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Tunable poly(methacrylic acid-co-acrylamide) nanoparticles through inverse emulsion polymerization

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Abstract

Environmentally responsive biomaterials have played key roles in the design of biosensors and drug delivery vehicles. Their physical response to external stimuli, such as temperature or pH, can transduce a signal or trigger the release of a drug. In this work, we designed a robust, highly tunable, pH-responsive nanoscale hydrogel system. We present the design and characterization of poly(methacrylic acid-coacrylamide) hydrogel nanoparticles, crosslinked with methylenebisacrylamide, through inverse emulsion polymerization. The effects of polymerization parameters (i.e., identities and concentrations of monomer and surfactant) and polymer composition (i.e., weight fraction of ionic and crosslinking monomers) on the nanoparticles' bulk and environmentally responsive properties were determined. We generated uniform, spherical nanoparticles which, through modulation of crosslinking, exhibit a volume swelling of 1.77–4.07, relative to the collapsed state in an acidic environment. We believe our system has potential as a base platform for the targeted, injectable delivery of hydrophilic therapeutics. With equal importance, however, we hope that our systematic analysis of the individual impacts of polymerization and purification conditions on nanoparticle composition, morphology, and performance can be used to expedite the development of alternate hydrophilic nanomaterials for a range of biomedical applications.

Keywords

inverse emulsion polymerization; pH responsiveness; intelligent hydrogels; swelling

INTRODUCTION

Hydrogel nanoparticles, or nanogels, interact uniquely with the physiological environment; these interactions are tunable through careful determination of the gel's composition and surface chemistry. Because of these unique material-host interactions, nanogels have received extensive research interest in biosensing,¹ drug delivery,² and regenerative medicine applications.³ Nanogels are especially useful when they can overcome existing shortcomings of current systems. In drug delivery, for example, nanoparticles will preferentially accumulate in the kidneys,⁴ lymph nodes,⁴ or liver⁵ depending on their size and geometry. In cancer drug delivery in particular, researchers consistently seek nanoparticles with hydrodynamic diameters of <200 nm to capitalize on the enhanced permeation and retention (EPR) effect.⁶ In each of these cases, the nanogel overcomes a previous shortcoming in biodistribution, enabling accumulation and increased delivery of loaded or encapsulated payload at the desired site.

Nanogel chemistry is also paramount for the purpose of function and utility. Polyionic nanogels are particularly interesting, as these nanoparticles with non-neutral surface charge are generally stable in aqueous solutions and exhibit predictable responses around their pKa. Our group and others have extensively studied the use of polyanionic and polycationic nanogels for the pH-responsive drug delivery of small molecules and macromolecular therapeutics in the treatment of cancer, ulcerative colitis, and numerous other diseases.^{7–10} Each of these systems functions by entrapping the payload in a buffer condition where therapeutic nanomaterial interactions are favorable, followed by a predictable shift toward unfavorable interaction in the destination's physiological environment. Alternate nanogel systems include temperature-responsive monomers such as *N*-isopropyl acrylamide, which allow for triggerable swelling and release profiles in response to hypothermic conditions.¹¹ Metal nanomaterials are also frequently included within nanogels, of a variety of composition, to act as transducers and enable responsiveness to light or alternating magnetic fields.¹²

Nanogels are typically synthesized by miniemulsion polymerization techniques, where the polymerizing monomers and crosslinker are present in nanodroplets suspended in nonsolvent and stabilized by surfactant.¹³ Because of this design, uniform spherical particles are formed, which is especially advantageous in medical applications where consistency and reproducibility are paramount. However, miniemulsion polymerizations rely on the hydrophobicity of growing nanogels to partition in the organic phase. Therefore, it can be somewhat problematic if one wants to make a hydrophilic nanogel to encapsulate and deliver hydrophilic payloads.

In the present work, we employ an inverse emulsion polymerization technique to generate uniform nanoparticles consisting of bisacrylamide-crosslinked acrylamide and methacrylic acid. We selected this composition for a number of reasons. First, methacrylic acid's pH-responsiveness is useful for drug delivery applications that include physiological transitions between neutrality and acidity (i.e., the gastrointestinal tract, tumor microenvironments).¹⁴ Second, in a survey of the literature, there was no existing method for the fabrication of acrylamide-co-methacrylic acid nanomaterials. Similar literature reports have explored

biological applications of acrylamide-co-methacrylic acid microparticles and/or films;¹⁵ or alternatively acrylamide-co-acrylic acid for upper critical solution temperature responsive drug delivery.^{16,17} Therefore, we know that copolymers of methacrylic acid and acrylamide could potentially interact with biological systems in a unique and productive way, if they could be readily produced at the nanoscale.

This work was approached with a number of set design goals. First and foremost, we wanted to yield a lowpolydispersity nanogel of methacrylic acid and acrylamide that had a hydrodynamic diameter of <200 nm, so that it could be useful for injectable drug delivery in the future. Second, we wanted to make a fundamental contribution to the field in analyzing the optimization of every relevant aspect of the polymerization and purification schema, as we believed these findings will help others streamline the process of alternate nanogel development for diverse applications. Finally, we sought to test the limits of our system's pH-responsiveness, providing insight into its likely behavior *in vitro* or *in vivo* while simultaneously verifying that it does not substantially respond to other environmental factors.

We hypothesized that poly(acrylamide-co-methacrylic acid) nanogels would exhibit predictable and recoverable pH-responsive swelling around the pKa of methacrylic acid, tunable through modulation of the acidic monomer fraction and crosslinking density. Additionally, we posited that there will be a range of operating conditions: a set ionic strength, surfactant identity, surfactant concentration, and monomer concentration, which yields optimal and reproducible nanogels. We believe the approach documented herein, which takes a critical materials-characterization focus, is especially important for biomaterials scientists as it lays the groundwork for developing new polymeric biomedical materials.

MATERIALS AND METHODS

Materials

All reagents and solvents were purchased from Sigma Aldrich or Fisher Scientific and used as received. Ultrapure water (18.2M Ω) was obtained from a Barnstead GenPure purification system from Thermo Scientific.

Instrumentation and analysis

Dynamic light scattering (DLS) and zeta potential measurements were obtained with a ZetaSizer Nano-ZS (Malvern). TEM imaging was conducted on a FEI Tecnai Transmission Electron Microscope operating at 80 kV. FTIR spectra were acquired on a Nicolet is10 FT-IR spectrometer, and analyzed in OMNIC software (Thermo Scientific). A Hanna Instruments HI 902 potentiometric titrator was utilized for autotitration of nanoparticle suspensions.

Synthesis of hydrogel nanoparticles

Hydrogel nanoparticles were prepared by inverse emulsion polymerization, adapted from Zhang et al.¹⁸ All values in parentheses represent final concentration in the prepolymer

solution of the base formulation. Acrylamide (Aam) (4.47M), methacrylic acid (MAA) (0.656M), and methylenebisacrylamide (BIS) (0.428M) were dissolved in ultrapure water, along with 25 mL of N,N,N',N'-tetramethylethylenediamine. The pre-polymer solution was sonicated for 10 min to ensure a uniform aqueous phase (aqueous phase total volume = 1.682 mL). Surfactants dioctyl sulfosuccinate (AOT) (30.3 mM) and Brij-30 (151.4 mM) (1:5 molar ratio) were dissolved in 30 mL of hexane to form the organic phase (organic phase total volume = 32.26 mL). The organic phase was stirred rapidly (500 rpm) until all of the surfactant dissolved. The aqueous phase was then added to the organic phase to form the pre-polymer emulsion.

Following 30 min of nitrogen purging to remove dissolved oxygen, an initiator solution of ammonium persulfate (APS) (100 mg/mL) in ultrapure water was purged with nitrogen for 10 min and 50 μ L were injected into each prepolymer emulsion. Reactions were allowed to proceed with constant stirring (500 rpm) at room temperature for 2 h (Fig. 1). Following polymerization, synthesized nanoparticles were precipitated by breaking the emulsion with an equal volume of ethanol, and then collected via centrifugation at 3200 g for 5 min. Precipitation was repeated two additional times in 50 mL of ethanol prior to further purification.

Purification by ionomer collapse

In our initial syntheses, we selected an ionomer collapse purification schema. This schema was selected because it was previously successful in purifying alternate nanogels containing methacrylic acid.⁷ Ionomer collapse relies on the super-collapsing of ionic gels in media with a low dielectric constant. Because of the polyacidic nature of our P(MAA-co-AAm) nanogel, basic conditions (0.5N NaOH) were used to charge to gel, while complexation with the sodium counterions enabled collapse and collection in low dielectric constant conditions. Unreacted monomer and remaining surfactant were removed by six cycles of ionomer collapse. Each cycle consisted of resuspending the nanogels in 15 mL of 0.5N sodium hydroxide solution, vortexing to suspend the polymer pellet, and collapse by the addition of 35 mL acetone. The collapsed nanogel pellet was collected by centrifugation at 3200g for 5 min. Particles were then dialyzed against ultrapure water for >96 h (frequent water changes) for solvent exchange, and were lyophilized to yield a white nanoparticle powder.

Impact of inverse emulsion parameters on nanoparticle size

Inverse emulsion polymerizations were prepared as described above, with the independent modulation of one of the following variables—monomer concentration, surfactant concentration, surfactant ratio, and ionic strength of the aqueous phase. Monomer concentration was varied from 3.33 to 6.67M, maintaining the relative molar ratios of MAA, AAm, and BIS. Surfactant molar ratio was varied from 1:2.26 to 5:1 Brij 30 to AOT, maintaining a constant molar concentration of surfactant (195 mM in 30 mL hexane). Total surfactant concentration was adjusted from 94.1 to 263.3 mM, maintaining the 5:1 Brij 30:AOT ratio. Ionic strength of the aqueous phase was adjusted between 0 and 162.7 mM (0 to 1 $\times$) with phosphate buffered saline (PBS). Following the synthesis and purification of each nanogel formulation, nanoparticle size was measured using DLS. All DLS samples

were prepared by diluting the collapsed nanogels into ultrapure water. Measurements were taken in triplicate, with each measurement representing an average of >10 runs.

Optimization of formulation

The methacrylic acid content and degree of crosslinking were independently manipulated. Methacrylic acid content was varied from 5.8 to 34.6 mol % of the total monomer in the pre-polymer solution. Methylenebisacrylamide was adjusted from 0.9 to 10 mol % of the monomer feed to modulate crosslinking. In both modifications of polymer composition, the total molar concentration of all monomers was kept constant. All nanoparticles were purified by ionomer collapse, dialyzed against ultrapure water for >96 hours with frequent water changes, and lyophilized. With this optimization step, the range of magnitude and sharpness of pH-responses that can be obtained with the nanoscale P(MAA-co-AAm) system is observed.

Impact of ionomer collapse conditions on the polymer backbone

Initial nanogel characterization led to questioning the stability of acrylamide, within the P(MAA-co-AAm) copolymer, in the basic conditions of ionomer collapse. Hydrolysis of poly(acrylamide) during extended incubation in acidic or basic media is well known,¹⁹ and we wanted to determine the extent to which ionomer collapse's base incubation steps modify pendant amide groups in our copolymer. To assess the impact of incubation in basic media, nanoparticles were incubated in 0.5N NaOH solution for between 0 and 244 h following precipitation in ethanol (no ionomer collapse). At each time increment, a nanoparticle sample was removed, neutralized with 1N HCl, and dialyzed for >96 h (frequent water changes). After lyophilization, relative nanoparticle composition was determined via FTIR. FTIR spectra were obtained on dried powders from 4000 to 680 cm⁻¹. Absorbance spectra are presented as the average of 64 scans, with background subtracted. For comparison, we compare these FTIR spectra to one obtained for nanoparticles purified by ionomer collapse.

We also quantified the carboxylic acid content of each nanogel sample by potentiometric titration. For composition analysis by potentiometric titration, 10 mg of dried nanoparticles were suspended in 5 mM KCl, adjusted to pH >7. Particles were then titrated with 0.01 M HCl until an equilibrium pH of 3.0 was achieved. The normality of the NaOH titrant was established by titration with a standardized HCl buffer. Data from titration experiments are presented on a reverse x-axis (equivalent NaOH added, rather than HCl added) to establish a consistent starting point (pH = 3) and make the trend in carboxylic acid—pH buffering visually apparent.

Purification by precipitation and gradient dialysis

Following ethanol precipitation (3× total), nanoparticles were dialyzed against an excess water:ethanol gradient (1:1 two times, 3:1 two times, 7:1 two times, 15:1 two times, pure water two times). Following dialysis, nanogels were lyophilized to obtain a dry particle powder. For comparison, the FTIR spectra of nanogels purified by this method was also compared to those obtained in the base-incubation experiments and following ionomer collapse purification.

Nanogel morphology and surface charge

For TEM experiments, nanoparticles were suspended in ultrapure water at 1 mg/mL, cast on a carbon-coated grid, and stained with 2% uranyl acetate. Representative images are presented, to illustrate the uniformity of particle dispersity and morphology. Some aggregation on the grid was observed, but can be explained by the dehydration of the swollen nanogels during grid preparation and staining. Zeta potential measurements were taken using the Malvern Zetasizer, as described above, with nanogels suspended in 0.13 PBS and 2 mg/mL. Zeta potential measurements provide insight into both methacrylic acid content and nanogel stability in aqueous media.

Dynamic, environmentally responsive swelling

Following purification and dialysis, select nanoparticle formulations were subjected to titration, coupled with DLS, to assess the pH-responsive swelling properties. In each case, a 2 mg/mL suspension of nanoparticles in 1× PBS was pH-adjusted to pH 5.8. After this initial adjustment, the suspension was incorporated into an autotitration apparatus, which enables constant stirring of the sample, circulation to the cuvette for DLS analysis, and access to titrants (1M HCl, 0.1M HCl, 0.1M NaOH). The titrator gradually decreased the pH of the nanoparticle suspension, in increments of 0.3, until a final pH of 2.5 was reached. At each pH increment, three independent size measurements were taken, prior to the subsequent pH adjustment. Once a pH of 2.5 was reached, the titrator returned the pH to 8.0 in the same 0.3 pH unit increment, again allowing for three independent size measurements at each increment. This method captured the kinetics of both swelling and collapse, to parse any differences between the two processes. Similarly, select nanoparticle formulations were subjected to heating coupled with DLS to observe if temperature had any effect on nanoparticle swelling. Nanoparticles were suspended at 2 mg/mL in 1× PBS and pH-adjusted to pH = 7.4. The temperature of the sample was increased from 25 to 60°C in increments of 5°C, where the hydrodynamic diameter was measured at each step. The nanoparticles' size was compared to that measured in the collapsed state measured by pH-responsive swelling experiments to determine the swelling ratio.

Cytotoxicity studies

L929 murine fibroblast cells and RAW 264.7 murine macrophages were cultured according to ATCC specifications, and maintained in high glucose Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 2 mM L-glutamine, 1% penicillin-streptomycin, and 10% heat-inactivated fetal bovine serum (FBS). Prior to toxicity experiments fibroblasts or macrophages were seeded in uncoated 96-well microplates at a density of 10,000 cells/well and given 24–48 h to reach approximately 80% confluency.

Nanoparticles were incubated, in the presence of cells, in low serum (2% FBS) phenol red free medium supplemented with L-glutamine and penicillin-streptomycin. Nanoparticles, synthesized as described, were irradiated with UVlight for 20 min in a laminar flow cabinet, dissolved in medium at 5 mg/mL concentration, and sonicated briefly to promote uniform dispersion. Further dilution in phenol red free, low-serum medium achieved subsequent nanoparticle stocks for cell exposure.

After cells reached proper confluency, seeding medium was promptly removed by inverting the microplate. Nanoparticle suspensions, at a concentration range up to 5 mg/mL, were distributed pseudo-randomly in each microplate to minimize bias due to addition order or microwell location. For MTS assays, which assessed cell metabolic activity, nanoparticle suspensions were removed by plate inversion after a 24-h cell-nanoparticle incubation and cells were washed two times with sterile Dulbesco's phosphate buffered saline (DPBS). MTS was diluted 1:6 in low-serum medium and 120 μ L were added to each well (90 min incubation at 37°C). MTS-metabolism was quantified via absorbance at 490 nm with the absorbance at 690 nm subtracted as background. Percent viability was calculated relative to positive (untreated) controls, and 2% lysis buffer (Life Technologies) in low-serum medium (assay negative control). For LDH assays, 100 μ L of assay reagent were added to each microwell, containing cells and nanoparticles preincubated for 24 hours. LDH incubation was 10 min in duration at room temperature, following which 50 μ L of stop solution were added to each well. Membrane integrity was calculated relative to positive (untreated) and negative (2% lysis buffer, 30-min incubation) controls via fluorescence (excitation 560 nm, emission 590 nm).

RESULTS

Nanoparticle synthesis and characterization

Nanoparticles were successfully synthesized through inverse emulsion polymerization. By isolating each nanoparticle in a surfactant micelle during polymerization, nanoparticles were uniform in conformation and had a low degree of polydispersity, with a measured diameter of 78.8 ± 0.8 nm and a polydispersity of 0.143 ± 0.011 following purification, prior to lyophilization.

In titration experiments, as the pH decreased through the pKa of methacrylic acid, the nanoparticles collapsed, as evidenced by the decreasing hydrodynamic diameter and swelling ratio [Fig. 2(a)]. This swelling behavior is attributable to the protonation of carboxylic acids from methacrylic acid, leading to an efflux of counterions and associated solvent, as well as hydrogen bonding and elevated hydrophobicity associated with the protonated state. Titrating the pH back to original, neutral levels demonstrated complete recovery of the original swollen size, 2.73 times larger than the collapsed state by volume. As expected, no significant temperatureresponsive swelling behavior was observed [Fig. 2(b)]. TEM analysis revealed that uniform, spherical nanoparticles were produced [Fig 2(c-e)]. Some aggregation on the carboncoated grid was observed [Fig 2(d)], which we are confident, in consideration of the light scattering and titration results, was an artifact of grid preparation and staining rather than an indication of nanoparticle aggregation behavior.

Impact of polymerization parameters

Each polymerization parameter impacted the final nanoparticle diameter in a different manner. All data are presented as the hydrodynamic diameter plus or minus the standard deviation of the nanoparticle size distribution. Increasing the concentration of surfactant in the continuous organic phase decreased the resulting nanoparticle diameter, approaching a lower size threshold of approximately 60 nm [Fig. 3(a)]. Modulating the ratio of co-

surfactants demonstrated that increasing the amount of ionic surfactant (AOT), relative to non-ionic surfactant (Brij-30) created larger nanoparticles [Fig. 3(b)], while the converse generated smaller nanoparticles. Increasing monomer concentration in the aqueous phase increased nanoparticle size until monomer concentration reached $6.3M$, after which the emulsion became unstable during polymerization, resulting in the formation of macro-scale polymer during synthesis [Fig. 3(c)]. As monomer concentration decreased, micelle constraints limited a decrease in particle size to 60 nm, the same threshold observed when surfactant concentration was modulated [Fig. 3(c)]. Ionic strength of the aqueous phase was varied to mimic physiological ionic strengths, and did not impact nanoparticle size [Fig. 3(d)].

Impact of NaOH incubation of polymer backbone

Potentiometric titration curves shown here [Fig 4(a)] were obtained following nanoparticle incubation at varying lengths in sodium hydroxide solution, neutralization, dialysis, lyophilization, and resuspension in 5 mM KCl solution with pH adjustment to pH = 3. A greater equivalent volume of 0.01N NaOH was required to titrate an equivalent mass of nanoparticles (10 mg) through the equivalence point for methacrylic acid (observed pH ~ 4.8) as the length of base incubation increased. This increase in carboxylic acid content, relative to acrylamide, was reinforced by zeta potential [Fig. 4(b)] and FTIR [Fig. 4(c)] data, shown here. Zeta potential is presented as the effective surface charge of nanoparticles in 0.1x PBS at pH = 7.4, plus or minus the standard deviation of the zeta distribution. Increased incubation time in 0.5N NaOH led to more negatively charged nanoparticles. Composition analysis by FTIR showed that increased incubation time in base led to decreased intensity of the acrylamide carbonyl peak at 1650 cm^{-1} while an intensifying peak at 1550 cm^{-1} , which can be attributed to carbonyl stretching in deprotonated carboxylates [Fig. 4(c)]. This FTIR result, in combination with the shift in potentiometric titration and decrease in zeta potential with increased 0.5N NaOH incubation time suggests that purification in basic conditions likely leads to hydrolysis of acrylamide in the polymer backbone. The additional polymer modification through purification of these nanoparticles was determined to be undesirable and necessitated the development of a new purification approach.

Polymer purification methods

Purification by ionomer collapse, which included six separate incubations in 0.5N NaOH and dialysis from a basic condition, resulted in significant acrylamide hydrolysis, as evidenced by the FTIR spectra (Fig. 5). The FTIR spectra obtained for nanoparticles purified by ionomer collapse was most similar to that obtained from nanoparticles incubated in 0.5N NaOH for 74–142 h. On the other hand, nanoparticles which were purified by dialysis against a water:ethanol gradient did not exhibit detectable acrylamide hydrolysis (Fig. 5).

Particle properties for different polymer composition

As crosslinking percentage increased, a dampened swelling response (lower magnitude of volume swelling) was observed, while the methacrylic acid content of the nanoparticles did not impact the magnitude of the pH-swelling response [Fig. 6]. The 0.9% crosslinked particles were 4.07 times larger by volume in their swollen state compared to their collapsed state; by comparison, 10% crosslinked nanoparticles were only 1.77 times larger in the

swollen state compared to their collapsed state. Nanogels with intermediate crosslinking percentages exhibited swelling behavior between the extrema. Increasing the methacrylic acid content in the pre-polymer solution, however, did lead to gradual decreases in nanoparticle size, likely due to interaction with the anionic surfactant cocktail. At 7.7% crosslinking (held constant for all modulations of methacrylic acid content), increasing the methacrylic acid content decreased the particle diameter from 85.57 nm in 5.8% MAA particles to 60.65 nm in 34.6% MAA. For all nanoparticles in this methacrylic acid sequence, the volume swelling ratio was found to be 2–2.5, as compared to the nanoparticles' collapsed state.

Nanoparticle cytotoxicity

MTS and LDH cytotoxicity experiments were conducted at varying concentrations of nanoparticles on murine fibroblasts and macrophages. For both cell types, nanoparticles at all tested concentrations had no impact on the metabolic activity of the cells [Fig. 7(a,c)]. Similarly, nanoparticles did not affect the membrane integrity of either cell line [Fig. 7(b,d)].

DISCUSSION

Inverse emulsion polymerization for the development of hydrophilic biomedical materials

In this work we explored how different parameters of an emulsion polymerization will impact the size of hydrogel nanoparticles, and demonstrated that each explored parameter has a different effect on particle size. When varying surfactant or monomer concentration, a lower threshold for particle size was observed at ~60 nm. Modulation of surfactant ratios led to decreased stability of micelles when the molar ratio of co-surfactants was shifted toward AOT. Surfactant ratios were modified to understand how the cosurfactants impacted micelle stability, and thus micelle size, as part of understanding how the micelle controls nanoparticle size. While it was expected that increasing Brij-30 content in an AOT-Brij-30 mixed micelle increased micelle size based on previous studies,²⁰ the opposite effect was observed in this experiment. However, the identified previous study did not include the other components of our system that influence micelle diameter (monomers, crosslinker, and so forth). Increasing the ionic strength of the aqueous represented physiological conditions. Physiological ionic strength would facilitate the addition of biological components in native conformations to the polymerization, enabling co-encapsulation of biomolecules for a drug delivery application, or molecular imprinting for the development of high-affinity sorbents, in the future. Furthermore, we have shown that these nanoparticles are non-toxic to cells, with no impact on either cell metabolic activity or membrane integrity.

Overall, this inverse emulsion polymerization is a robust process that produces uniform biocompatible nanoscale hydrogels. Understanding how different parameters of the polymerization individually and collectively influence both the hydrodynamic diameter and dynamic swelling response allows for a customized polymerization schematic to produce nanoparticles of a target size for a range of applications. This approach perfectly lends itself to the design of hydrophilic injectable biomaterials for sensing or drug delivery. As discussed in the introduction, nanoparticles for injectable drug delivery systems must be larger than 5.5–8 nm in diameter to avoid renal clearance and remain in circulation for a

relevant amount of time.²¹ Additionally, for targeted chemotherapeutic delivery, nanoparticles can be designed to take advantage of the EPR effect, where particles under 200 nm will favorably accumulate in the leaky vasculature of tumors.⁶ Our system offers a dynamic range of spherical nanoparticles that satisfy these design criteria.

Insights into purification

Base-mediated ionomer collapse is commonly used for the purification of nanoparticles, synthesized by emulsion at the lab-scale in order to remove surfactant from the formed nanoparticles by promoting the portioning of uncharged surfactant in an organic phase.⁷ However, in this study we observed that extended incubation of polymer in basic solution, like those necessary for this purification, leads to a compositional change of the polymer. FTIR analysis coupled with potentiometric titration and zeta potential measurements revealed that acrylamide's pendant amide groups were being hydrolyzed during ionomer collapse. In response to these results, we developed an alternate purification schema (water:ethanol gradient dialysis). With this new purification, we observed complete surfactant and unreacted component removal, but a dramatic decrease in acrylamide hydrolysis.

Tunable hydrogel systems for diverse pH responses

Modification of polymer composition demonstrated the versatility of this crosslinked poly(MAA-co-AAm) co-polymer system, in terms of generating a range of magnitude in pH response. Modulating the crosslinking density of the system led to a decreased swelling response when the pH of a nanoparticle suspension was modulated. This type of response is expected according to the Flory-Rehner theory, which describes the swelling ratio as a function of multiple polymer properties.²² As the amount of crosslinking increases, there will be fewer monomers between each crosslink, decreasing the molecular weight between crosslinks, which then decreases the swelling ratio. Variations of methacrylic acid content, on the other hand, do not change the magnitude of the swelling response, but do shift the hydrodynamic diameter more generally. This decrease in nanoparticle size can be explained by interactions between the methacrylic acid in the aqueous phase and the surfactants in the surrounding micelle, which together dictate nanoparticle diameter.

Methacrylic acid content in the polymer is interesting, not only because of its impact on pH-responsive swelling, but also because the pendant carboxylic acid groups are amenable to bioconjugation chemistries. These postsynthesis reactions can introduce hydrophobic moieties for improved drug loading and retention,²³ positively charged groups to serve as sites for catalysis,²⁴ or biological ligands for improved protein separation or sensing.²⁵

Summary

In this work, we developed and characterized a polymerization scheme that produces uniform, biocompatible nanoparticles with intelligent pH-responsive behavior. Our systematic emulsion optimization provides insight into the extent to which different synthesis and purification conditions influence nanoparticle properties. We present a new approach for nanoparticle purification that removes excess monomer and surfactants without hydrolysis of functional groups. Finally, network composition was varied to generate

nanoparticles with a magnitude range of pH-responsive swelling. We believe that the hydrogel nanoparticles developed in this work can serve as the base formulation for a pH responsive hydrogel system. While many nanogels exist as drug delivery systems, this system can capitalize on passive and directed targeting mechanisms while demonstrating environmentally responsive behavior to address many of the issues identified in previous nanoparticle drug delivery systems, including targeted delivery,²⁶ controlled release,^{26,27} and biocompatibility.²⁸ Our contribution shines light on the different engineering parameters in both inverse emulsion polymerization and the purification of the nanoparticles produced thereof. It is our hope that this can illuminate important design considerations, and expedite the development of unique and useful hydrophilic nanomaterials by others in the future.

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FIGURE 1.

Co-monomers methacrylic acid and acrylamide were dissolved in an aqueous solution with bisacrylamide crosslinker, and were added to an organic hexane phase containing co-surfactants Brij-30 and dioctyl sulfosuccinate (AOT). Nanogels were polymerized by free radical polymerization within aqueous droplets suspended in the organic phase, facilitating control of aggregation and particle size. Excess monomers and surfactants were removed through ethanol precipitation followed by ionomer collapse, followed by dialysis against ultrapure water for solvent exchange before lyophilization.

FIGURE 2.

(a) pH responsiveness of 7.7% BIS P(MAA-co-Aam) nanoparticles was measured at 2 mg/mL in 1× PBS. Nanoparticles collapsed as pH decreased and were able to return to their original size when pH was increased, demonstrating recoverable pH responsiveness. (b) Temperature's effect on nanoparticle swelling was negligible (2.5% BIS nanogels shown). (c–e) Representative TEM images of P(MAA-co-Aam) nanoparticles.

FIGURE 3.

Various parameters of the polymerization were individually manipulated to explore their impact on nanoparticle size. (a) Increasing the concentration of surfactant produced smaller nanoparticles. Size reached a minimum of 60 nm, as 0.26 and 0.3 M surfactant formulations produced similarly sized nanoparticles. (b) Smaller nanoparticles with a lower polydispersity were achieved with an increase in Brij 30 surfactant relative to AOT. (c) Increasing monomer concentration produced larger nanoparticles until the emulsion became unstable at monomer concentrations >6.3 M. A similar size minimum of 60 nm was observed for 2.8–4.5 M monomer formulations. (d) Ionic strength of the aqueous phase did not impact nanoparticle size. (All graphs $n = 3$, represented as mean $\pm$ SD of the nanoparticle distribution. Hydrodynamic diameters measured in the swollen state).

FIGURE 4.

Incubation of P(MAA-co-Aam) nanoparticles in 0.5N NaOH showed distinct changes in nanoparticle composition with longer incubation. (a) Potentiometric titration revealed an increased number of acidic groups present in the polymer, as base incubation was extended. (b) Longer NaOH incubation led to more negatively charged nanoparticles. (c) FTIR spectra were obtained for each incubation time and normalized to each spectrum's largest peak. Increased incubation time showed to rise of peak at 1550 cm^{-1} , representing the deprotonated carboxylate.

FIGURE 5.

FTIR spectra of P(MAA-co-Aam) NPs purified against a water/ ethanol gradient and via ionomer collapse were obtained and normalized to each spectrum's largest peak. A distinct peak at 1550 cm^{-1} is present for ionomer collapse purified particles that is not present in nanoparticles purified through dialysis against a water/ethanol gradient.

FIGURE 6.

Dynamic pH responsive swelling of P(MAA-co-Aam) nanoparticles for varying (a) degrees of crosslinking and (b) amount of methacrylic acid. As crosslinking percentage decreased, particles showed a greater swelling response. Increasing methacrylic acid concentrations resulted in a decrease in particle size without significantly affecting volume swelling. ($n = 3$, mean $\pm$ SD of the nanoparticle distribution).

FIGURE 7.

Nanogels were incubated with L929 murine fibroblasts and RAW 264.7 murine macrophages. Following 24 h incubation, viability was quantified in terms of metabolic activity (a,c) and membrane integrity (b,d). Cytotoxicity was negligible in both cell models, as evidenced by both viability metrics. (* $p < 0.05$, relative to control, $n = 6-24$).