nucleation and subsequent morphological evolution are highlighted. Subsequently, PISA formulations that use other polymerization methods [atom transfer radical polymerization (ATRP), nitroxide-mediated polymerization (NMP), ring-opening metathesis polymerization (ROMP), and ring-opening polymerization (ROP) of N-carboxyanhydrides (NCAs)] are summarized in detail. Finally, more exotic PISA formulations are emphasized: these are based on organotellurium-mediated living radical polymerization (TERP), living anionic polymerization (LAP), addition-fragmentation chain transfer (AFCT) polymerization, reversible complexation-mediated polymerization (RCMP), and cobalt-mediated radical polymerization (CMRP), or utilize a comonomer that undergoes radical ring-opening polymerization (rROP). This review is concluded with a perspective on the status and potential of PISA.

Keywords: polymerization-induced self-assembly, block copolymers, nanoparticles, dispersion polymerization, emulsion polymerization
Introduction

As polymer nanoparticles become more applicable, the demand for their efficient synthesis grows. Polymer synthesis methods that induce nanoparticle formation are highly desired. Various polymerization techniques that enable in situ nanoparticle preparation are precipitation polymerization, dispersion polymerization, and emulsion polymerization. The former two utilize miscible monomers; the latter two utilize a polymer stabilizer or surfactant. These three techniques are based on a growing insoluble polymer chain or network that is stabilized by various means, and therefore, remain dispersed in the preparation media. An intrinsic limitation of these methods is that the resulting nanoparticle morphology is mainly spherical, lacking diversity in nanoparticle morphology. Furthermore, in the last two cases, stabilizer polymers or surfactants are not always desired in the final polymer product, and removal is often difficult.

Other commonly used nanoparticle preparation methods are polymerization-induced self-assembly (PISA), and the more traditional postpolymerization solvent switch approach. Compared with the previously discussed approaches, these two methods utilize block copolymers consisting of a soluble block and an insoluble block. The resulting spherical particles can, therefore, also be seen as polymer micelles; higher order morphologies can also be achieved. To some extent, PISA is a type of dispersion (or emulsion) polymerization—an insoluble polymer block grows from a soluble (or immiscible) monomer. In contrast, conventional dispersion or emulsion polymerization utilizes a separate polymer stabilizer or surfactant for the preparation of latex particles. These stabilizers are, therefore, not chemically attached to the “core-forming” polymer. PISA is based on the chain extension of an initial soluble precursor block, which acts as a steric stabilizer, with a second insoluble polymer block that forms the nanoparticle core in situ. This polymerization reaction triggers diblock copolymer self-assembly once a sufficiently high degree of polymerization (DP) of the core-forming block is attained. PISA is efficient because polymer synthesis and assembly occur simultaneously. Furthermore, PISA can be performed at a range of final polymer concentrations (5–50% w/w). The most commonly observed nanoparticle morphologies obtained from PISA are spheres, worms, and vesicles. As far as we are aware, the first example of such a synthetic approach was reported in 2002, and its term “PISA” first appeared in the academic literature in 2009.

The other frequently-used block copolymer self-assembly method, the solvent switch approach, is a well-established post-polymerization procedure that is based on the dropwise addition of a selective solvent to a dilute block copolymer solution. The addition of this selective solvent leads to the insolubility of one of the polymer blocks. This process triggers block copolymer self-assembly into various nanoparticle morphologies once a certain solvent ratio is attained. Subsequently, extensive dialysis is required to remove the initial good solvent. This approach is generally conducted at a polymer concentration of ~1–3% w/w and might, therefore, seem inefficient compared with PISA. However, the solvent switch method allows a wider variety of polymers than PISA since this method is independent of the polymerization technique. In other words, the desired polymer is not always easily accessible with a polymerization technique that is well established for PISA. Hence, the solvent switch method is still widely used for the preparation of various application-oriented functional nanoparticles. Indeed, this also means that the development of new PISA protocols with different polymerization methods is highly desired.

Here, the principles and characteristics of PISA are described with the focus on several polymerization methods that allow various types of monomers to be utilized. Reversible addition-fragmentation chain transfer (RAFT)-PISA is discussed first since this is unarguably the most well-established PISA method. General RAFT-PISA principles and nanoparticle characteristics are highlighted. Thereafter, the characteristics of other PISA formulations, based on other polymerization techniques, including atom transfer radical polymerization (ATRP), nitroxide-mediated polymerization (NMP), ring-opening metathesis polymerization (ROMP), and ring-opening polymerization (ROP) of N-carboxyanhydrides (NCAs), are discussed and compared. Subsequently, more exotic and novel PISA formulations are highlighted. These are based on organotellurium-mediated living radical polymerization (TERP), living anionic polymerization (LAP), addition-fragmentation chain transfer (AFCT), reversible complexation-mediated polymerization (RCMP), cobalt-mediated radical polymerization (CMRP), and radical ring-opening polymerization (rROP). This review concludes with a perspective on PISA, highlighting its current potential and recent scientific trends. We would guide the reader to other reviews for a more in-depth discussion of the recent trends in RAFT-PISA and ROMPISA, and an assessment of general PISA reaction conditions suitable for various monomer species. PISA can produce applicable nanoparticles that are functional or reactive, this is discussed in a recent review from Delaître and coworkers. These reviews also provide comprehensive lists of reported PISA formulations; such a list is therefore not given in this review.

RAFT-PISA

RAFT polymerization is the most studied polymerization method for PISA. A range of morphologies can be obtained when using both RAFT dispersion and RAFT emulsion polymerization. Furthermore, this method
allows nanoparticle cross-linking\textsuperscript{17} and can be performed in polar solvents, such as water\textsuperscript{14,16}; various organic solvents and alcohols\textsuperscript{19-22}; and in nonpolar solvents, such as n-alkanes\textsuperscript{15,23} and mineral oil.\textsuperscript{1,24} RAFT-PISA can also be performed in ionic liquids and supercritical CO\textsubscript{2}\textsuperscript{25,26}.

A well-known example of an aqueous RAFT dispersion polymerization, and certainly, the most-studied, is the poly(glycerol monomethacrylate)-block-poly(2-hydroxypropyl methacrylate) (PGMA-b-PHPMA) composition (Figure 1a).\textsuperscript{14,27,28} Here, PGMA forms the soluble stabilizer block, and PHPMA forms the insoluble nanoparticle core. Nonpolar RAFT-PISA formulations are less well-known. A good example of such formulations is the poly(lauryl methacrylate)-block-poly(benzyl methacrylate) (PLMA-b-PBzMA) composition in various n-alkanes (Figure 2c),\textsuperscript{15} where the PLMA blocks act as an oil-soluble stabilizer and the PBzMA blocks from the insoluble nanoparticle core. Commonly observed nanoparticle morphologies are spheres, worms, and vesicles. All three morphologies are observed in polar and nonpolar media. Interestingly,
these three morphologies can also be obtained via RAFT-PISA methods that are based on aqueous emulsion polymerizations.16,31

RAFT-PISA formulations are also well-known for the “PISA phase diagrams” (Figures 1b and 1c) (N.B., these diagrams are not related to the traditional physical phase diagrams that consist of the three physical states of matter (solid, liquid, and gas)). From these PISA phase diagrams it is clear that both the stabilizer and core-forming block DP (or block volumes) can be altered to target the desired nanoparticle morphology reproducibly. This aspect is often referred to as the packing parameter. However, it is essential to realize that the same copolymer composition can form different nanoparticle morphologies when the solid content is varied (total polymer concentration). This can be explained by the morphological nanoparticle evolution mechanism during RAFT-PISA, which is generally limited to spherical nanoparticles when conducted at low concentrations (as described later in this review).

There are essential differences between nanoparticles prepared via RAFT-PISA in water and nonpolar media: nanoparticles in water can have lower critical solution temperature (LCST) characteristics,29,32–35 while nanoparticles in nonpolar media tend to have upper critical solution temperature (UCST) characteristics.30,34,37 In other words, the insoluble core-forming block of PGMA-b-PHPMA nanoparticles in water, and PLMA-b-PBzMA nanoparticles in oil, becomes partially solvated at specific temperatures. The nanoparticle cores with LCST characteristics in water become progressively plasticized on cooling, while nanoparticle cores with UCST characteristics in nonpolar media become progressively plasticized on heating.

Core plasticization is well documented for nanoparticle worms obtained from RAFT-PISA. Such worm dispersions form a viscous gel when prepared at sufficiently high polymer concentrations. It is believed that worm entanglements cause this viscous state. However, the percolation theory can also explain this phenomenon.38 Alterations in temperature can trigger core plasticization, this can lead to reversible morphological transitions to lower-order morphologies: cooling PGMA-b-PHPMA worms induce a morphological worm-to-sphere transition; the same transition is observed on heating PLMA-b-PBzMA worms (Figures 2a and 2b). Interestingly, this morphological change is characterized by a gel-to-liquid transition. The temperature at which the dispersion no longer forms a worm gel is defined as the critical gelation temperature (CGT).6 Such worm gels are also concentration-dependent: diluting these dispersions can lead to a free-flowing fluid once the critical gelation concentration (CGC) is attained, owing to reduced interworm contacts.38 The reversible nature of the thermally induced worm-to-sphere transition also disappears when the initial worms are sufficiently diluted.50 This suggests that worm regeneration via sphere–sphere fusion is inhibited at low particle concentrations. The morphological worm-to-sphere transition can be prevented by cross-linking the initial worms.39 Indeed, this modification leads to a worm gel that is incapable of forming a free-flowing dispersion upon heat treatment at high solid contents.

A reversible vesicle-to-worm transition can also be achieved according to this principle—resulting in an abrupt increase in viscosity.32,40 It is also noteworthy that pH-responsive nanoparticles have been prepared by aqueous RAFT-PISA that are capable of morphological transitions.41–44 These transitions are based on polymer end groups that become charged upon the addition of acid or base, leading to higher solvation of the nonionic polymer stabilizer block.

Finally, thermally induced morphological transitions are also observed for more complex nanoparticles. The thermal behavior of nanoparticles prepared with an inherently different aqueous PISA protocol was reported recently by Cai and co-workers in 2020.45 In contrast to the previously discussed formulations, this aqueous PISA formulation is based on the chain extension of a macro-molecular chain transfer agent (macro-CTA), poly[N-(2-hydroxypropyl)methacrylamide], with a charged core-forming monomer in the presence of an oppositely charged homopolymer. The resulting nanoparticles are polyelectrolyte complexes. This approach is also known as polymerization-induced electrostatic self-assembly (PIESA). Conform to the previously discussed aqueous PGMA-b-PHPMA formulation, and a reversible morphological sphere-to-worm evolution could be realized on heating (Figure 2a). An irreversible worm-to-vesicle transition was also achieved by altering block DP of the charged core-forming block. This transition was attributed to the dehydration of the stabilizer block, as a result of the formation of hydrogen bonds between the core-forming block and the stabilizer block, instead of core plasticization.

Morphological evolution during RAFT-PISA (from chains to spheres, to worms, to vesicles)

Despite the differences in temperature-responsive behavior, polar and nonpolar RAFT-PISA formulations have similar characteristics in terms of in situ morphological evolution. Generally, this morphological evolution proceeds from dissolved polymer chains to spheres, to worms, to vesicles.46 Herein, each morphological transition is discussed separately.

Polymer chain-to-sphere transition

RAFT-PISA generally proceeds as follows: a soluble polymer precursor block (also known as macro-CTA) is prepared via solution polymerization. After purification, this
macro-CTA is chain extended with a monomer to form a soluble diblock copolymer. The second polymer block becomes progressively insoluble as the polymerization proceeds. Polymer assembly (or nucleation) occurs once a critical core-forming block DP is achieved. Multiple dissolved diblock polymer chains aggregate during this process and form nascent spherical nanoparticles. Here, the second insoluble block forms the nanoparticle core, and the soluble block acts as a steric stabilizer for these initial nanostructures.

One might consider that nucleation leads to premature termination of the RAFT polymerization. However, quite the opposite is observed. Enhanced first-order reaction kinetics is a well-established phenomenon that characterizes nucleation during RAFT-PISA.\textsuperscript{16,24,28,33,47–50} It is presumed that this increase in the reaction rate is caused by a local high monomer concentration within the nascent spherical nanoparticles. In other words, unreacted monomer is likely to diffuse into the nanoparticle cores and stimulate the polymerization reaction. Such small molecule diffusion processes are common. Solvent molecules are also capable of diffusing into a nanoparticle core.\textsuperscript{36,37,51}

New insights into this nucleation-induced increase in the reaction rate were recently reported.\textsuperscript{37} Poly(stearyl methacrylate)-block-poly(2,2,2-trifluoroethyl methacrylate) (PSMA-b-PTFEA) spheres were prepared in n-tetradecane (Figure 3a); a dispersion that can become highly transparent [refractive index (RI)-matched] depending on the applied temperature. This formulation is suitable for studying nucleation during RAFT-PISA since detailed reaction kinetics can be obtained by in situ \textsuperscript{19}F NMR (Figures 3b, 3c, and 3d). A \textsuperscript{19}F NMR spectrum comprises two distinct peaks that represent the fluorinated monomer and the growing fluorinated core-forming block. The polymer peak area in the \textsuperscript{19}F NMR spectrum continues to increase during the polymerization; however, the intensity (peak height) of this peak changes since it describes the polymer nucleation process. A reduction in peak intensity corresponds to the aggregation of polymer chains into nascent spherical nanoparticles. The obtained reaction kinetics for this formulation clearly shows three distinct kinetic reaction regimes. A very modest increase in reaction kinetics is observed after 60 min; an obvious increase is observed after 115 min. Each reaction regime is described as follows: growing dissolved diblock copolymer chains, spheres with nascent micelle cores, and spheres with compact micelle cores. Here, it is suggested that a fraction of the solvent is present within the growing nanoparticle cores, and that this solvent fraction reduces as polymerization proceeds, which would then delay the second larger increase in the reaction rate. Also, similar three-stage reaction kinetics are observed for aqueous PISA formulations.\textsuperscript{53,54} However, more research is desired to explain this phenomenon further.

It is important to consider that the self-assembled spheres obtained from various RAFT-PISA formulations are not “frozen” nanostructures. A recently published paper from Armes and co-workers\textsuperscript{36} described a fast copolymer chain-exchange process between spherical nanoparticles prepared by PISA in nonpolar media. This work showed that a larger DP of the insoluble core-forming block and a higher temperature lead to a faster copolymer chain-exchange process between spherical nanoparticles. This study was conducted on the final nanoparticle spheres (post-PISA). However, it is likely that nanoparticle core-solvation by monomer and hot solvent aids this copolymer chain-exchange process during PISA.

A different study showed that heating PLMA-b-PBzMA polymer spheres in n-dodecane causes a fraction of block copolymer chains to become molecularly dissolved.\textsuperscript{36} Indeed, this causes a reduction in the aggregation number (number of polymer chains per nanoparticle). This study also showed that diblock copolymer spheres with larger core-forming block DPs tended to become less solvated and do not produce molecularly dissolved block copolymers on heating. It is likely that copolymer chain-exchange can also be observed for nanoparticles obtained from aqueous PISA formulations.\textsuperscript{51} However, as far as we are aware, this has, unfortunately, not yet been established. Aqueous polymer nanoparticles are likely to have interesting dynamic characteristics since they can exhibit LCST-characteristics.

Other aspects that need to be considered during the early stages of RAFT-PISA are that the aggregation number and spherical nanoparticle diameter increase as RAFT polymerization proceeds. This characteristic of RAFT-PISA is well established with in situ small-angle X-ray scattering (SAXS) studies on an aqueous emulsion polymerization,\textsuperscript{16} an aqueous dispersion polymerization,\textsuperscript{54} and a nonpolar dispersion polymerization.\textsuperscript{37} The increase in the number of polymer chains per nanoparticle cannot be explained merely by copolymer chain exchange between spherical nanoparticles because this process does not change the aggregation number when in equilibrium. There are three possible reasons for this phenomenon: (1) a fraction of diblock polymer chains is molecularly dissolved during the early stages of PISA. As the core-forming block DP increases, block copolymers become progressively more insoluble and insert in the nascent diblock copolymer nanoparticles. This theory can be further rationalized with the molecular weight distribution (MWD) of copolymer chains: chains with shorter DPs are more likely to be molecularly dissolved. (2) Sphere–sphere fusion causes the formation of larger spherical nanoparticles with a doubled number of copolymer chains. After fusion, the preservation of the
Figure 3 | (a) Molecular structure of PSMA-b-PTFEMA, prepared via RAFT dispersion polymerization in n-tetradecane. (b) In situ $^{19}$F NMR studies during the PISA synthesis of PSMA-b-PTFEMA spheres. The TFEMA monomer peak area increases upon polymerization. The resulting polymer peak intensity decreases after diblock copolymer self-assembly (also known as nucleation). After that, polymerization occurs within the monomer (and solvent) swollen nanoparticles. (c) Three separate kinetics reaction regimes are observed during the polymerization of TFEMA. (d) Normalized PTFEMA $^{19}$F NMR peak intensities plotted against TFEMA monomer conversion confirm three separate reaction stages. Reprinted with permission from ref 37. Copyright 2018 American Chemical Society. PSMA-b-PTFEMA, poly(stearyl methacrylate)-block-poly(2,2,2-trifluoroethyl methacrylate); RAFT, reversible addition-fragmentation chain transfer; PISA, polymerization-induced self-assembly.
spherical morphology might be aided by monomer and solvent fractions within the nanoparticle core. (3) Nanoparticles might be extremely dynamic during the early stages of PISA; all block copolymer chains might be capable of constant rearrangement, leading to the most energetically favorable arrangement. It is very likely that all three possible mechanisms are to some extent responsible for the increased aggregation number during RAFT-PISA. More research is required to further establish this characteristic of RAFT-PISA. Furthermore, it would be interesting to evaluate if this phenomena can be observed for PISA with other polymerization techniques.

**Sphere-to-worm transition**

Elongated structures (worms) can be observed when the core-forming block DP increases after the initial spherical particles are obtained. It is believed that this sphere-to-worm transition is caused by multiple sphere–sphere fusion processes. Indeed, it is difficult to imagine how such elongated structures would originate from a process other than sphere–sphere fusion such as copolymer chain exchange. This hypothesis is supported by the inability to attain this elongated structure when using a sufficiently long stabilizer block during RAFT-PISA. In this case, it is very likely that a thicker nanoparticle corona prevents sphere–sphere fusion. Instead of aggregating, spherical nanoparticles with a sufficiently long stabilizer block grow in diameter as the core-forming block becomes longer.

Another argument that supports the sphere–sphere fusion mechanism is the concentration dependency of the copolymer on the sphere-to-worm evolution during RAFT-PISA. Worms are obtained only at sufficiently high polymer concentrations. A low concentration leads to the formation of solely spherical nanoparticles that grow in diameter when the core-forming block DP increases. Interestingly, fusion processes have also been observed at low polymer concentrations for nanoparticles prepared via RAFT-PISA and other techniques. However, these fusion processes are supported by specific repeat units, or by introduction of a second population diblock copolymer spheres with shorter core-forming block DP.

Worms can also be obtained as a final temperature-responsive morphology, as discussed earlier (Figure 2a and 2b). It is noteworthy that this worm phase usually occupies a narrow region in the PISA phase diagram for various formulations. However, it is possible to increase the worm-shaped nanoparticle dimensions by preparing a polymer with a longer stabilizer and core-forming block DP for specific formulations. This leads to longer and thicker worms. Interestingly, the CGT is lower for the worm gels, which consist of longer total block DPs.

Finally, it is important to consider that the sphere-to-worm evolution is the most crucial step in the formation of vesicular nanoparticles during RAFT-PISA. Indeed, it is challenging (if not impossible) to obtain the subsequent intermediate octopi, jellyfish, and vesicular nanostructures if worms are not initially formed.

**Worm-to-vesicle transition**

Targeting a sufficiently high core-forming block DP can lead to the formation of vesicular nanoparticles, provided that a sufficiently short stabilizer block DP is used (as discussed in the previous section). Transmission electron microscopy (TEM) studies of samples obtained during RAFT-PISA, quenched at intermediate monomer conversions, revealed various intermediate PGMA-b-PHPMA nanostructures in water (Figure 4). These data imply that the worm-to-vesicle transition is based on the coalescence of branched worms, to form octopi structures. These structures tend to curve and form nanoparticles.

Figure 4 | The morphological worm-to-vesicle evolution during RAFT-PISA. TEM images correspond to the aqueous RAFT dispersion polymerization of PGMA_{47}-b-PHPMA_{200} diblock copolymers at intermediate HPMA conversion. Scale bars correspond to 100 nm. Reprinted with permission from ref 46. Copyright 2011 American Chemical Society. TEM, transmission electron microscopy; RAFT, reversible addition-fragmentation chain transfer; HPMA, 2-hydroxypropyl methacrylate.
with a jellyfish morphology, which then wrap up and enclose to create the final vesicle morphology. It is very challenging to obtain the intermediate octopi and jellyfish morphology as final structures, which are not plasticized by the remaining monomer, and thus, are generally not found in RAFT-PISA phase diagrams as a pure phase. However, they have been detected with TEM as intermediate morphology during RAFT-PISA in polar and non-polar media.46,47

After the vesicles are formed, polymerization continues within the monomer-swollen membrane. SAXS analysis has shown that a further chain extension of the core-forming block thickens the vesicle membrane—the vesicular diameter remains constant.47,51,54 In other words, the vesicle membrane grows inward, leading to a reduced interfacial area; hence, minimizing the system’s free energy. It is also noteworthy that such vesicle membranes consist of somewhat interdigitated polymer chains, compared with the preceding worm membranes. It is also noted that such vesicles have unique morphological aspects that can be considered a disadvantage of the so-called ‘octopus morphology’ as a functional form. The vesicular nanoparticle dimensions can be adjusted during PISA. A study from Yuan and co-workers58 showed that this could be achieved by adjusting the polymer topology. In this study, a poly(N,N-dimethylaminoethyl methacrylate) (PDMA)-based macro-CTA was chain extended with PBzMA and stearyl methacrylate (SMA) [or 2-(perfluoroctyl)ethyl methacrylate (FMA)] in ethanol at 15% w/w. The incorporation of this second core-forming monomer led to a core-forming block with a brush-like nature. Adjusting the ratio between the core-forming blocks allowed adjustments in vesicle diameter. The variation in the packing parameter was determined to be responsible for this effect. Large compound micelles, large compound vesicles, hexagonally packed hollow hoops, and nanoporous spheres can also be prepared using this approach.59

Other higher-order morphologies have also been observed for specific PISA formulations. For example, hollow tubes can be prepared; these likely evolve from a vesicle-vesicle fusion process.60–62 A lamella morphology has also been observed.62 Nevertheless, more research is required to gain more insight into the formation mechanism and the required reaction conditions to obtain such higher-order morphologies.

Disadvantages of RAFT-PISA

In summary, RAFT-PISA is a formidable technique for the in situ preparation of dispersed nanoparticles in various media. However, this technique has its limitations. A aspect that can be considered a disadvantage of the so far discussed PISA formulations is that they require thermal initiators (reaction temperatures are ~70–90 °C). Recently, a considerable amount of work was dedicated to light-controlled radical polymerization reactions at ambient temperatures.63 Judiciously chosen photoinitiators allow such polymerization reactions in the presence of light, while the absence of light pauses the polymerization reaction. Such initiator systems have been investigated for RAFT-PISA.64–67 However, it is important to consider how temperature affects the nanoparticle morphology during PISA.68,69 PISA generally performs better above the glass transition temperature ($T_g$) of the core-forming polymer block.3 Albeit, photo-PISA at room temperature is more suitable for the preparation of nanoparticle hybrids that consist of proteins and synthetic block copolymers. This is because a modest reaction temperature inhibits the denaturation of biomolecules.70–72

Nanoparticles obtained from RAFT-PISA contain sulfur-containing polymer end groups that are located within the nanoparticle cores. These are potentially harmful and cause intrinsically colored dispersions. Furthermore, these polymer end groups give an undesired odor to the polymers. Fortunately, convenient techniques have been developed to degrade the polymer chain ends from nanoparticles prepared via RAFT-PISA in polar73,74 and nonpolar media.37 In contrast, these end groups’ stability is desired during RAFT-PISA to maintain control over the polymerization reaction. For this reason, aqueous RAFT-PISA is generally performed under acidic or neutral conditions; RAFT end groups are susceptible to hydrolysis above pH 7.75,76

These limitations can be avoided when using polymerization techniques that avoid sulfur-containing end groups, as discussed in the rest of this review. Furthermore, different polymerization techniques, such as ROP, also allow other monomer species for PISA, including NCAs. This enables the preparation of biocompatible and biodegradable nanoparticles. Although some examples are known,77–79 such characteristics are challenging to achieve with RAFT-PISA.

ATRPISA

A controlled living radical polymerization method that competed with RAFT polymerization over the last decade is ATRP. PISA was attempted with both polymerization methods, but RAFT polymerization proved to be more successful.

There are several reasons why ATRP is less suitable for PISA than RAFT. For example, ATRP utilizes a copper catalyst, which forms an undesired potential toxic impurity in the polymer product.80 For this reason, nanoparticles obtained from ATRPISA are less suitable for biomedical/pharmaceutical applications. Copper removal is possible; for example, silica column chromatography could be used after nanoparticle cross-linking.81 Another
recent study described a method that utilizes a “Cu scavenger”, followed by filtration, on a polymer solution. Nonetheless, such procedures are demanding and would require cross-linked nanoparticles.

There are other disadvantages of ATRPISA which arise from using a metal catalyst. For example, ATRP metal complexes are often vulnerable to oxidation. Fortunately, different ATRP methods were developed to improve upon this limitation and to allow this polymerization reaction with low copper concentrations. Such methods are known as: simultaneous reverse and normal initiation (SR&NI)-ATRP; activators regenerated by electron transfer (ARGET)-ATRP; supplemental activators and reducing agent (SARA)-ATRP [also known as single-electron transfer living radical polymerization (SET-LRP)]; electrochemically mediated (e)-ATRP, and initiators for continuous activator regeneration (ICAR)-ATRP.83

Another factor that potentially complicates ATRPISA is that both the activator and deactivator (Cu–ligand and Cu–ligand) need to be present in the growing nanoparticle core where the polymerization takes place.80 Diffusion of these species into the nascent nanoparticle cores might be difficult, owing to its insoluble character.

Initially, a series of PISA reactions were reported using conventional ATRP in alcohol/water mixtures, using a relatively large amount of metal catalysts. The first example of ATRPISA was reported by Pan and co-workers in 2007.84 Subsequently, Armes, Lewis and their co-workers showed in 2010 that ATRPISA could be used to prepare nanocages81 and nanolatexes85 in alcohol/water mixtures. Later, examples utilize more advanced ATRP methods. For instance, Cunningham and co-workers86 prepared polysaccharide-based nanoparticle spheres using SARA-ATRP in 2015. This was achieved by grafting methyl methacrylate from an alginate macroinitiator in a methanol/water mixture.

A recent example of ATRPISA was reported by Matyjaszewski and co-workers in 2016;60 here, ATRPISA was attempted with a reduced copper concentration. This was achieved by employing the ICAR-ATRP method. A poly[oligo(ethylene glycol) methyl ether methacrylate] (POEOMA) macroinitiator was chain extended with PBzMA in ethanol at room temperature and 65 °C. The effects of catalyst concentration, radical initiators, target PBzMA DP, solids content, and temperature were investigated for this dispersion polymerization. High monomer conversions and relatively narrow MWDs were obtained. As commonly observed in RAFT-PISA, a two stage semi-logarithmic plot was obtained for these ATRPISA formulations. This suggests that the nanoparticles became monomer swollen after nucleation.

Only spherical nanoparticles and worm-like aggregates were obtained from this PISA formulation at room temperature and 65 °C. Like RAFT-PISA, this morphology is dependent on the block DPs and solids content. Only spheres are formed at low polymer concentrations. Spheres with diameters of ~300 nm and worms were observed at higher final polymer concentrations. In contrast to the samples obtained at 65 °C, the worms obtained at room temperature had short fractal-type connected-bead morphology. This suggests that these elongated structures are formed by a sphere–sphere fusion process. The observed differences between the ATRPISA formulations at the two investigated reaction temperatures (room temperature and 65 °C) were attributed to the $T_g$ of the core-forming PBzMA block ($T_g = 54 ^\circ$C). The melt-like state of the nanoparticle cores likely allows core solvation by solvent and monomer at 65 °C. This aspect was expected to promote sphere–sphere fusion, leading to a smooth worm surface. In contrast, the glassy nature of the nanoparticle core at room temperature (below $T_g$) inhibits fusion and leads to a connected-bead-like morphology.

Annealing the samples prepared at room temperature to 65 °C led to the morphological evolution from either spheres-to-worms or worms-to-vesicles. Interestingly, the same polymer compositions that were initially prepared via ATRPISA at 65 °C did not form this vesicular nanostructure. Again, this difference was attributed to the $T_g$ of the core-forming PBzMA block. It was speculated that copolymer chains are more likely to reorganize during PISA when conducted above the core-forming block $T_g$. PISA below the $T_g$ of the core-forming block leads to morphologies with glassy cores in a metastable state. Therefore, heating the latter formulations leads to polymer rearrangement to form morphologies that exhibit a more energetically favorable polymer arrangement.

The same formulation (POEOMA-b-PBzMA) was also investigated by Boyer and co-workers64 using photo RAFT-PISA at room temperature. Interestingly, spheres, worms, and vesicles formed when acetonitrile was used as a cosolvent, or in the presence of a large fraction of unreacted monomer. This can plasticize the nanoparticle cores. These observations further confirm the suggestions made by the former group. Besides, chain mobility and the effect of $T_g$ during PISA were evaluated by various groups.3,52 Generally, it appears that PISA performs better when conducted above the $T_g$ of the core-forming block.

A direct comparison between ICAR-ATRPISA and RAFT-PISA was reported by Zhang and co-workers in 2017.85 Poly(2-hydroxypropyl methacrylate)-block-poly (benzyl methacrylate) (PHPMA-b-PBzMA) spheres and worms were formed in methanol/water mixtures (80:20) at 65 °C. High BzMA monomer conversions were achieved with RAFT-PISA and ATRPISA, no vesicles were obtained from these dispersion polymerizations. In both cases, a reaction rate increase was observed at ~40% monomer conversion. This implies that the ATRP catalyst does not influence the onset of nucleation. Interestingly, ICAR-ATRP gives rise to larger nanoparticles than RAFT.
The authors speculated that this is because of a “salting-out” effect that arises from the salt-like nature of the ATRP catalyst.

Another comparison between RAFT-PISA and ATRP-PISA was made by the same group in 2019.88 Here, poly(ethylene glycol)-block-polystyrene, PEG-b-PS, nanoparticles were prepared in alcoholic media. This study showed that ICAR-ATRPISA led to larger MWDs and less well-defined morphologies, compared with RAFT-PISA. It was believed that the salt-like nature of the ATRP catalyst and the broad MWD were responsible for this drastic difference in nanoparticle morphology. In another study, Zhang and co-workers89 showed the possibility to prepare well-defined vesicles via ICAR-ATRP. This formulation is based on the chain extension of a PEG macroinitiator with styrene and utilizes PEG as a solvent.

Finally, a most recent ATRPISA contribution was reported by Zetterlund and co-workers in 2019.90 This formulation comprised chain extension of poly(dimethylsiloxane) and BzMA in supercritical CO2. TEM images of the poly(dimethylsiloxane)-block-poly(benzyl methacrylate) (PDMA-b-PBzMA) nanoparticles suggested the presence of spheres, worms, and possibly, vesicles.

In summary, ATRPISA has potential, but it seems that RAFT-PISA can be utilized to prepare identical nanoparticles with better control over polymer molecular weight and nanoparticle morphology. Nevertheless, the examined ATRPISA formulations provided valuable insights into the true nature of PISA.

NMPISA

NMP proceeds via reversible homolytic dissociation of terminal alkoxyamine groups; thus, all growing polymer chains behave as radical initiators directly.91,92 NMP has shown promising results in terms of PISA. Early examples date back to 2005; Charleux and co-workers93,94 reported spherical nanoparticle formation from the chain extension of poly(sodium acrylate) with styrene and n-butyl acrylate (BA) in the water at 20% w/w. These dispersion polymerizations yielded spherical nanoparticles.

Later, in 2007, the Charleux and co-workers95 showed that NMPISA could be used to prepare cross-linked and uncross-linked poly(sodium acrylate)-block-poly(N,N-diethylacrylamide) spheres. Such spherical nanoparticles exhibit an LCST, cooling the uncross-linked dispersion causes the nanoparticles to dissolve. Gradual heating to the original temperature stimulated polymer self-assembly. Despite the reversible character of this transition, the spherical nanoparticles obtained after this thermal cycle had a significantly larger diameter. As discussed in the previous section, morphologies via PISA do not necessarily have the most energetically favorable diblock copolymer arrangement. Similar observations were made by Zhang and co-workers96 who compared RAFT-PISA with the solvent switch method.

Furthermore, Charleux and co-workers97 reported in 2009 that the poly(sodium acrylate)-block-poly(4-vinylpyridine) composition allows self-assembly into well-defined spheres, worms, and vesicles. Here it is worth to emphasize that all the above NMPISA formulations are emulsion polymerizations that were conducted at 120 °C (3 bar N2) and allowed high monomer conversions.

RAFT-PISA evolved at approximately the same time as NMPISA. Despite the early start of NMPISA, time has proven that RAFT-PISA was more fruitful over the following 10 years, based on the overwhelming number of RAFT-PISA publications. The widespread use of NMPISA was likely to be hindered by its harsh reaction conditions (high temperature and pressure). However, recent advantages allowed this polymerization technique to be conducted under more modest conditions.98,99 It would, therefore, be worthwhile to further explore the possibilities with NMPISA. Furthermore, it is worth to consider if the high temperature and pressure, that are generally required for NMP, benefit PISA. Besides, it is known that an increase in temperature can lead to nanoparticle core solvation and enhanced chain mobility. Thus, the harsh polymerization conditions might contribute to the formation of higher-order morphologies during the discussed emulsion polymerizations. It is typically challenging to obtain higher-order morphologies using a PISA formulation based on a RAFT emulsion polymerization, which is generally conducted at significantly lower temperatures at ambient pressure.97

Nevertheless, a judicious selection of monomers allows lower reaction temperatures during NMPISA. Copolymerization of styrenic monomers in the core-forming and stabilizer block allowed the preparation of P(MAA-co-sodium 4-styrene sulfonate)-b-P(MMA-co-styrene) at 90 °C (3 bar).98 However, the monomer conversions of these emulsion polymerizations were ~75%, but allowed the formation of spheres, worms, and vesicles.

Another example where styrene was used as a comonomer is the poly[poly(ethylene oxide) methyl ether methacrylate-co-styrene]-block-poly(n-butyl methacrylate-co-styrene) NMPISA formulation.99 The polymerization of this aqueous emulsion was conducted at 85 °C (ambient pressure) and allowed the preparation of various higher-order morphologies. Interestingly, spheres, worms, and vesicles can be targeted when adjusting the salt content of this PISA formulation. It was believed that this is the result of the PEO chains’ responsiveness toward this addition. Indeed, salt additions can change the cloud point of this specific polymer block; the aqueous solubility of PEO is based on hydrogen bonding, this can be disrupted by variation in temperature, salt concentration, and pH.100-102

NMPISA can also be performed on silica particles.103,104 Here, a PEO-based macro-alkoxyamine-initiator is adsorbed onto the surface of silica nanoparticles. The subsequent chain extension by emulsion polymerization leads to the preparation of silica particles coated with
(or incorporated in) various block copolymer morphologies. This is, however, not further discussed in this review. Compared with RAFT-PISA, an advantage of NMPISA is that the resulting nanoparticles do not bear sulfur-containing end groups. However, it seems that the demanding reaction conditions of NMPISA restricted the development of this technology.

**ROMPISA**

PISA is not limited to radical chemistry. An emerging PISA procedure utilizes ring-opening metathesis (ROM) for the in situ preparation of nanoparticles (Figure 5a). This approach is based on the synthesis of polynorbornenes using metal catalysts such as a Grubbs catalyst. The resulting block copolymer has an interesting chemical structure that contains cyclic groups and olefin linkages.

ROMPISA is a fast process, on the timescale of several minutes, that can be performed at room temperature, and has good functional group tolerance. In addition, ROMPISA can be used to prepare nanoparticles in an oxygen-rich environment, in contrast to the previously discussed radical PISA methods, and allows organic solvents or water. The earlier generations of metal catalysts for metathesis were susceptible to water and oxygen, but recent advances overcame these limitations. These recent examples utilize a Brønsted or Lewis acid to promote the water solubility of the catalyst. However, catalyst activity and water solubility remain challenging factors for ROMPISA. Also, there is a limited number of suitable monomers, which are generally expensive. Herein, we aim to provide a brief overview of ROMPISA. For a more detailed description, we refer to a more detailed summary recently written by Varlas et al.

In 2010, Xie and co-workers reported the first example of ROMPISA in toluene. A first-generation Grubbs catalyst was used to prepare spherical diblock copolymer nanoparticles with diameters of ~140–200 nm. This was achieved with the chain extension of poly[2,3-bis(2-bromoisobutyryloxy)methyl]-5-norbornene] (PBNBE) using oxanorbornene dicarboxylic acid dimethyl ester (ONBDM). This one-pot approach suffered from incomplete monomer conversions, the yield ranged from 60% to 84%. The same group showed that this method allowed the preparation of triblock copolymers by incorporating a poly[exo-N-(cinnamoyloxyethyl)-7-oxanorborn-5-ene-2,3-dicarboximide] (PCONBI) block into this formulation. This addition allowed nanoparticle crosslinking upon UV-irradiation. Interestingly, the monomer conversions were reduced with the introduction of each new segment during the preparation of PBNBE-b-PCONBI-b-PONBDM. Monomer conversions for each chain extension were 90%, 50%, and 20% for the first, second, and third block, respectively. It was believed that nucleation inhibited the polymerization from reaching high monomer conversions. Also, it was speculated that active polymer end groups became trapped within the nanoparticle cores after nucleation. The monomer was unable to diffuse into the nanoparticle cores, thereby hindering the polymerization reaction.

Subsequent organic ROMPISA formulations were conducted in solvent mixtures by other groups. Choi and co-workers chain extended poly(N-cyclohexyl-exo-norbornene-5,6-dicarboximide) (PChNDI) with a cyclic polysulfane monomer (CPM) using a third-generation Grubbs catalyst in dichloromethane (DCM) and tetrahydrofuran (THF). High monomer conversions could be achieved, and the resulting sulfur-rich polysulfane-bearing norbornenes (PChNDI-b-PCPM) formed spherical nanoparticles. Increasing the core-forming block DP yielded larger spheres with diameters of around 29–52 nm and higher nanoparticle refractive indices (RI = 1.54–1.65).

Le et al. incorporated stable TEMPO radicals into spherical nanoparticles with diameters ranging from 10 to 110 nm, using a similar one-pot protocol in organic media and a first-generation Grubbs catalyst. First, a soluble polymer was prepared in THF from oligoethylene glycol-modified norbornene (NOEG) and...
TEMPO-functionalized norbornene (NTEMPO). This soluble first block (PNOEG-co-PNTEMPO) was chain extended with norbornene (Nor) in ethanol/THF mixtures to drive polymer self-assembly. The final polymer (PNTEMPO-co-PNOEG-b-PNor) was achieved in high yield and predominantly formed spheres. Both polymerization steps proceeded extremely fast (2 and 5 min). Subsequently, these nanoparticles were employed successfully for the reduction of various small-molecule alcohols. Favorably, these nanoparticles could be reused after recovery via centrifugation without losing their catalytic activity. Furthermore, the low cytotoxicity of these nanomaterials possibly allows usage as scavengers of reactive oxygen species (ROS) in biomedical applications.

The possibility of preparing higher-order morphologies (spheres, worms, and vesicles) via ROMPISA in organic media (DMF/MeOH mixtures) was reported by Gianineschi and co-workers in 2017. A third-generation Grubbs catalyst was used for the preparation of a poly[norbornenyl oligo-(ethylene glycol)] homopolymer that was subsequently chain extended with a peptide-substituted norbornene monomer. The variation of the block DPs allowed the final morphology to be tuned accurately.

ROMPISA is not limited to organic media: O’Reilly and co-workers reported a wide range of suitable monomers for aqueous ROMPISA in 2018 and 2019. The similarities of ROMPISA to RAFT-PISA were highlighted with the preparation of a PISA phase diagram. Furthermore, recent examples showed that aqueous ROMPISA could also be used for the preparation of peptide-based nanoparticles and drug-loaded nanoparticles.

ROMPISA can also be used for the chain extension of polynorbornenes with cyclooctatetraenes in organic media, as reported by Choi and co-workers in 2017. This results in a polycetylene core-forming block, which is insoluble in almost any solvent and unstable in air because of the strong π–π interactions of the conjugated backbone. These PISA formulations allowed the formation of nanoparticles with diameters of around 4 nm, nano-caterpillars, and aggregates. The same group showed that this approach is suitable for a “one-shot PISA” procedure. Here, a mixture of monomers (a norbornene derivative and cyclooctatetraene) reacts to form block copolymers, owing to the large difference in reactivity. Block copolymer self-assembly occurs once a critical polycetylene DP is attained. A similar ROMPISA approach can also be performed in aqueous media. Cunningham and co-workers reported the block copolymer nanoparticle preparation from PEG-substituted norbornene and 1,5-cyclooctadiene. Here, the resulting polyolefin block forms the nanoparticle core. These latter two formulations can also be classified as in situ nanoparticleization of conjugated polymers (INCP).

ROMPISA can be used to prepare metal-containing polymer nanoparticles, as Tang and co-workers demonstrated in 2019. Such nanoparticles were obtained from the chain extension of polynorbornene with 1,1′-(2-butenyl)ferrocenedicarboxylate and 1,1′-(2-butenyl) ruthenocenedicarboxylate. Various nano-objects (spheres and platelets) were achieved according to this protocol. Here, solvophobicity and crystallization were the driving forces for nanoparticle self-assembly. Therefore, this process is also known as ring-opening metathesis polymerization-induced crystallization-driven self-assembly (ROMPI-CDSA).

Finally, a very recent ROMPISA study from O’Reilly and co-workers showed a morphological evolution mechanism based on an in situ vesicle-vesicle fusion process that forms tubular polymersomes. These formulations utilize norbornene imides that are either functionalized with PEG methyl ether or with a tertiary amine, as stabilizer block; norbornene imide ethylene glycol mono-methyl ether is used as a core-forming block. This aqueous dispersion polymerization allowed the formation of vesicles that fuse during PISA to form tubular polymersomes (Figures 5b and 5c). This study also showed that this fusion process is prevented when an amine-based stabilizer block was employed, owing to the associated charge. However, the addition of enough salt to screen the positive charge allowed the formation of tubular polymersomes with this stabilizer block during ROMPISA. It is worth mentioning that a similar vesicle-vesicle fusion process was previously observed for a RAFT-PISA formulation conducted in PEG. Here, it was speculated that the solvent’s viscous nature contributed to vesicle-vesicle fusion. In addition, it is known that stimulus-responsive tubular polymersomes can be obtained from a RAFT-PISA protocol based on an aqueous dispersion polymerization.

In summary, ROMPISA is an emerging method for the preparation of various nanoparticle morphologies under mild conditions. In contrast to the previously discussed radical PISA methods, ROMPISA utilizes cyclic monomers that give rise to an alternative polymer structure with exciting characteristics that could prove useful in the years to come.

ROPISA with NCA-Monomers

Poor biodegradability is a major disadvantage of the nanoparticles obtained from the previously discussed radical- and metathesis-PISA formulations. Besides, the oxygen sensitivity of radical chemistry and the requirement of specific chemicals such as CTAs, for RAFT polymerization, or metal complex, for ATRP and ROMP, make these PISA procedures more complicated. Limitations of the previously discussed PISA methods were resolved recently by Du and co-workers in 2019.
novel route for the preparation of biodegradable nanoparticles via the ROP of NCA-monomers for PISA was developed (Figure 6). Here, a PEG45-NH2 macroinitiator was chain extended with phenylalanine (Phe)-NCA in THF at 10 °C. High NCA monomer conversions (97%) were achieved within 8 h. Conform to RAFT-PISA, the core-forming block DP and the solid content can be altered to target specific nanoparticle morphologies; spheres formed at 10% w/w, vesicles were achieved when targeting a longer core-forming block DP at 20% w/w. The first-order reaction kinetic plot of these reactions did not exhibit the separate stages that are commonly observed during PISA (see Figure 3c). This indicated that no monomer reservoir is formed within the nanoparticles during PISA. The authors suggest that the excellent solubility of the Phe-NCA monomer in THF contributes to a constant reaction rate. Another interesting aspect of this PISA formulation is that the reaction rate should be faster than the hydrolysis rate. This was achieved by using an initial macroinitiator concentration close to the critical micelle concentration (CMC). Another interesting aspect of this formulation is that only solid contents below 13% w/w were allowed. Higher concentrations led to low monomer conversions and the formation of a soft gel. Furthermore, Fourier transform infrared (FTIR) spectral analysis showed that a significant amount of the polypeptide block adopted an α-helix and β-sheet conformation. These interesting features were likely related to the minimal formation of spherical morphology during this PISA formulation and should be considered for the design of future NCA-based PISA formulations.

**Other PISA Formulations**

There are examples of more exotic PISA formulations based on other polymerization techniques such as TERP, LAP, AFCT, RCMP, and CMRP, or utilize a comonomer that allows rROP.

An early PISA formulation based on TERP was reported by Minami and co-workers in 2009. This was before the term "PISA" appeared in the literature. Here, a poly (methacrylic acid) TERP-agent was chain extended with BA in water. This emulsion polymerization led to the formation of PMAA\textsubscript{30-b-PBA}\textsubscript{x} nanoparticles at 2-8% w/w. Gravimetry indicated that full monomer conversions could be achieved. Subsequent studies showed that this approach was suitable for preparing PMAA\textsubscript{30-b-PBA}\textsubscript{x} and PMAA\textsubscript{30-b-PBA}\textsubscript{x} triblock copolymer spheres in water. In all cases, spherical nanoparticles were obtained. Particle diameter and dispersity could be varied by adjusting the temperature, stirring rate, and TERP agent.

A very recent PISA procedure that utilizes a very early living polymerization technique was reported by Wang...
and co-workers in 2020. LAP was used for PISA to prepare nanoparticles from polysisoprene-block-polystyrene (Pi-b-PS) and poly(p-tert-butylstyrrene)-block-polystyrene (PtBS-b-PS). Well-defined spheres, worms, and vesicles were targeted accurately in heptane by adjusting the DP and solids content (10–30% w/w). Furthermore, the addition of divinylbenzene at the end of the polymerization led to nanoparticle core-crosslinking. Indeed, this approach enabled new possibilities for PISA. Unfortunately, it does not circumvent the intrinsic problems associated with this sensitive polymerization technique; rigorous, anhydrous conditions are required, and this polymerization method is restricted to a relatively narrow range of vinyl (or cyclic) monomers.

The inherent need for a polymerization’s “livingness” during PISA was challenged by Zetterlund and co-workers in 2017. AFCT polymerization was attempted during PISA, leading to sulfur-free nanoparticles. This polymerization technique is very similar to RAFT polymerization. The essential difference is that the addition rate of growing polymer chains to AFCT agents is slow; fast addition (or deactivation) rates give rise to the control of a RAFT polymerization. Here, the deactivation rate during AFCT-PISA was stimulated using a sufficiently low monomer concentration, so that deactivation was favored above propagation. More precisely, a PEGn-methacrylate (PEGnMA) was used to prepare a macro-AFCT-agent. PISA was achieved by chain extending this macro-AFCT-agent with BzMA in ethanol. The resulting polymer, PEGnMAy-b-PBzMAx, formed nanoparticles when the PBzMA attained a sufficiently high DP. Spheres, worms, and vesicles were obtained; however, this method requires further optimization since low monomer conversions (generally <40%) were achieved.

Another PISA method that provides sulfur-free nanoparticles was reported by Goto and co-workers in 2018. RCPMISA was used to prepare PMAA-b-PMMA nanoparticles in ethanol at 60 °C. This polymerization technique was based on the reversible deactivation of a propagating polymer chain with iodine, NaI was used as a catalyst. Block copolymer chains assembled in situ into spheres, worms, and vesicles at around 7% w/w. A maximal monomer conversion of ~80% was obtained. Furthermore, nanoparticle core-crosslinking was achieved using ethylene glycol dimethacrylate (EGDMA). A similar procedure based on a light-driven bromide-iodine transformation was reported for an in situ generation of an iodine initiator around the same time. The subsequent polymerization at room temperature led to the in situ formation of spheres and vesicles.

A somewhat unusual PISA formulation that produces charged nanoparticles was reported by Detrembleur and co-workers in 2017. Here, CMRP was used to prepare block copolymer spheres from imidazolium-based poly(ionic liquid)s in water. High monomer conversions were achieved for both the initial solution polymerization and the subsequent aqueous dispersion polymerization. Spherical nanoparticles with hydrodynamic diameters of 48 and 144 nm were obtained. Subsequently, anion exchange reaction, followed by purification by precipitation, led to the final polymer product: a solid polymer material functionalized with bis(trifluoromethylsulfonyl)imide anions (Tf2N·). Such functionalization is desired for their subsequent use in solid polyelectrolytes in electronics because it enhances the electrochemical stability, leading to higher dissociation constants and materials with reduced \( T_g \).

Finally, a smart modification of an existing nonpolar RAFT-PISA formulation was reported by Nicolas and co-workers in 2019. A fraction of cyclic ketene acetal (CKAs) were copolymerized (by RAFT-based rROP) with BzMA during the chain extension of a PLMA macro-CTA in heptane. This RAFT-PISA procedure led to the formation of well-defined spherical nanoparticles with diameters of around 40–500 nm that contained ester linkages within the nanoparticle core. A polymer film was made from this dispersion by evaporating the solvent in vacuo, which could be degraded with an aqueous KOH solution. This approach clearly shows potential; however, it would be interesting to examine if a PISA method can be developed that is solely based on the polymerization of CKAs.

**Conclusion/Perspective**

Over the last decade, PISA has equipped conventional emulsion and dispersion polymerization methods with the ability to prepare various block copolymer nano-objects at relatively high polymer concentrations. These traditional polymerization methods were initially restricted to spherical nanoparticles. Originally, PISA was limited to a small selection of suitable monomers, particularly in aqueous solution. Currently, PISA can be performed with various polymerization techniques and a range of monomers. It is well known that RAFT polymerization is a formidable technique for PISA. However, other polymerization methods can certainly be used to improve upon the current technologies. It is known that RAFT-PISA has allowed the preparation of nanoparticles that are suitable in various medical applications, including long-term stem cell storage and drug delivery. Known nonmedical applications include the usage of nanoparticles in engine oils as lubricant and as a viscosity modifier. Nonetheless, improvements can be made. Future PISA methods, with alternative polymerization techniques, will likely allow the efficient preparation of functional nanoparticles (or nanomedicines) that are currently only accessible via the less efficient solvent switch method. For example, efficient synthesis is beneficial for nanoparticles used in antibacterial products, treatment of diabetes, drug delivery, magnetic resonance imaging (MRI), and so forth.
New delicate methods to accurately target desired morphologies during PISA are desired to fully utilize the potential of this powerful technique. Currently, various morphologies are obtained by altering the solid content and the block DP of the stabilizer or core-forming polymer. However, recent research showed that RAFT-PISA, in the presence of homopolymer and nonreactive block copolymers, can lead to multicomponent higher-order morphologies. Furthermore, seeded PISA protocols also offer routes toward various novel nanoparticle morphologies. For example, incorporation of a polymer block with liquid crystal characteristics allows an unusual morphological evolution.

Other PISA principles, such as INCP, are also emerging. Here, the π-π interactions between growing polymer chains induce polymer self-assembly. Additionally, CDSA could become an invaluable tool for the preparation of dispersed nanoparticles. Another developing PISA principle utilizes electrostatic interactions. Here, photo-RAFT polymerization is used for a chain extension of a nonionic macro-CTA with the ionic monomer in the presence of an oppositely charged homopolymer. This approach allowed the preparation of spheres, worms, and vesicles. This principle is known as PIESA. Repeat units of opposite charge can also be introduced as (or within) a third block, leading to spheres, worms, vesicles, and lamellas. Moreover, it is worth mentioning that Cai and co-workers recently established a well-considered seeded RAFT-PIESA protocol that allowed the preparation of multicompartmentalized micelles, nanosheets, and nanocages. This work is based on the synthesis of a positively charged diblock copolymer in the presence of an oppositely charged nonreactive diblock copolymer sphere. Here, the initial anionic spheres act as a charge template for PIESA. The efficient preparation of multicompartimentalized nano-objects with well-defined subdomains undoubtedly highlights the potential of this approach.

Future PISA methodologies will likely utilize proteins to achieve desired reaction conditions, as reported by Zhang and co-workers in 2018. More specifically, enzymes can be used to deoxygenate reaction mixtures, which, subsequently, initiate various radical polymerization reactions. Furthermore, proteins can be encapsulated into vesicular nanoparticles during PISA. This approach leads to nanoparticles that are, for instance, capable of enzyme catalysis. Also, membrane proteins can be inserted into the vesicle membrane during PISA in the presence of surfactants.

The discussed development of new PISA protocols clearly enables the preparation of more complex homogeneous nano-objects. It is worth to investigate the in situ self-assembly mechanism of these next-generation nanoparticles with elaborate techniques such as SAXS. This analysis method enables in situ assessment of a dispersed nanoparticle morphology. Furthermore, the introduction of internal phase-separated nanoparticle domains could establish an interesting platform for small-angle neutron scattering (SANS) studies to examine the in situ self-assembly process and the behavior/characteristics of nanoparticles obtained from PISA. More specifically, a key advantage of SANS is that it allows contrast variation experiments (i.e., adjustment of the neutron scattering intensity of various domains within a nanoparticle). For example, it is possible to diminish the neutron scattering intensity of individual sections of a nanoparticle, such as an entire nanoparticle core, by using semideuterated solvent mixtures.

Finally, post-PISA procedures are emerging that allow the preparation of a new class of materials. For example, thermally induced copolymer chain exchange between nanoparticles allows the formation of nanoparticles with a distribution of two chemically/isotopically distinct block copolymer chains, or with two different chain lengths. It is known that such a process is able to yield various nanostructures. Even anisotropic nanoparticle can be prepared from an initial mixture of dispersed spheres with two distinct diameters, that were prepared separately by RAFT-PISA. Another post-PISA method utilizes the advantages of RAFT-PISA (i.e., efficient polymer synthesis) for the subsequent preparation of various bulk morphologies. These bulk morphologies are obtained directly from post-PISA polymer isolation. Finally, the preparation of composite nanomaterials also introduces interesting new possibilities. For example, nanoparticles can readily be occluded in situ into calcite crystals. Clearly, PISA is a fruitful approach for efficient preparation of a large variety of nanoparticles; various new nanoparticle possibilities can be expected to develop over the next decade.

Conflict of Interest
There is no conflict of interest to report.

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