

## Synthesis and Characterization of Black Currant Selenium Nanoparticles (Part I)

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### ABSTRACT

The present study aimed to synthesize selenium nanoparticles (SeNPs) using aqueous extract of black currant as a reducing agent. The green synthesized black currant selenium nanoparticles (BCSeNPs) were identified by color change. The characterization of SeNPs was achieved by Ultraviolet-visible (UV-VIS) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction analysis (XRD), and Fourier transform infrared spectroscopy (FTIR). These tests were used to detect: stability, morphology, size, crystalline nature, and functional groups present on the surface of BCSeNPs. The results revealed appearance of the brick-red color indicating the specific color of selenium nanoparticles, and UV-Vis spectroscopy showed band absorbance at 265 nm of intense surface plasmon resonance manifesting the formation and stability of the prepared BCSeNPs. The SEM image showed the prevalence of spherical selenium nanosized, XRD at 2 $\theta$  revealed crystalline selenium nanoparticles, the size was in the average of 18-50 nm. Furthermore, FTIR revealed the presence of functional groups of the plant which act as stabilizing and reducing agents. In conclusion, the aqueous black currant extract can act as a reducing and capping agent to synthesize BCSeNPs in nano-scale size by a simple method.

**Keywords:** Black currant extract, selenium, nanoparticles, XRD, SEM, FTIR

### Introduction

Nanotechnology sciences allow to improve the experimental practice of the nanoscale constituent's preparation with exceptional possessions (1). Today, the production of nanoparticles (NPs) using biosynthetic techniques has been considered as a valuable method with increased attraction (2, 3). SeNPs could be chemically (4) or physically (5) synthesized. Moreover, it can even be obtained by the biological way, using microorganisms or extracts of different plants (6, 7). Chemically synthesized SeNPs were prepared by reduction of the selenious acid solution using ascorbic acid in the presence of polysaccharides such as acacia gum; glucomannan

as well as carboxy methyl cellulose (8, 9). Besides, SeNPs could be synthesized via different Gram-negative (10, 11), Gram-positive (12) bacteria or fungi (13, 14). In the green synthesis of SeNPs, many plant extracts were used as reducing agents including tea extract (15), *Allium sativum* (16), ferulic acid (17), and *Clausena dentata* plant leaf extract (18). Besides, spherical SeNPs (size range of 13-18 nm) can be synthesized using dried fruit extract of *Vitis vinifera* (19). However, many disadvantages due to using chemical or physical methods of SeNPs have been identified, such as extreme temperature, high pH, environmental pollution, as well as they are very expensive methods (20, 21). On the contrary, using plant extract (green method) for biosynthesis of nanomaterials has been pointed to possess many advantages compared to biological methods, e.g. it is inexpensive and no special conditions were required (6, 22). According to great amount of active ingredients that act as inducing agents in the black currant, such as flavonoids, polyphenol, lignin's, and sugar, this study aimed to synthesize and characterize selenium nanoparticles (SeNPs)

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through a biogenic method using aqueous black currant extract.

### Materials and Methods

Black currant without seed (*Vitis vinifera*) was purchased from the local market of AL-Diwaniya, Iraq. The rating of black currant was done by the Ministry of Agriculture/ Stat Board for seed testimony in Abu Graib/ Baghdad.

The biogenic synthesis and characterization of SeNPs depended on using black currant aqueous extract without seeds. About 100 gm of black currant without seed was washed with distilled water several times and dried at 40°C (incubator) and grinded by blender. A solution of 10 gm of black currant in 100 mL distilled water was prepared and stirred at 45-50 °C for 10-20 min by magnetic hotplate stirrer, and then left at room temperature overnight. The solution was centrifuged at 1000 rpm for 15 min. Whatman® filter paper No.4 was used for separation and filtration of the supernatant. Centrifugation and filtration were repeated twice (23,24). Green synthesis of SeNPs using black currant aqueous extract was prepared as described by (25-27). The 0.1 M of sodium selenite was mixed with black currant extract in a volume ratio of 1:2 with adjustment of reaction medium pH to 8 by using 1 N of NaOH. The mixture was stirred via magnetic stirrer (600 rpm) at 60-65 °C for 12 hours in the darkness. The solution was kept overnight, and observed for the formation of red brick color. Color changes occurred due to the reduction of NaHSeO<sub>3</sub> to selenium nanoparticles (BCSeNPs). The formation of SeNPs was markedly determined by the appearance of a red brick color. Separation of precipitate from the total solution was done by using high speed cold centrifuge at 15,000 rpm/30 min at 4 °C. The obtained pellet was then resuspended in deionized water and centrifuged again under the same conditions. This process was repeated three times to remove the organic impurities that exist in BCSeNPs colloid and dried incubator at 45 °C. Characterization of black currant SeNPs (BCSeNPs) was performed by: Ultraviolet-visible spectroscopy (UV-VIS), in which the bio-reduction property of black currants in the synthesis of SeNPs was investigated by UV-VIS spectrum (Metertech SP-8001 Taiwan), which is a significant technique to authenticate the formation and stability of BCSeNPs in the colloid

as described by (28, 29). Furthermore, SEM technique (SEM-Tescan Vega III, Czech) was used to investigate the shape and size of the synthesized BCSeNPs as described by (30), and X-ray diffraction (Shemadzu-6000 Japan) was also used to determine the crystalline amorphous of BCSeNPs as describe by (31, 32). Fourier-transform infrared spectroscopy (Shemadzu-8400s, Japan) was used for detecting the functional groups in the biological compound involved in the reactions of nanoparticles synthesis as described by (33, 34).

### Results and Discussion

Synthesis and characterization of BCSeNPs revealed brown color after 30 min and changed gradually to red-brownish color after 72 hours, later, the color became more stable (Figure 1). The biogenic synthesized SeNPs were proven via conversion of the colorless selenious acid to brick red color due to the possibility of conversion of multi-oxidized forms of selenium (Se<sup>-2</sup>, Se<sup>+2</sup>, Se<sup>+4</sup> and Se<sup>+6</sup>) to Se<sup>0</sup> (35). Encapsulations of Se<sup>0</sup> to organic molecules from the black currant give stabilization to these nanoparticles. The color change upon the addition of selenium selenite to the black currant extract was considered as an indication of SeNPs synthesis (36). This result has been previously obtained by many researchers using different types of green plants (37-40). The characteristic color was attributed to the excitation of the surface plasmon resonance (SPR) (41). The high ability of plants extracts to synthesize SeNPs was attributed to their high content of phenol and flavonoids, in addition to potent reduction capacity (39). The optical absorbance of synthesized BCSeNPs was measured using UV-Vis spectroscopy. An absorption peak between 265-370 nm confirms the presence of BCSeNPs (Figure 2). This absorbance indicated the formation of conjugated compounds, which appeared as indicator to the formed SeNPs. The bio-reduction property of black currants in the synthesis of SeNPs was investigated by UV-VIS spectrum, which is a significant technique to authenticate the stability and formation of BCSeNPs in the solution. The most well-defined peak at 265 nm represents the SPR of BCSeNPs. The current result is in agreement with the previous studies (42-44) using different types of bacteria and fungi. Concerning SPR, it has been reported that the smallest molecules give the highest peak at UV

spectroscopy between 280-320 nm (19). The current result was correlated with UV-visible spectra of SeNPs synthesized using orange peel aqueous extract (peak 265.5 nm) and the leaf of *Petroselinum crispum* (peak 270 nm) (45, 46).

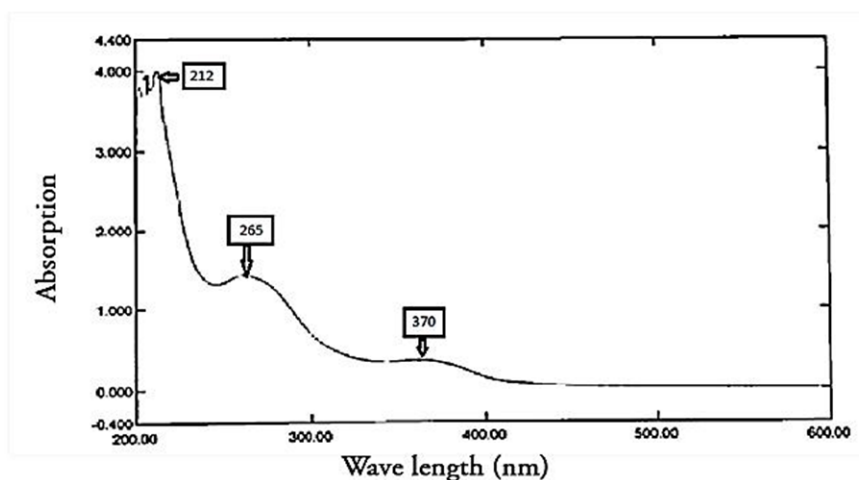
While Menon and his colleagues (39) synthesized SeNPs using *Zingiber officinale* reducing agent, where the peak value was between 370 and 420 nm that represent SPR. The spherical shape of BCSeNPs was observed according to SEM image with a diameter ranged from 18 to 50 nm with an average diameter size of  $27.10 \pm 1.56$  nm in an electron microscope (Figure 3; Histogram 1). The green synthesized SeNPs using black currant extract showed spherical amorphous NPs with different sizes between 18-50 nm. Spherical SeNPs were synthesized in a range of 30-50 nm (47, 48) and 97 nm size (49). Spherical shaped SeNPs was also documented by (50) and (39) with size range of 50-100 and 100-150 nm, respectively. Another author reported 30–40 nm as a range size of SeNPs (51). Black currant polyphenols were found to play a great role in particles size and the final structure of SeNPs dependent on pH, selenium salt molarity, reaction temperature, and reaction time, this result coincides with that of another study (52). In addition, SeNPs aggregation was prevented by organic compounds present in the blank current extract leading to stabilizing SeNPs (53). The usage of raisin or dried *V. vinifera* in the green synthesis of SeNPs was attributed to its contents of phenols and flavonoids, sugar, iron, calcium and potassium and some vitamins (54). In the current study, a pattern of X-ray diffraction peaks at theta angle-2 value of 14.421, 17.941, 24.542 and 29.972, respectively, correspondent to hkl values of 110, 101, 102, and 100 crystal planes, were observed (Figure 4). According to the results of XRD analysis in the current study, the physical characteristic of the particles in the prepared compounds is spherical and crystallized nanoparticles. The size of crystal was in a range of 18 to 50 nm. To clarify the crystalline nature of selenium nanoparticles, XRD analysis was applied, where the result showed different broad diffraction peaks at the lower angle, which goes in line with

(55) and (56) using different plant extracts and with (19) and (57) using raisin.

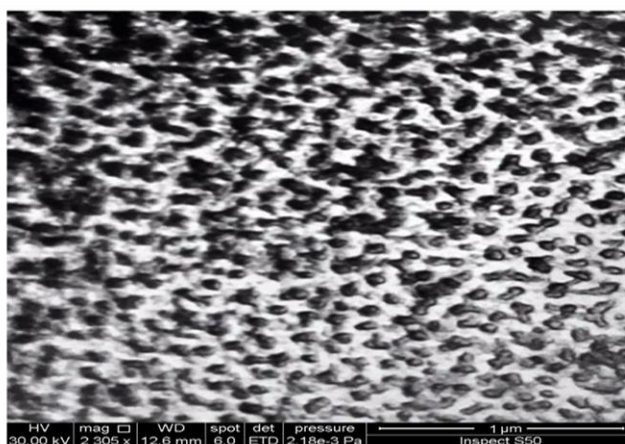
F-TIR spectroscopy for selenium nanoparticles (Figure 5) showed a distinct peak of BCSeNPs at  $3352.39 \text{ cm}^{-1}$  corresponding to OH: NH due to Stretch Vibration in Amide A. While an absorption peak at  $2931.90 \text{ cm}^{-1}$  corresponded to C-H in  $-\text{CH}_2$  in aliphatic compounds, the band at  $1608.69 \text{ cm}^{-1}$  indicated  $\text{NH}_2$  in primary amides. The peak at  $1514.17 \text{ cm}^{-1}$  is due to NH in secondary amides (Amide II). The peak at  $1359.86 \text{ cm}^{-1}$  attributed to the C-H bending in alkanes. However, the peaks at  $1066.67$  and  $1035.81 \text{ cm}^{-1}$  confirmed C–O, C–C Stretching Vibrations, C–O–H, C–O–C bending vibrations in polysaccharides, protein and polyesters. C–X stretching in alkyl halides causes a band at  $871.85$  and  $835.21 \text{ cm}^{-1}$ . The bands at  $590.24$  and  $547.80 \text{ cm}^{-1}$  were due to C–N–C bending in amines (58-60). The appearance of many peaks in green synthesized SeNPs indicates different vibrations due to different IR absorption, which is in accordance with (61, 62). Also, the characterization of selenium nanoparticles from *V. vinifera* using FTIR was clarified by (19), who demonstrated the presence of different peaks due to the occurrence of different active components of this plant, which acts as reducing agent. As well as, the presence of lignin in raisin could be claimed by (63), where lignin could be responsible for stabilization of the nanoballs, and this was previously suggested by (64). Chen and his colleagues (65) documented that in green synthesis of SeNPs, a high concentration amount of sugars present in dried *V. vinifera*, such as fructose and glucose, could act as reducing agents. Many amorphous and crystalline forms of selenium can be exist, however the structure, size and shape of SeNPs depend on temperature, concentration, PH, as well as the nature of biomolecules (42, 66). In conclusion, it seems that the best conditions for synthesizing selenium nanoparticles using aqueous black currant extract as a reducing agent were under alkaline pH (pH 9) and range of temperature between (60-65 °C). This technique can be considered as a good source to synthesize stable SeNPs in lesser time and cost.



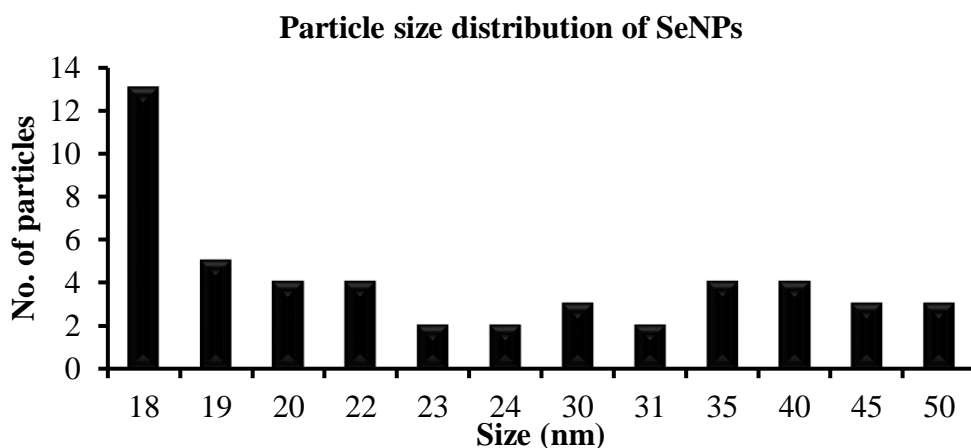
**Figure 1.** Changing the color after the reduction of sodium selenite to BCSenNPs using black currant extract. **A:** Image shows black currant aqueous extract, **B:** Image showed mixture of sodium selenite and black currant extract, **C:** Image shows BCSenNPs after 48-72 hour, **D:** Image shows BCSenNPs after centerfication



**Figure 2.** UV-Vis spectroscopy shows absorbance of selenium nanoparticles made from sodium selenite with black currant extract in PH 9



**Figure 3.** SEM image (1µm) of the selenium nanoparticles made from sodium selenite with black currant extract in percentage 1:2, v: v ratio in PH 9



Histogram 1. Diameter distributions of SeNPs

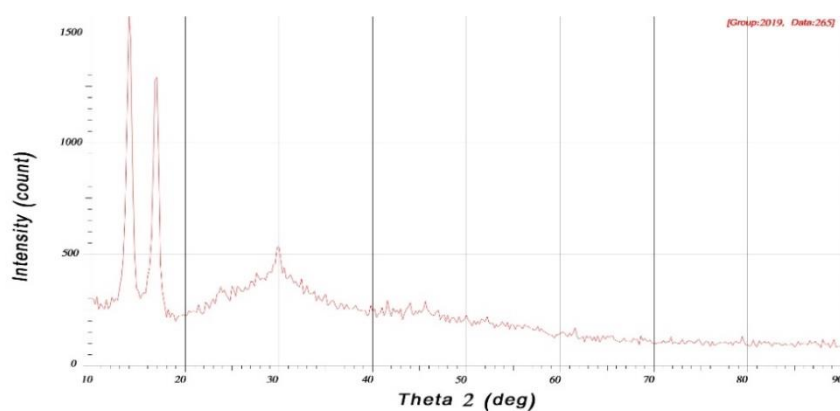


Figure 4. X-ray diffraction pattern for selenium nanoparticles made from sodium selenite with black currant extract in PH 9

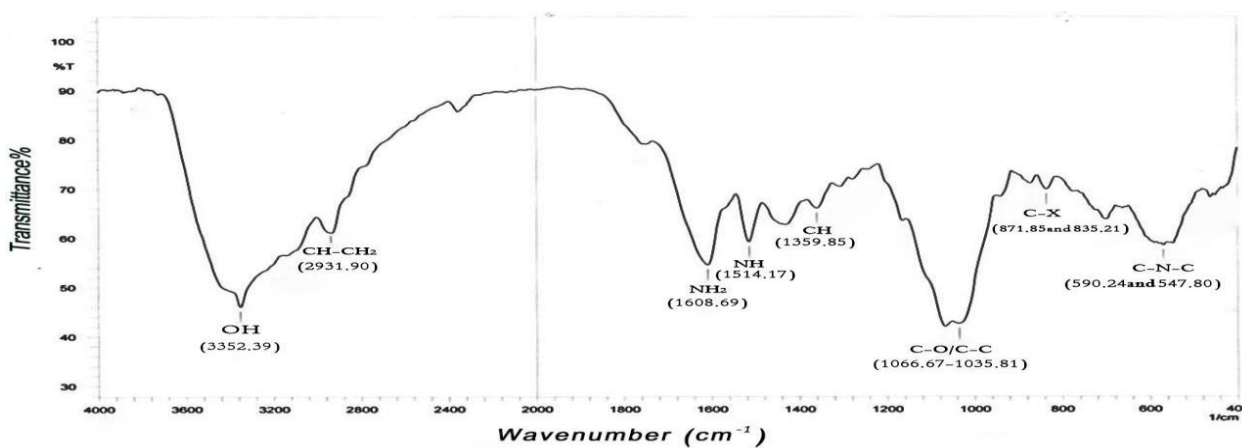


Figure 5. FT-IR spectroscopy for selenium nanoparticles made from sodium selenite with black currant extract in PH 9

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**Conflicts of interest**

The authors declare that there is no conflicts of interest.

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## تصنيع وتشخيص جسيمات السيلينيوم النانوية باستخدام الزبيب الأسود (الجزء الاول)

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### الخلاصة

هدفت الدراسة الحالية الى تصنيع جسيمات السيلينيوم النانوية باستخدام المستخلص المائي للزبيب الأسود كعامل اختزال. التصنيع الحيوي لجسيمات السيلينيوم النانوية المرتبطة مع الزبيب الأسود تم تمييزه بواسطة تغيرات اللون. تم تشخيص جزيئات السيلينيوم النانوية باستخدام التحليل الطيفي المرئي للأشعة فوق البنفسجية ، تقنيات الفحص المجهرى الإلكتروني , تحليل حيود الأشعة السينية , التحليل الطيفي بالأشعة تحت الحمراء. تم استخدام هذه الاختبارات لمعرفة مايلي على التوالي و الثبات، الشكل، الحجم، الطبيعة البلورية والمجاميع الفعالة الموجودة في جسيمات السيلينيوم النانوية المصنعة باستخدام الزبيب الأسود. أظهرت النتائج ظهور لون أحمر قرميدي المميز لجسيمات السيلينيوم النانوية، اما مطياف الأشعة فوق البنفسجية- المرئية أظهرت الامتصاصية عند الحزمة 265 نانومتر من رنين البلازمون المكثف على السطح، والذي يتجلى في تكوين واستقرار جسيمات السيلينيوم النانوية المحضرة، أظهرت صورة المجهر الإلكتروني انتشار بلورات السيلينيوم الكروية، وكان حيود الأشعة السينية عند الزاوية 2 كشف جسيمات السيلينيوم النانوية البلورية، الحجم كان بحدود 18-50 نانومتر. علاوة على ذلك، فقد كشف فحص التحليل الطيفي بالأشعة تحت الحمراء ظهور المجاميع الفعالة للنبات والتي تعمل كعوامل استقرار واختزال. نستنتج من الدراسة ان مستخلص الزبيب الأسود المائي يعمل كعامل اختزال يستخدم في تخليق جسيمات السيلينيوم النانوية بحجم نانو وطريقة بسيطة.

الكلمات المفتاحية: مستخلص الزبيب الأسود، السيلينيوم، الجسيمات النانوية، التصنيع الحيوي.