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*Research Article*

# Preparation and Effect of Selenium Nanoparticles/Oligochitosan on the White Blood Cell Recovery of Mice Exposed to Gamma-Ray Radiation

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Owing to their excellent bioavailability, high bioactivity, and low toxicity, selenium nanoparticles (SeNPs) are emerging nanomaterials. In this study, SeNPs with a size of ~41.8 nm were synthesised by $\gamma$-irradiation using oligochitosan (OCS) as the stabiliser. As-synthesized SeNPs/OCS were characterised by UV-Vis spectroscopy, transmission electron microscopy, and energy-dispersive X-ray (EDX) analysis. Results revealed that the as-obtained SeNP/OCS powder exhibits high purity. The SeNP/OCS solution's stability test results indicated that the SeNP/OCS solution stored at 4°C exhibits good stability for 60 days. The SeNP/OCS solution was unstable at ambient temperature, and SeNP/OCS exhibited agglomeration after about 15 days. SeNP/OCS products recovered the total white blood cells of $\gamma$-ray irradiated mice. The SeNP/OCS product, which was synthesised by a green approach, with high purity and efficient recuperation of white blood cells, can be used potentially as a functional supplement to assist cancer radiotherapy patients.

## 1. Introduction

As the most threatening disease to human life, cancer is the leading cause of death worldwide. According to the International Agency for Research on Cancer (IARC), in 2018, more than 18 million new patients and about 9.6 million cancer-related deaths were estimated worldwide [1]. Radiotherapy and chemotherapy are still considered to be optimum approaches for cancer treatment. However, these approaches also cause several undesirable side effects, particularly, a drastic reduction in the number of blood cells, such as white blood cells, red blood cells, and platelets; this reduction can lead to anaemia and infections caused by opportunistic microorganisms in people with a compromised immune system [2, 3]. Among the essential micronutrients required for human beings to maintain good health, selenium has attracted considerable attention due to its high biological activity, especially cancer prevention and immune enhancement [4–6].

However, the margin of activity and toxicity of seleno compounds is extremely narrow. Recently, compared to other selenium compounds, selenium nanoparticles (SeNPs) have been reported to exhibit outstanding biological properties and low toxicity [7, 8]. Besides, SeNPs exhibit

unique properties, including anticancer, antioxidant, and antiviral activities [9, 10]. Furthermore, SeNPs play a key role in regulating immune responses as well as effects of immune-related diseases, especially neutrophil disorders. Yazdi et al. [11] have reported that after 30 days of oral supplementation of SeNPs, the number of neutrophils and lymphocytes significantly increases, especially in mice exposed to X-ray radiation at doses of 2 Gy and 4 Gy. Thus, SeNPs have been proposed as a potential agent for use as a nutritional supplement to reduce the harmful side effects of radiotherapy or chemotherapy and increase cancer patients' immune system. Besides, SeNP supplementation already has been demonstrated to prevent and decrease the incidence of cancers, such as lung, mammary, colon, liver, and prostate cancers [12, 13]. Several methods have been employed to synthesise metal nanoparticles, such as chemical reduction methods using ascorbic acid, glutathione, and hydrazine hydrate as reducing agents [7, 8, 14, 15], biological methods using bacterial biomass as the reducing agent [4, 9], and gamma Co-60 irradiation using sodium dodecyl sulphate, dextran, and ethanol as the stabilisers and free-radical capture agent, respectively [16, 17]. In particular, irradiation is considered to be effective for synthesising SeNPs due to its following advantages: the reaction is performed at room temperature, high-purity SeNPs are obtained due to the absence of reductant residues, the SeNP particle size can be easily controlled by adjusting the dose and dose rate, and large-scale production is possible.

Meanwhile, oligochitosan (OCS) is the degradation product of chitosan, mainly prepared by chemical, biological, and $\gamma$-irradiation methods [18]. $\gamma$-irradiation has attracted considerable attention due to several advantages, including product purity, reliable control of the molecular weight by adjusting the dose and favourable for large-scale production [18, 19]. OCS exhibits higher activities, including immunomodulatory, antioxidant, and antitumor, and an increased number of physiological functions than initial chitosan due to its low molecular weight, good solubility, and low viscosity [18, 20]. A combination of SeNPs and OCS may increase the immunomodulation function via a synergistic or combined effect. Besides, similar to other polysaccharides such as alginate, dextran, and gelatin, OCS comprises electron-rich functional groups such as–$NH_2$ and–OH groups, which sufficiently stabilise SeNPs through coordinate bonds and electrostatic interactions [21].

In this study, OCS was used to stabilise SeNPs synthesised by gamma Co-60 irradiation. Besides, the effect of the oral administration of SeNPs/OCS and OCS on the recovery of white blood cells (WBCs) of mice exposed to $\gamma$-ray radiation at a harmful dose level was investigated.

## 2. Materials and Methods

*2.1. Materials.* Pure selenium dioxide ($SeO_2$) was obtained from Merck (Germany). A 3% ($w{\cdot}v^{-1}$) OCS solution was supplied from the Research and Development Center for Radiation Technology (VINAGAMMA), with a deacetyl degree of ~85% and an Mw of ~5000 g $mol^{-1}$. Other pure-grade chemicals were used, and distilled water was used throughout the experiments.

*2.2. Preparation of SeNPs/OCS by $\gamma$-Irradiation.* SeNPs were synthesised by the $\gamma$-ray irradiation of $H_2SeO_3$ as described by Hien et al. [16] with some modifications. In brief, a required amount of $SeO_2$ was dissolved in a 1% ($w{\cdot}v^{-1}$) OCS solution to prepare a 2.5 mM selenous acid ($H_2SeO_3$) solution described in

$$SeO_2\,(s)+H_2O\,(l) \longrightarrow H_2SeO_3\,(aq). \quad (1)$$

For the synthesis of SeNPs, the $SeO_3^{2-}$/OCS solution was irradiated using a Gamma irradiator SVST-Co60/B at a dose of 20 kGy, with a dose rate of 1.3 kGy $h^{-1}$ measured by a dichromate dosimetry system [22].

*2.3. Characterisation and Stability of the SeNP/OCS Solution.* Absorption spectra of OCS and the resulting SeNP/OCS solutions were recorded on a UV-Vis spectrophotometer (UV–2401PC, Shimadzu, Japan). The size of the prepared SeNPs was determined by transmission electron microscopy (TEM) images (JEM1010, JEOL, Japan) and statistically calculated from ~300 particles [16]. The SeNP/OCS powder was prepared by the spray drying of a 2.5 mM SeNPs/1% OCS solution with an ADL311 spray dryer (Yamato, Japan). The selenium content of the SeNP/OCS powder was determined by energy-dispersive X-ray (EDX) spectroscopy on a JEOL 6610 LA instrument. The particle size change determined the stability of the SeNP/OCS solution with the storage time.

*2.4. Effect of SeNP/OCS and OCS Supplementation on the WBC of Irradiated Mice*

*2.4.1. Animal.* Forty-five mice between six and eight weeks of age and between 27 and 30 g in weight were purchased from the National Institute of Malariology-Parasitology-Entomology, Ho Chi Minh City, Vietnam. Experimental mice were taken care of with usual water and food and kept in plastic cages with a 12 : 12 hour light and dark cycle during the experimental period [11]. Experimental procedures were conducted following the approved protocol for the care and use of laboratory animals set by the University of Science, Vietnam National University, Ho Chi Minh City, Vietnam.

*2.4.2. $\gamma$-Ray Irradiation of Mice.* Forty-five mice were subjected to $\gamma$-ray irradiation at a dose of 3 Gy for the whole body using a Gamma Chamber 5000 BRIT (India) with a dose rate of 1.25 kGy $h^{-1}$. After irradiation, mice were divided into three groups. The first group was supplemented with OCS (2 mg·$day^{-1}$), the second group was supplemented with SeNPs (20 $\mu$g·$day^{-1}$) + OCS (2 mg·$day^{-1}$), and the last group (the control group) was augmented with phosphate buffer saline (PBS). The oral supplementation of the samples was performed during 40 consecutive days for all groups.

*2.4.3. Blood Sampling and Total White Blood Cell Analysis.* Blood sampling was conducted at different intervals (0, 10, 20, 30, and 40 days) after OCS, SeNP, and PBS oral supplementation, as well as after stopping the oral supplementation of the above solutions of 30 days. Blood was sampled from the heart of mice under anaesthesia by ether inhalation. Next, ~1 mL of blood from a mouse in each group was collected in an appropriate blood collecting tube containing anticoagulating agents. The first blood sampling (0 days) was conducted 1 day before $\gamma$-ray irradiation. Finally, the total white blood cells were analysed by Nihon Kohden automated haematology analyser (Japan).

*2.5. Statistical Analysis.* The results were displayed as mean ± SD. The difference among groups was analysed by one-way ANOVA, followed by Dunnett's multiple comparison test, involving Origin 8.5 and SPSS 16.0. Statistically significant differences were considered as $p < 0.05$.

## 3. Results and Discussion

*3.1. Characteristics of SeNP/OCS Solution.* SeNPs were prepared by gamma Co-60 irradiation at a dose of 20 kGy using 1% OCS as the stabiliser, according to Hien et al. [16]. Figure 1 shows the UV-Vis spectra of OCS, ion, selenium, and SeNP/OCS solutions and the colour of the solution and TEM images of SeNPs. The colour of the irradiated $H_2SeO_3$/OCS solution changed from yellow-orange to orange-red, indicating the formation of SeNPs.

SeNPs were formed by water radiolysis products ($e^-$, H·) to reduce $Se^{4+}$ to $Se^\circ$. However, the UV-Vis spectrum shown in Figure 1(a) of SeNPs did not exhibit typical absorption peaks. SeNPs with a size of less than 100 nm did not exhibit an absorption peak ($\lambda_{max}$) in the UV-Vis region (200–800 nm), and the absorption peak was observed for SeNPs with a diameter of greater than 100 nm [23]. In particular, absorption peaks were observed at 550 nm and 680 nm for SeNP sizes of 146 nm and 240 nm, respectively [23]. The TEM images and size distribution of SeNPs in Figures 1(b) and 1(c) revealed spherical SeNPs, with a calculated average diameter of ~41.8 nm.

Figure 2(a) shows the FTIR spectra of OCS and SeNPs/OCS. Absorption peaks typical of OCS and SeNPs/OCS, such as–OH, $-NH_2$, $-CH_2$, –C=O, and (1 ⟶ 4) $\beta$-glycoside, were observed, and their typical shape was maintained. However, the typical peaks shifted significantly. Especially, $-NH_2$ or CO–NH bending vibrations shifted from 3468 $cm^{-1}$ to 3383 $cm^{-1}$ and 2926 $cm^{-1}$ to 2923 $cm^{-1}$, and -C=O stretching vibrations shifted from 1632 $cm^{-1}$ to 1600 $cm^{-1}$ and moved from 1590 $cm^{-1}$ to 1578 $cm^{-1}$ for N–H vibration in the FTIR spectra of OCS and of SeNPs/OCS, respectively, indicative of the complexation between selenium and OCS via the Se–O bond, leading to the improved colloidal stability of SeNPs/OCS and confirming the protective role of OCS for SeNPs [24]. The typical and shifted peaks in FTIR spectra are consistent with those reported previously [16, 25, 26].

Figure 2(b) shows typical XRD patterns of SeNPs/OCS: a broad characteristic peak was observed at $2\theta$ of 19.15°, corresponding to amorphous phases of OCS [27]. SeNPs/OCS exhibited diffraction peaks at 27° (100), 31° (101), 42° (110), 45° (102), and 47° (111), attributed to the crystal structure of Se nanoparticles [28]. This XRD spectrum of SeNPs/OCS agrees with those reported in the study by Yin et al. [28] and Fresneda et al. [29], where different methods synthesise SeNPs.

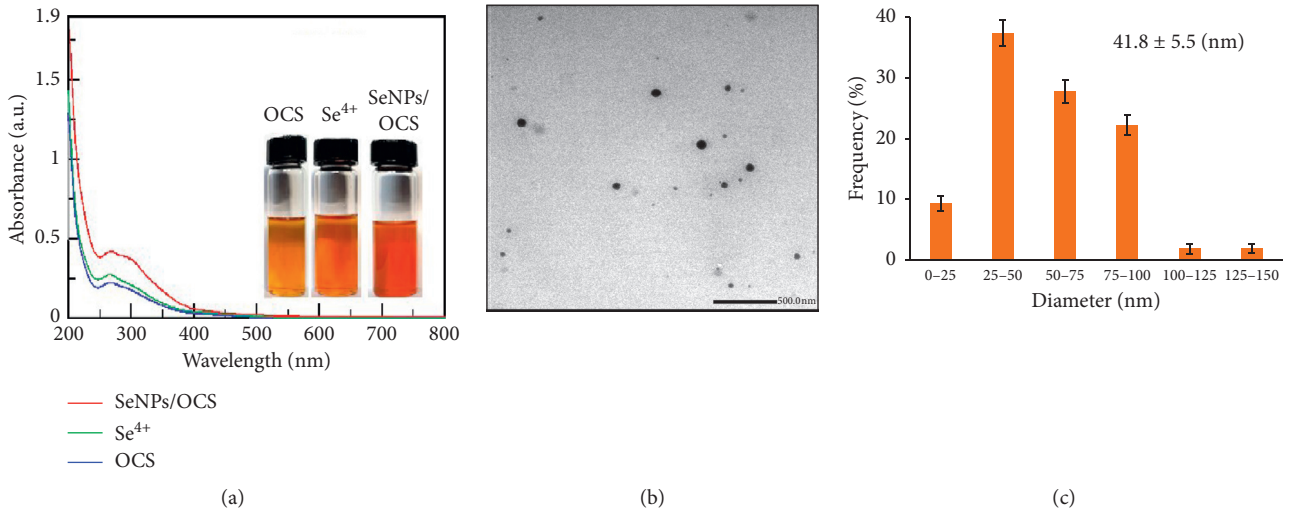
*3.2. Stability of the SeNP/OCS Solution during Storage Time.* Stability of a colloidal nanoparticle solution plays a vital role in applying a nanoparticle solution, and it depends on various factors such as concentration, molecular weight, and type of stabilisers, pH and dielectric constant, etc. [16]. In particular, the temperature strongly affects the stability and properties of the colloidal nanoparticle solution. At low temperatures, the aggregation of nanoparticles significantly decreased, while at high temperatures, the nanoparticles easily aggregated to form a larger size. The increase in the nanoparticle size at high temperatures can be explained by the Brownian movement [25]. The change in the colour of a colloidal solution for storage is evidence for the enlarged particle size.

Figure 3 shows the change in the SeNP/OCS solution colour during storage. Results indicated that at low temperatures (4°C), the SeNP/OCS solution colour remained almost unchanged over 60 days. Meanwhile, at 27°C, the solution colour markedly changed from light red to dark orange, and coagulation was observed after 25 days of storage.

Figure 4 shows the TEM images and the size distribution of the SeNP/OCS solution: spherical SeNPs were observed, with calculated average diameters of 41.8, 50.9, and 51.9 nm at storage times of 0, 30, and 45 days, respectively, at 4 °C. At 27°C, the SeNP size increased more rapidly than that at 4°C. The SeNP particle size dramatically increased from 41.8 nm (0 days) to 115.1 nm and 125.8 nm for storage time of 30 and 45 days, respectively (Figure 5). On the 45$^{th}$ day, SeNPs coagulated, and the particle size cannot be determined by TEM images.

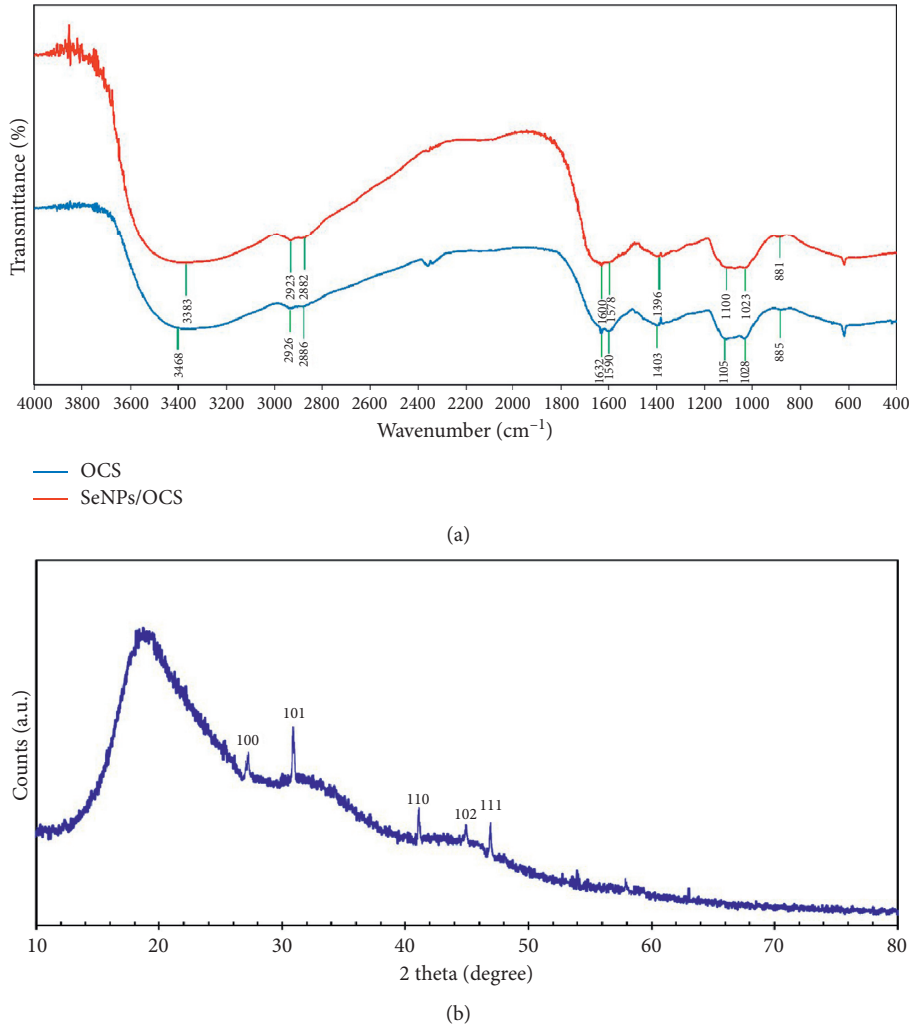
Similar results have been reported by Bai et al. [25]: SeNPs stabilised by 1% chitosan also become unstable after storage for more than 28 days and turned to black bulk after 42 days of storage at 25°C. The Bai group (2017) has reported that aggregation leading to size enlargement appears unavoidable in an aqueous solution. It was challenging to conserve the colloidal SeNP solution for a long time. Kong et al. [30] and Zhang et al. [7] also have independently reported that sialic acid or gum arabic can stabilise SeNPs for at least 30 days.

*3.3. Preparation of SeNP/OCS Powder by Spray Drying.* From the above results, the appropriate temperature to store the SeNP/OCS solution was 4°C. However, preserving the SeNP solution at low temperature was not always convenient for either storage or transportation. Therefore, there are some difficulties related to the commercial applications of




(a) (b) (c)

Figure 1: UV-Vis spectra of OCS, selenium ion, and a SeNP/OCS solution (a), TEM image (b), and the size distribution of a SeNP/OCS solution (c).




(a)

(b)

Figure 2: FTIR spectra (a) and XRD patterns (b) of OCS and SeNP/OCS solution.

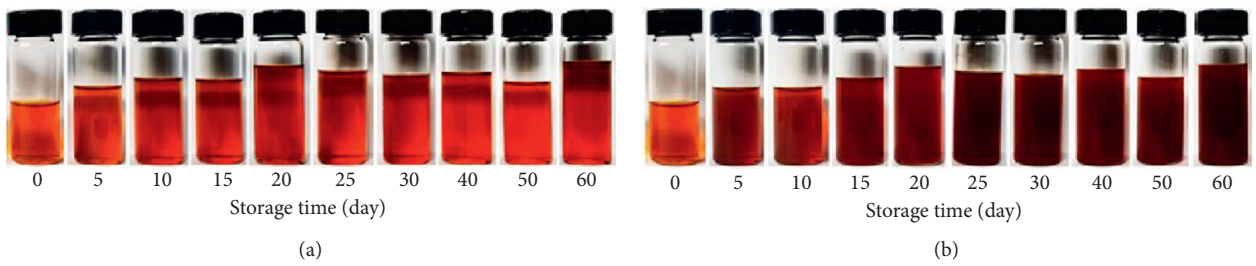



Figure 3: Colour of the SeNP/OCS solution stored at 4°C (a) and 27°C (b) for 0 to 60 days.

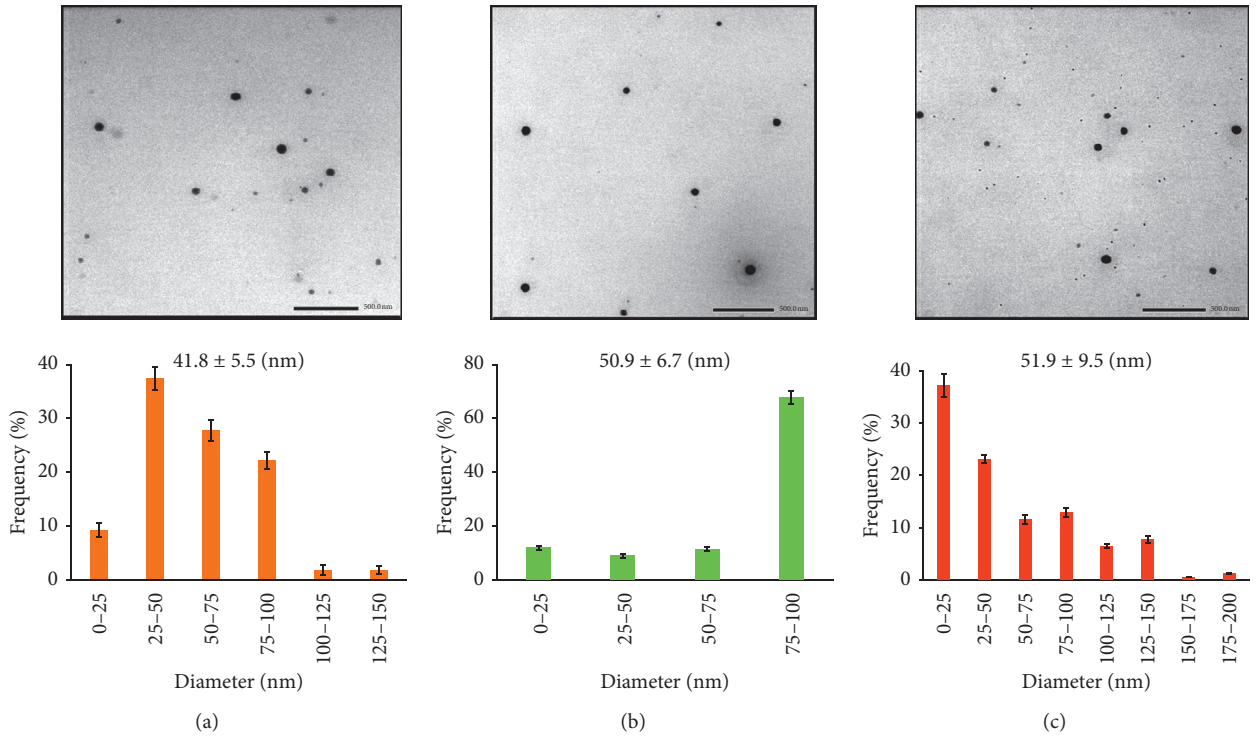



Figure 4: TEM image and size distribution of SeNPs/OCS stored at 4°C at different times: 0 days (a), 30 days (b), and 45 days (c).

the SeNP/OCS solution, especially for biomedical and pharmaceutical applications, if they are not sufficiently stable at ambient temperature. To overcome the limitations mentioned above and expand applications' scope, the SeNP powder was prepared by spray drying. Furthermore, Hien et al. [16] have reported that SeNP powder can be suitably sterilised by radiation to apply intravenous administration.

The SeNP/OCS powder prepared by spray drying in Figure 6(a) exhibited an orange colour, and the EDX spectrum in Figure 6(b) revealed that the obtained SeNP/OCS powder product is of high purity, comprising selenium (4.53%), carbon (50.22%), and oxygen (45.25%).

Figure 7 shows the TEM image and size distribution of the SeNP/OCS powder: with the change in the SeNP/OCS solution to the powder form, the particle size of SeNPs slightly increased from 41.8 nm to 43.8 nm. Hien et al. [16] also reported a similar result: the particle size of SeNP/dextran powder was slightly changed compared to that of the original SeNP/dextran solution.

*3.4. Recovery of the WBC of Irradiated Mice.* Along with chemotherapy, radiation therapy or radiotherapy remains a practical approach for treating several types of cancer [1]. However, this treatment causes unwanted adverse side effects in cancer patients, including early and late side effects. One of the side effects is apoptosis of bone marrow (BM) stem cells and BM stromal cell damage. These side effects can lead to a decrease in the WBC count, which is crucial for the body to fight infection [3]. In this study, the effect of oral supplementation of OCS and SeNPs/OCS on irradiated mice's WBC recovery rate was investigated.

As can be observed in Figure 8, the total WBCs in all three groups of irradiated mice, which were even orally supplemented with OCS, SeNPs/OCS, and PBS, respectively, were significantly decreased during the ten days after irradiation. However, the total WBC recovery to normal levels in irradiated mice that were orally supplemented with OCS and SeNPs/OCS for 20 days was clearly observed. Besides, results indicated that the oral supplementation of SeNPs/

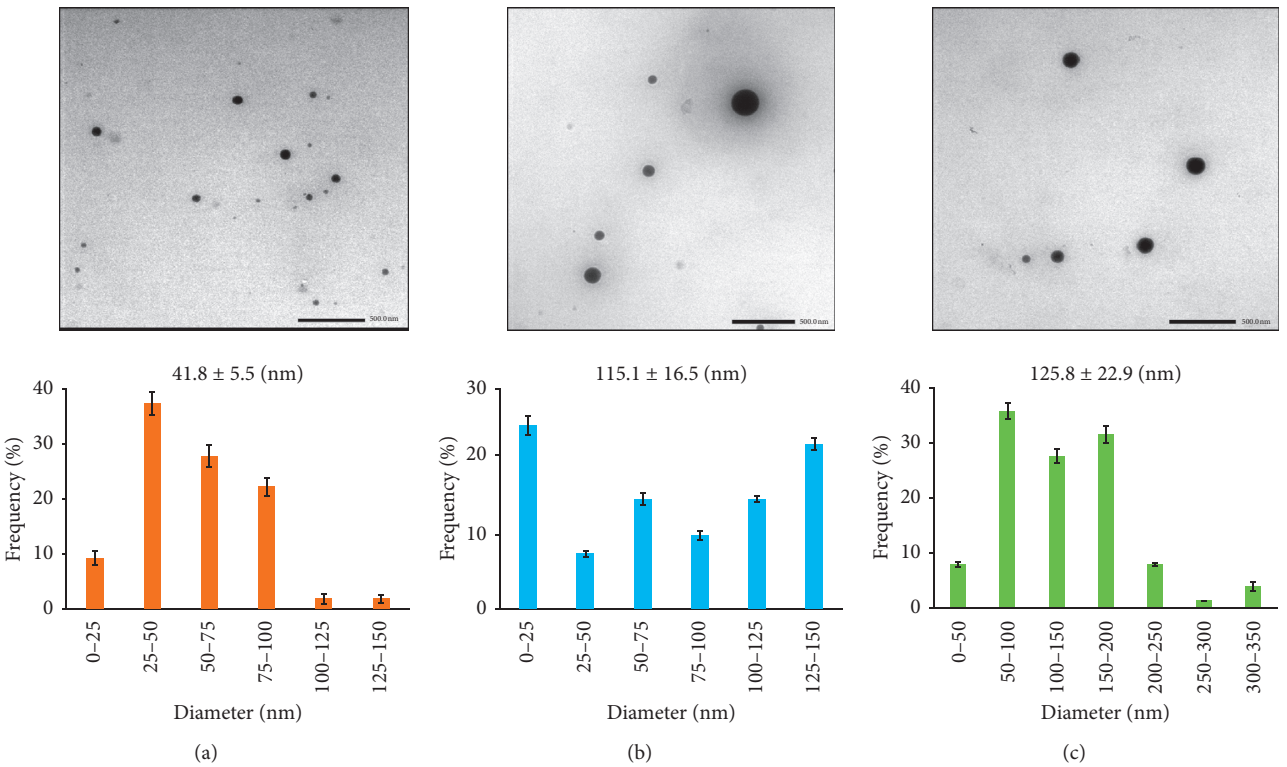



Figure 5: TEM images and size distribution of SeNPs/OCS stored at 27°C at different times: 0 days (a), 15 days (b), and 30 days (c).

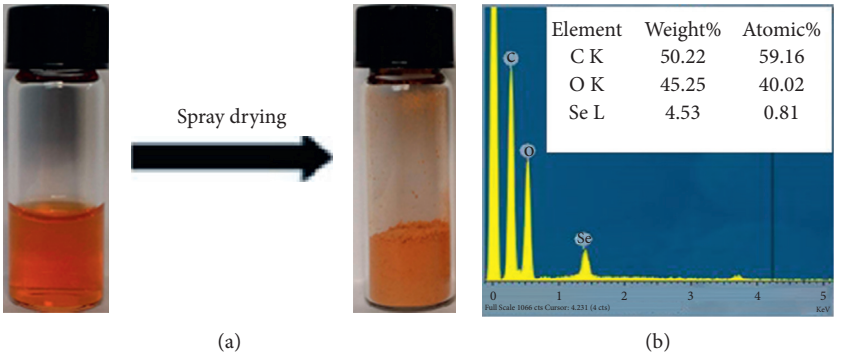



Figure 6: SeNPs/OCS in powder form prepared by spray drying (a) and EDX spectrum of the SeNP/OCS powder (b).

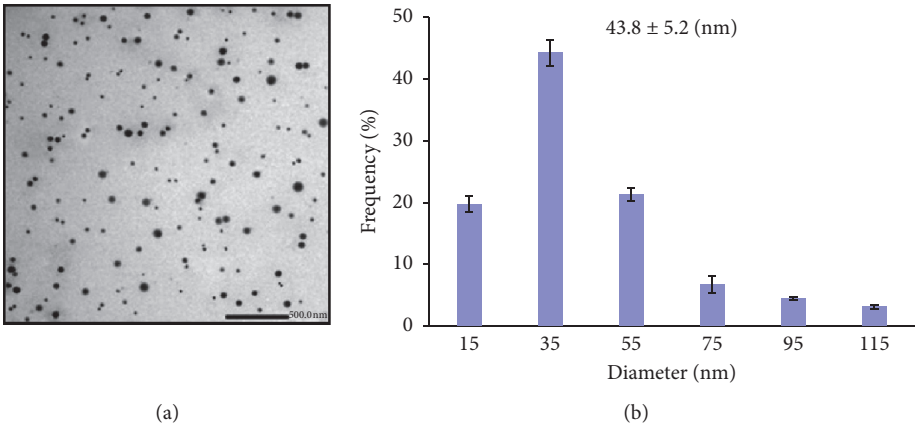



Figure 7: TEM image and size distribution of the SeNP/OCS powder.

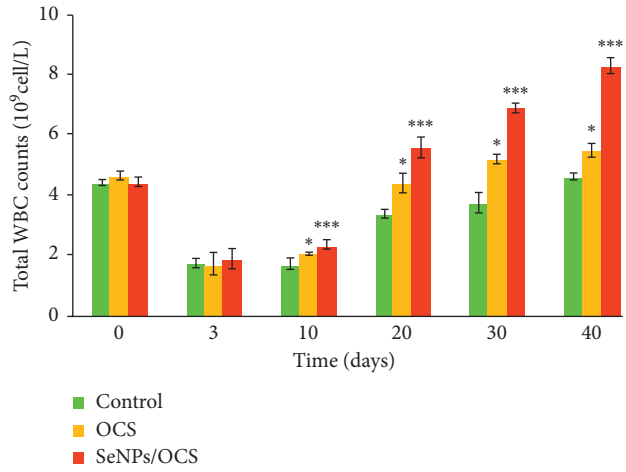



Figure 8: Total WBC counts in irradiated mice, which were orally supplemented with OCS, SeNPs/OCS, and PBS buffer (control group) for 40 days, as well as after stopping oral supplementation for 30 days. $^{*}p < 0.05$, significant difference to the control group; $^{**}p < 0.05$, significant difference of SeNPs/OCS to the OCS-supplemented group.

Table 1: Characterisation of some synthesised SeNPs.

| Size (nm) | Bioactivity | Stability | Reference |
|---|---|---|---|
| $35.6 \pm 7.5$ | MIC value is 330 $\mu$g/mL for *E. coli* | Several weeks | [31] |
| 36–95 | $LD_{50}$ is 62.3 mg Se/kg mouse | 28 days | [25] |
| $50 \pm 6.5$ | Scavenging free radicals at 0.1–4.0 mg/mL | 30–120 days | [24] |
| 20–60 | $LD_{50}$ is 92.1 mg Se/kg mouse | — | [32] |
| 50–103 | $LD_{50}$ is 258.2 mg Se/kg mouse | 28 days | [33] |
| 90–105 | $IC_{50}$ is $11.57 \pm 3.6$ $\mu$g/mL (antitumor) | — | [34] |
| 20–150 | WBC recovery is ~30 days | — | [11] |
| $41.8 \pm 5.5$ | WBC recovery is ~14 days | 30–45 days | This study |

OCS compared with OCS is better for WBC recovery. In particular, in the test group, SeNPs/OCS took less than 20 days for the complete recovery of WBCs.

As predicted, the time for the recovery of the total WBCs to normal levels in irradiated mice supplemented with SeNPs/OCS is 14 days. In contrast, the time for the recovery of the total WBCs to normal levels for the control group supplemented with PBS was 40 days. Moreover, the mice's total WBCs supplemented with OCS and SeNPs/OCS after stopping the supplementation of OCS and SeNPs/OCS for 30 days already returned to normal levels. Yazdi et al. [11] also reported that the total WBCs of irradiated X-ray mice supplemented with SeNPs (100 $\mu g \cdot day^{-1}$) are recovered after 30 days. Accordingly, the time for the recovery of the total WBCs to normal levels in our study was less than that reported in the survey by Yazdi et al. [11]. This result can be explained by the combined or synergistic effect between SeNPs and OCS. Table 1 lists the comparison between the SeNPs synthesised in this study with those synthesised previously, in addition to their stabilities and bioactivities.

## 4. Conclusion

SeNPs with a concentration of 2.5 mM and a diameter of ~41.8 nm stabilised by a 1% OCS solution were successfully synthesised by gamma Co-60 ray irradiation. Low temperature (4°C) was favourable for storing the SeNP/OCS colloidal solution. Also, highly pure SeNP/OCS powder, convenient to use, was prepared from the SeNP/OCS solution by spray drying. Compared to OCS, SeNPs/OCS exhibited a higher recovery for the total WBCs. The results of this study revealed that SeNPs/OCS could be used potentially for the recovery of the total WBCs, particularly in cancer patients who have undergone invasive radiotherapy.

## Data Availability

The data used to support the findings of this study are included in the manuscript.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

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