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BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Chemistry

COLLOIDAL SELENIUM NANOPARTICLES FUNCTIONALIZED WITH SOME POLYMERS: SYNTHESIS, CHARACTERIZATION, AND APPLICATIONS

MASTER OF SCIENCE

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Ibrahim A. M. AHMED

ABSTRACT

COLLOIDAL SELENIUM NANOPARTICLES FUNCTIONALIZED WITH SOME POLYMERS: SYNTHESIS, CHARACTERIZATION, AND APPLICATIONS

MSC THESIS
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(xiv + 63)

Selenium nanoparticles (Se NPs) have attracted attention due to their small size, low toxicity, antimicrobial activity, and many other properties and widespread uses. In addition to these advantages, it is important to enrich and functionalize Se NPs with some capping agents to further improve their properties and increase their applicability. Therefore, in this thesis, the synthesis and characterization of Se NPs using natural biopolymers chitosan (Ch) and sodium alginate (Alg) and synthetic polymers polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) and their potential to be used as an antibacterial agent were systematically studied. In addition, the effects of capping agents on the physicochemical properties of Se NPs were investigated. The synthesis of Se NPs was carried out by reduction of sodium selenite (Na_2SeO_3) as the selenium source with ascorbic acid (VC) as the reducing agent in the presence of polymer solutions (Ch, Alg, PVA, and PVP), which contained quercetin or not. The formation, shape, and size of SeNPs were evaluated using ultraviolet-visible (UV-Vis) and Fourier transform infrared (FTIR) spectroscopies and scanning electron microscopy (SEM). The SEM analysis revealed that the Se NPs were spherical, and their diameters changed depending on the polymer used. While the presence of functional groups of the coating substances in Se NPs was determined by FTIR analysis, it was observed from XRD measurements that Se NPs had an amorphous structure. Zeta potential measurements showed that Se NPs were stable, and their surface charge varied with the functional groups of the polymer. The antibacterial activity of all SeNPs was determined against *S. aureus* bacterium by broth microdilution method. The results of the microdilution assay revealed that all Se NPs examined had highly significant antibacterial activity.

KEYWORDS: Selenium nanoparticles, Capping agent, Chitosan, Alginic acid sodium salt, PVA, PVP, Quercetin, SEM, Antibacterial activity.

ÖZET

BAZI POLİMERLERLE FONKSIYONELLEŞTİRİLMİŞ KOLLOİDAL SELENYUM NANOPARÇACIKLAR: SENTEZ, KARAKTERİZASYON VE UYGULAMALARI

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Selenyum nanoparçacıkları (Se NP'ler) küçük boyutları, düşük toksisiteleri, antimikrobiyal aktiviteleri ve diğer birçok özelliği ve ayrıca yaygın kullanımları nedeniyle dikkat çekmektedir. Bu avantajlara ek olarak Se NP'lerin bazı kaplama ajanları ile zenginleştirilmesi ve işlevselleştirilmesi, özelliklerinin daha da geliştirilmesi uygulanabilirliğinin arttırılması açısından önemlidir. Bu nedenle, bu tezde, doğal biyopolimerler olan kitosan (Ch) ve sodyum aljinat (Alg) ile sentetik polimerlerden polivinil alkol (PVA) ve polivinilpirolidon (PVP) kullanılarak Se NP'lerin sentezi, karakterizasyonu ve bunların antibakteriyel ajan olarak kullanılma potansiyelleri sistematik olarak incelenmiştir. Ayrıca kaplama ajanlarının Se NP'lerin fizikokimyasal özellikleri üzerindeki etkileri araştırılmıştır. Se NP'lerin sentezi, quercetin içeren veya içermeyen polimer çözeltilerinin varlığında, selenyum kaynağı olarak sodyum selenitin askorbik asit (indirgeyici madde) ile indirgenmesiyle gerçekleştirildi. SeNP'lerin oluşumu, şekli ve boyutu, ultraviyole görünür (UV-Vis) ve Fourier dönüşümü kızılötesi (FTIR) spektroskopileri ve taramalı elektron mikroskobu (SEM) kullanılarak değerlendirildi. SEM analizi, Se NP'lerin küresel şekilli olduğunu ve kullanılan polimere bağlı olarak çaplarının değiştiğini ortaya çıkardı. Se NP'lerde kaplama maddelerinin fonksiyonel gruplarının varlığı FTIR analizi ile belirlenirken, XRD ölçümlerinden Se NP'lerin amorf bir yapıya sahip olduğu gözlemlendi. Zeta potansiyeli ölçümleri Se NP'lerin kararlı olduğunu ve yüzey yüklerinin polimerin fonksiyonel gruplarına göre değiştiğini gösterdi. Tüm SeNP'lerin antibakteriyel aktivitesi, sıvı mikrodilüsyon yöntemiyle *S. aureus* bakterisine karşı belirlendi. Mikrodilüsyon testinin sonuçları, incelenen tüm Se NP'lerin oldukça önemli antibakteriyel aktiviteye sahip olduğunu ortaya çıkardı.

ANAHTAR KELİMELER: Selenyum nanoparçacıkları, Kaplama ajanı, Kitosan, Aljinik asit sodyum tuzu, PVA, PVP, Kuersetin, SEM, Antibakteriyel aktivite.

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LIST OF ABBREVIATIONS AND SYMBOLS

Au NPs	: Gold nanoparticles
Ag NPs	: Silver nanoparticles
Pt NPs	: Platinum nanoparticles
Pd NPs	: Palladium nanoparticles
Se NPs	: Selenium nanoparticles
Se	: Selenium
CN NTs	: Carbon nanotubes
TiO_2 NPs	: Titanium dioxide nanoparticles
Ch	: Chitosan
Alg	: Alginic acid sodium salt (sodium alginate)
PVA	: Polyvinyl alcohol
PVP	: Polyvinylpyrrolidone
Q	: Quercetin
UV-Vis	: Ultraviolet-Visible
FTIR	: Fourier-transform infrared
XRD	: X-ray Diffraction
SEM	: Scanning electron microscopy
ICP-OES	: Inductively Coupled Plasma Optical Emission Spectroscopy
Na_2SeO_3	: Sodium selenite
CH_3COOH	: Glacial acetic acid
MHB	: Mueller Hilton broth
TSB	: Tryptic soy broth
VC	: Ascorbic acid
TSA	: Tryptic soy agar
°C	: Degree Celsius
rpm	: Revolution per minute
min	: Minute
mL	: Milliliter
µL	: Microliter
mM	: Millimolar
D	: Diameter
mV	: Millivolt

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DEDICATION

This humble work is dedicated to the souls of my late brothers and sisters, whom I was waiting to meet after this trip is over.

1. INTRODUCTION

Nanotechnology is the science that includes studies on the development and use of materials, systems, and devices at nanoscale, approximately in the range of 1-100 nm (1–3). These materials or systems exhibit unique properties from their macro-scale counterparts in terms of their optical, electrical, photoelectric, magnetic, mechanical, chemical, and biological properties (3). Nanoscale materials with unique properties and multi-purpose uses are called nanomaterials. The term nanomaterials comes from the Greek word "nano" meaning "very small" or "dwarf" and is used to refer to materials of extremely small size (4). The nanomaterials, comprising nanoparticles, nanocomposites, nanotubes, nanowires, or other nanoscale structures, each of which has different properties compared to their bulk counterparts, attract attention with their potential applications (5). Among these, nanoparticles generally include aggregates of individual entities or particles with nanoscale dimensions that are a part of larger materials. Nanoparticles of polymers, some metals, metal oxides, and carbon-based materials are widely known (4,5). With the developing technology, especially gold (Au), silver (Ag), platinum (Pt) and palladium (Pd) metal nanoparticles have found considerable interest in biomedicine applications (5–8). However, despite their medical benefits, metal nanoparticles are more costly to synthesize and have cytotoxic effects that limit their use (8). On the other hand, the synthesis of selenium nanoparticles (Se NPs) derived from selenium (Se) is both more economical and more likely to improve their biological properties (9). Hence, it is well known that pure Se in nano form has better biocompatibility and lower toxicity compared to its various organic and inorganic forms (10). This has enabled Se NPs to have a promising potential as antioxidant, antimicrobial, antifungal, and anticancer agents and drug carriers in applications, and many studies have supported these properties (3,4,10–16). Considering all these advantages, the synthesis, characterization, and applications of Se NPs still maintain their importance and related studies continue to increase.

Throughout this chapter, general information about nanomaterials, nanoparticles, especially Se NPs, and some theoretical information about their synthesis, characterization, and applications are mentioned in detail.

1.1 Nanomaterials

For a substance to be classified as nanomaterial, its size must be less than 100 nm in at least one direction (3,4,7,17,18). While the properties of a substance are limited in the bulk state, its properties change significantly in the nanoscale. Therefore, reducing the size of substances leads to the emergence of many new physicochemical properties and their potential usage areas increase, meaning that the properties of substances are greatly affected by their size. These size-dependent properties arise from the high surface area/volume ratio, quantum effects, and changing chemical, electronic, optical, mechanical, thermal, and magnetic properties (4,18,19). Due to their distinctive properties and functionality, nanomaterials find wide use in medicine, electronics, cosmetics, pharmaceutics, etc. (7,18). For instance, they have important applications in drug delivery, imaging, and surgical procedures due to their similarity in size to biological molecules such as proteins, receptors, DNA, and RNA, and being relatively smaller than cells (5,20,21). Since the field of nanoscale materials encompasses a wide range of industrial applications and scientific research, it is important to investigate the underlying factors that contribute to the unique properties and characteristics exhibited by such materials for a comprehensive understanding of their implications in the field of nanotechnology and nanoscience (22).

Nanomaterials can exist in a variety of forms including single, composite, aggregated, or clustered, and shapes with spherical, cylindrical, or asymmetric, etc. (1,4,7). Therefore, they can be classified according to various parameters, including their chemical composition, origin (natural or synthetic), material types (such as carbon, inorganic, organic, or composite-based), and dimensions (4,5,7). Nanomaterials can occur naturally or be synthesized through chemical, mechanical, physical, or biological processes in a variety of forms including powders, buckyballs, tubes, wires, colloids, rods, clusters, thin films, etc. (3,5,7). Furthermore, it is possible to redesign synthesized nanomaterials using various methods into preferred shapes and provide functionality for specific applications. Although many methods are used to obtain nanomaterials, there are generally two basic approaches, which are bottom-up and top-down (Figure 1.1), for their production and synthesis (3–5). Both strategies have advantages and disadvantages in terms of the properties of the nanomaterial and the application areas to be used. The bottom-up approach, which promotes the production of nanomaterials through nucleation and growth, starting from the atomic or molecular levels, along with self-assembly processes, provides precise control over the properties and functions of the resulting product (3,4,13). This method has a high potential for producing nanomaterials such as nanotubes, nanoparticles, and monolayer structures.

Another way to obtain nanomaterials with the desired properties is the top-down approach, in which it is aimed to obtain nanomaterials from large structures or bulk materials using mechanical or chemical processes (3,4,13). This approach is widely used in the production of microelectronics and the semiconductor industry with its advantages such as efficiency and production of large quantities of nanomaterials.

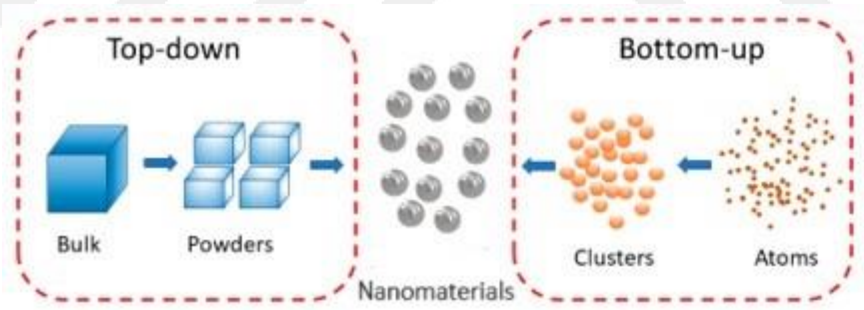

Figure 1.1. Schematic representation of the bottom-up and top-down approaches to produce nanomaterials.

1.2 Nanoparticles

Nanomaterials and nanoparticles are often used interchangeably, but there are some differences in their meanings (23). Nanomaterials is a broader term that encompasses a variety of materials at the nanoscale, including nanoparticles, nanotubes, nanowires, and other nanoscale structures (3). Each of these materials has unique properties and related applications. Therefore, nanomaterials include but are not limited to nanoparticles. Both terms refer to nanoscale dimensions, but they differ in emphasis and scope. Nanoparticles include specific particles of very small amount of an element, component, or material in the nanoscale range (3,4,7). Common examples are metal-based nanoparticles (Au, Ag), semiconductor nanoparticles (Se), polymeric nanoparticles, and carbon-based nanoparticles (e.g., carbon nanodots, carbon nanotubes, and graphene) (4). Nanoparticles can exhibit a wide variety of structures, including spheres, disks, cubes, rods, stars, and more complex geometries (5,24,25). These structures often vary depending on the synthesis method used and specific application requirements (3,5,7,25). Determining and controlling the structure and shape of nanoparticles is important to reveal their potential in various applications. The same approaches (bottom-up and top-down, Figure 1.1) are used in the synthesis of nanoparticles as in nanomaterials (4,5,7).

1.3 Selenium Nanoparticles

Selenium ($_{34}Se$), discovered by J.J. Berzelius, a Swedish chemist, in 1817, is a metalloid element in the group of chalcogens (VIA/16) that has the properties of both metals and non-metals (26–28). Although it is rarely found in the earth's crust, especially in rocks and soil, its concentration is relatively higher in certain food sources such as nuts, tuna, and salmon (26,29,30). Selenium is rarely found in pure form. It has several allotropic forms, both amorphous (non-crystalline) and crystalline. Brick-red in powder is amorphous (a-Se) form of selenium (31–33). Monoclinic selenium (m-Se) is a crystalline form with red in color and has Se_8 rings (29,33). It exists in three allotropic (α, β, and γ) forms. Trigonal selenium (t-

Se) is metallic gray to black and is the most stable crystalline form having helical chains (33). Selenium exists in a variety of structures and oxidation states (+6, +4, +2, 0, -2), including the inorganic compounds selenate (Na_2SeO_4), selenite (Na_2SeO_3), elemental (Se_8), and organic compounds selenocysteine and selenomethionine (27,30,33–36). Selenium, an important component of seleno-proteins, each containing selenocysteine in their active centers and seleno-compounds found in the human body, plays a vital function in many biological processes such as DNA synthesis, reproduction, metabolic activities, thyroid hormone regulation, and oxidative stress (35,37,38). So, it is a nutritional trace element necessary for cellular and metabolic functioning in living organisms. Besides, Se compounds serve as antifungal, potent antioxidant, antimicrobial, anticancer, and anti-aging agents and are also known to protect humans against muscle weakness and wasting (39–42). Therefore, selenium is extensively used in pharmaceuticals and gains importance in the effective fight against diseases. In general, elemental selenium (Se^0) is attracting more attention due to its low toxicity, good bioavailability, and improved absorption abilities compared to alternative forms of selenium (Se^{+6}, Se^{+4}). However, Se^0 is difficult to use in the food and pharmaceutical industries due to its poor water solubility and toxicity in high doses. Therefore, solutions are sought to reduce the toxicity and increase the effectiveness of selenium in applications. The enhancement of solubility for a chemical can be achieved through the reduction of particle size to the nanoscale and therefore increasing its specific surface area. The desired and promising solution here has been achieved thanks to nanotechnology, i.e., through the forming of nanoparticles. Likewise, selenium ions can be converted into selenium nanoparticles (Se NPs) with an oxidation number of zero (Se^0) to increase its potential for applications (43). Se NPs are a type of nanomaterial composed of elemental selenium. In the context of results obtained from experiments, it has been suggested that elemental nanosized selenium may serve as a more advantageous alternative to conventional selenium sources (44). In recent years, Se NPs have attracted attention among many metallic and metal oxide nanoparticles. Se NPs play an important role in the fields of medicine, biology, food industries, environmental studies, electronics manufacturing, energy production, and agriculture (45–50). They have various properties that make them useful due to their high biological activity and low toxic effects for a wide range of applications, including anticancer, antimicrobial, antioxidant, biocompatible, and photocatalytic (41,49,51–62). In addition, the semiconductor properties of SeNPs make them valuable for many applications such as photocells, photocopiers, photometers, and xerography, and their importance in renewable energy devices is also increasing (63,64). The advantages and disadvantages of some nanoparticles, including Se NPs, are summarized in Table 1.1.

Table 1.1. Comparison of selenium nanoparticles (Se NPS) with other nanoparticles.

Selenium nanoparticles (Se NPs) (44,65)	Gold nanoparticles (Au NPs) (66,67)	Silver nanoparticles (Ag NPs) (68,69)	Carbon nanotubes (C-NTs) (70,71)	Titanium dioxide nanoparticles (TiO_2 NPs) (72,73)
Biocompatible, antioxidant, anticancer, antimicrobial, and tunable properties	Biocompatible, tunable properties	Antimicrobial, tunable properties	Strong, lightweight, electrically conductive	Photocatalytic activity, biocompatible
Can be expensive to produce	Can be expensive to produce	Can be toxic to cells at high concentration	Can be toxic to cells	Can be toxic to cells at high concentrations

<u>Biocompatibility</u>: Se NPs are generally more biocompatible than other similar nanoparticles such as Ag NPs and C-NTs.

<u>Biological activity</u>: Since Se NPs show antibacterial, anticancer and antioxidant activities, they have the possibility of being used in many applications such as drug delivery and cancer treatment.

<u>Tunable properties</u>: By adjusting the composition, size and shape of Se NPs, their optical, electrical, and catalytic activity properties can be improved and thus can be used in various fields.

1.3.1 Some Methods Used in Selenium Nanoparticle Synthesis

Due to their obvious benefits such as biocompatibility, biological activity, reduced toxicity, and lower dosage requirements, Se NPs have potential for many applications (74,75). Therefore, the selection of synthesis technique is of great importance for the correct use of this potential. It is very important to obtain the desired properties and preserve these properties for a successful synthesis, that is, to produce advanced nanoparticles. The choice of the synthesis method of Se NPs affects many aspects such as shape, size, composition, and nanoparticles' surface properties (76,77). Colloidal forms of SeNPs cause some problems with stability, which partially limits their commercialization potential. In general, various factors such as pH, reaction time, temperature, and chemical composition affect the shape, size, and stability of SeNPs (36,77). By choosing both the mentioned factors and the appropriate synthesis method, the properties of Se NPs can be optimized. Thus, Se NPs with different physicochemical and biological properties can be produced. Basically, three types of synthesis methods stand out, these are chemical, physical, and biological methods (36,44,77).

Chemical methods: This method allows the synthesis of Se NPs with high precision, in desired sizes and as monodisperse, by the controlled reduction of selenium precursors. So, thanks to this controlled reduction, Se NPs can manage their size, shape, and surface properties. The basic approach is to reduce selenium precursors to elemental selenium using substances such as sodium borohydride or ascorbic acid as reducing agents (36,44,56,75). It is important to add capping agents to control the nucleation and growth of nanoparticles during synthesis, prevent their aggregation, and increase their stability. Surfactants and polymers are generally used as capping agents to control the synthesis process and obtain monodisperse Se NPs (44,78). The synthesis of Se NPs can also be carried out by enabling chemical reactions using hydrothermal process (75,79). In the hydrothermal process, a solution containing a selenium precursor with or without reducing agent is sealed inside a closed tank and heat can be applied (36,79). Reduction or decomposition of the precursor under the influence of both pressure and temperature leads to the successful formation of Se NPs (79).

Chemical methods offer versatility in tailoring the properties of Se NPs according to specific requirements for various fields, including medicine, catalysis, and electronics. However, there is potential for toxicity resulting from by-products or waste during the synthesis process, which can be considered a disadvantage.

Physical methods: While physical methods offer some advantages, such as precise control over morphology and size, reduced chemical contamination, and the ability to produce high-purity selenium nanoparticles, they also have some shortcomings, such as the need for special equipment and the difficulty of tailoring the properties nanoparticles. Synthesis of Se NPs using physical methods involves some techniques that utilize physical principles. Sonochemical (ultrasonic treatment), laser ablation, pulsed laser ablation and microwave irradiation are physical techniques frequently used to synthesize SeNPs (63,75,80–82). The basis of the sonochemical method is to obtain Se NPs by creating cavitation bubbles in the solution using ultrasonic waves and breaking down the selenium precursor in the solution with the help of these bubbles. This technique is simple and inexpensive, as well as having the capacity to produce nanoparticles in a certain size range (81). In the laser ablation method, which is another physical approach, the selenium precursor in a medium is evaporated using a continuous laser beam. Then, the formation of nanoparticles is achieved by condensing the resulting vapor. Similarly, another technique that uses pulsed laser beam to achieve the same result is pulsed laser ablation. The microwave irradiation process requires the use of microwaves to induce thermal energy in a solution containing a selenium precursor. Microwaves cause the solvent to heat rapidly, leading to the reduction or decomposition of the precursor and subsequent formation of nanoparticles (83). Both techniques can produce nanoparticles with a limited size range and high purity (81,84).

Biological methods: Recent studies have shown that the biosynthesis method is a more preferred approach due to its ability to reduce chemical waste and be a sustainable and environmentally friendly approach (49,65,85,86). This method enables the reduction and conversion of selenium ions into Se NPs by biological sources such as yeast, fungi, plant extracts, bacteria, and mold

(28,65,75,87). These bioresources are rich in flavonoids, phenols, amino acids, glycosides, saponins, alkaloids and steroids that play a role in the reduction of selenium salts (65). Although the biological method is preferred over chemical and physical methods because it is more sustainable and does not require special equipment, it has limitations in some parameters such as the shape, size, synthesis time, and efficiency of SeNPs.

1.3.2 Capping Agents for Selenium Nanoparticle

As mentioned before, nanoparticles expand their impact areas when their sizes are between 1 and 100 nm. However, they tend to come together due to their small size and therefore increased surface energy. This tendency to aggregate not only negatively affects the biological and physical properties of nanoparticles but can also harm the environment. To overcome these problems, capping agents are used. These substances prevent the aggregation of nanoparticles by the help of steric hindrance effects and/or electrostatic repulsion and maintain the stability of the nanoparticle formulation (88). Capping agents have a very important role in ensuring that nanoparticles have the desired physical, chemical, and biological properties (89,90). For instance, they can determine the size, shape, charge, surface of nanoparticles and their pharmacological effects such as antibacterial, antioxidant, antifungal, anticancer and antidiabetic (27,91). In addition, nanoparticles can be used to increase the loading capacity of diagnostic and therapeutic agents and to deliver them.

In general, when Se NPs appear as orange-colored, they are smaller in size, exhibit strong stability, and are more effective. Se NPs, whose activities, sizes, and stability are known to be affected by various factors, begin to transform over time into larger gray/black aggregates that exhibit biological inertness and are thermodynamically stable. Various chemicals called capping agents, such as polysaccharides, synthetic polymers, proteins, and amino acids can be used to prevent the aggregation of Se NPs, increase their durability, and stabilize them

(47,56,90,92). Among these, polysaccharides, which biopolymers composed of sugar units, are the most preferred in Se NP synthesis due to their various chemical and unique structural properties (47,79,93–95). Polysaccharides (i.e., chitosan, alginic acid, etc.) are biocompatible, exhibit stability, and can naturally decompose (96). Therefore, they can be used as reducing agents, effective stabilizers in the synthesis of Se NPs, and contribute to the functionalization of NPs. Selenium nanoparticles can form stable complexes with hydrophilic groups present in polysaccharides via non-covalent interactions, such as hydrogen bonding, electrostatic interactions, and van der Waals forces (88). This interaction not only increases the biocompatibility, stability, antimicrobial, and antioxidant properties of SeNPs but also facilitates the controlled release of them.

In addition, synthetic polymers, such as polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP), are also crucial in the production of Se NPs (41,97–99). They work as capping and stabilization agents, which aids in the controlled synthesis of Se NPs and prevents them from aggregating. As a result, a well-dispersed and stable colloidal system is formed. Additionally, these polymers can also act as reducing agents by enabling the reduction of selenium ions into elemental selenium. The PVP and PVA-assisted synthesis method is simple, cost-effective, environmentally friendly, and biocompatible, making it ideal for drug delivery, imaging, and catalysis applications.

Furthermore, there are some molecules added to increase the effectiveness of Se NPs. These are small organic molecules that mostly have antibacterial, anti-inflammatory, and antioxidant properties. Quercetin, acetylcholine, and caffeic acid are some of the most notable (100–104).

In this section, some information is also given about the coating agents used in the thesis.

Chitosan (Ch): Chitosan is a polysaccharide derived from the deacetylated form of chitin, which is commonly found in the exoskeletons of marine organisms such as shrimp, oysters, and crabs, Figure 1.2, (105). Since chitosan is a biocompatible, biodegradable, and non-toxic biopolymer, it has a wide variety of uses in the medical, cosmetic, and pharmaceutical industries. In medicine, it has a function as a hemostatic and wound-healing agent, an artificial membrane for kidney filtration, and a component material for contact lenses (105–107). In the pharmaceutical industry, chitosan is used for many purposes such as diluent, sustained release vehicle, binder, disintegrant, film-forming agent, gel matrix and microencapsulation material for many different types of drugs (108). In addition, it is also used in cosmetics, skin cares and hair care products (109).

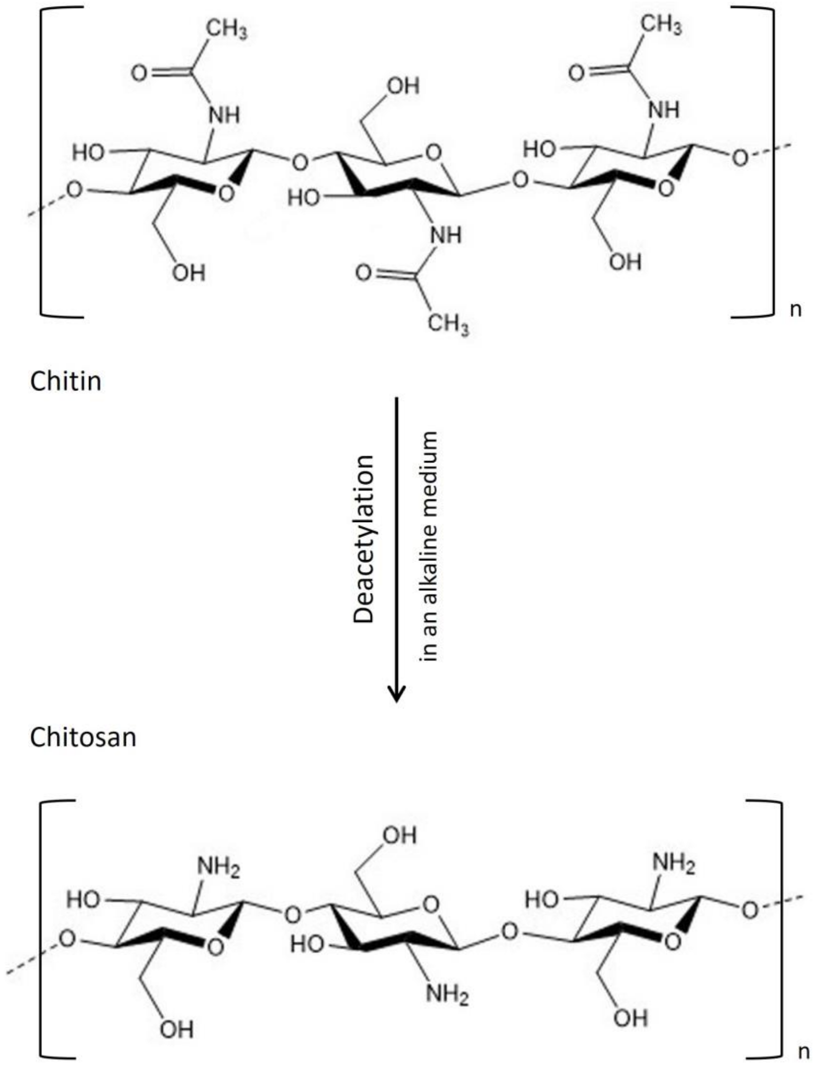

Figure 1.2. Structure of chitin and chitosan (deacetylated chitin).

Chitosan possess functional groups, amino and hydroxyl, that can interact with the surface of Se NPs and prevent their growth and aggregation, making it an ideal capping and stabilizing agent for Se NPs (40,79,95). The stabilized Se NPs with chitosan offer numerous advantages such as more stable, biocompatible, and less toxic, making them ideal for utilization in biomedical applications (40,47). For instance, chitosan-stabilized Se NPs can be more effective against cancer cells in cancer treatment due to their improved properties (110). Moreover, Se NPs can serve as a tool for targeted drug delivery to specific cells and increase the efficiency of drug delivery and their therapeutic potential (111).

Sodium alginate (Alg): Sodium alginate, also known as alginic acid sodium salt, is a biopolymer that is obtained from alginic acid; a polysaccharide found naturally in marine algae, Figure 1.3. This soluble salt can be used in various of industrial applications. In addition, biocompatibility, biodegradability, and non-toxicity of sodium alginate make it an advantageous option for biomedical applications (112,113). Furthermore, sodium alginate can increase viscosity, facilitate emulsification, promote stability, and exhibit chelating properties. The ability of sodium alginate to form complexes with metal ions makes it advantageous in removing heavy metals from wastewater and increasing the bioavailability of vital minerals (112,114). Additionally, thanks to its film-forming capacity, it allows the production of durable and flexible films, allowing them to be used for encapsulation and packaging applications.

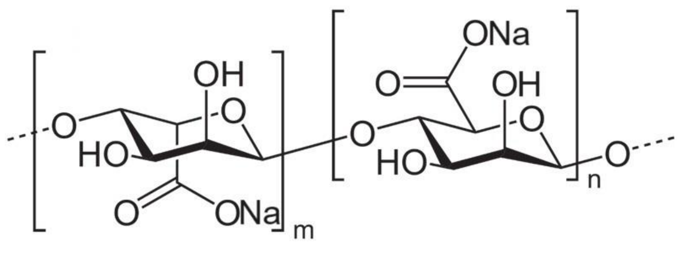

Figure 1.3. Structure of alginic acid sodium salt.

Sodium alginate has been the subject of many studies as a potentially effective capping and stabilization agent for Se NPs. In these studies, sodium alginate was shown to be effective in preventing the aggregation of colloidal Se NPs and increasing their stability (115). Sodium Alginate can form a protective barrier around the SeNPs and prevent their aggregation and precipitation by stabilizing the SeNPs through electrostatic interactions between the carboxyl groups ($-COO^{-}$) and the positively charged surface of the Se NPs.

Polyvinyl alcohol (PVA): Polyvinyl alcohol (PVA), synthesized through the hydrolysis of polyvinyl acetate (116), is a synthetic polymer and can be transformed into films, fibers, and gels, Figure 1.4. It is used in different industries, including commercial, medical, adhesives, textiles, packaging, and pharmaceuticals due to its unique combination of biocompatibility, water solubility, biodegradability, adhesive attributes, chemical resistance, and film-forming capability, properties (116,117). For instance, it is a valuable and indispensable polymer for drug delivery systems due to its low toxicity, compatibility with various substances, and its ability to form hydrogels (116).

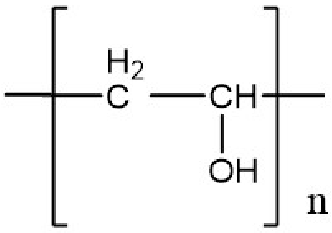

Figure 1.4. Structure of polyvinyl alcohol (PVA).

In addition, there are many studies providing evidence that PVA contributes to the controlled formation and stability of nanoparticles by serving as a stabilization and capping agent in the synthesis of Se NPs (41,83,97,98). Electrostatic interactions between the hydroxyl groups ($-OH$) of PVA and the positively charged surface of Se NPs can play a crucial role in stabilizing NPs, preventing their tendency to aggregate and precipitate in solution. The biodegradability of PVA serves to reduce its environmental impact, while its film-forming capacity allows the production of robust and flexible films suitable for encapsulating Se NPs and regulating their release. Moreover, the adhesive properties of PVA allow Se NPs to be attached to various surfaces such as electrodes or implants for different purposes (41).

Polyvinylpyrrolidone (PVP): Polyvinylpyrrolidone (PVP) is a synthetic polymer derived from the N-vinylpyrrolidone monomer by polymerization and subsequent hydrolysis of vinyl acetate, Figure 1.5. Containing functional groups such as −C=O, −C−N, and $-CH_2$ enables it to dissolve in water, alcohol, and various organic solvents (118). PVP is used as an auxiliary material in several industrial applications due to its distinctive properties such as biocompatibility, water solubility, film formation, binder, and adhesive abilities (119). For instance, in the pharmaceutical industry, PVP serves as a solubilizing agent that aids in the formulation of drug delivery systems and increases the solubility of poorly soluble drugs, and in the food industry, it acts as a thickening, stabilizing, and clarifying agent of sauces and other foodstuffs (119).

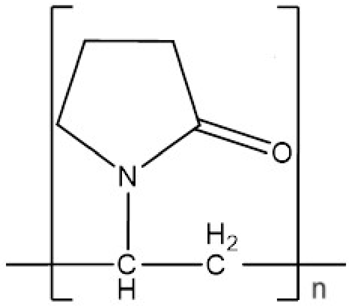

Figure 1.5. Structure of polyvinylpyrrolidone (PVP).

Additionally, the presence of different functional groups of PVP (such as −C=O, −C−N, and $-CH_2$) and its unique properties allow it to be used in Se NPs synthesis and stabilization. The stabilizing effect of PVP is attributed to the strong interactions occurring between the functional groups and positively charged surface of Se NPs, its large molecular weight, and the entanglement of its chains, which hinders the mobility of SeNPs and reduces the possibility of aggregation.

Thanks to these interactions, a defensive barrier is formed around the SeNPs, preventing their tendency to aggregate and precipitate out of solution. The biodegradability, biocompatibility, and non-toxic properties of PVP contribute to Se NPs, making it suitable for different applications (120).

Quercetin (3,3',4',5,7-pentahydroxyflavone) (Q): Quercetin, a natural flavonoid found in various fruits and vegetables, has many biological and pharmacological properties that are antiviral, antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anticancer, lipid-lowering, and wound healing (69), Figure 1.6. The distinct properties of quercetin have enabled many studies to be conducted on the synthesis of new structures with quercetin and their biological properties (102,121). Furthermore, quercetin has potential as a stabilizing and capping agent in the synthesis of Se NPs, either together with polymers or separately (51,102). The combination of Se NPs with quercetin also confers additional biological functions to the nanoparticles, making it a promising candidate for applications in medicine and biotechnology. The interaction between quercetin and Se NPs is attributed to the presence of −OH and −C=O groups on quercetin with selenium atoms present on the surface of the nanoparticle. These interactions show a high degree of effectiveness, preventing the aggregation of nanoparticles (77). Research is ongoing to elucidate these complex interactions and design Se-quercetin NPs for biomedical applications.

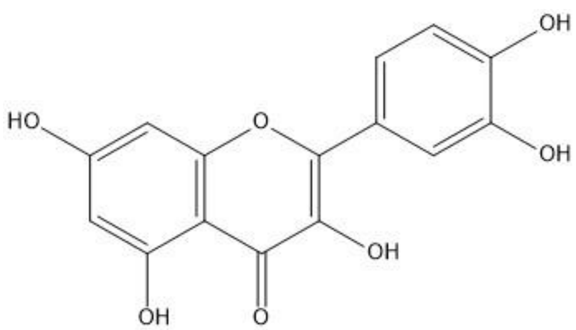

Figure 1.6. Structure of quercetin.

1.3.3 Characterization Methods for Selenium Nanoparticles

Se NPs have attracted great attention due to their properties and potential for use in various fields. Comprehensive characterization of SeNPs and a complete understanding of their physicochemical properties are of considerable importance for the specific tailoring of their synthesis to specific applications. Various techniques are used to characterize SeNPs. These techniques are briefly summarized below. The working principles of these techniques are explained in the materials and methods section of the thesis.

Ultraviolet-Visible Absorption (UV-Vis) Spectroscopy:

UV-Vis spectroscopy is a method that can provide valuable information about particle size and electronic structure from measuring the light absorbed by Se NPs (47,79). Observation of a distinct absorption peak at about UV region indicates that Se NPs production has occurred (86).

Fourier-Transform Infrared (FTIR) Spectroscopy:

FTIR can distinguish many functional groups present on the surface of Se NPs, including capping agents or molecules adsorbed to the surface (47,79). These data can be used to understand the interactions between Se NPs and their surrounding environment (93,122).

X-ray Diffraction (XRD) Analysis:

XRD can be used to examine the structure of Se NPs (47,79). The observation of sharp diffraction peaks reveals the presence of crystalline structure of Se NPs, while the absence of these diffraction peaks confirms their amorphous nature (55,86,93).

Scanning electron microscopy (SEM):

SEM reveals the morphology of SeNPs (123). High-resolution images from the SEM allow determination of the size distribution and shape of SeNPs (47).

Zeta potential Analysis:

Measurement of zeta potential plays an important role in determining the surface charge of Se NPs (93,123,124). Additionally, it can provide information about the stability of NPs and their interactions with other substances (55,95). A high zeta potential, whether positive or negative, is indicative of a stable distribution of Se NPs (95).

1.3.4 Applications of Selenium Nanoparticles

Se NPs have great potential in academic fields and industrial applications due to their large surface area, small size, and tunable properties. Especially, functionalized Se NPs have attracted interest as drug carriers and therapeutic agents due to their antioxidant, anti-inflammatory and anticancer abilities (41,125–127). Unlike traditional antibiotics, which have been associated with the development of bacterial resistance, the use of Se NPs may offer a potentially more effective option against bacterial infections (115). Interaction of Se NPs with membranes of bacterial cells can damage cellular components and disrupt bacterial metabolic processes. Therefore, they have a broad spectrum of activity against microorganisms, reduced likelihood of bacterial resistance, compatibility with biological systems, and their ability to naturally degrade (128). In addition, Se NPs play an important role in adsorbing and eliminating heavy metals, organic pollutants and radioactive pollutants from water and soil, thanks to their surface area and complex formation ability (75). Furthermore, due to their ability to improve nutrient uptake and stress tolerance in plants, Se NPs can be used to promote plant growth and increase crop yield (129). The potential of Se NPs to increase soil fertility and their capacity using as pesticides and fungicides offers an alternative to traditional chemical methods and promotes sustainable agricultural methodologies (129). Additionally, their uses, including solar cells, sensors, and catalysts, have been extensively researched (28,63,81). Se NPs can be expected to play an increasingly important role in various industries as knowledge about their properties and potential emerges.

2. AIM AND SCOPE OF THE STUDY

Selenium nanoparticles (Se NPs) have been widely studied due to their widespread applications such as antibacterial agent, drug delivery, biomedical imaging, and environmental remediation (10,41,75,102,122,130,131). SeNPs have a high surface area to volume ratio, are biocompatible, can penetrate cell membranes and target specific tissues. Since Se NPs, like other nanoparticles, tend to aggregate, stabilizing them is especially important for the applications of Se NPs. As is known, the simplest method to stabilize all nanoparticles, including Se NPs, is to cover and functionalize them with a capping agent such as a polymer or surfactant (76,90,126,132). This method prevents aggregation by reducing the interactions between Se NPs. Functionalization of Se NPs with various substances not only helps maintain the stability of the nanoparticles, but also helps in the conjugation of nanoparticles with other systems such as biomacromolecules, increasing their effectiveness and reducing their side effects (28,76,133). Thus, Se NPs become ideal for many applications. There are many studies reporting the use of various capping agents, such as surfactants, polymers, or various biocompatible substances, to functionalize Se NPs (41,55,83,90,122–124,126,127). In these studies, functionalized Se NPs has been synthesized and characterized, and their potential in various application areas has been revealed.

Although there are several studies in the literature on the use of polymers as capping agents in Se NP synthesis, there is no systematic study comparing the synthesis, characterization and antibacterial properties of Se NPs obtained using different polymers. Therefore, one of the aims of this thesis is to synthesize selenium nanoparticles (Se NPs) by reducing sodium selenite (Na_2SeO_3), a selenium precursor, using ascorbic acid (VC) as a reducing agent in the presence of natural biopolymers such as chitosan (Ch) and alginic acid sodium salt (Alg) and synthetic polymers such as polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP). Another aim of this study is to investigate the contribution of quercetin (Q), a flavonoid with antibacterial and antioxidant properties, to the properties of Se NPs. To achieve this, in addition to samples without quercetin (Ch-Se, Alg-Se, PVA-Se, PVP-Se), that is, only polymer functionalized, Se NPs containing both polymer and quercetin (Ch-Se-wQ, Alg-

Se-wQ, PVA-Se-wQ, PVP-Se-wQ) will also be synthesized. Finally, it is aimed to characterize all Se NPs using various techniques and to reveal their potential as antibacterial agents.

3. MATERIALS AND METHODS

3.1 Chemicals

All chemicals, including chitosan (medium molecular weight: 190,000–310,000 Da, 75–85% deacetylated), quercetin (≥95%), sodium selenite (Na_2SeO_3, purity 99%), dimethyl sulfoxide (DMSO, ≥99.0%), polyvinyl alcohol (PVA, MW of 31,000), , glacial acetic acid (CH_3COOH), polyvinylpyrrolidone (PVP K-30, MW of 40,000), L(+) ascorbic acid (VC, purity 99%), and alginic acid sodium salt (sodium alginate) were purchased from Merck, Sigma, Aldrich, Sisco Research Laboratories Pvt. Ltd., Carlo Erba and Alfa Aesar of high purities. All chemicals were used as received. Milli-Q water obtained by the purification system was used to prepare aqueous solutions. For antibacterial activity test, Mueller Hilton broth (Becton Dickinson, Sparks, MD, USA), tryptic soy agar and tryptic soy broth were purchased from (Bacto) Sigma Aldrich, Germany.

3.2 Synthesis of Colloidal Selenium Nanoparticles

The chemicals were weighed using a Mettler AT261 analytical balance with an accuracy of ±0.00001 g. Heidolph MR Hei-Standard magnetic stirrers and vortex (ISOLAB) were used during the sample preparation and synthesis. The obtained nanoparticles were collected by ultracentrifuging (Hitachi, Himac CP100 wx).

Selenium nanoparticles (Se NPs) functionalized with four different polymers were synthesized using the chemical method. First, 0.5% solutions of each polymer (sodium alginate, PVA and PVP) were prepared in 25 ml of distilled water. Due to the limited solubility of chitosan in water, 25 ml of 2% acetic acid solution was used. Each polymer solution was prepared in two ways, one with quercetin and the other without quercetin. Samples without quercetin were mixed on a magnetic stirrer for approximately one hour until completely dissolved, and for chitosan for twenty-four hours. For samples containing quercetin, they were mixed in a magnetic stirrer for approximately two hours to ensure that quercetin was mixed with the polymer. Since the amount of quercetin is added in excess in samples containing quercetin, excess quercetin was precipitated with the help of the ultracentrifuge (10000 rpm/10 min). The same procedure was applied to samples without quercetin. The supernatant of both samples, i.e., including those with and without quercetin, were filtered using a 0.45 μm filter. 18 mL of each of these solutions, which were first centrifuged and then filtered, were taken, and placed in the reaction vessel. While the solutions were mixed at 1400 rpm at room temperature (~25 ºC), 1 mL of 25 M Na_2SeO_3 was added to each sample and waited for 30 min to ensure that it was mixed well. After 30 min, 1 mL of 100 M ascorbic acid was added dropwise to each sample, and all samples were allowed to react for 2 hours (except chitosan, that required 24 hours). At the end of this period, Se NPs were ultracentrifuged to collect at different speeds and times depending on the polymer used (Chitosan: 14000 rpm/45 min, sodium alginate: 14000 rpm/30 min, PVA: 14000 rpm/15 min, PVP: 14000 rpm/15 min). Each collected sample was washed with Milli-Q water and centrifuged again. At the end of centrifugation, all collected Se NPs were diluted with Milli-Q water. The Figure 3.1 briefly schematizes the synthesis protocol.

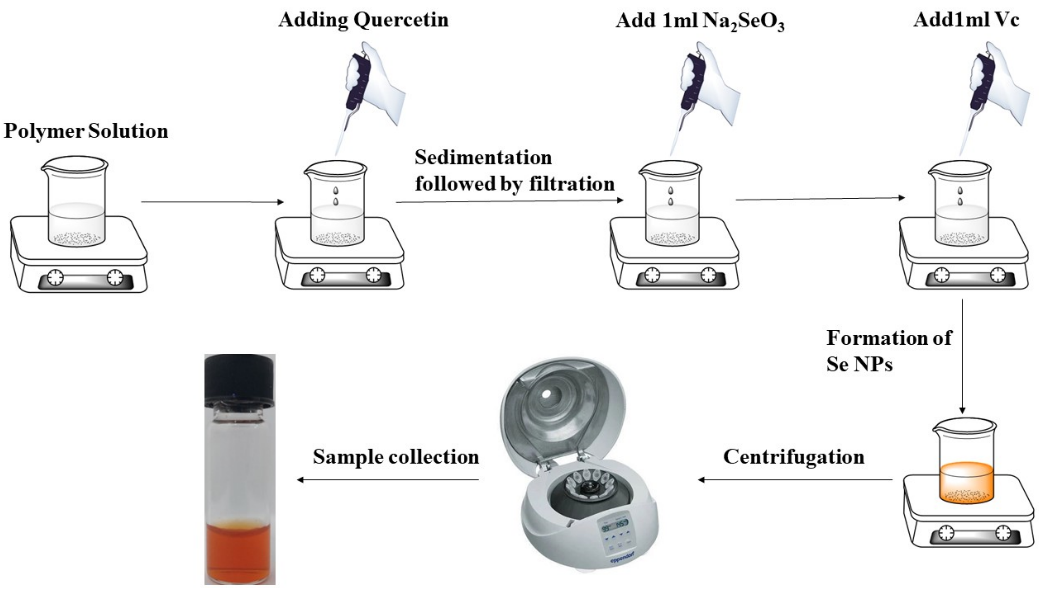

Figure 3.1. A brief schematic representation of Se NPs synthesis.

3.3 Characterization of Colloidal Selenium Nanoparticles

The characterization analysis of the polymer functionalized selenium nanoparticles was performed by various techniques. In this section of the thesis, information is given about characterization techniques for selenium nanoparticles, their working principles, and the purposes for which these techniques are used.

3.3.1 Ultraviolet-Visible Absorption Spectroscopy

Ultraviolet-visible absorption (UV-Vis) spectroscopy is a fundamental analytical technique used for both qualitative and quantitative analysis, that is, to determine structure and concentration and to facilitate reaction monitoring in various environments (134). Therefore, it is an important tool in many scientific fields such as environmental science, pharmaceutical chemistry, and biochemistry etc. This method relies on the interaction of electromagnetic radiation in the UV and visible regions with sample. When light is sent to a sample by a light source, how much of the light passing through the sample absorbed (or transmitted) is measured relative to a reference or blank (134,135). When the sample absorbs UV-Vis radiation, electrons are exited from lower energy levels to higher energy levels. As a result of the absorption of this energy, a characteristic peak appears in the UV-Vis spectrum (135). At this characteristic peak, specific wavelengths corresponding to maximum absorbance can provide useful information about the sample under study. Additionally, the Beer-Lambert law, which relates the absorbance of light to the concentration of the sample, the path length of the sample cell, and the molar absorptivity of the compound at a given wavelength, can be applied to determine the concentration of the sample (134–136).

UV-Vis absorption measurements are also widely used as a technique for the characterization of nanoparticles because they can provide information about their surface characteristics, stability, aggregation, size, and concentration (78,136,137). Therefore, in this thesis, UV-Vis measurements were taken to characterize the Se NPs. A UV–Vis double-beam spectrophotometer (Hitachi U-2900) with a quartz cell (1.0 cm path) were used for the absorption measurements,

Figure 3.2. The spectra of all Se NPs were recorded in the wavelength range of 200–800 nm using water as the reference solvent at room temperature (RT).

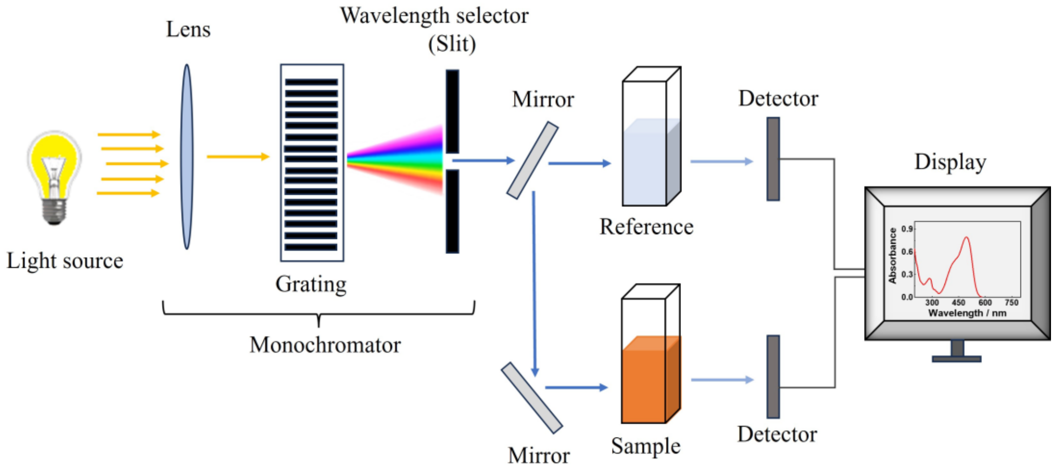

Figure 3.2. A simple schematic representation of the main components in a double-beam UV-Vis spectrophotometer.

3.3.2 Fourier-Transform Infrared Spectroscopy

In the fields of molecular spectroscopy and material characterization, Fourier-transform infrared (FTIR) spectroscopy is a valuable and widely used method. The focus of FTIR spectroscopy is the vibrational transitions of chemical bonds and is based on measuring the absorption frequency of its interaction with infrared radiation at frequencies matching the vibrational modes (136,138). Unlike traditional scattering techniques, FTIR increases the speed, accuracy, and sensitivity of data collection by simultaneously recording the entire infrared spectrum using an interferometer. FTIR spectroscopy finds applications in a wide range of disciplines, including chemistry, biology, pharmaceuticals, material sciences, and environmental sciences due to its capacity to clarify molecular structures, identify chemical components, and investigate a variety of chemical processes (136,139).

FTIR spectroscopy is one of the main methods used in the characterization of nanoparticles as it can reveal details about their functional groups and chemical properties (136,138,139). It can provide critical information regarding the stability, reactivity, surface modifications, and the chemical composition of nanoparticles. For this purpose, this method was utilized to characterize the synthesized Se NPs. FTIR spectra of all starting chemicals and SeNPs were recorded using a Perkin Elmer Spectrum Two FTIR ATR (Attenuated Total Reflectance) spectrophotometer in the range of 4000–400 cm^{-1}, Figure 3.3. First, freshly synthesized Se NPs were dropped onto a glass substrate and dried under vacuum in a dark environment at room temperature. Then, solid Se NP powders were scraped from the glass surface and placed on the ATR and the spectrum was obtained.

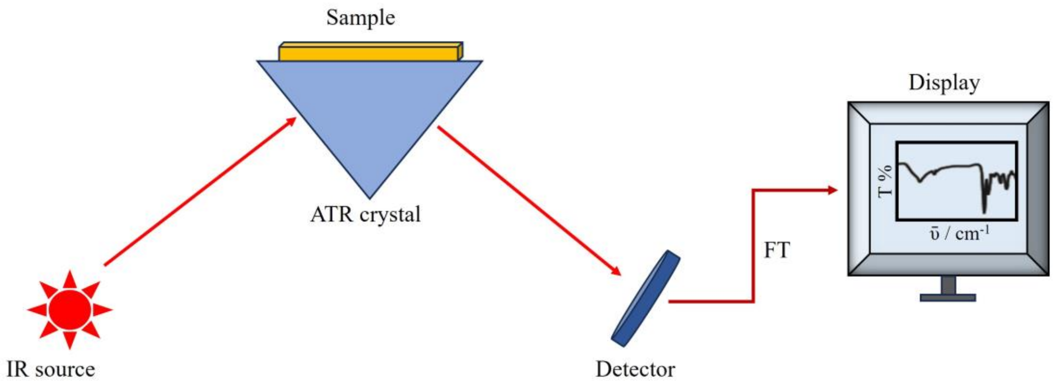

Figure 3.3. A schematic representation of the main parts of FTIR ATR spectrophotometer.

3.3.3 X-Ray Diffraction Analysis

X-ray diffraction (XRD) is an important technique used to identify and characterize complex structures such as crystals, nanomaterials, and biomolecules. Therefore, this technique is highly needed in a wide variety of scientific fields such as material science, chemistry, geology, biochemistry, and drug development (140,141). This technique provides detailed information about the spacing between crystal planes of crystal samples, unit cell parameters and the positions of their atoms, as well as information about the morphology of the formulation, that is, crystalline, semi-crystalline or amorphous structures (18,140,141). For instance, crystalline materials exhibit sharp, well-defined diffraction peaks, whereas amorphous materials do not (140).

XRD is based on the basic principle of wave interference and analysis of XRD diffraction patterns as defined in Bragg's law (140,141). A monochromatic X-ray is directed at the sample and this beam interacts with the electron clouds surrounding the atoms in the sample, Figure 3.4. Scattered X-rays propagate in different directions and interfere with each other constructively or destructively. When the path difference matches the exact number of wavelengths, constructive interference results in a diffraction pattern with distinct points or peaks at certain angles, corresponding to crystallographic planes in the sample. By measuring the angles and intensities of these diffraction points, the distance between crystal planes can be determined, information about the crystal structure, unit cell parameters and atomic positions can be provided.

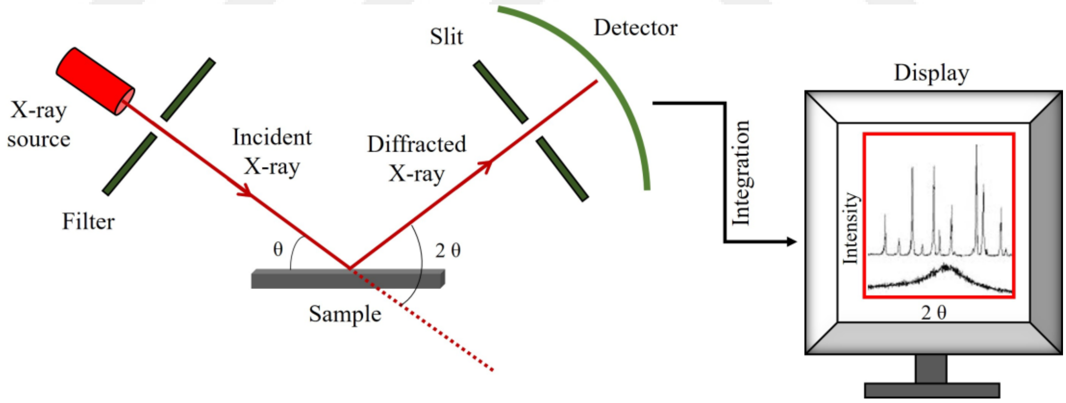

Figure 3.4. A schematic diagram for the XRD technique.

The morphology of Se NPs was analyzed by XRD measurements. First, Se NPs were dropped onto a glass substrate. It was then left to dry under vacuum at room temperature. XRD spectra of these samples were collected with the help of a Rigaku Multiflex diffractometer with Cu K_{α} radiation (λ = 1.5418 Å).

3.3.4 Scanning Electron Microscopy

Scanning electron microscopy (SEM) gives high-resolution images obtained by scanning the surface of a sample with a high-energy electron beam (18,142). Images providing high-resolution surface details are produced due to the emission of secondary electrons and backscattered electrons as a result of the electron beam hitting the sample, Figure 3.5. This method is used to obtain detailed information about the surface topography, shape, size, and composition of a sample (142). It is therefore an important tool in research and industrial applications requiring surface characterization and visual inspection in material science, nanotechnology, biology, geology, and many other disciplines.

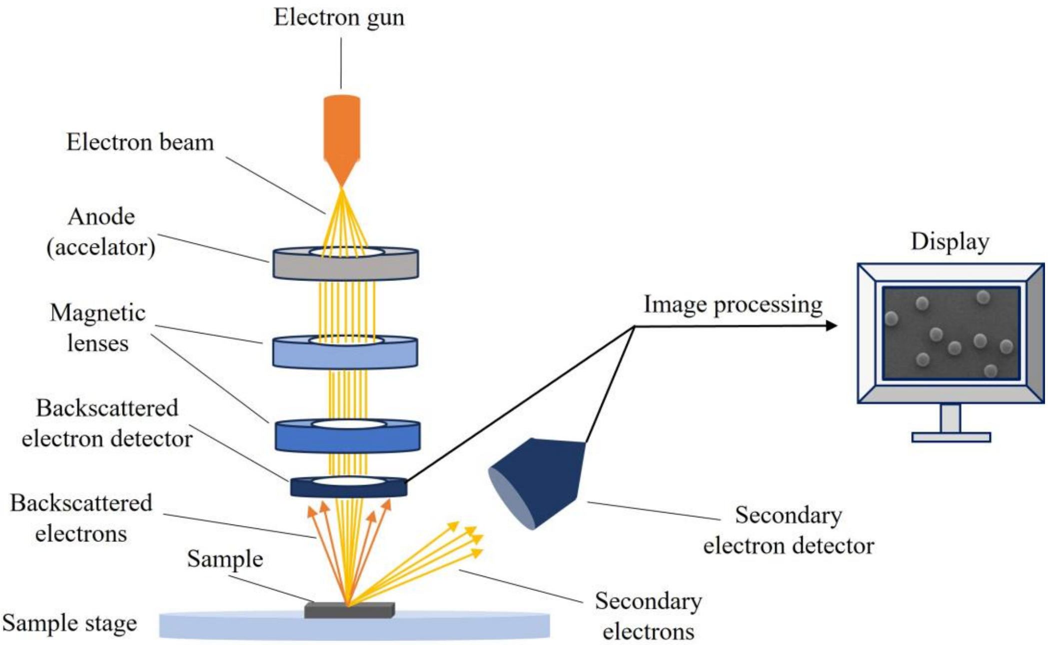

Figure 3.5. A schematic representation of the main parts of SEM.

The shape and size analysis of SeNPs were carried out by SEM technique. The SEM samples were prepared by dropping a dilute solution of SeNPs onto a silicon wafer. After thoroughly drying the samples, SEM measurements were obtained by using a FEI Quanta FEG-250 electron microscope operating at 20 kV.

An Image-J software program was used to analyze the SEM images and determine the size of the nanoparticles.

3.3.5 Zeta Potential Analysis

Zeta potential is considered an important parameter in colloid and surface science and gives information about the stability and behavior of colloidal dispersions (17,143). The zeta potential arises from the electrical double layer formed at the interface between the particle surface and the surrounding fluid (17,144). This potential affects the stability of the dispersion; Particles with higher zeta potential generally exhibit greater electrostatic repulsion, preventing aggregation, sedimentation, and coagulation (123,143). Therefore, zeta potential measurements are important to understand and control stability of the colloidal systems (143). Zeta potential values allow researchers to optimize formulations in various fields such as pharmaceuticals, cosmetics, water purification, and nanotechnology. The magnitude and sign of the zeta potential, which is the basic indicator of colloidal stability, is important in adapting materials to specific applications.

In zeta potential measurements (Figure 3.6), an electric field is applied to the sample, causing charged particles to move toward oppositely charged electrodes (17). As the particles move, they displace surrounding ions and create a shear plane, forming a liquid layer that moves in the opposite direction. At the same time, a laser beam is directed at the sample. Light scattered by moving particles is perceived at a certain angle. The Doppler shift in scattered light is analyzed to provide information about the speed and direction of the particles (electrophoretic mobility) (17). From this mobility, the zeta potential is calculated with the help of some equations and the device presents this value as zeta potential.

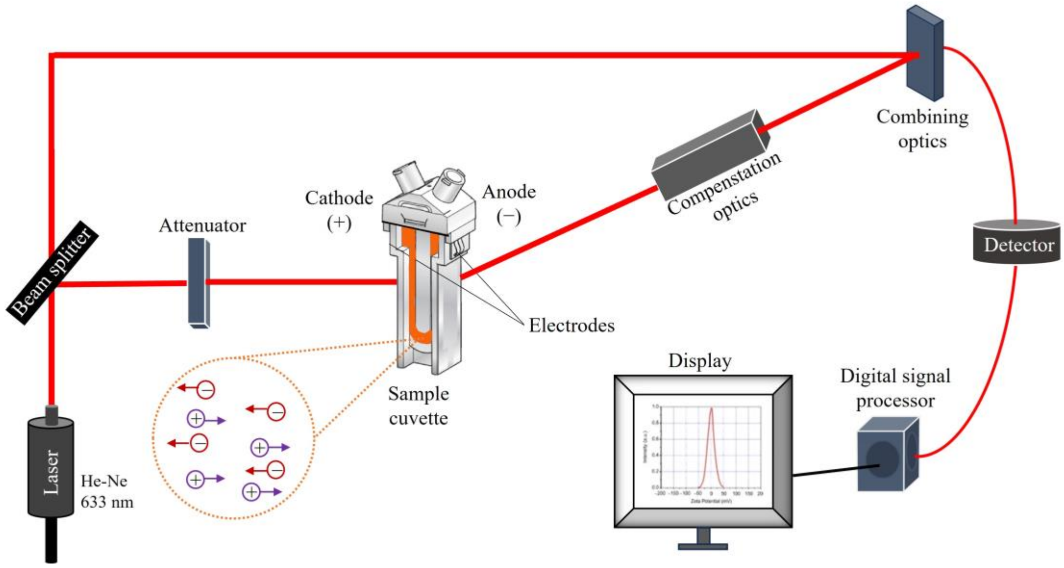

Figure 3.6. A schematic representation of typical components for zeta potential measurements.

Zeta potential measurements were taken to determine both the stability and surface charge of the synthesized Se NPs. These measurements were performed with a Malvern Nano–Z instrument at 25 ± 0.1 ºC. The data was taken as the average of three independent measurements from10 runs.

3.3.6 Inductively Coupled Plasma Optical Emission Spectroscopy

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is a powerful and versatile technique widely used in elemental analysis (136). In this method, the sample is aerosolized with a nebulizer, Figure 3.7. The aerosol is introduced into an inductively coupled plasma (a high-temperature ionized gas) powered by a radiofrequency electromagnetic field (136). Plasma is used to atomize and excite the elements in the sample. The resulting atoms are excited to higher energy states due to the high temperature in the plasma. As they return to their ground state, they emit light at characteristic wavelengths associated with each element. The emitted light passes through a spectrometer, and an emission spectrum specific to the elements is obtained. The characteristic emission spectra of each element are used to identify the elements present in the sample and to determine the composition and concentration of the sample by comparison with known standards.

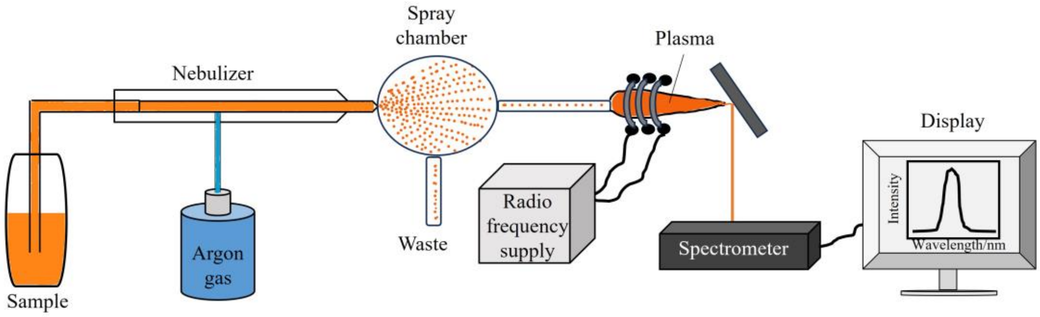

Figure 3.7. Schematic representation of typical components of ICP-OES measurements.

ICP-OES is an extremely sensitive method. It can detect the elemental composition of the sample even at very low concentrations. Therefore, this method is an important tool in elemental analysis in various fields such as environmental chemistry, pharmaceuticals, metallurgy, and materials science, as well as in research, quality control, and process optimization (136).

In general, determination of the amount of selenium in Se NPs is important in order not to exceed toxic levels in applications. ICP-OES analysis is a widely used analytical method that provides quantitative data on selenium concentration in Se NPs (9,122,128). Determination of Se concentration of Se NPs was carried out with a Perkin Elmer Avio 220 Max ICP-OES instrument. The Se concentrations of stock solutions of Se NPs are given in Table 3.1

Table 3.1. Se concentrations (mg L^{-1}) in stock solutions of functionalized Se NPs obtained from ICP-OES measurements.

Ch-Se	Ch-Se wQ	Alg-Se	Alg-Se wQ	PVA-Se	PVA-Se wQ	PVP-Se	PVP-Se wQ
316.347	300.383	485.694	288.179	1465.456	1071.518	1146.707	397.355

3.4 Antibacterial Test for Colloidal Selenium Nanoparticles

Firstly, lyophilized *Staphylococcus aureus* (*S. aureus*) bacteria disc (BD, Microtrol Discs-Becton Dickinson Laboratories, France) was placed in test tubes containing 2 ml of Tryptic Soy Broth (TSB) and incubated at 37 ºC overnight. After incubation, a bacteriological loop of broth was seeded onto Tryptic Soy Agar (TSA) plates and incubated at 37 ºC for 2 days. After 2 days, a single colony was taken and streaked onto a new TSA plate for pure culture and incubated for an additional 2 days at 37 ºC. Then, 1-2 loops of pure culture for each bacterial strain were transferred into 10 ml of Tryptic Soy Broth (TSB) in a test tube. The turbidity of each suspension was adjusted to achieve turbidity visually comparable to the 0.5 McFarland standard (1.5×10^8 cfu/ml) (145,146).

The lowest concentration of an antimicrobial agent is the minimum inhibition concentration (MIC) which prevents visible growth of a microorganism in broth microdilution susceptibility test (41). The antibacterial activities of the Se NPs in aqueous solution were evaluated against *S. aureus* (ATTC 25923) pathogenic bacterial strain via broth microdilution method with some modifications (55,130). Mueller Hinton Broth (MHB) was utilized in accordance with the National Committee for Clinical Laboratory Standards' microdilution plate procedure (147). For the antimicrobial activity test, 200 µL of double-strength MHB and 200 µL of each Se NPs at different ppm were added to each well from the first (1^{st}) to the sixth (6^{th}) column of a 96-well microplate, Figure 3.8. From columns seventh (7^{th}) to twelfth (12^{th}), one row contained 200 µL of single-strength MHB, and the other row contained nanoparticles prepared by the same procedure as in columns 1–6, to measure the absorbance of nanoparticles without pathogenic bacterial culture as blank. Then, 10 µL of pathogenic bacterial culture (the adjusted OD_{600} bacterial suspension; 10^8 cfu/mL) was transferred to each well from 1st column to 6th column and to the control wells containing 200 µl of the medium broth. Microplate was incubated at 37 ˚C for 18-24 hours. After the incubation, the MIC values were read as the lowest concentrations of Se NPs which inhibited visible growth of a microorganism in the wells. The experiments were performed as three replicates.

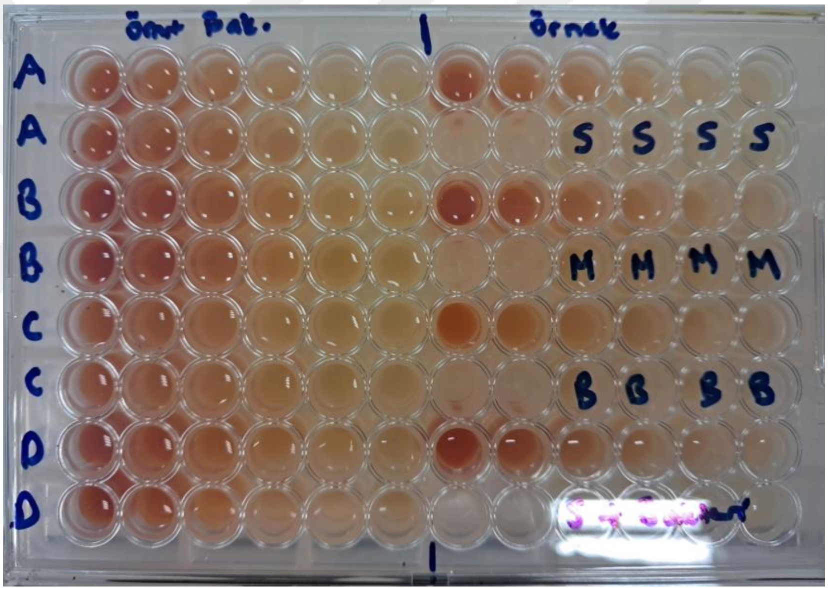

Figure 3.8. Appearance of the 96-well microplate with Se NPs for antibacterial test.

4. RESULTS AND DISCUSSION

4.1 Synthesis of Colloidal Selenium Nanoparticles

The selenium nanoparticles (Se NPs) were synthesized using the chemical method, which is the simplest and most common method (28,31,36,75,79). Sodium selenite (Na_2SeO_3) was used as the selenium precursor, ascorbic acid ($C_6H_8O_6$) as the reducing agent and water as the solvent. Se NPs were obtained by reducing Se^{+4} ions to Se^0 with the help of ascorbic acid at room temperature. Four different polymers, two of which were biopolymers (chitosan and alginic acid sodium salt) and the other two were synthetic polymers (polyvinyl alcohol and polyvinylpyrrolidone) were used to functionalize and stabilize the Se NPs. Additionally, quercetin was also used in the preparation of Se NPs to further increase its effectiveness in biological applications. Thus, two different samples, one containing quercetin and the other without quercetin, were obtained. The colorless solutions turned orange during the reaction, indicating the formation of Se NPs.

4.2 Characterization of Colloidal Selenium Nanoparticles

This section presents the characterization results of Se NPs obtained by various techniques.

4.2.1 Ultraviolet-Visible Spectroscopy Measurements

UV-Vis absorption spectroscopy is one of the most widely-used techniques to characterize colloidal Se NPs (41,47,75,79). When Se NPs are formed, Se^{4+} in SeO_3^{2-} ions are reduced to Se^0; therefore, a characteristic absorption peak appears on the higher energy side of the spectrum showing the formation of nanoparticles (9,47,79,96). For this purpose, UV-Vis absorption measurements were taken in aqueous media at room temperature to characterize synthesized colloidal Se NPs. UV-Vis spectra of Se NPs in the range of 200 to 800 nm are shown in Figures 1–4.

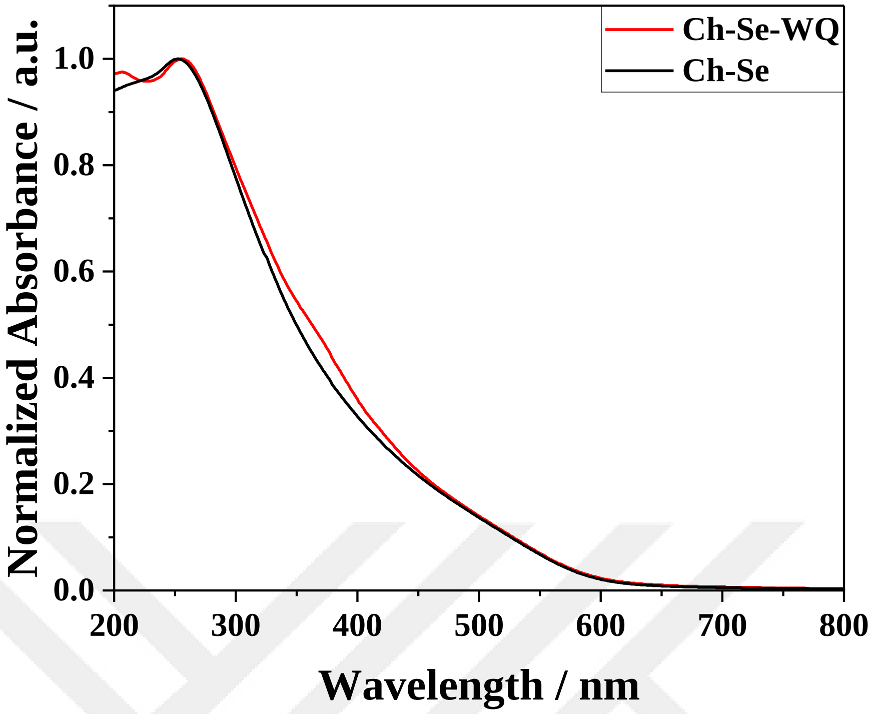

Figure 4.1. UV–Vis spectrum of chitosan-selenium NPs with (Ch-Se-wQ) and without (Ch-Se) quercetin.

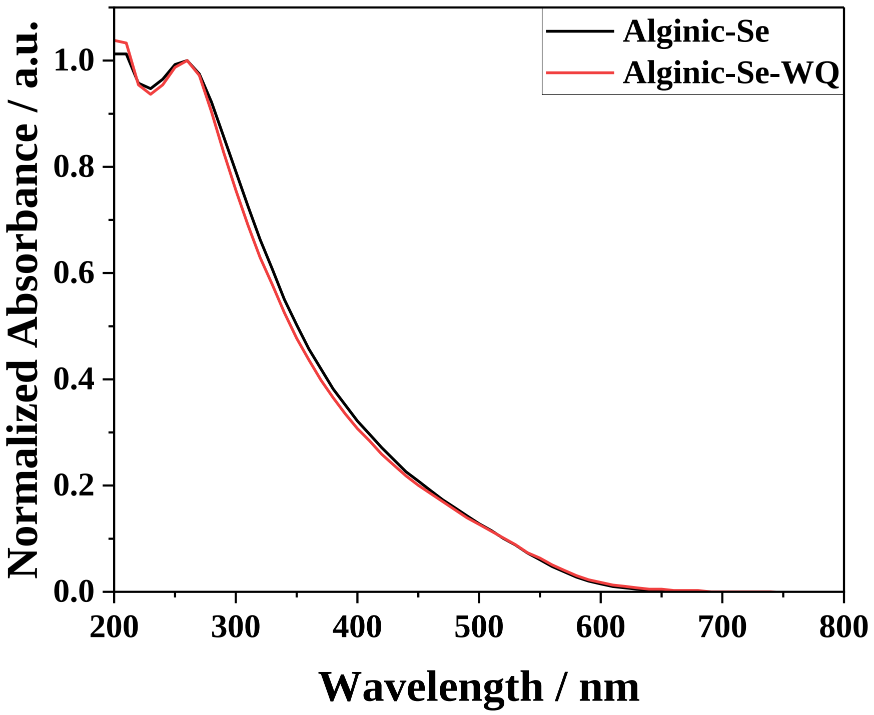

Figure 4.2. UV–Vis spectrum of alginic acid sodium salt-selenium NPs with (Alg-Se-wQ) and without (Alg-Se) quercetin.

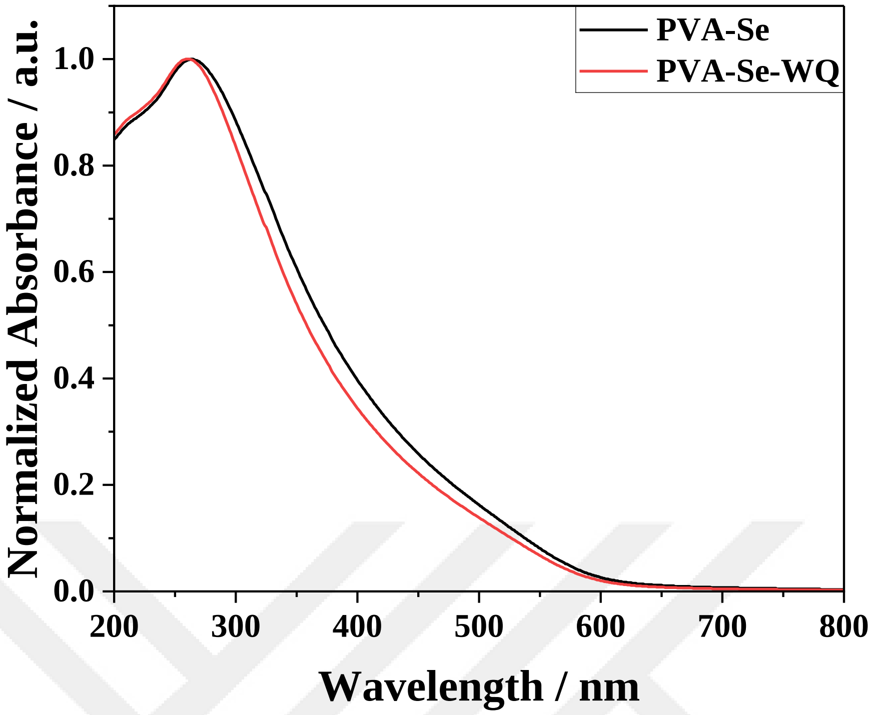

Figure 4.3. UV–Vis spectrum of polyvinyl alcohol-selenium NPs with (PVA-Se-wQ) and without (PVA-Se) quercetin.

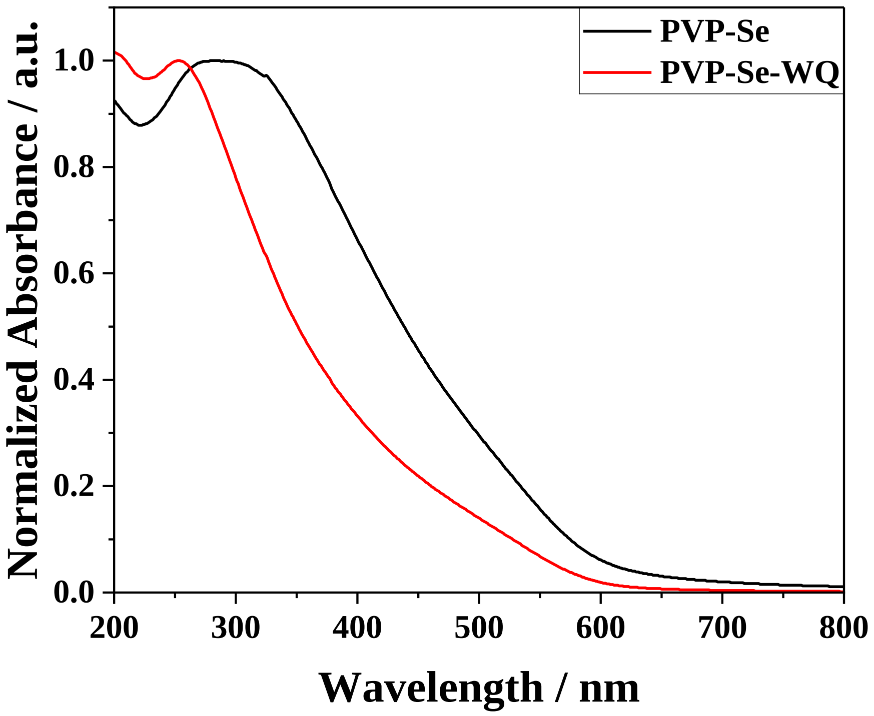

Figure 4.4. UV–Vis spectrum of polyvinylpyrrolidone-selenium NPs with (PVP-Se-wQ) and without (PVP-Se) quercetin.

As shown in Figures, the absorption spectra of the Se NPs exhibited broad absorption bands at varying wavelengths depending on the capping agent used (chitosan, alginic acid sodium salt, polyvinyl alcohol and polyvinylpyrrolidone). Ch-Se and Ch-Se-WQ displayed maximum absorption peaks at about 253 and 256 nm, while Alg-Se and Alg-Se-WQ showed absorption maxima at 257 and 259 nm, respectively, Figures 1 and 2. As displayed in Figure 3, the absorption maxima for PVA-Se and PVA-Se-WQ were observed at 263 nm and 260 nm, respectively. On the other hand, for PVP-Se and PVP-Se-WQ, the absorption maxima were located at 281 and 253 nm, respectively, Figure 4. It was shown that except for Se NPs obtained with PVP, the difference between the wavelengths observed in Se NPs with and without quercetin is around 2-3 nm and are quite close to each other. However, the wavelength observed for Se NPs functionalized with PVP and containing quercetin (PVP-Se-WQ) is significantly lower than that of without quercetin (PVP-Se). This observation is anticipated to be due to the higher solubility of quercetin in PVP than other polymers, thus limiting the size of Se NPs during synthesis. This interaction resulted in a phenomenon known as a blue shift in the absorption wavelength, i.e. from 281 nm to 253 nm. As it is known, small-sized particles are expected to have high energy and absorb light at lower wavelengths, so UV-Vis absorption spectra can provide information about the size of nanoparticles (47,79). Based on this, the size of the PVP-Se-WQ sample was expected to be smaller than that of PVP-Se. As expected, the results obtained from the SEM were found to confirm the UV-Vis results (see section 4.2.4 Scanning Electron Microscopy Measurements).

4.2.2 Fourier-Transform Infrared Spectroscopy Measurements

Fourier-transform infrared (FTIR) spectra all samples were obtained in the range of 400-4000 cm^{-1}. Firstly, the FTIR spectra of all starting materials, that are polymers, capping agents, reducing agent and selenium precursor, were performed to reveal their functional groups. Figure 4.5 shows the FTIR spectra of chitosan, alginic acid sodium salt, polyvinyl alcohol, and polyvinylpyrrolidone, ascorbic acid, sodium selenite, and quercetin.

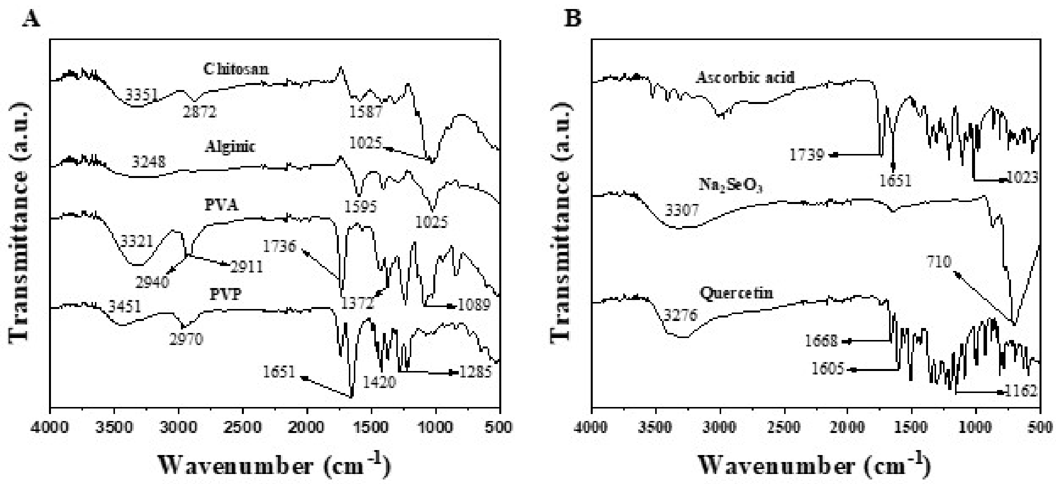

Figure 4.5. FTIR spectra of starting materials (A) chitosan, alginic acid sodium salt, polyvinyl alcohol, and polyvinylpyrrolidone, (B) ascorbic acid, sodium selenite, and quercetin.

The FTIR spectrum of chitosan exhibited broad absorption band at 3351 cm^{-1} corresponding to O-H stretching vibrations. In addition, the absorption bands for chitosan at 2872, 1587 and 1025 cm^{-1} were attributed to C-H stretching, N-H bending, C-O stretching vibrations, respectively, Figure 4.5 (A). The spectrum of alginic acid sodium salt showed characteristic absorption bands at 3248, 1595, and 1025 cm^{-1} corresponding to O-H, C=O, and C-O stretching vibrations, respectively, Figure 4.5 (A). The spectrum of PVP displayed absorption bands at about 3451, 2970, 1651, 1420, and 1285 cm^{-1}, which were attributed to O-H stretching, C-H stretching, C=O stretching, C-H bending, and C-N stretching vibrations, respectively, Figure 4.5 (A). The absorption bands at nearly 3321, 2940, 2911, 1736, 1372, and 1089 cm^{-1} in the spectrum of PVA represented O-H stretching, C-H stretching, C=O stretching, C-H bending, and C-O stretching vibrations, respectively, Figure 4.5 (A). In the spectrum of quercetin, characteristic absorption bands at 3276, 1668, 1605, and 1161 cm^{-1} was assigned to O-H, C=O, C=C (aromatic ring), and C-O stretching vibrations, respectively, Figure 4.5 (B). According to the spectrum of Na_2SeO_3 the absorption bands were at 3307 and 710 cm^{-1} belonged to O-H stretching and O-Se-O bending vibrations, Figure 4.5 (B). As shown in Figure 4.5 (B), the FTIR spectrum of ascorbic acid demonstrated that the absorption bands were 1739, 1651, and 1023 cm^{-1} for C=O stretching vibrations of lactone carbonyl, C=C stretching vibrations, and C-O stretching vibrations, respectively.

The presence of functional groups on the surface of nanoparticles due to the interaction of capping agents with Se NPs can be determined by measuring vibrational frequencies by FTIR spectroscopy (9,41,47,75,79,95,96,124,148). Therefore, FTIR measurements were performed to identify the interaction between polymers, chitosan, alginic, PVA, PVP, and Se NPs. Figures from 4.6 to 4.9 shows the FTIR spectra of functionalized Se NPs. As shown in Figure 4.6, Ch-Se-WQ and Ch-Se displayed absorption bands at 3241 and 3225 cm^{-1}, corresponded to O-H stretching vibrations which were similar with the peak of pure chitosan at 3351 cm^{-1}. A red shift was observed in the absorption peaks of hydroxyl groups of chitosan for Ch-Se-WQ (~110 cm^{-1}) and Ch-Se (~126 cm^{-1}), indicating of the interaction between the -OH groups and surface atoms of Se NPs.

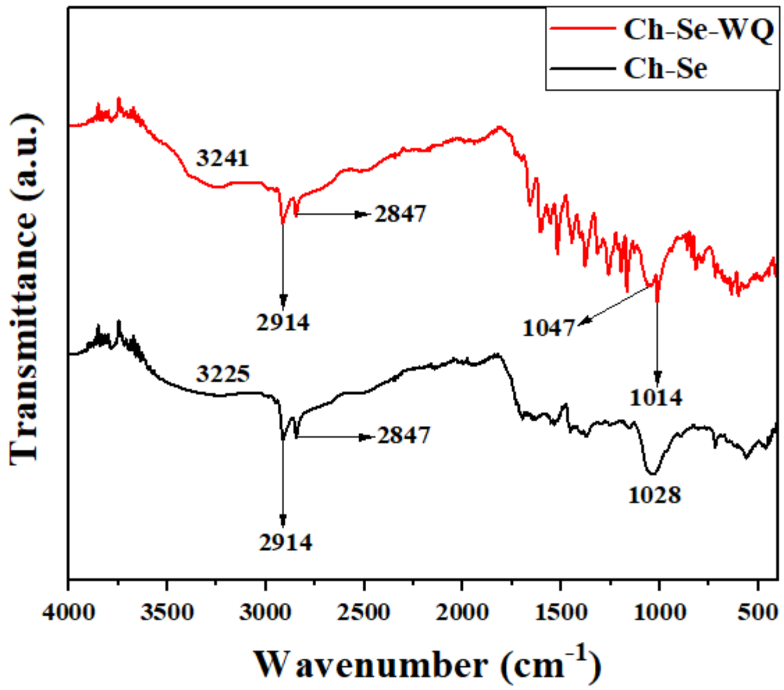

Figure 4.6. FTIR spectra of chitosan-selenium NPs with (Ch-Se-wQ) and without (Ch-Se) quercetin.

As displayed in Figure 4.7, the absorption bands at 3235 (Alginic-Se-WQ) and 3270 cm^{-1} (Alginic-Se) assigned to O-H stretching vibrations shifted compared to that of alginic acid sodium salt (3248 cm^{-1}). However, the absorption band of the carbonyl (C=O) group appeared at 1595 cm^{-1} for alginic acid sodium salt blue shifted to 1613 (with quercetin) and 1648 cm^{-1} (without quercetin) for Alginic-Se NPs. Similar behavior was also observed for the absorption band of C-O stretching vibrations from 1025 cm^{-1} to 1034 and 1048 cm^{-1}, respectively. As stated in the literature, these results revealed that the blue or red shift in the absorption bands was caused by the interaction of the surface atoms of Se NPs with the functional groups of chitosan and alginic acid sodium salt (47,57,75,96,124,148).

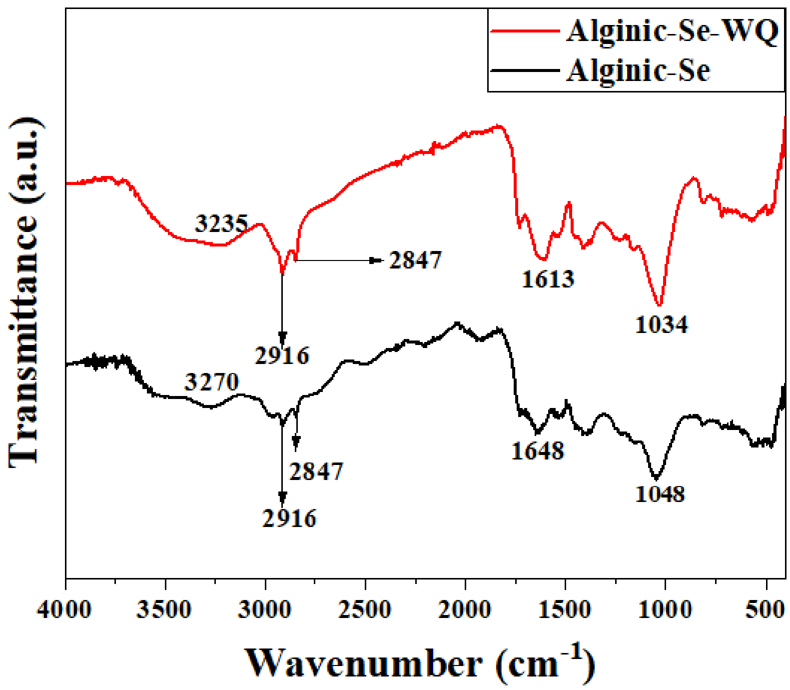

Figure 4.7. FTIR spectra of alginic acid sodium salt-selenium NPs with (Alg-Se-wQ) and without (Alg-Se) quercetin.

The FTIR spectra of PVA-Se-WQ and PVA-Se exhibited broad absorption bands at about 3269 and 3181 cm^{-1}, respectively, corresponding to O-H stretching vibrations, Figure 4.8. The absorption bands at 2901 cm^{-1} for PVA-Se-WQ and

2914 cm^{-1} for PVA-Se were attributed to C-H stretching vibrations. The characteristic peak at 1714 cm^{-1} for PVA-Se-WQ and 1719 cm^{-1} for PVA-Se were related to C=O stretching vibrations. However, the absorption peak of hydroxyl group at 3321 cm^{-1} in PVA was red-shifted to 3269 cm^{-1} in PVA-Se-WQ and 3181 cm^{-1} in PVA-Se, implying the bonding interaction between O-H groups in PVA and Se NPs.

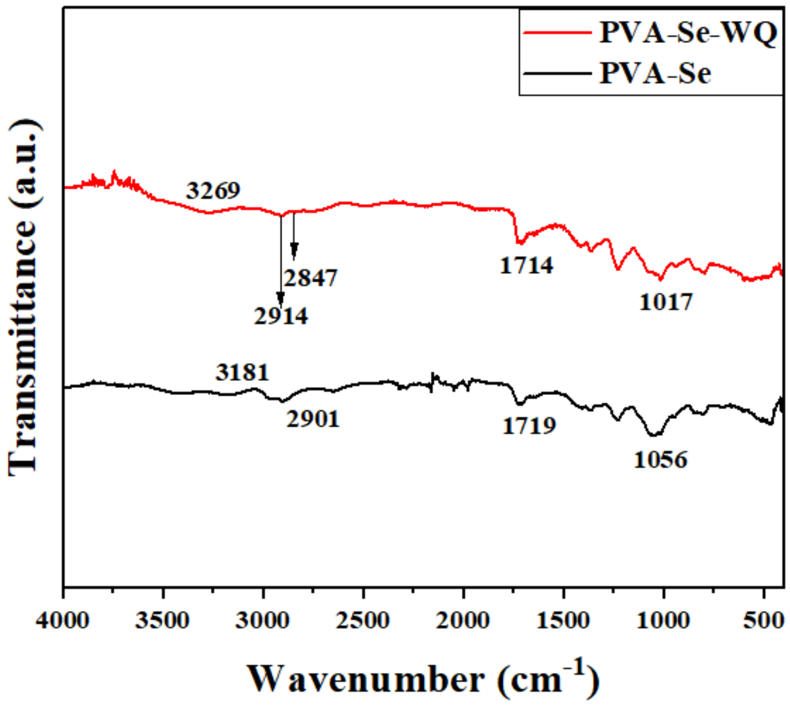

Figure 4.8. FTIR spectra of polyvinyl alcohol-selenium NPs with (PVA-Se-wQ) and without (PVA-Se) quercetin.

The O-H stretching vibration peaks of PVP-Se-WQ and PVP-Se were located at 3197 and 3412 cm^{-1}, respectively, Figure 4.9. While the absorption bands at 2911 and 2913 cm^{-1} were attributed to C-H stretching vibrations, the absorption band at 1648 cm^{-1} was related to C=O stretching vibration. As with other polymers, the red shift in the absorption peaks indicated that the carbonyl (C=O) and hydroxyl groups (O-H) of PVP interacted with SeNPs.

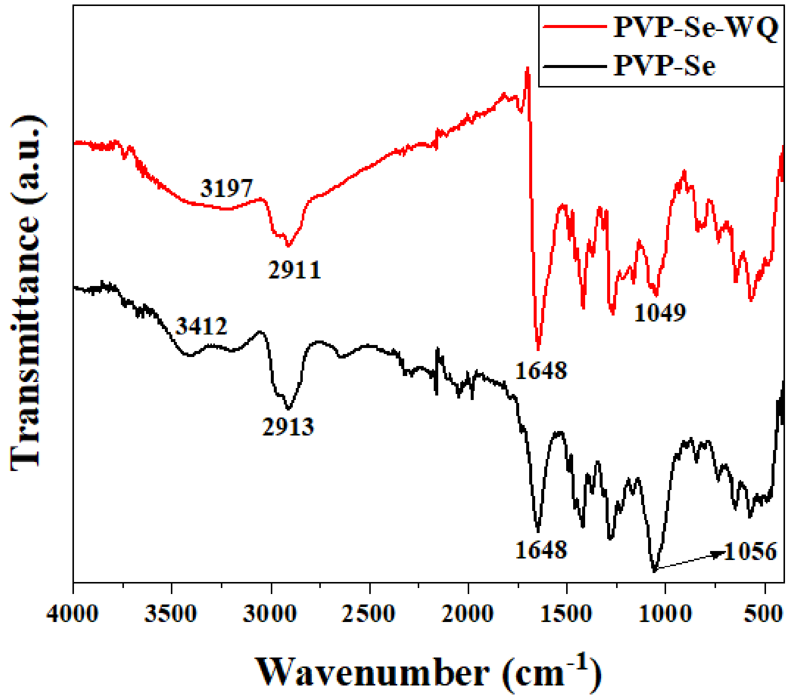

Figure 4.9. FTIR spectra of polyvinylpyrrolidone-selenium NPs with (PVP-Se-wQ) and without (PVP-Se) quercetin.

4.2.3 X-Ray Diffraction Measurements

The absence of Bragg's peaks in the XRD spectra of Se NPs functionalized using polymers (chitosan, alginic acid, PVP, and PVA) with and without quercetin indicated that the Se NPs are amorphous, Figures 4.10 - 4.13. It turned out that all synthesized Se NPs exhibited broad peaks similar to those obtained in the literature indicating the amorphous nature of the Se NPs (55,75,78,79,86,123,124,148,149). Moreover, this can also confirm presence of capping agents and the formation of nanosized particles (75,149). This amorphous nature may have several potential benefits, such as increased antibacterial and antioxidant activity and increased biocompatibility.

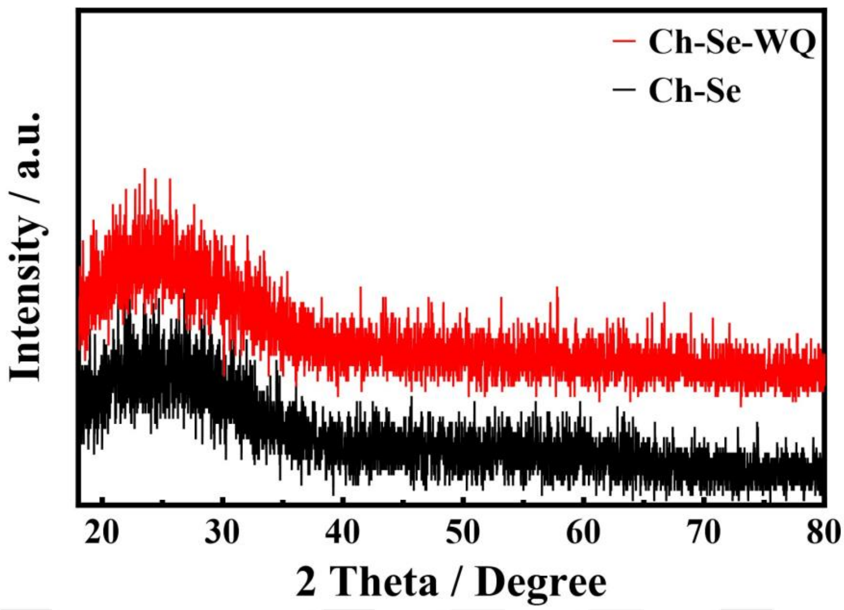

Figure 4.10. XRD spectra of chitosan-selenium NPs with (Ch-Se-wQ) and without (Ch-Se) quercetin.

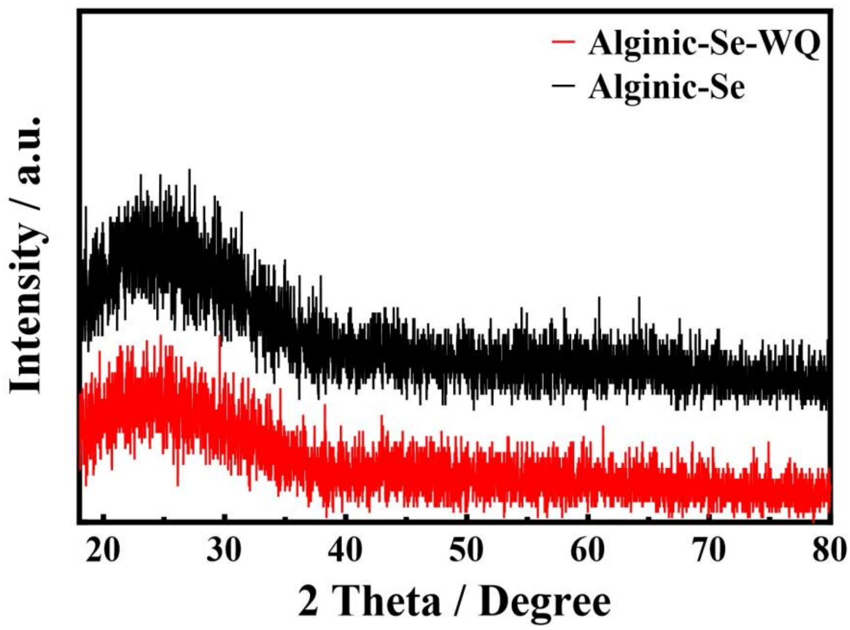

Figure 4.11. XRD spectra of alginic acid sodium salt-selenium NPs with (Alg-Se-wQ) and without (Alg-Se) quercetin.

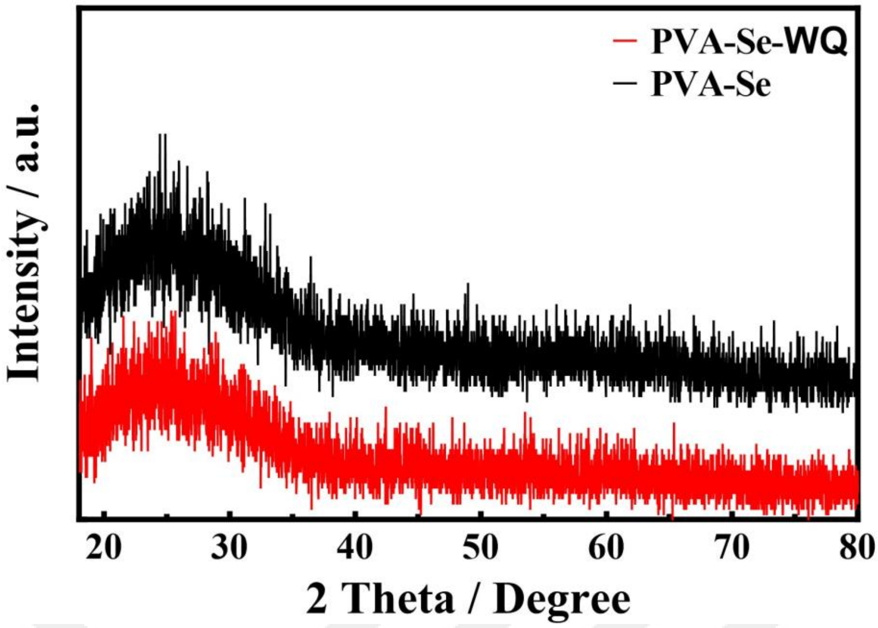

Figure 4.12. XRD spectra of Polyvinyl alcohol-Selenium NPs with (PVA-Se-wQ) and without (PVA-Se) quercetin.

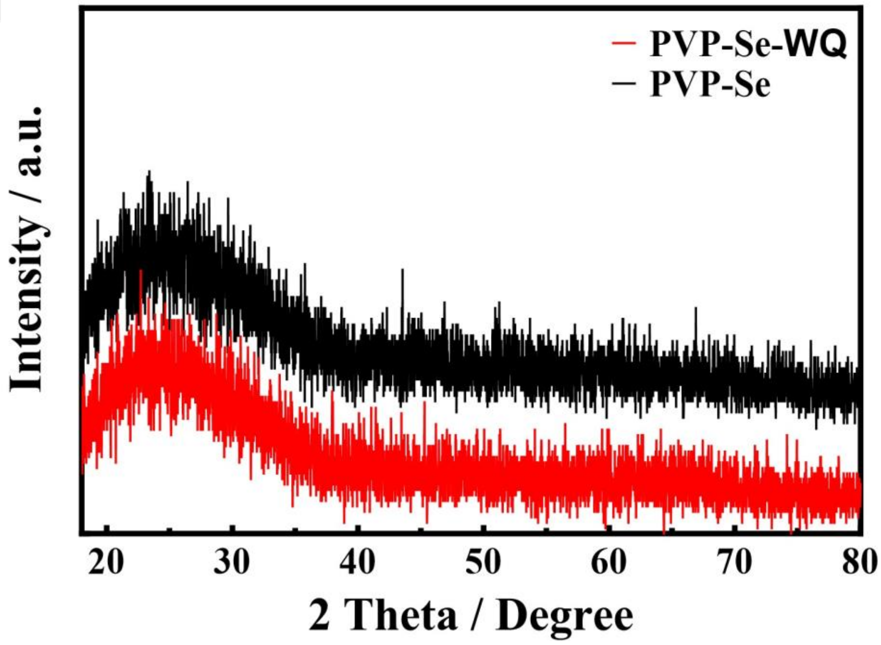

Figure 4.13. XRD spectra of Polyvinylpyrrolidone-Selenium NPs with (PVP-Se-wQ) and without (PVP-Se) quercetin.

4.2.4 Scanning Electron Microscopy Measurements

Using scanning electron microscopy (SEM) technique, it was investigated how the capping agents (i.e., chitosan, alginic, PVP and PVA) affected the size and morphology of Se NPs with and without quercetin. Figures from 4.14 to 4.21 show the SEM images and the size distribution of the Se NPs stabilized with different polymers. It was observed that all synthesized Se NPs were well dispersed, in spherical shapes and different sizes (41,47,75,78,95,148).

The size distribution and the mean size of Se NPs stabilized with different polymers and quercetin was determined using Image J program from SEM images. The mean size of Ch-Se-wQ was 62.51 ± 9.04 nm, while the mean size of Ch-Se was 58.74 ± 5.75 nm, Figures 4.14 and 4.15. As shown in Figures 4.16 and 4.17, the mean sizes of Alg-Se-wQ and Alg-Se were found to be 65.93 ± 6.63 and 69.96 ± 8.52 nm, respectively. Similarly, it was obtained that the mean sizes of the PVA-Se-wQ and PVA-Se were 64.18 ± 5.1 and 70.51 ± 7.35 nm, respectively. On the other hand, the mean size of the PVP-Se-wQ was 47.13 ± 6.95 nm, while the mean size of the PVP-Se was 87.48 ± 3.04 nm; This can be attributed to the good solubility of quercetin in PVP compared to other polymers. This may restrict the size of Se NPs due to the interaction between quercetin and PVP, which may change the surface properties of the nanoparticles and affect their size. As a result, it can be said that quercetin has a slight to significant effect on the size of the SeNPs, depending on the polymer used. The decreasing trend both in the mean size and the size distribution of Se NPs with quercetin (except chitosan) is in good agreement.

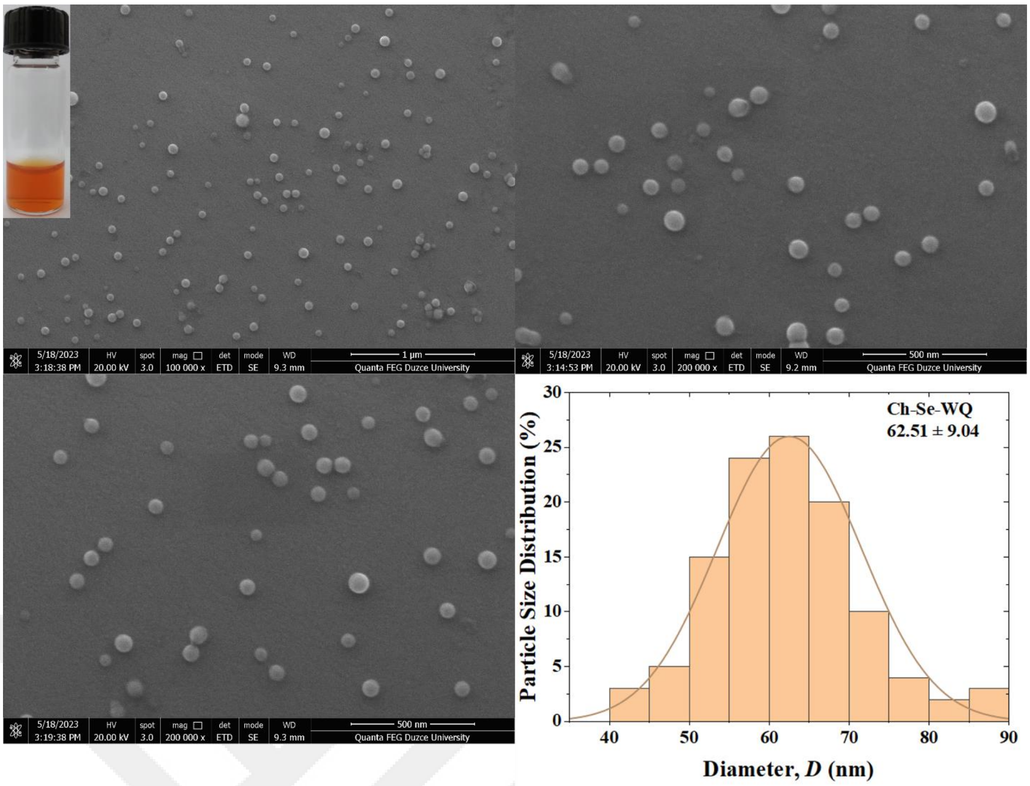

Figure 4.14. SEM images and size distribution of chitosan-selenium NPs with quercetin (Ch-Se-wQ).

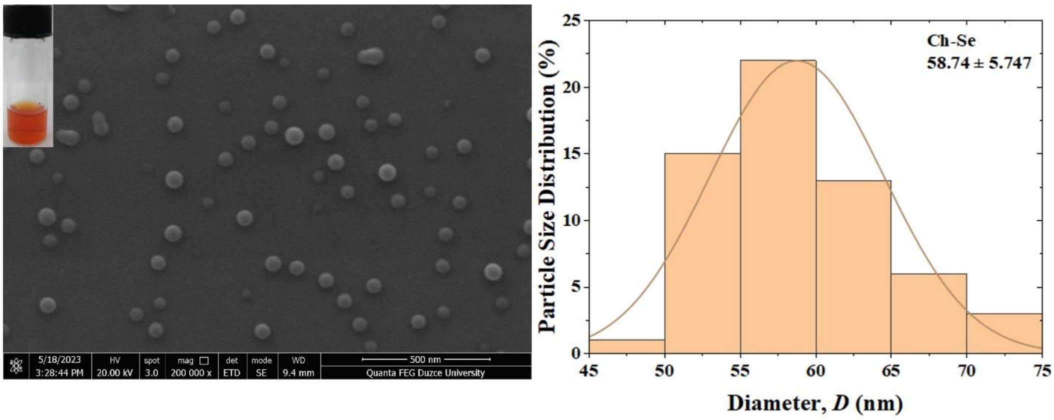

Figure 4.15. SEM image and size distribution of Chitosan-Selenium NPs without quercetin (Ch-Se).

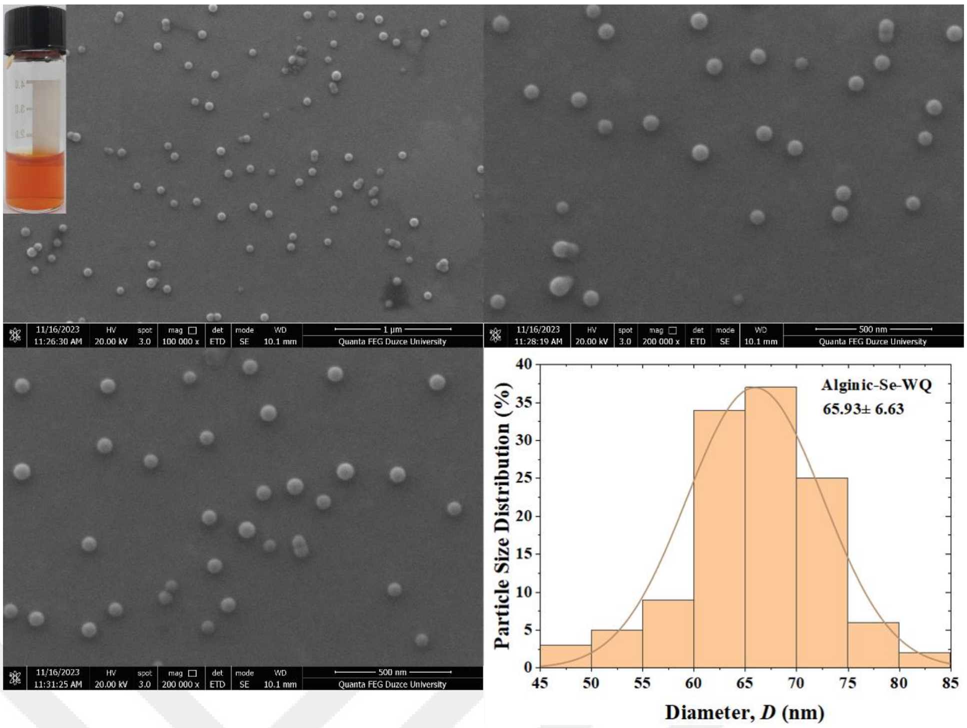

Figure 4.16. SEM images and size distribution of alginic acid sodium salt-selenium NPs with quercetin (Alg-Se-wQ).

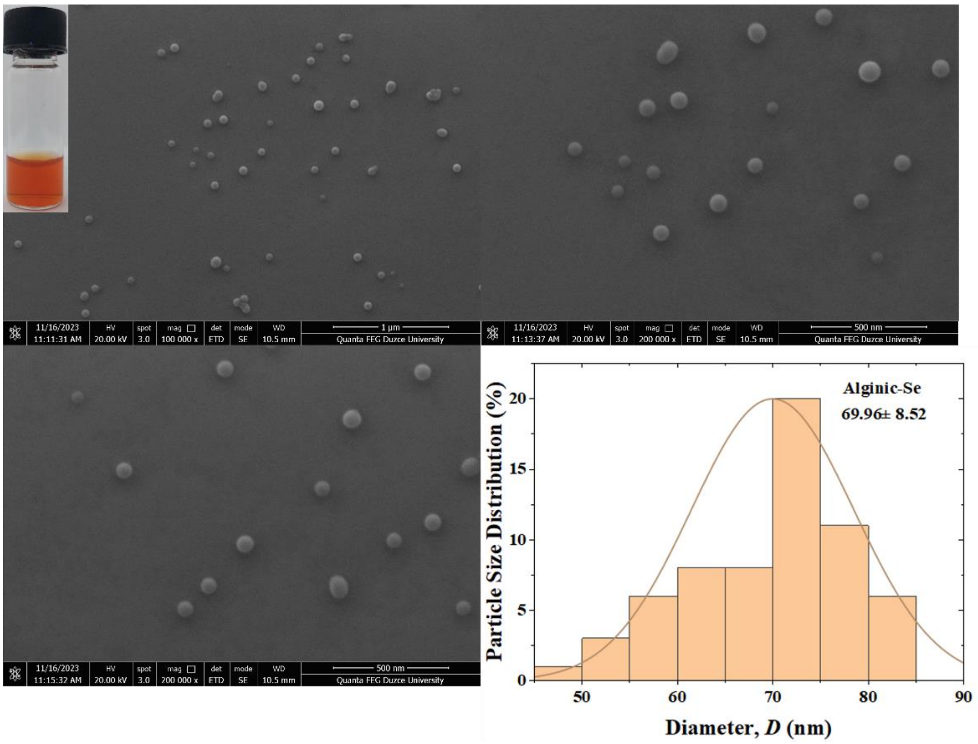

Figure 4.17. SEM images and size distribution of alginic acid sodium salt-selenium NPs without quercetin (Alg-Se).

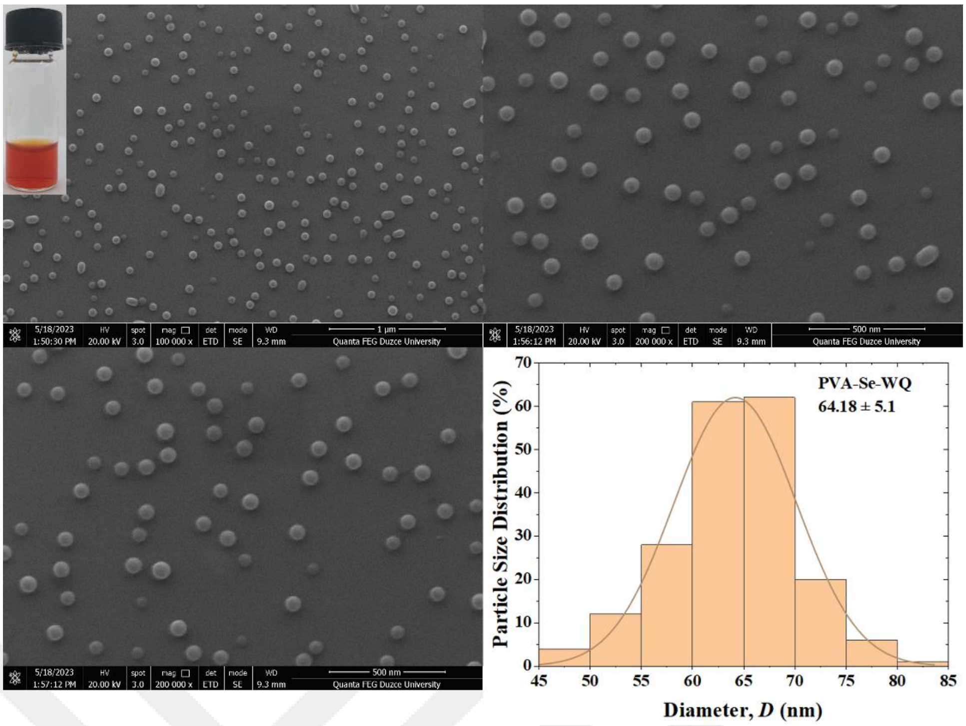

Figure 4.18. SEM images and size distribution of polyvinyl alcohol-selenium NPs with quercetin (PVA-Se-wQ).

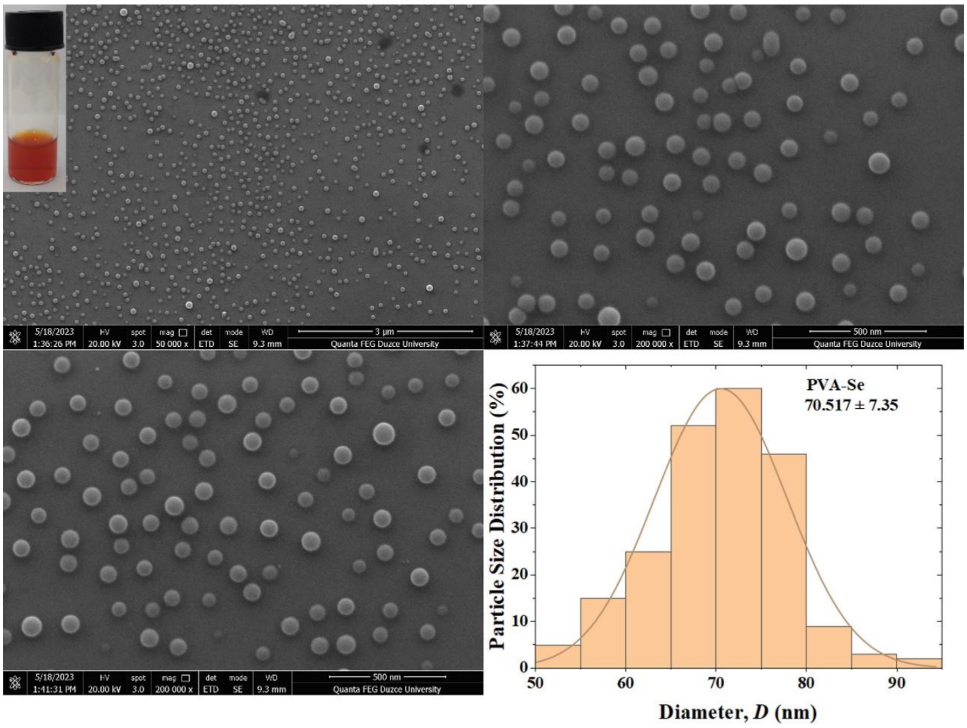

Figure 4.19. SEM images and size distribution of polyvinyl alcohol-selenium NPs without quercetin (PVA-Se).

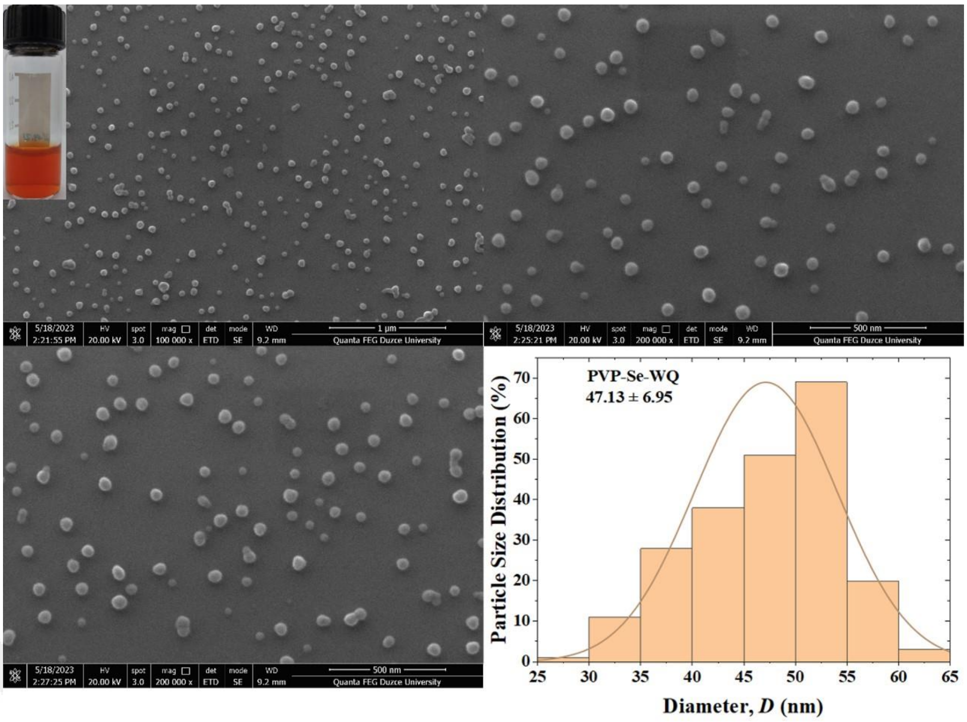

Figure 4.20. SEM images and size distribution of polyvinylpyrrolidone-selenium NPs with quercetin (PVP-Se-wQ).

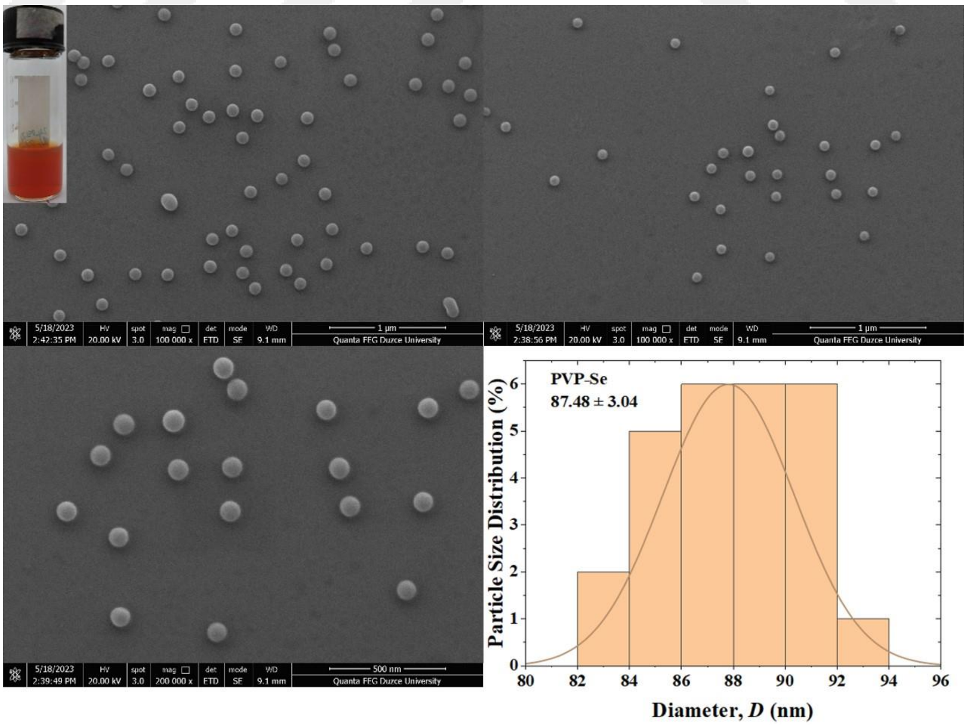

Figure 4.21. SEM images and size distribution of polyvinylpyrrolidone-selenium NPs without quercetin (PVP-Se).

4.2.5 Zeta Potential Measurements

In this study, zeta potential measurements were utilized to determine the stability and possible surface charge of Se NPs (18,150). Zeta potentials of the synthesized Se NPs were obtained by redispersing the NPs in pure water. In general, the zeta potential of nanoparticles depends on the effective electrical charge on the surface, the composition of the particles and the dispersion medium properties (17,123). Depending on the type of functional groups on the nanoparticle surface, positive zeta potential indicates that the particles are positively charged, while negative zeta potential indicates that the particles are negatively charged (42,151,152). Additionally, nanoparticles with a zeta potential higher than +30 mV or lower than −30 mV are considered stable colloidal suspensions (57,149). Table 4.1 shows the zeta potential values of Se NPs functionalized with some polymers and enriched with quercetin.

Table 4.1. Zeta potential values of Se NPs functionalized with polymers and with or without quercetin.

Se NPs	Zeta potentials / mV	Se NPs	Zeta potentials / mV
Ch-Se-wQ	+38.0	PVA-Se-wQ	−5.55
Ch-Se	+37.2	PVA-Se	−17.4
Alg-Se-wQ	−58.9	PVP-Se-wQ	−30.0
Alg-Se	−44.1	PVP-Se	−28.9

As can be seen from Table 4.1, chitosan- Se NPs with or without quercetin have a positive zeta potential above +30 mV. However, Alginic- Se NPs with or without quercetin, have a negative zeta potential below −30 mV. Therefore, these samples can be considered as stable colloidal suspensions. On the other hand, the zeta potential values of PVA- Se NPs are lower than ±30 mV both in the presence and absence of quercetin. In addition, the zeta potential values of PVP- Se NPs are at the limit. Therefore, it can be predicted that the nanoparticles will tend to lose their stability and aggregate in the long term.

4.3 Antibacterial Activity Measurements

Se NPs were evaluated for their antibacterial potential against *S. aureus* (ATTC 25923). The minimum inhibitory concentration (MIC), which is the lowest concentration to inhibit the growth of an organism (41,153), of various Se NPs were measured, Figure 4.22 and 4.23. After comparing the growth in each well with the growth control, MIC values were determined to be the concentration that suppresses 80% of the growth in each well. Results from the microdilution assay showed that all tested Se NPs solutions exhibited high antibacterial activity. For PVP-Se-wQ with a concentration of ~50 µg/mL showed the best inhibition (over 80%) against *S. aureus*. Additionally, high inhibition (76%) of PVP-Se-wQ was also found at lowest concentration (~6 µg/mL). On the other hand, PVP-Se, Alg-Se-wQ and Ch-Se-wQ showed 80% inhibitions approx. at same concentrations (~36 µg/mL) when compared with other nanoparticles. Besides, another high inhibition (75%) was observed with low concentration (~5 µg/mL) of Ch-Se-wQ nanoparticles. Similarly, Ch-Se nanoparticles showed 76% inhibition at a low concentrations (~5µg/mL). PVA-Se showed 80% inhibition even at its lowest concentration (~23 µg/mL). Similarly, PVA-Se-wQ showed 70% inhibition at its lowest concentration (~17 µg/mL) in this assay.

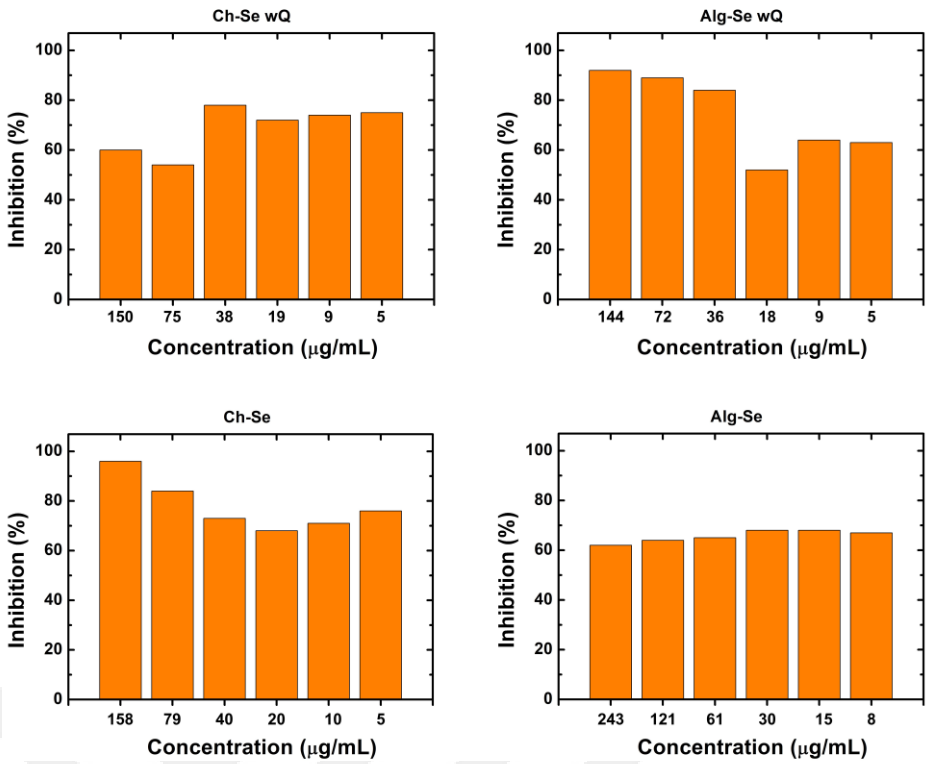

Figure 4.22. Inhibition (%) assay results for *S. aureus* with different concentrations of Ch-Se-wQ, Ch-Se, Alg-Se-wQ, and Alg-Se NPs, respectively.

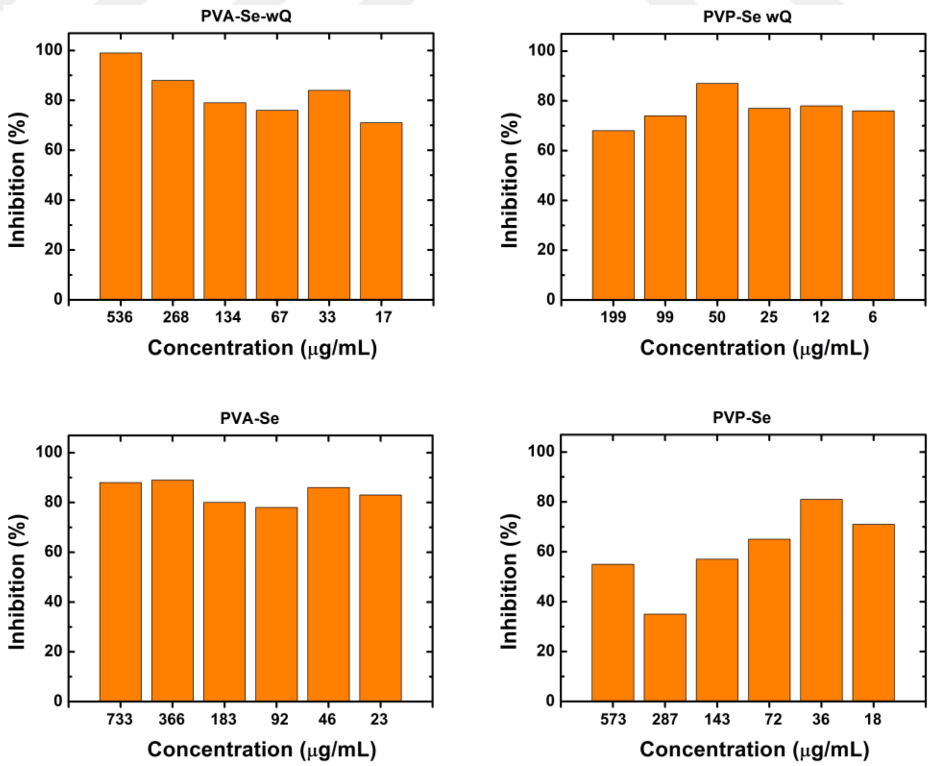

Figure 4.23. Inhibition (%) assay results for *S. aureus* with different concentrations of PVA-Se-wQ, PVA-Se, PVP-Se-wQ, and PVP-Se NPs, respectively.

5. CONCLUSIONS AND RECOMMENDATIONS

In this study, the synthesis of colloidal selenium nanoparticles (Se NPs) was successfully carried out with the help of chemical method and using polymers as capping and stabilizing agents. A total of four polymers were used in the production of Se NPs: two natural polymers, chitosan (Ch) and alginic acid sodium salt (Alg), and two artificial polymers, polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP). In addition, these syntheses were carried out with the addition of quercetin (Q), which is thought to have a positive effect on the effectiveness of Se NPs functionalized with polymers. Se NPs functionalized with polymers (and with Q) were characterized with the help of various techniques such as UV-Vis, SEM, XRD, Zeta potential and FTIR spectroscopy.

UV-Vis measurements showed a characteristic peak with a maximum in the range of 200-300 nm for all Se NPs, consistent with the literature. According to the SEM results, it was observed that all Se NPs had spherical shapes and a homogeneous distribution. The average particle size of Se NPs was found to vary between 47.13 nm and 87.48 nm. This result demonstrated that the use of polymers/quercetin as coating and stabilizing agents in the process effectively regulates the size and distribution of nanoparticles. When Se NPs obtained with quercetin were compared to those prepared without quercetin, it was observed that quercetin led to smaller average particle size for all Se NPs except chitosan. Among them, PVP had the highest difference in particle size between nanoparticles with and without quercetin. It was concluded that this significant difference in particle sizes for PVP-Se (87.48 nm) and PVP-Se-wQ (47.13 nm) could be due to the higher solubility of quercetin in PVP compared to the other polymers used. According to the XRD results, it was confirmed that Se NPs have an amorphous structure, which has been shown in many studies in the literature. Zeta potential results revealed that chitosan samples with and without quercetin exhibited a positive zeta potential. However, other Se NPs, prepared with Alg, PVP and PVA (containing with or without quercetin) showed a negative zeta potential. In addition, it was observed that almost all Se NPs, except PVA, had a high zeta potential value and were quite stable. Thus, it was understood that the polymers successfully stabilized the nanoparticles and prevented them from agglomeration. Finally, the antibacterial activities of all Se NPs were tested against *S. aureus* bacterium, and the results from microdilution assay showed that all tested samples exhibited high antibacterial activity. According to the preliminary results from these experiments, it was concluded that colloidal Se NPs functionalized with Ch, Alg, PVA and PVP in the presence or absence of quercetin may show promising therapeutic abilities against some pathogenic bacteria. It will be important to conduct in vivo studies to understand the location of nanoparticles in the cell and their mechanism of action.

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