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One-step synthesis of platinum nanoparticles loaded in alginate bubbles

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Abstract

Composite particles with multifunctions have been extensively utilized for various applications. Bubble particles can be applied for ultrasound-mediated imaging, drug delivery, absorbers, cell culture, etc. This study proposes a one-step strategy to obtain Pt nanoparticles loaded in alginate bubbles. A needle-based droplet formation was used to generate uniform alginate particles about 2 mm in diameter. The hydrolysis reaction of NaBH4 was utilized to produce gaseous hydrogen and then trapped within alginate particles to form bubbles. The Pt4+ mixed with alginate solution was dropped into the reservoir to react with reducing NaBH4 and hardening CaCl2 to form Pt nanoparticles-alginate composite bubbles. Results indicate that the size of bubbles decreases with the CaCl2 concentration (1% ~ 20%), and size of bubbles increases with the NaBH4 concentration (1 ~ 20 mM). The advantages for the present approach include low cost, easy operation, and effective production of Pt nanoparticles-alginate composite bubbles.

Keywords: Platinum; Bubbles; Alginate; Nanoparticle

Background

The metal nanoparticles (NPs) are powerful products of nanotechnology, providing broad variety of applications in life science [1,2]. For example, drug delivery, cellular imaging, and biosensing have been extensively described [3-6]. The chemical versatility of metal NPs holds the potential to outclass in a number of applications [2]. These unique properties and applications of metal NPs are well reviewed [7-9]. Platinum is used in various applications such as catalysts in many organic reactions [10,11], preparation of organic dyes [12], and biomedical applications [13,14]. For example, the Pt NPs were employed for successful photothermal treatment of Neuro 2A cancer cell by using irradiation with 1,064 nm near-infrared pulse wave and the Nd YAG laser set at 3 W for 480 s. The Pt NPs increased 9°C in temperature leading to effective photothermal killing of cancer cells [15].

The Pt composite materials have gained much attention due to their good multifunctions [16,17]. Pt NPs-chitosan composite particles have been extensively studied over the last decade [18,19], and Pt NPs-chitosan composite bubbles are one of the most emerging and intriguing topics [20,21]. Bubble particles have import features entrapping air bubbles inside. Due to their low density, bubble particles can float on liquid surface for specific applications. They can also be applied as novel vehicles for ultrasound-mediated imaging and targeted drug delivery followed by burst release [22-27]. Besides, bubble particles can be utilized as absorbers to facilitate adsorption of substrates due to a high-surface area. Pt NPs-chitosan composite bubbles can be applied in controlled release and tissue engineering; however, chitosan carrier substrates will disintegrate and dissolve in acid solution such as gastric juices. Therefore, Pt NPs-chitosan composite bubbles are limited in acidic condition. Fortunately, alginate polymer provides a solution to overcome this problem. Alginate polymer has a dense structure to pass the acid solution. To our best knowledge, Pt NPs-chitosan composite bubbles are seldom reported in literatures, and they can provide applications for wide pH ranges.

By extending our previous works to prepare uniform alginate particles [28-31] and alginate bubbles [32], this work further develops a novel one-step method to fabricate composite Pt NPs@alginate bubbles through a simple chemical reaction. The Pt NPs and bubbles...
within alginate particles are investigated and characterized. The manufactured alginate products will provide great promise for multifunctional applications.

Methods
Materials
Algic acid sodium salt (Na-alginate, brown algae with viscosities 150 cp and 350 cp in 2% (w/v) solution at 25°C) and dihydrogen hexachloroplatinate (IV) hexahydrate, ACS, Premion, 99.95% were obtained from Alfa Aesar (Johnson Matthey Company, London, UK). Sodium borohydride (NaBH$_4$) was purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA), and calcium chloride (CaCl$_2$) was obtained from J.T. Baker (J.T. Baker Chemical Company, Phillipsburg, NJ, USA). All chemicals and solvents were of analytical reagent grade.

Mechanism of bubbles formation
The gas source is from the hydrolysis of NaBH$_4$ as following reaction [33]:

NaBH$_4$ + 2 H$_2$O $\rightarrow$ NaBO$_2$ + 4H$_2$.

The NaBH$_4$ hydrolysis is spontaneous, and gaseous H$_2$ generation continues with the hydrolysis reaction. Due
to the density difference, generated H\textsubscript{2} bubbles move upwards in the reservoir solution. After a dropwise addition of Na-alginate solution into the reservoir, gas bubbles were entrapped within alginate particles to be alginate bubbles. One alginate particle can hold many numbers of bubbles by random. After 30 min, alginate bubbles were collected by filter, washed twice with 30 mL dd-H\textsubscript{2}O, and finally collected and characterized.

Preparation of Pt NPs@alginate bubbles

Na-alginate (0.08 g dissolved in 2 mL of deionized water) and 2 mM platinum salt solution from dihydrogen hexachloroplatinate hexahydrate were mixed homogenously to be Pt\textsuperscript{4+} mixed Na-alginate (Pt\textsuperscript{4+}-Na-alginate) solution. As shown in Figure 1, Pt\textsuperscript{4+}-Na-alginate solution loaded in the syringe (TERUMO\textsuperscript{®} syringe, 3 mL; Terumo, Tokyo, Japan) was extruded from the needle tip by a KDS230 syringe

Figure 3 Alginate bubbles with different NaBH\textsubscript{4} concentrations. (A and E) 1 mM NaBH\textsubscript{4}; (B and F) 5 mM NaBH\textsubscript{4}; (C and G) 10 mM NaBH\textsubscript{4}; (D and H) 20 mM NaBH\textsubscript{4}. Alginate in (A to D) and (E to H) are 150 and 350 cp, respectively. All scale bars are 2 mm.

Figure 4 SEM images of alginate bubbles with different NaBH\textsubscript{4} concentrations. Surface (A to C) and cross-section (D to F). (A and D) 0 mM NaBH\textsubscript{4}; (B and E) 10 mM NaBH\textsubscript{4}; (C and F) 20 mM NaBH\textsubscript{4}. 
pump (KD Scientific Inc., Holliston, MA, USA). Under a constant injection rate, Pt⁴⁺-Na-alginate solution was broken up to form a series of isolatable Pt⁴⁺-Na-alginate droplets at the tip of the needle. The liquid in the receiving collector was filled with CaCl₂ and NaBH₄ for crosslinking alginate (by CaCl₂) and generating Pt NPs (by NaBH₄) and bubbles (by NaBH₄), respectively. Pt⁴⁺-Na-alginate droplets are gelled in situ to be Ca-alginate particles when contacting Ca²⁺ ions. The NaBH₄ plays a dual functional. One is a reducing agent to reduce Pt⁴⁺ to be Pt NPs by a chemical reduction reaction. The other one is gaseous H₂ generation by a hydrolysis reaction. When the Pt⁴⁺-Na-alginate droplets immersed in the receiving collector, the Pt NPs@alginate bubbles are generated.

**Characterization**

An optical microscope system (TE2000U, Nikon, Lewisville, TX, USA) and a USB digital microscope (UPG621, UPMOST Technology Corp., Taipei, Taiwan) were utilized to observe the morphology of the collected particles. To minimize selection bias, a total of more than 50 individual particles were analyzed to ensure statistical representation. X-ray diffraction (XRD, D2 Phaser, Bruker AXS Gmbh, Germany) patterns were obtained at room temperature by using Cu K-α radiation (30 kV/10 mA) with a range of 2θ = 20° ~ 80°, and a scanning rate of 4° min⁻¹. Laser Raman spectroscopy was obtained using a Renishaw Microscope Raman Spectrometer (Renishaw plc., Gloucestershire, UK) from 200 to 1,100 cm⁻¹ at room temperature. The 785-nm line of the laser was used as the excitation source, with the capability of supplying 300 mW. The morphology of the particle composites was analyzed using a scanning electron microscope (SEM, S-3400, Hitachi Ltd, Tokyo, Japan) and

**Figure 5** TEM images and the electron diffraction pattern of Pt nanoparticles. (A-C). TEM images of Pt nanoparticles with different magnifications. (D) Electron diffraction pattern of Pt nanoparticles.

**Figure 6** XRD patterns of Pt@alginate particles prepared from different alginate.

![Figure 6](https://example.com/figure6.png)
a transmission electron microscope (TEM, FEI Tecnai G2 20 S-Twin; FEI Company, Hillsboro, OR, USA) equipped with a METEK (PV 97–56700 ME) X-ray energy dispersive spectrometer (METEK Meteorologische Messtechnik GmbH, Elmshorn, Germany).

Cell viability test
The viability of the control and the treated cells were evaluated using 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay with human breast adenocarcinoma MCF-7 cells (1 × 10^4/well) seeded in a 96-well microtiter plate with a 100 μL culture medium treated with various amounts of Pt NPs@alginate bubbles. After 1 day exposure, a 200-μL MTT solution was added to react with the cells for 4 h. After removal of the medium, 100 μL DMSO was added and examined at 595 nm using a microplate reader (Multiskan Ascent, Thermo Electron Corporation, Vantaa, Finland). The control group in the untreated well was considered to be 100%.

Figure 7 Raman patterns of different Pt compounds.

Figure 8 Cytotoxicity induced by Pt@alginate bubbles on MCF-7 cells. Alginate is 150 cp (A and B) and 350 cp (C and D). The concentrations of CaCl₂ are 10% (A and C) and 20% (B and D).
Table 1 Particle morphology of chitosan and alginate immersed in different solutions

| Material | Solution          | Immersion time (hour) | 0     | 0.5   | 1     | 2     |
|----------|-------------------|-----------------------|-------|-------|-------|-------|
| Chitosan | Gastric juice (pH 1.2) |                      | ![](image1) | ![](image2) | ![](image3) | ![](image4) |
|          | PBS (pH 7.81)     |                      | ![](image5) | ![](image6) | ![](image7) | ![](image8) |
|          | Intestinal juice (pH 9.02) |                 | ![](image9) | ![](image10) | ![](image11) | ![](image12) |
| Alginate | Gastric juice (pH 1.2) |                      | ![](image13) | ![](image14) | ![](image15) | ![](image16) |
|          | PBS (pH 7.81)     |                      | ![](image17) | ![](image18) | ![](image19) | ![](image20) |
|          | Intestinal juice (pH 9.02) |                 | ![](image21) | ![](image22) | ![](image23) | ![](image24) |
Results and discussion

Pt NPs@alginate bubbles
Alginate is a kind of polysaccharide from marine brown algae. A variety of fundamental properties such as excellent biodegradability and biocompatibility make alginate a very attractive material for applications. Alginate has been applied in diverse areas [34-36] including serving biomedical materials for drug delivery and tissue engineering, and being adsorbent materials for elimination of heavy metals or organic pollutants [37]. Due to acid dissolution, conventional Pt NPs@chitosan bubbles have constraint applications for limited pH conditions. Therefore, it is needed to develop Pt NPs@alginate bubbles for wide pH applications.

Figure 2 shows the effects of CaCl₂ concentration on Pt NPs@alginate bubbles. Results indicate that the size of the bubbles decreases with the CaCl₂ concentration. The difference between the two alginate materials with distinct viscosities was not significant. The size of bubbles reaches 1 mm at 1% CaCl₂, but only 0.4 mm at 20% CaCl₂. The reason may be attributed to a lower crosslinking rate of alginate in a low CaCl₂ concentration. The alginate pregel allows entrapped small bubbles merging into lager bubbles before gel network (solidification) formation in a low CaCl₂ concentration.

Table 1 Particle morphology of chitosan and alginate immersed in different solutions (Continued)

| Pt@alginate bubbles | Gastric juice (pH 1.2) | PBS (pH 7.81) | Intestinal juice (pH 9.02) |
|---------------------|------------------------|---------------|--------------------------|

Figure 3 shows the effects of NaBH₄ concentration on Pt NPs@alginate bubbles. The results indicate that the number of bubbles within an alginate particle increases with NaBH₄ concentration, but there is no significant difference between two alginate materials. There are no obvious bubbles in the low 1 mM NaBH₄ due to the little amount of entrapped gas. Furthermore, the bubbles are well dispersed within the particle, but their size is not significantly varied with different NaBH₄ concentrations. This phenomenon resulted from the high viscous alginate matrix to retard the fusion of bubbles.

Reduction reaction of Pt salts by reducing agents such as borohydrides and citrates is one of the convenient methods to prepare Pt NPs [38]. This study demonstrates a proof-of-concept approach for encapsulating the Pt NPs and bubbles into alginate particles utilizing simple reduction and hydrolysis reactions. Produced Pt NPs@alginate bubbles combined the characteristics of Pt NPs and bubbles. The composite bubble particles can provide wide applications, such as smart vehicles for ultrasound-mediated imaging and targeted drug delivery, and effective absorbers and catalysts for decomposing pollutants. In the future, this proposed strategy to formulate Pt NPs@alginate bubbles can also be applied for synthesis of other composite materials.
Characterization
Figure 4 shows SEM images of Pt NPs@alginate bubbles. The exterior and interior morphologies of alginate particles obtained from different NaBH₄ concentration are compared. In absence of NaBH₄ there is no bubbles formation and the morphology is smooth and intact. For 10 and 20 mM NaBH₄ ridges and cavities are found at particle surface and interior, showing entrapped bubbles.

The TEM images shown in Figure 5 with different magnifications reveal that synthesized Pt NPs were nearly spherical and well dispersed in the Ca-alginate particle. The electron diffraction pattern of Pt NPs were indexed as (111), (220), and (222), indicating the polycrystalline characteristic. Figure 6 shows the XRD pattern of synthesized Pt NPs. Four distinct peaks at 39.6, 46.1, and 67.9 correspond to the crystal planes (111), (200), and (220) of cubic Pt NP structure, respectively. This result agrees with the finding in the electron diffraction data. Figure 7 is the Raman spectrum of different Pt substrates. There are different Raman patterns for Pt⁴⁺ and Pt. Compared to nonionic Pt, ionic Pt⁴⁺ shows more splits between 300 cm⁻¹ and 350 cm⁻¹. The Raman pattern of Pt NPs agrees with Pt NPs@alginate bubbles, and Pt⁴⁺ is consistent with Pt⁴⁺@alginate solution.

Cytotoxicity
Figure 8 shows the cytotoxicity test of Pt NPs@alginate bubbles. The MCF-7 cells were used to test the cytotoxicity. Four kinds of alginate particles varying from alginate viscosity and CaCl₂ concentration were tested. After a 24-h exposure to alginate particles ranging from 5 to 1,000 μg/mL, the cell viability was assayed. Results show that there was no significant difference among the control (without adding alginate particles) and the samples. Furthermore, the differences among the four kinds of alginate particles were rather indistinguishable. These results ensure the low cytotoxicity of prepared particles on the MCF-7 cells. Therefore, Pt NPs@alginate bubbles obtained in this study can be safely applied for biomedical applications in the future, such as the scaffold for cartilage tissue engineering [39].

Particle morphology
Table 1 shows the particle morphology of chitosan and alginate materials in different pH conditions. The three particles, chitosan, alginate, and NPs@alginate bubbles, were compared along the immersion time. The results indicate that chitosan particles disintegrated in acid solution after 1 h immersion but the alginate material still had an entire particle shape. Although alginate displayed swelling in alkaline solution, the particles still remained. Therefore, NPs@alginate bubbles can provide more applications for wide pH ranges than conventional NPs@chitosan bubbles.

Conclusions
This paper developed a facile method to synthesize platinum nanoparticles within alginate bubbles. Sodium borohydride was utilized to generate platinum NPs and gaseous hydrogen by reduction reaction and hydrolysis reaction, respectively. Bubbles entrapped within around 2-mm alginate particles increased with the borohydride concentration and alginate viscosity. This proposed one-step method to prepare Pt NPs@alginate bubbles has advantages of low cost, easy operation, and effective pore formation. Compared with conventional Pt NPs@chitosan bubbles, Pt NPs@alginate bubbles provide more applications for wide pH ranges.

Competing interests
The authors declare that they have no competing interest.

Authors’ contributions
CHY designed the study. WTW performed the entire search. AMG contributed to the discussion of the results. KSH and YSL wrote the manuscript and made the same contribution. All authors read and approved the final manuscript.

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