Songklanakarin J. Sci. Technol.
41 (6), 1396-1403, Nov. - Dec. 2019

http://www.sjst.psu.ac.th

Original Article

Investigation of biodegradable chitosan nanoparticles incorporated with Gd-DTPA prepared by ionotropic gelation for magnetic resonance imaging

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Received: 12 March 2018; Revised: 11 August 2018; Accepted: 22 August 2018

Abstract

A nanoparticle based magnetic resonance imaging (MRI) contrast agent was studied using the biopolymer chitosan. (Gd-DTPA)-conjugated chitosan nanoparticles were prepared by a one-step ionotropic gelation method at chitosan concentrations of 0.5 and 1.0 mg/mL. The nanoparticles were characterized by scanning electron microscopy and the particle size, zeta potential, and polydispersity index (PDI) were measured by dynamic light scattering. The results showed that (Gd-DTPA)-conjugated chitosan nanoparticles were homogeneous and the size had a narrow distribution based on the PDI which was in the range of 0.1–0.3. The mean±SD of the Gd content was found to be 6.2±1.5 mg/g particle. The *in vitro* release study along with UV-vis spectra showed no free Gd ions released from the prepared nanoparticles. The results confirmed that the binding of Gd-DTPA to chitosan did not exert any influence on the nanoparticle size or zeta-potential.

Keywords: nanoparticles, chitosan, Gd-DTPA, MRI contrast agent

1. Introduction

Biodegradable polymers play a key role in biomedical fields as carrier systems because of their biocompatibility, non-toxicity, and biodegradability properties (Nagpal, Singh, & Mishra, 2010; Peer *et al.*, 2007; Ulery, Nair, & Laurencin, 2011). Chitosan, which is a natural polysaccharide prepared by the N-deacetylation of chitin, has been widely used in food processing and bioengineering industries (Zhao *et al.*, 2011). Chitosan is composed of N-acetyl-D-glucosamine and D-glucosamine units with one amino group and two free hydroxyl groups in the repeating glucosidic residue (Şenel, & McClure, 2004). According to its unique polymeric cationic character, the available amino and hydroxyl groups of chitosan provide the applicable reacting sites in many areas and make it easy for chemical modifications. In addition, the availability of free amino groups of chitosan allows it to react with many negatively charged surfaces such as a cell membrane, mucus lining, and also with other anionic polymers because of the negatively charged sialic acid residues (Kunjachan, Jose, & Lammers, 2014).

Due to its unique biological and chemical properties, chitosan has great potential in a range of biomedical applications as a carrier for controlled drug and gene delivery. The use of biodegradable polymers as drug delivery systems can overcome the stability issues of certain drugs and minimize the side effects of drug molecules because of the controlled biodistribution behavior (Moghimi, Hunter, & Andresen, 2012). Furthermore, it is non-toxic and can interact with polyanions leading to complex and gel formations (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Kim & Rajapakse, 2005). Besides drug delivery applications, chitosan has also been used in enzyme immobilization, tissue engineering, and wound-healing formulations according to its gel-forming capability, high adsorption capacity, and inherent pharmacological properties including antibacterial, antifungal, and antitumor activity (Dash, Chiellini, Ottenbrite, & Chiellini, 2011).

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Recently, chitosan has been employed in the development of nanocarriers as polymeric coatings to provide a steric barrier to prevent nanoparticle agglomeration and avoid opsonization (Morachis, Mahmoud, & Almutairi, 2012; Wang *et al.*, 2011).

Over a few decades, magnetic resonance imaging (MRI) has become a powerful imaging modality for cancer diagnosis (Farraher *et al.*, 2006; Morrow, Waters, & Morris, 2011). The gadolinium ion (Gd^{3+}) and polyaminocarboxylate ligands are one of the most widely used contrast agents in MRI (Rebizak, Schaefer, & Dellacherie, 1997). The MRI contrast is generated by the paramagnetism of the Gd ions, resulting in enhancement of signal intensity and improvement of the contrast between magnetically similar but histologically distinct tissues (Sharma *et al.*, 2007; Tang *et al.*, 2013). However, it is well known that free Gd^{3+} ion is toxic as it can carry the risk of nephrogenic systemic fibrosis, particularly in kidney impaired subjects (Bui *et al.*, 2010; Rogosnitzky & Branch, 2016). However, Gd^{3+} ions can be chemically stabilized by chelation to develop Gd (III) complexes composed of a sufficient Gd concentration to achieve a tumor-specific accumulative property and to improve their clinical safety profile during treatment (Ichikawa *et al.*, 2014). To avoid the high toxicity of the free form of the Gd^{3+} ion, the most extensively used Gd (III) complex contrast agents are Gd-chelates with polyaminocarboxylate ligands such as diethylenetriaminepentaacetic acid (DTPA) and 1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (DOTA) (Park *et al.*, 2009). These Gd-based contrast agents, such as gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) (Magnevist®), Gd-DTPA-BMA (Omniscan®), and Gd-DOTA (Dotarem®), have been widely used in clinical imaging for cancer diagnosis because they have shown excellent enhancement of the signal intensity at the tumor site for identification of pathologic tissue and maintain enough imaging time due to the Gd paramagnetic properties (Bui *et al.*, 2010; Liu & Zhang, 2012). However, after administration, Gd chelates are rapidly cleared from the intravascular space for rapid renal clearance and nonspecific biodistribution (Liu & Zhang, 2012). To overcome this limitation, Gd chelates are formulated with a variety of materials, such as polysaccharides, phospholipids, poly(L-lysine), proteins, and polyamines to increase the residence time in the blood, reduce non-specific distribution, and provide targeted deliver to the tissues (Liu & Zhang, 2012).

Gd-loaded nanoparticles can be achieved with ideal physicochemical and pharmacological properties that include low toxicity, high water solubility, thermodynamic and kinetic stability, high relaxivity. Furthermore, Gd-loaded nanoparticles have great potential in tumor diagnosis such as passive targeting properties, prolonged imaging time, and enhancement of the contrast (Liu *et al.*, 2011; Yang & Chuang, 2012). Recently, several nanomaterials have been studied as targeted contrast agents in MRI for cancer diagnosis and therapy (Blasiak, Veggel, & Tomanek, 2013; Wang, 2011). Previous research studies reported that chitosan was used as a biopolymer for the preparation of Gd-DTPA microparticles for neutron capture therapy using the emulsion-droplet coalescence method (Saha *et al.*, 2006; Tokumitsu, *et al.*, 1999). More recently, Zhang *et al*. reported the preparation of hyaluronic acid-chitosan nanoparticles to deliver Gd-DTPA for MR cancer imaging (Zhang *et al.*, 2015). In general, the hyaluronic acid-chitosan nanoparticles have been prepared by multistep ionotropic gelation by carbodiimide reaction. In the present study, the modified chitosan nanoparticles with Gd-DTPA were prepared by a one-step ionotropic gelation process with dextran as the MRI contrast agent for cancer diagnosis. The particle size, zeta potential, and morphology of the particles were characterized. This work was also developed to improve its *in vitro* stability which in turn investigated the potential of chitosan nanoparticles containing Gd-DTPA as an MRI contrast agent.

2. Materials and Methods

Low molecular weight chitosan (70 kDa, the degree of deacetylation 75%–85%) and Gd-DTPA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Arsenazo III (2, 20-[1,8-dihydroxy-3,6-disulfonaphthylene]-2,7-bisazobisbenzenearsonic acid) was purchased from Sigma-Aldrich (Steinheim, Germany). Glacial acetic acid was purchased from Merck (Darmstadt, Germany). Water was deionized using a Milli-Q water purification system (Millipore). All other chemicals were analytical grade and used as received.

2.1 Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared by an ionic gelation method adopted by Calvo *et al.* (1997). Briefly, low molecular weight chitosan (LMWCS) was dissolved in Milli-Q water containing 5% (w/v) acetic acid to a concentration of 0.5 mg/mL. Dextran sulfate (molecular weight ~4000) was dissolved in Milli-Q water to a concentration of 0.1–0.5 mg/mL. The pH of the chitosan and dextran solutions was adjusted to 5. Subsequently, 4 mL of the dextran solution was added to 10 mL chitosan solution under continuous stirring for 2 h. The reaction was performed at room temperature. The resulting chitosan nanoparticles were opalescent suspensions. The suspensions were then dialyzed against Milli-Q water over night using Pierce SnakeSkin® pleated dialysis tubing (molecular weight cut-off (MWCO)=10,000), changing the water three times. The chitosan nanoparticles retrieved from the dialysis tubes were stored at 4 °C for further analysis.

2.2 Modification of chitosan nanoparticles with Gd-DTPA

For the preparation of the modified chitosan nanoparticles with Gd-DTPA (Gd-DTPA-Chitosan nanoparticles [NP]), LMWCS was dissolved in Milli-Q water containing 5% (w/v) Gd-DTPA to concentrations of 0.5 and 1.0 mg/mL. Dextran concentration was varied to 0.1, 0.3, and 0.5% (w/v). The pH of the chitosan and dextran solutions was adjusted to 5. The dextran solution (4 mL) at each concentration was added to 10 mL of 0.5 and 1.0 mg/mL chitosan, respectively. The solutions were continuously stirred for 2 h at room temperature.

The resulting chitosan nanoparticles with Gd-DTPA (Gd-DTPA-Chitosan NP) were then dialyzed against Milli-Q water using Pierce SnakeSkin® pleated dialysis tubing (MWCO=10,000) as previously described and stored at 4 °C for further analysis.

2.3 Characterization of Gd-DTPA-Chitosan NP

2.3.1 Particle size and polydispersity

The mean diameter and polydispersity of the resulting nanoparticles were measured by dynamic light scattering. For the measurements, 10 µL of the sample were diluted with 1 mL Milli-Q water. The resulting nanoparticles were characterized using a Zetasizer 3000 (Malvern, UK). Samples were prepared from three independent experiments and measured in triplicate.

2.3.2 Surface charge

The zeta-potential of the particles was assessed using Laser Doppler micro-electrophoresis in a palladium electrode dip cell (Malvern Instruments, Malvern, UK). For the measurements the samples were diluted 1:50 with purified water. The scattering angle was 173º and the temperature was set to 25 °C. Samples were prepared from three independent experiments and measured in triplicate.

2.3.3 Particle morphology

The nanoparticle morphology was studied using scanning electron microscopy (SEM) using a Hitachi S 4500 (Japan). The sample specimens were prepared by suspending the nanoparticles in Milli-Q water, allowed to dry over 6 h at room temperature, and then sputtered with gold one to two times for 60 s. Subsequently, SEM was performed using an accelerating voltage of 15–20 kV and secondary electron detection mode. The images were digitally photographed using the Digital Image Processing System 2.6 program.

2.4 Determination of Gd concentration

The Gd (III) content was determined by atomic absorption spectroscopy (AAS) using a Perkin Elmer Zeeman AAS 4110 ZL spectrometer (Perkin Elmer Company, USA). The nanoparticle suspensions were dissolved in diluted nitric acid prior to analysis and the Gd content was calculated by comparison with standard calibration curves.

2.5 Colorimetric assays

The colorimetric assay of the arsenazo III/Gd^{3+} complex was used for the quantitative analysis of excess gadolinium (Nagaraja *et al.*, 2006). In the presence of transition metals such as Gd^{3+}, the arsenazo III/Gd^{3+} complex was detected by a strong visible absorption at 660 nm while the free dye showed a strong visible absorption at 548 nm. The arsenazo III/Gd^{3+} complex was prepared by mixing the solution of Gd-DTPA-CS (0.4 mM) and stock solution of arsenazo III (0.4 mM) in water. The free Gd^{3+} was prepared by Gd $Cl_3 \cdot 6H_2O$ (2 mg/mL) in water and mixed with a stock solution of arsenazo reagent. All samples were analyzed by using a UV-vis spectrophotometer (Hitachi, Japan).

2.6 Release study of Gd

The prepared nanoparticles (4 mg) were placed in 2 mL of either Milli-Q water or phosphate buffer pH 7.4 in Eppendorf tubes and kept at 37 °C in an incubator shaker with continuous shaking at 550 rpm. Several samples in Eppendorf tubes were kept for analysis of Gd-DTPA release at predetermined intervals (0.5, 1, 2, 4, 24 h). At each time point the tubes were taken out and centrifuged at 16,100 g for 8 min. The supernatant was collected to determine the concentration of Gd by AAS. The Gd release was expressed as the percentage of the cumulative release.

2.7 Statistical analysis

Data were reported as mean±SD determined by at least three independent experiments. The statistical analysis of samples was performed using SigmaPlot 11.0 by one-way analysis of variance (ANOVA) using the Student-Newman-Keuls test. The differences were considered to be statistically significant at $P<0.05$ (∗) and $P<0.01$ (∗∗).

3. Results and Discussion

3.1 Preparation and characterization of chitosan nanoparticles

Chitosan nanoparticles were prepared by the ionotropic gelation technique. The nanoparticles were formed via the electrostatic interaction between positively charged amino groups on chitosan chains and negatively charged polyanions such as tripolyphosphate and dextran (Singh & Mishra, 2013). This simple and mild preparation method was applied to produce chitosan nanoparticles in the absence or presence of a stabilizing agent under aqueous environment at room temperature. It was reported that the size and surface charge of chitosan particles can be controlled by varying the ratio of chitosan and stabilizer (Abanese *et al.*, 2012; Calvo *et al.*, 1997).

In order to study the effects of chitosan and dextran concentrations on particle size and distribution, two concentrations of chitosan (0.5 mg/mL and 1.0 mg/mL) with three different concentrations of dextran (0.1%, 0.3%, and 0.5%) were set for nanoparticle formation. As determined by dynamic light scattering, the prepared chitosan nanoparticles had average diameters of 330–360 nm, the polydispersity was 0.1–0.3, and the zeta-potential was in the range of 20–30 mV. The results of all formulations produced homogeneously dispersed chitosan nanoparticles with small sizes and low PDI values which can be prepared by a simple and easily synthetic method. However, there were no statistically significant differences in particle size or PDI among these nanoparticle formulations ($P>0.05$). Therefore, the concentration of chitosan and dextran did not affect the particle size and PDI in this study. Although it was reported that the nanoparticle size and formation resulted from the chitosan concentration, there was no consistency in size distribution among these studies (Katas & Alpar, 2006). It was reported that in the preparation of chitosan nanoparticles by ionic gelation reactions with tripolyphosphate (TPP) the particle size differed when the nanoparticle formation parameters remained predominantly unchanged (Masarudin *et al.*, 2015). This revealed that the concentrations of chitosan and TPP were not a key parameter in the particle size and distribution in nanoparticle formation. In order to determine the particle stability, the PDI value indicated the nanoparticle stability and uniformity of formation (Masarudin *et al.*, 2015). A sample with a wide range of size

distribution has a higher PDI value and lower particle stability. In this study, the PDI of all formulations was found to be smaller than 0.3 which indicated monodisperse nanoparticles with greater particle stability.

The zeta potential value reflects the nanoparticle colloid stability. Nanoparticles with a zeta potential greater than +30 mV or less than −30 mV provide enough repulsion forces to avoid particle aggregation which suggests a higher stability of the nanoparticles (Nallamuthu *et al.*, 2013). These preparations showed zeta potentials of the modified nanoparticles in the range of 20–30 mV providing nanoparticles with high stability. These results were consistent with previous reports that the zeta potentials of chitosan-TPP nanoparticles were in the range of 24–28 mV (Rampino *et al.*, 2013). Therefore, all formulations of chitosan nanoparticles were considered for further modification with Gd-DTPA.

3.2 Chemical modification of chitosan nanoparticles with Gd-DTPA (Gd-DTPA-Chitosan NP)

Chitosan has been frequently used in bioimaging to encapsulate bioactive substances and contrast agents because of its excellent non-toxicity, bioactivity, and hydrophilic properties. In this study, the modified chitosan nanoparticles with Gd-DTPA are potential contrast agents for MRI. Figure 1 illustrates the Gd-DTPA-conjugated chitosan nanoparticle formation (Gd-DTPA-Chitosan NP). The DTPA-complex was bound to the particles by the interactions between the positively charged amino groups on the chitosan chains and the oppositely charged polyanions of the DTPA either before or after the particle formation (Ichikawa *et al.*, 2014).

The diameters of the modified nanoparticles were slightly higher than those without modification, while maintaining a low polydispersity index (PDI <0.3) and varied within the range of 350–400 nm and the zeta potential of all the modified nanoparticles was in the range of 25–40 mV. The Gd-DTPA-Chitosan NP was prepared using different amounts of chitosan (0.5 mg/mL or 1.0 mg/mL) but exhibited similar physicochemical parameters (Figure 2). The results confirmed that the association of Gd-DTPA to chitosan nanoparticles did not exert any influence on the nanoparticle size or zeta-potential. As previously mentioned, the Gd-DTPA molecules can be incorporated into the chitosan nanoparticles through the electrostatic interaction between negatively charged carboxylic groups and positively charged amino groups of chitosan molecules (Ichikawa *et al.*, 2014). The SEM image of the Gd-DTPA-Chitosan NP prepared with 1.0 mg/mL chitosan and 0.1% dextran is shown in Figure 3. The particles possessed a spherical shape and smooth surface morphology.

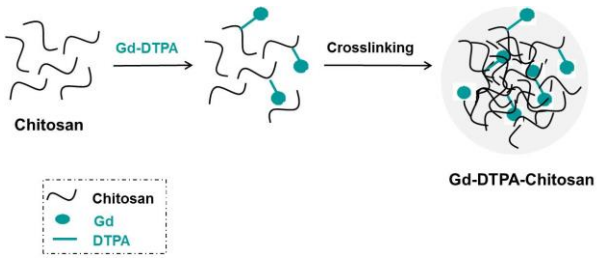

Figure 1. Schematic representation of the preparation of Gd-DTPA-Chitosan NP.

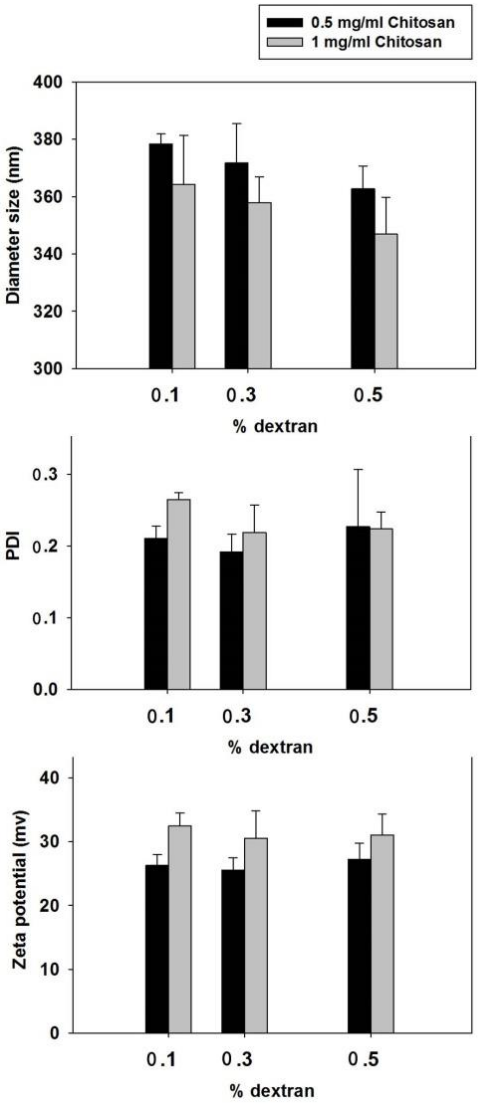

Figure 2. Particle diameter, PDI, and zeta potential of Gd-DTPA-Chitosan NP prepared by 0.5 and 1.0 mg/mL chitosan with 0.1, 0.3, and 0.5% dextran in distilled water. Results are expressed as mean±SD (n=3).

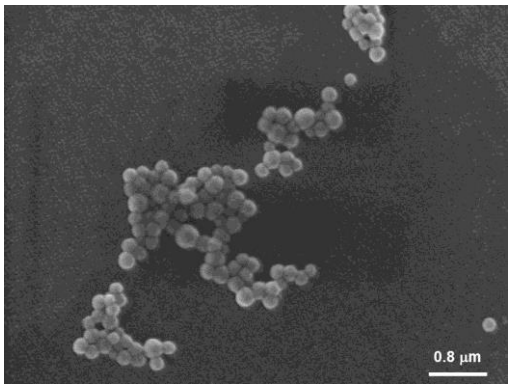

Figure 3. SEM image of the Gd-DTPA-Chitosan NP prepared by 1.0 mg/mL chitosan using 0.1% dextran.

3.3 Gd concentration

The Gd (III) concentration in the chitosan nanoparticles was determined by AAS and compared in terms of mg Gd per g nanoparticles (mg/g NP). The resulting nanoparticles were prepared by 0.5 mg/mL chitosan to obtain the final concentrations of Gd as 5.7±1.1 mg/g NP, 6.6±1.4 mg/g NP and 6.5±0.8 mg/g NP using 0.1, 0.3, and 0.5% dextran, respectively (Figure 4). The final concentrations of Gd prepared by 1.0 mg/mL chitosan were 8.1±1.5 mg/g NP, 6.3±0.6 mg/g NP, and 4.1±0.8 mg/g NP for 0.1, 0.3, and 0.5% dextran, respectively. It was reported that the Gd-loaded nanoparticles with concentrations of Gd (III) in the range of 4–10 mg Gd per g NP were applicable for MRI to provide continuous enhancement of the signal intensity in the liver and improve the detection of the hepatocellular carcinoma in animal models (Korkusuz *et al.,* 2103). In this study, Gd-DTPA-Chitosan NP were achieved with a similar Gd concentration. In comparison with the dose of Gd used in humans, relatively high doses (0.03–0.1 mmole/kg body weight) of Gd chelates produce sufficient MR images due to the wide-spread tissue and cell distribution (Bui *et al.,* 2010). The Gd content in the Gd-DTPA-Chitosan NP prepared using 1.0 mg/mL chitosan and 0.1% dextran was slightly higher than other formulations.

Based on the results, an increase in the dextran concentration from 0.1% to 0.5% led to an increase in Gd concentration for Gd-DTPA-Chitosan NP prepared by 0.5 mg/mL chitosan but a decrease in Gd concentration for that of NP prepared by 1.0 mg/mL chitosan. Therefore, the efficiency of incorporating Gd varied with the chitosan and dextran concentrations. At low chitosan and dextran concentrations, the dextran could easily induce nanoparticle formation and promote electrostatic interaction equilibrium between the Gd-DTPA and chitosan, which in turn increased the Gd content. At higher chitosan and dextran concentrations, the dextran could easily form complexes with chitosan which resulted in greater turbidity of the nanoparticle suspension and particle aggregation. This steric hindrance could restrict the ability of the Gd-DTPA to incorporate the nanoparticles and, in turn, decrease the Gd content (Jaszberenyi *et al.,* 2007). Since dextran can easily form complexes with chitosan, excess dextran

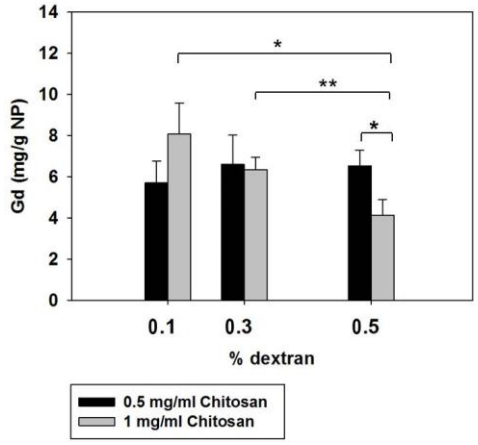

Figure 4. Concentration of Gd in Gd-DTPA-Chitosan NP prepared by 0.5 and 1.0 mg/mL chitosan with 0.1, 0.3, and 0.5% dextran. The results are expressed as mean±SD (n=3).

can impair the stability of chitosan nanoparticles and decrease the drug incorporation efficiency (Lu *et al.,* 2009). In addition, the Gd concentrations of Gd-DTPA-Chitosan NP prepared by 0.5 mg/mL chitosan using 0.1% dextran exhibited no significant difference compared to 1.0 mg/mL chitosan using the same concentration of dextran ($P<0.05$). Interestingly, the Gd-DTPA-Chitosan NP prepared by 1.0 mg/mL chitosan and 0.1% dextran demonstrated a significant difference compared to 0.5% dextran ($P<0.05$). The results of these characterizations indicated that the modified nanoparticles prepared by 1.0 mg/mL chitosan treated with 0.1% dextran showed the highest Gd concentration and turned out to be the optimum formulation which was used as a model for further study.

3.4 Characterization of colorimetric assays

The binding of Gd-DTPA to chitosan was confirmed by the colorimetric arsenazo assay. In the presence of transition metals such as Gd^{3+}, the arsenazo III/Gd^{3+} complex was detected by a strong visible absorption at 660 nm while the free dye showed a strong visible absorption at 548 nm. The absence of Gd in free or complexed form was confirmed by the arsenazo assay for gadolinium after dialysis of the nanoparticle suspension (Gouin & Winnik, 2001).

As shown in Figure 5, the free arsenazo III solution without gadolinium is presented in red (A). The development of color from red (A) to purple (B) indicated that the gadolinium ions were fully chelated. For the purification of Gd-DTPA-Chitosan NP, the nanoparticles were thoroughly washed by dialysis and the supernatant was evaluated for the absence of Gd-DTPA which would indicate complete removal of excess Gd-DTPA. The arsenazo III solution containing free gadolinium ions is presented in green (D). This result indicated the absence of free gadolinium ions in Gd-DTPA-Chitosan NP, demonstrating the binding of Gd-DTPA and chitosan. Despite a number of studies that examined the potential of Gd-based contrast agents for MRI, recent studies reported that the toxicity of Gd-based contrast agents resulting from the dissociation of gadolinium ions from the chelated complexes can lead to the development of nephrogenic systemic fibrosis and other adverse biological effects (Rogosnitzky & Branch, 2016). Therefore, the evaluation of complete elimination of free gadolinium from the nanoparticle suspension was necessary due to the high toxicity of free gadolinium ions. The results from Figures 5D and 5E confirmed the binding of Gd-DTPA and chitosan nanoparticles and showed no free Gd^{3+} ions distributed from the prepared Gd-DTPA-Chitosan NP. For this reason, chitosan nanoparticles can be used as a core or coating material to conjugate with these imaging agents to produce non-toxic and stable gadolinium-chitosan nanoparticle contrast agents.

3.5 Release profile of Gd

An *in vitro* study of Gd release from the Gd-DTPA-Chitosan NP was performed in Milli-Q water and phosphate buffer solution (PBS) at pH 7.4 and 37 °C. Less than 1.2% of Gd was released from the particles in both Milli-Q water and PBS over a period of 24 h (Figure 6). This possibly resulted from the stable covalent bonding of the Gd-DTPA to the chitosan nanoparticles. The release of Gd in Milli-Q water and PBS pH 7.4 showed a similar profile over time; however,

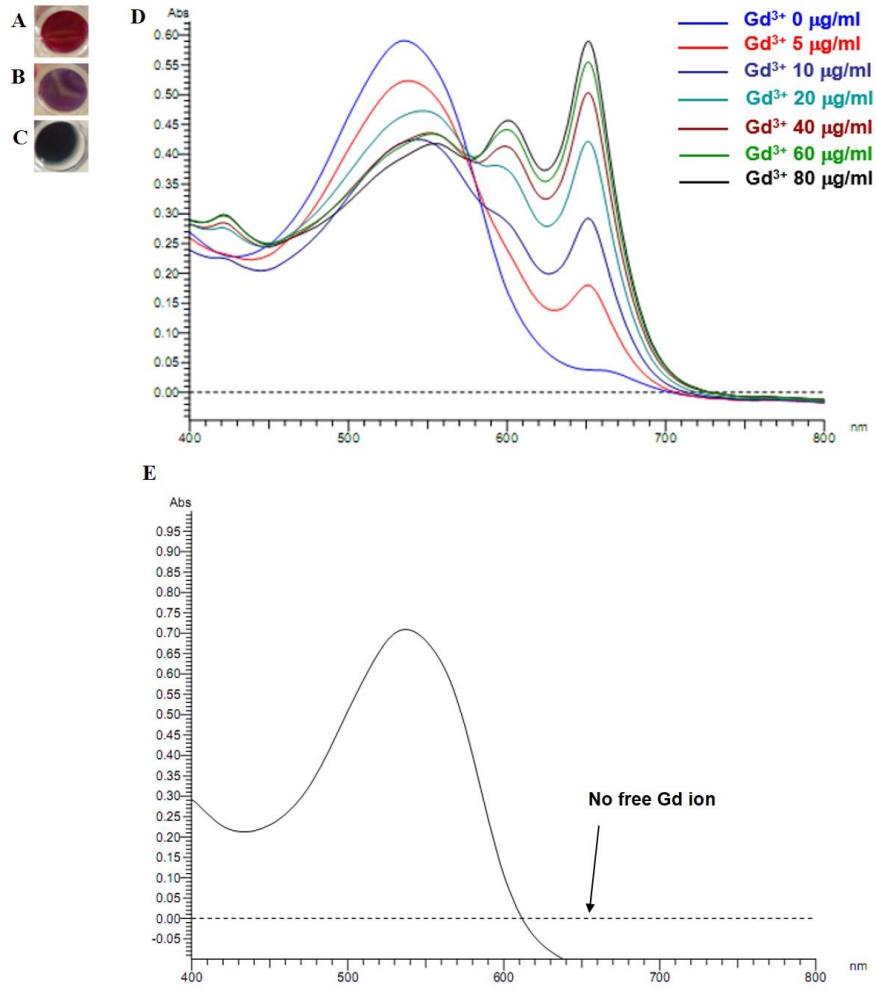

Figure 5. Colorimetric analysis of gadolinium chelation by arsenazo assay. (A)-(C) Color development of arsenaso reagent with or without Gd complexes: (A) arsenaso reagent, (B) arsenaso reagent with Gd-DTPA-Chitosan NP, (C) arsenaso reagent with free Gd^{3+} ions. (D) UV-vis spectra of arsenaso reagent containing Gd^{3+} ions at different concentrations. (E) UV-vis spectrum of arsenaso reagent containing Gd-DTPA-Chitosan NP prepared by 1.0 mg/mL chitosan with 0.1% dextran.

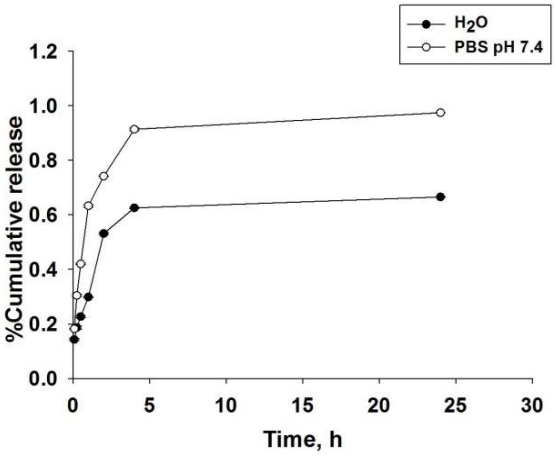

Figure 6. Release profile of Gd from the Gd-DTPA-Chitosan NP prepared by 1.0 mg/mL chitosan with 0.1% dextran in Milli-Q water and PBS pH 7.4 at 37 °C.

lower levels of Gd were released in the Milli-Q water compared with those in PBS. Interestingly, less than 1.2% of Gd was released from the nanoparticles in both media within the first 4 h and this amount did not considerably increase even after 20 h. This finding confirmed the stability of the prepared nanoparticles which resulted in the strong interaction of Gd-DTPA to chitosan. An *in vivo* investigation is required to evaluate the cytotoxicity and efficiency of this Gd-DTPA-Chitosan NP as an MRI contrast agent for the detection of tumors.

4. Conclusions

Paramagnetic Gd-DTPA-Chitosan NP were successfully prepared by a one-step ionotropic gelation method. The reaction was carried out using a simple method under an aqueous environment without the use of a toxic crosslinker. The nanoparticles were achieved with a spherical shape and narrow size distribution. The particle sizes of the prepared Gd-DTPA-Chitosan NP prepared by 0.5 and 1.0 mg/mL chitosan were in the range of 300–400 nm. The zeta potential of the

particles was found to be 25–40 mV. The stability of the Gd-DTPA-Chitosan NP was greatly influenced by their particle size and surface charge. The release study indicated that less than 1.2% of Gd-DTPA was released from the nanoparticles during 24 h. Chitosan nanoparticles have available positively charged sites for interaction with negatively charged DTPA providing better binding efficiency. Therefore, the Gd-DTPA-Chitosan NP are promising candidates as magnetic contrast agents for MRI.

Acknowledgements

The authors would like to thank the Faculty of Biotechnology, Assumption University for providing all of the necessary facilities and support for this project.

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