Synthesis of monodisperse spherical AgNPs by ultrasound-intensified Lee-Meisel method, and quick evaluation via machine learning

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A B S T R A C T

Due to the high reactivity of Ag+ and uncontrolled growth process, the AgNPs produced by conventional Lee-Meisel method always exhibited larger particle size (30–200 nm) and polydispersity morphology (including spherical, triangular, and rod-like shape). An ultrasound-intensified Lee-Meisel (UILM) method is developed in this study to environmental-friendly and controllable synthesize monodisperse spherical AgNPs (∼3.7 nm). Effects of Ag: citrate ratio (1:3 or 5:4), ultrasound power (300 to 1200 W) and reaction time (4 to 24 min) on the physical–chemical properties of AgNPs are investigated systematically. The transmission electron microscope (TEM) images, UV–Vis spectra, average particle size, zeta potential and pH value all demonstrate that crystallization and digestive ripening processes are facilitated in the presence of ultrasound irradiation. Therefore, both chemical reaction rate and mass transfer rate are enhanced to accelerate primary nucleation and inhibit uncontrolled particle growth, leading to the formation of monodisperse spherical AgNPs. Moreover, a machine learning approach - Decision Tree Regressor in conjunction with Shapley value analysis reveal the concentration of reactants is a more important feature affecting the particle.

1. Introduction

Silver nanoparticles (AgNPs) have been recognized as promising drug delivery agents, catalyst, and nanometallo-antibiotics, because of their unique catalytic, optical, electronic and wide spectrum antimicrobial effects [1,2]. Lee-Meisel method is one of the widely used approaches to synthesize AgNPs by using biocompatible trisodium citrate (Na3CA) as the reductant and stabilizer, and the thin capping citrate layer could be further replaced by other functional moieties to improve the AgNPs performance [3–5]. However, due to the high reactivity of Ag+ and uncontrolled growth process in water at high temperatures, the AgNPs obtained by the conventional Lee-Meisel method exhibited large particle size (30–200 nm) and diverse morphologies (including sphere, triangle, rod-like shapes) as well as the broad polydispersity [6,7]. It is reported that the antimicrobial efficiency of AgNPs was highly inhibited by the larger particle sizes [8]. Besides, as silver nanoplasts exhibited higher toxicity than silver nanospheres, polydisperse morphology also increases ecotoxicity of AgNPs [9]. Therefore, it is necessary to produce monodisperse spherical AgNPs with small size to improve antibacterial activity and reduce ecotoxicity.

Over the last few decades, different research groups have modified Lee-Meisel method to prepare AgNPs with smaller particle size and monodispersity morphology. To facilitate nucleation and inhibit uncontrolled particle growth processes, most of the studies employed large amount of toxic reductant (such as sodium borohydride, formaldehyde, acetaldehyde, hydroquinone etc.) instead of trisodium citrate. Patil et al. prepared AgNPs (3–7 nm) by using excess amount of sodium borohydride (NaBH4) as reductant, and intensifying AgNPs synthesis process in microreactor [10]. Similarly, Baber et al. reduced AgNO3 by NaBH4 within a coaxial flow reactor. The size of AgNPs could be controlled from 3.1 ± 1.6 nm to 9.3 ± 3.0 nm through adjusting flow rate, surfactant concentration and precursor concentration [11]. Agnihotri et al. precisely controlled nucleation and growth kinetics during the synthesis process to produced AgNPs ranging from 5 to 100 nm. NaBH4 and Na3CA
were regarded as primary and secondary reductant, respectively [12]. Wu et al. designed a helical microreactor to continuously synthesize AgNPs with tuneable size (4.6 to 10.63 nm). Excess amount of NaBH₄ was used in study, the molar ratio between NaBH₄ and AgNO₃ was 6:1 [13]. However, according to U.S. National Library of Medicine and previous study, sodium borohydride (NaBH₄) has been characterized as irritant, flammable, acute toxic and health hazard [14]. Dissolving NaBH₄ in water can produce sodium hydroxide and hydrogen, which are corrosive material and flammable gas, respectively. Both NaBH₄ and hydrogen gas are easily ignited by the heat generated from this reaction. Although the using of toxic reductants contribute to the rapid generation of small and monodispersd AgNPs, toxic raw materials and residues will pose potential risks for operators and environment.

Ultrasonic-irradiation intensified synthesis method is a promising technology which can effectively enhance turbulence intensity and intensify micromixing in solution so as to achieve stronger turbulent shear stresses [15–17]. Such ultrasonic intensification can be applied in industrial manufacturing process and material synthesis because of its fast, cost-effective and environmental-friendly features [18,19]. Ultrasound-intensified crystallization, or sonocrystallization, is able to improve crystal purity and product homogeneity, shorten crystallization time and enhance process stability [20,21]. When the liquid is irradiated by ultrasound, ultrasonic wave can induce acoustic cavitation and generate a large number of cavitation micro-bubbles. Instantaneous collapse of the cavitation micro-bubbles can create extremely high pressure (up to 10¹⁵ K s⁻¹) in the cavitation zone, leading to the chemical and physical changes of the solution [22]. Furthermore, the enhanced turbulence intensity, micromixing level, as well as turbulent shear stresses play more important role in transferring energy to chemical bonds and driving chemical reactions during manufacturing process, and influence the physical–chemical properties and yield of as-synthesized products [23]. Recently, it has been demonstrated that strong local shear stress generated by turbulence can promote the yield of platelet to enable clinical scale manufacturing process, and influence the physical
central properties of nanocomposites and nanomedicine are also influenced dramatically by the competition between fluid dynamics and self-assembly performance [25–27]. With the help of ultrasound irradiation, Ag⁺ could be reduced to AgNPs (around 8 nm) without using of any reductant and surfactant [28].

Hence, an ultrasound-intensified Lee-Meisel method is developed in this study to produce monodisperse spherical AgNPs with small particle size. The effects of Ag-citrate ratio, ultrasound intensity and reaction time on AgNPs formation are systematically illustrated. In the presence of ultrasound irradiation, turbulence intensity, micromixing level, and turbulent shear stress are all improved, leading to the enhancement of crystallization and digestive ripening processes. To support our proof of concept, the underlying AgNPs formation mechanism is further explored, and find that ultrasound irradiation can facilitate primary nucleation during sonocrystallization and inhibit uncontrolled particle growth during ultrasound-intensified digestive ripening process. Moreover, a machine learning approach is applied to develop a model to investigate the relationship and importance of initial reactant concentration and ultrasound intensity with the size of AgNPs.

2. Experimental section

2.1. Synthesis of AgNPs

In this work, chemicals were supplied from Sinopharm Chemical Reagent Co., Ltd. Silver nitrate (AgNO₃, 99%) and sodium citrate (Na₃CA, 99%) were dissolved into DI water to prepare AgNO₃ (5 mM) and Na₃CA (500 mM) aqueous solutions.

In the first step, AgNPs were prepared by three methods to investigate the influences of sonocrystallization and ultrasound-intensified digestive ripening. The first type of AgNPs (AgNPs-UILM) was synthesized by ultrasound-intensified Lee-Meisel method. The schematic diagram is shown in Fig. 1 to describe this synthesis process. Briefly, 800 mL of DI water was placed into water tank and heated to boiling (99.8 ± 0.4 °C). The water tank was covered with preservative film and polyethylene film to prevent the loss of water and heat. Then AgNO₃ aqueous solution (5 mM, 40 mL) was stirred (300 rpm) in a 100 mL conical flask which was covered by aluminum foil and heated to boiling in water bath. Subsequently, Na₃CA aqueous solution (500 mM, 0.32 mL) was injected into the boiling AgNO₃ aqueous solution rapidly. Thus, the molar ratio between AgNO₃ and Na₃CA is 5:4. Meanwhile, the mixed solution was irradiated by a FS-1500 T ultrasonic wave piezoelectric vibrator (0–1500 W, 13 mm in diameter). The ultrasound resonance frequency was maintained at 20 kHz, while the ultrasound intensity was precisely controlled at different level (including 300, 600, 900 and 1200 W). The value of temperature in water tank and conical flask was recorded every 2 min by thermometer. After 24 min, the solution was transferred into a centrifuge tube (50 mL) covered with aluminum foil, and cooled down in cold water with ice. After that, the obtained sample was kept at 4 °C in fridge. The second and third type of AgNPs (AgNPS-LMDR and AgNPS-LM) was prepared according to the conventional Lee-Meisel method based on previous research [3]. AgNO₃ solution (5 mM, 40 mL) was stirred with a magnetic Teflon-coated bar (300 rpm), and heated to 100 °C. Then Na₃CA aqueous solution (500 mM, 0.32 mL for AgNPS-LMDR and 1.2 mL for AgNPS-LM) was injected into AgNO₃ solution and reacted for 24 mins. The difference between AgNPS-LMDR and AgNPS-LM is that the molar ratio between AgNO₃ and Na₃CA is 5:4 for AgNPS-LMDR sample, but the molar ratio between AgNO₃ and Na₃CA is 1:3 during the synthesis of AgNPS-LM.

After demonstrating the influences of sonocrystallization and ultrasound-intensified digestive ripening, the effect of reaction time was further investigated to reveal how ultrasound irradiation affected the formation progresses of AgNPs. Moreover, to investigate the relationship among initial reactant concentration, ultrasound intensity and the size of AgNPs, a machine learning approach was applied to develop a model for the problem studied. The conditions of experiments applied with different initial reactant concentration and ultrasound intensity are shown in Table S1.

As changing of the solution volume and location of ultrasound horn may affect ultrasound power inside the conical flask, the power of ultrasound inside the conical flask was further evaluated. The experimental setup used to measure the power of ultrasound is shown in Figure S1a. The distance between ultrasound horn and the side of conical flask was kept at 1.0 cm. Besides, the volume of water in conical flask and water tank was 40 mL and 800 mL, which was the same with AgNPs synthesis. The water in conical flask and water bath was maintained at 25 °C by magnetic stirrer in the beginning, then irradiated by ultrasonic wave piezoelectric vibrator with different power (300 W, 600 W, 900 W and 1200 W). The value of temperature in water bath and conical flask was recorded every 2 min by thermometer. The
temperature increased as a function of time along the reaction process is shown in Figure S1b, and the power of ultrasound dissipated inside the conical flask was calculated by the following equation [29]:

\[ P = \frac{mC_p\Delta T}{dt} \]

where \( m \) is the mass of water (0.04 kg in conical flask and 0.8 kg in water tank), \( C_p \) is the specific heat capacity of water (4.2 kJ/kg-K), \( \Delta T \) is the temperature change per second.

The applied ultrasound power, temperature difference (\( \Delta T \)) after ultrasound irradiation for 24 min, and calculated calorimetric power are shown in Table S2. It is clear to see that the calculated calorimetric power in conical flask is much lower than applied ultrasound power. Most of ultrasound power might be transferred to kinetic energy and sound.

2.2. Characterisation

The morphology of the AgNPs was measured by transmission electron microscopy (TEM, FEI Tecnai F20, U.S.). The particle size distribution and zeta potential of the AgNPs were determined by dynamic light scattering (DLS, Anton Paar Litesizer 500, Austria). The ultra-violet–visible spectra was performed on Agilent Cary 60 spectrophotometer. The pH value of synthesized AgNPs samples was measured by a pH-meter (Metler Toledo FiveEasy Plus, Switzerland).

2.3. Antibacterial activity of synthesized AgNPs

To study the antibacterial activity of the materials against Gram-negative Escherichia coli (E. coli, ATCC 25922) and Gram-positive Staphylococcus aureus (S. aureus, ATCC 25923), the culture dishes and medium were sterilized before antibacterial test. The monoculture bacteria was cultured in 100 mL of Luria-Bertani (LB) (yeast extract 5 g, tryptone 10 g, tryptone 10 g L\(^{-1}\), and sodium chloride 10 g L\(^{-1}\)) and shaken at 37°C/200 rpm for 12 h. After that, the bacteria was centrifuged and washed three times with PBS (pH 7.4) and further diluted to about 10\(^3\) CFU mL\(^{-1}\) with normal saline (0.9% NaCl solution). The bacterial concentration was estimated with OD600 = 0.1. Next, the synthesized AgNPs suspension (5 mM) was added to 5 mL bacterial suspension and incubated in a shaker at 37°C for 12 h. The treated bacterial solution was diluted 10\(^3\) times with normal saline. Finally, 50 µL of bacterial diluent was evenly coated on LB agar plate, and the number of CFUs was calculated after incubation for 20 h at 37°C.

2.4. Photothermal effect of synthesized AgNPs

The photothermal effect of synthesized AgNPs samples was evaluated by a fiber-coupled diode laser system (PSU-H-LED, MLD-III-808–5 W, China). The AgNPs samples (100 µg/mL, 1.0 mL) synthesized by conventional Lee-Meisel method, and ultrasound-intensified Lee-Meisel method were firstly prepared with DI water and stored in centrifuge tube (5.0 mL). Besides, DI water (1.0 mL) was regarded as a control. Then these samples were irradiated by an 808 nm laser (2.0 W cm\(^{-2}\)) for a period of 10 min. The value of temperature was recorded every 30 s with a thermal imaging camera (FLIR-E64501, FLIR System, Estonia).

2.5. Machine learning

A machine learning approach was applied in this work to evaluate the relationship among initial reactant concentration, particle size and ultrasound intensity. The features were analyzed using the Decision Tree Regressor model in scikit-learn (v0.19.1) in Python (v3.7). The data were split randomly into training and testing sets with 70% in the training set and 30% in the testing set. This process was simulated 10 times and the Mean Absolute Error (MAE) performance metric was recorded. While the model was being simulated, we captured the Sharply value [30] as feature importance.

3. Results and discussion

3.1. Effect of ultrasound irradiation on the preparation of AgNPs

The effect of ultrasound irradiation on crystallization and digestive ripening processes, as well as physico-chemical properties of AgNPs were investigated systematically when reaction time and initial Ag\(^+\) concentration were maintained at 24 min and 5 mM, respectively. Besides, the effect of ultrasound intensity on the size and size distribution of AgNPs was investigated systematically when keeping the molar ratio of AgNO\(_3\) : sodium citrate at 5:4, and varying the ultrasound intensity from 300 to 1200 W. The morphology, particle size distribution, and color of AgNPs suspension prepared by conventional Lee-Meisel method (AgNPs-LM), Lee-Meisel method with digestive ripening (AgNPs-LMDR), and ultrasound-intensified Lee-Meisel method (AgNPs-UILM) were shown in Fig. 2. According to the TEM image (Fig. 2a), the AgNPs-LM particles were highly agglomerated, and exhibited polydisperse morphology (including spherical, rod, and triangular shape) with larger particle size and broader distribution (105.9 ± 30 nm). This might be caused by slow nucleation, unexpected secondary nucleation, and uncontrolled particle growth, which were the main drawbacks of conventional Lee-Meisel method. When the experiment condition was silent, and changing the molar ratio between AgNO\(_3\) and Na\(_2\)Citrate from 1:3 to 5:4, the AgNPs with rod and triangular shape cannot be observed from TEM images (Fig. 2b). The AgNPs-LMDR showed near-spherical shape with larger average particles size (14.5 nm) and broader particle size distribution (ranging from 4 to 25 nm), which was similar with previous report [31]. When applying ultrasound irradiation during the formation of AgNPs (Fig. 2c-f), it was clear to see as the increasing of ultrasound intensity, the size of AgNPs maintained a decreasing tendency in the beginning. A significant decrease of particle size (from 14.5 ± 10 nm to 3.7 ± 2 nm) could be obtained with the increasing of ultrasonic power from 0 W to 600 W. The smallest AgNPs with most uniform morphology was observed in Fig. 2d. However, further increasing ultrasound power from 600 W to 1200 W resulted in an increase of AgNPs size from 3.7 ± 2 nm to 25.2 ± 18 nm, indicated that keep increasing the ultrasound power might not always lead to the reduction of the average size of AgNPs (Fig. 2g). Besides, the color of AgNPs-LM suspension (Fig. 2h) was almost transparent even after 24 min reaction. In contrast, AgNPs-LMDR suspension exhibited brown color. Then the color of AgNPs suspension became to orange, light brown and blackish green as the changing of ultrasound intensity. Therefore, ultrasound intensity with 600 W could be considered as critical level of ultrasonic energy in this work. Lower amount of Ag\(^0\) nuclei was produced below this ultrasonic energy critical level due to the inhibition of sonocrystallization and ultrasound-intensified digestive ripening processes. On the contrary, both sonocrystallization and ultrasound-intensified digestive ripening might be enhanced if the ultrasound intensity used in the experiment was much higher than this critical level. However, the Ag\(^0\) nuclei and AgNPs were merged together at extremely high instantaneous temperature created by ultrasound irradiation, leading to the formation of larger AgNPs. Consequently, higher ultrasound power had negative effects on the synthesis of AgNPs.

According to TEM and PSD analysis, AgNPs-LM, AgNPs-LMDR and AgNPs-UILM-600 W samples were chosen to reveal the effects of sonocrystallization and ultrasound-intensified digestive ripening. Fig. 3 exhibited the differences among these three AgNPs samples in terms of UV–Vis spectra, zeta potential and UV–Vis spectra value. As shown in Fig. 3a, four peaks around 350 nm (Peak A), 380 nm (Peak B), 420 nm (Peak C) and 490 nm (Peak D) could be observed in the UV–Vis spectra of AgNPs-LM. The maximum absorbance intensity at Peak B was 0.83, while the full-width at half-maximum (FWHM) of AgNPs-LM was around 258 nm. The lowest maximum absorbance intensity and the highest FWHM value implied that AgNPs prepared via conventional Lee-Meisel method had polydisperse morphology and larger particle size [32]. In contrast, the maximum absorbance intensities of AgNPs-LMDR and AgNPs-UILM-600 W samples were chosen to reveal the effects of sonocrystallization and ultrasound-intensified digestive ripening.
W were much higher than those of AgNPs-LM, which were 2.48 and 2.39, respectively. It was important to notice that two peaks around 350 nm and 460 nm were shown in the UV–Vis spectra of AgNPs-LMDR, but there was only one peak around 350 nm in the UV–Vis spectra of AgNPs-UILM-600 W. In addition, the FWHM value of AgNPs-LMDR and AgNPs-UILM-600 W was 193 nm and 86 nm. This result indicated that reducing the ratio between Ag ion and citrate ion contributed to improve the monodisperse of AgNPs. Applying ultrasound irradiation could further reduce particle size and narrow particle size distribution of AgNPs.

Regarding the zeta potential, all AgNPs suspensions possessed negative surface charge (Fig. 3b). The AgNPs-LM sample exhibited the highest negative zeta potential with the broadest distribution (−23.6 ± 19.0 mV). In comparison, the zeta potential of AgNPs-LMDR and AgNPs-UILM-600 W was −39.0 ± 6.2 mV and −47.3 ± 2.3 mV, respectively. As expected by theory, the value of zeta potential could influence dispersion and aggregation processes, and determined the stability of nanoparticles and colloids in solution [33]. Nanoparticles tended to be more stable as the increasing of zeta potential absolute value. Therefore, AgNPs-LM sample was highly agglomerated. Both UV–Vis spectra and zeta potential predictions were matched well with TEM and PSD observation (Fig. 2). The pH value of three AgNPs suspension were tested and shown in Fig. 3c, which was 6.38 ± 0.03 (AgNPs-UILM-600 W), 6.61 ± 0.07 (AgNPs-LMDR), and 6.72 ± 0.08 (AgNPs-LM), respectively. The difference of pH value is explained in Section 3.3.

3.2. Effect of reaction time on the preparation of AgNPs

To investigate the effect of reaction time and explain the formation mechanism of AgNPs prepared by different methods, the experiments were conducted by varying reaction time from 4 to 24 min. The
corresponding UV–Vis spectra, particle size distribution and photographs reveal the difference of as-synthesized AgNPs dispersed in aqueous solution (Fig. 4). Besides, as the experiments were repeated for three times, the average FWHM value and particle size of AgNPs prepared with different reaction time were shown in Fig. 4g and 4 h respectively. Fig. 4a exhibited the UV–Vis spectra of AgNPs-LM from 4 to 24 min. As mentioned before, there were four peaks around 350 nm (Peak A), 380 nm (Peak B), 420 nm (Peak C) and 490 nm (Peak D) in the UV–Vis spectra of AgNPs-LM. The intensities of these peaks all improved as increasing of reaction time. The extinction peak at 477 nm was maximized after reaction for 20 min. After that, the peak absorbance of spectra at 477 nm decreased from 1.48 to 0.88 a.u. during 20 to 24 min. Meanwhile, the position of Peak A and B maintained at the same position, but Peak C becomes broader and red-shifted from 477 nm (20 min) to 492 nm (24 min). Besides, the FWHM value improved significantly from 192 nm (20 min) to 258 nm (24 min). As the formation of larger AgNPs did not contribute to the absorption band signal [31], the reduced spectra intensity, red-shifted peak position, and increased FWHM value implied Ostwald ripening phenomenon. Uncontrollable growth of AgNPs was facilitated by the shrink of small AgNPs, leading to the formation of AgNPs with larger particle size and polydisperse morphology [31].

In contrast, two peaks could be observed in the UV–Vis spectra of AgNPs-LMDR (Fig. 4b). When the reaction time increased from 4 to 24 min, the absorbance of Peak B raised from 0.43 to 2.37 a.u., and the peak position shifted from 414 to 455 nm. Meanwhile, the FWHM reduced from 208 nm (4 min) to 172 nm (24 min). Similarly, two peaks could be observed clearly in the UV–Vis spectra of AgNPs-UILM-600 W from 8 to 16 min (Fig. 4c). After 16 min, the weaker peak around 350 nm disappeared, and the main peak intensity grew rapidly to 2.86 a.u. Moreover, the center of the extinction peak shifted from 429 nm to shorter wavelength (421 nm). The value of FWHM also decreased significantly from 151 nm (4 min) to 86 nm (24 min). According to previous research, the only one peak at around 420 nm in UV–Vis spectra indicated the existence of Ag nanospheres [31], while reduced FWHM value corresponded to uniform and monodisperse particles [34]. Therefore, the blue-shifted peak position and decreased FWHM observed in the UV–Vis spectrum of AgNPs-UILM-600 W indicated that the ultrasound-intensified digestive ripening could effectively reduce particle size and narrow size distribution of AgNPs.

Fig. 4d-4f, as well as Fig. 4h, exhibited the relationship between particle size distribution and reaction time. According to Fig. 4d, the AgNPs-LM showed the widest distribution and largest particle size. It reduced from 865 nm (4 min) to 31 nm (20 min), then increased dramatically to 105.9 nm (24 min). This could be contributed to Ostwald ripening process which caused by the excess of citrate ion. In comparison, the average particle size of AgNPs-LMDR maintained an increasing tendency, and grew from 1.2 nm (4 min) to 14.5 nm (24 min). For AgNPs-UILM-600 W, the PSD (Fig. 4f and 4 h) showed a similar tendency with its UV–Vis spectra. Under ultrasound irradiation, the average particle size increased from 1.2 nm to 5.2 nm as the increasing of reaction time from 4 to 20 mins, then decreased to 3.7 nm after 24 min. Therefore, the particle size distribution results were consistent with UV–Vis analysis, which demonstrated that digestive ripening helped to reduce particle size. Ultrasound irradiation could further inhibit uncontrolled secondary nucleation and particle growth, leading to improved morphology uniformity and reduced particle size.

The color of AgNPs suspension was shown in Fig. 4i. AgNPs-LM kept transparent even after 24 min. On the contrary, the color of AgNPs-UILM-600 W and AgNPs-LMDR suspensions after reaction for 4 min was...
light yellow and yellow, respectively, indicating the initial formation of Ag nuclei occurs within 4 min after the addition of citrate ion. As the increasing of ultrasound irradiation time, the color of AgNPs-LMDR was turned to taupe after 24 min, while AgNPs-UILM-600 W suspension was changed to red brown.

3.3. Formation mechanism of AgNPs

Based on the experimental results and theoretical analysis, the main formation process could be divided into three main stages, including generation of Ag$^0$ nuclei, formation of Ag clusters, and growth of AgNPs [35]. In UILM method, these three stages were all influenced by ultrasound irradiation. Besides, the formation of monodispersed spherical AgNPs was attributed to two main processes, including sonocrystallization and ultrasound-intensified digestive ripening.

The formation mechanism of AgNPs was shown in Scheme 1 and 2. In terms of chemical effects, several chemical reaction mechanisms were proposed to explain the reduction of Ag$^+$ to Ag$^0$. The first reaction mechanism was that, large amount of highly reactive free radicals (R$^\cdot$), including H$^\cdot$ and OH$^\cdot$, was produced by thermal dissociation of water molecules under ultrasound irradiation. These free radicals were further rearranged, resulted in the producing of H$_2$, H$_2$O$_2$, and H$_2$O. Due to the initial weak alkaline condition caused by the dissolution of sodium citrate, the reduction radicals and H$_2$O$_2$ could be regarded as reducing agent, leading to the reduction of Ag$^+$ and formation of Ag$^0$ [36–38]. Apart from that, the second reaction mechanism was the reduction of Ag$^+$ to Ag$^0$, as well as the creation of H$^\cdot$ and oxygen, can be achieved by sodium citrate solution. Subsequently, dicarboxy acetone and free electrons were produced through the oxidation of citrate ion. As mentioned above, ultrasonic irradiation could create special reaction condition with extremely high temperature and pressure. Dicarboxyacetone could further dissolve in this reaction condition, and produced acetone and pyrolysis radicals, which were used as reducing agent to prepare Ag$^0$ [39]. Thus, the reduction of Ag$^+$ to Ag$^0$ could be also achieved by sodium citrate, citrate ion and pyrolysis radicals [40]. These chemical reactions were represented in Scheme 1.

Moreover, it was clear to see the formation of Ag$^0$ involve consumption of OH$^\cdot$ and production of H$^\cdot$, especially during ultrasound irradiation. Therefore, AgNPs-UILM-600 W suspension exhibited the lowest pH value (Fig. 3c). Besides, two adjacent carbonyl groups of one citrate molecule were adsorbed on the surface of AgNPs through coordination effect. Meanwhile, the other carbonyl group extended outward, which made the surface of AgNPs exhibit negative charges [41].

Apart from chemical effects, it was also important to mention the influence of fluid dynamics created by ultrasound irradiation on the formation of uniform and spherical AgNPs. Previous studies demonstrated that ultrasound irradiation could create large amount of microbubbles. Such imploding bubbles could be considered as small microreactors which generated powerful hydraulic shocks and acoustic streaming. As no solid existed in solution at the beginning of the experiment, the reaction system was considered as homogeneous reaction. The bursting of the microbubbles which caused by ultrasound induced hydraulic shocks and acoustic streaming in the solution. The symmetrical implosion bubbles produced micro-streaming and eddies that facilitated the flow regime transition from laminar flow to turbulence, and enhanced turbulent energy dissipation rate. Thus, the diffusion rate and micromixing intensity among these eddies were remarkably improved by the turbulence produced by ultrasonic irradiation. In addition, it was believed that micromixing intensity, as well as mass and heat transfer rate could be improved dramatically in turbulence. The collisions among molecules were also enhanced significantly by turbulence, leading to the reduction of induction time, and increasing of both chemical reaction rate and primary nucleation rate. Therefore, large amount of Ag$^0$ nuclei could be produced by sonocrystallization within extremely short time.

When Ag$^0$ nuclei was produced, the system became to heterogeneous, and induced secondary nucleation and particle growth processes. In addition, Oswald ripening or digestive ripening (or inverse Oswald method) would occur depending on the molar ratio between Ag ion and citrate ion. According to TEM images, UV–Vis spectra and particle size distribution results, Oswald ripening tended to happen when the molar ratio between AgNO$_3$ and Na$_2$Cit was 1:3. Thus, AgNPs sample with polydisperse morphology, larger particle size, and broader size distribution was produced due to the slow nucleation and uncontrolled Oswald ripening processes. When reducing the molar ratio between AgNO$_3$ and Na$_2$Cit from 1:3 to 5:4, digestive ripening would occur and facilitate the production of uniform sized AgNPs from highly dispersed polydispersed colloid. Previous study demonstrated that the dispersion efficiency of the mechanical stirring was much weaker than that under ultrasonic irradiation at lower ultrasound intensity [42]. Thus, micro-jetting produced by the asymmetrical implosion bubbles prevented agglomeration among Ag$^0$ nuclei, inhibited uncontrollable particle growth, and broke larger AgNPs into small fragments during ultrasound-intensified digestive ripening. Comparing with digestive ripening, fragmentation played dominated role rather than agglomeration under ultrasound irradiation. More nucleation sites for new Ag$^0$ nuclei were produced by these fragments. Besides, the high temperature and pressure environment created by the implosion of cavitation bubbles might melt part of AgNPs. The enhanced mass transfer rate also increased collision velocity and possibility between new-generated Ag$^0$ nuclei and existed AgNPs. These effects were responsible for uniform particle growth process, and the formation of AgNPs with smaller size, narrower particle size distribution, and spherical morphology during the ultrasound-intensified digestive ripening process. According to the results shown in Fig. 4 and formation mechanism described in Scheme 2, it might be concluded that the initial formation of Ag nuclei could occur...
within 4 min after the addition of citrate ion. Generation of Ag clusters and growth of AgNPs processes might happen from 4 to 20 min. Oswald ripening, digestive ripening, and ultrasound-intensified digestive ripening mainly occurred after reaction for 20 min.

3.4. Photothermal and antibacterial effects of AgNPs

The photothermal and antibacterial effects of AgNPs-LM and AgNPs-UILM-600 W were further evaluated. As shown in Fig. 5a-b, after NIR irradiation, laser energy could be rapidly and effectively transformed to heat by AgNPs-UILM-600 W. The maximum temperature of core area increased from 21.9 to 57.6°C within 10 mins. Meanwhile, the temperature increasing rate of AgNPs-LM was lower than AgNPs-UILM-600 W. After laser irradiation at 808 nm for 10 min, the temperature of AgNPs-LM sample rose from 23.5 to 41.4 °C in 10 mins. In contrast, the DI water exhibited slightly change in temperature (8.5 °C) under the same irradiation power and time. These results implied that applying ultrasound-intensified Lee-Meisel method was helpful to produce AgNPs with better photothermal performance.

E. coli and S. aureus were chosen in this study to evaluate the antibacterial ability of AgNPs samples. As shown in Fig. 5c and 6d, the bacteria colonies treated with AgNPs-UILM-600 W revealed the fewest grown colonies among all samples, around 99.5% of E. coli and 98.6% of S. aureus could be eliminated by AgNPs-UILM-600 W, verifying its greatest antibacterial ability which caused by its smallest particle size.

3.5. Machine learning for quick and efficient evaluation of particle size

To investigate the relationship among initial reactant concentration, ultrasound intensity, and average particle size of AgNPs, and reveal which factor plays more important role for the determination of particle size, a machine learning approach-Decision Tree Regressor (DTR) was applied to develop a model for the problem studied. Fig. 6a showed the experimental results and relationship among initial AgNO₃ concentration (C_Ag), ultrasound intensity and particle size. The size of the points corresponded to the ultrasound intensity. If C_Ag < 12 mM, the particle size steadily grew with C_Ag and lighter UI lead to larger size in general. If C_Ag > 1400 mM, the particle size increased abruptly and proportional to ultrasound intensity, whereas ultrasound intensity was negatively correlated with particle size when particle size was small (e.g. < 100 nm). After that, the initial reagent concentration and ultrasound intensity were used as features (independent variable) in the DTR, while the size of AgNPs was regarded as target (dependent variable). Performance metric of Mean Absolute Error (MAE) = -0.45 was achieved as the mean of 10 simulations using tuned hyper-parameters. To get a generalized model and avoid overfitting, the minimum samples per leaf was set to 3. This could be observed in Fig. 6b which showed the predicted vs actual particle size. It could be seen that the DTR fitted the data well for small particle size (<100 nm), but not for the three extreme values (particle size ≥ 100 nm). The fitted DTR was illustrated in Fig. 6c, where the node represented the experimental condition, the percentage of samples covered and the value of the particle size. The extreme values were filled in a dark colour, suggesting C_Ag > 1250 mM resulted in 20% of samples with particle size value equaled to 489.85 nm. Next, the importance of initial reagent concentration and ultrasound intensity on the particle size was also studied, and the results were presented in Fig. 6d, which suggested the initial reactant concentration played a more important role than ultrasound intensity on determining particle size.

4. Conclusions

Spherical and monodisperse AgNPs are controllable prepared by ultrasound-intensified Lee-Meisel method (UILM). The effects of Ag: citrate ratio, ultrasound intensity and reaction time are systematically investigated in this work to reveal the formation mechanism of AgNPs during sonocrystallization and ultrasound-intensified digestive ripening processes. Besides, a machine learning approach-Decision Tree Regressor is applied to investigate the relationship among initial reactant concentration, ultrasound intensity, and average particle size of AgNPs, and reveal which factor plays more important role for the determination of particle size. The conclusions reached as the results of the current study are as follows:

(1) During the formation of AgNPs, reducing the molar ratio of Ag:
citrate can induce digestive ripening instead of Oswald ripening. Moreover, ultrasound irradiation can further inhibit uncontrolled secondary nucleation and particle growth processes, leading to the formation of AgNPs with improved morphology uniformity and reduced particle size.

(2) Increasing the applied ultrasound intensity in the UILM can reduce micromixing time, and improve both mass transfer rate and overall reaction time, which can produce AgNPs with smaller primary particle size, as well as monodisperse and spherical particle shape. However, excess-increasing the ultrasound intensity will have a negative impact, consequently resulting in an increase in the average grain size.

(3) Ultrasound irradiation can influence the formation process of AgNPs through fluid dynamics and chemical effects. Thus, three main stages, including generation of Ag\(^+\) nuclei, formation of Ag clusters, and growth of AgNPs, are all affected by sonocrystallization and ultrasound-intensified digestive ripening.

(4) Machine learning approach - decision tree and Shapley value explanation is applied to study the relationship among initial reactant concentration, particle size and ultrasound intensity. The results revealed that the concentration of AgNO\(_3\) is a more important feature affecting particle size.

CRediT authorship contribution statement

Bin Dong: Conceptualization, Methodology, Writing - original draft.
Ning Mu: Methodology. Mengjun Wang: Methodology. Zonghua Xiao: Methodology. Lin Dai: Methodology. Zhixiang Wang: Writing - review & editing, Funding acquisition. Dechun Huang: Investigation. HONGLIANG QIAN: Writing - review & editing. Wei Chen: Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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