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Emerging investigator series: polymeric nanocarriers for agricultural applications: synthesis, characterization, and environmental and biological interactions

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Polymeric nanoparticles represent one major class of nanomaterials that has been proposed to improve the sustainability of agricultural operations by delivering organic agrochemicals such as pesticides more efficiently. Polymeric nanoparticles can improve efficiency through improved targeting and uptake, slow release, and lower losses of the chemicals, while also conferring the benefits of biodegradability and biocompatibility. This review provides a tutorial to environmental nanotechnology researchers interested in initiating research on the development and application of polymeric nanocarriers for delivery of agrochemicals, including pesticides and growth promoters for crops and antibiotics for livestock. In particular, this review covers the wider suite of methods that will be required beyond those typically used for inorganic metal or metal oxide nanoparticles, including synthesis of custom polymeric nanocarriers and characterization and tuning of agrochemical loading and release profiles. Benefits of polymeric nanocarriers are then discussed in terms of the physicochemical properties and fate and transport behaviors that contribute to higher efficiency and lesser environmental impacts compared to traditional (non-nano) formulations. Finally, opportunities for environmental nanotechnology researchers to collaborate with material scientists, microbiologists, and agricultural scientists to optimize the development of polymeric nanocarriers for agriculture are discussed.

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Environmental significance

Sustainable nanotechnology for agriculture encourages the development of nanomaterials that will reduce the reliance on traditional chemicals, such as pesticides for crops or antibiotics for livestock, or the environmental impact of agrochemicals. While the environmental nanotechnology community has developed significant knowledge on the applications and impacts of inorganic nanoparticles, an opportunity exists to expand upon the applications of polymeric nanocarriers that can improve the efficiency of delivery of traditional agrochemicals while also providing advantages of biocompatibility and biodegradability. This Tutorial Review provides an overview of the synthesis, characterization, and fate and transport of polymeric nanocarriers as alternatives to inorganic nanoparticles, along with the potential benefits of polymeric nanocarriers over traditional agrochemicals.

1. Introduction

Nanotechnology is emerging as a means to improve the sustainability of agricultural operations. The general use of nanomaterials (both inorganic and organic or polymeric) for agriculture has recently been reviewed to provide a general understanding of the opportunities and research priorities,[1–5] as well as a critical evaluation of the efficacy of nano-enabled pesticides and fertilizers relative to conventional formulations.[6] Here, this review focuses specifically on polymeric "nanocarriers," in which active ingredients are loaded into or onto a polymeric nanoparticle. While polymeric nanocarriers have extensively been considered for human drug delivery applications, this review highlights the agricultural applications of polymeric nanocarriers for crops (pesticides, plant growth promoters, *etc.*) and livestock (specifically, antibiotic delivery). In these applications, polymer nanoparticles can improve the efficiency of application of active ingredients by enhancing the aqueous dispersibility and bioavailability of hydrophobic active ingredients, conferring targeting properties,

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and extending the effective lifetime of the active ingredient (*e.g. via* slow release, enhanced adhesion to leaves or roots, or protection from degradation). Polymeric nanomaterials can also serve as more sustainable alternatives to inorganic nanoparticles when biocompatible and biodegradable polymers are selected that are expected to minimize the potential for ecotoxicity.

This tutorial review aims to serve as a primer for environmental researchers to initiate new research on the application and development of polymeric nanocarriers for agricultural applications. Given the extensive experience developed in the environmental nanotechnology community with inorganic nanoparticles, special considerations that are required for the study of polymeric nanomaterials as compared to inorganic nanoparticles are emphasized. First, methods for synthesis of polymeric nanocarriers and approaches to optimize the synthesis are presented. Then, important characterization needs for polymeric nanoparticles loaded with active ingredients are discussed. Finally, mechanisms for the delivery or release of active ingredients, environmental fate, and biological effects of polymers nanocarriers, and how these mechanisms inform the design of the nanoparticles, are presented. The integration of knowledge on synthesis, characterization, behavior, and effects is expected to lead to advances in the development of polymeric nanocarriers as a beneficial technology for agriculture and the environment.

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Sheyda Shakiba is currently a Ph.D. student in Civil and Environmental Engineering at the University of Houston. Her research focuses on studying the interaction of biomolecules with polymeric and metal oxide nanoparticles, as well as investigation of different approaches to obtain release profiles of drug loaded polymeric nanoparticles especially by use of asymmetric flow field-flow fractionation.

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Carlos E. Astete, Ph.D. is Associate Professor Research in the Biological and Agricultural Engineering Department at Louisiana State University and LSU Agricultural Center. Dr. Astete has developed an extensive research area in the field of polymeric nanoparticles designed for bioactive delivery and chemical functionalization for targeting applications in the health, agriculture, and food. The systems designed increases bioavailability, diminish side effects, and prolongs action of bioactives. The systems designed involve natural and biodegradable synthetic polymers with entrapment of hydrophobic and/or hydrophilic bioactives that are currently used in order to study the effects and interactions of nanosystems in biological entities.

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2. Synthesis of polymeric nanocarriers

Research and development teams in both industry and academia will likely need to develop expertise in synthesizing materials in-house during the development of new polymeric nanocarriers for ultimate application by farmers. Currently, polymeric nanoparticles have limited commercial availability, with those available for purchase limited to a few common synthetic polymer types (*e.g.* polystyrene and poly(lactic-*co*-glycolic acid) (PLGA)). Furthermore, the pure polymeric nanoparticle typically does not serve as an "active ingredient." Rather, an active ingredient (*e.g.*, a pesticide, hormone, or antibiotic) must be loaded into the nanoparticle during the synthesis of the particle. Hence, considering the number of polymer types multiplied by the number of agrochemical types that may be of interest, new materials for research and development purposes will require custom syntheses. This issue is in contrast to the relatively widespread commercial availability of inorganic (metal and metal oxide) nanoparticles, where the nanoparticle itself confers the "active" properties (*e.g.*, fungicidal copper nanoparticles) and does not need to be loaded during the synthesis with an active ingredient. Here, we introduce common materials and synthesis methods for polymeric nanoparticles, as well as approaches to optimize the synthesis parameters to obtain desired nanoparticle properties.

2.1. Common polymeric materials for agricultural applications

Research published over the last 10 years indicates that the preferred natural polymers for food and agricultural applications are chitosan,[7,8] zein,[9–14] and alginic acid.[15–17] Also, some biocompatible and biodegradable synthetic polymers, such as PLGA, are of interest to confer new properties to the delivery systems, and as a platform to develop new biomaterials, for example by linking synthetic and natural polymers. In addition to the active ingredient that will be loaded into the nanoparticle, other ingredients that are frequently added include surfactants (*e.g.*, poly(vinyl alcohol) or Tween) to impart colloidal stability to the nanoparticles, as well as a cryoprotectant (*e.g.* mannitol or trehalose) to preserve material integrity during lyophilization. Finally, the addition of an oil can be used to form a "nanocapsule" structure, where the oil forms a liquid core at room temperature surrounded by a polymer shell and can be used to carry poorly soluble active ingredients.

2.2. General approaches for optimization of nanocarrier synthesis

Optimization of the synthesis of polymeric nanocarriers typically revolves around obtaining a desired particle size with low polydispersity, good colloidal stability, and high loading or entrapment efficiency of the active ingredient. Entrapment efficiency is defined as the percentage of active ingredient added in the synthesis that is incorporated into the nanoparticles, while loading refers to the concentration of active ingredient in the nanoparticles (typically expressed as a weight percent). Smaller sized nanoparticles with narrow size distributions can be achieved by tuning the ratio of ingredients (polymers, surfactants, and active ingredients)[18–23] and/or the forces imparted (*e.g.* by shear, impact, sonication, or high pressure homogenization) during or immediately after the synthesis.[19,22,24,25] Colloidal stability is determined by Derjaguin Landau Verwey Overbeek (DLVO) interaction energies, similarly to inorganic nanoparticles, and hence charged polymers or surfactants can be utilized to confer electrostatic stabilization.

Entrapment efficiency and loading are optimized by selection of materials that favor incorporation of the active ingredient into the nanoparticle during synthesis. Optimal loading conditions can be identified experimentally by varying

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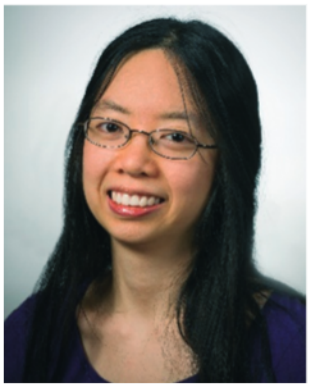

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Stacey M. Louie, Ph.D. is an Assistant Professor in Civil and Environmental Engineering at the University of Houston. Her research covers the environmental implications and applications of inorganic and polymeric nanomaterials, with a specific focus on developing new methods to characterize the dynamic interactions of nanomaterials with small molecules, natural organic matter, and biomolecules and the effects of organic surface coatings on their aggregation, transport, and reactivity. She seeks to apply these tools to gain a mechanistic understanding of the fate and behavior of nanomaterials in complex media in both natural environments and engineered systems.

ingredient concentrations, *e.g.* using factorial design.[20,21,26] Specific interactions, such as electrostatic complexation interactions[27–30] or covalent bonding (conjugation) of the active ingredient to the polymer,[31] can be used to increase loading. However, loading is more typically achieved through partitioning of the active ingredient into the polymer phase *versus* the solvent, *e.g.* based on the hydrophobicity or polarity of the active ingredient and polymer.[32] Models have hence been developed to explain or predict *a priori* the active ingredient loading based on thermodynamic parameters such as the Flory–Huggins interaction parameter[33] or Hansen solubility parameter,[34] or using universal functional activity coefficient (UNIFAC) methods that account for the chemical structure of the active ingredient, polymer properties (including the glass transition temperature), and partitioning of active ingredient into surfactant micelles.[35] However, agreement between experimental data and these models is rarely evaluated and would be useful in future studies.

2.3. Synthesis methods for polymeric nanocarriers

Synthesis methods for polymeric nanoparticles can be divided into two categories: bottom-up techniques that involve *in situ* polymerization, and top-down techniques that involve steps such as mixing or emulsification with external energy input. The first approach involves organic chemical synthesis in the presence of solvents, initiators, and other potentially toxic agents. The separation and purification steps add extra cost that limit its uses in food and agriculture. The top-down techniques use natural or synthetic polymers to form particles in the nanometer size range and surfactants, needed to stabilize the system. The active components are entrapped in the core of the polymeric matrix, adsorbed on the surface, or both depending on the chemical nature of the polymer, surfactant, active component, and other additives. The top-down techniques require less solvents and chemicals in general, and have been adopted for various food and agricultural applications based on the safety of materials, versatility offered in delivery of both hydrophobic and hydrophilic bioactives, and ease of scale up.

This section will focus on top-down techniques used to make biocompatible, biodegradable polymeric nanoparticles, which can be easily functionalized as required by the application, using low cost, versatile and scalable processes (Table 1). The method chosen to synthesize polymeric nanoparticles depends on the type of polymer, surfactant, and active component. Usually, nanoprecipitation or emulsion evaporation techniques are preferred for hydrophobic polymers; these techniques call for the use of organic solvents such as alcohol, acetone, or ethyl acetate. Other techniques such as ionic gelation, *e.g.* attraction between oppositely-charged amine and carboxylic groups of two polymers, or double-emulsion evaporation are employed for more hydrophilic polymers and bioactives ingredients. Fig. 1 and Table 1 show a summary of techniques used in the agricultural nanotechnology literature, chemicals needed, and the main characteristics of the synthesized polymeric nanoparticles. Notably, the majority of studies reporting polymeric nanoformulations produced particles with diameters between 100 nm to 1000 nm rather than the generally accepted size definition of nanoparticles having sizes from 1 nm to 100 nm. Here, we follow the convention of the literature in using the "nanoparticle" terminology and discuss the effect of size in subsequent sections.

2.3.1. Emulsion evaporation. The emulsion evaporation technique has been widely used in the biopharmaceutical area based on the solubility of hydrophobic synthetic polymers (such as PLGA) and many drugs in organic solvents. The technique involves two phases: an organic phase with the dissolved hydrophobic active ingredient and polymer, and an aqueous phase containing surfactant. The selection of materials for the polymer and surfactant can be optimized to obtain high surface charge (*e.g.*, zeta potential higher or lower than 30 and −30 mV, respectively) and hence high electrostatic repulsive forces associated with a longer stability in aqueous suspension.[36] The phases are mixed with further droplet size reduction by high shear forces, such a sonication or homogenization, followed by the evaporation of the solvent. Freeze-drying is applied to obtain a formulation in powder form. The final drying step will assure a long-term stability of the formulation, especially for nanoparticles made using polymers that degrade by hydrolysis. In food and agriculture, this method is less common because of cost restrictions and applicability of synthetic polymers. Nonetheless, the biodegradable and biocompatible family of polymers poly(ε-caprolactone) (PCL) were reported to be suitable for delivery of atrazine herbicide by Pereira *et al.* (Table 1).[37]

2.3.2. Nanoprecipitation or solvent displacement. The nanoprecipitation or solvent displacement technique is suitable for polymers soluble in water-miscible organic solvents such as acetone, methanol, ethanol, and other polar solvents. Usually, the active component is dissolved in the organic phase, and the mixing of phases is performed under strong stirring. Next, the solvent is evaporated with a rotary evaporator under vacuum for 1 to 3 hours, or at room temperature under stirring for 12 to 24 hours, similarly to the evaporation step in the emulsion evaporation technique. It is important to remove 100% of the organic solvent to avoid toxicity, altered release profile of active components, and changes in the nanoparticle stability over time.

Several examples of applications of this method to produce polymeric nanocarriers for agriculture are available in the literature. For example, polycaprolactone (PCL) polymer was used to entrap essential oils from *Zanthoxylum rhoifolium* (Rutaceae) as a pesticide.[38] In another approach, the herbicides atrazine and ametryn were entrapped in PCL nanocapsules.[39] Capric and caprylic acid oils (Myritol 318) were dissolved in the organic phase together with the herbicides and the hydrophobic surfactant Span 60, while the surfactant Tween 80 was dissolved in the aqueous phase.[39–41]

Other studies reported on the formation of zein nanoparticles capable to deliver pesticides for soybeans and

Table 1 Major synthesis methods for polymeric nanoparticles

Methods	Polymer	Active ingredient	Solvent	Other comp.	Size (nm)	Zeta (mV)	PDI	pH	EE (%)	Application	Process	Ref.
Emulsion–evaporation	PCL	Atrazine	Dichloromethane	Myritol, PVA	365 to 520	−23 to −26	>0.200	NR	93%	Increase efficiency of A.I. tested in *Brassica* sp. and *Zea mays*	Sonication	37
	PCL	Carbendazim and tebuconazole	Acetone, chloroform	Myritol, PVA	300 to 700	−20 to −30	0.120–0.200	NR	>99%	Controlled release of A.I. tested in *P. vulgaris* seeds	Sonication	53
Double-emulsion	Carboxymethyl cellulose	Clodinafop-propargyl	Dichloromethane	Sodium dioctyl sulfosuccinate, PVA	150 to 350	−37.4	NR	NR	90%	Reduce toxicity of A.I. tested in wheat	Sonication	49
Nanoprecipitation	Zein	NA	Acetone	DMAB	100 to 300	+35	0.205	6.2	NA	Biodistribution of nanoparticles in soybeans and sugarcane	Microfluidizer	10, 11
	PCL	Essential oils	Acetone	Span 60, Tween 80	450 to 460	−23 to −26	NA	4.5–5	96 to 99%	Increase solubility and protection of A.I. tested in tomatoes	Mixing, evaporation	38
	PCL	Atrazine, ametryn	Oil, alcohol	Tween 80, Span 60, Myritol 318	200 to 300	NA	NA	NA	83%	Delivery and toxicity to algae and microcrustacean	Mixing, evaporation	39
Ionic gelation	Chitosan	NA	Water	STPP, acetic acid	181	−23 to −26	0.31	45	NA	Inhibition against *F. Graminearum* in wheat	Mixing	48
	Sodium alginate	Gibberrellic acid plant hormone	Water	$CaCl_2$	392 to 545	−27 to −31	0.26–0.36	4–5	100%	Stabilization and increase efficiency of A.I.	Mixing	42
	Chitosan	Gibberrellic acid plant hormone	Water	STPP	188 to 430	+17 to +27	0.3–0.4	4–5	97%	Increases release times of A.I. compared to sodium alginate nanoparticles	Mixing	42
	Chitosan	Hexaconazole	Water, ethanol	STPP, Tween 80	100 to 600	+35	0.3–0.6	4.9	73%	Less toxic and more efficient A.I. in nanoparticles compared to commercial formulation	Mixing	7
	Chitosan	NA	Water	STPP	233	+21	0.3	4–5	NA	Concentration dependent inhibition of germination and plant growth	Mixing	47
Other techniques, *e.g.* crosslink	Carboxymethyl chitosan, 93% DA	Methomyl	Water	Azidobenzaldehyde	78 to 99	−17 to −23	0.101–0.124	4–6	94 to 97%	Better stability and controlled release of A.I. tested against armyworm on red kidney beans foliage	Mixing	50

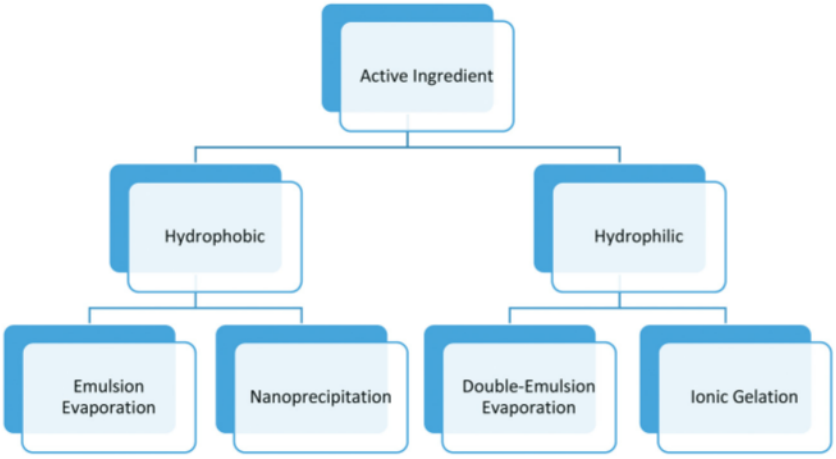

Fig. 1 Schematic of common methods for entrapment of active ingredients.

sugarcane using the same technique.[10,11] A cationic surfactant was used to promote ionic interaction between the polymeric nanoparticle and the plant tissue, especially with the roots, imparting a positive zeta potential of +81 ± 4 mV at pH 6.[10,11]

2.3.3. Ionic gelation. The technique uses the ionic interactions between polymer and oppositely charged molecules to form a gel. When chitosan (a cationic polymer at pH under 6) is used, a negatively-charged gelling agent must be added to promote ionic interactions and formation of aggregates. The addition of gelling agent must be performed slowly, usually drop-by-drop under strong stirring. Also, the active component is usually hydrophilic and the pH of the aqueous phase must be controlled to avoid aggregates formation at more basic pH. For chitosan, the pH is kept under 5–6 to avoid precipitation based on the amine group ionization. After the mixing of the gelling agent (usually an anionic agent), centrifugation is generally performed to collect the particles with entrapped active components. Several studies were reported on forming chitosan particles with entrapped pesticides by ionic gelation.[42–47] Sodium tripolyphosphate (STPP) is commonly used as the anionic gelling agent. These types of particles were formed for delivery of gibberellic acid hormone (GA_3),[42] paraquat, an herbicide,[43,45] atrazine,[40] and hexaconazole as a fungicide.[7] In all these studies, chitosan was solubilized under acidic pH (4–5), most commonly in the presence of acetic acid. The hormone, herbicide, or fungicide was mixed in with the chitosan solution to form particles in the 100–500 nm size range, of a positive charge, with high entrapment efficiency of the bioactives (70–80%). Empty chitosan nanoparticles can also be of interest to synthesize as they have been shown to be effective for treatment against Fusarium head blight (FHB) disease caused by *Fusarium graminearum* in wheat,[48] although empty chitosan nanoparticles with STPP were also shown to inhibit germination of *Zea mays*, *Brassica rapa*, and *Pisum sativum* at high concentration.[47]

An alternative polymer suitable for formation of nanoparticles by ionic gelation is alginic acid. Alginic acid is negatively charged and can be crosslinked by calcium ions or alternatively used in combination with cationic chitosan. For example, Kumar *et al.* studied the entrapment of water-soluble a neonicotinoid insecticide (acetamiprid) in sodium alginate.[46] Similarly, alginic acid was used to entrap GA_3 hormone,[42] and polylysine in chitosan alginate particles.[44]

2.3.4. Double emulsion method. Double emulsion evaporation method involves first formation of a water/oil (W/O) emulsion where the bioactive ingredient is dissolved in the water phase, followed by formation of a (W/O)/W emulsion. This method allows for entrapment of more hydrophilic bioactives, whereas single emulsion is more suitable for entrapment of hydrophobic bioactives. The use of carboxymethyl cellulose (CMC) to synthesize nanoparticles capable to entrap a water-soluble herbicide (clodinafop-propargyl) required the use of the double emulsion technique; the mean particle size ranged from 100 nm to 245 nm and the entrapment efficiency ranged from 4 to 94% depending on the amounts of CMC and surfactants used in the synthesis.[49]

2.3.5. Other techniques. Other approaches used to form polymeric nanoparticles involve chemical modification of natural polymers such as chitosan, or formation of amphiphilic copolymers suitable for delivery of agrochemicals. In the first example, chitosan chemical modification was performed followed by photo-crosslinking.[50] More specifically, carboxymethyl chitosan with a 93% degree of deacetylation was linked to azidobenzaldehyde to form an amphiphilic polymer. After mixing at room temperature, the photo-crosslinkable carboxymethyl chitosan was washed with ethanol, and the resuspended polymer in deionized water was separated by centrifugation. Next, the modified chitosan was mixed with the insecticide methomyl and the aqueous suspension was sonicated. Finally, the suspension was exposed to UV light for 5 min with further centrifugation to remove free methomyl. The suspension was dried to form a fine powder. The mean size of the nanoparticles ranged from 78 nm to 99 nm, with a negative zeta potential from −17 mV to −23 mV and entrapment efficiency ranging from 94% to 97%. Crosslinking significantly slowed the insecticide release

relative to the non-crosslinked samples, and the crosslinked nanoparticles also showed improved insecticidal efficacy relative to the control (free methomyl).[50]

An interesting new star amphiphilic copolymer was formed from poly(aspartic acid) and polysuccinimide (PSI). The amphiphilic properties of the copolymer allow its self-assembly in water and entrapment of the synthetic plant hormone naphthaleneacetic acid (NAA). The copolymer degrades at basic pH, providing pH-controlled release properties, of importance considering the basic environment of plant phloem (pH 8 to 8.5). The release profiles confirmed that a minimum amount of NAA (<20%) was released at pH 7 compared to almost 75% of NAA at pH 8.5 in 24 hours.[51] Alternatively, PSI nanoparticles can be prepared by dispersing PSI polymer in dimethylformamide and 2-aminoethoxyethanol, and dialyzing against DI water to precipitate the nanoparticles, followed by freeze-drying.[51,52] The polymeric nanoparticles showed a mean size of 20.6 nm and minimal toxic effects on plant tissue with no negative effect on soil microbial growth.[52]

3. Characterization of polymeric nanocarriers

Comprehensive characterization is a critical need to explain or predict the behavior and efficiency of nano-enabled agrochemicals.[6] Fig. 2 summarizes important properties to characterize. Notably, additional characterization will be needed beyond what has been specified in previous "minimum characterization" guidance that was developed for inorganic nanoparticles.[54–57] In particular, the loading and release behavior of active ingredients within the polymer matrix,[58] as well as the composition and phase of the polymer itself, are needed. Furthermore, the internal structure of the polymeric nanoparticle will be important to explain the release or retention of active ingredients within the particle under environmental conditions. These special considerations are emphasized hereafter.

3.1. Size, morphology, internal structure, and surface charge

Particle size and surface charge are well known to be key factors in the fate and biological interactions of nanoparticles. Following the same methods of surface charge evaluation for inorganic nanoparticles, electrophoretic light scattering (ELS) is typically used to determine the electrophoretic mobility, which is converted to zeta potential using the Smoluchowski, Hückel, or Henry equations.

A tutorial review by Patterson *et al.*[59] covers the application of scattering techniques and microscopy to characterize the size and morphology or structure of self-assembled polymeric nanomaterials, which is also generally relevant to other polymer nanomaterials. Briefly, morphology or structural information can be acquired using a combination of dynamic light scattering (DLS) to obtain the hydrodynamic radius, R_h, together with static light scattering (SLS) for the radius of gyration, R_g. The relationship between R_g and R_h depends on particle morphology and can hence be used to deduce the shape (*e.g.* rod or spherical) or structure (*e.g.*, hollow or filled spheres) of the nanoparticles.[59] Microscopy techniques, including transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM), can also determine both size and important structural characteristics. For example, Ye *et al.* developed photolabile 2-nitrobenzyl succinate (NBS) – carboxymethyl chitosan (CMCS) micellar nanoparticles for pesticide delivery, in which the NBS forms a photodegradable core within a crosslinked CMCS shell.[60] Using TEM imaging, photodegradation of the NBS core could be deduced by the observed transformation of the micellar structures to hollow nanocapsules.

Polymeric particles can present new challenges to microscopy characterization methods relative to inorganic nanoparticles. Notably, organic nanomaterials will show lower contrast relative to the background, so the nanoparticles may need to be stained for improved imaging by TEM.[59,61] The use of high energy microscopy techniques such as TEM is also prone to cause beam damage to polymeric nanoparticles that must be considered.[61] For example, a diminishment in the measured size of latex particles of up to 29% over time in

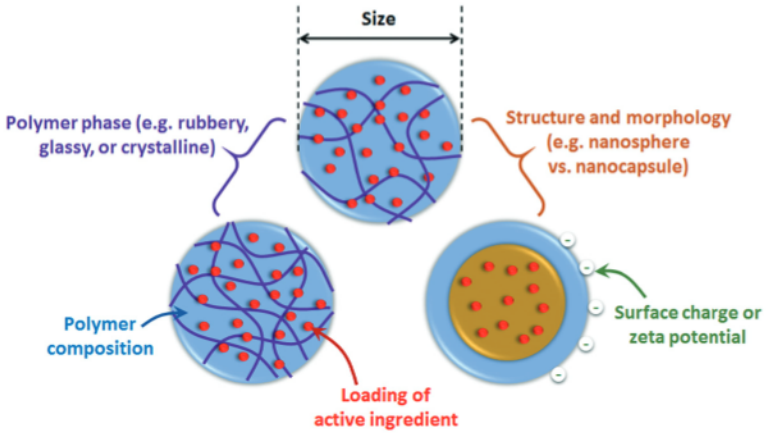

Fig. 2 Important physicochemical properties to characterize for polymeric nanocarriers.

TEM measurements was attributed to degradation under the high energy electron beam.[62] Drying artifacts will also be particularly significant for polymeric nanoparticles in conventional TEM, SEM, or AFM analysis, where sample dehydration can result in shrinking of the nanoparticles or bursting of hollow nanocapsules. Advanced methods such as cryo-TEM may be required to preserve the hydrated structure,[63] which can be particularly useful to visualize swelling and shrinking of polymer nanoparticles, *e.g.* for thermoresponsive polymers.[64] While AFM imaging can be performed in a liquid cell, the nanoparticles must be firmly attached to the substrate such that they are not removed by contact with the AFM tip.[59] Furthermore, since the forces imparted by the AFM tip during contact mode can deform soft polymeric materials, intermittent contact or tapping modes may be required.[65,66]

Direct characterization of the volume density profile of the polymer matrix typically requires the use of advanced methods. Small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) are useful to determine the internal radial structure of polymeric nanoparticles, as well as the core–shell structure of polymeric nanoparticles comprised of two polymers or block copolymers, as reviewed by Ballauff.[67,68] For nanoparticles with multiple components, either a combination of SAXS and SANS or contrast matching of each polymeric component in SANS (by deuteration of the polymers) can be applied to better distinguish the structure of each individual component.[67] Such detailed characterization can be important to understand the encapsulation and release of active compounds in the polymer particles, as has been demonstrated for drug delivery nanoparticles.[69] Overall, while each sizing method has advantages and limitations, the combined analysis of information from several different sizing methods (more than may be required for inorganic nanoparticles) is recommended to acquire a complete understanding of not only the size but also the structure of polymeric nanocarriers.

3.2. Phase and phase transitions of the polymeric matrix

The phase (*e.g.* rubbery, glassy, or crystalline) and phase transition temperatures of the polymer matrix can be critical to characterize for polymeric nanocarriers, because a phase change will strongly affect the release rate of active ingredients from the matrix, as discussed in section 4. Crystallinity can be evaluated by X-ray diffraction (XRD) and has previously been applied to confirm crosslinking in polymeric nanoparticles, *e.g.*, for chitosan nanoparticles after binding of cyclodextrin (which was used to enhance loading of hydrophobic pesticides),[27] or alginate nanoparticles crosslinked with calcium for pesticide delivery.[70] Differential scanning calorimetry (DSC) provides further information on the glass transition temperature (T_g) and melting temperatures of the polymer and the active ingredient. Finally, thermogravimetric analysis (TGA) provides the thermal degradation profile of the nanoparticles as well as quantitative information on the mass composition, provided the degradation temperatures of different components are distinct and represent a significant mass percent of the particle.

Strong interactions between active ingredients and the polymer can result in shifts or disappearance of phase transition or thermal degradation temperatures in the loaded nanoparticles compared to the individual components. For example, a change in the melting temperature of the pure active ingredient has been suggested to indicate successful dispersion of antibiotics[71,72] and herbicides[30,31,73] in an amorphous state throughout the nanoparticles. T_g of the polymeric matrix can also be affected by the presence of the active ingredient, depending on the size, structure, hydrophilicity, and amount of loaded ingredient.[18,74] For example, Stloukal *et al.* reported that T_g decreased with increasing loading of an herbicide, metazachlor, in poly(lactic acid) nanoparticles.[18] Therefore, it will be important to evaluate phase transition temperatures on each specific sample, rather than relying on reference data for bulk materials, to predict temperature-dependent release behavior for polymeric nanocarriers.

3.3. Chemical composition of polymer and active ingredients

Spectroscopic methods, particularly attenuated total reflectance-Fourier-transform infrared (ATR-FTIR) spectroscopy, are frequently performed to confirm the polymer composition, as well as the presence of active ingredient if the loading is above the detection limit and has distinct spectral features from the polymer. A strong interaction between the polymer and active ingredient may also be deduced from changes in the peak intensity, peak location, or peak broadening of functional groups participating in the interaction. For this analysis, the spectrum of the loaded nanoparticle should be compared to not only the "empty" nanoparticle and pure active ingredient controls, but also a "physical mixture" of the active ingredient and empty nanoparticles to confirm whether or not spectral changes are attributable to entrapment within the nanoparticle.

Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), and proton and carbon nuclear magnetic resonance spectroscopy (^{1}H NMR and ^{13}C NMR, respectively) are less commonly applied but can provide further information beyond ATR-FTIR spectroscopy. An advantage of Raman over ATR-FTIR spectroscopy is the significant reduction in interferences from liquid water;[75] hence, Raman spectroscopy has recently been shown to be capable of obtaining spectra of individual drug-loaded PLGA nanoparticles in combination with optical trapping.[76] XPS and NMR can further provide information on structure: for example, Celasco *et al.* reported the use of depth profiling XPS and angle-resolved XPS to distinguish the organization of poly(ethylene glycol) copolymers in nanosphere *versus* nanocapsule structures,[22] and ^{1}H NMR has been applied to understand the mobility of drug molecules in liposomes or solid lipid nanoparticles.[77,78]

Additional research is needed that applies these methods not only to the as-synthesized nanocarriers, but also after exposing the nanoparticles to environmental conditions, such

as light exposure or biodegradation. For example, Chen *et al.* applied FTIR and ^{1}H NMR spectroscopy to confirm the proposed pH-dependent hydrolysis of polysuccinimide (PSI) groups for targeted plant phloem delivery of plant hormones.[51] Ye *et al.* also demonstrated the use of ^{1}H NMR to confirm the formation of photolytic products in micellar carboxymethyl chitosan nanoparticles with 2-nitrobenzyl modification for photo-responsiveness.[60] Mass spectrometry is also applied to identify polymer degradation products, *e.g.* for PLGA.[79] *In situ* (flow cell) ATR-FTIR methods have previously been used to monitor adsorption[80–86] and chemical reactions or degradation[87–89] of organic coatings on inorganic nanoparticles; these methods would be interesting to apply for polymeric nanocarriers to further evaluate the kinetics of transformation or degradation and hence understand their long-term fate and interactions in the environment.

3.4. Quantification of the loading and release of active ingredients in simple media

The loading and release rate of active ingredients from nanocarriers are key factors in assessing or predicting their efficiency. Two approaches can be used (Fig. 3): either the concentration of ingredients remaining inside the polymeric matrix is measured, or the released ingredients are quantified. Regardless of the chosen approach, separation of nanoparticles from the matrix (which includes the released ingredients) is required.

Traditional quantification of loading or release involves separation of the nanoparticles and dissolved materials *prior* to measurement (Fig. 3b). In some separation methods (*e.g.*, ultracentrifugation or centrifugal ultrafiltration), release may be overestimated due to the force applied during the separation process or time required to process the sample.[90] If the nanoparticles are needed for further analysis, another drawback is the possibility for poor recovery. Dialysis is a gentler separation process, but slow diffusion of dissolved ingredients through the dialysis membrane may result in underestimation of the true release rate.[90] In addition, the released compounds are significantly diluted in the dialysate, which may require the use of high nanoparticle concentrations to achieve measurable results (however, if "sink" conditions are maintained on the dialysate side, release rates are still representative of diluted conditions *e.g.* as would occur when diluting a formulation for use in the field). After separation, released ingredients in the filtrate, dialysate, or supernatant can be easily quantified by high performance liquid chromatography (HPLC) or batch UV-vis spectrophotometry. To quantify the entrapped ingredient, addition of an organic solvent is often required to extract compounds from the polymeric matrix or dissolve the polymeric nanoparticle. In both measurements, the presence of dissolved polymer or other reagents can interfere with the analysis, and hence it is important that high recovery is confirmed in spike recovery tests or appropriate corrections are made, *e.g.* by the method of standard additions instead of quantifying against external standards.

Direct quantification of entrapped ingredients within an intact nanoparticle without a need for pre-separation can provide advantages to the traditional approach described above.

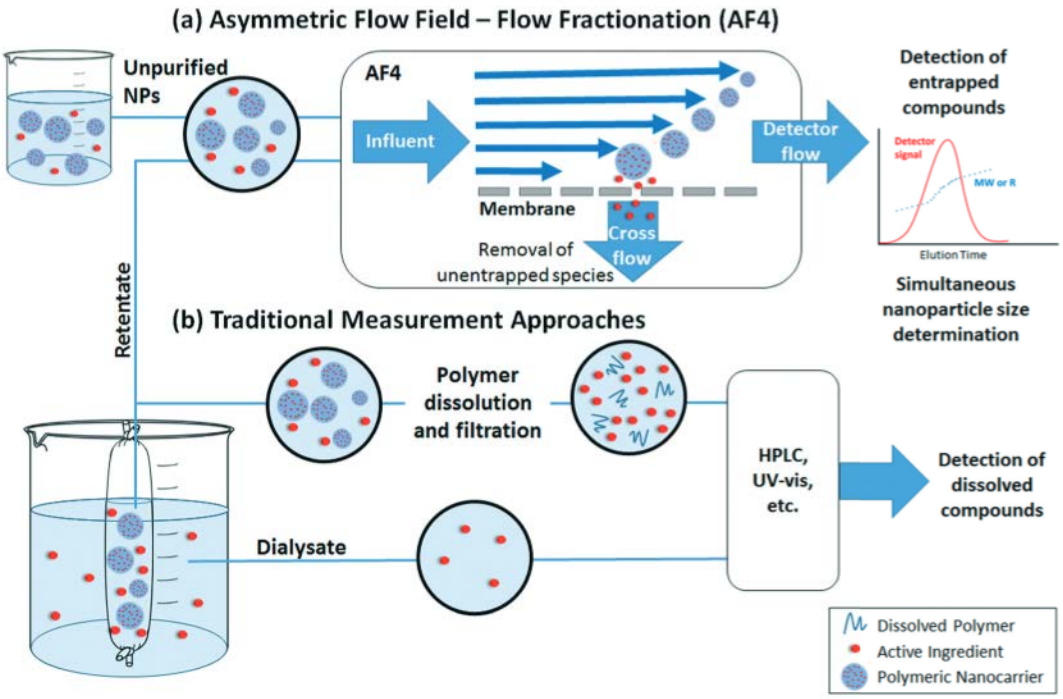

Fig. 3 Methods for measurement of active ingredient loading and release by (a) asymmetric flow field – flow fractionation (AF4) or (b) traditional measurement approaches.

However, such approach requires the compound of interest to have a distinct property (*e.g.* fluorescence or UV-vis absorbance) from the polymer and minimal matrix interference. Asymmetric flow field – flow fractionation (AF4) (Fig. 3a) is a relatively new approach that can eliminate sample processing steps and provide simultaneous particle characterization together with quantification of loading. In this method, the nanoparticles are focused in an AF4 channel at the beginning of the analysis; incidentally, the nanoparticles are also separated from dissolved species (which pass through a cross-flow membrane) during this step. Hence, no pre-separation steps are required as in traditional measurement approaches. Thereafter, the nanoparticles are separated by size (diffusion coefficient) in the AF4 channel, enabling size-resolved detection and characterization by downstream detectors. Based on the choice of detectors, quantitative information about the loading (*e.g.*, using the UV-vis absorbance or fluorescence of the active ingredient), as well as the concentration and size distribution of the nanoparticles, can be obtained. Sources of error include the potential for entrapped ingredients to be washed out during the focus step,[91–93] the need to correct for any particle scattering contributions to the signal used,[94,95] as well as the possibility for interactions of the active ingredient within the nanoparticle to change its spectral properties.[96] Despite these issues, AF4 with online UV-vis detection has successfully been applied by Hinna *et al.* to quantify a porphyrin drug within liposomal nanocarriers,[94,97,98] by Iavicoli *et al.* to quantify the binding of antimicrobial peptides to liposomes,[99] and by Fraunhofer *et al.* to quantify oligonucleotide loading on gelatin nanoparticles.[100]

Dialysis and AF4 can be successfully performed in aqueous matrices containing dissolved humic substances or biomolecules as well as other ingredients that may comprise the matrix of a commercial formulation. Chromatographic methods such as AF4 can even probe interactions between the nanoparticles and matrix components. For example, Holzschuh *et al.* have applied A4F to separate liposomes from human plasma and to evaluate lipid and drug transfer from the liposomes.[93] However, to our knowledge, most studies evaluate release in only simplified media (deionized water at a specified pH, possibly with a background electrolyte). Interactions with natural molecules present in soil porewaters, as well as other solutions that may be co-applied (*e.g.* fertilizer solutions[101]) should be considered in future studies.

3.5. Detection and characterization in complex matrices

The application of polymeric nanoparticles in soils, plants, and animals introduces the significant challenge of finding a carbon-based material in a highly complex matrix full of other organic carbon species and solid or particulate material. Measuring active ingredient release rates will also be highly challenging. Incorporation of a probe compound, such as a fluorescent tag[10,11,102] or radiolabeled polymers, in the nanoparticle is often used to identify the particle by imaging or other methods. Otherwise, the nanoparticles would need to be isolated from the media due to the severe interferences. However, extraction processes are likely to be either ineffective or likely to disrupt the nanomaterial or the partitioning of the active ingredient. Kah *et al.* highlight the difficulty in measuring release in soils and suggest that release may only be possible to evaluate through indirect methods.[6] One such method that has been applied for pesticides[103,104] is to assume that degradation of the active ingredient occurs only upon release. Then, the total remaining (undegraded) active ingredient in the soil can be extracted into organic solvent at several time points for measurement by HPLC, and the rate of degradation is measured for the pure (unentrapped) pesticide and the nano-formulation. Models that incorporate both the release rate and degradation rate of released compound can then be fitted to estimate the release rate.

4. Mechanisms for release of active ingredients

The release profile of the active ingredient from the polymer matrix will be critical in designing or predicting the behavior of the overall nanoparticle, *e.g.* controlled, slow release for prolonged application, or stimuli-responsive release for timed or targeted delivery of active compounds.[1] Release can occur by Fickian diffusion, swelling or relaxation of the polymer (promoting more rapid diffusion), and surface or bulk erosion (degradation) of the nanoparticle.[105] An initial "burst" release is also commonly observed. Major factors affecting the release rate are illustrated in Fig. 4 for the diffusion and relaxation mechanisms (which do not involve decomposition of the polymeric nanoparticle) and Fig. 5 for the erosion mechanisms (in which polymer degradation leads to release).

4.1. "Burst" release

Burst release refers to the phenomenon in which an initial rapid release of active ingredient occurs prior to slow release, and can be undesirable if an initially high concentration of active ingredient is not tolerable for the application of interest.[105] A burst release phenomenon would indicate a higher concentration of active ingredient residing on or near the surface of the nanoparticles after synthesis, with smaller nanoparticles (higher surface area to volume ratio) demonstrating more significant burst releases, as shown by Stloukal *et al.* for poly(lactic acid) (PLA) nanoparticles loaded with an herbicide, metazachlor.[18] The use of a nanocapsule structure or a coating around the surface of the nanoparticles has been suggested to suppress the rapid initial "burst" release that is often observed for nanospheres.[106]

4.2. Release by diffusion through the polymer matrix and nanoparticle swelling/relaxation

In Fickian diffusion, active ingredients will diffuse from regions of high concentration inside the nanoparticle to low concentration outside the nanoparticle following Fick's second law. Because of the dependence of release rate on the

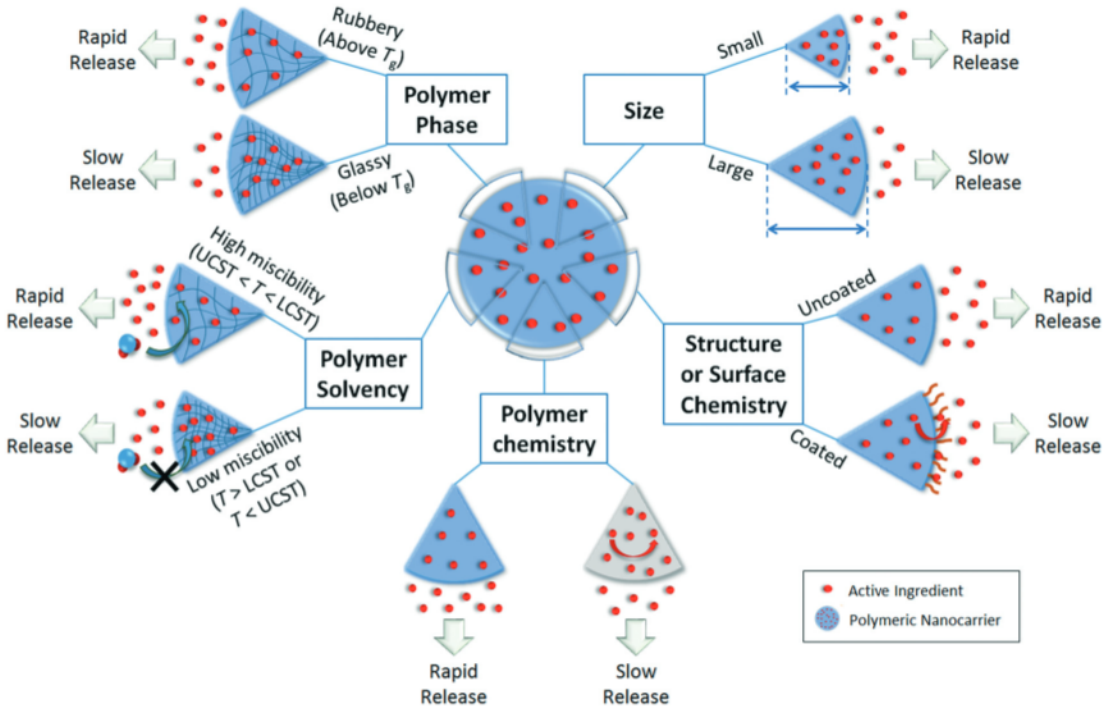

Fig. 4 Diffusive release of active ingredients from polymeric nanocarriers, and effects of material properties and environmental conditions on the release rate. In addition to the size and chemistry of the particles, the polymer phase and polymer solvency can strongly affect the diffusion rate of the active ingredient and can vary with the temperature relative to the glass transition temperature (T_g) and the upper or lower critical solution temperature (UCST or LCST, respectively) of the polymeric nanoparticle.

concentration gradient, release would occur more rapidly when the nanocarriers are diluted, *e.g.* upon dilution of a solid or concentrated formulation by growers prior to application, or during rainfall or irrigation events. Release by Fickian diffusion can be slowed by increasing the nanoparticle size (*i.e.* increasing the distance across which the active ingredient must diffuse). For example, in addition to a reduced burst release, Stloukal *et al.* also observed slower release of metazachlor by diffusion from PLA nanoparticles as the size increased.[18] Increased cross-linking has also been reported as a successful strategy to delay diffusion by decreasing the porosity or increasing the tortuosity through the polymer matrix, as shown for a methomyl pesticide loaded into azidobenzaldehyde–carboxymethyl chitosan nanocapsules before and after crosslinking of the polymer.[50]

Swelling or relaxation of the polymeric nanoparticle will cause faster release of active ingredients as they dissolve into the infiltrating solvent (typically an aqueous medium) and transport more rapidly out of the relaxed polymer matrix through the solvent-filled pores. This mechanism is referred to as "Case II" transport, and can be distinguished from Fickian diffusion by modeling the release profile. For example, the empirical Korsmeyer–Peppas model[107] (eqn (1)) is frequently applied to distinguish release mechanisms:

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

where M_t/M_∞ is the fraction of drug released at time t, k is a rate constant, and the exponent n is representative of the release mechanism. For spherical particles, n = 0.43 corresponds to Fickian diffusion as the rate-limiting phenomenon, n = 0.85 corresponds to case II transport (relaxation is rate-limiting), and $0.43 < n < 0.85$ corresponds to "anomalous transport," which can arise from a combination of diffusion and relaxation.[108]

Polymer swelling and relaxation can be strongly affected by environmental factors, such as temperature, and hence be exploited to achieve triggered or stimuli-responsive release in agricultural applications.[109] Important temperatures of note are the upper critical solution temperature (UCST) and lower critical solution temperature (LCST), between which the

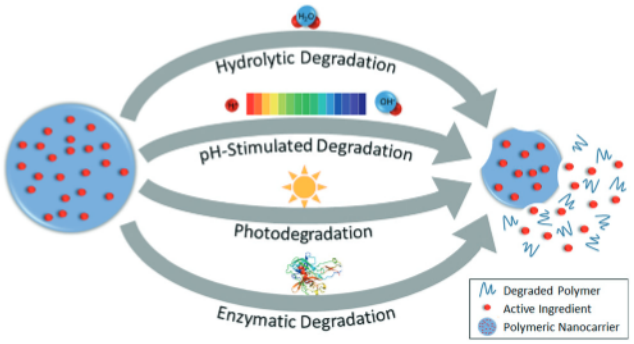

Fig. 5 Release of active ingredients from polymeric nanocarriers by degradation of the polymeric matrix.

polymer is miscible with the solvent. For example, poly-*N*-isopropyl acrylamide (PNIPAm) is a well-known temperature-sensitive polymer that swells at temperatures below its LCST of 32 °C. Grafting of the PNIPAm polymer onto polydopamine (PDA) nanoparticles has hence been shown to lead to temperature-dependent release of a pesticide, emamectin benzoate, with faster release at lower temperature attributable to swelling of the PNIPAm below the LCST.[110]

Another important thermal property is the glass transition temperature (T_g), describing the phase transition of the polymer from glassy (rigid) below T_g to rubbery (flexible) above T_g. Lappe *et al.*[74] showed that for DL-PLA, L-PLA, and PLGA nanocarriers, primarily burst release of adsorbed drugs on the nanoparticle surface occurred at temperatures below T_g. On the other hand, at temperatures higher than T_g, higher release of the entrapped drugs occurred.[74]

To further slow the release of an active compound below the rate of Fickian diffusion or the swelling/relaxation rate, materials can be selected such that the active ingredients have more favorable interactions with the components of the nanoparticle matrix relative to the solvent. For example, when Campos *et al.* compared the release of two pesticides, carvacrol and linalool, co-loaded in β-cyclodextrin-functionalized chitosan nanoparticles, faster and more extensive release of the more hydrophilic linalool ingredient was observed.[111] Grillo *et al.* compared the release rates and profiles of three herbicides, ametryn, atrazine, simazine, from nanocapsules with a PCL shell and an oil core.[41] The slower release of ametryn compared to atrazine was attributed to the higher affinity of ametryn with either the PCL shell or oily interior of the nanocapsule. The release was slowest for simazine, which was proposed to occur because of hydrogen bonding between simazine and the PCL shell of the nanoparticles, which is blocked by the methyl groups present on atrazine and ametryn.[41]

Similarly, different structures or compositions of the nanoparticle have been proposed to tune the release kinetics. Nanocapsules or vesicles comprised of a shell surrounding a core of a different composition have been suggested to provide slower release profiles than homogeneous nanospheres;[106] however, release profiles were similar for atrazine loaded into PCL nanocapsules compared to PCL nanospheres.[37] Therefore, tuning the chemistry of the coating or shell around the nanoparticle may be a more promising strategy to delay release, as opposed to developing nanoparticles comprised of the same material in different nanocapsule or nanosphere structures. For example, for pesticide delivery, the addition of a polyurea coating onto imidacloprid-loaded PDA microcapsules[112] or a chitosan coating onto deltamethrin-loaded beeswax solid lipid nanoparticles[113] delayed the release relative to the uncoated nanoparticles. Sun *et al.* also reported that high entrapment of a pesticide, methomyl, in carboxymethyl chitosan nanocapsules was primarily attributable to adsorption of the methomyl to the polymer, rather than partitioning into the aqueous interior of the nanocapsules.[50] Additional characterization and predictive models to localize interactions between the active ingredient and the specific components of the nanoparticle would be useful to better predict *a priori* materials that can be used to develop nanoparticles with a desired release rate.

4.3. Degradation of nanoparticles

Release can be accelerated or triggered by chemical, physical, or biological degradation of the nanoparticle. This degradation can proceed by hydrolysis with water, or it can require a specific stimulus, such as a change in pH or temperature, light exposure, or enzymatic activity, to occur (Fig. 5).[109]

In hydrolytic degradation, water participates in a cleavage reaction of vulnerable bonds such as esters, degrading the polymer chains and then leading to loss of mass from the nanoparticle.[114] For instance, PLGA nanoparticles show slow degradation that occurs by bulk erosion *via* hydrolysis of ester bonds; after the initial hydrolysis, faster degradation is catalyzed by the increasing water penetration and formation of carboxylic groups.[115,116] Nano-sized PLGA shows faster hydrolytic degradation than micro-sized PLGA because of the higher surface area to volume ratio (*i.e.* higher accessibility to water), as well as the greater ease for polymer degradation products to diffuse out through the polymer matrix.[117] The degradation rate can also be tuned by adjusting the composition of a nanocarrier such that the proportion or accessibility of labile bonds is modified. For example, the rate of hydrolysis of nanoparticles composed of mixtures of PLGA and poly(L-lactic acid) or solely of PLGA with different ratios of lactic acid to glycolic acid, decreases with increasing lactic acid content: the methyl side groups on the lactic acid impart steric hindrance inhibiting the hydrolysis of the ester bonds[118] while the glycolic acid groups have higher bound, reactive water content.[119] On the other hand, incorporating methoxy poly(ethylene glycol) (mPEG) in PLGA nanoparticles leads to faster degradation of the nanoparticles,[120] since the mPEG increases the hydrophilicity of the nanoparticle and hence accessibility for hydrolysis.[121]

Polymer degradation can be acid- or base-catalyzed, enabling pH-responsive release. For example, solid lipid nanoparticles have been synthesized with acetal groups that are cleavable under acidic conditions (*e.g.*, pH 6.5) for targeted release of vancomycin antibiotics at acidic infection sites.[122] In plants, the pH is higher in the phloem than other regions,[123] and hence pH-sensitive PSI-based nanoparticles have been proposed for triggered release of active compounds in the phloem. For example, Chen *et al.*[51] suggested the use of poly(aspartic acid-*co*-succinimide) polymeric nanoparticles for targeted delivery of a synthetic plant hormone, naphthaleneacetic acid (NAA), to the phloem of plants. These nanoparticles are stable under neutral conditions. In contrast, at pH 8.5, the PSI units of the nanoparticles are hydrolyzed to polyaspartate, resulting in more rapid release of the NAA.[51] Similarly, the release of two model compounds, coumarin 6 (ref. 52) and Nile Red,[124] from PSI-based

nanocarriers occurs more rapidly at basic pH, with slightly faster release of Nile Red under hydrolytic conditions for smaller nanoparticles with higher surface area.[124] Functionalization of the PSI with hydrophobic hexylamine was able to prevent base hydrolysis and dye release,[124] providing another option to tune the release behavior by tuning the penetration of solvent carrying reactive species into the polymer matrix.

The pH can also affect the physical stability of the nanoparticle when the polymer is a weak acid or base, such that the charge and electrostatic interactions will depend on pH. For example, Lin *et al.* developed nanoparticles from feather keratin and carboxymethyl cellulose (CMC) loaded with a pesticide, avermectin.[28] While diffusion was Fickian at lower pH, the release rate became faster and non-Fickian transport at higher pH. The faster release was proposed to be caused by the transition of the keratin to negative charge at higher pH, resulting in electrostatic repulsion with the negatively-charged CMC and dissociation of the nanoparticles.

Stimuli-responsive release can also be achieved using photosensitive polymers. For example, UV-labile core–shell or micellar nanoparticles were developed by conjugating nitrobenzyl compounds to carboxymehtyl chitosan[60] and poly(ethylene glycol) (PEG)[125] polymers. These nanoparticles were loaded with diuron and 2,4-dichlorophenoxyacetic acid (2,4-D) herbicides, respectively, and demonstrated to exhibit UV-triggered release. Further study on light-activated nanoparticles would be interesting for applications of nanoparticles in sunlit environments, such as foliar delivery of agrochemicals.

Finally, the activity of enzymes such as proteases, glycosidases and phosphatases can induce the degradation of nanoparticles. For example, Chawla *et al.* found that the degradation of PCL nanoparticles increases dramatically in the presence of lipase enzyme in comparison with enzyme-free phosphate buffered saline.[126] They proposed that the hydrophilicity of the enzyme prohibits movement into the hydrophobic interior of the nanoparticle, so enzymatic hydrolysis occurs at the surface of nanoparticle where the enzyme adsorbs.[126] Another study by Fu *et al.* showed more rapid and extensive degradation of zein nanoparticles and release of an entrapped antibiotic, ciprofloxacin, in the presence of trypsin than collagenase or enzyme-free phosphate buffered saline.[127] *In vitro* enzymatic degradation of chitosan nanoparticles by lysozyme was also reported by Hou *et al.*[128] Akagi *et al.* demonstrated that the enzyme-mediated degradation of poly(γ-glutamic acid) (γ-PGA) nanoparticles by γ-glutamyl transpeptidase (γ-GTP), which is a common enzyme found in wide range of organisms, is more rapid than hydrolytic degradation.[129] In addition, enzymes such as pronase, protease, cathepsin B, and lipase, all of which may be present in *in vivo* systems, have also been reported to induce degradation of γ-PGA by cleaving the amide bond of the polymer.[130] Given the wide variety of enzymes present in *in vivo* systems and the variety of enzymatic activities demonstrated in these studies, additional research is needed to fully understand and develop a generic mechanism to predict the enzymatic degradation behavior of polymeric nanoparticles.

5. Environmental fate and biological effects

The fate, transport, bio-uptake, and biological effects of the polymeric nanoparticles and their associated active ingredients must all ultimately be optimized in order to develop a successful technology that improves the desired function of the active ingredient (compared to non-nano formulations) while having minimal adverse effects in the environment. Potential mechanisms for polymeric nanocarriers to play this role are highlighted below.

5.1. Fate, transport, and uptake of polymeric nanocarriers and their associated active ingredients

For agricultural applications, the goal of using a polymeric nanocarrier is often to reduce the overall quantity of agrochemicals needed, which can be achieved by improved targeting or uptake of the active ingredient or protecting the active ingredient from degradation (Fig. 6).

Enhanced photostability and reduced volatility of the active ingredient have been demonstrated across a variety of polymer types, as summarized in Table 2, and would reduce the quantities of pesticides required as well as the need for reapplication over time. Furthermore, the enhanced stability afforded by the nanoparticles enables the use of more sustainable active ingredients, such as botanical oils, that would be prone to degradation or volatilization in their unentrapped form.[21,38,131,132] Polymeric nanoparticles can also be designed to enhance the adhesion or uptake of agrochemicals, particularly for foliar applications (Table 2). For example, bio-inspired polydopamine and polycatechol-coated nanoparticles have been proposed for enhanced adhesion of pesticides to plant leaves.[133,134] Few studies are available that directly demonstrate plant uptake, likely due to the challenges in detecting polymeric nanoparticles within plants, but recent studies using fluorescently-labeled nanocarriers have shown promising results for foliar uptake of PCL nanoparticles (up to 345 nm in diameter) and root uptake of zein nanoparticles (135 nm).[10,102] For comparison, the typical upper size limits summarized by Lv *et al.* for inorganic nanoparticles are up to 140 nm for root uptake, with foliar uptake by stomatal pathways having a largely unknown size limit with uptake of up to ≈50 nm reported thus far.[135] Additional uptake studies on the variety of other polymer types that have been proposed as well as across a range of sizes are needed to identify the ideal nanoparticles for agrochemical delivery.

Subsequent to field application, the effect of the polymeric nanoparticles on the transport of the agrochemicals from soils is also of interest, given the problems of surface water and groundwater pollution from agricultural runoff. Varying results have been observed in the literature regarding whether entrapment or encapsulation enhances or reduces

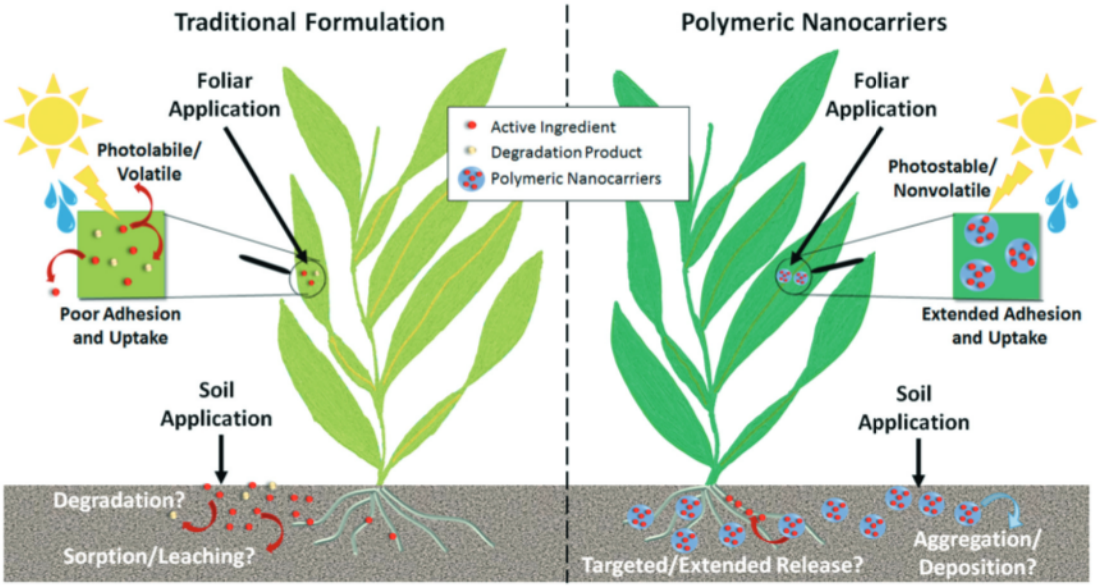

Fig. 6 Improved efficiency of agrochemicals achieved by enhanced uptake (by targeting or adhesion) and protection of active ingredients against degradation. Polymeric nanocarriers will also change the fate and transport of agrochemicals in soils, altering leaching profiles and environmental exposures.

release of the agrochemicals from soils. For example, loading of carbendazim and tebuconazole fungicides into polymeric and PCL nanocapsules and solid lipid nanoparticles resulted in diminished leaching from soils compared to commercial, non-nano formulations;[53] on the contrary, Grillo *et al.* and Silva *et al.* reported lower sorption of paraquat to soils when loaded into chitosan/tripolyphosphate and alginate/chitosan nanoparticles, respectively,[30,45] and Pereira *et al.* reported deeper penetration of atrazine into soil columns when loaded into PCL nanocapsules and nanospheres.[37] Chen *et al.*, Kah *et al.*, and Petosa *et al.* have each found that the transport or deposition of polymeric nanocarriers and their associated active ingredients (*e.g.* drugs or herbicides) varies widely with the type of polymer as well as the environmental conditions (*e.g.*, water chemistry and soil type).[104,136,137] The possibility for naturally occurring macromolecules such as natural organic matter, proteins, and polysaccharides to adsorb to the nanoparticles and change their transport behavior should also be considered. While Grillo *et al.* reported that aquatic humic substances did not affect the colloidal stability of paraquat-loaded chitosan nanoparticles,[45] Chen *et al.* observed a significant effect of the interaction of negatively-charged humic acids on the deposition of positively-charged poly(caprolactone-*b*-ethylenimine) (PCL-PEI) nanoparticles onto silica surfaces, consistent with charge neutralization and reversal.[136]

In summary, to fully describe the transport behavior of active ingredients carried by polymer nanoparticles, not only the aggregation and deposition behavior of the nanoparticle, but also the kinetics of release and the sorption behavior of the active ingredient, must all be taken into account. Hence, transport models can be more complex than those previously developed for inorganic nanoparticles (without an active ingredient loading), and a large suite of additional studies will likely be needed to develop such models.

5.2. Effectiveness for agricultural applications

For crop growth and protection, polymeric nanoparticles have been proposed to deliver plant growth promoters and pesticides, including insecticides, herbicides, and fungicides. For livestock and aquaculture, polymeric nanocarriers may also be used to deliver antibiotics. As summarized in Table 3, many types of polymeric nanoparticles or nanocapsules have been developed using biocompatible or biodegradable materials (*e.g.*, alginate, chitosan, zein, PEG, and PCL) to deliver both conventional synthetic herbicides, insecticides, and fungicides as well as unconventional, botanically derived oils as more sustainable alternatives.

For plant growth promoters, herbicides, insecticides, and fungicides, the nano-formulations typically show similar to improved efficiency compared to the unentrapped active ingredient. A possible mechanism for the improved efficiency is the ability for the nanoparticles to provide targeted or enhanced delivery, *e.g.* by designing nanoparticles that encourage the adhesion or uptake of the active ingredients by the target organism.[133,134] In contrast, hydrophobic insecticides can have potential flaws of poor solubility, which can reduce their targeting efficiency to less than 1%.[141] Nanoparticles have also been highlighted to perform particularly well over extended time durations,[132,142–144] *e.g.* by stabilizing the active ingredient against degradation or providing slow release properties, which reduces the overall quantity of pesticides required.

A limitation of the currently available data is that only ten of twenty-six studies identified in the literature compare the

Table 2 Effects of polymeric nanocarriers on the fate and uptake of active ingredients (A.I.)

Function	Polymer (nanoparticle diameter in parentheses)	Active ingredient	Benefits conferred by polymeric nanoformulations	Ref.
Photostability of active ingredients	PCL (450 to 465 nm by DLS)	Essential oils (insecticides)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 7 h exposure to UV-A and UV-C light	38
	Chitosan/gum arabic (≈200 nm)	Geraniol (insecticide)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 7 d exposure to UV (365 nm) light	21
	Zein (143 to 172 nm by DLS)	*R*-Citronellal, geraniol (insecticide)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 7 d exposure to UV (365 nm) light; photoprotection more apparent for geraniol than citronella	131
	Polydopamine (215 nm by TEM)	Avermectin (insecticide)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 78 h exposure to UV light	133
	Poly(styrene-*co*-methacrylic acid) – polycatechol (102 to 122 nm by DLS)	Avermectin (insecticide)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 96 h exposure to UV light	134
	Feather keratin – carboxymethylcellulose (≈390 nm by DLS)	Avermectin (insecticide)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 30 h exposure to UV light	28
	PLA (680 to 4600 nm by DLS)	λ-Cyhalothrin (insecticide)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 72 h exposure to UV (365 nm) light	138
	Beeswax solid lipid nanoparticles, with or without chitosan coating (≈200 to 230 nm by DLS)	Deltamethrin (insecticide)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 72 h exposure to UV-B light	113
	Polyacrylate (≈80 nm by DLS)	Emamectin benzoate (insecticide)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 9 h exposure to simulated sunlight	139
	PLGA (600 nm by laser particle size distribution analysis)	Pyraclostrobin (fungicide)	Enhanced photostability compared to unentrapped A.I., evaluated over up to 1 h exposure to UV light	140
Volatility of active ingredients	Zein (234 to 282 nm by DLS)	Cinnamaldehyde, eugenol, and geraniol (insecticides)	Reduced volatility compared to unentrapped A.I., evaluated for 120 d storage duration	132
Adhesion and uptake by plants	Polydopamine (215 nm by TEM)	Avermectin (insecticide)	Attachment to cotton and corn leaves from aqueous suspension, with and without water washing	133
	Poly(styrene-*co*-methacrylic acid) – polycatechol (102 to 122 nm by DLS)	Avermectin (insecticide)	Attachment to cucumber and broccoli leaves after spraying, drying, and washing	134
	PCL (256 to 345 nm)	Atrazine (herbicide)	Uptake through stomata, particularly in hydathode regions, and vascular transport in *Brassica juncea*	102
	Zein (135 nm)	None	Root uptake and translocation in sugar cane plants	10
	Zein (135 nm)	None	Association of nanoparticles with roots, with possible uptake and translocation, in soybean plants	11

Notes: A.I.: active ingredient; PCL: poly(ε-caprolactone); PLA: poly(lactic acid): PLGA: poly(lactic-*co*-glycolic acid).

activity of the nano-formulation to a commercial formulation. Of these studies, three report no significant difference for the nano-formulation,[19,25,145] and two report improvement only under specific conditions (*e.g.* at longer durations[142] or with an adhesive coating on the nanoparticles[134]). As noted by Kah *et al.*,[6] to truly demonstrate an advantage of the nano-formulation over currently available alternatives, several commercial formulations that may differ in composition (*e.g.* solution, suspension, or emulsion; with or without polymeric ingredients) should be compared. The majority of nano-carrier studies available report "nano"-formulations with diameters between 100 nm and 1000 nm, and more information is also needed to evaluate whether particle sizes <1000 nm truly confer additional benefits (*e.g.* enhanced uptake or targeting) that would justify their use over micron-sized polymeric particles (which would be expected to provide slower and more extended release). The two studies that compare microemulsions or water-dispersible granules report no improvement using the nano-formulation[25] or improvement only after adding an adhesive surface coating on the nanoparticles.[134] Additional studies providing side-by-side characterization of particle size (for suspensions or emulsions) and

Table 3 Potential benefits of polymeric nanocarriers for agricultural applications

Purpose	Polymer (nanoparticle diameter in parentheses)	Active ingredient (A.I.)	Target species	Activity against target species	Representative concentration		Cytotoxic, phytotoxic, or ecotoxicological effects	Ref.
					Free A.I.	NP A.I.		
Plant growth hormones	γ-Polyglutamic acid/chitosan (134 nm by DLS)	Gibberellic acid	*Phaseolus vulgaris*	Enhanced germination and development compared to unentrapped A.I.		[a]0.7 and 2.1 μg g^{-1} of seeds	Not evaluated	177
	Alginate/chitosan (450 nm by DLS); chitosan/tripolyphosphate (195 nm by DLS)	Gibberellic acid	*Phaseolus vulgaris*	Increased leaf area only for alginate/chitosan nanoparticles compared to unentrapped A.I.; shoot and root growth similar		[a]0.037% and 0.05%	Not evaluated	42
	Alginate/chitosan (450 nm by DLS); chitosan/tripolyphosphate (195 nm by DLS)	Gibberellic acid	*Solanum lycopersicum*	Enhanced root/shoot growth and fruit production compared to unentrapped A.I., particularly for the alginate/chitosan nanoparticles		[a]0.0005 to 0.005 mg ml^{-1}	Not evaluated	165
Herbicide	Alginate/chitosan (378 nm by DLS) and chitosan/tripolyphosphate (479 nm by DLS)	Imazapic and imazapyr (co-loaded)	*Bidens pilosa*	Similar herbicidal activity to the unentrapped A.I. (evaluated at 400 g ha^{-1})		[a]n/a (no significant difference)	Lower cytotoxicity and genotoxicity to Chinese hamster ovary cells and *Allium cepa* seedlings, compared to unentrapped A.I.	169
	Chitosan/tripolyphosphate (300 nm by DLS)	Paraquat	*Brassica* sp.	Similar herbicidal activity to the unentrapped A.I. (evaluated at 2 kg ha^{-1})		[a]n/a (no significant difference)	Less pronounced phytotoxicity to non-target *Zea mays* plants; lower cytotoxicity and genotoxicity to Chinese hamster ovary cells and *Allium cepa* seedlings, compared to unentrapped A.I.	45
	Chitosan/tripolyphosphate (282 nm by DLS)	Paraquat	n/a	n/a	n/a	n/a	Lower toxicity to *Pseudokirchneriella subcapitata* (algae) than unentrapped A.I.	43
	Alginate/chitosan (200 to 1000 nm by DLS)	Clomazone	n/a	n/a	n/a	n/a	Similar hepatotoxicity to *Lithobates catesbeianus* (bullfrog tadpoles) compared to unentrapped A.I.	178
	Lignin (150 to 190 nm by NTA)	Diuron	*Brassica rapa*	Similar or greater leaf chlorosis and mortality compared to unentrapped A.I.; similar efficiency to commercial formulation		[a]2.5 mg/pot compared to unentrapped A.I.	Not evaluated	19
	Pectin nanocapsules (164 nm by DLS)	Metsulfuron methyl	*Chenopodium album* in wheat crop (*T. aestivum*)	Reduced weed biomass compared to commercial formulation		[a]50 mg L^{-1} (with 6.3% herbicide loading); total dose not reported	Lower cytotoxicity to Vero cell lines, compared to commercial formulation	20
	PCL nanocapsules	Atrazine	*Brassica juncea*	Similar reduction in shoot		[a]n/a (no	Not evaluated	145

Table 3 *(continued)*

Purpose	Polymer (nanoparticle diameter in parentheses)	Active ingredient (A.I.)	Target species	Activity against target species	Representative concentration		Cytotoxic, phytotoxic, or ecotoxicological effects	Ref.
					Free A.I.	NP A.I.		
	(241 nm by DLS)			growth compared to commercial formulation (evaluated at 0.1 and 1 mg mL^{-1})		significant difference)		
	PCL nanocapsules (483 nm by DLS) and wfi 1nanospheres (409 nm by DLS)	Atrazine	*Brassica* sp.	Greater inhibition of seedling emergence by nanocapsules and nanospheres compared to unentrapped A.I.		[a]2.5 kg ha^{-1}	No effect on non-target crops (*Zea mays*); reduced genotoxicity to *Allium cepa* at some concentrations	37
	PCL nanocapsules (size not reported)	Atrazine	*Bidens pilosa*	Higher mortality of weeds at lower dose compared to commercial formulation		[a]200 g ha^{-1}	Higher short-term (17 d) toxicity to non-target *Glycine max* (soybean) plants, but gradual recovery over 60 d	179
	PCL nanocapsules (260 nm by DLS)	Atrazine	*Amaranthus viridis* and *Bidens pilosa*	Inhibition of root and shoot growth at lower dose compared to commercial formulation		[a]200 and 2000 g ha^{-1}	Not evaluated	180
	PCL nanocapsules (size not reported)	Atrazine	n/a	n/a	n/a	n/a	No effect of atrazine (commercial or nanocapsule) to *Zea mays* L. at 0.1 mg mL^{-1}; reduction in photosynthesis for 1 to 2 days with nanocapsule at 1 mg mL^{-1} but recovery by 4 days after application	181
	PCL nanocapsules (260 nm by DLS)	Ametryn, atrazine, and simazine	n/a	n/a	n/a	n/a	Reduced DNA damage in Comet assay, reduced genotoxicity to *Allium cepa* compared to unentrapped A.I.	41
	PCL nanocapsules (200 to 300 nm by DLS)	Ametryn, or atrazine	n/a	n/a	n/a	n/a	Lower toxicity to algae, higher toxicity to *Daphnia similis*, and lower cytotoxicity to lymphocytes than unentrapped A.I.	39
	PCL nanocapsules, chitosan/tripolyphosphate, and SLNs (≈250 to 370 nm)	Atrazine, simazine, and/or paraquat	n/a	n/a	n/a	n/a	Higher toxicity to *C. elegans* for both empty and loaded nanocarriers compared to unentrapped A.I.	182
	mPEG-PLGA (≈90 nm by DLS)	Metolachlor	*Oryza sativa*, *Digitaria sanguinalis*, *Arabidopsis thaliana*	Inhibited seedling growth (unentrapped A.I. was not evaluated)	Not evaluated	[a]0.1 mg L^{-1}	Lower cytotoxicity to MC3T3 preosteoblast cells than unentrapped A.I.	183
Insecticide or insect repellent	Alginate (150 nm by DLS)	Imidacloprid	Leafhoppers	Lesser efficacy in reducing pest population over short duration (7 d), but improved efficacy at		[a]0.145 mg L^{-1} (0.02 mg m^{-2} total dose)	Lower cytotoxicity to Vero cell lines than commercial formulation	142

Table 3 *(continued)*

Purpose	Polymer (nanoparticle diameter in parentheses)	Active ingredient (A.I.)	Target species	Activity against target species	Representative concentration		Cytotoxic, phytotoxic, or ecotoxicological effects	Ref.
					Free A.I.	NP A.I.		
				longer durations (9 to 15 d), compared to commercial formulation				
	Carboxymethyl chitosan nanocapsules with aqueous core (90 to 99 nm by DLS)	Methomyl	Armyworm larvae	Higher larvicidal activity compared to the unentrapped A.I.		[a]50 and 100 mg L^{-1} in spray	Not evaluated	50
	Chitosan with β-cyclodextrin functionalization (175 to 246 nm by DLS)	Carvacrol or linalool (separately loaded)	*Tetranychus urticae*	Higher repellency, and higher acaricidal activity and hindrance of oviposition, compared to the unentrapped A.I.		[a]1.56 mg cm^{-2} of leaf area	Not evaluated	27
	Chitosan/gum arabic with β-cyclodextrin functionalization (226 nm by DLS)	Carvacrol and linalool (co-loaded)	*Helicoverpa armigera*, *Tetranychus urticae*	Higher insecticidal activity, and higher acaricidal activity and hindrance of oviposition for *T. urticae*, compared to the unentrapped A.I.		[a]1.25 mg ml^{-1}	Lower cytotoxicity to pulmonary (v79) and mouse fibroblast (Balb C-33) cell lines, and lower phytotoxicity to *Zea mays*, than unentrapped A.I.	111
	Polyacrylate (≈80 nm by DLS)	Emamectin benzoate	*Helicoverpa armigera*	Improved efficacy for larva mortality over 72 h compared to unentrapped A.I.		[a]1%	Not evaluated	139
	PCL (450 to 465 nm by DLS)	Essential oils	*Bemisia tabaci*	Reduction in eggs and nymphs compared to pyriproxyfen 1% insecticide (unentrapped essential oils not evaluated)	Not evaluated	[a]1%	Not evaluated	38
	PEG (≈230 nm by DLS)	Garlic oil	*Tribolium castaneum*	Improved insecticidal activity over 5 month duration compared to unentrapped A.I.		[a]640 mg kg^{-1} of rice for 5 months	Not evaluated	144
	PEG copolymer (initial size 10 to 20 nm by TEM, followed by formation of microcapsules)	Imidacloprid	*Glyphodes pyloalis*	Higher efficiency for larva mortality compared to unentrapped A.I., especially over longer durations (2 to 5 d)	[b]Time- and assay-dependent (*e.g.* 60 mg L^{-1} at 5 d)	[b]Time- and assay-dependent (*e.g.* 7 mg L^{-1} at 5 d)	Not evaluated	143
	PEG–PLA (150 nm by DLS)	λ-Cyhalothrin	*Aphis craccivora*	Similar aphid mortality compared to commercial emulsion or microemulsion	[b]0.27 mg L^{-1}	[b]0.26 mg L^{-1}	Not evaluated	25
	Unknown polymer (commercial formulation separated into ≈250 nm and ≈2200 nm fractions)	λ-Cyhalothrin	n/a	n/a	n/a	n/a	Lesser tremors in embryonic *Danio rerio* for unentrapped A.I. compared to all polymeric formulations; otherwise similar sublethal impacts and mortality for all A.I. exposures	184
	Poly(styrene-*co*-methacrylic acid) – polycatechol (102 to 122 nm by DLS)	Avermectin	Aphids	Improved efficiency with adhesive polycatechol functionalization compared to commercial emulsification and	[b]10.1 to 12.4 mg L^{-1} on cucumber; 124.6 to 150.3 mg L^{-1}	[b]4.3 mg L^{-1} on cucumber; 55.4 mg L^{-1} on broccoli for	Not evaluated	134

Table 3 *(continued)*

Purpose	Polymer (nanoparticle diameter in parentheses)	Active ingredient (A.I.)	Target species	Activity against target species	Representative concentration		Cytotoxic, phytotoxic, or ecotoxicological effects	Ref.
					Free A.I.	NP A.I.		
				water-dispersible granule formulations; nanoformulations without polycatechol showed similar or lower efficiency than commercial formulations	on broccoli for commercial formulations	catechol-functionalized NPs		
	Zein (143 to 172 nm by DLS)	Geraniol or *R*-citronellal	*Tetranychus urticae*	Better insect repellent activity for geraniol nanoformulation compared to unentrapped A.I. at shorter times (*e.g.* 8 h and 24 h)		[a]0.5 and 5 mg ml^{-1}	Similar or lower cytotoxicity and phyotoxicity to pulmonary fibroblast permanent cell line (v79) and fibroblast cell line (3 T3) and *Phaseolus vulgaris*, respectively, than unentrapped A.I.	131
	Zein (234 to 282 nm by DLS)	Cinnamaldehyde, eugenol, or geraniol	*Tetranychus urticae*, *Chrysodeixis includes*	Lesser insect repellency to *T. urticae* at short time (2 h) compared to unentrapped A.I., but improved repellency after longer times because of sustained release; lower mortality and sublethal effects to *C. includes*		[a]5 mg ml^{-1}	Lower cytotoxicity to pulmonary fibroblast permanent cell line (v79) and fibroblast cell line (3 T3) than unentrapped A.I.	132
	Zein (288 nm by DLS)	Neem oil	n/a	n/a	n/a	n/a	Lower chromosomal damage to *Allium cepa* and lower toxicity to *C. elegans* than commercial formulation; no significant long-term effect on soil bacterial community for N cycling	170
Fungicide	Chitosan/tripolyphosphate (100 nm by DLS)	Hexaconazole	*Rhizoctonia solani*	Better antifungal activity at moderate concentration compared to commercial formulation		[a]1 mg L^{-1}	Similar or lower cytotoxicity to Vero cell lines than commercial formulation	7
	Chitosan/pectin (129 nm by DLS)	Carbendazim	*Aspergillus parasiticus*, *Fusarium oxysporum*	Better antifungal activity compared to both unentrapped A.I. and commercial formulation		[a]0.5 mg L^{-1}	Lower phytotoxicity to *Zea mays*, *Cannabis sativa*, and *Lycopersicon esculentum* than unentrapped A.I.; no bacterial inhibition against *E. coli* or *S. aureus* for both nano- and unentrapped A.I.	23
	PCL nanocapsules; SLNs (479 to 472 nm by DLS)	Carbendazim and tebuconazole (co-loaded)	n/a	Not evaluated	n/a	n/a	Lower phytotoxicity to *Phaseolus vulgaris* for PCL nanocapsules than for SLNs or commercial formulation; cytotoxicity	53

Table 3 *(continued)*

Purpose	Polymer (nanoparticle diameter in parentheses)	Active ingredient (A.I.)	Target species	Activity against target species	Representative concentration		Cytotoxic, phytotoxic, or ecotoxicological effects	Ref.
					Free A.I.	NP A.I.		
							can be lower or higher than commercial formulation, depending on cell line (3T3, MC3T3, or HeLa)	
Antibiotic	*O*-Carboxymethyl chitosan (200 nm by DLS)	Tetracycline	*Staphylococcus aureus*	Higher survival of *S. aureus*-infected THP-1 and HEK-293 cells *in vitro*, compared to unentrapped A.I., but similar MIC for *S. aureus* in broth culture	[c]0.2 to 0.4 mg L^{-1}	[c]0.3 to 0.6 mg L^{-1}	No significant cytotoxicity to NIH-3T3, L-929 and HEK-293 epithelial cell lines or THP-1 monocytic cells	185
	Chitosan/tripolyphosphate (≈20 to 50 nm by SEM)	Cefazolin	Multi drug resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* & extended spectrum beta lactamase (ESBL) positive *Escherichia coli*	Improved inhibition *in vitro* compared to unentrapped A.I.	[d]n/a (no zone of inhibition observed)	[d]200 μg mL^{-1} for all species	Not evaluated	163
	Chitosan/tripolyphosphate (220 nm)	Ceftriaxone	Ceftriaxone-resistant strains of *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA)	Improved antibacterial efficacy compared to unentrapped A.I. *in vitro* and *in vivo* (neutropenic mouse thigh model)	[d]n/a (no zone of inhibition observed)	[d]0.1 mg mL^{-1} for both species	>80% viability of MCF-7 cells	164
	Chitosan/tripolyphosphate (≈200 to 220 nm by DLS)	Ceftriaxone	*Salmonella typhimurium*	Improved antibacterial efficacy to *S. typhimurium*-infected Caco-2 and J774.2 cells compared to unentrapped A.I.		[a]50 μg mL^{-1}	Reduced hemolysis compared to unentrapped A.I.	158
	Chondroitin sulfate (CS)/chitosan; dextran sulfate (DS)/chitosan (180 nm by DLS)	Chloramphenicol	*Salmonella* paratyphi A	Lower antibacterial activity *in vitro* than unentrapped A.I., but improved intracellular efficacy for DS nanoformulation in RAW 264.7 macrophage cells and *ex vivo* efficacy in chicken intestine model	[c]3 μg mL^{-1}	[c]120 μg mL^{-1} and 80 μg mL^{-1} for CS and DS, respectively; [a]4 × MIC used in *ex vivo* tests	Minimal hemolysis and cytotoxicity to IEC-6, VERO, and NIH-3T3 cell lines	159
	Polyacrylate (25 to 40 nm by DLS)	Penicillin	*Staphylococcus aureus* & methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* (MSSA and MRSA, respectively)	Antibacterial activity maintained against *S. aureus* and MRSA	[c]0.012 μg mL^{-1} for *S. aureus* and 16 μg mL^{-1} for MRSA	[c]2 μg mL^{-1} for *S. aureus* and 2 μg mL^{-1} for MRSA	No significant cytotoxicity to human dermal fibroblast cells	162
	PLGA (130 to 353 nm by DLS)	Ciprofloxacin	*Escherichia coli*	Similar or slightly lower antibacterial activity *in vitro* against *E. coli* than unentrapped A.I.; significantly improved activity in *in vivo* model (dialysis tubing) because of reduced drug	[c]0.05 μg mL^{-1}	[c]0.05 μg mL^{-1}; [a]25 mg kg^{-1} for *in vivo* test	Not evaluated	186

Table 3 *(continued)*

Purpose	Polymer (nanoparticle diameter in parentheses)	Active ingredient (A.I.)	Target species	Activity against target species	Representative concentration		Cytotoxic, phytotoxic, or ecotoxicological effects	Ref.
					Free A.I.	NP A.I.		
				washout				
	PLGA (300 nm by DLS)	Ciprofloxacin	*Staphylococcus aureus* & *Pseudomonas aeruginosa*	Similar efficacy of single dose of nanoformulation to repeated doses of unentrapped A.I. over 6 d because of sustained release	[c]0.5 μg mL^{-1} for *S. aureus* and 0.25 μg mL^{-1} for *P. aeruginosa*	[c]0.5 μg mL^{-1} for *S. aureus* and 0.125 μg mL^{-1} for *P. aeruginosa*	Not evaluated	161
	PLGA (102 nm by DLS)	Enrofloxacin	*Escherichia coli* & *Staphylococcus aureus*	Similar or slightly lower antibacterial activity *in vitro* than unentrapped A.I.	[c]0.031 mg L^{-1} for *E. coli* and 0.083 mg L^{-1} for *S. aureus*	[c]0.024 mg L^{-1} for *E. coli* and 0.128 mg L^{-1} for *S. aureus*	Significantly reduced cytotoxicity to IPEC-J2 cells	187
	PLGA (289 to 299 nm by DLS)	Gentamicin (modified with anionic surfactant)	*Brucella melitensis*	Improved inhibition in *in vitro* macrophage infection test and significantly better reduction of infection in mice, compared to unentrapped A.I.		[a]1 mg L^{-1} for *in vitro* macrophage test and 100 μg per mouse for *in vivo* test	No observed toxicity to mice	188
	PLGA (240 to 360 nm by DLS)	Gentamicin	*Pseudomonas aeruginosa*	Poorer efficiency *in vitro*, but improved efficiency at extended duration against biofilms (36 h) and *in vivo* (96 h), relative to unentrapped A.I.	[c]1.5 μg mL^{-1}	[c]3 μg mL^{-1}; [a]0.08 mg mL^{-1} for 36 h biofilm test and 0.4 mg kg^{-1} dose for mouse studies	Not evaluated	160
	PLGA (230 nm by DLS)	Rifampicin	Methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*	Improved antibacterial activity against MRSA and similar activity against *B. subtilis* compared to unentrapped A.I.; no improved activity against *P. aeruginosa* or *E. coli*	[c]0.008 μg mL^{-1} for *S. aureus*, 0.06 μg mL^{-1} for *B. subtilis*	[c]0.002 μg mL for *S. aureus*, 0.06 μg mL^{-1} for *B. subtilis*	Not evaluated	189
	PLGA (243 nm by DLS); mPEG-PLGA (150 nm by DLS)	Ofloxacin	*Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*	Improved bacterial uptake and antibacterial activity compared to unentrapped A.I.; inhibition of antibiotic resistance development in *B. subtilis*	[d]25 μg per agar plate – smaller zone of inhibition (19.2 mm) with growth of resistant colonies by 60 h	[d]25 μg per agar plate – larger zone of inhibition (22.8 mm) with no growth of resistant colonies by 60 h	Not evaluated	190
	PLGA : PCL (80 : 20) (230 to 360 nm by DLS)	Doxycycline	*Escherichia coli* (DH5α)	Improved antibacterial efficiency compared to unentrapped A.I.	[c]6 mg L^{-1}	[c]4 mg L^{-1}	Not evaluated	191
	Zein (500 to 2000 nm)	Ciprofloxacin	*Escherichia coli*, *Staphylococcus aureus*	Lower bacterial adhesion compared to empty zein particles or glass surface; continuous and slow release of drug to inhibit bacterial growth over 6 d	Not evaluated	Not measured (particle film was deposited for evaluation)	Not evaluated	127

Notes: A.I.: active ingredient; PCL: poly(ε-caprolactone); (m)PEG: (methoxy)poly(ethylene glycol); PLA: poly(lactic acid): PLGA: poly(lactic-*co*-glycolic acid); SLNs: solid lipid nanoparticles.[a] Indicates the concentration where a significantly improved effect was reported relative to the unentrapped A.I. or commercial formulation. [b] Indicates the lethal concentration to achieve 50% mortality (LC_{50}) of the A.I. [c] Indicates the minimum inhibitory concentration (MIC) of the A.I. [d] Indicates the A.I. concentration resulting in a measurable zone of inhibition.

release rates for both the commercial and nano-formulations are needed to better understand the benefits or lack thereof of the nano-formulation.

Polymeric nanoparticles have also been proposed and developed to deliver antimicrobial agents.[146–148] Livestock have particularly been highlighted as a reservoir for the development of antibiotic-resistant microorganisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA),[149] that could potentially be transmissible to humans.[150,151] Nanoparticles have been suggested as a possible route to mitigate the proliferation of such antibiotic-resistant microorganisms by reducing the overall quantities of antibiotics required by improving the efficiency of delivery or by restoring the efficacy of the antibiotic.[152–157] While few studies are currently available that specifically target development of antibiotic-loaded nanoparticles for livestock and aquaculture applications, research on the development of nanoparticles loaded with antibiotics relevant to livestock are summarized in Table 3. Similarly to the agrochemicals, mechanisms for improved antibiotic efficiency include their targeting properties or enhanced uptake, leading to improved intracellular activity toward pre-infected cells,[158,159] as well as sustained release allowing for single doses to be effective over long durations (*e.g.*, several days)[127,160] and potentially eliminate the need for repeated doses of traditional (non-nano) formulations.[161] Interestingly, polymeric nanocarriers have also been found to restore the effectiveness of antibiotics against antibiotic-resistant organisms. For example, drug resistance to β-lactam drugs, such as penicillin and methicillin, is conferred by the production of β-lactamase enzymes that degrade the antibiotics. By incorporating these drugs into polymeric nanoparticles, *e.g.* penicillin-loaded polyacrylate nanoparticles,[162] cefazolin-loaded chitosan nanoparticles,[163] or ceftriaxone-loaded chitosan nanoparticles,[164] the drug is effectively shielded from enzymatic degradation to restore its antibiotic efficacy against resistant strains such as methicillin resistant *Staphylococcus aureus* (MRSA).

Finally, we note that a major hurdle that must be overcome for commercialization of the nanocarriers is the implementation of larger scale field trials that evaluate the ultimate improvements in endpoints of interest to farmers (*e.g.* crop or animal yield). The survey of literature in Table 3 shows that a variety of endpoints are evaluated across different studies, including seedling emergence, root and shoot growth, weed growth or mortality, and insect deterrence or mortality for crop applications, or minimum inhibitory concentrations, cell survival, or antibiotic resistance development for antibiotics. One recent study showed improved crop productivity (tomato production) in a field study for plant growth hormones delivered by nanocarrier,[165] and additional field studies are needed to make a convincing case for investments leading to commercialization of nanocarrier formulations.

5.3. Environmental and biological implications

The use of agrochemicals has spurred concerns over potential hazards associated with their application, *e.g.* cytotoxicity against nontarget species or ecotoxicity. Therefore, many studies on the development of nanocarriers have also investigated whether nano-formulations would exacerbate the cytotoxicity, phytotoxicity, or ecotoxicity compared to traditional formulations, or whether these side effects will be mitigated.

Cytotoxicity can be triggered upon penetration of cell membranes and leakage of important intracellular components and generation of reactive oxygen species (ROS), which eventually leads to oxidative stress, cell inflammation, and damage to intracellular components like mitochondria, protein, and DNA.[166] While cytotoxicity of polymeric nanoparticles has been reported, *e.g.* for smaller nanoparticles higher surface area and bioavailability[167] or PLGA-PEG nanoparticles with needle-shaped morphologies that could disrupt the lipid bilayer membrane,[168] polymeric nanoparticles typically have low cytotoxicity and furthermore, polymeric nanocarriers are often reported to minimize the cytotoxicity of agrochemicals and antibiotics (Table 3), hence providing a substantial benefit in improving not only the efficiency of the active ingredient but also improving their safe use.

Several studies have also demonstrated benefits of polymeric nanocarriers to reduce the toxicity of synthetic pesticides toward nontarget crop species (*e.g.*, *Zea mays* or *Phaseolus vulgaris*)[23,45,53,111,131] or environmental test organisms such as *C. elegans*, *Allium cepa*, and *Pseudokirchneriella subcapitata* (algae).[41,43,169,170] For applications in complex matrices, effects of nano-formulations on the microbiome are also of interest, given the key role of the microbial community in carbon and nutrient cycling in soils or the utilization of food and regulation of gastrointestinal diseases for oral drug delivery in animal health applications.[171] Nano-microbiota interactions have been studied for metal and metal oxide nanoparticles such as silver nanoparticles[172,173] or zinc oxide (ZnO), cerium oxide (CeO_2), and titanium dioxide (TiO_2) nanoparticles,[174] where the nanoparticles did not significantly impact the microbiome composition. However, new studies may be required for polymeric nanocarriers, particularly those carrying active ingredients with known microbial activity, *e.g.* antibiotics. In such cases, the use of a nano-formulation compared to traditional formulations may change the nano-microbiota interaction due to the changes in the site of the gastrointestinal tract in which the antibiotics are delivered, and hence differences in the types of gut microbiota impacted.[175] In soils, recent work conducted by Maruyama *et al.*[169] showed a slight change in the soil microbiome when chitosan/tripolyphosphate nanoparticles loaded with imazapic and imazapyr herbicides were applied,[169] where the ratios of nitrogen-fixing bacteria may be reduced and denitrifying bacteria may increase. Pascoli *et al.* reported that the application of neem oil-loaded zein nanoparticles as pesticides did not significantly change the relative number of genes associated with nitrogen-fixing or denitrifying bacteria after 30 d.[170]

The currently available studies generally suggest that short-term environmental hazards posed by polymeric nanocarriers can be minimal or even alleviated relative to

unentrapped or commercial formulations of active ingredients. Some commonly used polymers such as PLGA/PLA are FDA-approved for human drug delivery and expected to pose minimal environmental risk. However, studies are needed to evaluate the rate of polymer degradation in agricultural applications, the recalcitrance and accumulation of the polymer and any additives or byproducts, and potential toxicity of degradation products. For example, PVA (often used as a surfactant) has been reported to have limited degradation only by specific microorganisms despite being considered "biodegradable", and hence its longevity in soils is unknown.[176] To our knowledge, long-term soil studies of polymeric nanocarriers have not been conducted thus far to evaluate the consequences of repeated applications over longer durations.

6. Challenges and opportunities for future research

This review has demonstrated the potential environmental benefits of polymeric nanocarriers in agricultural applications, as well as many examples thus far of the successful synthesis of these materials and methods to characterize these materials in order to understand their behavior and effectiveness for the desired application. As shown in the literature, the function of the polymeric carrier to provide targeting or enhanced uptake, protect the active ingredient until it is delivered to its target, and slowly release the active ingredient over extended durations can be key to the improved efficiency of these nanomaterials compared to traditional formulations.

Based on the current literature, several major challenges and research questions can be identified to develop polymeric nanocarriers with optimal effectiveness. First, while extended release is one of the main benefits of nano-formulations, a more quantitative or systematic consideration of extended release has yet to be achieved. For example, a better consensus or practical guidance on the duration of release that would be desirable for various applications (*e.g.* crop protection, antibiotic delivery, *etc.*) will be critical for researchers to develop materials with appropriate release profiles. Alternatively, studies that specifically evaluate or report the dose and duration at which a single application of slow release nano-formulation is equivalent to repeated applications of non-nano-formulations will be useful to better quantify the benefits of using nano-formulations over current practices. Life-cycle analyses (LCA) have been proposed[192] and can incorporate information when available on the tradeoff between upstream resource costs to produce the nano-formulations and benefits of reducing the overall amount of active ingredients needed or improving agricultural yield. However, the potential ecological and safety benefits of nano-formulations conferred through the reduction in cytotoxicity or ecotoxicity of the active ingredient or reduced proliferation of antibiotic resistant organisms should also be considered and will be difficult to incorporate in LCA approaches.

Quantitative structure–function relationships to predict biological responses of polymeric nanocarriers from their physicochemical properties and other key phenomena, such as release or degradation rates, are also needed. Prior studies have postulated that enhanced efficiency of the nanocarriers is tied to their slow release, targeting, and protective capabilities of the active ingredient. Hence, new models that correlate spatial distribution or temporal release profiles of the nanocarrier and active ingredient to the biological effects (*e.g.* pesticide or antibiotic efficiency) are expected to be extremely useful to understand how to better design the nanocarriers. However, gathering experimental data to parameterize these models is non-trivial because of the variety of tools needed to comprehensively characterize polymeric nanocarriers, as well as a lack of satisfactory methods to directly measure the localization of the nanocarriers and release of their active ingredients *in vivo* or in the field. Machine learning represents an alternative "black-box" approach to correlate nanocarrier properties to biological endpoints. However, as discussed in a review by Jones *et al.* for biomedical effects of drug delivery nanoparticles,[193] machine learning approaches are currently challenged by limitations in the quantity and completeness of data relative to the high number of potential predictive parameters, as well as an imbalance in the types of nanocarriers (*e.g.*, PLGA) with available data. Hence, these is a higher risk for the models to be overfitted or biased toward the samples in the training data, which would result in poorer predictive capability for other nanocarriers.

Finally, once a design goal has been defined based on properties of the nanocarriers needed to achieve the desired efficiency of the active ingredient, synthesis of nanomaterials that meet the design goal may be non-trivial. Again, a major challenge is presented by the large number of experimental factors that contribute to the nanoparticle properties, including size, structure (*e.g.* phase and solvency), and loading capacity, as well as the release behavior (rate and mechanism) of the active ingredient from the nanoparticle. Optimization of the synthesis can successfully be conducted on an individual basis for each polymer and active ingredient type, as in factorial design studies,[20,21,26] but this approach requires significant time and effort. Predictive approaches have been proposed: either first principles approaches to predict particle properties and release rates from thermodynamic models[33–35] and molecular dynamics simulations,[194–197] or machine learning approaches to develop correlations from existing data sets.[194,198–201] In both cases, studies have been limited to either modeling a limited number of polymers for several types of active ingredients, or *vice versa*. Experimental validation studies are needed to evaluate whether the proposed first principles tools can be applied a broader set of combinations of material types and experimental conditions. Machine learning approaches also require larger data sets with thorough characterization, as discussed above. Therefore, it is

currently unknown whether any single tool will successfully predict the synthesis materials and conditions that are most likely to provide a favorable outcome across a broad variety of polymers and active ingredients. Furthermore, the limited accessibility of predictive modeling tools to experimentalists hinders progress toward validation against new experimental data, updating the tools to incorporate new data, or application of the tools to test their capabilities for design of new nanocarriers with a desired set of properties.

Considering the integrated nature of these challenges, the development of polymeric nanocarriers presents a great opportunity for multi-disciplinary collaborations between synthetic and analytical chemists, environmental engineers and microbiologists, and agricultural scientists and engineers. Such collaborations will advance our understanding of how environmental nanotechnology can enhance the portfolio of technologies for agricultural applications and spur the development of new materials and predictive tools to achieve the maximum benefit from these technologies.

Conflicts of interest

There are no conflicts to declare.

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