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Development of multiple-layer polymeric particles for targeted and controlled drug delivery

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Abstract

The purpose of this work was to develop multilayered particles consisting of a magnetic core and two encompassing shells made up of poly (*N*-isopropylacrylamide) (PNIPAAm) and poly(*D,L*-lactide-co-glycolide) (PLGA) for targeted and controlled drug delivery. Transmission electron microscopy confirmed that multilayered particles were obtained with PNIPAAm magnetic nanoparticles embedded within the PLGA shell. Factorial analysis studies also showed that the particle size was inversely proportional to the surfactant concentration and sonication power and directly proportional to the PLGA concentration. Drug-release results demonstrated that these multilayer particles produced an initial burst release and a subsequent sustained release of both bovine serum albumin (BSA) and curcumin loaded into the core and shell of the particle, respectively. BSA release was also affected by changes in temperature. In conclusion, our results indicate that the multilayered magnetic particles could be synthesized and used for targeted and controlled delivery of multiple drugs with different release mechanisms.

Keywords

Biodegradable; Dual drug release; Multilayered particles; Temperature sensitive

Although magnetic nanoparticles have been used as magnetic resonance imaging (MRI) contrast agents and in hyperthermic treatment for cancer cells,^{1–5} their application in targeted and controlled drug delivery has been limited. One reason for this is because magnetic nanoparticles made of pure iron oxide cannot be loaded with drugs for controlled release. Thus, the incorporation of magnetic nanoparticles with polymers and other biocompatible materials such as poly(*D,L*-lactide-co-glycolide) (PLGA), polystyrene, hyaluronic acid, and chitosan^{6–13} has been used to increase their biocompatibility and ability for controlled drug delivery. Magnetic nanoparticles encapsulated with PLGA were prepared in this respect, allowing the PLGA to be loaded with a hydrophobic drug.^{8,10,14} The drug was then released by degradation of the PLGA polymer. In addition, thermosensitive polymers such as poly(*N*-isopropylacrylamide) (PNIPAAm) have been incorporated with magnetic cores to provide a temperature-sensitive drug release mechanism.¹⁵ These nanoparticles can be loaded with a

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From the Clinical Editor: Authors demonstrate the synthesis of multilayered particles consisting of a magnetic core and two encompassing shells made up of poly (*N*-isopropylacrylamide) (PNIPAAm) and poly(*D, L*-lactide-co-glycolide) (PLGA) for targeted and controlled drug delivery. The presented results indicate successful synthesis and application for targeted and controlled delivery of multiple drugs with different release mechanisms.

hydrophilic drug and guided to the treatment site by an external magnetic field. Then, an external electromagnetic device can be used to locally raise the temperature above the polymer's lower critical solution temperature (LCST); consequently, the polymer structure collapses and releases the drug. In general, polymer-coated magnetic particles consist of core-shell based nanostructures.^{8-9,12-14} The magnetic core allows a magnetic-based targeting mechanism, serves as an MRI contrast agent, and produces a heat source, whereas the shell is used to enhance the particle biocompatibility while allowing drug-loading and release.

The objective of our research was to develop dynamic nanoparticles capable of providing a dual drug-delivery mechanism. To reach our goal, multilayered nanoparticles (MLNPs) with a magnetic core and two shells made up of temperature-sensitive polymers (PNIPAAm) and biodegradable polymers (PLGA), as shown in Figure 1, were synthesized. PNIPAAm was immobilized onto the magnetic nanoparticles using a coupled silane agent and free radical polymerization of the *N*-isopropylacrylamide monomer. The resultant PNIPAAm magnetic nanoparticles were then encapsulated with PLGA by a double emulsion solvent evaporation technique using poly(vinyl alcohol) (PVA) as a surfactant as described previously.¹⁰⁻¹⁶⁻¹⁹ The morphology, size, and size distribution of these multilayered nanoparticles were determined by transmission electron microscopy (TEM) and dynamic light scattering (DLS) technology. The effect of different factors including surfactant PVA concentration, PLGA concentration, and sonication power on the particle size were determined using factorial analysis. The iron particle concentration, drug loading and release from 300-nm particles (the smallest particles obtained from factorial analysis) were also studied.

Methods

Materials

Poly(D,L-lactide-co-glycolide) (PLGA, 50/50; Birmingham Polymers, Pelham, Alabama), *N*-isopropylacrylamide (NIPAAm, 97%; Sigma-Aldrich, St. Louis, Missouri), docusate sodium salt (AOT; Sigma-Aldrich), sodium dodecyl sulfate (SDS, 99%; Sigma-Aldrich), *N,N*-methylenebisacrylamide (MBA; Sigma-Aldrich), potassium persulfate (KPS, 99+%; Sigma-Aldrich), dichloromethane (DCM; Merck KGaA, Gibbstown, New Jersey), vinyltrimethoxysilane (VTMS, 98%; Sigma-Aldrich), and poly(vinyl alcohol) (PVA, 87% to 89%; Sigma-Aldrich) were used as obtained.

Preparation of magnetic nanoparticles

Magnetic nanoparticles were synthesized in our lab using a co-precipitation method of ferrous and ferric salts in the presence of a basic solution and the surfactant AOT.^{2-9,20} In brief, ferric chloride hexahydrate and ferrous chloride tetrahydrate (2:1) were dissolved in 600 mL de-ionized (DI) water. After purging the solution with argon gas, 0.36 g AOT in 16 mL hexane was added, and the solution was heated to 85°C. At this temperature, NaOH (7.1 M) was added. After a 2-hour reaction period, particles were washed extensively with ethanol and then centrifuged at 25,000 rpm for 45 minutes. The magnetic nanoparticles were dried in a vacuum oven.

Preparation of VTMS-coated magnetic nanoparticles

The magnetic nanoparticles were coated with VTMS via acid catalyst hydrolysis followed by electrophilic substitution of ferrous oxide on the surface of the magnetic nanoparticles.^{21,22} In brief, 0.48 mL VTMS was hydrolyzed using 3% vol/vol acetic acid in the 99% ethanol solution. Then, 0.074 g magnetic nanoparticles were dispersed by sonication at 100 W for 30 minutes in this solution. The VTMS-coated magnetic nanoparticles were then obtained after 24 hours of vigorous mechanical stirring at room temperature. The product was excessively washed with a mixture of water/ethanol (1:99 vol:vol) to remove unreacted components. The

VTMS-coated magnetic particles were collected using a magnet and were dispersed in water before the next step.

Immobilization of PNIPAAm on the surface of magnetic nanoparticles

VTMS-coated magnetic nanoparticles were used as a template to polymerize NIPAAm as previously described.^{23,24} In brief, 0.028 g VTMS-coated magnetic nanoparticles, 0.15 g N-isopropylacrylamide (NIPA), 0.0131 g *N,N*-methylenebisacrylamide (BIS; a cross-linking agent), and 0.041 g SDS (a surfactant) were sonicated in 100 mL cold water for 30 minutes. The mixture was then heated to 70°C, and 0.078 g of KPS was added to initiate the reaction. The solution was stirred under argon for 4 hours. The product was purified several times with DI water using a magnet to isolate and collect PNIPAAm-coated magnetic nanoparticles.

Encapsulation of PNIPAAm magnetic nanoparticles with PLGA

PNIPAAm magnetic nanoparticles were encapsulated with PLGA using a double-emulsion solvent evaporation method similar to that used to encapsulate bare iron oxide nanoparticles (or magnetic nanoparticles) and proteins.^{8,10,17,25} Initially, 15 mg PNIPAAm magnetic nanoparticles were dispersed in 300 µL DI water, and this particle dispersion was added to 3% wt/vol PLGA in 3 mL DCM. The solution was then sonicated for 30 seconds at 55 W using a sonicator (3000; Misonix Inc., Farmingdale, New York) to obtain the primary water in oil (w/o) emulsion. The emulsion solution was added dropwise into a 2% wt/vol PVA solution, and the mixture was then sonicated for 2 minutes at 55 W power to obtain a w/o/w emulsion. After stirring overnight to allow solvent evaporation, our multilayer magnetic nanoparticles were collected using a magnet, washed several times with DI water to remove bare PLGA particles, and then centrifuged at 15,000 rpm for 20 minutes using an ultracentrifuge (LM100; Beckman, Fullerton, California) to remove residual PVA.

Size and morphologic characterization of the MLNPs

The obtained MLNPs were characterized for size and morphology using TEM imaging and light dispersion methods, respectively. For TEM, nanoparticles were placed on a plasma activated grid and observed using the transmission electron microscope (1200; JEOL, Tokyo, Japan) to determine the size and morphology of the particles. The size and size distribution of the nanoparticles were also determined by a DLS method (Nanotracs, Microtracs Inc., Montgomeryville, Pennsylvania).

Effects of different factors on particle size

Factorial studies were conducted to evaluate the effects of different factors on the particle size. Statistical analysis software Designexpert (version 11; Statease, Minneapolis, Minnesota) was used for the analysis. A half-factorial experiment (runs instead of 8) for 3 factors was designed. The three factors (independent variables) included PVA concentration (2% and 5% wt/vol), PLGA concentration (2% and 3% wt/vol), and sonication power (35 W and 55 W). The evaluated response (dependent outcome) was the particle size. The resulting factorial design is shown in Tables 1 and 2. To evaluate the effects of factors on particle size, particles were synthesized for each run, and their sizes were analyzed using TEM and DLS. After the analysis, particles of 300-nm size obtained for run no. 3 were used for later studies.

Determination of iron oxide concentration

Iron oxide concentration was determined by digesting the particles with HCL and applying spectrophotometric methods on these samples.²⁶ In brief, 2 mg MLNPs were suspended in 1 mL DI water, and 1 mL 30% vol/vol HCL was added to the particles. The solution was incubated at 40°C for 2 hours to initiate breakdown. One milliliter of 1% wt/vol ammonium persulfate was added to oxidize the ferrous ions present in the above solution to ferric ions. In

all, 1.0 mL potassium thiocyanate (0.1 M) was added to this solution and shaken for 15 minutes to form the ironthiocyanate. After incubation, the absorption of this solution was read at a wavelength of 540 nm and compared with standards to get the amount of iron oxide presented in the particles.

Drug loading and release studies

Bovine serum albumin (BSA) and curcumin were used as hydrophilic and hydrophobic model drugs, respectively. BSA (1 g) was first loaded into PNIPAAm magnetic nanoparticles (60 mg) that were suspended in 20 mL DI water. The solution was left on a shaker for 3 days at 4°C to let the drug absorb into the particles. These loaded PNIPAAm magnetic nanoparticles were used to form the multilayer nanoparticles. Second, curcumin was loaded by mixing 9 mg curcumin with 90 mg PLGA in 3 mL DCM during the emulsion phase to form the multilayer nanoparticles as described above. Thus, curcumin was embedded in the PLGA layer while BSA was loaded into the inner PNIPAAm layer.

Loading efficiency was calculated indirectly. Here, the amount of drug left in the supernatant after removing the drug-loaded particles was determined. This amount was subtracted from the initial concentration of drug to get the percentage of loading efficiency:

$$\text{Percentage loading efficiency} = \frac{(\text{Original concentration} - \text{Supernatant concentration})}{\text{Original concentration}} \times 100.$$

To study the amount of drug released at a certain time, the drug-loaded particles were suspended in a known amount of PBS. Samples were dialyzed at 4°C and 37°C, respectively, and the dialysate was collected and replaced by fresh PBS at regular intervals of time up to a maximum of 3 weeks. The collected samples were stored at -20°C until analysis. The samples were analyzed for the BSA release amount using BCA protein assays (Pierce, Rockford, Illinois) following the manufacturer's instructions. To determine the released curcumin, the supernatant samples were mixed with ethanol at a 1:1 ratio to completely ensure the curcumin solubility. The absorbance of samples was read at 490 nm, and the curcumin solutions with various concentrations were used to obtain a standard curve. The accumulative release of BSA at 4°C and 37°C and for curcumin at 37°C was plotted versus time to get the drug release profile of the particles.

Results

Size and morphologic characterization of the particles

As shown in Figure 2, our multilayer particles obtained from initial PVA concentration of 2% wt/vol, PLGA concentration of 3% wt/vol, and sonication power of 35 W are in the range of 500 to 1000 nm using TEM. The MLNPs have a solid spherical morphology with core-shell structure and a nonuniform rough surface.

Effect of PVA, PLGA concentration, and sonication power on the multilayer nanoparticle size

The size of our multilayer particles depends on the droplet size formed during the emulsion step, thus it can easily be reduced by varying the factors like sonication power and surfactant concentration.²⁷ Therefore, these factors were selected to study their effects on the synthesized particle size in our study. The average size of the particles measured using TEM was compared with that using DLS (Figure 3).

A half normal probability plot was obtained using Designexpert software to demonstrate the relative importance of these factors. The absolute values of effects are represented on the x-

axis as squares, and estimates of errors are represented as triangles (Figure 4, A). Also, as seen Figure 4, B–D , the response surface diagrams were developed using Designexpert to study the relationship between the factors involved in the formulation process such as surfactant concentrations, sonication power, and PLGA concentrations with the particle size.

As seen in the half-normal probability plot (Figure 4, A), the most important factors that affected the particle size were sonication power and PVA concentration. These two factors have a negative effect on particle size, meaning that increasing the sonication power and PVA concentration decreases the particle size. On the other hand, PLGA concentration is the least important factor and has a positive effect on particle size. The response surface diagrams were developed using Designexpert to study the relationship between the factors and particle size. Figure 4, B shows that at a PLGA concentration of 2.5% wt/vol, it is evident that increasing the sonication power (from 35 W to 55 W) and the PVA concentration (from 2% to 5% wt/vol) has the largest effect on reducing particle size. At a PVA concentration of 3.5% wt/vol (Figure 4, C) and a sonication power of 45 W (Figure 4, D), the particles size increased slightly with an increase in PLGA concentration (from 2% to 3% wt/vol). Both sonication power (Figure 4, B and C) and PVA concentration (Figure 4, B and D) changed the particle size significantly when compared with PLGA concentration (Figure 4, C and D).

Iron oxide concentration of multilayered nanoparticles

The iron oxide concentration of the samples from run no. 3 was measured to quantify the magnetic particles to the polymer ratio of the resultant MLNPs. Weight/weight of the iron oxide concentration in the multilayered particle varied from 70% to 75%, with the remaining 25% to 30% being made of PNIPAAm and PLGA. The high iron oxide content of 70% to 75% is due to the higher density of iron oxide compared with the polymer and the presence of nonencapsulated magnetic nanoparticles, which cannot be eliminated. Bare polymeric particles are eliminated by the magnetic extraction process during synthesis.

Drug-release profile of the multilayered nanoparticles

BSA and curcumin were selected as hydrophilic and hydrophobic model drugs, respectively, because they can easily be quantified. BSA was loaded into the PNIPAAm layer with a loading efficiency of 65%, whereas curcumin was incorporated in the biodegradable PLGA shell with a loading efficiency of 49.5%. As seen in Figure 5, the curcumin and BSA release profile of the nanoparticles was characterized by an initial burst release followed by a sustained release. Also, as seen in Figure 5, B, the percent of cumulative release of BSA at 37°C was significantly higher than at 4°C suggesting that the BSA released altered with changes in temperature.

Discussion

In this study, we have systematically developed a novel multilayered particle that consists of a PNIPAAm magnetic core and a PLGA shell. Our study shows that the particle size can be controlled by varying the PVA concentration and sonication power. Iron oxide concentration studies indicate that magnetic particles are successfully encapsulated by PNIPAAm and PLGA. We were also able to successfully load the particles with BSA and curcumin, models for hydrophilic and hydrophobic drugs, respectively. Particles provided a sustained release of curcumin throughout the 2 weeks of study, whereas the BSA release was characterized by an initial burst release followed by a sustained release. The BSA release at 37°C was also significantly more than that at 4°C, suggesting a temperature-responsive drug release.

Apart from the metallic/polymer core-shell nanoparticles, multilayered particles formed by various materials have been investigated for use as potential drug carriers.^{6,28,29} In one sample, multilayered particles formulated by anionic interactions of various materials are prepared by

sequential deposition of interacting polymers onto colloidal particle templates.^{6,28} A single drug can be loaded into the particle's hollow shell, and the particle size and stability can be easily manipulated given the stepwise formation of the capsules. In contrast, our multilayered particles are formed by covalently binding the polymer shell onto the magnetic core using a silane coupling agent followed by a standard emulsion technique. These nanoparticles are capable of carrying two drugs with good loading efficiencies, and the magnetic core provides for a magnetic-based targeting mechanism. The size of these particles can be controlled by varying the formulation factors.

The effect of PVA concentration, sonication power, and PLGA concentration on particle size was determined using factorial analysis. Our observations are consistent with other studies showing that the size of the particles prepared by emulsion methods can be effectively controlled by varying surfactant concentration and sonication speed.^{27,30,31} These results suggest that the size of the formed emulsion droplets depends mainly on the surfactant concentration and homogenization speed/power in emulsion methods. The equation for nanoparticle size in terms of actual factors was obtained from factorial analysis. The predicted nanoparticle size can be calculated for any combination of individual factors within the range provided in Table 1 using the following equation:

$$\text{Particle size (nm)} = 624.71 - 32.17 \times (\text{PVA concentration}) + 25.50 \times (\text{PLGA concentration}) - 4.63 \times (\text{Sonication power}).$$

Drug-release studies were done to determine the drug loading and release profile of MLNPs. The loading efficiency of curcumin into the PLGA shell was approximately 49.5%, which is consistent with other studies^{14,31,32} where hydrophobic drugs were incorporated into PLGA particles using an emulsion method.

The loading efficiency of BSA into the PNIPAAm layer was approximately 65%, which is less than that obtained in other studies^{8,9} where the hydrophilic drug was incorporated into PNIPAAm-based magnetic nanoparticles. The lower BSA loading in our study might be due to the formulation process where some BSA was released from the PNIPAAm magnetic nanoparticles during encapsulation with PLGA.

The release characteristics of curcumin from our nanoparticles showed a burst effect within 1 day, followed by a sustained release for the remaining 13 days with approximately 35% of the total encapsulated drug being released. As shown in Figure 5, *A*, about 14% of the total drug (curcumin) encapsulated was released within 12 hours due to a burst effect. This might be due to the drug being adsorbed onto the particle. This result is consistent with other studies of drug-loaded PLGA particles.^{14,32} As shown in Figure 5, *B*, the percentage of cumulative release of BSA at 37°C was significantly higher than that at 4°C. This is consistent with previous studies¹⁵ where drug released from the PNIPAAm magnetic nanoparticles was studied and is indicative of the temperature-sensitive drug release from the PNIPAAm layer. About 28% of BSA was released due to the initial burst effect followed by a sustained release after 12 hours. This is contrary to previous studies on PNIPAAm-based drug delivery systems where even a higher amount of drug was released due to a burst effect that lasted for longer periods of time.¹⁵ The low BSA release might be due to the inability of BSA released from the PNIPAAm layer to pass through the outer PLGA layer. Thus, the release of both model drugs might be dependent on the degradation of PLGA. Also, an initial burst BSA release can be partly attributed to the release of BSA from the PNIPAAm magnetic particles attached to the surface of our multilayer nanoparticles.

Though we were successful in developing multilayered particles that can be loaded with two drugs by encapsulating PNIPAAm magnetic nanoparticles with PLGA, the applicability of our

multilayer particles for drug delivery may have some limitations. As seen in the TEM images, not all of the PNIPAAm magnetic nanoparticles were encapsulated, and few were present on the particle surface. The initial burst release of BSA seen in Figure 5 is also due to the presence of BSA-loaded PNIPAAm magnetic particles on the surface of our multilayered particle that are not encapsulated in the PLGA shell. The attachment of the PNIPAAm magnetic nanoparticles onto the outer surface can be attributed to the interaction between the polymers and the magnetic attraction between the particles. This would affect the particle size and hinder the drug-release mechanism as observed during the drug-release studies. Also, the presence of the PNIPAAm magnetic particles on the surface reduces the number of carboxylic groups available for future conjugation with the targeting moieties. Another limitation is that a few of the PNIPAAm magnetic nanoparticles were encapsulated in the PLGA shell instead of a single particle encapsulation, which would affect the particle size. In the future, we will try to minimize the unencapsulated PNIPAAm magnetic particles by careful adjustment of the parameters, and we will investigate methods to improve our MLNPs.

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Figure 1.
Scheme of our multilayered particles.

Figure 2.
Transmission electron microscope image of multilayered particles.

Figure 3.

Size of the particles for factorial design studies measured by TEM and DLS. Run 1: 2% PVA, 3% PLGA, and 35 W sonication power. Run 2: 5% PVA, 2% PLGA, and 35 W sonication power. Run 3: 5% PVA, 3% PLGA, and 55 W sonication power. Run 4: 2% PVA, 2% PLGA, and 55 W sonication power.

Figure 4. (A) Half-normal plot showing the effect of factors on the particle size. (B–D) Three-dimensional surface plot showing the effect of factors on the particle size.

Figure 5.
(A) Drug-release studies: curcumin release. (B) Drug-release studies: BSA release.

Table 1

Variables used for half factorial experimental design

Factors	High	Low
PVA concentration	60 mg (5% wt/vol)	24 mg (2% wt/vol)
PLGA concentration	90 mg (3% wt/vol)	60 mg (2% wt/vol)
Sonication power	55 W	35 W

Table 2

Half factorial experimental variables used for preparation of multilayered particles

Experiment no.	PVA concentration, %	PLGA concentration, %	Sonication power, W
1	2	3	35
2	5	2	35
3	5	3	55
4	2	2	55