Research Article

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Biosynthesis and antioxidation of nano-selenium using lemon juice as a reducing agent

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Abstract: Nano-selenium was synthesized using lemon juice as a reducing agent. The experiments showed that pH value affected greatly the shape and the size of the prepared nano-selenium. At pH 9, lemon juice could reduce 50 mmol/L of selenite ions to nano-selenium with particle size between 50 and 90 nm, which was spherical and well dispersed. Lemon juice acted as both a reducing agent and a stabilizer in the synthesis of nano-selenium, in which the chemical interaction between biomolecules and the nano-selenium surface was the basis for the stable existence of nano-selenium. The selenite concentration influenced the formation of nano-selenium, and a low selenite concentration was beneficial to obtain small particles. The achieved nano-selenium exhibited a strong antioxidant activity positively related to concentration. The comparative study showed that the antioxidation of nano-selenium is weaker than that of vitamin C but higher than that of lemon juice.

Keywords: nano-selenium, lemon juice, antioxidation *in vitro*, UV-Vis

1 Introduction

Selenium is an essential micronutrient for human health. Selenium deficiency can lead to a significant increase in the incidence rates of cardiovascular diseases, cancer,

Kashin-Beck disease and viral infection. Selenium supplementation can help prevent these diseases [1–4].

In the past few decades, the phenomenon of selenium deficiency in human body has appeared worldwide [5,6]. The safe dose range of selenium in organism is very narrow. It is easy to produce toxicity due to overdose, which limits the application of traditional selenium compounds [7]. Compared with traditional selenium compounds, nano-selenium has unique physical and chemical properties, high activity, and low toxicity [8,9]. Therefore, the synthesis and biological effects of nano-particle selenium (SeNPs) have been widely concerned [10–12]. It is expected that SeNPs will become a new selenium nutritional supplement and treatment drug. Therefore, it is of great significance to study the preparation of nano-selenium.

There are many methods for the preparation of SeNPs, including the physical method [13], the chemical method [14], and the biosynthesis method [15–18]. In the physical method, mechanical actions are often used including friction, extrusion, shear, impact, ultrasound, and other treatment of solid raw materials to prepare nano-selenium, or sublimation condensation is adopted to change the intermolecular force of selenium to prepare nano-selenium. This method is simple and fast, but it has strict requirements for equipment conditions, the purity of the product is low, and the particle size is not easy to control.

In the chemical method, nano-selenium is prepared by the oxidation–reduction reaction [19]. Vitamin C, sodium sulfite (Na_2SO_3), sodium thiosulfate, hydrazine, and other chemical reagents are commonly used as reducing agents. Selenite, selenate, or selenium dioxide is used as a selenium source and as an oxidant. They are reduced to nano-selenium. In the process of oxidation–reduction, the particle size can be controlled by adding templates or changing reaction conditions. Bartůňek et al. [20] used sodium selenite (Na_2SeO_3) as a selenium source, surfactant sodium dodecyl sulfate as a template, polysorbate 80 as a particle size regulator, and ascorbic acid as a reductant to prepare 44–70 nm nano-selenium. By the chemical method, the raw materials are easy to obtain, and the reaction can take place at the atomic or

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molecular level. The particle size, shape, and crystal form can be easily realized and controlled. But the disadvantages are that the preparation process is complex, and the conditions are harsh. In addition, the reducing agent, template agent, and stabilizer used in the synthesis are often toxic, and the waste water produced has secondary pollution to the environment.

Compared with the chemical method, the biological method can avoid the use of toxic chemical reducing agents and stable dispersants [21,22]. The raw materials are easy to obtain. To a large extent, it can meet the concept of green environmental protection, hence attracting people's attention [23]. Biological synthesis includes plant and microbial synthesis, among which microbial synthesis is more applied [24]. Rasouli [25] synthesized spherical selenium nanoparticles with particle sizes between 50 and 250 nm by yeast *nematode* yeast in cells. The prepared particles have biological characteristics of resistance and antioxidant activity of *Candida* (a pathogenic bacteria). Tan et al. [26] isolated an aerobic *actinomyces* strain, *Streptomyces* sp. ES2-5, from the selenium ore soil. It was inoculated into a trypsin soybean agar plate, and SeO_3^{2-} was reduced to nano-selenium in the cell by glutathione. The reduced nano-selenium was released by mycelium fragmentation or fragmentation. The particle sizes were between 50 and 500 nm. It has also been reported that SeNPs was synthesized by *Bacillus cereus* [27], and the spherical SeNPs with particle sizes of 150–200 nm were obtained.

In addition, the purification process is complex. However, when a plant extract solution is adopted, it is easy to prepare and purify the synthesized SeNPs with the intervention of biological macromolecules. No strict aseptic operation and expensive equipment are required. The obtained nano-selenium has excellent characteristics of typical biosynthetic products, such as biocompatibility, antibacterial, and anticancer [28]. So, the biosynthesis of SeNPs mediated by plant biomolecules is more attractive [29,30]. Ye et al. [31] prepared SeNPs using green tea nano-aggregates as templates and ascorbic acid to reduce Na_2SeO_3 . The synthesis conditions were controlled to be the concentration of green tea nano-aggregates of 500 mg/L, the ratio of ascorbic acid to sodium selenite being 8:1 (mmol:mmol), and the reaction taking place at 40°C for 1 h. Thus, the red spherical nano-selenium with a diameter of 50 nm was prepared. The achieved nano-selenium could inhibit the proliferation of cancer cell lines HCT 116 and MDA-MB-231. Mulla et al. [32] used water extract of *Azadirachta indica* leaves to react with sodium selenite solution at 37°C to prepare nano-selenium. He found that the reaction time was an

important condition to control the particle size of the achieved nano-selenium. The longer the reaction time is, the smaller the nanoparticle is. The prepared nano-selenium had good cell compatibility. Dhivya et al. [33] used the water extract of cassia seeds and sodium selenite solution to synthesize amorphous spherical SeNPs with the particle sizes of 80–100 nm, which have not only inhibit bacteria and fungi but also inhibit the growth of human breast cancer cells. Liang et al. [34] obtained 15–20 nm spherical SeNPs by reaction of *Ocimum tenuiflorum* leaf extract and sodium selenite solution. Under the influence of these small-sized nano-selenium particles, the crystal structure and shape of calcium oxalate changed, which provides a new method for the treatment of urinary calculi.

In summary, it is the best way to synthesize SeNPs by plant extract, and the key to the biosynthesis of nano-selenium is the redox process of reducing substances in plant extract with selenite or selenate. The important factors affecting the biosynthesis of SeNPs are concentration, temperature, and pH value, of which the pH value is most important. The pH value can even determine whether the reaction can take place. The influences of temperature, concentration of selenite, and ratio of ingredients on the synthesis of nano-selenium have been reported in the previous literature [32,33]. But the influence of pH value on it has been rarely reported yet.

With this in mind, in this study, lemon juice was used as a raw material to prepare nano-selenium. The synthesis mechanism was explored. The influences of pH value, concentration of selenite, and ratio of ingredients on the reaction were investigated. The structure and properties of nano-selenium were characterized by XRD, SEM, and FTIR. The antioxidation of nano-selenium was tested.

2 Materials and methods

2.1 Materials

A fresh lemon fruit was taken, washed with water for 3–4 times, and peeled. After juicing the pulp, the liquid was centrifuged and filtrated with a 0.45 μm filter paper. The obtained lemon juice was stored in a refrigerator for subsequent use. Selenium dioxide (SeO_2 , AR) was purchased from Aladdin Industrial Co., Ltd., and it was prepared with deionized water to the required concentration for test design. Ammonia ($\text{NH}_3\cdot\text{H}_2\text{O}$, 25% (w), AR) was purchased from Tianjin Tianli Chemical Reagent Co.,

Ltd., and it was prepared with deionized water to the required concentration for test design.

2.2 Preparation of nano-selenium

2.2.1 Preparation and UV-Vis tracking of nano-selenium

A 40 mL of lemon juice filtrate and 10 mL of selenite solution were taken and placed in a 50 mL beaker. Ammonia solution was added drop by drop under magnetic stirring. The color of the solution changed gradually from colorless to orange red. At pH 9, the beaker was under ultrasonic vibration. After 60 min, the solution was stirred magnetically at room temperature and left standing, and then, the orange red suspension was centrifuged. The obtained nano-selenium particles were cleaned with purified water for three times and finally subjected to freeze-drying for 48 h. Thus, nanometer selenium powder was obtained. In the preparation of nano-selenium, when the mixed solution of lemon juice and selenite was adjusted to pH 9 by ammonia water, the reaction solution was taken to be used as a test sample every 30 min. UV-Vis (UV-Vis, Unico Instrument, Shanghai) was used to track the formation of nano-selenium and to detect its surface plasmon resonance (SPR) absorption spectrum.

2.2.2 Factors affecting the formation of nano-selenium

2.2.2.1 Effect of selenite concentration on the formation of nano-selenium

The concentrations of 0.1, 1, 10, 50, and 100 mmol/L aqueous selenite solution were prepared. They were placed in the shade for standby. Lemon juice and selenite solutions of different concentrations were mixed at the volume ratio of 4:1 (v/v). After ultrasonic vibration for 60 min, the mixture was stirred at room temperature by magnetic force. The color change during the reaction was observed. UV-Vis tracking analysis was used to analyze the effect of selenite concentrations on the synthesis of nano-selenium by the absorption intensity at the absorption peak position of SPR.

2.2.2.2 Effect of pH value on the formation of selenite

Ten parts of 20 mL lemon juice were taken, and they mixed respectively with 5 mL selenite solution (50 mmol/L) at room temperature. In one part, ammonia water was not

added (pH 2.3 of the original solution). In nine other parts, ammonia water was added to adjust the pH values to 4, 5, 6, 7, 8, 9, 10, 11, and 12. Ten parts of the mixture solutions were vibrated under magnetic force after ultrasonic vibration. The color change of 10 samples of the mixed solution was observed, and the influence of pH value on the formation of selenite was discussed by the absorption intensity of UV-Vis at the absorption peak position.

2.3 Chemical composition and structure characterization of nano-selenium

The colloidal solution obtained from the reaction of lemon juice with selenite was detected by UV-Vis to trace the reduction of selenite ion and the synthesis of SeNPs in the reaction system. X-ray diffraction (XRD; D/max-2200PC, Rigaku) was used to analyze the composition and the structure of the obtained SeNPs powder. Zetasizer NANO-ZS90, US Canta instruments, Inc., was applied to characterize the nano-selenium particle size and their distribution. TEM (FEI G2 F20 S-Twin, America FEI) was used to observe the surface morphology of nano-selenium. FT-IR (VECTOR-22, Bruker Corporation of Germany) was used to analyze the surface chemical action and to discuss the formation mechanism of nano-selenium.

2.4 Antioxidant analysis of nano-selenium

The scavenging effect of nano-selenium on the superoxide anion radical was determined by pyrogallol auto-oxidation. Hydroxyl radical scavenging was tested by salicylic acid. DPPH radical scavenging was tested by ultraviolet [35].

3 Results and discussion

3.1 The formation of nano-selenium and its UV-Vis spectrum

Figure 1 shows the UV-Vis spectra of selenite, lemon juice, and their mixture system. The detection results show that selenite does not absorb UV and visible light, while lemon juice has certain absorption in UV light area, which indicates that lemon juice may contain polyphenols. This is also the chemical basis for the strong reducibility of lemon juice. Research shows that when

Figure 1: UV-Vis spectra of selenite, lemon juice, and selenite-lemon juice system.

the nanoparticles are spherical or nearly spherical, the absorption peak of UV-Vis appears only with a single SPR resonance, whereas anisotropic particles will show two or three SPR resonances in accordance with their shapes [36]. In this study, there is a single absorption peak near 400 nm in the mixture system of selenite and lemon juice, indicating the formation of nano-selenium (SeNPs), and the obtained SeNPs has a spherical or spheroid-like surface morphology. The single peak is relatively wide, which indicates that the particle size distribution of nano-selenium is relatively wide, and the particle size uniformity is poor. The reaction solution containing nano-selenium looks orange red, which indicates that nano-selenium is of red color. With the proceeding of the reaction, the color of the synthesis system gradually turns red due to the surface plasmon resonance (SPR) of nano-selenium. As the reaction goes on, the red color appears and deepens, showing that the tetravalent selenium is continuously reduced and the nano-sized selenium microcrystals grow, forming more nano-sized selenium.

Figure 2 shows the UV-Vis spectra of nano-selenium at different times during the synthesis. Clearly, nano-selenium can be formed in 5 min with absorption at 381 nm. In 30 min, and the absorption peak intensity increases, indicating the increase of nano-selenium. The absorption peak position remains unchanged, indicating that the particle sizes are basically the same. Afterward, nano-selenium forms rapidly and grows up. After 60 min, the absorption peak of nano-selenium is shifted to red and the absorption intensity also increased, meaning that the amount of nano-selenium and the particle size increase too. Nano-selenium has the size effect, and the

Figure 2: UV-Vis trace synthesis spectrum of the nano-selenium synthesis system.

newly formed nanometer selenium particles are very small and with strong surface free energy. So, the generation of plasma resonance requires high-energy light wave, i.e., the short length wave. With the growth of nanoparticles, the particle size becomes larger, the surface free energy decreases, and the plasma resonance formation requires absorbing the long length wave. It can be inferred from Figure 2 that the reaction time is one of the most important factors affecting nano-selenium particle sizes and the synthesis amount. When the reaction lasts for 5 min, small-sized nano-selenium particles are formed. The corresponding absorption peak is located at a short wave of 381 nm. When the reaction time is 60 min, large-sized nano-selenium particles are formed, and the absorption peak is located at a long wave of 400 nm. In addition, the absorption intensity of nano-selenium particles synthesized within 60 min is enhanced, the light absorption range extends to the long-wave area, and the absorption line moves up.

3.2 Effect of selenite concentration on the formation of nano-selenium

Figure 3 shows the effects of different selenite concentrations on the formation of nano-selenium at pH 9. Only a single SPR resonance band appears in the absorption peak of UV-Vis, indicating that the formed nano-sized particles are spherical or spheroid like [36].

It is also found that the same absorption curve can be obtained under three kinds of high concentrations, which shows that spherical nano-selenium particles can be obtained within the designed concentration ranges

Figure 3: SPR absorption spectra of UV-Vis nano-selenium at different selenite concentrations.

under alkaline conditions. The largest absorption occurs at 50 mmol, indicating that the largest amount of nano-selenium formed. Therefore, at pH 9, 50 mmol is the optimal concentration of selenite for the synthesis of nano-selenium.

Figure 3 also shows that when the concentration of selenite is 0.1 mmol/L, a small-sized nano-selenium particle is obtained, and its corresponding absorption peak is located at 380 nm. When the concentration of selenite is increased to 1 mmol/L, the obtained nano-selenium particle size is lightly larger than that of the particles synthesized from 0.1 mmol/L. The absorption peak is red shifted to 384 nm (slightly longer than 380 nm). In addition, the absorption intensity of the obtained nano-selenium particle synthesized from 0.1 mmol/L is smaller than 0.5. However, the absorption intensity of the particle synthesized from 1 mmol/L is slightly larger than 0.5, implying the increased synthesis amount. When the concentration of selenite exceeds 1 mmol/L, the absorption peak of the prepared nano-selenium particle is red shifted to 400 nm, and its absorption intensity increases to about 0.8, indicating that the particle size and the synthesis amount of nano-selenium increase with the increasing concentration of selenite. A low selenite concentration is beneficial to obtain small nano-selenium particles.

3.3 Effect of pH value on the synthesis of nano-selenium

According to the UV-Vis spectrum of lemon juice, there is a strong absorption peak near 378 nm, from which it can

be inferred that lemon juice may contain polyphenols, carboxylic acids, sterols, flavonoids, and other reducing substances. Most of the molecules contain alcohol or phenol hydroxyl groups. For example, hypericum contains 8 hydroxyl groups, rutin contains 10 hydroxyl groups, naringin dihydrochalcone contains 9 hydroxyl groups, and chlorogenic acid contains 6 hydroxyl groups. Their oxidation reactions can be expressed as follows:

The corresponding Nernst equation is as follows:

$$E_{R-(OH)/R=O} = E_{R-(OH)/R=O}^0 + (0.05915) \log \frac{[R-(OH)_n]}{[R=O]^n [H^+]^n}, \quad (2)$$

where R refers to the chemical group connected by the phenolic or alcohol hydroxyl of the reducing substances in lemon juice, which can be benzene ring, saturated or unsaturated hydrocarbon, etc. The reduction reaction of SeO_3^{2-} is expressed as follows:

The oxidation potential $E_{Se(IV)-/Se(0)}$ is positively correlated with the value of the 6th power of hydrogen concentration $[H^+]$. However, for the reducing substance $R-(OH)_n$ in lemon juice, its reducibility is negatively correlated with the value of the n th power of hydrogen concentration $[H^+]$. As analyzed earlier, the n value of reductive substances in the lemon juice is often greater than 6. Therefore, the effect of acidity, i.e., pH, on the reducibility of reductive substances in the lemon juice is more obvious. In other words, alkaline conditions are conducive to improving the reducibility of lemon juice in favor of SeNPs formation. This is the reason why vitamin C and polyphenols are easily oxidized by air under alkaline conditions, whereas they are relatively stable under acidic conditions. Because of this, vitamin C and polyphenols are usually extracted under acidic conditions.

Figure 4 shows the effects of pH value on the synthesis of nano-selenium. When the pH value of the reaction system is less than 6, including the natural pH value of lemon juice (pH 2.3), the synthesis reaction cannot take place. However, under the alkaline condition of pH 9, SeNPs can be successfully formed. As mentioned earlier, in the acidic condition, although selenite has a strong oxidation, the reductive substances such as vitamin C and polyphenols in lemon juice have weak reducibility and high stability. In this case, it is impossible to reduce the tetravalent selenium. At this time, the absorption peak in the reaction system is the same as that in the

Figure 4: Effects of pH value on the formation of nano-selenium.

lemon juice, indicating that nano-selenium cannot be synthesized in the acidic condition.

3.4 Composition and structure characterization of nano-selenium

3.4.1 XRD examination of nano-selenium

Figure 5 is the XRD pattern of the nano-selenium powder synthesized by lemon juice. There is a specific diffraction peak in the range of 20–30° at the angle of 2θ, which is basically consistent with the diffraction peak of JCPDS card number 65-1290. It can be inferred that the obtained particles are selenium. The diffraction peak in the pattern is very wide, indicating that the synthesized nano-selenium particles are very small in size, poor in crystallinity, and amorphous. The possible reason for the formation of amorphous particles is that there is a biomolecular coating of polyphenols, flavonoids, vitamins, and other biological macromolecules in lemon juice (as the analysis presented in Section 3.4.2). The macromolecules contain carboxyl, hydroxyl, and other chemical groups. The groups have high electronic density or coordination ability. However, the newly formed micro nano-selenium is a simple substance selenium, its valence electron structure is $4s^2 4p^4$, 4d orbital is all empty, and nano-selenium particles have very high surface free energy, showing the surface effect of nanomaterials. They adsorb the biological macromolecules on their surface, blocking and hindering the growth of crystals, and hence, amorphous substances are produced.

Figure 5: XRD pattern of nano-selenium synthesized from lemon juice.

3.4.2 FT-IR analysis of nano-selenium

Figure 6 shows the FTIR spectra of lemon juice before and after the synthesis of nano-selenium. It can be seen from the spectrum that there is a strong absorption peak of pure lemon juice at 3420.46 cm^{-1} before synthesis, which is caused by the stretching vibration of the N–H bond in amide group. After the synthesis of nano-selenium, the absorption of this peak in the curve becomes weak and is red shifted to 3433.14 cm^{-1} , which shows that N–H bond complexes with selenium ion. At 1728.62 cm^{-1} , it is the C=O stretching vibration absorption of flavonoids and amides. At 1402.84 and 1221.92 cm^{-1} , the

Figure 6: FTIR spectra of lemon juice before (a) and after (b) SeNPs was synthesized.

Figure 7: TEM images of nano-selenium.

absorption occurs due to the C–N stretching vibration of aromatic amino group or the –C–N stretching vibration. The absorption is red shifted or disappears at 1402.84 and 1221.92 cm^{-1} after the synthesis of nano-selenium, indicating that the complexation takes place between C–N or –C–N group and selenium ions [37].

At 1633.63 and 1068.37 cm^{-1} in the curve of lemon juice is the stretching vibration absorption peak of C–OH from protein and polyphenol in lemon juice and that of C–H from olefin [38]. After the synthesis of nano-selenium, it is red shifted from 1633.63 to 1647.14 cm^{-1} , indicating that alkenes may undergo substitution, oxidation, or electron-induced effects. At 1068.37 cm^{-1} , the absorption peak is red shifted to 1074.30 cm^{-1} , indicating that C–OH of protein and polyphenol is oxidized. Therefore, FTIR analysis shows that in the synthesis of nano-selenium, amido group, amino group, carbonyl group, and polyphenol compounds in lemon juice play the role of reduction, dispersion, and protection. The 889.3 cm^{-1} peak in the lemon juice before synthesis and 789.21 cm^{-1} peak in the lemon juice after synthesis disappeared, which may be due to the oxidation of the aldehyde group, while the 594.60 cm^{-1} peak in the lemon juice disappearing after synthesis of nano-selenium may be caused by partial removal of amine or carboxyl group [39].

3.4.3 Surface morphology of nano-selenium

Figure 7 shows the TEM images of nano-selenium synthesized by lemon juice. It can be observed that the nano-

selenium particles have a regular spherical structure, which is consistent with the result of the symmetrical single peak by the UV-Vis analysis. The sizes are between 50 and 90 nm.

3.5 Formation mechanism of nano-selenium

It has been reported that lemon contains polyphenols, vitamins, proteins, esters, flavonoids, etc. [40]. Polyphenols, vitamin C, and other substances contain multiple hydroxyl groups, which have strong reducibility and can reduce the tetravalent selenium ions to selenium. Amino and carbonyl groups in biomacromolecules have a strong complexation effect on selenium and selenium ions. They can be wrapped on the surface of nano-selenium and play a role of dispersion and protection. The essence of dispersion protection is that the surface free energy of nano-selenium particles is reduced by the encapsulation of biomacromolecules. The nano-selenium particles become stable, and it is difficult for them to agglomerate.

After the tetravalent selenium is reduced to selenium atoms by lemon juice, the selenium atoms may have two competing actions: one is that the atoms are very small, the surface free energy is very large, and many atoms gather to form microcrystals; the other is that the selenium atoms form complex selenium with the biological macromolecules in lemon juice. In the early stage of the selenium atom formation, the first kind of action may be strong. A single selenium atom is very small, but its surface free energy is very high. The aggregation of multiple selenium atoms can greatly reduce the surface free energy and become stable after the formation of particles. For a single selenium atom or several selenium atoms, when it forms complex bonds or adsorption force with biological macromolecules, the energy released by the system will be less than that released by the polymerization between selenium atoms.

In addition, the formation of complex bonds between biological macromolecules and single selenium atom or several selenium atoms requires the rotation and folding

Figure 8: The mechanism of SeNPs formation.

Figure 9: Scavenging effects of vitamin C, lemon juice, and nano-selenium on free radicals: (a) O_2^- , (b) DPPH, and (c) OH .

of chains, which has a large steric hindrance. Therefore, it can be inferred that in the early stage of the formation of nano-selenium, the aggregation of selenium atoms is dominant. As the aggregation of atomic selenium becomes

Figure 10: Comparison of scavenging effects of nano-selenium on O_2^- , DPPH, and OH radicals.

larger, the combination with biological macromolecules becomes smooth. The formation of a large number of coordination bonds makes the energy released by the system exceed the energy released by the aggregation between selenium atoms. When the nano-selenium surface is covered with biological macromolecules, the nano-selenium particles become stable due to the protection of biological macromolecules. It is not easy to aggregate and grow up. This protective effect can be understood as follows: the polar groups of biological macromolecules combine with the nano-selenium surface, while the nonpolar or weak polar groups of biological molecules are distributed around the outer layer of the coated nano-selenium particles. There exists a weak hydrophobic force between the particles, among which weak polymerization is formed. Finally, multiple small nano-selenium particles form a large nano-selenium particle. Further approach of the particles will lead to the increase of the repulsion force. Therefore, nano-selenium particles can exist stably. The formation mechanism of nano-selenium is shown in Figure 8.

In the previous study on the preparation of selenium nanoparticles with dispersants or stabilizers, scholars have proved that Se can interact with $-\text{NH}_2$, $-\text{COOH}$, $-\text{SH}$, and $-\text{OH}$ groups on polysaccharides, proteins, and other molecules [41,42], which is consistent with the analysis results above.

3.6 Antioxidant analysis

The antioxidation of a substance is often tested by its scavenging effect on free radicals. The scavenging rate

is used to measure the strength of antioxidation. Vitamin C contains four hydroxyl groups, and it is a polyhydroxy compound. It is a well-known strong antioxidant. In this study, Vitamin C (Vc) was used as a control to determine the scavenging capacity of lemon juice and nano-selenium to O_2^- , OH , and DPPH free radicals to characterize their antioxidant properties. The results are shown in Figure 9.

It can be seen from Figure 9 that nano-selenium has a strong scavenging ability to O_2^- , OH , and DPPH free radicals. The scavenging rate is positively related to the concentration of nano-selenium. Compared with Vc, the scavenging rate of lemon juice and nano-selenium to free radicals is weak, while the scavenging ability of nano-selenium to free radicals is stronger than that of the lemon juice.

To further analyze the scavenging rule of nano-selenium on free radicals, Figure 10 compares the scavenging ability of nano-selenium on O_2^- , DPPH , and OH free radicals.

Figure 10 shows that the scavenging ability of nano-selenium on them ranks in order of $\text{DPPH} > \text{OH} > \text{O}_2^-$. The reason may be related to the degree of electron deficiency of three kinds of free radicals and also be related with the molecular weight of free radicals as well as the difficulty of their movement in solution.

From the viewpoint of atomic structure, the valence electron structure of selenium is $4\text{S}^24\text{P}^4$. After losing 2, 4, and 6 electrons, it has the stable structure of valence electron orbital, i.e., $4\text{S}^14\text{P}^3$, $4\text{S}^24\text{P}^0$, and $4\text{S}^04\text{P}^0$, showing +2, +4, and +6 oxidation states. In this process, nano-selenium shows strong reducibility, so the electron transfer occurs when it encounters free radicals with strong oxidation. As a result, free radicals are removed. For different free radicals, the scavenging rate is different because of their different covalent structures and oxidations.

4 Conclusions

Lemon juice was used to reduce selenite to prepare nano-selenium. Major conclusions are drawn as follows:

- (1) Nano-selenium can be prepared at room temperature. The achieved product is of good dispersibility and high stability. Lemon juice is acted as both a reducing agent and a stabilizer in the synthesis of nano-selenium.
- (2) When the concentration of selenite is 50 mmol/L and pH 9, nano-selenium particles with the particle size

of 50–90 nm can be synthesized. The morphology looks spherical.

- (3) The nano-selenium synthesized from lemon juice has strong scavenging ability to superoxide anion radical, hydroxyl radical, and DPPH radical. The scavenging rate is weaker than vitamin C, but stronger than lemon juice.

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Author contributions: Yang Hui: planned the research work and guided the research group to do experiments, look up literature, finished the work, and responsible for the entire work, from title to references; Su Wen: mainly did experiments, collected materials, and wrote experiment reports under Yang Hui's guidance; Wang Chuang: mainly responsible for the organization of the experiments under Yang Hui's instruction.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The data used to support the findings of this study are included within the article and are available from the corresponding author upon request.

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