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Preliminary Study of Citric Gadolinium Encapsulated by Silica-Nanoparticles for Cancer Contrast Agent

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Abstract Gadolinium is a rare earth element (REE) which is widely used in contrast agent, as coordination compounds, since it enhances the quality of Magnetic Resonance Imaging (MRI). However, its coordination compound, Gd-DTPA, is able to cross blood brain barrier and potentially releasing toxic free gadolinium ions. Gadolinium citrate encapsulated by silica nanoparticles (Gd-citrate@SiO_2-NPs) can be an alternative to Gd-DTPA. Therefore, this study was aimed to synthesize Gd-citrate@SiO_2-NPs and determine the encapsulation efficiency of gadolinium citrate. Gadolinium citrate was encapsulated using sol-gel Stöber method. The resulting Gd-citrate@SiO_2-NPswere further characterized using Particle Size Analysis (PSA) and X-ray Fluorescence (XRF). Encapsulation efficiency was determined by visible spectrometry method. The results showed that the obtained Gd-citrate@SiO_2-NPsare in the nano-size of 79.3 nm with polydispersity index of 0.459.Meanwhile encapsulation efficiency was 75.24%.

Keywords contrast agents, core-shell Gd-$C_6H_5O_7$@SiO_2, Computed Tomography Scan (CT-Scan), sol-gel Stöber's method

1. Introduction

Gadolinium is a member of rare earth elements (REEs). Due to its strong magnetic property, gadolinium is commonly used as a main component of contrast agents for magnetic resonance imaging (MRI) [1]. Nowadays, predominant contrast agents are coordination compounds of gadolinium, such as $[Gd(DTPA)(H_2O)]^{2-}$and $[Gd(DOTA)(H_2O)]^{-}$. Nevertheless, both contrast agents cause allergy and their efficiency and sensitivity are low [2]. Thus, a new contrast agent needs to be developed.

In our previous research [3], we synthesized gadolinium-mesoporous silica in the effort to develop a gadolinium-based contrast agent. However, a stability testing showeda gadolinium ion (Gd^{3+}) release from the mesoporous silica in a considerable amount of 6.32 x 10^{-2}mg.Free Gd^{3+} should be avoided in contrast agents for clinical use, since it is toxic for human body [4].

Another mode of gadolinium-based contrast agent is encapsulated gadolinium citrate in silica nanoparticles (Gd-citrate@SiO_2-NPs).Encapsulation of gadolinium in silica nanoparticles offers many advantages. It prevents the release of the toxic Gd^{3+} ions. Silica nanoparticles have porous surfaces which only allow water molecules to easily penetrate, but not other bigger molecules. Moreover, the surfaces of silica nanoparticles are malleable for

functionalization. Therefore, Gd-citrate@SiO_2-NPs are expected to prevent the release of toxic Gd^{3+} ions, while maintaining their functionality as a contrast agent. In this study, we synthesized Gd-citrate@SiO_2-NPsand evaluated the efficiency of gadolinium encapsulation. Results of this study is a valuable foundation for further development of Gd-citrate@SiO_2-NPs as an MRI contrast agent.

2. Methods

Synthesis of Gd-citrate@SiO_2-NPs

Gd-citrate solution was obtained by mixing 0.6207 g $GdCl_3.6H_2O$ in a measuring flask of 10 mL, followed by the addition of 0.9793 g $Na_3C_6H_5O_7$,1.13 mL NH_4OH solution 25%, and water up to the mark. The amount of 200 µLGd-citrate solution was mixed and stirred with 15 mL water and 35 mL ethanol in a beaker glass of 100 mL. While stirring, 100 µL TEOS 99% was added. After 5 minutes, 1.48 mL ammonia solution 25% was added and stirred for 16 hours at room temperature.

Encapsulation efficiency

Encapsulation efficiency of gadolinium was determined using a visible spectrophotometry method through complexation with Alizarin Red S (ARS).Standard solutions of Gd-ARS of 10.0, 12.0, 14.0, 18.0, 24.0, and 26.0 ppm were prepared by pipetting 6.0, 7.0, 9.0, 12.0, and 13.0 mL Gd(III) solution of 100 ppm to different measuring flask of 25 mL. After pH of each solution adjusted to 4 by the addition of sodium acetate buffer, 1.0 mL ARS of 1000 ppm was pipetted into every measuring flask. Water was added up to the mark and each solution was shaken. Blank solution was prepared using the same way without the addition of Gd(III) solution of 100 ppm. The wave length at maximum absorbance (λ_{max}) of Gd-ARScomplex was determined at a concentration Gd(III) of 12 ppm. Absorbance of each standard was measured by visible spectrophotometer at λ_{max}. Limit of detection (LoD) and quantification (LoQ) were determined using residual standard deviation of the response and the slope from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [5]. To determine concentration of Gd-citrate which did not encapsulated, 0.3 mL Gd-sitrat@SiO_2-NPs was pipetted into a measuring flask of 25 mL and treated in the same way to standard solutions. Encapsulation efficiency of gadolinium was calculated by subtracting the concentration of Gd-citrate used in synthesis with that not encapsulated.

Particle size analysis

Particle size of the obtained Gd-citrate@SiO_2-NP colloid was measured using SZ-100 Horiba which required 0.5 mL sample. Run duration was set 60 s. The measurement used ND filter and gate time of 100% and 2.56 µs, respectively. Form distribution applied for the calculation of particle size was polydisperse.

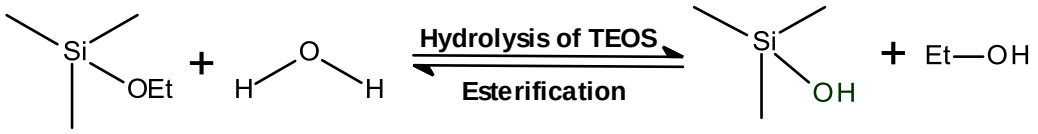
2. Results and Discussions

Synthesis of Gd-citrate@SiO_2-NP and analysis of its particle size

The use of Gd-citrate in this study was to obtain considerable sizes of complexes that can be trapped in silica nanoparticles. In a basic condition, the fully deprotonated state of citric acid is predominant species which act as a polydentate ligands and form cross-linked Gd(III) oligomers [6]. Sizes of such Gd(III) oligomers are bigger than poresof silica nanoparticles which prevent the release of toxic Gd^{3+} ions. Additionally, Gd-citrate complexes have high solubility in water and, thus, help to produce SiO_2-NPs containing homogenous Gd-citrate complexes.

Gd-citrate solution was prepared by mixing gadolinium(III) chloride, sodium citrate, and ammonia in water. Gadolinium(III) chloride gives an advantage in solution preparation since it easily dissolves in water. Subsequently, 200 µL solution stock of Gd-citrate was added to 15 mL water, followed by the addition of 35 mL ethanol with stirring. Water has an important role in hydrolysis reaction during sol-gel process, whereas ethanol help to prevent further agglomeration of formed silica, where the condensation reaction of TEOS to silica following Scheme 3.1-3.3. Stirring was performed to homogenized Gd-citrate complexes in the mixture. The stirring process was also

conducted during the gentle addition of TEOS to the mixture. Such treatment was to spread TEOS in the mixture, maximizing the formation of silica particles in nano sizes.



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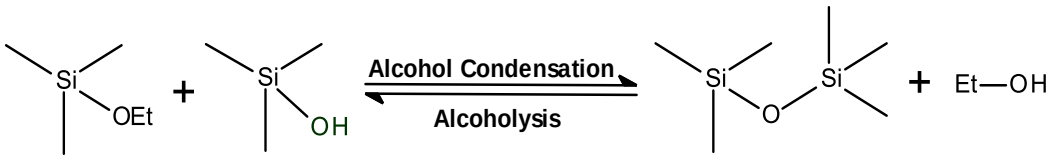

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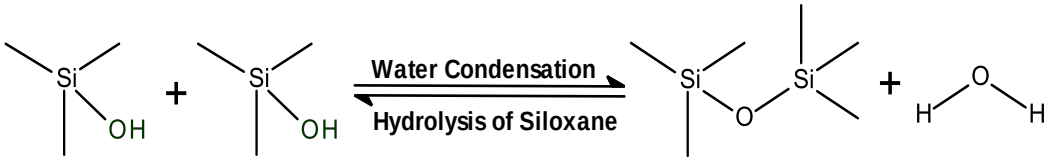

(3.3) [7]

After 5 minutes of stirring, 1.48 mL ammonia solution 25% was added to the mixture and stirred rigorously for 16 hours at room temperature. According to [8], condensation of TEOS to silica is optimum at basic condition of pH 9.The obtained Gd-citrate@SiO_2-NP colloid was then subjected for further characterizations.

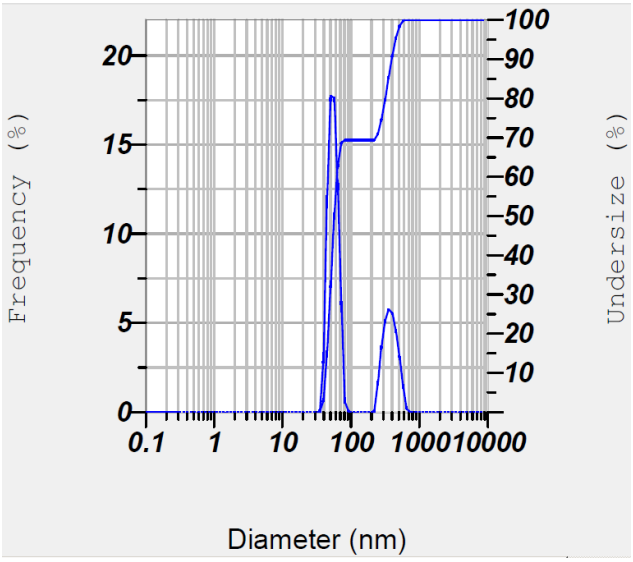

Figure 1: Particle Size Analysis of the obtained Gd-sitrat@SiO_2-NPs

Particle size analysis of the obtained Gd-citrate@SiO_2-NP colloid showed two peaks. The first peak is in a nano size range, whereas the second peak is in a micro size range (Fig. 1). The percentage of the first peak is 69% which is higher than that of the second peak (31%) (Table 1). Therefore, the obtained Gd-citrate@SiO_2-NP colloid was predominantly in nano size ranging from 42.6 to 82.3 nm, with a median and average of 57.1 and 79.3 nm, respectively. Moreover, particle size analysis revealed that Gd-citrate@SiO_2-NP colloid has a polydispersity index (PI) of 0.459. Such PI value indicates that the obtained Gd-citrate@SiO_2-NP colloid has a low tendency to flocculate, becoming a sediment.

Table 1: Particle size analysis results of the synthesized Gd-citrate@SiO_2-NPs

Histogram Operations	
Size (median)	: 57.1 nm
Mode	: 48.5 nm
% Cumulative (2)	: 10.0 (%) – 42.6 nm
% Cumulative (6)	: 50.0 (%) – 57.1 nm
% Cumulative (8)	: 69.0 (%) – 82.3 nm
% Cumulative (10)	: 100.0 (%) – 580.4 nm
Cumulant Operations	
Z-Average	: 79.3 nm
PI	: 0.459

Encapsulation efficiency of Gd-citrate

Gd-citrate complexes potentially release free Gd^{3+} ions which are toxic to human body. Therefore, concentration of Gd-citrate complexes which were not encapsulated by silica nanoparticles should be determined. In this study, such Gd-citrate complexes were analyzed using a visible spectrophotometry method using ARS. The pH of both standard solutions and Gd-citrate@SiO_2-NP sample was adjusted to 4, since at this condition ARS form a stable complex with Gd^{3+}. From measurement, ARS and Gd-ARS have very different λ_{max} values of198 and 527 nm respectively, which mean excess ARS not interfering the response of Gd-ARS.

Calibration curve for analysis of free Gd-citrate complexes was depicted in Fig. 2. The calibration curve has a good quality with determination coefficient (R^2) of 0.991. For method verification, we also determined limit of detection (LoD) and quantification (LoQ) from the calibration curve based on residual standard deviation of the response andthe slope [5]. The respective LoD and LoQ for the curve were 3.0 and 8.0 ppm, which mean the method could not measure concentration of Gd-citrate bellow 8.0 ppm. Extrapolation of sample response to the calibration curve revealed that 25 ppm of Gd(III) remains outside nanoparticles which can be removed using size exclusion or ion exchange chromatography.

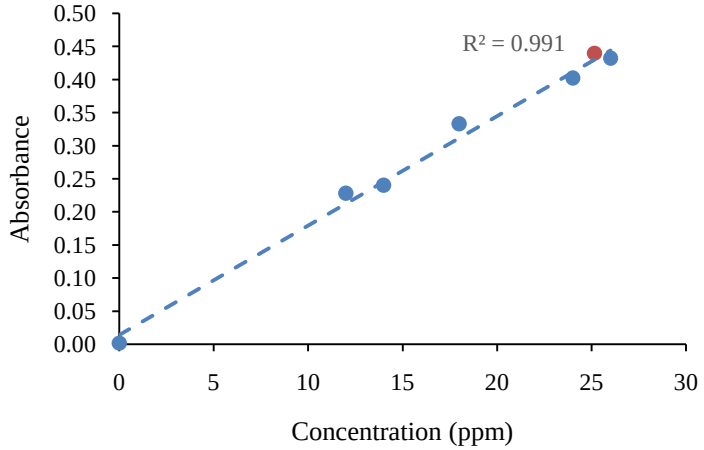

Figure 2: Calibration curve for the determination of Gd-citrate complexes which were not encapsulated by silica nanoparticles. Blue-dashed line (---) denotes the calibration curve, whereas blue (●) and orange (●) dots indicate data points of Gd-ARS standard solution and sample. Measurement was performed at 527 nm

Since we knew concentration of Gd-citrate remains outside nanoparticles, level of Gd-citrate inside silica can be determined as written in Method section (Encapsulation efficiency). The level of Gd(III) trapped is 75.24%. This

shows that encapsulation of Gd-citrate using sol-gel Stöber method is potential to generate a MRI gadolinium-based contrast agent.

Conclusions

We have synthesized Gd-citrate@SiO_2-NP, from Gd-citrate solution and TEOS through sol-gel Stöber method, with average nanoparticle size of 79.3 nm. The obtained Gd-citrate@SiO_2-NPs make 69.0% of the total formed silica. Meanwhile, encapsulation efficiency in the synthesis of Gd-citrate@SiO_2-NPs is 75.24 %. Our results are valuable for further development of Gd-citrate@SiO_2-NPs as an MRI contrast agent. The yield of Gd-citrate@SiO_2-NPs and encapsulation efficiency can be improved in further studies, using response surface method of Boz-Behnken or central composite design.

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